Soybean Research for Sustainable Development

WSRC 11
World Soybean Research Conference
18 – 23 June 2023
Vienna | Austria

Abstracts

World Soybean Research Conference 11

18-23 June 2023, Vienna, Austria

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Editors

Soybean Research for Sustainable Development

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18-23 June 2023
Vienna, Austria

University of Natural Resources and Life Sciences, Vienna, Austria

Editors
Johann Vollmann, Marjana Vasiljević, Leopold Rittler, Jegor Miladinović, Donal Murphy-Bokern

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Foreword

At present, the world community is facing substantial changes of different nature which have a considerable impact on the global food and nutrition situation: Man-made climate change is becoming clearly visible and is negatively affecting food security, while disruptions and changes in global supply chains also contribute to increased food insecurity as well as economic instability. The soybean stands in the middle of these processes, as it is and continues to be the most important protein and oilseed crop of the world, which is utilized for the production of livestock feeds, numerous human food components, and non-food products. Due to a global rise in the consumption of meat and dairy products during previous decades, the massive expansion of soybean production was partly associated with increased greenhouse gas emission, deforestation, and loss of biodiversity. Because of this, present soybean production is experiencing harsh criticism from science and civil society.

Therefore, the timely motto of the World Soybean Research Conference 11 is "*Soybean research for sustainable development*". As research and development are the major engine behind any progress in crop production, agricultural research is urgently needed to mitigate the negative effects of climate change and to minimize the impact of soybean on the environment towards a fully sustainable soybean production in the near future. This includes a reduction of production inputs through improvement of agronomic and genetic input efficiency, new pathways in livestock feed uses, and finally an enhanced direct utilization of soybeans in human foods. The five major themes of the conference are (i.) breeding-genetics-genomics-biotechnology, (ii.) food-feed-nutrition, (iii.) agronomy-physiology-agrotechnology, (iv.) weeds-diseases-pests, and (v.) business-policy-market issues, which will be elaborated in over 30 scientific parallel sessions, 4 plenaries and 2 poster sessions. The versatility of approaches to sustainable soybean production is well reflected by miscellaneous contributions from fields such as genetic diversity, genomics-assisted soybean breeding, genome editing, stress and climate change mitigation, novel developments in soy-foods and feeds including chemistry, nutrition and health, agro-technology and data-driven crop and soil management, organic soybean production, seed science, the soybean rhizosphere, progress in the management of weeds, diseases and pests, as well as policy and environmental sciences.

The WSRC 11 organizers have received over 500 scientific contributions, and the most outstanding scientists from all major soybean producing countries including China, the United States, Argentina, Canada, Brazil, India, Ukraine, France, Serbia, Japan and South Korea will share and discuss their latest results. In addition, reports from all world regions with significant soybean production will provide unique insights into specific issues of soybean cropping and utilization. Dedicated workshops have been organized to address specific needs such as soybeans for Africa, or soybean breeding for organic farming. In the time since the last World Soybean Research Conference, huge progress has been achieved in the utilization of genomic information, in data-driven agronomic decision-making as well as in various other aspects of soybean biology including the interaction with stress factors. Thus, the conference aims at fostering the exchange of new knowledge and stimulating collaborative research among the world scientific community. Apart from oral presentations, poster sessions and an industry exhibition provide unique opportunities for personal interaction between the conference participants. Posters in particular are covering a very wide range of specific topics and research questions, and therefore poster sessions are a major source of exchange and scientific inspiration.

I am grateful to all conference chairpersons, members of the international scientific committee, members of the WSRC continuing committee and to all scientific contributors for their excellent inputs to compile an outstanding program, which delivers both a comprehensive overview as well as numerous specific highlights and new landmarks of soybean research. I also thank all sponsors, industry representatives and exhibitors for their valuable support of this conference. And most particularly, I acknowledge the help of the two WSRC organizing teams of the earlier conferences to be held in Savannah, GA, United States and Novi Sad, Serbia which had to be cancelled due to natural disasters; both teams were generously sharing their experience and preparation work they had carried out before.

During the historical Vienna World Exposition of 1873, soybean made its first legendary appearance on the international stage. In 2023 - 150 years later - the soybean community is back in Vienna. I wish all participants of the World Soybean Research Conference 11 many inspiring encounters and helpful insights for meeting the challenges ahead of us.

Johann Vollmann
Chair, International Scientific Committee, WSRC 11

Vienna, June 18, 2023

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Soybean Lifetime Achievement Awards

The World Soybean Research Conference 11 is recognizing outstanding achievements in soybean research to the following award-winning individuals from the different WSRC regions:

Region I
Thomas E. Carter, Jr.
Randall L. Nelson

Region II
Ademir Henning
Eligio Morandi (*in memoriam*)

Region III
Ruzhen Chang
Philips Varghese (*in memoriam*)

Region IV
Keisuke Kitamura
Arnold Schori

Region I: **Thomas E. Carter, Jr.**

Dr. Thomas Carter, former Research Leader & Research Geneticist, USDA-ARS and Professor at North Carolina State University, has made significant contributions to the field of soybean breeding and genetics, with a career spanning over four decades. Throughout his tenure, Dr. Carter has published 170 scientific papers and released 66 soybean varieties/germplasm, making him a prominent figure in the field. Dr. Carter is a highly recognized leader in genetic diversity. His 113-page monograph on the subject is the first comprehensive review of diversity in soybean. He was the first to show evidence that advancements in U.S. soybean breeding was limited due to insufficient genetic diversity. His findings revealed that the common practice of mating closely related varieties was creating a genetic bottleneck. To address this issue, Dr. Carter spearheaded research efforts to incorporate Asian varieties into breeding programs, tapping into a reservoir of yield genes. This initiative resulted in a significant shift among U.S. soybean breeders, leading to the development of high-yielding lines, some of which are among the highest yielding lines in the South. Dr. Carter's contributions extended beyond genetic diversity. He discovered the first field-proven drought-tolerant soybean and successfully utilized it to produce drought-tolerant progeny. This breakthrough led to the release of N8002, the first cultivar with drought tolerance traits. Additionally, Dr. Carter made important discoveries and validations of QTLs associated with the slow-wilting trait, bridging theoretical science with applied field research. Recognized for his outstanding achievements, Dr. Carter was named the USDA South Atlantic Scientist of the Year in 2010. His research paper defining and describing the genetic base of North American soybeans ranks among the top 10 most cited papers in Crop Science. Beyond his research, Dr. Carter has been a dedicated educator and mentor of 14 graduate students and 13 postdocs. Dr. Thomas Carter's contributions have significantly impacted soybean research and breeding efforts.

(Istvan Rajcan and Zenglu Li)

Region I: **Randall L. Nelson**

Dr. Randall Nelson, a former Supervisory Research Geneticist and Curator of the USDA Soybean Germplasm Collection, research leader at Agricultural Research Service (USDA-ARS) and professor at University of Illinois, made extraordinary contributions to the field. During his 28-year tenure, he significantly expanded the collection from 12,964 to 22,232 accessions and successfully merged the Northern and Southern Collections in 1991. Dr. Nelson introduced computerization to the collection's operations, greatly enhancing efficiency. His leadership and vision led to the establishment of a core collection, enabling researchers to focus their studies. He pioneered the use of DNA markers to evaluate germplasm and played a crucial role in characterizing 20,087 annual accessions with SoySNP50K chips, marking the first extensive genotyping of a major collection. Dr. Nelson's efforts made the USDA Soybean Germplasm Collection the most thoroughly documented and widely utilized collection worldwide. He excelled in utilizing exotic germplasm in breeding, driving the modern breeding efforts to enhance yield. As a result, 75% of public breeders and major commercial companies employ lines developed by him. His work is vital to the soybean industry, as the current gene pool relies heavily on only 30 ancestors, resulting in limited genetic diversity and impeding genetic improvement. Dr. Nelson's innovative approaches led to the release of 22 high-yielding lines derived from 30 accessions, profoundly impacting commercial soybean breeding in the United States. Furthermore, he explored the potential of perennial Glycine species and wild soybean in developing experimental lines that outperformed their soybean parent. Dr. Nelson's contributions to genetic diversity research are reflected in his 145 refereed articles and 5 book chapters. In summary, Dr. Randall Nelson's leadership, creativity, and innovative use of germplasm have made a significant and lasting impact on soybean breeding and genetic diversity research.

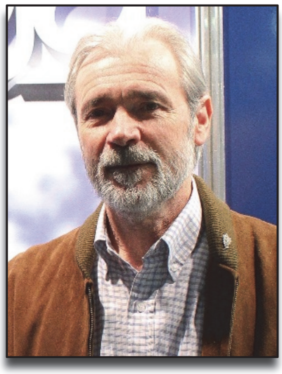
(Istvan Rajcan and Zenglu Li)

Region II: **Ademir Henning**

Dr. Ademir Henning, an accomplished Agricultural Engineer, has made significant contributions to soybean seed and plant pathology in Brazil. Graduating from the Federal University of Paraná, Brazil, in 1973, he pursued further education, earning a Master's degree in Phytopathology from Mississippi State University and a Ph.D. from the University of Florida in the field of soybean seed pathology. Dr. Henning began his career overseeing improved soybean seed production in Paraná in 1974 and joined EMBRAPA (Brazilian Agricultural Research Corporation), a renowned research organization, in 1976. From 1979 until his retirement in 2023, he developed important research projects and coordinated the Seed Pathology Laboratory at Embrapa Soja in Londrina, Paraná. Throughout his career, Dr. Henning conducted impactful research, leading to key public policies such as the official recommendation of fungicide for soybean seed treatment. Additionally, he played a pivotal role in modifying legislation by developing an alternative packaging for soybean seed storage, permitting the use of braided polypropylene (raffia) bags for seed storage and commercialization. Dr. Henning has been an active advocate of technology transfer, dedicating himself to the training and development of soybean seed professionals and students through the "DIACOM" course. Over the course of his career, he facilitated an impressive 71 theoretical and practical training courses on tetrazolium tests and seed pathology, both nationally and internationally. Dr. Henning's expertise extends to publishing scientific articles, documents, books, and book chapters on seed pathology and treatment. He mentored many undergraduate and graduate students in the field, fostering the next generation of experts. Recognized for his leadership, Dr. Henning actively participated in various soybean seed organizations, including serving as President of the Brazilian Association of Seed Technology (ABRATES) for two terms and holding positions in state and national commissions and sub-commissions related to soybean seeds.

(Ricardo Abdelnoor)

Region II: **Eligio Morandi (*in memoriam*)**



Eligio Natalio Morandi, born in 1946 in Cañada Rosquin, Argentina, was a prominent figure in the field of Plant Physiology. He obtained his degree from the Faculty of Agronomy at the National University of La Plata and later joined the Faculty of Agricultural Sciences at the National University of Rosario, where he dedicated his entire teaching and research career to the Plant Physiology Department. He furthered his studies at Michigan State University. Throughout his career, Eligio demonstrated a strong commitment to mentoring. He trained and supported over twenty researchers, interns, and graduate students, providing them with knowledge, ideas, equipment, and manuscript reviews. He played a guiding and supportive role in their development, leaving a lasting impact on their paths. Eligio achieved national and international recognition in Plant Physiology. He served as Principal Researcher of the National Science and Technology Research Council (CONICET) and held positions as president and vice president of the Argentine Society of Plant Physiology. He authored and coauthored numerous articles published in prestigious journals and actively participated in conferences and scientific congresses. His academic focus centered on the ecophysiology of soybean. He was a founding member of PROSOJA, an organization that brought together soybean breeders and researchers in Argentina. Additionally, he played a crucial role in the Argentine Soybean Chain Association (ACSOJA) and represented Region II in the WSRC Continuing Committee. Beyond his professional accomplishments, Eligio was known for his loyalty and unpretentious personality. He approached research projects with unwavering optimism and determination, consistently overcoming obstacles. He had a remarkable ability to convince others that what seemed impossible could be achievable. Eligio's impact extended beyond academia; he was a true friend to those around him. Those fortunate enough to have worked alongside him cherish his legacy as a university professor who nurtured intellectual growth. He remained dedicated to his work until the end, and his presence and legacy will endure, transcending physical boundaries and defying reality itself.

(Daniel Ploper and Julio Ferrarotti)

Region III: **Ruzhen Chang**

Professor Ruzhen Chang, former director of the Soybean Specialty Committee in the Crop Science Society of China and professor at the Chinese Academy of Agricultural Sciences, made great contributions to the research of soybean germplasm. He led the collection of >5,000 wild soybean accessions, which deepened the research on wild soybeans in China. He also developed a plan for collection, preservation, and evaluation of >20,000 cultivated soybean accessions in China and introduced over 1,000 isogenic lines and genetic materials from other countries, making China the largest preservation country of soybean germplasm in the world. Professor Chang also directed the establishment and utilization of the Chinese soybean core collection, which greatly supported the construction of the first wild soybean pan-genome and discovery of numerous genes for the traits of importance. He also devoted himself to the innovative uses of soybean germplasm and developed a number of elite lines and varieties with excellent characteristics. The soybean variety Zhongpin 661 that he developed has become the core ancestral parent in the soybean breeding programs in the Huang Huai Region of China, which has contributed to the development of more than 19 excellent new varieties. With his great contributions to soybean research, Professor Chang won 10 national and provincial awards and he published 10 book chapters, and authored >100 research papers in peer-reviewed journals over the last 45 years.

(Xiaobing Liu)

Region III: **Philips Varghese (*in memoriam*)**

Dr. Philips Varghese, a Soybean Breeder and Scientist-D at MACS - Agharkar Research Institute in Pune, Maharashtra, India, has devoted his career to soybean research. After receiving his post-graduate degree in 1982 from Savitribai Phule Pune University, Pune, he began his career in the Department of Genetics and Plant Breeding at Agharkar Research Institute, Pune - a renowned institute under the Department of Science & Technology, Government of India. He was awarded a Ph.D. degree in Botany as an in-service candidate in 1994 from the same University. He has worked on many crops such as wheat, sunflower, gram, winged bean, and French bean, among others. Later, he focused on breeding soybeans for high yield, disease resistance, oil content, and heat insensitivity. During his tenure, he handled five major projects on soybean crop improvement. He developed and released seven high-yielding, disease-resistant, and non-shattering types of soybean varieties suitable for mechanical harvest. Some of his developed varieties include the high-yielding and popular 'MACS 1188', the drought-tolerant 'MACS 1281', the early-maturing, widely adaptable, and high-yielding 'MACS-1460' and 'MACS-1520', and the high-yielding, non-shattering variety 'MACS-1520' for the Central Zone of India. Recently, he successfully used molecular marker-assisted breeding to develop and release the next generation soybean variety 'MACSNRC1667', which is trypsin-free, benefiting both farmers and the soy-food industry. These varieties have been released for cultivation throughout India, leading to the popularization of soybean among farmers, and are being cultivated by farmers with high demand for their seeds every year. He was involved in the collection, evaluation, and management of soybean germplasm, as well as the production of quality nucleus and breeder seeds of soybean for supply to seed multiplying organizations in India.

(Gyanesh Kumar Satpute)

Region IV: **Keisuke Kitamura**

Dr. Kitamura is a soybean researcher who worked at Iwate University, the Agricultural Research Center of the Ministry of Agriculture, Forestry, and Fisheries, and the National Institute of Crop Science, NARO, before becoming a professor at Hokkaido University in 2003. In the 1980s and 1990s, he worked on improving soybean proteins at Iwate University and the Agricultural Research Center. As a result, he discovered many protein mutants, including a soybean lipoxygenase deletion mutant, and elucidated their inheritance patterns. He also bred a soybean cultivar called "Yumeyutaka," which lacks two of the three soybean seed lipoxygenases, and succeeded in producing soy milk without the beany flavor for the first time in the world. The results of this research later led to the creation of the first soybean deficient in all lipoxygenases. In addition to his work on protein improvement, he has also led research on soybean isoflavone. He found that seed isoflavone content varies among varieties and that isoflavone content varies significantly with temperature during the ripening period. His findings provide the theoretical basis for producing soybeans with high isoflavone content in cold climates. Furthermore, he worked at Hokkaido University to improve the functional components of soybeans, finding genetic resources with high α-tocopherol and lutein content and elucidating their mode of inheritance. Inspired by his work, many researchers around the world are now working on improving the composition of soybeans. By improving their composition, the uses of soybeans are expected to expand dramatically and contribute to improving the quality of human life. He retired from Hokkaido University in 2010, but his students and associates are now at the core of soybean research worldwide.

(Makita Hajica)

Region IV: **Arnold Schori**

(Photo credit: Agroscope)

Dr. Arnold Schori has made valuable contributions towards a better adaptation of early maturity soybeans to Central European growing conditions. Arnold Schori was born in Switzerland in 1957. He received his PhD from ETH Zurich (Swiss Federal Institute of Technology in Zurich), Switzerland in 1994. His doctoral research focused on the contribution of genetic improvement to increasing cold (chilling) tolerance of soybean during the reproductive stage. Dr. Schori successfully identified genotypes capable of compensating for the loss of flowers and small pods after a cold spell through a reduced abscission rate and asynchronous development of lateral racemes. He demonstrated that the asynchronous blooming type is closely linked with tawny pubescence. This discovery led to the establishment of an effective system for selecting soybean varieties with cold stress tolerance. From 1985 onward, Arnold Schori worked in soybean breeding at Agroscope, the Swiss Confederation's center of excellence for agricultural research, located in Changins. Throughout his professional career, he had various responsibilities, including wheat and triticale breeding, as well as genetic resources management. Despite these additional duties, Dr. Schori managed to maintain a successful soybean breeding program that was originally initiated by Nestlé in 1981 in response to the global protein crisis of the 1970s. The program utilized starting materials that were partly sourced from Canada and Sweden which had their origins in far east Russia, where they had been adapted to the challenging conditions of Sakhalin, Hokkaido and Kourils, some west Pacific islands. Dr. Schori's soybean cultivars, characterized by their productivity and early maturity, have significant relevance in many high-latitude countries such as France, Germany, Poland, Austria, Switzerland and others. He also worked on soybean food quality and developed cultivars with reduced lipoxygenase activity for improved taste of soy-food products. His cultivars have made a substantial contribution to the development of soybean production and adaptation in central Europe and beyond. Furthermore, his selection methods for cold tolerance could potentially serve as a model for future approaches to developing abiotic stress tolerance, such as drought or heat tolerance.

(Johann Vollmann and Claude-Alain Bétrix)

Plenary presentations

Opening: Soybean research for sustainable development

European soybean

Soybean in agricultural systems

Regional soybean reports

Optimization and minimization towards sustainable soybean production

Jingyuan Xia[1]

[1]*Plant Production and Protection Division (NSP), Food and Agriculture Organization of the United Nations (FAO), Rome, Italy*

Opening plenary

Soybean (*Glycine max* [L.] Merr.) is an important oil-bearing crop, which was originally domesticated in northeastern China. Soybeans have been part of the Asian diet for millennials and are consumed in many forms, while in the Americas and Europe the crop is mainly used to produce edible oil and animal feed. The production of soybean reached 371 million tons in 2021, making it the most important legume crop in the world. Soybean is a multi-functional crop, which provides food, feed, fertilizer and fuel (biofuel), as well as supports livelihoods in particular for smallholder farmers. Therefore, soybean contributes directly to achieve multiple Sustainable Development Goals (SDGs), such as Zero hunger (SDG 2), Good health and well-being (SDG 3) and Responsible consumption and production (SDG 12).

The soybean production is, currently, facing with five major challenges: 1) the diminished arable land resource, because expansion of soybean production has caused server losses of forests and savannahs; 2) low use efficiency of resource, such as 60 percent of applied N remains unused or lost from soil; 3) poor resilience to stresses as some soybean cropping systems are more vulnerable to external shocks from pests and diseases, as well as droughts and floods; 4) weak agricultural extension services, which result in low adoption rate of good agricultural practices; and 5) ineffective enabling environment, including the inadequate investment and lack of coherent policies and regulatory frameworks to support farmers who desire to adopt new technologies.

The key strategy for promoting sustainable soybean production is: to optimize all the positive aspects of soybean cropping systems while minimize all the negative positive aspects of the systems. These should include: 1) increasing in recourse use efficiency in particular the water and fertilizers; 2) building more resilient and diversified soybean cropping systems; 3) promoting application of innovative technologies such as alternative for mineral fertilizers; 4) fostering context specific agricultural extension services, such as Farmer Field Schools (FFS); and 5) creating enabling environment covering national framework and strategies, relevant international code of conduct, standards for good agricultural practices, incentive mechanism, and financial support.

Key technologies for sustainable soybean production cover: 1) adaption of improved varieties and production and supply of good quality seeds; 2) development and adoption of optimized soybean cropping systems resilient to biotic and abiotic stresses; 3) promotion of integrated pest and disease management; 4) enhancement of soil health management; and 5) large-scale extension of precision fertigation.

The key elements to promote future sustainable soybean production are composed of: 1) strategic planning based on priciples of optimatzation and minimization; 2) technical synergy at global regional and national levels; 3) multi-stakeholder engagement including research and academia, extension and education, NGO, private sector and development organizations; 4) knowledge sharing among different stakeholders; and 5) international cooperation, such as international guidance, international platform (network), international standards, and international working groups and coordination mechanism.

Keywords: Food security and nutrition, legumes, livelihoods, sustainable production

Classical and modern technologies used world-wide for sustainable soybean production

Istvan Rajcan[1]
[1]*University of Guelph, Guelph, Canada*

Opening plenary

Soybean (*Glycine max* [L.] Merr.) is the most important grain legume crop and the most widely grown oilseed with a substantial global economic impact. Soybean is grown primarily for protein in the seed which makes it an excellent source of animal feed and human food. The high quality oil adds to the range of uses, including for industrial purposes. Although global soybean production has grown over twofold in the past decade, climate change, shifting weather patterns, and the growing global human population are expected to pose major challenges to developing environmentally resilient, high-yielding cultivars that are capable of withstanding ever increasing biotic and abiotic stresses. Sustainable soybean production depends on the use of a range of technologies within cropping systems and agronomy programs as well as modern tools such as genomic and high throughput phenotyping that are used in support of soybean breeding. A review of different technologies currently used to enhance sustainable soybean production will be provided along with specific research examples.

Keywords: Soybean production, climate change, agronomy, breeding, research, technology

Production and processing of soybean in Europe

Leopold Rittler[1]
[1] *Donau Soja, Vienna, Austria*

European soybean

Europe is re-discovering grain legumes to diversify cropping systems. This is supported by growing consumer interest in locally-resourced food, enterprising farmers, and innovative plant breeders. European soybean production (incl. European part of Russia) increased recently from approximately 2.2 to 4.7 million ha (2011 – 2022). This contribution provides an overview of the status of soybean production and processing in Europe and outlines possible growth potentials. In 19th century, the soybean was introduced from China to Europe where it grows well in most of the main cropping regions. The Danube river catchment stretching from southern Germany, across Austria and along a basin with highly fertile chernozem soils is particularly relevant. Production is commonly linked to dedicated soy-based value chains where there is a premium for crop produce of known origin grown to European Union production standards. Corporate social responsibility in the food and feed sector is an important driver for the soybean development.

The place of soybean in the European agricultural research system

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European soybean

This presentation will reflect on Europe's agricultural research system. Technical progress from national research set up after 1945 and public policy through the CAP (established in 1962) supported increased agricultural production. By 1980, these state-based structures faced changing and international societal goals. Against this background, Europe's Framework Programmes have fostered a European Research Area since 1984. At nearly 100 billion euros, the current Framework Programme (FP, Horizon Europe) is one of the largest investments in civilian research world-wide. FPs have fostered very active and open international agricultural research and innovation communities. As part of the reform of Europe's Common Agricultural Policy (CAP), European agricultural research investment is now linked to the CAP through the European Innovation Partnership (EIP Agri/EU CAP Network). Since about 2000 in particular, EU investment has become increasingly oriented towards innovation and European policy goals, currently most clearly set out in the Farm to Fork Strategy and the Biodiversity Strategy under the European Green Deal. There is no European research programme for soybean as such, but the EU has played a decisive role in investing in research for legume-supported cropping systems and value chains now including soybean. We now have a very active, competitive, and well-connected soybean research and innovation community in Europe.

Changing protein patterns

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European soybean

The expected developments on protein usage and production in Europe will be highlighted. Turbulent times will have its impact on the position of protein utilization and production all over the globe. Not only the world shows turbulent times, but also Europe is very turbulent, as the Ukraine crisis is impacting raw material supply for a longer period now already. In combination with the ambitions of the EU Green Deal, development of regenerative agricultural techniques and the de facto non-GM policy of Europe is leading to the fact that geopolitical considerations concerning food self sufficiency are on the agenda again. And one of the important raw materials in the middle of this is soybean. Not in all areas of Europe is protein cropping that profitable, but from governmental and political movements it is clear it will be stimulated. Not so much the direct support of it will stimulate the production, but many regulations are stimulating soy and other protein production. But, without a proper plan protein production in Europe will be develop slowly, although society does have high expectations of it. Some of the aspects of such a plan will be highlighted.

Soybean breeding in Europe: methods, sources, and utilization

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European soybean

In past few decades, soybean breeders in Europe have been successful in producing a large number of commercial varieties using conventional breeding methods, most of all single seed descent (SSD) method with various modifications. As a supplement to conventional breeding methods, number of breeding tools like high-throughput phenotyping, marker assisted selection, genomic selection, and others, are used to speed up the breeding process. Low genetic diversity is a major obstacle to soybean improvement. Therefore, projects between Europe and China, centre of origin of soybean, that involve germplasm exchange are intensified in recent years. Utilization of wild soybean species, especially perennial ones, bears a huge, still unused potential for improving soybean breeding process. Increased demand for non-GMO soybeans, led to intensifying of soybean research throughout Europe which resulted in the number of companies that offer commercial soybean varieties. The oldest and best-known among them are Lidea from France, Institute of Field and Vegetable Crops from Serbia, Agroscope from Switzerland, RAGT from France and a number of other companies which initiated soybean breeding programs later.

Keywords: Soybean, breeding, cultivar

Potentials and challenges of regenerative farming systems for climate change adaptation in crop production

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Soybean in agricultural systems

Regenerative farming aims to enhance soil health via nature-oriented management solutions that fosters microbial processes underlying essential soil ecosystem functions. In order to benchmark the ability of regenerative farming to restore properties and function of arable soils towards a natural state, we performed an on-farm assessment of a range of soil biological and physical descriptors in a pairwise comparison of conventional standard vs. soil-health oriented pioneer farming vs. natural reference sites at 20 pedo-climatically different locations in Austria. We could demonstrate that via enhancing easily degradable organic matter inputs (e.g. due to intense cover cropping and intercropping), pioneer farmers increased a number of microbial descriptors, particularly in coarse-textured soils, thereby approaching a state comparable to non-agriculturally used reference soils. Among others, these microbial properties induced changes in soil hydraulic properties with high relevance for climate change adaptation, such as the amount of plant available water. However, there was only a minor change in stable organic carbon that is expected to support climate change mitigation via soil carbon sequestration. Our results suggest that management elements characterizing regenerative farming systems primarily induce an increase in microbial cycling of organic matter, thereby driving significant improvements in soil structural properties with relevance to climate change adaptation. However, expectations on the extent of climate change mitigation via “carbon farming” has to be taken with care, even for the case of advanced regenerative farming systems aiming to restore the soil health state of undisturbed natural soils.

Soybean within the no-till system: a farmers' perspective

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Soybean in agricultural systems

One of the great challenges of agriculture today - if not the greatest - is the transition towards production models with less environmental impact, capable of sustaining and increasing the production of food, fiber and energy in a context of climate change, and of give guarantees of this to a population that demands it. It is possible to identify 3 key factors in achieving this goal. The first is the systemic view, where each productive establishment is approached as a "whole" and each input, technology and crop interacts with the others, generating effects on each other. When soybeans are conceived as an element within a rotation intensification and diversification plan managed under no-tillage, a balanced nutrition strategy and integrated pest management, it can contribute to the system as a whole in aspects such as: improving the balance of carbon and the nitrogen content, biological and physical fertility of the soils, reduction of the environmental impact and soil erosion, increase of the potential and stability of the yields of the sequence and of the economic margins. A second factor is the inclusion of the producer as a key actor in the development, adaptation and spreading process of any innovation that seeks a more sustainable agriculture. Aapresid has been implementing collaborative innovation projects for 30 years based on: 1) the exchange of experiences between producers at the local level; 2) the identification of common problems; 3) testing solutions in real production scenarios, where the producer allies with science and companies; and 4) the application and scaling of these solutions anticipating future scenarios. This methodology was what allowed, for example, the expansion of no-till technology in Argentina, which today occupies 90% of the national agricultural area and positions the country as a leader in soil protection against erosion. The third factor is the development of mechanisms that provide consumers with guarantees of sustainable origin of food, fiber and energy. In the soybean chain, the focus of the discussion is on no deforestation. It is time to give seriousness to the debate and go further, putting broad aspects such as the carbon footprint on the table. Along this path, scientific support in the development of solutions as well as the adoption of serious certification schemes must be non-negotiable conditions.

Defining regenerative agriculture and opportunities for soy

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Soybean in agricultural systems

Regenerative agriculture is, mostly simply defined, a method of farming that improves the environment and regenerates the soil whilst producing a crop. But what that means in practice is not clearly defined and is open to interpretation. Through discussion of regenerative agriculture techniques and a review of what measures of success - or regeneration - might look like, we can draw conclusions about what opportunities regenerative agriculture could bring to soy production. As part of this it is essential to consider the impact of customer demand and the changing policy environment, particularly in relation to agricultural (Scope 3) emissions, which places pressure on retail markets to demonstrate the sustainability of their agricultural supply chains. Understanding what claims soy growers could make about their agricultural practices, and what outcome measures should be used to correctly identify positive impact and avoid claims of 'greenwashing' is also critical in assessing the opportunities offered by regenerative agriculture.

United States report

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Regional soybean reports

Soybean is an important crop in the United States (US) and only second behind maize in value and planted area. During the 2021 crop season, soybean was planted on over 35.3 million hectares, yielded an average of 3.5 metric tons ha^{-1}, produced 121 million metric tons, and had a value of $46 billion. Increases in soybean production in the US during recent decades were largely driven by improvements in grain yield. During the twenty-year period between 2003 to 2022, yields in the US increased at a rate of over 46 kg ha^{-1} $year^{-1}$. This rate is considerably greater than observed during the period from 1924 to 2022, which was 24 kg ha^{-1} $year^{-1}$. These yield increases are mostly driven by the genetic improvement of cultivars, which is fueled by investment in breeding by the commercial seed industry. Other general trends observed in the US include a general decrease in seed protein concentration and an increase in oil concentration over years as breeders have selected for increased yield. US soybean production is geographically shifting north and west with production expanding in the states North and South Dakota, which have become major soybean producers. Soybean remains largely a commodity crop although there continues to be interest in cultivars with value added traits such as altered fatty acids and high protein. Approximately 95% of the soybean area in the US is planted with GMO seed.

Canadian soybean regional update

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Regional soybean reports

The Canadian soybean industry has experienced remarkable growth since its introduction in 1893. By 2022, the cultivation area and production of soybeans have expanded to exceed 2.1 million hectares. The majority of soybean cultivation takes place in Ontario, Quebec, Manitoba, with smaller areas in Saskatchewan and the Maritimes. While genetically modified (GM) herbicide-tolerant crops dominate soybean production, particularly in the western provinces, a noteworthy portion, ranging from 20 to 25%, in Ontario and Quebec is dedicated to non-GM soybeans. These non-GM soybeans are primarily grown as identity preserved (IP) food-grade crops for export markets. Ontario and Quebec have developed a solid market strategy and established a reputable presence in European and Asian markets, enabling them to sustain a consistent demand for non-GMO Canadian soybeans. The cooler climate in these regions contributes to higher protein content in the soybeans, further enhancing their desirability. The development of soybean seeds in Canada benefits from a vibrant research sector consisting of both private and publicly funded plant breeders. This collaborative effort ensures that Canadian growers have access to a diverse range of soybean varieties, each tailored to specific environmental conditions, resistant to diseases, and tolerant of pests. This discussion will delve into the production, research, and innovation within the Canadian soybean industry, as well as explore the opportunities and challenges it faces in the current landscape.

Update and perspectives on the Argentine soybean chain

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Regional soybean reports

The Argentine agro-industrial complex represented 70% of the country exports, with soybeans being the main export complex, with 42% of it (INDEC,2022). Soybeans celebrate 22 years as the country's main exports complex, maintaining first place in global exports of soybean meal, soybean oil, being third in grain export and holding and important position in the biodiesel market .The grain is mainly exported to China, with many markets in soybean meal and concentrated in oil, in meal, Vietnam, Indonesia, Italy Spain, Poland and Malaysia stand out, with the EU accounting for 33%, and in soybean oil, India is the prominent market. More that 85% of the production of soybean is exported. Santa Fe province, at the gran Rosario city area, concentrated 80% of the crushing capacity, with 90% of the crushing of oilseed and more of the export of industrial soybean product. Plants can industrialize 70.4 million tonnes per year. The use of the active installed capacity of the oil industry would reach 61% in 2022. In the last 6 years, soybean acreage has been reduced by 20%, due to high export taxes and mainly relative to others crops. Soybean unit yields have been affected by several years of drought and the highest planting rate after wheat. The technical capacity to grow is intact. Despite not having resolved on a reasonable system on capture of value in the seed, and the breeding programs have been reduced, in 2022 the record for registering new cultivars was reached. The 98 % of the seed is GMO, and the genetic gain is maintained. Argentina is the first country to have specific regulations for products obtained through new breeding technologies. The main successes of soybean production are 90% zero-till, soil cover, crop rotation, multiple crops by year, precision agriculture, improved seeds, new molecules for agrochemical solutions, integrated pest management, satellite imaging, logistics innovations such as silobags, post-harvest management, efficient plant nutrition and precision weed management. Soybean in Argentine has a Carbon Neutral Program and soybean flow monitoring systems that provide evidence of risk in land use promoting planet's biodiversity. We believe that we have given sufficient evidence of the efforts that are made in the sustainable production of soybean.

Keywords: Soybean meal, soybean oil, soybean chain

Soybean production in Brazil: a report of sustainable growth, challenges and opportunities

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Regional soybean reports

Since 2019, Brazil is the leading soybean-producing country in the world, with 40% of global production. Brazil has doubled its production since 2013, with a 58% increase in crop area and a 26.6% boost in yield. Brazil is also the major soybean exporter, being China and the European Union the primary markets. In 2022, Brazil exported 78 Mt, corresponding to US$46.5 billion. Brazilian scientists were responsible for the “tropicalization of soybeans”, and technological advances have been paramount for the protagonism of Brazilian production. Examples are the use of direct sowing and biological fixation of nitrogen, in over 95% of the area. The observation of the recommendation for correction and fertilization, along with the use of pest and disease management constitute important tools. Soybean breeding programs – including new genetic tools – allowed the availability of new cultivars, with important traits like improved yield, resistance or tolerance to herbicides and to major soybean pests. The most important challenges are weather constraints, modulated by Global Changes and specifically by El Niño and La Niña cycles, which directly affect water availability to the crop. Pest management is a factor needing constant improvement in order to assure sustainable production systems. Other issues that need to be addressed are the continuous increment of the cost of inputs, at rates over the international soybean prices; and the adequate logistics, to keep pace with the continuous rising of soybean production. National yield contests demonstrated potential soybean yields close to 9 t/ha, compared to the national yield average of 3.8 t/ha, in 2023. The pursuit of yield increment is crucial for the sustainability of soybean production, by reducing the necessity for expanding cultivation areas and decreasing the use of inputs by each soybean ton produced while maintaining a good profitability for the growers.

Survey of the French soy industry

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In France, soybean production, which is GM-free, has grown significantly since 2010, with acreage increasing more than threefold. However, the supply of soybean, and more widely of vegetable proteins, is still way behind demand and acreage seems to stall for 5 years around 400 000 tons per year. Therefore, the French government launched a Protein Plan in order to enhance further development of protein crops. This plan has been an opportunity to study the French soy sector, both dedicated to feed and food. This work is based on almost 70 semi-directive interviews of chain operators, from breeders to processor and users. Associated with a producer-destined survey lead by the technical institute Terres Inovia, it will allow to display a comprehensive scheme of the French soy sector, at least on quantitative and qualitative scales. First, the study shows most crushing industries (≃ 40% of the outlet) are structured at a local level, and often associated to cooperatives for their supply and to feed manufacturers for their sales. Almost 100% soy cake produced is then used regionally to feed animals responding to quality specifications. Second, it reveals soyfood industries (≃ 10% of the outlet) totally supply themselves in France, with higher requirements on traceability and quality. Finally, as new crushing industries will start shortly, the biggest challenge will be to avoid supply tensions. Two leads can be pursued: capturing export flows (which currently represent a third of the production) and developing the acreage. A part of the study lead in a new production region (North-West) showed there are still technical and economic issues to address. Therefore, in the years to come, price will be determining for the development of soybean production. The dynamic variety selection will also be a lever as it will make better margins possible in new regions thanks to higher yields.

Current status and prospect of soybean consumption and supply in China

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As an ancient crop in China, soybean plays a major role in safeguarding protein and edible oil supply to the modern world. Since 1996 when China became a net importer, soybean demand in China has increased dramatically, driven by the growing need for feed, food, and edible oil. In 2022, China consumed 113 million tonnes of soybean, of which over 85% was used for crushing. In the harvest year of 2021/2022, China consumed 68.9 million tonnes of soymeal and 16.7 million tonnes of soy oil, accounting for 72.4% and 45.6% of protein meal and vegetable oil consumption in the country, respectively. About 16 million tonnes were used for food purposes, of which tofu and other fresh soy food processing consumed about 40%, and the percentage for liquid and soymilk powder was about 10% and 20%, respectively. In recent years, significant efforts have been made to develop domestic soybean production in China, resulting in a record output of 20.3 million tonnes in 2022. However, low self-sufficiency (<18%) and heavy dependence on the south (>60%) and north (>30%) Americas remain as big challenges. In 2022, China imported 91.1 million tonnes of soybeans (nearly 60% of the total global export), of which 59.7% was from Brazil and 32.4% from the US. It is projected that soybean consumption in China will continue to grow leading up to 2030, with an increase of about 40 million tonnes compared to 2022. China needs to take holistic measures to increase soybean production capacity, improve soybean use efficiency, and maintain international trade orders based on WTO rules to safeguard the soybean supply. Collaboration with Europe (the second soybean consumer in the world) and other partners is required to achieve a global move towards sustainable production, secure supply, and efficient use of soybean and protein.

Keywords: China, soybean, consumption, supply, challenge

Theme A:
Breeding, genetics, genomics, and biotechnology

	Sessions and workshops *(chair)*
A1	Soybean pangenome and genetic diversity *(Henry Nguyen)*
A2	Innovations in breeding technology *(Jegor Miladinović)*
A3	Genomics-assisted breeding *(Zenglu Li, Xia Li)*
A4	Seed composition *(Kristin Bilyeu, Jeong-Dong Lee)*
A5	Soybean genome editing *(Robert Stupar)*
A6	Breeding for abiotic stress and climate change *(Volker Hahn)*
A7	Germplasm collections and evaluation *(Li-Juan Qiu)*
A8	Breeding for biotic stress *(Istvan Rajcan, Khalid Meksem)*
W2	Introduction to SoyBase (soybase.org), the Soybean Breeder's Toolbox *(Rex Nelson)*
W5	Organic soybean breeding (ECOBREED) *(Vuk Djordjević, Uroš Žibrat)*
	Posters A: Breeding, genetics, genomics, and biotechnology

Global soybean variation map for enhanced haplotype-based trait mapping

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A1: Soybean pangenome and genetic diversity

Soybean serves as a major source of protein and oil globally. Insights into soybean domestication and identifying major trait linked loci accelerates genomics-based breeding. Here, we present a global variation map of 26.84 million single-nucleotide polymorphisms (SNPs) and 8.41 million insertions-deletions for 1252 soybean individuals, including 763 landraces, 294 cultivars, 30 breeders' lines and 159 wild (*Glycine soja*) accessions representing different maturity groups. We deployed a machine learning (VQSR from GATK) SNP filtering approach to obtain a high-quality SNP set for QTL discovery. These high-quality variations were imputed across 21,618 accessions genotyped previously with higher accuracy. Imputed data was used to identify markers associated with important traits using genome-wide association analysis. This variation map serves as a resource for identifying haplotypes linked with distinct phenotypes. The genome-wide deleterious mutations resulting in decreased fitness were identified. Genotype imputation with the variation map increases the mapping resolution for genome-wide association analysis. The variations (SNPs, InDels, structural variations) identified across diverse set of soybean lines were utilized for the pangenome analyses. The haplotype analysis revealed the impact of the interaction of copy number variants of the rhg1 and Rhg4 genes on broad-based resistance to soybean cyst nematode. We further utilized the pangenome resource to clone a major natural variation (WLT1) in waterlogging tolerance and RSA plasticity in soybean. Subsequently, the evolution and selection history of WLT1 was reveal by the pangenome-based phylogenetic analysis. Resources from this study will aid haplotype-based trait mapping in soybean and the identified deleterious mutations could be potential targets for gene-editing.

Evaluation and harnessing of diversity in soybean using the omics-based high-throughput strategies

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A1: Soybean pangenome and genetic diversity

The application of omics-based high-throughput strategies has revolutionized soybean genetic research. In this study, we utilized these advancements to investigate various aspects of soybean, including domestication, adaptation, trait dissection, and data sharing. Firstly, we employed cost-effective next-generation sequencing to analyze the genome sequence of 2,214 soybean accessions, revealing a comprehensive soybean evolutionary route. This involved the expansion of annual wild soybean (*Glycine soja* Sieb. & Zucc.) from southern China, followed by domestication in central China, and subsequent expansion and local breeding selection of its landraces. Notably, we observed that the genetic introgression in soybean landraces was mostly derived from sympatric rather than allopatric wild populations during the geographic expansion. Furthermore, we collected time-series canopy coverage data using an unmanned aircraft system for a diverse panel of soybean varieties, providing a more comprehensive understanding of trait dynamics regulation compared to single time-point measurements. By genome-wide association study of the time-series data, we successfully identified known flowering time and plant height quantitative trait loci (QTLs) reported in previous studies of adult plants. Additionally, we discovered novel QTLs associated with canopy coverage, with a notable bias towards early developmental stages. We also inferred the speed of canopy closure, an additional dimension of canopy coverage, from the time-series data, as it may represent an important trait for weed control. Lastly, we developed SoyFGB v2.0 (https://sfgb.rmbreeding.cn/), a website dedicated to sharing genotyping and phenotyping data. This platform serves as a valuable resource for researchers and breeders, offering tools to facilitate genetic studies and breeding efforts effectively. Overall, our studies demonstrate the power of combination of omics-based approaches in soybean research, providing valuable insights into its evolution, trait genetics, and the establishment of a collaborative data-sharing platform.

Keywords: Evolution, introgression, unmanned aircraft system, dynamic regulation, SoyFGB v2.0

Enhancing wild germplasm utility with telomere-to-telomere assemblies and pangenomic graphs

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A1: Soybean pangenome and genetic diversity

Wild progenitors of a crop often contain valuable alleles, but wide crosses using this divergent germplasm present numerous challenges to molecular breeding and genotyping. For example, sequencing-based genotyping uses a reference genome for short read alignment. Bias for reference alleles and other associated alignment issues frequently result in missing data, biased calls, and "phantom" variants. In addition, the true gene content segregating in the population is obscured. Pangenomic graphs are one bioinformatic solution to these single reference problems. We have applied pangenomic graphs to structured populations across a range of species and divergence levels. We are currently characterizing a *Glycine soja* x *Glycine max* chromosomal substitution panel. Both parental genomes have been sequenced with long and accurate reads that enable telomere-to-centromere assembly even prior to scaffolding. The complete genomic knowledge of this panel allows us to not only improve genotyping accuracy but to evaluate potentially deleterious mutations much more effectively. In turn, this information can supplement models trained solely on yield data.

Introducing SoyMAGIC: an 8-parent multi-parent advanced generation inter-cross population for soybean genetic studies and breeding activities

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A2: Innovations in breeding technology

Multi-parent advanced generation inter-cross (MAGIC) populations have emerged as powerful genetic platforms for studying complex traits and generating novel germplasm for breeding endeavors. By crossing and intercrossing multiple genetically diverse parent lines over several generations, these populations yield highly diverse and recombinant offspring. In this presentation, I present the first 8-parent MAGIC population in soybean, SoyMAGIC, which comprises over 700 recombinant inbred lines spanning from MG0 to MG3.5. SoyMAGIC was established through intercrossing eight elite soybean lines that segregate for a wide range of desirable characteristics, including seed quality and agronomic traits. The population has been genotyped using genotyping-by-sequencing method, resulting in the construction of a comprehensive linkage map spanning 3,770.75 cM and containing over 12,000 SNP markers. The exceptional characteristics of SoyMAGIC, including its indefinite lifespan and potential for use by multiple laboratories for diverse genetic studies and breeding activities, make it a valuable resource for the soybean research community. As such, we are committed to making SoyMAGIC readily available to interested researchers, further advancing our understanding of the genetic architecture of complex traits in soybean and facilitating the development of improved soybean varieties through accelerated breeding efforts.

Tilling by sequencing to improve seed traits in soybean

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A2: Innovations in breeding technology

In the year 1999-2000, we developed the first soybean EMS mutagenized populations to be used for TILLING (Targeting induced local lesions in genomes) at Southern Illinois University. The technology was applied successfully to the isolation and characterization of the major soybean cyst nematode disease resistance genes, the Rhg1(GmSNAP18) and Rhg4 (GmSHMT08) genes (Liu et al. 2012 and 2017). We optimized the platform since the year 2018 by using TIILLING by target capture-sequencing (TILLING by Sequencing+) as a tool to speed up the process of finding mutations in genes underlying economically important traits in soybean. Several lines with high protein, sucrose, tocopherols, and oil were characterized and are now available for releases to improve soybean seed traits. (Lakhssassi et al. 2020, 2021, Zhou et al., 2021).

Digital solutions in plant breeding

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A2: Innovations in breeding technology

Just like all sectors of life, agriculture and plant breeding are becoming increasingly digitalized and ever more data must be managed and handled. At Wintersteiger we support breeders in the transition to the digital age. The backbone of this objective is our breeding software Easy Breed.
The flexible and user-friendly data management software for plant breeding and field trials helps you to successfully develop new crop varieties.

Easy Breed supports you throughout the entire breeding process. From crossing, over trial design and scoring to statistical analysis and selection. Easy Breed communicates with machinery and software inside and outside of the Wintersteiger world, e.g., with the GPS field trial planning software 'Mini GIS', trial planters, scoring software like Smatrix and Field Book, drones and picture analysis software from Delair and Alteia as well as with harvesters. This way data is automatically integrated into the Easy Breed database. Time-consuming and error-prone data processing steps are replaced by validated integration routines.

Data collected over different trials, locations and years can be easily consolidated, analyzed, visualized, and presented in reports or graphics. The result: an effective and transparent selection of promising genotypes for the subsequent breeding process.

A special strength of the software is its flexibility: If you want to integrate special analyses, visualizations and reports, you can do so any time. Easy Breed uses the open-source software R and offers interfaces that allow you to make use of your preferred analysis pipelines as well as visualization and reporting methods.

Keywords: Data management, plant breeding, digitalization

Applied genomics for identification of causative mutations for accelerated soybean breeding and improvement

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A3: Genomics-assisted breeding

The genome-wide association study (GWAS) is a powerful method to identify a causative mutation (CM) that underlies a phenotype change. GWAS has led to successful discoveries in many cases but failed to predict traits of complicated genetics or high complexity. As a statistical method, GWAS is critically dependent on the data set size, the genotype quality and the phenotype frequency. For soybean, there is a great amount of publicly available genomic data already gained from independent studies that could be utilized for CM identification and thus, accelerate soybean improvement. The hypothesis of our applied genomics approach is that the existing genotype data can be leveraged in an empowered GWAS strategy to identify CM with higher confidence, even for complicated traits. We developed an applied genomics methodology for CM identification encompassing three novel concepts: Synthetic Phenotype, Accuracy Calculation and Curated Panel of Diverse Soybean Resequenced Accessions (Soy1066). As a result, we created a Synthetic phenotype to CM strategy (SP2CM) and developed a set of supportive genomic tools that are available either online (soykb.org/tools) or can be downloaded for standalone use. The tools enable an easy-to-use creation of a Synthetic Phenotype from user-provided genotype data or the curated panel of accessions (AccuCalc); the analysis of direct correspondence between variant positions in the curated panel (AccuTool); and the exploration of haplotype context (SNPViz). By utilizing the SP2CM strategy, we validated CMs for a palette of cloned genes that represent the most common scenarios (quantitative, qualitative, proportional, disproportional and rare phenotypes). Importantly, we discovered new genes/CMs involved in seed coat pigmentation, soybean cyst nematode resistance and seed protein content. We created a hub for soybean applied genomics (soykb.org/soyhub/) that enables CM identification for providing more precise and thus accelerated soybean breeding and improvement.

Keywords: GWAS, resequencing, genotyping, genomics assisted breeding, causal gene, causative mutation

Using genomic data and machine learning to predict the most promising crosses

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A3: Genomics-assisted breeding

With the advent of low-cost genome-wide genotyping tools for many crops, there is a growing interest in leveraging these to predict the phenotype based solely on the genotype. While these methods have been widely adopted in very large breeding programs run by multinational seed companies, this is not the case in smaller public and private breeding programs. In this work, we sought to explore the use of genomic prediction methods to help breeders identify crosses that are more likely to produce improved lines by predicting progeny performance for various key traits. To assess whether genomic prediction of cross performance could help breeders simultaneously improve multiple traits, yield and maturity were predicted for 60,000 soybean crosses. These predictions were then compared with the persistence of 101 biparental crosses throughout the selection process. Truly superior crosses were those that produced progeny lines that reached advanced stages in the breeding process (registration trials and commercialization). All but 2 of the 22 superior crosses retained by breeders had been predicted to display above-average mean yield within different maturity windows. At the opposite end of the spectrum, 96.2% of all crosses predicted to produce progeny with a below-average mean yield within a specific maturity window were eliminated during selection. Our results therefore suggest that by making crosses predicted to produce progeny (i.e. meeting target requirements for multiple key traits), breeders could either achieve the same genetic gains with fewer resources or invest the same resources on a more promising set of crosses and thereby achieve greater gains. In addition, more recently, we have explored the use of machine learning approaches to further improve the accuracy of our genomic predictions and preliminary results will be presented.

Keywords: Genomic prediction, superior crosses, multi-trait selection, genetic gain, machine learning

New technologies enabling design-centered products at Bayer

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A3: Genomics-assisted breeding

Megatrends such as a growing world population, climate change and evolving consumer preferences has driven the need for innovation in agriculture. In order to address these growing megatrends, Bayer Crop Science has transitioned from Breeding 3.0, which was focused on increased operational efficiency and improved data collection to Precision Breeding, which is a more focused on utilizing data to align our breeding efforts and decisions to our customer needs. We have charged ourselves with leveraging data to increase the speed, accuracy, and efficiency of our breeding strategy and go from Selecting the Best to Designing the Best. To accelerate the achievement of these goals, we have split our breeding organization into Product Design and Product Development. Product Design is centered on population improvement while Product Development centers around line level evaluation and placement. Technological investments in protected culture, automated robotic seed packaging, and high-throughput phenotyping will also be crucial for us to deliver the best product we can to our farmer customers around the world.

Genomics-enabled breeding design and selection to improve the rate of genetic gain in soybean

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A3: Genomics-assisted breeding

Improving yield is a primary soybean breeding goal, as yield is the main determinant of soybean's profitability. Cultivar development is a high complexity, long term process that involves many selection and evaluation steps. Within the breeding process, selection of the best cross combinations among parental genotypes prior to crossing and best progeny for yield evaluation is one of most important elements. To increase genetic gain and efficiency of breeding pipelines, this research aimed to evaluate and develop predictive, genomic tools for soybean cultivar development. Two predictive tools were investigated, genomic selection and cross prediction. Genomic selection was carried out using a set of elite soybean breeding material from an established cultivar development program. Multiple statistical models, validation methods, and SNP marker sets were utilized. It was found via empirical validation that extended genomic BLUP was the most effective of the genomic selection models and use of the SoySNP3k marker set was preferable compared to the SoySNP1k or 6k marker set under cross validation, inter-environment evaluation, and empirical validation. In optimal cross prediction, multiple methods for evaluating crosses were tested using empirical data with various training sets and SoySNP3k and 6k marker sets. Extended genomic BLUP was found to be the most effective statistical model to use, with a 0.4 prediction accuracy under breeding program conditions. Marker number had a significant impact on predictive accuracy with the SoySNP6k marker set preferable of the two marker sets tested. Deployment of the cross prediction and genomic selection in an applied breeding program will be presented. These genomic tools can aid soybean breeders in making more informed selection decisions and lead to accelerated genetic gain.

Keywords: Soybean breeding, SNP markers, cross prediction, genomic selection, genetic gain

Differential gene expression of higher latitudes in soybean

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A4: Seed composition

Over the past two decades soybeans grown in western Canada (higher latitudes) have persistently had lower seed protein than those grown in eastern Canada. To understand the discrepancy in seed protein content between soybean grown in higher latitudes compare to the lower ones, RNA-seq and differential expression analysis have been investigated. Ten soybean genotypes, ranging from low to high in seed protein content, were grown in four locations across eastern (Ottawa) and western Canada. Differential expression analysis revealed 34 differentially expressed genes encoding *Glycine max Sugars Will Eventually be Exported Transporters* (GmSWEETs), including paralogs GmSWEET29 and GmSWEET34 (AtSWEET2 homologs) that were consistently upregulated across all ten genotypes in each of the western locations over three years. GmSWEET29 and GmSWEET34 are likely candidates underlying the lower seed protein content of western soybeans. GmSWEET20 (AtSWEET12 homolog) was downregulated in locations with higher latitudes land may also play a role in lower seed protein content. These findings are valuable for improving soybean agriculture in regions with higher latitudes (i.e., Western Canada growing regions), establishing more strategic and efficient agricultural practices.

Keywords: Glycine max, SWEET, sugar transport, RNA-seq, transcriptomics

A single amino acid mutation in a transcriptional repressor increases oil and protein content in soybean

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A4: Seed composition

Climate change is a growing concern, especially the increase of CO_2 release from use of fossil fuels. To help reduce carbon emission, demand for renewable biofuels is increasing. This is driving an increased demand for plant oil production especially for soybean oil, which is the second largest oil source for renewable biofuel production. To discover new genes for increasing oil in soybean, we have identified high oil mutants by high throughput single seed screening. One of these high oil mutants has been fully characterized and the mutant gene has been mapped, cloned, and validated by CRISPR gene editing. The mechanism of this novel transcriptional repressor, which regulates oil accumulation in soybean, and its application in high oil soybean development will be presented.

Identification of a potential gene for elevating ω-3 concentration and its efficiency for improving the ω-6/ω-3 ratio in soybean

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A4: Seed composition

This present study was to identify a novel candidate gene that contribute to the elevated α-linolenic acid (ALA, ω-3) concentration in PE2166 from mutagenesis of Pungsannamul. Major loci qALA5_1 and qALA5_2 were detected on chromosome 5 of soybean through quantitative trait loci mapping analyses of recombinant inbred lines. With next generation sequencing of parental lines and Pungsannamul, and recombinant analyses, a potential gene, Glyma.05g221500 (HD) controlling elevated ALA concentration was identified. HD is a homeodomain-like transcriptional regulator that may regulate the expression level of microsomal ω-3 fatty acid desaturase (FAD3) genes responsible for the conversion of linoleic acid into ALA in the fatty acid biosynthetic pathway. In addition, we hypothesized that combination of mutant alleles, HD and either of microsomal delta-12 fatty acid desaturase 2-1 (FAD2-1), could reduce the ω-6/ω-3 ratio. In populations where HD, and FAD2-1A and FAD2-1B genes were segregated, combination of a hd allele from PE2166 and either of the variant FAD2-1 alleles were sufficient to reduce the ω-6/ω-3 ratio in seeds.

GmJAZ3 interacts with GmRR18a and GmMYC2a to regulate seed traits in soybean

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A4: Seed composition

Seed weight is usually associated with seed size and is one of the important agronomic traits that determine yield. Understanding of seed weight control is limited especially in soybean plants. Here we show that GmJAZ3, a gene identified through gene co-expression network analysis, regulates seed-related traits in soybean. Overexpression of GmJAZ3 promotes seed size/weight and other organ size in stable transgenic soybean plants likely by increasing cell proliferation. GmJAZ3 interacted with both GmRR18a and GmMYC2a to inhibit their transcriptional activation of cytokinin oxidase gene GmCKX3-4, which usually affects seed traits. Meanwhile, the GmRR18a binds to the promoter of GmMYC2a and activates GmMYC2a gene expression. In GmJAZ3-overexpressing soybean seeds, the protein contents were increased while the fatty acid contents were reduced compared to those in the control seeds, indicating that the GmJAZ3 affects seed size/weight and compositions. Natural variation in JAZ3 promoter region was further analyzed and Hap3 promoter correlates with higher promoter activity, higher gene expression and higher seed weight. The Hap3 promoter may be selected and fixed during soybean domestication. JAZ3 orthologues from other plants/crops may also control seed size and weight. Taken together, our study reveals a novel molecular module GmJAZ3-GmRR18a/GmMYC2a-GmCKXs for seed size and weight control, providing promising targets during soybean molecular breeding for better seed traits.

Keywords: Soybean, GmJAZ3, seed size, seed weight, response regulator

Fine mapping and cloning of the major seed protein QTL on soybean chromosome 20

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A4: Seed composition

Soybean [*Glycine max* (L.) Merr.] seed contains high levels of both protein and oil. Many QTL controlling soybean seed protein content have been identified, but alleles of the cqSeed protein-003 QTL on chromosome 20 exert the greatest additive effect. The high protein allele for this QTL is present in both cultivated and wild soybean (*Glycine soja* Siebold & Zucc.) germplasm. Our objective was to fine map this QTL to enable positional-based cloning of its underlying causative gene(s). Fine mapping was achieved by developing and testing a series of populations in which the chromosomal region surrounding the segregating high vs. low protein alleles was gradually narrowed, using marker-based mapping of recombinant events. The resultant 77.8 kb interval was directly sequenced from a *G. soja* source and compared to the reference genome to identify structural and sequence polymorphisms. An insertion/deletion variant detected in Glyma.20G85100 was found to have near-perfect concordance with high / low protein allele genotypes inferred for this QTL in parents of published mapping populations. The indel structure was concordant with an evolutionarily recent insertion of a TIR transposon into the gene in the low protein lineage. Seed protein was significantly greater in soybean expressing an RNAi hairpin down-regulation element in two independent events relative to control null segregant lineages. We conclude that a transposon insertion within the CCT domain protein encoded by the Glyma.20G85100 gene accounts for the high / low seed protein alleles of the cqSeed protein-003 QTL.

Identification of the genes controlling yield and seed quality in soybean

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A4: Seed composition

Seed size is one of the most important agronomic traits determining the yield of crops. Cloning the key genes controlling seed size and pyramiding their elite alleles will facilitate yield improvement. To date, few genes controlling seed size have been identified in soybean, a major crop that provides half of the plant oil and one quarter of the plant protein globally. Here, through a genome-wide association study of over 1800 soybean accessions, we determined that natural allelic variation at GmST05 (Seed Thickness 05) predominantly controlled seed thickness and size in soybean germplasm. Further analyses suggested that the two major haplotypes of GmST05 differed significantly at the transcriptional level. Transgenic experiments demonstrated that GmST05 positively regulated seed size and influenced oil and protein contents, possibly by regulating the transcription of GmSWEET10a. Population genetic diversity analysis suggested that allelic variations of GmST05 were selected during geographical differentiation but have not been fixed. In summary, natural variation in GmST05 determines transcription levels and influences seed size and quality in soybean, making it an important gene resource for soybean molecular breeding.

Chromosome engineering for crop improvement

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A5: Soybean genome editing

CRISPR–Cas is a powerful technology with wide-ranging applications from gene discovery to commercial product development. However, this tool has been almost exclusively used for gene knockouts and gene deletions, with a few examples of specific edits and targeted gene insertions. One of the new applications of CRISPR–Cas9 technology is associated with targeted chromosomal rearrangements, including large deletions, duplications, inversions and translocations. This approach allows QTL mapping, somatic recombination and unlocking genetic variations unavailable through conventional plant breeding, thus providing opportunities for development of new varieties with improved phenotypes. Specific examples of chromosome engineering application will be presented.

The first two elite soybean varieties genetically edited in South America

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A5: Soybean genome editing

Genome editing is one of the new tools known as new breeding techniques (NBTs) and has shown the potential to contribute to genetic breeding of soybeans and other crops. It allows the generation of new genetic combinations and superior allelic variants that are not possible or rare to obtain by conventional breeding methods, or that would require many years or generations to accumulate several natural mutations. We used genome gene editing to modify the genome of two GDM elite soybean varieties with high yield and good agricultural adaptation for a nutritional component and an agronomic trait. The first edited soybean has a genetic modification that increases the nutritional value of the soybean meal by reducing the proportion of raffinose and stachyose soluble sugars. The expected value is the elevation of the metabolizable energy of feed for monogastric animals and better digestibility for humans. The second edited soybean had a gene that controls the plant response to drought and heat abiotic stress and may have a positive impact on the stability of soybean yield. We also obtained the exemption of GMO regulation of both varieties in Brazil and Argentina, and the exemption of the first in Colombia. These countries demonstrated to have an efficient and modern regulatory framework for genome edited organisms. With the exemptions, these organisms can be planted and commercialized in these countries as if they were conventional soybean varieties. The possibility of managing the edited soybean varieties as conventional soybean varieties facilitates their final evaluations and eventual further commercialization, all without bringing any health, environmental, or phytosanitary risk. Our technical and regulatory experience provided us evidence that genome editing will help GDM and other seed companies to continue developing soybean products for farmers and customers of the different markets around the world, contributing to food production and sustainability.

Keywords: Gene editing, CRISPR, soybean

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Accelerating gene discovery in soybean through genome editing

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A5: Soybean genome editing

Genome editing has revolutionized the ability to effectively modify plant genomes in the absence of efficient homologous recombination mechanisms that exist in other organisms. Soybean is recalcitrant to transformation and thus, plants with stable gene edits are costly and take a long time to produce. Moreover, soybean is allotetraploid and editing paralogous genes are often necessary to obtain observable phenotype(s). To address these issues, we established a highly efficient dual gRNA CRISPR/Cas9 system which we employed in our functional genomic studies. A large number of quantitative trait loci (QTLs) governing important agronomic traits in soybean have been identified, but the causatives genes remain unknown for the majority of these QTLs. To facilitate gene discovery, we employed forward genetics methods combined with CRISPR/Cas9 to identify genes governing important agronomic traits in soybean. Examples of genes identified through this approach will be discussed.

Understanding nutrient uptake and resilience mechanism to water deficit conditions through gene-editing in soybean

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A5: Soybean genome editing

Bioavailability, uptake, and spatial distribution of essential and toxic mineral elements are pivotal factors that govern crop growth, development, and productivity. Soybean is a major leguminous crop globally, and yield losses are commonly attributed to various abiotic factors, including nutrient deficiencies or the influence of toxic minerals in the soil. Therefore, understanding the molecular basis of differential mineral element uptake, translocation, and accumulation in soybean is vital for developing improved cultivars. We performed high-throughput mineral element profiling of a diverse set of soybean germplasm and identified several lines that show two to tenfold differences in various mineral nutrient uptake, which could serve as important genetic resources for germplasm development. Haplotype analysis uncovered the allelic diversity and the potential candidate genes underpinning the element mobilization from source to sink tissues. Functional characterization of major genes involved in mineral nutrient uptake was performed using CRISPR/Cas9 and their potential role in mineral and water-deficit condition was established. Using single nuclei RNA-sequencing (snRNA-seq), we established a transcriptional map of nutrient accumulation of soybean leaf at a single-cell resolution and identified unique cell clusters. This analysis suggests that nutrient uptake induces significant changes in the transcriptome of soybean leaf.

Genomic regions associated with C13 ratio and plasticity for improving water use efficiency in soybean

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A6: Breeding for abiotic stress and climate change

Drought is the most significant abiotic stress limiting soybean yield, and the vast majority (>90%) of soybean acreage is rainfed. Traditional breeding to improve drought tolerance by selecting for yield has been ineffective. An alternative approach is to measure and deploy water use efficiency (WUE), however selecting for constitutively high WUE would likely limit seed yield when soil moisture is plentiful. An ideal genotype would be plastic and able to increase WUE when soil moisture is limiting but have relatively low WUE when soil moisture is plentiful. Determining WUE for large populations is exceedingly difficult, but the isotopic ratio of C13 to C12 in plant tissue is tightly correlated with WUE. We performed association mapping for C13 ratio, using two panels of maturity group 4 soybean. Panel 1 consisted of 205 genotypes across seven irrigated and four drought environments; Panel 2 consisted of 373 genotypes across four environments. Above ground plant tissue was collected between flowering and beginning seed fill, dried, and analyzed for C13 ratio. Plasticity was quantified as the slope from regressing C13 ratio of individual genotypes against an environmental index calculated based on the mean within and across all environments. Our study aims were : (1) identify genotypes able to change C13 ratio in response to the environment, and (2) associate genomic regions with plasticity and overall C13 ratio. We quantified plasticity response and identified entries with high plasticity and high WUE. Association mapping identified 19 SNPs associated with plasticity and 39 SNPs associated with C13 ratio. In total, 71 candidate genes were identified with annotations related to transpiration, water conservation, rooting, and stomatal complexes. The SNPs associated with plasticity and C13 ratio we identified, as well as associated alleles, are valuable resources towards improving soybean drought tolerance without limiting yield under high water availability.

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Genetic diversity of heat stress tolerance in soybean

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A6: Breeding for abiotic stress and climate change

Despite the current and projected challenges associated with global rising temperatures, studies on heat stress tolerance in soybean [*Glycine max* (L.) Merr.] are limited. Soybean yield is correlated with increasing temperatures in the seed filling stage until 30°C, followed by a sharp decline with each additional degree. With the projected increase in growing season temperatures, heat tolerant varieties are the ideal solution to maintain soybean production; however, soybean response to heat stress in vegetative and reproductive stages is unclear. This study aimed to determine genetic variation for heat stress in a soybean diversity panel consisting of 450 accessions. A replicated factorial experiment with two temperature regimes, optimal (OT) at 28°C and high temperature (HT) at 35°C, was conducted. At 28 days after planting, data on chlorophyll content and canopy temperature, and dry shoot and root biomass were collected. Additional photosynthetic traits were added to data collection in 2022. This created three sets of measurements: (i) traits in OT, (ii) traits in HT and (iii) heat stress index (I) that combines the responses from optimal and heated chamber. Heat stress index was calculated using (T^HT-T^OT)/T^OT, where T is the trait raw value. This enabled study of genetic variation among genotypes in OT, HT and I. Root fresh biomass ($P=0.99$) showed no significant difference between temperature treatments. All remaining physiological and photosynthetic traits did show significant difference ($P<0.05$) between temperatures. In 2021, all indexed traits were genotypically significant. Results suggests that soybean has the necessary genetic variation to utilize in cultivar development. Ongoing work is utilizing field deployable heat chambers to study response of a sub-set of lines for heat stress during seed filling stage. Additional analyses are on-going for genome wide association studies and gene expression studies.

Legume biotechnology-reducing impact of climate change on soybean

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A6: Breeding for abiotic stress and climate change

The world's population is increasing at a rate to require a doubling of food production from the same amount of arable land in the background of climate change threatening our food security. Moreover, heat waves associated with climate change seriously threaten crop productivity. Crop seed yield depends on the success of reproduction. However, reproductive development is most vulnerable to heat stress conditions. Perception of heat and its conversion into cellular signals is a complex process.

Soybean is a major oilseed legume crop used for human and animal feed, possessing a unique nitrogen-fixing ability leading to its use in rotation agriculture for replenishing soil nitrogen composition. Thus, soybean breeding programmes continuously aim to develop improved cultivars for better regional adaptability. Expanding soybean cultivation requires optimization of environmental and development responses suitable for changing climate regimes.

Our lab used an integrated bioinformatic and experimental approach to address this gap in our knowledge. Soybean is a paleopolyploid legume with multiple gene copies. Our study also revealed the functional divergence of flowering genes in soybean and identified key transcription factors that could affect phenotypes related to plant morphology and reproductive stages in soybean. We will present our exciting work on the thermotolerance of soybean, showing reduced loss of heat stress-induced seed yield in soybean plants at the flowering stage by influencing multiple attributes and can be used as a gene target for soybean improvement programmes to ensure future food security.

Adaptation of European cultivars to severe drought and moisture-favourable conditions

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A6: Breeding for abiotic stress and climate change

The changing climate emphasizes the importance of breeding soybean cultivars with greater drought tolerance. This study assessed grain yield (GY) and other agronomic and physiological traits of 72 early-maturing (MG 000/0+) and 72 intermediate-maturity (MG I-/II) European cultivars under managed drought stress and moisture-favourable conditions during the reproductive phase, to highlight opportunities and optimal selection strategies for drought tolerance improvement. The material was grown in a phenotyping platform composed of eight large (24.0 m x 1.6 m x 0.8 deep), bottomless containers filled with sandy loam soil, equipped with a rainout shelter and a double-rail irrigation bloom. We adopted an alpha-lattice design with four replications per condition, with plant density of 42 plants m-2. Stress and favourable conditions implied soil moisture kept at 80% and within 10-30% of the available water, respectively. On average, drought stress reduced GY by 66% (0.94t ha^{-1} vs. 2.76t ha^{-1}). Both early and intermediate maturity groups revealed large genotype × environment interaction across conditions ($P < 0.01$), with fairly modest genetic correlation for cultivar GY responses across environments ($r_g = 0.59$ and 0.53 for early and intermediate cultivars, respectively). Both maturity groups revealed greater genetic variation for yielding ability (expressed as genetic coefficient of variation, CVg) under favourable conditions than under stress (21.8% vs. 16.3%, averaged across groups), probably due to limited historical effort in terms of selection environments and useful genetic resources that was paid to selection for drought tolerance in soybean. Our results highlighted the crucial importance of selecting under drought stress (given the modest rg value across conditions) and carefully identifying suitable parental germplasm by thorough screening of commercial cultivars and exotic or less conventional genetic resources, when targeting drought stress environments. Additional information on phenology, plant biomass and physiological traits contributed to define a breeding strategy for stress environment.

Keywords: Drought tolerance, maturity group, genotype × environment interaction, genetic correlation, yielding ability

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GWAS analysis reveals key loci associated with drought and water logging tolerance and root trait architecture in soybean

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A6: Breeding for abiotic stress and climate change

Abiotic stresses such as drought and water logging have been identified as a key factor reducing soybean yield. Genome-wide association and gene expression studies were conducted to identify key genes and markers associated with drought tolerance, water logging tolerance and root trait architecture. GWAS panel consisting of 300 diverse soybean accessions were phenotyped for morpho-physiological traits under control and drought and water logging stress condition for three years during 2019-2021. Genotyping by Sequencing (GBS) of GWAS panel identified 66300 SNPs distributed all over 20 chromosomes and were used for association analysis. GWAS analysis revealed closely located significant SNPs on chromosome 4 (Position: 50500762, 52098616 and 53008801) and chromosome 16 (32371663, 33082976 and 33283177). Key candidate genes related to Myb family, cell signaling gene (GPCR), salicylic acid synthesis, oxidative stress, auxin and ABA synthesis pathway were identified. Further, GWAS analysis was conducted for water-logging related traits such as Foliar Damage score (FDS), Plant survival Rate (PSR), Stem elongation Rate (SER) and Adventitious Root Ratings (ARR). Candidate genes related to oxidative stress, auxin and ABA synthesis pathway were identified in genomic region on chromosome 13 (SNP Position 34048347) and chromosome 15 (52423961). GWAS analysis was also conducted for root system architecture related traits such as Root Length (RL), Root Diameter (RD), Root Volume (RV) and Root Dry Weight (RDW) and candidate genes related to cell signalling gene, auxin transport, Phosphate starvation, oxidative stress and ABA synthesis pathway were identified on chromosome 7 (SNP 5599213) and chromosome 20 (49984086). The key genes are being validated using gene expression and functional genomics studies. The SNPs associated with key traits will be useful in molecular breeding programme for soybean improvement.

Building bridge between genomics and phenomics: discovering favorite alleles of Chinese soybean collections for breeding

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A7: Germplasm collections and evaluation

Soybean is an important crop as a main source of plant protein and oil in China, its consumption has been increasing tremendously in recent years. It is necessary to discover elite alleles for soybean improvement. Among more than 30,000 soybean accessions reserved in National Genebank in China, genetic diversity were evaluated and a series of core collections were established by joining phenotyping and limited genotyping data, such as SSR and resequencing data. Then seven representative accessions were selected for building the pan-genome of wild soybeans that could be look insight into the evolution; diversity and domestication of soybean by de novo sequencing. Based on the genomic variations, a series of DNA chips was developed, including ZDX (600K), ZDX1 (200K), and ZDX2 (5K). The majority of accessions were genotyped with ZDX1 chip. Based on the functional loci, some accessions with favorite allele were found out. Meanwhile, combing with agronomic traits, GWAS (genome-wide association study) was carried out in over 20,000 accessions, a number of loci were identified, including either reported genes such as leaf shape, flower color, oil content, and SCN resistance etc, or novel loci. Compared with phenotyping identification, genome wide genotyping with ZDX1 chip could speedy discovering more elite accessions with desired trait. The accessions with favorite traits or elite alleles become the important source of parental lines in breeding program for germplasm enhancement and cultivar development.

Genetic diversity in early maturity Chinese and European elite soybeans: A comparative analysis

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A7: Germplasm collections and evaluation

Soybean (*Glycine max* [L.] Merr.) is an essential legume crop providing high-quality vegetable protein and oil, and is crucial for global food security. China, considered as the birthplace of soybeans, has numerous landraces and genetic resources, making it rich in soybean genetic diversity. In the past 100 years, Asian soybeans spread to all over the word and founded the modern soybean production. However, because of regional breeding projects and adaptation, the genetic background of present-day elite soybean cultivars is getting narrow. Therefore, a study was conducted to analyze the diversity of early maturity Chinese and European elite soybeans using a high-throughput functional SNP array and an SSR marker set. The results showed a clear differentiation between Chinese and European elite cultivars, with similar levels of genetic diversity in both populations. Both Chinese and European cultivars exhibited unique SSR alleles, indicating that selection for specific adaptation can preserve genetic variation. The European population was structured by maturity groups, which was less evident in the Chinese population. The structure analysis revealed that European cultivars are based on two major ancestral lines, whereas Chinese elite soybean cultivars trace back to more ancestral lines, pointing to the rich natural soybean diversity of China. Joint analysis of selective sweep and Fst among European varieties and Chinese varieties stored in Chinese gene bank identified 124 selected regions, with 16 regions distributed over 15 and 10 chromosomes in Chinese and European collections, respectively. Additionally, 35 and 26 selected regions were identified in newly introduced European soybeans and stored collections, respectively, and distributed over 13 and 10 chromosomes. The study confirms that long-term selection under divergent environmental and agronomic conditions can produce specific diversity, which could be utilized to enhance both Chinese and European soybean breeding in the future. Overall, this study highlights the importance of preserving and utilizing soybean genetic diversity, especially in light of the current and future challenges faced by global agriculture.

Keywords: Genetic diversity, plant introduction, elite cultivar, plant breeding, genetic structure

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Development of genotyping tools for soybean genetics research and breeding selection

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A7: Germplasm collections and evaluation

The limited number of recombinant events in recombinant inbred lines suggests that for a biparental population with a limited number of recombinant inbred lines, it is unnecessary to genotype the lines with many markers. For genomic prediction and selection, previous studies have demonstrated that only 1000-2000 genome-wide common markers across all lines/accessions are needed to reach maximum efficiency of genomic prediction in populations. For early generation breeding selection or plant variety property protection, fewer markers are required. Evaluation of too many markers will not only increase the cost but also generate redundant information. We developed soybean assays, BARCSoySNP6K, 3K and 1K containing 6K, 3K and 1K markers, respectively, which were carefully chosen from the SoySNP50K assay based on their position in the soybean genome and based on the analysis of haplotype blocks, polymorphism and genotyping quality of approximately 20,000 accessions including soybean landraces, North American cultivars, and wild soybeans. The final selection of 1K, 3K, 6K SNPs from the SoySNP50K assay still captures most of the diversity represented by 50K DNA differences and provides high-quality genotypic data and enough power of characterizing genotypes to accommodate different research purposes. The assays are proven as excellent tools for detecting quantitative trait loci, genomic selection and assessing genetic relationships after genotyping thousands of samples. In collaboration with soybean researchers across the U.S., we identified genomic regions controlling important traits including resistance to phytophthora root rot, sudden death syndrome and aphid, drought resistance, etc and performed genomic selection of seed composition in soybean. The assays are commercialized and being used by soybean breeders and geneticists and the list of SNPs in the assay is an ideal resource for SNP genotyping by targeted amplicon sequencing.

Keywords: SNP assays, single nucleotide polymorphisms, haplotype block, QTL mapping, genomic selection

Allele mining for yield and agronomic traits using a diverse GWAS panel made up of modern Canadian and Chinese cultivars and the progeny of their crosses

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A7: Germplasm collections and evaluation

Despite mounting concerns regarding the narrowness of the genetic base of soybean (*Glycine max* (L.) Merr.) in North America and the challenges that it may pose in the changing global environment and climate, exotic germplasm remains seldom used by breeders owing to various concerns. The objective of this study was to evaluate a Genome-Wide Association Study (GWAS) genomic diversity panel of 200 soybean genotypes for seed yield, seed quality, and agronomic trait performance. The GWAS panel consisted of lines derived from several generations of bi-parental crosses between modern Canadian and Chinese cultivars (CD–CH), Canadian cultivars (CD), and Chinese cultivars (CH) from Northeast China were evaluated at Elora and Woodstock, ON, in 2019 and 2020. In the combined analysis of variance, the CD–CH group showed a significant increase in seed yield, although the performance of this group was otherwise comparable or inferior to the adapted elite Canadian cultivars. Genotyping-by-sequencing (GBS) was conducted and yielded almost 32 K high-quality SNPs. GWAS was conducted using Fixed and random model Circulating Probability Unification (FarmCPU) model on the following traits: seed yield, seed protein concentration, seed oil concentration, plant height, 100 seed weight, days to maturity, and lodging score that allowed to identify five QTL regions controlling seed yield and seed oil and protein content. A candidate gene search identified a putative gene for each of the three traits. The results of this GWAS study provide insight into potentially valuable genetic resources residing in modern Chinese cultivars that breeders may use to further improve soybean seed yield and seed quality traits.

New molecular tools in support of breeding for *Phytophthora sojae* resistance in soybean

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A8: Breeding for biotic stress

Phytophthora sojae is arguably one of the most important pathogens of soybean worldwide. A common method of control resides in the introgression of specific resistance genes (Rps) in elite material. These Rps genes have a gene-for-gene relationship with avirulence (Avr) genes of *P. sojae*, which triggers defense reactions. Over the years, some Rps genes have lost their efficacy as a result of adaptation by the pathogen leading to new alleles of Avr genes whose product is no longer recognized by Rps proteins. This problem is compounded by the rapid evolution of Avr genes and the fact that very few Rps genes have actually been precisely identified and cloned. Following extensive surveys of *P. sojae* isolates throughout soybean growing areas in Canada, we were able to define SNP haplotypes for the alleles of Avr genes associated with the most commonly deployed Rps genes, Rps1a, Rps1b, Rps1c, Rps1k, Rps3a, and Rps6. Based on this information, we have developed molecular tools that can specifically identify each haplotype of these six Avr genes found in the isolates of *P. sojae* recovered in soybean fields. We have further been able to identify markers that will discriminate isolates on the basis of their virulence toward the newly identified Rps11. These tools can be easily exploited by breeders to introgress a proper Rps gene and farmers to use cultivars carrying a gene known to be effective in a given region. In parallel, we have used RenSeq to precisely define Rps regions and genes, a method that has allowed a much clearer understanding of many of the reported Rps genes along with a finer resolution of their position in the soybean genome. Once optimized, this approach should facilitate introgression of Rps genes already in use and allow deployment of new sources of resistance against Phytophthora root rot.

Breeding for resistance to soybean diseases in Michigan

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A8: Breeding for biotic stress

Sclerotinia stem rot (white mold), phytophthora root rot, and soybean cyst nematode (SCN) are historically and continue to be the major soybean diseases in Michigan. Soybean sudden death syndrome (SDS), pythium seed and root rot, and *Phytophthora sansomeana* were recently identified in Michigan that caused yield losses. A summary of genetic resistance to most of these diseases from the research at Michigan State University and the improvement of soybean resistance to these diseases will be presented.

Synergistic R gene interactions in gene pyramids enhance resistance to soybean aphids

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A8: Breeding for biotic stress

Soybean aphids (*Aphis glycines*) are important insect pests that cause significant yield loss in untreated susceptible soybean cultivars. Control strategies rely heavily on insecticide treatments, which increase production costs and have undesirable environmental impact. Host-plant resistance (HPR) is an important management option, and several Resistance to *Aphis glycines* (Rag) genes have been identified. However, rapid aphid evolution can lead to biotypes able to overcome HPR. Thus, novel sources of resistance and deployment strategies are needed. We screened a subset of the core collection of the USDA Soybean Germplasm Collection and identified nine new soybean accessions carrying different levels of resistance that had not been previously reported. SNP analyses and a genome-wide association study revealed candidate resistance genes in genomic regions different from known Rag gene loci, increasing the resources available for breeding strategies. Pyramiding different resistance genes into one plant genotype confers enhanced resistance at the phenotypic level, but the molecular mechanisms underlying this effect are not well-understood. We assessed how the combination of Rag genes enhances the soybean resistance to aphid infestation, by characterizing differential transcriptional responses of soybean genotypes carrying individual Rag genes (Rag1 or Rag2) and the Rag1/Rag2 pyramid. We observed a strong synergistic interaction between Rag1 and Rag2, defined as genes differentially expressed only in the pyramid genotype. Our analysis suggests that a network of interacting TF integrates signaling from Rag1 and Rag2 to produce the unique Rag1/Rag2 response. Downstream effectors of the pyramid resistance response included rapid phytochemical production, cell wall strengthening, and repression of photosynthesis. The synergistic interaction between Rag1 and Rag2 in the Rag1/Rag2 genotype can explain its enhanced resistance phenotype. Understanding molecular mechanisms that support enhanced resistance in pyramid genotypes could facilitate more directed approaches for crop improvement.

Using the SoyBase database to facilitate soybean genome exploration

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W2: Introduction to SoyBase (soybase.org), the Soybean Breeder's Toolbox

In this workshop, we will present some of the uses of the SoyBase genetic and genomic data to explore the soybean genome. Exploratory case studies will be presented both from observations of genetic and genomic maps and from experimental results. We will also demonstrate how the SoyBase Database could be used to further refine candidate genes from a list of genes that were identified by various genetic and genomic studies. We will also introduce the workshop participants to our Data Store, which contains genomic sequences and gene annotations and markers from more than 50 soybean and other Glycine species as well as the genomic sequences from over 20 other cultivated and wild legume genera. Features of our sister database, the Legume Information System (LIS), for the exploration of other legume species will also be presented.

SoyBase.org: integrate genetics, genomics and breeding data to advance soybean research

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SoyBase (https://soybase.org) is the USDA-ARS soybean resource hub for the soybean community, by providing a comprehensive collection of data, analysis tools, and links to external resources of interest to soybean researchers. SoyBase partners with other plant data resources (eg. LegumeInfo, PhyloGenes, Phytozome and SoybeanBase) to provide links to data sets not housed at SoyBase. Currently, SoyBase hosts seven annotated genomes from multiple *Glycine max* and *Glycine soja* cultivars, including the most recent Williams 82 genome (Wm82_ISU01.a2.v1). The soybean reference genome (Glyma.Wm82) GBrowse has numerous data tracks available to view including genome organization, gene annotation and expression, markers, methylation and sequence variants (SoySNP50K and GmHapMap projects). SoyBase is an actively curated database, with new data regularly being incorporated, including gene expression, GWAS QTLs, and genome sequences and annotations. The latest set of tools added to SoyBase include the Pan-Genome Sequence Search and the SequenceSever for both nucleotide and protein BLAST. The SequenceSever includes more output options and the ability to download all the hits as FASTA files and alignment of the hit and tabular reports of the hits. SoyBase provides easy access to download data including SoySNP50K marker data, QTL positions, pan-genome datasets, genome, CDS and protein sequences. A major goal of SoyBase is to assist researchers in discovering important trait, genomic, and genetic information within the vast amount of data available.

Keywords: Data, soybean, SoyBase, genetics, breeding

GTA genotyping: an integrated genome-wide and targeted amplicon genotyping platform for a combined genomic selection and marker assisted selection

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Following the publication of NanoGBS by Torkamaneh et al. (2020) which has proven that the miniaturization of GBS and increased multiplexing leads to a substantial decrease in per-sample cost, and with the continuous objective of reducing the genotyping costs and develop eco-friendly approaches, two other components have been proposed to further develop the NanoGBS+ platform:

First, in order to decrease per-sample cost by increasing the multiplexing capacity on a single sequencing run, we have (i) investigated the choice of restriction enzymes to reduce the sequencing coverage and (ii) determinate the minimal read count required while maintaining a good density and distribution of the SNPs through the genome. Using DepthFinder, PstI a less frequent cutter than ApeKI, the methylation sensitive NsiI and insensitive MspI were selected and tested to construct 3D-GBS libraries. As expected, 3D-GBS data provided highly qualitative genotyping with better marker distribution uniformity than GBS for a lower cost per sample. Moreover, we have determined that 100K reads seems suitable for GWAS and genomic selection/prediction whereas 200K seems more relevant for QTL mapping.

Second, the development of an AmpSeq procedure adapted to the GBS library preparation in order to target trait-specific markers with high-agronomic values but missed by the GBS. By designing primers containing a RE compatible with the GBS library preparation, we were able to produce amplicons suitable for the ligation with the GBS-adaptors. All amplicons were amplified in a single multiplex PCR reaction, sequenced and all markers of interest were successfully genotyped.

By improving the multiplexing capacity, we will ultimately be able to continuously decrease the cost of the genotyping per sample while the integration of the AmpSeq will provide crucial information for breeders as well as screening analysis as CRISPR.

AccuCalc: An easy-to-use Python package for soybean genomics and breeding

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Soybean is one of the most important commodities worldwide. Therefore, a lot of genotyping data was produced over the past years that can serve the purpose of being a model for the development of genomic analyses, strategies and tools for more precise soybean breeding and accelerated soybean improvement. Genome-wide association study (GWAS) is a statistical method that associates a genotype with a phenotype and thus, can identify candidate genes. Even though it is a widely used method, its potential to discover a causative mutation (CM) is overestimated. This is caused by the fact that GWAS is data set size sensitive and quality of input data dependent, regardless of a fitting model under the hood of the analysis. Also, the complexity of multi-genic traits complicates GWAS results interpretation [1]. To improve GWAS efficiency in identifying a true CM, we developed an empowered "GWAS to Genes" strategy that we named Synthetic phenotype to causal mutation, SP2CM [2]. The tools that facilitate the utilization of SP2CM strategy for soybean GWAS-driven discoveries are available at SoyKB.org [3] for a curated panel of resequenced soybean accessions. To enable the utilization of SP2CM for other soybean data sets and even for other crops and species, we developed an easy-to-use Python package called AccuCalc [4]. AccuCalc formats Synthetic phenotype from a user-provided genotype data in variant calling format (vcf) or HapMap format (hmp) and thus, serves as a valuable tool for direct correspondence (Accuracy, [2]) calculation between the Synthetic phenotype and every variant position in the genotype data. AccuCalc can also calculate Accuracy for any selection of binary phenotype that a user provides. Further, a user-provided GWAS result in a tab-delimited format can be used as an additional input file and thus, the AccuCalc analysis output enriched by *p*-values can be visualized as an Accuracy accentuated Manhattan. AccuCalc Python package facilitates an easy-to-use GWAS results evaluation and easier interpretation and is freely available on GitHub (https://github.com/Biovja/AccuCalc).

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The allele catalog tool: A web-based interactive tool for allele discovery and analysis

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The whole-genome re-sequenced (WGRS) data availability has increased drastically as the sequencing technologies are improved over time. Nonetheless, conducting research with WGRS data without the enhancement of other data and tools is highly challenging. Hence, an interactive web-based Allele Catalog Tool that focuses on exploring the coding region allelic variations and connecting metadata and phenotype data has been developed. The Allele Catalog Tool is powered by our Allele Catalog datasets of soybean, Arabidopsis, maize, rice, and poplar, generated using our variant calling pipeline (SnakyVC) and the Allele Catalog pipeline (AlleleCatalog). The variant calling pipeline is developed to parallelly process sequencing data to generate the Variant Call Format (VCF) files, and the Allele Catalog pipeline takes VCF files to perform imputations, functional effect predictions, and assembles alleles for each gene to generate Allele Catalog datasets for the Allele Catalog Tool. The main features of the Allele Catalog Tool include data query, visualization, categorical filtering, and download functions. Users' queries return results that are summarized in tabular format by categorical description and genotype results of the alleles for each gene. The categorical information is specific to each species; additionally, metadata is also provided in modal popups. The genotypic information contains variant positions, reference or alternate genotypes, functional effect classes, and amino-acid changes. Besides that, the queried results can also be downloaded for other research purposes. The Soybean Allele Catalog Tool is hosted on the SoyKB website, while the Allele Catalog Tool of other organisms is hosted on the KBCommons website. Researchers can use this tool to identify novel alleles of genes and select plant accessions to improve selective breeding strategies and agricultural traits of plants. It has been designed in such a way that similar tools can be quickly implemented for any organisms with the availability or generation of similar WGRS datasets.

Keywords: Variant calling pipeline, allele catalog pipeline, allele catalog tool, alleles in gene, data visualization

Bioinformatics tools and frameworks for translational research

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Rapid multi-omics data production in research has enabled efficient data analytics and utilization to advance biological research. Adopting strategies such as data mining, web services development, and machine learning on the multi-omics data allows researchers to gain valuable information about underlying genomic and molecular mechanisms which can ultimately apply in plant breeding and phenotype improvements. Therefore, translational genomics tools and data integration platforms have been developed and hosted on our web-based informatics frameworks, Soybean Knowledge Base (SoyKB) and Knowledge base Commons (KBCommons). The SoyKB and KBCommons bridge translation genomics and multi-omics data for assistive breeding. These frameworks consist of analytics tools including multiple gene/metabolite expression hierarchical clustering, omics expression viewer, pathway viewer, and others powered by Cyverse and high-performance computing systems. Besides that, state-of-the-art tools such as Allele Catalog Tool, Genomic Variation Explorer (GenVarX), SNPViz v2.0, Integrative Multi-omics Pathway Resolution (IMPRes), and G2PDeep are integrated into the frameworks to support various aspects of translational research. The Allele Catalog Tool provides query, visualization, and download capabilities for users to investigate coding region allelic variations with genotype data, phenotype data, and metadata. The GenVarX toolsets that compose the promoter search section and copy number variation (CNV) section allow users to validate and annotate genotypes according to phenotype differences. Furthermore, SNPViz v2.0 can link genome-wide association studies (GWAS) results to genes, perform haplotype analysis, and present phylogeny trees with identified haplotype blocks. The IMPRes tool uses transcriptomic, proteomics, and metabolomic data on a background network of pathways and protein-protein-interaction (PPI) to understand pathway connectivity and support *in silico* hypothesis building. Lastly, the deep-learning-based G2PDeep is for quantitative genotypes to phenotypes model training and predictions using multi-omics data. With the availability of these tools via easy-to-use web-based frameworks, researchers can easily apply these to multi-omics datasets and advance their translational research for any organism.

Keywords: Soybean knowledge base, knowledge base commons, multi-omics data integration, data visualization

Assembly and utilization of a single haplotype reference genome for soybean

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This report details the assembly of a new reference genome of the soybean genotype Williams 82 and its use in comparative genomics. The genome was derived from sampling a single plant of Williams 82, known as the sub-line 'Williams 82-ISU-01' (Wm82-ISU). The genome was assembled using Pacific BioSciences HiFi reads and integrated into chromosomes using HiC. The Wm82-ISU genome adds 59.5 Mb of sequence and reduces contig number from 9,202 to 36 total contigs. The new annotation includes a significant amount of full-length cDNA sequencing which has reduced the gene count from 52,872 to 48,387. Williams 82 was derived from backcrossing genotype Kingwa into the background of Williams, leading to heterogeneous introgressed segments that persists in modern Williams 82 sub-lines. The Wm82-ISU assembly shows clean Kingwa introgression segments, reflecting its derivation from a single sub-line DNA source. In addition to Wm82-ISU assembly, we also assembled the genome of soybean line 'Fiskeby III,' a rich resource for abiotic stress resistance genes. We used these assemblies to study the genomic variation between 'Fiskeby III' and the Wm82-ISU reference within a fine-mapped QTL for iron deficiency chlorosis resistance, revealing candidate sequence polymorphisms that may be underlying the QTL variation.

Implementation of molecular selection of soybean towards adaptation to Polish agroclimatic conditions

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European countries are highly dependent on soybean meal imports and look for an increase a domestic production of plant-based protein. Climate change due to global warming caused more favorable thermal conditions for soybean in Poland. However, soybean cultivation under long-day photoperiod (up to 17 hours) and during relatively short growing season requires efficient reselection of adapted germplasm from domesticated resources. In the present study, a PCR array has been implemented, targeting early maturity (E1-E4, E7, E9 and E10), pod shattering (qPHD1) and growth determination (Dt1 and Dt2) genes. This array was optimized for routine screening, excluding Dt2 markers due to low reliability, and subsequently used for genotyping of soybean germplasm diversity panel (204 accessions) subjected to 3-year field survey of phenology, morphological and yield components traits. Genotyping of soybean diversity panel with the PCR array revealed 41 allelic combinations when simple classification to recessive or dominant alleles was applied, whereas when diversity among dominant or recessive alleles was taken into account, the number of identified combinations raised to 98. All genes except E9 and E10 revealed significant correlations with at least three traits. The most remarkable correlations were those identified for days to flowering with E7 and E1 genes (0.42 and 0.41), days to maturity with E4 and E7 (0.48 and 0.40), and plant height with Dt1 and E4 (0.46 and 0.43). Seed yield was positively influenced by wild alleles of Dt1 (22.1% of mean percentage change as compared to domesticated allele), E3 (15.6%) and E4 (9.8%), whereas thousand grain weight was negatively influenced by E2 (-9.9%).To summarize, the study showed a huge genetic diversity of tested germplasm (almost every second genotype had a different combinations of alleles) and demonstrated high applicability of PCR array for molecular selection of soybean towards adaptation to Polish agroclimatic conditions.

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Progress in soybean grain yield and grain quality breeding at the Agricultural Institute Osijek, Croatia

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The primary objective of the conventional, GMO-free soybean breeding programme at the Agricultural Institute Osijek (AIO, Croatia) is creating high-yielding and high-grain quality soybean cultivars in maturity groups (MG) 00 to I, stable and adaptable in a variety of European environmental conditions. The contribution of the AIO soybean breeding program to the development, stability and improvement of soybean production in Croatia has been fundamental since its beginnings in the 1970s. Along with breeding for increased grain yields and grain quality traits such as protein and oil content, in the last decade, breeding efforts have been focused on nutraceutical grain quality as well, aiming to make European soybean more appealing to the diverse processing industry as well as profitable enough to satisfy European farmers. The specific aims were to increase grain yield, protein and oil contents, total isoflavone and oleic acid contents and to decrease the unfavourable linolenic acid content and galactooligosaccharides. The breeding efforts were evaluated in a three-year preliminary trial comparing 12 elite breeding lines, chosen based on their superior phenotypic and agronomic performances during selection, with respective standard AIO cultivars leading in commercial production. All genotypes were divided into three sets depending on the MG: 00, 0, I. Field trials were set up as a randomised complete block design with three replications at the AIO (Osijek, Croatia), and all analyses were performed at the AIO laboratory. The most progress was determined in MG 00, which had the highest average increase in grain yield, grain proteins and total isoflavones (15.9%, 2.8% and 68%, respectively) and the highest average decrease in linolenic acid (13.5%). MG 0 breeding lines resulted in the highest average oleic acid increase (10.3%) and galactooligosaccharides decrease (13.8%). These results confirm that soybean grain yield and grain quality progress can be achieved with conventional, GMO-free breeding. However, as all tested traits are greatly influenced by environmental conditions, their stability needs to be evaluated in more than three environments.

Keywords: Grain yield, grain quality, nutraceuticals, conventional breeding

Data strategies, techniques, principles, and practices: harnessing the power of genomics and phenomics for accelerating soybean improvement

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Soybean breeding has made great strides in the last decade due to the increased availability and accessibility of high-throughput technologies in different omics, specifically phenomics and genomics. The combination of phenomics and genomics information allows soybean breeders to develop new varieties better adapted to different climates (target environment), with exponential improvement in yields and disease resistance. However, one of the major ongoing challenges soybean breeders face is to efficiently organize, manage, and analyze large and complex datasets of phenomics and genomics and successfully translating them to soybean breeding programs. To address this challenge, soybean breeders have recently used several data strategies such as machine learning, data integration methods, and predictive analytics) to facilitate data processing in phenomics, genomics, and breeding datasets. Yet, it is still unclear how far and to what extent different data strategies have come to help soybean breeding in the last decade. Here, we provide an overview of genomics and phenomics technologies, with descriptions of the key data types and associated tools. We will also explore various data strategies that can be employed to leverage the power of genomics and phenomics, including data integration, analysis, principles, and practices for developing successful data-driven soybean breeding programs, including how to identify and prioritize breeding targets/traits, methods for generating and managing high-quality data, and best practices for data storage and sharing. In general, the overall goal is to explore the opportunities and challenges associated with integrating different data strategies of phenomics and genomics datasets and outline potential strategies to maximize the effectiveness of genomic and phenomic data in soybean breeding for moving forward to sustainable agriculture practices ensuring food safety for the future.

Genetic basis and selection of phytochemical variation in wild soybean

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Glyceollins, a family of phytoalexin induced in legume species, play essential roles in responding to environmental stresses and in human health. However, little is known about the genetic basis and selection of glyceollin induction. We employed a metabolite-based genome-wide association (mGWA) approach to identify candidate genes involved in glyceollin induction from genetically diverse and understudied wild soybeans subjected to soybean cyst nematode stress. Eight SNPs on chromosomes 3, 9, 13, 15, and 20 showed significant association with glyceollin induction. Six genes close to one of the significant SNPs (ss715603454) on chromosome 9 fell into two clusters, and they encode enzymes in the glycosyltransferase class within the phenylpropanoid pathway. Transcription factors (TFs) genes, such as MYB and WRKY were also found within the linkage disequilibrium of the significant SNPs on chromosome 9. Epistasis and a strong selection signal were detected on the four significant SNPs on chromosome 9. Gene clusters and transcription factors may play important roles in regulating glyceollin induction in wild soybeans. Additionally, as major evolutionary factors, epistatic interactions and selection may influence glyceollin variation in natural populations.

Keywords: Epistasis, gene cluster, mGWAS, phytoalexin, transcription factors

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DNA hypomethylation potentiates the expression of stress-responsive genes in salt stress priming in soybean

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Plants that have experienced certain abiotic stress may gain tolerance to a similar stress in subsequent exposure. This phenomenon is known as priming. Previously, we have reported that the soybean cultivar (C08) had enhanced salt tolerance after priming. The contribution of histone modifications to the reprogramming of transcriptional responses suggested that other epigenetic features may also be important players in salt stress priming. Therefore, it was hypothesized that DNA methylation plays a key role in regulating salt-stress responses in priming in soybean. In soybean leaves, salt stress priming was found to induce global DNA hypomethylation, as indicated by the whole-genome bisulfite sequencing (WGBS) data. While the transcript level of DNA methyltransferase-encoding genes remained unchanged, the total activity of DNA methyltransferase was reduced. Overall, the hypomethylated genes (hypo-DMGs) were modified by higher levels of active histone mark (H3K4me3) and lower levels of repressive histone mark (H3K4me2). Further analysis of the transcriptional responses of hypo-DMGs identified a gene cluster with higher expression upon high salt stress. The ABA-responsive element (ABRE)-like motif was significantly enriched in the promoter regions of these genes, suggesting that DNA hypomethylation may facilitate the transcriptional responses mediated by ABA signaling. To support the importance of DNA demethylation, the DNA methyltransferase inhibitor 5-azacytidine was applied to pretreat soybean seedlings. After high salt stress, the non-primed seedlings were severely wilted, whereas the 5-azacytidine-treated seedlings did not show the wilting phenotype, suggesting that DNA hypomethylation could enhance salt tolerance in soybean seedlings. This research reveals the key regulatory role of epigenetics features in salt stress priming in soybean.

Keywords: Priming, epigenetics, DNA methylation, histone modification, salt stress

Acknowledgements: This work was supported by grants from the Hong Kong Research Grants Council Area of Excellence Scheme (AoE/M-403/16) and the Lo Kwee-Seong Biomedical Research Fund.

Identification of novel candidate gene associated with sensitivity to phytotoxicity of etofenprox in soybean

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Phytotoxicity is caused by the interaction between plants and a chemical substance, which can result in critical damage to plants. Understanding the molecular mechanism underlying plant-chemical interaction is essential to manage pests in crop fields and avoid phytotoxicity of plants by insecticides. Some Korean soybean cultivars including Danbaek and Kwangan showed sensitivity to phytotoxicity of etofenprox (PE), resulting in leaf shape shrinkage damage and finally critical yield loss. Genomic region responsible for sensitivity to phytotoxicity of etofenprox (PE), controlled by a single dominant gene, was detected by constructing high density genetic map using recombination inbred lines (RILs) in soybean. The genomic region of ~80 kbp containing nine genes was identified on chromosome 16 using high-throughput single nucleotide polymorphism (SNP) genotyping system using two different RIL populations. Using re-sequencing data of 31 genotypes, nonsynonymous SNPs were identified in three genes, encoding glycosylphosphatidylinositol -anchored protein (GPI-AP), resulted in critical structure disruption on active site of the protein. This structural variation of GPI-AP may change various properties of the ion channels which are the target of pyrethroid insecticide including etofenprox. This is the first study to identify the candidate gene and develop SNP markers associated with PE. This study would provide genomic information to understand mechanism of phytotoxicity in soybean and functionally characterize the responsive gene.

Keywords: Phytotoxicity, etofenprox, soybean, Axiom® 180K SoyaSNP array

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Transgenic soybean expressing a gene from *Amaranthus hybridus* shows cross-resistance to ALS-inhibiting herbicides

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This abstract describes the development oftransgenic soybean events expressing a herbicide-resistant gene from a weed previously characterized in Argentina. Thus, a mutated ALS (W574L) gene, isolated from an ALS-herbicide resistant *Amaranthus hybridus* plant, was isolated and cloned under the CaMV35S promoter and NOS-ter in a vector for *Agrobacterium tumefaciens* transformation. Eighteen transgenic soybean events (cv. Williams 82) were obtained in the Plant Transformation Facility from Iowa State University (USA), using hypocotiles for infection and the bar gene as selectable marker. Molecular detection by PCR of the T1 and T2 progenies from each event allowed us to identify two events with a single-copy transgene integration and homozygous. T3 generation of transgenic events allowed us to amplify the number of seeds for further experiments. Once stabilized both transgenic lines (C and E), plants from the T4 generation were grown in a growth chamber in controlled environmental conditions. Proteins extracts from five plants were assayed to check ALS activity in the presence of different doses of chlorimuron (from 0 to 1000 nM), imazetapyr (from 0 to 1000 uM) and diclosulam (from 0 to 640 nM), keeping the transgenic plants a high activity according to the resistance. In the same way, ten plants from each event were sprayed with commercial formulations of each herbicide, adjusted to different doses of chlorsulfuron, imazapyr, diclosulam, bispyribac sodium and fluorcarbazone (0, 2.8, 5.6, 8.4, 11.2, and 20 uM). Negative control was theno-transgenic genotype, and the CD250 commercial variety with the same mutation was the positive control. No differences were found between the transgenic lines and the commercial positive control at the highest doses tested. Plants were conducted to maturity in the growth chamber, and no penalty was detected neither in the plant phenotype or yield, compared with a non-transgenic control from the same variety.

Keywords: Transgenic soybean, herbicide resistance, ALS

Acknowledgements: We thank the Plant Transformation Facility from Iowa State University for developing the transgenic events. The financial support came from ANPCYT-MINCYT and ASACTEI from Argentina. Facultad de Ciencias Agrarias UNR and Coop Santa Rosa conducted all experiments.

High multiple shoot regeneration from embryonic axes of elite germplasm from soybean

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Plant breeding pursues the goal of increasing productivity and quality for any crop. Ag-biotechnology offers new tools, such as transgenesis and gene editing, for these purposes. Both require an in-vitro culture system that allows the regeneration of fertile plants, being this a bottleneck of the process. Soybean (and particularly, elite germplasm) is recalcitrant to regenerate plants by somatic embryogenesis, but organogenesis protocols have been alternatively developed. In this abstract, we describe an improvement in the protocol to regenerate multiple shoos from apical meristem from the embryonic axes of elite varieties (previously cited by Reich el al -2008- Nat Protocols, 3: 410-18). Meristems were isolated from mature seeds and cultured in MS medium with 5 mg/L BAP overnight. Then, they were transferred to the same medium without plant regulators, under 300 umol 4000K-LED light tubes with 16 h-photoperiod. At the time a shoot was developed from the meristem, it was isolated and cultured individually in an MS medium. The meristem initially treated with BAP continued growing in the same medium for at least 50 days. An efficiency of 10-20 shoots regenerated from each initial apical meristem of 20 commercial varieties was observed. Individual shoots were transferred to an MS medium supplemented with 0.5 mg/L NAA to induce rooting, and then acclimated and conducted to maturity in a plant growth chamber. This improved procedure of isolating shoots from the apical meristem of embryonic axes shows the following advantages: a) it is applicable to elite germplasm, b) the initial material is mature seeds, c) the meristem may be genetically modified by either Agrobacterium or biolistic transformation, and d) the multiple developed shoots may be transferred to a selective regeneration medium sequentially, according to the time they are differentiating.

Keywords: Plant regeneration, organogenesis, multiple shoots, genetic engineering, elite germplasm

Acknowledgements: The financial support came from UNR, ASACTEI, and Coop Criadero Santa Rosa from Argentina. Facultad de Ciencias Agrarias UNR and Coop Criadero Santa Rosa conducted all experiments.

Expression profiling of PAP3, BZIP, and P5CS genes in soybean under drought stress conditions

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Under drought stress, a signaling system induces the expression of specific genes to alleviate the harmful effects of drought stress. The BZIP gene is a transcription factor in the signaling abiotic stresses and plays a role in the regulation of responses to different stresses in plants. The P5CS gene controls the activity of the proline-5-carboxylate synthase enzyme that is involved in proline synthesis in drought stress conditions. In this research, the effect of drought stress was investigated on the expression of GmPAP3, GmBZIP and GmP5CS genes in two soybean cultivars, Williams (tolerant) and L17 (susceptible). Total RNA was isolated from leaves and roots of both nonstressed and stressed plants, and then cDNA was synthesized and used for real-time PCR. The housekeeping gene 18SrRNA was used to normalize data. Data analysis showed that the expression of GmPAP3, GmBZIP, and GmP5CS genes increased under drought stress. GmPAP3 and GmBZIP expressions were two-fold while GmP5CS expression was seven-fold greater in Williams than in L17. GmPAP3 and GmP5CS gene expressions were similar in leaves and roots, while GmBZIP expression was higher in roots than in leaves. In conclusion, the increased expression of these genes could be attributed to higher drought tolerance in cultivar Williams and it seems that transferring these genes into susceptible cultivars may enhance drought tolerance in soybean.

Keywords: GmPAP3, GmBZIP, GmP5CS, *Glycine max*, drought stress

Fine-mapping and identification of a restorer-of-fertility gene for the cytoplasmic male sterility in soybean

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The cytoplasmic male sterility/restorer-of-fertility(CMS/Rf) system is an important measure to utilize soybean heterosis. The Rf gene in this system directly affects the fertility and stability of hybrid lines. To date, there are relatively few reports on cloning and functional identification of Rf genes in soybean, which limits the breeding of new CMS lines and production of commercial hybrid soybean seeds. Therefore, in the present study, F_2 and F_3 segregating populations derived from the CMS line JLCMS5A crossed with the restorer line JLR2 were developed and used for Rf3 gene fine mapping. Genetic investigation indicated that the restorer line JLR2 was controlled by a single dominant gene, Rf3. By integrating bulk-segregant analysis and next-generation sequencing, a 4 Mb region on chromosome 9 was identified, which was most likely the target region harboring the candidate gene responsible for fertility restoration. This region was further narrowed down to 86.44 Kb via fine mapping in F_2 and F_3 populations using SSR, InDel, and dCAPS markers. This region contained 10 putative genes. Glyma.09G171200, which encodes a mitochondria-targeted pentatricopeptide repeat (PPR) protein, was proposed as the potential candidate for Rf3 using sequence alignment and expression analysis in restorer and CMS lines. Based on single-nucleotide polymorphisms in Glyma.09G171200, a CAPS marker co-segregated with Rf3 named CAPS1712 was developed. Finally, we constructed a phylogenetic tree, including 20 PPR proteins. The phylogenetic analysis revealed that the Glyma.09G171200 protein belongs to the PPR protein, clustered with the previously reported GmPPR576 protein from soybean. and the Glyma.09G171200 gene is consistent with most of the characteristics of Rf genes. Our results not only provided new Rf gene loci for "three-line" hybrid soybean breeding, but also provided technical support for assisted breeding and creation strong restorer lines with multi-restorer gene polymerization in production.

Phenotypic prediction of soybean based on the spectral reflectance spectra respond to drought stress

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Soybean is one of the most important crops in the world, but drought stress during their growth can significantly decrease yield and grain quality. In the present study, we analyzed relationship between reflectance spectra and several traits respond to drought stress to predict the phenotypes based on the spectral data. We cultivated ten soybean accessions in a greenhouse under four levels of watering conditions from normal watering (W1) to severe drought stress (W4). We measured time series data for the reflectance spectra of the top leaves and plant height, fresh and dry weight of the aboveground part, seed number, and seed weight on a weekly basis after the start of drought stress. We calculated second derivative average value in 5 nm intervals and performed significance difference tests to identify variations in the spectra respond to drought stress. In the W4 plot, a specific spectral variation was observed around 768-796 nm at 5-6 weeks after the stress applied, when the differences of phenotype due to stress became apparent. We considered these specific spectra to be the wavelength that respond to drought stress. Phenotypic prediction model constructed using partial least squares regression based on spectral data was evaluated its accuracy by correlation between measured phenotype data and predicted data. The plant height could not be accurately predicted from reflectance spectrum because it was not affected largely by drought stress. In contrast, the dry weight of the aboveground part and yield-related traits can be predicted with moderate accuracy. Therefore, we suggest that the model based on spectral reflectance spectra respond to drought stress can predict soybean growth under drought stress.

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Mutation in the PHD-finger protein MS3 restores the fertility of male-sterile plant lines under long-day conditions in soybean

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Male-sterile plants are used in hybrid breeding to improve yield in soybean [*Glycine max* (L.) Merr]. Developing the capability to alter fertility under different environmental conditions could broaden germplasm resources and simplify hybrid production. However, molecular mechanisms potentially underlying such a system in soybean were unclear. Here, using positional cloning, we identified a gene, MALE STERILITY 3 (MS3), which encodes a nuclear-localized protein containing a plant homeodomain (PHD)-finger domain. A spontaneous mutation in ms3 causing premature termination of MS3 translation and partial loss of the PHD-finger. Transgenetic analysis indicated that MS3 knockout resulted in nonfunctional pollen and none self-pollinated pods, and RNA-seq analysis revealed that MS3 affect the expression of genes associated with carbohydrate metabolism. Strikingly, the fertility of mutant ms3 can restore under long-day conditions. The mutant could thus be used to create a new, more stable photoperiod-sensitive genic male sterility line for two-line hybrid seed production, with significant impact on hybrid breeding and production.

Keywords: Fertility restoration, male sterililty, PHD-finger, photoperiod, soybean

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Combined physiological and transcriptomic analysis reveals key regulatory networks and potential hub genes controlling chilling tolerance during soybean germination

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Chilling is an important limiting factor for seed germination of soybean (*Glycine max* [L.] Merr.). To reveal the regulatory mechanism of chilling tolerance during the soybean germination stage, based on previous studies, the chilling tolerance strain R48 and chilling sensitive strain R89 in chromosome segment substitution lines (CSSLs) were selected for physiological index determination and transcriptome sequencing. Through the determination of physiological indexes, it was found that both strains responded to chilling stress, but the responses were different. Correlation analysis showed that osmotic adjustment substances, reactive oxygen species (ROS), and related enzymes of the ROS scavenging systems were interrelated. Gene Ontology (GO) enrichment and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment were performed on the differentially expressed genes obtained by transcriptome sequencing. It was found that terms or pathways related to flavonoids, unsaturated fatty acids, and abscisic acid were highly enriched. In addition, this study also used the gene expression data obtained by transcriptome sequencing to construct a weighted gene co-expression network, which produced four main modules. A correlation analysis showed that these main modules were correlated with physiological indicators. The five genes with the highest eigengene-based connectivity (KME) value in each module were selected as hub genes. This study has identified key pathways and key genes and used qRT-PCR to verify the expression of some of the important genes. The results show that the transcriptome sequencing data are reliable. This study lays a foundation for the analysis of the molecular regulatory mechanism of chilling tolerance during soybean germination.

Keywords: Soybean germination, chilling tolerance, transcriptome analysis, physiological analysis, hub genes

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Identification of key salt-tolerant genes using RNA sequencing analysis in cowpea (*Vigna unguiculata* L.)

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Cowpea [*Vigna unguiculata* L. Walp] is a herbaceous legume crop that is cultivated mainly in arid and semi-arid regions. However, the accumulation of soil salinity is caused by the physical and chemical characteristics of the soil in these areas. In this study, we performed a comparative transcriptome analysis using four cowpea germplasms with contrasting salt tolerance types. As a result, 1.14 billion short reads with an average length of 101 bp were obtained using the Illumina Novaseq 6000 platform. It was mapped to the cowpea reference genome, *Vigna unguiculata* v1.2, after a preprocessing process. Among numerous DEGs, we screened based on the salt tolerance pattern, and a total of 27 candidate genes were selected. This includes genes encoding late embryogenesis abundant and peroxidase family proteins. Then, reference sequencing was performed to narrow down the range of candidate genes. Most variations occurred in UTR and intron regions, but two candidate genes (Vigun_02G076100 and Vigun_08G125100) showed a significant relationship with salt tolerance in cowpea. The identified variations as a result of NGS include the missing gene and the cSNP with amino acid type substitution. The identified variations in each candidate gene have the potential as molecular markers in cowpea's salt tolerance breeding program.

Deciphering transcriptional and metabolic regulation involved in plant-microbe interaction in soybean using single-nuclei RNA sequencing (snRNA-seq) technology

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Securing higher crop yield is primarily dependent on use of synthetic fertilizers. However, these activities lead to fertilizer run-off with harmful effect on ecosystem, health, and economy. Fortunately, root-soil-microbe interactions, offers a sustainable-promise to overcome these challenges. Mounting evidence including our lab data has shown that Arbuscular-mycorrhizal-fungi (AMF) and rhizobium symbiosis improve nutrient uptake, and biomass in soybean. Despite these amazing mutualistic benefits, the complexity of signal reception, nodule development, nitrogen (N), and phosphorus (P) uptake at the 'sub-cellular level' is elusive in soybean. Additionally, limited information is available about P supplement and its puzzling negative effect on the capacity of mycorrhiza to colonize soy roots, hence affecting its utilization in the farmer's fields. Another reason for underutilization is, root-soil-microbe interactions are highly complex and involve a range of molecular, biological, and chemical processes. This study aims to understand these critical processes and provide solutions to improve nutrient uptake and N/P use efficiency in soybean. As a step forward, we developed a method to isolate high-quality nuclei from nodule tissues without the need for fluorescent-activated nuclei sorting and developed snRNA-seq libraries. Furthermore, we performed the metabolic analysis of 2-week-old soybean roots challenged with AMF and rhizobium.

Identification of QTL associated with salt tolerance in soybean mutant line induced by gamma-ray irradiation

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Salt stress is one of the major abiotic stresses that limit soybean yield and quality. In this study, we evaluated the salt tolerance of soybean mutant KA-1285 induced by gamma-ray irradiation and identified QTL associated with salt tolerance. Most of the salt tolerance-related traits in KA-1285 were similar to PI483463, salt-resistant germplasm. However, uniquely high chloride ion accumulation was induced in the roots of KA-1285. To identify QTL related to salt tolerance in KA-1285, a total of 169 $F_{2:3}$ lines from the 'Daepung' x KA-1285 were used for QTL analysis. The linkage map was constructed with a total of 1,452 SNP markers, and QTL was identified through interval mapping and the multi-QTL method. As a result, a major QTL associated with salt tolerance was identified with a high LOD score on chromosome 3. The results of this study explain the salt tolerance mechanism of the gamma-ray irradiation-induced mutant KA-1285. This will provide useful information for the soybean salt tolerance breeding program.

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Identification of QTLs related to high sucrose content in soybean

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Sucrose is one of the most important sweetness components that contributes to the flavor and taste of soybean products such as soy milk and tofu. The purpose of this study is to identify high sucrose-related quantitative trait loci (QTLs) using the two $F_{2:3}$ populations derived from Ilmi (high sucrose) x IT186230 (high sucrose) and IT186230 (high sucrose) x IT025668 (low sucrose). Sucrose content was determined with Glucoseoxidase/Invertase assay and SNP genotyping was conducted using SoySNP6K illumina BeadChip. In the 190 $F_{2:3}$ population derived from Ilmi x IT186230, three QTLs were identified on chr.11, chr.15, and chr17. In addition, one QTL located on chr.6 was detected in the 152 $F_{2:3}$ population derived from IT025668 x IT186230. In this study, identification of new sucrose-related QTLs will provide useful information for the development of new molecular markers.

Soybean-rhizobium symbiosis: Insights from omics studies

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Soybean develops root nodules for nitrogen fixation through symbiosis with rhizobium. Knowledge on soybean-rhizobium interactions is important for boosting biological nitrogen fixation for agricultural productivity and sustainability. *Sinorhizobium fredii* is a rhizobium adapted to alkaline–saline soils and is predominantly found in the North China Plain. In our previous study, the complete genome sequences of *S. fredii* CCBAU45436 and CCBAU25509 were obtained and novel genes contributing to symbiotic adaptations were identified from the transcriptome of these strains. The completion of the reference genomes facilitated the later proteomic study. Using a high-throughput Orbitrap system, Nodulation Outer Proteins (Nops), which are effectors affecting host specificity, were identified from the secretory proteome. In line with the role of secretory proteins in determining host specificity, a CCBAU25509 mutant defective in the type III secretion system and another mutant with its NopP replaced by the NopP from CCBAU45436 could nodulate the otherwise-incompatible soybean C08. To better understand the metabolic processes during nitrogen fixation, we reconstructed the metabolic network of CCBAU45436 and predicted 33 novel players of symbiosis. Incorporating the transcriptomes of CCBAU45436 bacteroids into the model revealed that the bacteroids in the cultivated soybean C08 had higher nitrogen fixation capacity than those in the wild soybean W05, and the cytochrome oxidase reaction was a determinant of this difference. Besides, the epigenetic features of soybean-rhizobium interactions were investigated. The histone modification H3K4me3, which plays a role in transcriptional activation, was shown to mark important genes for maintaining nodule functions. MiR399b, a microRNA upregulated in nodules when compared to roots, was shown to positively regulate Pi uptake and nitrogen fixation in nodules.

Keywords: Nodulation, symbiosis, *Sinorhizobium fredii*, biological nitrogen fixation, multi-omics

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MicroRNA 4* modulates nodulation in soybean by repressing a root-specific isopentenyltransferase

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MicroRNAs (miRNAs) are important regulators of plant biological processes. One soybean unique miRNA, miR4* was reduced by rhizobial infection and was supposed to be related to cytokinin biogenesis. From gain and loss of function analysis and promoter-GUS expression analysis, miR4* positively regulates lateral root emergence and root structures, and represses a root-specific ISOPENTENYLTRANSFERASE (GmIPT), both being expressed in the inner root cortex and nodule primordia. Upon rhizobial inoculation, miR4* was downregulated while GmIPT was upregulated. Overexpressing miR4* reduced the number of nodules in transgenic soybean hairy roots while overexpressing the wild-type GmIPT or a miR4*-resistant GmIPT mutant (mGmIPT) significantly increased the nodule number. The mechanism of miR4* and GmIPT functions was also linked to autoregulation of nodulation (AON), where miR4* overexpression repressed miR172c and activated its target, GmRIC1, turning on AON. Exogenous CK mimicked the effects of GmIPT overexpression on miR172c, supporting the notion that GmIPT regulates nodulation by enhancing root-derived CK. Our data, therefore, revealed a new miRNA-mediated regulatory mechanism of nodulation in soybean.

Keywords: miR4*, Isopentenyltransferase, GmIPT, cytokinin, nodule number, autoregulation of nodulation (AON), miR172c, soybean

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Assessment of drought tolerance response of soybean genotypes through agro-physiological and molecular breeding tools

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Drought tolerance capacity of sixty-four soybean accessions including released varieties, genetic stocks and five check varieties was evaluated during 2017 to 2018 under two different water regimes of water stress and non-stress, to identify the drought tolerant soybean accessions as well as agro-physiological traits contributing to drought stress tolerance. Results of two-year study revealed that water stress caused 713 to 1855 kg/ha average reduction in the yield as compared to non-stressed condition. Soybean seed yield was significantly higher (3056 kg/ha) under non stressed crop over water stressed crop (1557 kg/ha) and yield reduction in stressed crop was 41% and 57% for both years, respectively. On the basis of minimum yield reduction under stress RSC 10-46, TAMS 98-21, EC 241780, MACS 1281, HARDEE, MAUS 612, DS 9814, MACS 1460 and KDS 753 soybean accessions were observed as water stress tolerant while, EC 241695, LEE 54, MACS 1370, CAT 3466, PK 1029, VLS 75, AGS 228 and AMS 1002 were observed water stress sensitive. The traits contributing to total biomass viz., plant height, number of pods, number of branches, shoot-root dry weight at vegetative stage, and NDVI at vegetative as well as pod filling stage were associated with water-stress tolerance in soybean. Physiological traits viz., Relative leaf water content (RWC), Normalized vegetative index (NDVI) and Chlorophyll index were higher in non-stressed crop. Similarly, higher activities of the physiological traits viz., Photosynthetic rate (Pn), stomata conductance (Gs), intercellular CO_2 concentration (Ci) and transpiration rate (Tr) were observed under non stress condition. On the basis of drought tolerance indices, RSC 10-46, TAMS 98-21, HARDEE, MACS 1281 and EC 241780 were identified as the most tolerant accessions. These findings were confirmed by the cumulative rank due to drought tolerance indices. Cluster analysis using drought tolerance indices could differentiate RSC 10-46, TAMS 98-21, MACS 1281, HARDEE, EC 241780, DS 9814, MACS 1460, and MAUS 612 as water-stress tolerant accessions, hence, could be useful to identify the soybean accessions suitable for water-stressed conditions. Drought tolerant genotype RSC 10-46 was further studied for identification of the inventory of genes involved in drought tolerance mechanism in soybean. Various differentially expressed genes (DEG) were identified by RNA sequencing and the data was validated by qRT-PCR. Genes involved in antioxidant defense-system, plant hormonal response, and photosynthesis were among the major DEG.

Keywords: Agronomical, drought tolerant, molecular tool, physiological, water stress

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Developing a low-trypsin inhibitor soy variety through CRISPR/Cas9 and evaluating low-TI's fish feed to improve soy-based aquafeeds for Steelhead trout

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Trypsin inhibitor (TI), an abundant anti-nutritional factor in soybean seeds, causes low protein digestibility when raw soybeans are fed to animals. Similarly, despite soy meal is an alternative to fish meal due to its excellent protein profile, TI limits the amount of how much soybean can be incorporated into fish fed. This study goals were to: 1- edit genes contributing to TI content using CRISPR/Cas9 and develop molecular markers based on the mutant alleles, and 2- evaluate the performance of Steelhead trout fed a diet with low-TI soy meal. With the aid of KTI gene expression data, KTI1 (Glyma01g095000) and KTI3 (Glyma08g341500), were selected as the gene editing targets. A CRISPR/Cas9 construct for transformation on Williams 82 was developed. Results showed in the T0 seeds of transgenic plants, the gene editing of KTI1 has been all completed while of KTI3 has been partly finished. The TI content and activity declined more than 70% and 10% with knock-out of KTI1 alone and more than 90% and 30% with knock-out of both KTI1 and KTI3. We developed allele-specific markers that can amplify mutant alleles of kti1 or kti3. We also examined Steelhead trout fed with five different diets that varied in how much soy meal was present and the if the soy came from a low or high TI variety. The five diets were: fish meal control (no soy meal), two diets using commercial soy meal with high TI at 30% and 60% inclusion levels (SBM) and two diets with low-TI variety VT Barrack at 30% and 60% inclusion levels (VSBM). Fish fed with 30% SBM and 30% VSBM were found to have similar growth performances, while the 60% SBM and 60% VSBM were found to have lower growth than the other three diets with the 60% VSBM having the lowest growth.

Effect of maturity group on soybean root rot caused by *Fusarium graminearum*

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Fusarium graminearum Schwabe is one of the fungal pathogens in the *Fusarium* genus that causes root rot and seedling disease of soybean (*Glycine max* L.) in the United States. Typically, U.S. farmers will alternate soybean and maize (*Zea mays* L.) in a two-year crop rotation. However, rotation between soybean and maize may not help reduce the inoculum of *F. graminearum* in their field since isolates of *F. graminearum* isolated from corn can cause root rot of soybean. Currently, there are no commercial soybean cultivars available in the U.S., which has partial or complete resistance to *F. graminearum*. The objective of this study was to identify soybean accessions from the USDA soybean germplasm collection belonging to the maturity group (MG) 0 to V with resistance to F. graminearum in the greenhouse. Two hundred and forty-eight accessions (including the susceptible check, 'Williams 82') were screened to a single isolate of *F. graminearum* using the inoculum layer inoculation method in the greenhouse. At 21 days post-inoculation, root rot severity was evaluated on a 1-to-5 rating scale. Because the disease severity ratings data were ordinal, they were analysed by nonparametric statistics and relative treatment effects (RTE) of the ratings were calculated. Eight accessions belonging to MG I, II or IV were significantly less susceptible to F. graminearum when compared to 'Williams 82' based on 95% confidence intervals. Further, the effect of cultivar MG was determined on root rot severity, and it was determined that cultivars belonging to MG 0 or I had significantly greater RTE than cultivars belonging to MGs II, III, IV or V based on 95% confidence intervals. Our results indicate that the variation in susceptibility among soybean cultivars to *F. graminearum* should be considered by breeders during development of cultivars with root rot resistance.

Keywords: Fusarium, soybean, seedling disease, root rot

Antixenose determination to brown stink bug (*Euschistus heros*) in Brazilian soybean genotypes

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The soybean (*Glycine max* L.) is one of the main Brazilian crops. Among the pests capable of causing damage to this crop, the stink bug complex is the main one, causing severe losses in seed quality and productivity. To avoid the attack, antixenose resistance is an important plant mechanism. The goal of this study was to characterize the antixenose resistance in soybean to brown stink bugs (*Euschistus heros*). Thirteen genotypes were used: IAC 100, BRS 391, BRS 543 RR, BRS 1003 IPRO, LQ 8, RIL 227, RIL 186, RIL 109, CD 215, AS 3730, RIL 249, RIL 241, and RIL 7. Three pods of each entry at R6 stage were put in arenas covered with glass. Eighteen brown stink bugs 1:1 (male:female), fasted for 24 hours, were released in the center of the arena. The number of insects per entry (NIE), acicular trichome length (ATL), unciform trichome length (UTL), trichome density (TD), pod thickness (PT), and estimated attractive index (AI) were evaluated. The AI analysis considered (I) susceptible: RIL 7, RIL 109, BRS 543, RIL 227, RIL 249, LQ 8, and RIL 186; (II) neutral: BRS 1003 IPRO; (III) resistant: AS 3730, BRS 391, IAC 100 and RIL 241. BRS 543 and RIL 109 had the highest NIE and IAC 100 was the lowest. BRS 391 and IAC 100 had the highest TD. LQ 8 had the largest AT and UT, and BRS 391 and BRS 543 had the lowest AT and UT, respectively. The largest thickness was observed in BRS 391. TD showed a medium correlation with AT and UT (-0,51 and 0,51, respectively); UT showed a medium correlation with NIE (-0,55). AI had a high correlation with NIE (0,91). The antixenose characterization is important to develop resistant cultivars, and, due to the genetic resistance, reduce insecticide use.

Keywords: Antixenose, stink bug, resistance, mechanism

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Multifaceted phenotyping approaches toward QTL identification of drought tolerance of soybean (*Glycine max* L.) in the early vegetative stage

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Drought is one of the most significant environmental factors that reduce yield in soybean (*Glycine max* L.). Many studies about quantitative trait loci (QTLs) conferring drought tolerance in soybean have focused on the effects of water restriction on yield during the reproductive stages. More recently, the necessity of QTL studies to use physiological indices responding to drought at the early growth stages besides the reproductive ones has arisen due to the prevalent occurrence of drought throughout the soybean growing season. Thus, this study was conducted to identify QTLs associated with drought tolerance in soybean in the early vegetative stage. A recombinant inbred line (RIL) population derived from a cross between two different soybean cultivars, Taekwang (drought-sensitive) and SS2-2 (drought-tolerant), was grown in pots with the given amount of topsoil and subjected to drought stress. Drought tolerance in the RIL population was accessed via two phenotypic indices, wilting scores by visual inspection and relative water contents. Furthermore, we produced transpiration-related features drawn from profiles of transpiration rate monitored by the Arduino-based high-throughput phenotyping system we developed. A high-density bin map was constructed using single nucleotide polymorphism markers surveyed by the genotyping-by-sequencing method. The multifaceted phenotyping approaches and the high-density genetic bin map allowed us to identify multiple QTLs for drought tolerance of soybean in the early vegetative stages, which included a major QTL located on chromosome 10. Comprehensive Integration of multi-omics data has facilitated the elaborate dissection of genetic candidates responsible for the drought tolerance in soybean. Our results will provide primary insights into drought tolerance mechanisms in soybean and useful resources for the improvement of soybean varieties tolerant to drought stress.

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A genotypic inventory of modern European soybean germplasm and accessing genomics resources to the benefit of central European soybean breeding

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Expanded soybean cultivation in Central Europe during the last two decades has revived local breeding efforts but genomics has only played minor roles in this endeavor so far. The systematic genotypic analysis of local germplasm has great potential in fostering the investigation of local germplasm relationships and to provide an entranceway to effectively deploy global soybean genomics resources. To this end we genotyped 160 modern European varieties with the SoySNP50K array and investigated population structure and diversity in conjunction with a global soybean sample as represented by the USDA Soybean Germplasm Collection (N > 17,000). This contextualization revealed genetic similarity among modern varieties from Europe and early maturity germplasm from high-latitude regions in the USA and Canada, which is in accordance with anecdotal evidence on germplasm exchange between Europe and Northern America. We observed reduced genetic diversity despite a diverse assemblage of ancestry from Northeastern China and Japan in modern European germplasm. These results provide the first account of the genotypic composition of modern European germplasm in a global context. To facilitate the expansion of the genetic base of European breeding programs we screened a core collection (Haupt and Schmid, 2020) of diverse genebank accessions (N=160) in two common garden environments in northern and southern Germany for timely flowering and maturity. Flowering and maturity were delayed in northern Germany but highly correlated with observations from southern Germany and genebank legacy data. Common garden and legacy data phenotypes were further used to study the genetic base of flowering and ripening in early maturity accessions and revealed well-known and novel candidate loci. The obtained results will support the selection of suitable germplasm for pre-breeding and have demonstrated the value of incorporation of genebank genomics and genebank legacy data for central European soybean breeding.

Keywords: Central Europe, pre-breeding, genebank genomics, photoperiodic adaptation

Max Haupt and Karl Schmid; Combining focused identification of germplasm and core collection strategies to identify genebank accessions for central European soybean breeding; Plant, Cell and Environment; 43(6); 1421-1436; 2020; doi: https://doi.org/10.1111/pce.13761

A major locus governing flavanol contents in soybean seeds is independent of seed coat color

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(-)-Epicatechin (EC) and its dimer, procyanidin B2 (PB2) are primary forms of flavanols present in pigmented tissues of plant species with multiple health benefits as antioxidants. In soybeans (*Glycine* spp.), EC and PB2 exist only in black or brown seed coat but not in yellow one, which are end products of flavonoid biosynthesis pathway. However, their genetic regulation remains little disclosed in soybean. This study was performed to identify quantitative trait loci (QTLs) associated with soybean EC and PB2 contents in seeds. Using a recombinant inbred line population of IT109098 (greenish brown seed coat) and K7-113 (black seed coat), a high-resolution single nucleotide polymorphism-based linkage map was constructed via genotyping-by-sequencing. Measurement of EC and PB2 revealed both mapping parents had high levels of EC and PB2 and the population exhibited normal distribution. Additionally, color components of, L*, a* and b*, evaluated in seed coat, showed bimodal distribution in the progeny. We identified 2 QTLs governing seed EC and PB2 contents on chromosomes 02 and 10, consistently identified for the two years 2020-2021. Among them, a major QTL, qECPBB10 on Chr10 explained 23.5-44.4% of phenotypic variation and showed high LOD scores of 15.4-44.3. In qECPBB10, Glyma.10G209800 and Glyma.10G210000, encoding phenylalanine ammonia-lyase 2 and calcium-dependent lipid-binding family protein, respectively, are more likely to be candidate genes based on seed-specific expression and sequence variations. Remarkably, the loci for the flavanol contents were separately located on the QTLs conferring the L*, a* and b* values, indicating the novel QTL, qECPBB10 regulates flavanol contents in soybean seeds independently of seed coat coloration. This study will provide primary insights into flavanol biosynthesis in soybean and breeding high-flavanols cultivars.

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Variation in oligosaccharide contents in soybean mini-core collections of NARO genebank

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Sucrose, raffinose, and stachyose are major soluble oligosaccharides in soybean seeds, which are associated with food quality such as digestibility, taste, and bifidogenic effects. Composition of the three oligosaccharides is one of keys to developing soybean cultivars for various applications. In this study, a high-performance liquid chromatography was performed to determine oligosaccharide contents in seeds from the soybean mini-core collections of NARO genebank, whose whole genomes have been re-sequenced, to identify a germplasm having a unique profile of oligosaccharides and a DNA polymorphism affecting their profile. Soybean was cultivated in our experimental fields at Tsukuba, Ibaraki, Japan, in 2010 and 2021. Contents and ratios of seed sucrose, raffinose, stachyose, and oligosaccharides were significantly correlated between 2010 and 2021. The contents of seed sucrose, raffinose, and stachyose were ranged from 3.1 to 8.6, from 0.5 to 1.4, and from 1.1 to 4.5 g/100 g, respectively, in 2021. Among the 171 accessions examined, one accession showed remarkably low stachyose content, raffinose family oligosaccharides/sucrose ratio, and stachyose/raffinose ratio. This accession had a nonsense mutation in the raffinose synthase 2 (RS2) gene, resulting in premature termination of the gene product. Structural prediction by alphafold 2 suggested a complete loss of the domain involved in enzymatic activity of the gene product. Mutation resulting in a loss of function domain of RS2 has not been reported. We will also discuss other germplasms showing the unique profiles of oligosaccharides and the usefulness of alphafold 2 to evaluate gene mutations.

Genome-wide association study and genomic selection for soybean yield-related trait, quality and disease resistance for SCN and SMV

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Soybean [*Glycine max* (L.) Merr.] is a crop of great interest worldwide. Exploring molecular approaches to increase genetic gain of agronomic trait has been one of the main challenges for soybean breeders and geneticists. The objectives of this study were to conduct a genome-wide association study (GWAS), to identify single nucleotide polymorphism (SNP) markers, and to perform a genomic selection (GS) study for multiple complex quantitative traits in soybean, including yield-related trait such as maturity, plant height, and seed weight; quality traits such as protein, oil, and amino acid concentrations; disease resistance related traits such as tolerance of soybean biomass to soybean cyst nematode (SCN) infestation and Soybean Mosaic Virus (SMV) infestation. A genome-wide association study (GWAS) was performed using a Bayesian Information and Linkage Disequilibrium Iteratively Nested Keyway (BLINK) model. Genomic selection (GS) was performed using Bayesian Lasso (BL) and ridge regression best linear unbiased prediction (rrBULP). The results revealed that (1) 20, 31, 37, and 23 SNPs were significantly associated with maturity, plant height, seed weight, and yield, respectively; 22, 13, and 231 SNPs associated with protein content, oil content, and 15 with amino acids; 6, and 10 SNPs associated with SCN tolerance and SMV tolerance. (2) Candidate genes were found in the vicinity of the two SNPs with the highest significant levels associated with yield, maturity, plant height, seed weight, respectively. A 11.5-Mb region of chromosome 10 was associated with both yield and seed weight. fifteen SNPs localized near 14 candidate genes involving in amino acid metabolism. The new QTL genomic region on chromosome 6 (Chr6_18844283–19315351) included 7 candidate genes and the Hap.XAA at the Chr6_19172961 position was associated with high protein content. Genes around Glyma.06G134900, Glyma.15G097500.1, Glyma.15G100900.3, Glyma.15G105400, and Glyma.19G121200.1 are best candidates for tolerance index based on biomass reduction under SCN infestation in soybean. (3) The accuracy of GS was dependent on the trait heritability, the SNP set, training population size and population structure, and high accuracy indicates that these agronomic traits can be selected in molecular breeding through GS. The SNP markers identified in this study and other information were essential in establishing an efficient marker-assisted selection (MAS) and GS pipelines for improving soybean traits.

Soybean breeding program in eastern Mediterranean region in Turkiye

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The breeding program of soybean has been carried out from 1930 in Turkiye. Considering the cultivation area, production values and the use of soybean products, Turkey and the world countries there is a marked difference between them. Soybean cultivation area and production area has been limited economically entirely in our southern coasts (especially Çukurova). New varieties for different regions with high yields, will contribute an increase in soybean cultivation area and production in Türkiye.The main purpose of soybean breeding programs in Eastern Mediterranean Agricultural Research Institute to develop new varieties for high yield for farmers. Also, it has aimed to breed new varieties for animal feed. In recent years, the use of soybean as human food has become quite widespread. Because of that soybean studies focus on this subject too. Eastern Mediterranean Agricultural Research Institute (EMARI) has developed 6 soybean varieties. Two of them for forage soybean varieties. The results of soybean breeding program in EMARI for 2022, seed yield of genotypes varied between 2117-3829 kg ha-1. Oil ratio (%) varied between 21.4-22.9. Protein ratios of the genotypes ranged from 38.2-39.9 %.

Keywords: Soybean, variety, protein, breeding

Low-temperature pretreatment of soybean seeds enhances plant field performance and the content of free metabolites

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The main factors for crop production as soil, heat and the light regime are suitable for soybean cropping in Bulgaria. In the three-year period 2019-2021 we conducted an approach of pretreatment of soybean seeds with low temperature before sowing. The experiment was performed with two Bulgarian soybean cvs. (Avigea and Richy) compared to French cv. (Izidor). The cold pretreatment of soybean seeds with low temperature (2-5°C), was for two periods 12 days - called ‘treated control’ and 22 days - called ‘treated’. Our investigation was focused on how low-temperature pretreatment of soybean seeds later on affected plants morphology, the transcript level of selected genes, the quantity of free amino acids, fatty acids, and sugars detected in green and mature seeds collected from plants grown from “pretreated” and “non-treated” seeds. Expression and metabolomic profiles of leaves, green seeds and mature seeds were assessed by (qRT-PCR) and (GC/MS). Investigated approach of long-lasting low-temperature pretreatment of soybean seeds before sowing clearly indicated increased morphological indicators of plants grown from pretreated seeds. The metabolite profiles established during three consecutive years of free amino acids, sugars, and fatty acids in soybean grains (green and mature) were strongly affected by the applied approach and were significantly elevated in pretreated seeds compared to ‘non-treated’. The expression profiles obtained from selected AAs transporters clearly demonstrated a correlation between the expression profiles and the content of free AAs. The level of expression of the selected gene Invertase could serve as a marker for the level of free sucrose. The transcript profile of selected inorganic pyrophosphatase H+-PPase could be applied as a marker for the vigor of plants grown in the conditions of drought. Altogether, the collected results confirm the benefits of low-temperature pretreatment of seeds, which improve plants field growth and the quality of seeds.

A key gene for breeding high-yield soybeans of dense

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Plant height is an important factor that affects the lodging resistance and shade avoidance syndrome, thereby control the yield of soybean. At present, genes regulated soybean plant height were tend to accompany with reduced yield, resulting in a lack of available genetic resources. Here, we report a γ-ray induced dwarf mutant, compacted plant height (cph), and fine-mapping the dwarfism regulatory gene CPH. CPH encodes an E3 ubiquitin ligase, which interacts with STFs through its WD domain and degrades STFs, thus regulating the expression of two GA2ox genes. This CPH-STFs-GA module plays an important role in regulating plant height in soybean. Notablely, the dwarfing of cph mutant has no unfavorable effect on yield, providing a potential strategy for developing lodging resistant and high-yielding soybean varieties.

The AP2/ERF transcription factor TOE4b regulates photoperiodic flowering and grain yield in soybean

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Photoperiod-mediated flowering determines the phenological adaptability of crops, including soybean (*Glycine max*). A genome-wide association study (GWAS) identified a new flowering time locus, Time of Flowering 13 (TofF13), which defined a gene encoding an AP2/ERF transcription factor. This new transcription factor, which we named TOE4b, localized in the nucleus. TOE4b has been selected during soybean domestication and improvement. The existing natural variant TOE4bH4 was rare in wild soybean accessions but occurred more frequently in landraces and cultivars. Notably, TOE4bH4 improved high-latitude adaptation of soybean. TOE4b overexpression delayed flowering time by binding to the promoters of the key flowering integration factor genes FT2a and FT5a directly. Conversely, a gene-edited TOE4b knockout mutant exhibited earlier flowering. TOE4b overexpression lines boosted grain yield per plant in field trials by 60% in Guangzhou and 87% in Shijiazhuang through alterations in plant architecture that led to an increase in pod number by producing plants with shorter internodes, more internodes, and more branches. Our findings therefore identified TOE4b as a pleiotropic gene to increase yield potential in soybean, and these results provide a promising option for breeding a soybean variety with an idealized plant architecture that promotes high yields.

Keywords: Soybean, AP2/ERF transcription factor, TOE4b, photoperiodic flowering, yield, adaptability

Rhizobial type III effectors have multiple function on the nodule trait of soybean

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The plant can't grow without the uptake of N, however, biological nitrogen fixation is a method that can continuously supply N. The symbiotic nodulation process between legumes and rhizobia involves the conduction of many signaling molecules, among which the type III effectors secreted by the Type III secretion system of rhizobia (T3SS) is one of the main pivotal signaling molecules, which can affect the immune recognition process of plants and thus produce changes in nodulation. By studying the role of type III effector factors in symbiosis recognition, mining and studying related interacting genes in plants and explaining their immune recognition pathways, it is of great significance to explore the molecular mechanism of symbiosis between rhizobia and soybean and to cultivate new leguminous varieties with high nitrogen fixation efficiency. NopAA is a type III effect factor of rhizobium *Sinorhizobium fredii* HH103 with glycosyl hydrolase activity. The NopAA promoter region contains tts box conserved sequences. The subsequent expression analysis showed that NopAA could be induced and regulated by TtsI and Genistein, and its secretion might be used to decompose the cell wall of host cells, which was conducive to the entry of rhizobia into cells and conducive to the infection of rhizobia and the formation of nodules. Type III effector NopD of rhizobium also plays a positive role in the establishment of a symbiotic system. Identification of nodules from different germplasm resources shows that mutants nopD significantly reduce the number of nodules and dry weight of nodules in most varieties, and are closely related to the immune system of host plants. NodD promotes rhizobia infection by inhibiting the expression of a gene encoding an antiviral protein (PP2C). The type III effector NopL of rhizobium HH103, mainly acts on the nodular formation stage of soybean nodulation. While nopL is mutant positive and negative effects on nodulation were on different soybean germplasm. In addition, ectopic expression of nopL in tobacco, MAPK signal pathway was interfered. Type III efferent protein NopM is an E3 ubiquitin ligase. When rhizobium HH103 infects soybean, NopM can interact with immune-related GmMIP protein in soybean, and use GmMIP protein as the target protein for ubiquitination degradation, and then promote the occurrence of nodulation. Mutant nopM had a greater effect on the dry weight of root nodules, but less effect on the number of root nodules. Nodule identification of type III effector NopP on 10 soybean core germplasm resources showed that the number of nodules and fresh weight of nodules of mutant rhizobia were significantly lower than those of wild-type rhizobia, indicating that mutation of type III effector NopP would lead to decreased nodulation ability of rhizobia. The HH103ΩnopT mutant can increase the number of nodules and the dry weight of nodules in CSSL (chromosome segments substituted lines) population and RIL population, which plays a negative regulatory role in the establishment of a symbiotic system. However, in the process of infection, HH103ΩnopT mutant can cause obvious expression of a salicylic acid-related gene (GmPR1). These results indicated that the mutant nopT could inhibit the defense response of soybean with the infection of Rhizobium.

Natural variations at novel flowering regulatory module determine soybean adaptation

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Soybean (*Glycine max*) a photoperiod sensitive plant, the photoperiod flowering greatly determines soybean latitudinal adaptation and grain yield. Here we report the identification and characterization of Tof4a, which controlling flowering time at high latitudes. Tof4a encode E1-Like b (E1Lb), an ortholog of the soybean key flowering repressor, E1. Tof4a physically associates with the promoter of two FLOWERING LOCUS T (FT) genes thus delay flowering under long day conditions. An impairment allele of Tof4a was subjected to natural selection and contributed to the adaptation of wild soybean to high latitudes, then the very allele was introgressed to cultivated soybean and thereby drive the spread of cultivated soybean to extreme high latitudes. Our findings uncover the adaptation trajectory of soybean from its temperate origin to the high latitudes.

Tailoring coumarin biosynthesis for improved soybean protection

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Coumarins can protect plants from disease and abiotic stress. They are thus promising for sustainable crop protection. The nonhost resistance of *Arabidopsis thaliana* to the Asian soybean rust-causing fungus *Phakopsora pachyrhizi*, for example, is associated with the accumulation of the coumarin scopoletin. Soybean, by contrast, upon *P. pachyrhizi* attack does not accumulate scopoletin and is susceptible to Asian soybean rust. The phenylpropanoid pathway enzyme feruloyl-6' hydroxylase (F6'H1) is key to the biosynthesis of scopoletin and other coumarins in plants. To investigate whether one could exploit scopoletin for plant protection, we overexpressed AtF6'H1 in various plant species, including soybean. The transgenic plants hyperaccumulated scopoletin and its glycoside scopolin and were less susceptible to disease. Based on this promising finding we followed different strategies to enhance scopoletin's efficacy while avoiding detrimental effects of excess scopoletin. For example, we utilized coumarin-biosynthesis genes of various species to chemically modify scopoletin in transgenic plants. This led to the accumulation of varying scopoletin derivatives that differed in their antioxidative capacity, antimicrobial activity, and light stability. In addition, we identified a transporter protein that enhances the secretion of coumarins to the leaf surface. Exploiting coumarin secretion reduces the chance of detrimental effects of coumarins on the plant while simultaneously attacking pathogens on the leaf surface. In future work, we aim to control the activity of genes with a role in coumarin biosynthesis and secretion by spatial and temporal regulation of gene promoter activity. Our comprehensive approach aims at tailoring coumarin biosynthesis and accumulation to reduce soybean's sensitivity to Asian soybean rust.

Keywords: Coumarins, secondary metabolites, genetic engineering, crop protection, Asian soybean rust

GmTNL16 regulates immune response to phytophthora root rot in soybean

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Phytophthora root rot (PRR) caused by *Phytophthora sojae* (*P. sojae*) is a devastating disease of soybean. MicroRNAs (miRNAs) mediated gene silencing in plants is considered as an alternative defense system against invasion by pathogens miRNAs mediate NLR genes to regulate soybean resistance to disease. 5’-RACE was performed to validate the cleavage transcript target site of miR1510. We cloned and overexpressed GmTNL16 and silenced gma-miR1510 by short tandem target mimic (STTM) technology in soybean to uncover the roles of the module of GmTNL16-miR1510 in the interaction of soybean and oomycete. The miR1510 negatively regulates the resistance of soybean to *P. sojae* and verified that GmTNL16 is one of its valid target genes. GmTNL16, a TIR–NBS–LRR type R gene, was up-regulated in soybean during infection with *P. sojae*. The biomass of *P. sojae* in hairy roots the that overexpressing GmTNL16 was lower than that of control and the lesion length in hairy roots of silencing of gma-miR1510 were shorter than that of control, confirming the crucial roles of GmTNL16 in regulating the resistance of soybean to *P. sojae*. Furthermore, RNA-seq analysis was performed in the EV and OE-GmTNL16. Differential expression genes were obtained in the 12h-OE vs 0h-OE. Functional enrichment analysis revealed that the DEGs were significantly enriched in plant-pathogen interaction, plant hormone signal transduction and secondary metabolisms. The module of GmTNL16-miR1510 is participated in the defense response of soybean to resistance. The TIR-NBS-LRR gene GmTNL16 plays a positive role in *P. sojae* interactions.

Keywords: Soybean, *Phytophthora sojae*, gma-miR1510, NBS-LRR gene, GmTNL16

Acknowledgements: We thank Prof. Yuanchao Wang (Nanjing Agricultural University) for providing the *P. sojae* isolate P6497 and Prof. Dongdong Niu for providing the pFGC5941 vector. This work was supported by National Natural Science Foundation of China (32072082, 31301340), China Agriculture Research System of MOF and MARA, Jiangsu Collaborative Innovation Center for Modern Crop Production and Cyrus Tang Innovation Center for Seed Industry.

A nitrogen fixing symbiosis-specific pathway required for legume flowering

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Flowering time is crucial for plant survival and reproduction and is controlled by flowering pathways, including microRNA172 (miR172)-mediated age flowering pathway. Symbiotic nitrogen fixation (SNF) boosts legume growth and production in nitrogen-poor soils. It has long been assumed that fixed nitrogen promotes plant growth and increases reproductive success, but until now, the regulatory mechanism remains unknown. The observations that nodule number and nitrogen fixation peak when plants flower suggest a strong correlation between SNF and flowering time, but whether two traits are connected and how SNF and flowering time are coupled remain elusive. Previously we found that miR172c is induced significantly by rhizobia and maintains high level in the nodule, and this led us to hypothesize that symbtiotic miR172c moves to the shoot to promote flowering. To proof the hypothesis, we conducted a systemic study using combinatorial approach. We found a symbiotic flowering pathway that couples symbiotic and nutrient signals to the flowering induction pathway in legumes. We show that the symbiotic miR172c moves from nodules/roots to leaves to promote flowering; while fixed nitrogen is transported from the nodules/roots to the leaves to induce miR172c. Thus, symbiotic signal miR172c and nitrogen systemically and synergistically convey symbiotic and nutritional cues from nodules/roots to leaves to accelerate soybean (*Glycine max*) flowering. Furthermore, we found that the combinations of symbiotic miR172c and local miR172c elicited by fixed nitrogen and development in leaves with age-induced miR172 led to activation of florigen-encoding FLOWERING LOCUS T (FT) homologs (GmFT2a/5a) by repressing the flowing repressorTARGET OF EAT1-like 4a (GmTOE4a). Thus, FTs trigger reproductive development, which allows legumes to survive and reproduce under low-nitrogen conditions. Finally, we showed that this symbiotic specific flowering pathway is conserved in legumes. Together, our discovery has defined a legume specific flowering pathway that integrates symbiotic nodulation and flowering in legumes.

The GmXTH1 gene improves drought tolerance of soybean seedlings

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Drought stress is one of the significant factors that threatens productivity and seed quality of soybeans worldwide. Discovery and utilization of drought-tolerant genes in soybean breeding could help mitigate the drought stress to improve productivity of soybean cultivars. Combined transcriptomic and metabolomic analyses of soybean cultivar JN18 and its mutant M18 revealed that expression of the GmXTH1 gene was induced by drought stress, which was significantly associated with plant drought resistance. Xyloglucan endotransglucosylase/ hydrolase (XTH) is a key enzyme in plant cell wall remodeling, which affects plant growth and development, as well as stress tolerance. To understand the role of GmXTH1 gene in alleviating drought stress, the gene was cloned and transformed into soybean cultivar JN18. Using polyethylene glycol (PEG) 6000 to simulate the drought stress condition, the results of T4 transformed plants indicated that at the germination stage, the root length of the overexpression plants was 3.1 times longer than that of the interference expression plants. After overexpression of GmXTH1 gene, the total root length, surface area, root diameter, and total number of root tips of the transformed plants were significantly increased. It was hypothesized that these increases help improve the water intake and reduce the transpiration rate of water in leaves to improve drought resistance of soybean by reducing the accumulation of MDA and cell membrane damage, and producing more protective enzymes. With the increased drought stress, the expression level of GmXTH1 gene in soybean roots was increased from 56.4 to 120.4%. In the meantime, overexpression of GmXTH1 gene also increased the relative expression of JCVI-FLGm-14H24, by 80.3 and 12.5% in roots and leaves, respectively. These results demonstrated that overexpression of the GmXTH1 gene can positively regulate the expression of JCVI-FLGm-14H24, which in turn enriched the mechanism of GmXTH1 gene in soybean to respond to drought stress.

The B-type response regulator GmRR11d mediates systemic inhibition of symbiotic nodulation

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Symbiotic nodulation is imperative for the survival and productivity of soybean. Under low nitrogen conditions, soybean plants perceive lipochitooligosaccharide signals (nodulation factors [NFs]) released from rhizobia through NF receptors to initiate a molecular cascade to trigger nodulation. Symbiosis is energy consuming; therefore, soybean plants develop an autoregulation of nodulation (AON) to control the homeostasis of nodulation. In AON, nodulated roots produce peptides GmRIC1 (rhizobia induced CLAVATA3/ESR [CLE] peptide 1) and GmRIC2 that are transported from roots to shoots to activate their leucine-rich-repeat receptor kinases GmNARK (soybean nodulation autoregulation receptor kinase), and the GmNARK then inhibits further nodulation. However, the mechanism by which GmNARK suppresses nodulation remains largely unclear. It has long been proposed that shoot-controlled nodulation acts downstream of cytokinin (CK) signaling-mediated activation of nodule initiation, but the mechanisms are not fully understood. In this study, we conducted a systemic study using a combinatorial approach to answer the above scientific questions. We show that a B-type response regulator of CK, GmRR11d, mediates systemic inhibition of nodulation by AON and GmNARK in roots. GmRR11d is induced by rhizobia and low level cytokinin. Genetic evidence show that GmRR11d negatively regulates soybean nodulation. In addition, we found that GmRR11d can directly bind to GmNIN1a promoter to repress its expression, and it can also suppress the transcriptional activity of GmNSP1 on GmNIN1a to inhibit NF signaling that reduces soybean nodulation. Furthermore, GmRR11d positively regulates cytokinin response and its binding on the GmNIN1a promoter is enhanced by cytokinin. Intriguingly, rhizobial induction of GmRR11d and its function are dependent upon GmNARK. Thus, GmRR11d governs a transcriptional program associated with nodulation attenuation and cytokinin response activation essential for systemic regulation of nodulation. Together, our findings elucidate the molecular mechanism by which GmNARK suppresses further nodulation by simultaneously repressing NF signaling and increasing CK sensitivity in soybean.

Functional validation of a candidate gene contributing to root length in soybean through CRISPR/Cas9-mediated mutagenesis

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Soybean (*Glycine max*) exhibits a large phenotypic diversity with regards to its root system. In view of developing high yielding varieties best suited to overcome a range of abiotic stresses, selecting for superior root systems would be desirable. However, few genes involved in the development of the root system have been identified and characterized. Thanks to a prior GWAS analysis, SNP markers significantly associated with root length were identified on chromosome 3 and a single gene was located within the same linkage block (Glyma.03g065700). This candidate gene is an ortholog of the SCARECROW (SCR)/SHORT-ROOT (SHR) gene known to contribute to root length in *Arabidopsis thaliana*. To carry out a functional validation of this gene, we used CRISPR technology and hairy-root transformation with *Agrobacterium rhizogenes*. Two guide RNAs (gRNAs) targeting the first exon of the gene were designed and introduced into the pTRANS_230 vector under the control of the CmYLCV promoter. Multiple transgenic roots (expressing the RUBY reporter) were obtained (as well as non-transgenic control roots) using a modified one-step inoculation procedure. A broad range of reporter gene expression was seen among the different transgenic roots suggesting variation in the suitability of the genomic integration sites. The most intensely colored transgenic roots are typically extremely short whereas more lightly pigmented transgenic roots present root growth that appears to be less highly affected. Molecular analyses are underway to characterize the sequence of the candidate gene at the site of the two guide RNAs and these will be presented. Similarly, histological observations of transgenic roots (in contrast to control roots) will be presented. Through this work, we hope to identify an important gene controlling the length of the root system in soybean and provide breeders with a clear target for selection.

Keywords: Root system architecture, CRISPR, *Agrobacterium rhizogenes*, gene editing, functional validation

Identification of quantitative trait loci (QTLs) and candidate genes for seed sucrose and soluble sugar concentrations in soybean

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Soybean food quality is highly affected by sucrose/soluble sugar content. In this study, a RIL population constructed from ZD27 and HF25 with significantly different parental sucrose and soluble sugar contents was used as material. Using QTL mapping and transcriptome methods, we identified key QTLs and candidate genes affecting soybean sucrose and soluble sugar accumulation. In total, 16 QTLs were identified as affecting sucrose and soluble sugar, including a sucrose-associated major QTL qSU1901, which can be detected in multiple conditions and explained 10.6~13.2% phenotypic variation for sucrose. Within the qSU1901, we identified a B3 domain-containing protein transcript factor (Glyma.19G196600) as an important candidate gene regulating sucrose accumulation. Transcriptome analysis of the two parents revealed that key genes involved in sucrose and soluble sugar transport and metabolism were expressed at certain times as sucrose and soluble sugar accumulated. In the high sugar content variety ZD27, genes regulating sucrose synthesis direction enzyme (SPS) were up-regulated and sucrose decomposition direction enzyme (INV) were down-regulated. SWEETs and SUTs/SUCs sucrose transporters were highly expressed at certain times during seed development, which benefits sucrose accumulate. Based on WGCNA, we built a gene regulatory network (GRN) that correlated highly with sucrose and soluble sugar. There are four transcription factors (TF) (Glyma.04G028100, Glyma.05G095900, Glyma.11G239200, Glyma.12G076200) and forty associated genes in this GRN. Combining QTL and RNA-Seq, we narrowed down 233 candidate genes, including GmSPS1 gene (Glyma.17G109700) and 11 transcription factors. The QTLs and candidate genes identified in this study provide a better understanding of sucrose and soluble sugar regulation, as well as useful information for marker-assisted selection (MAS) in soybean quality improvement.

Keywords: Soybean, sucrose, soluble sugar, QTL, RNA-Seq

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Integrated single-nucleus and spatial transcriptomics captures transitional states in soybean nodule maturation

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Nodule development begins with the differentiation of cortex of infected root and involves interplays of both nodules and rhizobia. However, our understanding of cellular heterogeneity and developmental lineage of nodule is still limited. Here, we provide a transcriptomic atlas covering all major cell types of developing and mature soybean nodules and roots by integrating spatial and single-nucleus RNA-seq. We further found that transcriptional profiles of uninfected cells (UCs) in the central infection zone closely resembled that of cortex, while distinguished from infected cells (ICs). In central infected zone of nodule, we found that uninfected cells specialize into functionally distinct sub-groups during nodule development and meanwhile revealed a transitional subtype of infected cells with enriched nodulation-related genes. Overall, our results reveal a comprehensive transcriptomic landscape and cell-type-specific regulatory networks, providing valuable resources for understanding the molecular mechanism of legume-rhizobia symbiosis.

Identification of the genes underlying soybean resistant to SMV via chromosome segments substituted lines derived from wild soybean

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As an important source of plant protein and vegetable oil in the world, soybeans are often used in large quantities as the main raw materials for edible oil and animal feed, so the demand is huge. Soybean Mosaic virus is a kind of soybean virus that affects the quality and yield of soybean most. At present, the most effective way to prevent and control the spread of soybean mosaic virus is to cultivate resistant varieties. The most representative feature of soybean Mosaic virus on seed is seed mottling, which greatly affects the commodity value of soybeans. The most representative feature of soybean Mosaic virus on seed is seed mottling, which greatly affects the commodity value of soybeans. In previous studies, most scholars focused on adult-plant resistance of Mosaic virus and paid little attention to seed coat mottling resistance. In this study, chromosome segment substitution lines constructed by SN14 as the maternal parent and ZYD00006 as the paternal parent were used to screen candidate genes that determined the mottled seed grain. Two quantitative trait loci (QTLs) were identified on chromosome 17, and 18 genes were identified in this QTL region. The 18 differentially expressed genes (DEGs) in this region were screened by RNA-seq. The expression of five genes was associated with virus infection. Meanwhile, the effects of five differentially expressed genes on SMV resistance were investigated by Agrobacterium-mediated transient expression analysis. Glyma.17g238900 was found to encode a rice salt-sensitive 3-like protein (RSS3L) that inhibits the proliferation of SMV in *Nicotiana tabacum*. In addition, two non-synonymous single nucleotide polymorphisms (SNPs) were found in the encoding sequence of Glyma.17g238900 from wild soybean ZYD00006 (GsRSS3L), and these two amino acid mutants may be associated with SMV resistance. Therefore, GsRSS3L confers mottled resistance to soybean seed grains and participates in the anti-SMV mechanism of soybean.

Developing and deploying a UAS-based pipeline for determining maturity of soybeans

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Soybean varieties are categorized into relative maturity groups (MGs), which are estimates of photoperiod sensitivity and time to maturity. A variety's MG also equates to the approximate geographic zone that it is best adapted. Maturity is the most important trait that growers consider when deciding which cultivars to plant and is used in breeding programs as a covariate to draw meaningful comparisons among genotypes for traits of interest. Accurate phenotyping of maturity is an important task during line development but is a labor-intensive process. High-throughput phenotyping (HTP) of soybean maturity using unmanned aerial systems (UASs) provides an opportunity to reduce the human resources, time commitment, and error associated with manual maturity notes to support breeding efforts. A HTP pipeline for maturity will provide higher quality maturity data that will greatly improve breeders' ability to evaluate the performance of breeding lines and more efficiently develop elite cultivars for growers. Several studies have utilized spectral measurements, mainly with UASs, to estimate soybean maturity of lines from midwestern MGs (0–IV). However, these methodologies suffer from lack of public availability, scalability, and/or adoptability. The objective of this study is to develop an intuitive, accessible, and accurate HTP pipeline for determining the maturity of soybeans in later MGs VI–VIII, predominantly grown in the southeast region of US. Random forest (RF) models were developed to determine the maturity status of breeding plots based on zonal statistics derived from aerial imagery. Various train-test partitioning strategies indicated that the RF models exhibited promising and robust validation accuracies across plant materials of different maturities, genotypes, pedigrees, and breeding stages. However, plots that exhibited irregular canopy appearances or senescence patterns were the main sources of error. To account for these instances, future iterations will explore utilizing deep learning methodologies capable of learning image features important for maturity determination.

Keywords: High-throughput phenotyping (HTP), maturity group (MG), unmanned aerial systems (UASs), soybean

Identification of elusive *Phytophthora sojae* resistance gene in soybean through NLR gene targeted capture

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Exploitation of disease resistance genes in soybean (Rps), as a ubiquitous management method for *Phytophthora sojae*, is on the verge of an impasse. Few of the mapped resistance genes are commercially exploited and even fewer have been precisely identified. Therefore, little is known about identities or relationships among the 35 or so reported Rps genes, a hindrance preventing optimal introgression of new sources of resistance into elite soybean cultivars. Here, we describe an innovative approach using NLR gene enrichment Sequencing (RenSeq) coupled with NILs, and further confirmation through genetic mapping to study Rps genes. Through this method, we were able to precisely identify the elusive Rps3b gene on chromosome 7 rather than on chromosome 13 where it was originally reported to reside. Presumed allelic with Rps3a in 1985, Rps3b is part of an entirely distinct locus comprising 12 NLR genes spanning over 480 kb. Interestingly, this cluster bears similar features, both in terms of location and structure, with the recent description of Rps11. Extensive phenotyping of soybean lines carrying Rps3b resistance with contrasting *P. sojae* field isolates uncovered a perfect association with alleles of the Avr3b effector protein. Furthermore, our results show that only 8 and 19% of isolates could circumvent resistance conferred by Rps3b in Canada and Brazil, respectively, making the gene an invaluable new source of resistance for future breeding programs.

Identifying candidate genes responsible for soybean pod color using synthetic phenotype to causative mutation strategy

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Soybean pod color is a crucial trait for breeding because of its relatedness to important agronomical traits such as high nutritional values and stress resistance. Most importantly, the black pod color is related to higher protein content and resistance to pests. Pod color is controlled by two alleles – L1/l1 on chromosome 19, responsible for the accumulation of pigments perceived as the black color of pods, and L2/l2 on chromosome 3, responsible for brown color. Thus far, many genes have been suggested as candidate genes for L1 and L2 alleles, although the function of proteins encoded by the candidate genes has not been confirmed yet. Our objective was to identify new candidate genes using a novel approach – Synthetic phenotype to causative mutation (SP2CM) strategy [1]. We used GWAS to determine the region on chromosome 19 corresponding to allele L1/l1. With the use of the online Accuracy calculation tool (AccuTool), we suggested a candidate gene Glyma.19g120400 – a gene predicted as isopropylmalate synthase. A mutation in this gene explained the black pod color phenotype of *Glycine soja*. However, the mutation did not correspond fully with the loss of pod color pigments in *Glycine max*. Glyma.19g120400 may be the first factor responsible for the accumulation of pigments in pods, with a possible effect of other genes involved in the regulation of pigment synthesis in pods and the potential effect of pleiotropy and epistasis. In conclusion, using the SP2CM strategy, we were able to rule out previously suggested candidate genes and present new candidate genes for allele L1.

[1] M. Škrabišová et al. 2022, A novel Synthetic phenotype association study approach reveals the landscape of association for genomic variants and phenotypes, J Adv Res 42, 117–133. doi: 10.1016/J.JARE.2022.04.004.

Hyperspectral reflectance applications in soybean breeding research

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The implementation of novel high-throughput field phenotyping (HTP) methods is opening new perspectives to soybean breeding, gene characterisation, and germplasm evaluation. As an example, the collection of reflectance information from soybean field plots in visible and infrared wavelength ranges (hyperspectral reflectance) supports different approaches: (i.) Correlations between individual wavelengths and plant characters can be utilized for indirect measurement of traits. (ii.) Various spectral reflectance indices (SRI) can be calculated as novel plant traits; this includes plant characteristics related to total biomass accumulation, nitrogen metabolism, and canopy water status. (iii.) Information from whole spectra can be utilized for developing predictive regression models similar to NIRS-calibrations.

Examples of hyperspectral reflectance application in soybean:
Indirect determination of canopy nitrogen concentration, which reflects nitrogen uptake and symbiotic di-nitrogen fixation can be utilised in models for predicting grain yield and seed protein content. Measuring of canopy water content through spectral indices can illustrate differences in water related traits such as drought tolerance; thus, phenotypic effects such as individual QTL or the proposed effect of semideterminate vs. indeterminate stem termination (*Dt2*/*dt2*) on water use efficiency (WUE) can be analysed. Similarly, plant canopy pigment concentrations (chlorophylls, carotenoids) and their temporal dynamics can be analysed for differences between populations. Finally, various different indices describing biomass and yield development, nitrogen-related traits, pigments or water traits can be utilized together for multivariate description of germplasm populations; cluster analysis groupings obtained would reflect similarities in physiology including flowering/maturity time, biomass production or nitrogen accumulation traits.

Application of novel field phenotyping methods using either hand-held devices or drones and other carriers equipped with dedicated sensors can provide additional information helpful in selection, genetic research or germplasm characterisation. However, crop and target-specific implementation research is necessary for optimizing phenotyping procedures in order to generate accurate and reliable data.

Keywords: Carotenoids, di-nitrogen fixation, high-throughput field phenotyping, water use efficiency

High-throughput phenotyping for temporal screening of soybean canopy cover and height assessed in different environments

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The combined power of remote sensing and photogrammetry can be used to assess significant information about plant development. The canopy cover (CC) and height (HT) are important for defining the growth patterns of the plants and their reaction to different environmental conditions. The objective of this study was to utilize the technology of high-throughput phenotyping (HTPP) for the temporal screening of soybean CC and HT. The trial was set in 2020 and 2021 at the experimental fields of the Institute of Field and Vegetable Crops, Novi Sad, Serbia. In total, 206 soybean genotypes divided into early (ED) and late (LD) were grown in drought simulation environments. As a control, the same set of genotypes (EC and LC) was grown in favorable conditions. The CC and HT were determined from the images collected with the unmanned aerial vehicle (UAV). In both years, the photos were taken four times at approximately 274, 390, 706, and 917 growing degree days (GDDs) after emergence. The results showed that the genotypes grown in drought simulation environments had lower CC compared to the control in both years. This was especially pronounced at 274 and 390 GDDs. In these time points, depending on the year, the CC of genotypes from ED and LD groups was 16%–54% lower than the control. The unfavorable growing conditions also had a negative effect on the soybean HT. The drought reduced the HT of the plants within the ED and LD trial between 12% and 44% compared to the control. The results suggest that some genotypes were more tolerant to unfavorable conditions than others which can be very useful in the selection of drought-tolerant varieties. The study showed that HTPP can be successfully used for collecting important information about soybean development within different environments.

Keywords: Soybean, high-throughput phenotyping, UAV, canopy cover, height

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Cataloging SCN resistance loci in North American public soybean breeding programs

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Soybean cyst nematode (SCN) is a problematic pathogen of soybean responsible for annual yield loss exceeding $1 billion throughout North America. SCN screening and marker-assisted selection are routinely employed in North American public breeding programs for developing SCN-resistant varieties. Illumina array-based SoySNP6K genotyping and SCN bioassay screening offer a valuable resource to understand the landscape of SCN resistance by identification of significant marker associations. Breeding lines are screened with three HG types (HG 7, HG 2.5.7, and HG 1.2.5.7) and SCN virulence phenotypes are reported as female index (FI). Considering these resources, we chose to utilize genome-wide association studies (GWAS) to evaluate allelic diversity and identify loci associated with SCN resistance within Missouri soybean breeding programs and the Northern Uniform Soybean Tests (NUST). We conducted GWAS for individual HG types using multiple loci mixed modeling (MLMM) to test the association between FI and SNP variants in the curated populations of breeding lines. After the initial GWAS, we fixed the populations for the alternate allele of the most significant peak (Rhg-1) and re-ran the GWAS to identify minor effect loci. To identify candidate gene causal variants for the associated regions, we performed an analysis of direct correspondence between phenotypes and associated variant positions by accuracy calculation (AccuCalc) analysis. Within the Missouri soybean breeding programs, nine loci associated with SCN resistance were detected on chromosomes 2, 4, 8, 9, 11, 17, and 18. Additionally, four SCN resistance-associated loci were identified on chromosomes 6, 7, 18, and 20 within the NUST population. The GWAS identified peaks closely associated with previously known major genes, as well as peaks in novel regions. This study offers valuable insight into the diversity of SCN resistance loci in public soybean breeding programs which can be used to develop new and improved soybean cultivars with novel SCN resistance.

Genomic analysis of sclerotinia stem rot (caused by *Sclerotinia sclerotiorum*) resistance in Canadian soybean germplasm

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Soybean [*Glycine max* (L.) Merr.] is one of the most economically significant oilseed crops in Canada and globally. Sclerotinia stem rot (SSR) caused by *Sclerotinia sclerotiorum* [(Lib.) de Barry], is the fourth most important soybean disease in Canada. Managing SSR through cultural practices, biological control or fungicide application is either expensive, environmentally unsustainable, or ineffective. The most reliable and economical approach to reduce losses is by developing and using genetically resistant cultivars. There are no currently known soybean cultivars with high levels of SSR resistance. This study aims to identify quantitative trait loci (QTL) and candidate genes associated with SSR resistance in soybean grown in Canada. To achieve this, a panel of 193 soybean genotypes was assembled to ensure genetic diversity and adaptability to Canadian environments, within maturity groups 000 to 0. Genotypic data of 262,000 single nucleotide polymorphism markers was generated on the panel via genotyping-by-sequencing (GBS). The panel has also been phenotyped in controlled environments with preliminary results indicating significant variation for disease reaction. Genome-wide-association studies are being conducted to identify QTL associated with SSR resistance. Quantitative polymerase chain reaction (qPCR) and gene ontology analyses will be used to identify candidate genes conferring causal SSR resistance and compare them to previous reports as development of SSR resistance continues to be evasive. This study provides a basis for creating molecular tools that will allow breeders to improve SSR resistance in soybean varieties grown in Eastern Canada and northern US regions.

Keywords: Soybean, genomics, genetics, *Sclerotinia sclerotiorum*

Acknowledgements: University of Guelph Soybean Breeding Program Field Crew, Mohsen Yoosefzadeh Najafabadi, Christopher Grainger, Sue Couling.

The benefits of artificial light supplementation in soybeans

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Endless seeking by soybean breeders for innovations that could increase the genetic gain in their breeding programs such as multiple crop cycles year-round. Continuous nursery sites are generally located at lower latitudes, playing a strategic role in the process of generating new soybean varieties, as they allow breeders to run several generations during the calendar year. Because some genotypes are not naturally adapted for these regions, some strategies are needed to manipulate plant development. In view of this, the objective of this work was to find an efficient lighting system to allow breeding programs to work with soybean varieties of all relative maturities at lower latitudes. Different models of artificial lighting were tested, namely: Control, White LED, Yellow LED, Sodium Vapor and Full Spectrum LED. On top of this, four times of light supplementation were evaluated with constant and intermittent light pulses. Soybean varieties from a wide range of relative maturities (from 000 to 87) were used in this research and plant phenological stages, plant height, NDVI, yield (kg ha-1) and yield components were measured. The use of White LED lights associated with 1 hour of constant light with 3 pulses of intermittent light provided greater plant height, greater number of pods per plant and higher yield (kg ha-1) in the soybean varieties tested. This result allows breeding programs to conduct crossing blocks and segregating populations advancements for all relative maturities at lower latitudes, reducing the recycling time of elite lines.

Keywords: Glycine max, genetic gain, soybean cycles, maturity groups, LED lights

Evaluating soybean protein content and breeding strategies in the Americas

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Soybeans are a vital source of protein for animal feed but increasing seed protein concentration can be challenging due to its strong negative correlation with oil content and seed yield. There is not incentive for producers to grow high-protein soybeans with lower yield. In this study, we aimed to characterize the seed protein content of soybeans in different regions of Argentina, Brazil, and the United States, and to explore spatial patterns and different breeding strategies. GDM evaluates over 1.6 million plots annually and produces commercial lines that account for 75% market share in Argentina and Brazil and 40% globally. We sampled over 50,000 plots from the past two planting seasons and from two breeding stages of the GDM breeding program including the most relevant commercial checks of the industry in LATAM. Protein and oil concentration were measured using Near Infrared Spectroscopy and reported on a dry weight basis. We also analyzed the protein content obtained through a traditional breeding program and the improvements achieved through assisted backcross. Our results showed that, on average, Brazil and United States lines had higher protein content than Argentine lines. As previously reported, protein concentration decreased from north to south in Brazil and Argentina. However, lowest values for protein concentration were found in the Mid-South region of the United States compared to north, which was unexpected. Negative correlations between yield and protein concentration were found in all regions, but the magnitude of these correlations varied. In terms of breeding strategies, our results showed that a traditional breeding program can increase protein content without sacrificing yield, but at a slow rate. However, we obtained promising results using assisted backcross with an elite recurrent parent and a special donor with high protein.

Keywords: High protein, soybean breeding program, breeding strategies

Functional analysis of GmSTM17 and preliminary analysis of soybean regeneration mechanism

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Compared with other crops, soybean is one of the relatively difficult crops for genetic transformation. Most commercial soybean varieties are not ideal recipient materials for soybean genetic transformation. So how to obtain a stable and efficient regeneration system is also a prerequisite for genetic transformation. Compared with other regeneration systems, the organogenesis pathway using cotyledonous nodes as soybean explants has the advantages of short regeneration cycle, convenient material collection, and more fertile plants. It is now widely used in soybean genetic transformation. However, when this method is combined with Agrobacterium-mediated genetic transformation, it leads to mutual inhibition between clustered shoots, and clustered shoot primordia are not suitable for induction. This seriously affects the application of biotechnology in soybean breeding and variety improvement. The key to solving this problem lies in the excavation of the key genes of soybean regeneration and the analysis of the molecular mechanism, so as to break the bottleneck of genotype restriction. The research team obtained a soybean key regeneration gene GmSTM17 (SHOOT MERISTEMLESS) through the transcriptome, which encodes a nuclear protein and is specifically expressed in soybean stems. In situ hybridization showed that the GmSTM17 hybridization signal was in the central and peripheral layers of the shoot apical meristem. During soybean cotyledonary node organogenesis, the shoot induction and elongation ability of the GmSTM17 mutant was significantly improved compared with WT. In addition, GmSTM17 also significantly affected soybean plant height, pod number and grain number per plant. At the same time, the GmSTM17 interaction factor (GmESRI) was screened through the yeast screening library, and the direct interaction between GmSTM17 and GmESRI was verified by yeast two-hybrid verification, bimolecular fluorescence complementation and GST-Pull down. This study demonstrated that GmSTM17 is a key inhibitor of soybean cotyledon node organogenesis.

Keywords: Glycine max, GmSTM17, regeneration, gene function verification, GmESRI

Identification of candidate genes for pod color by bulked segregant analysis in soybean and wild soybean genomes

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Mature pod color (PC) was as an important character of soybean pod trait. The pod color changed constantly with reproduction and growth in soybean, and finalized until maturity stage. PC which was an important physiological character of pod formation. Identification of genes for PC could act as an important role in the research of soybean fruit development. Herein, we utilized the Suinong 26 (P_1, elite cultivar with deep yellow pod) and FF1235 (P_2, wild soybean with black pod) to construct F_2 population comprising 237 individuals. Two extreme pools were constructed with 40 individuals with deep yellow pods and black pods respectively to identify candidate genes involved in PC using bulk segregant analysis sequencing (BSA-seq). BSA-seq were performed twice with W82.a4 and W05.a1 as reference genomes, respectively. A total of 3980098 and 3220359 polymorphic SNPs were detected using two reference genomes, respectively. The SNP-index correlation algorithm detected one candidate genomic region (GmPC19) with a total length of 1.18 Mb and 2.05 Mb in Chromosome 19 based on W82 and W05 as reference genomes, respectively. GmPC19 was identified in 130.4 Kb interval which by QTL analysis which were performed using SNP markers within the candidate genomic region and F_2 population. Two genes residing within the genomic region of stable QTL were identified as potential candidates underlying PC trait based on the gene annotation and expression profiling data. This research provides positive information for map-based cloning of important gene involved in pod trait of soybean and wild soybean.

Keywords: Pod color, BSA-seq, QTL-mapping, wild soybean

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A new technology system of rapid soybean breeding in northeast China

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Conventional cross-breeding is an effective breeding method for developing superior soybean cultivars. The offspring progeny self-pollinated for several generations until the homozygous, stable offspring are selected for subsequent advanced experiments. In northeast China, soybean grows one season (generation) in one year, and the stable offspring usually takes more than 5 years. To shorten the time, most breeding units in northeast China plant the low generations of soybeans (mostly F_2-F_4 generation) in Hainan province for breeding in winter. In this way, 2-3 generations can be planted in a year. So the stable homozygous offspring can be accelerated gotten, and the breeding life cycle can be shortened. In the process of the low generations planting, 1 pod is picked from each offspring plant similar to SSD (single seed descent, SSD) method. The early breeding generations are not screened until after the high generation (more than the F_5 generation). This experiment combined with the technical measures of young embryo tissue culture (developing 35 days immature embryo seed), high temperature (more than 35°C), short-day sunshine (6h light/18h dark), short duration of high light intensity (20,000 Lux), increase planting density ($0.03m^2$>100 plants), etc. formed a set of a mature technology system to promote the formation of early maturation and dwarf. Soybeans dwarf into 1-2 pods per plant and mature in 60-70 days for 1 generation. In this way, large-scale addition can be carried out in local greenhouses or incubators, so that the soybean can be rapidly added for 5-6 generations in one year. This technology system makes soybean breeding faster than conventional breeding, saving more than 90% of the land area, and greatly reducing the cost.

Keywords: Rapid breeding, immature embryo seed, dwarf, short-day

Qualitative and quantitative traits associated genetic variability of soybean mutants for expedited varietal improvement program

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Soybean is one of the crops that Bangladesh needs to import in bulk range to fulfill its national demand. The longer maturity period of our existing soybean varieties is the main hindrance to utilizing this in our existing cropping system. The narrow genetic base of cultivated varieties and available soybean germplasm in Bangladesh limit our scope to utilize directly in the breeding program. Mutation breeding, one of the breeding techniques, provides a large genetic diversity from a single genotype. To broaden the genetic diversity, two registered soybean varieties Binasoybean-3 and Binasoybean-4 were imposed to different doses of gamma radiation. Two parental with one check varieties and 20 true breeding M5 mutants were selected based on their agronomic performance and were grouped into five different clusters. Maximum selection pressure was done during maturity period for protein and oil content in seeds. Finally, eight mutants were selected for further evaluation through various trials. From those selected eight mutants, mutant SM-03-15-5 was observed early maturing (within 90 days) containing 38% protein and 18.4% oil in seeds and considered to be registered soon as variety.

Screening of salt and alkali tolerance of wild soybean

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Soil salinity is one of adversity factors effecting plant productivity, development and growth in the world. As an important genetic germplasm resource for breeding cultivated soybean, wild soybean (*Glycine soja*) belonging to a relative of *G. max* is tolerant to various enviromental conditons such as salinity, drought and water deficit. In this study, the evaluation criteria of salt-alkali tolerance of wild soybean were first determined. The composition and concentration of saline-alkali solution are similar to that of saline-alkali soil in Heilongjiang Province. Then, the 1200 wild soybean resources collected in Northeast China were selected to identify for salt-alkali tolerance at the bud and seeding stage. Among them, 1% showed highly tolerant characteristic, 3.8% were tolerant, 5.9% were moderately tolerant, 11.5% were sensitive at the bud stage. In addtion, besides the highly sensitive resources, the other 270 (77.5%) resources were screened for salt-alkali tolerance at the seedling stage. The results showed that four wild soybean resources including ZKW032, ZKW048, ZKW084 and ZKW128, showed high tolerance to salt-alkali treatment at both the bud stage and seedling stage. Therefore, these four resources could be used as core parents in the subsequent creation of salt-alkali tolerance germplasm. These 270 materials have genomic information and can be used for further genomic analysis to identify the putative genes which related to salt-alkali tolerance of soybean. Further more, the leaf structure analysis by scanning electron microscope are under study. Thus, study on the relationship between the evolution of salt gland structure of wild soybean and the formation of soybean yield will be carried on and the technical system for identification of soybean yield and plant pattern will be established. The whole research results will play a decisive role in improving the saline-alkali tolerance of cultivated soybean.

Keywords: Wild soybean, salt-alkali tolerance

Functional analysis of soybean NFR5α and ROP6 genes using *Agrobacterium rhizogenes*-CRISPR/Cas9 system

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Agrobacterium rhizogenes-mediated hairy root transformation coupled with clustered regularly interspaced short palindromic repeat (CRISPR)/CRISPR-associated protein (CRISPR/Cas) system is the most efficient and fastest method for soybean “in root” gene functional analysis. This technique has been applied to validate the actual function of two candidate genes involved in soybean nodulation, including NFR5α and ROP6. For each gene, two specific gRNAs were designed to target their exon regions. Two-step *ex vitro Agrobacterium rhizogenes* inoculation technique was applied for transformation and hairy root induction. *A. rhizogenes* induced transgenic hairy roots were identified using applied reporter genes. Sanger sequencing of transgenic hairy roots approved the sequence disruption in the targeted regions of both genes. We examined nodules on transgenic and non-transgenic roots by rhizobial inoculation at 30 dpi. Obtained results revealed that nodulation was completely disrupted in transgenic hairy roots with mutated NFR5α and/or ROP6 genes. Our results demonstrate that both NFR5α and ROP6 are master genes of soybean symbiotic nodulation.

Keywords: Double-strand breaks, genome editing, guide RNA, mutation, symbiotic nodulation

Genome-wide association study (GWAS) for seed starch content in soybean

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Soybean (*Glycine max* (L.) Merr.) is one of the most important food resources. As soybeans are an excellent source of diets, they are used as an ingredient for nutritious foods and various traditional foods. Starch is an important nutrient as an energy source for humans and animals. Legumes such as mung beans, common beans and peas have about 34 to 55% starch content, while soybeans contain less than 1% of starch content in the seed. The high starch content in the seed is related to taste, flavor, and the softer texture, which improves the quality of processed foods. In this study, we identified the genomic regions associated with starch content by genome wide association study (GWAS) with 409 cultivated soybean collections which are publicly available for the next-generation-sequencing data. The mean starch content was 1.5% with a range of 0.4%-4.6% in 2020. The starch content averaged 1.3% with a range of 0.7%-2.5% in 2021. The starch content of core collections across two years averaged 1.4% and ranged from 0.4 to 4.6%. As a result of GWAS, the most significant SNPs were located on chromosomes 6 for the averaged starch content across two years. With haplotype analysis, three putative genes were in the linkage disequilibrium. We assumed that one of them may be a possible candidate gene because its function was associated with starch biosynthesis pathway. Further research will be required to determine this putative gene for starch content in this study. The information in this study will help to promote the use of soybean starch in breeding programs and the food industry.

Mining of resistance gene to pythium root rot of soybean and application in breeding

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Soybean root rot is a serious soil-borne disease, which causes great losses to soybean production. Pythium is main pathogen of this diseases. Due to the low separation ratio and the similarity of pathogenic characters with other species, there are few studies on the pythium root rot of soybean. We used high-density gene chips to scan the whole genome of two constructed recombinant inbred lines, 2QTLs were identified, which are closely linked to the resistance to rot disease, were located in No. 10 and 20 chromosomes respectively. The analysis showed that there was a significant allele variation in high-resistance wild soybean. Based on overexpression of genes in plant, Chromatin Immunoprecipition, Gene silencing technology, yeast two-hybrid system and some other methods. Functional mechanism of key genes is suspected to be illuminated signal transduction pathways in plant disease resistance and regulatory network and application potential of allele in wild soybean will be evaluated. These studies will evaluate its application value and provide new ideal and new strategy to breeding for pyramiding resistance genes in soybean and new germplasm will be created. This will promote the research of soybean molecular breeding and the development of new agricultural biotechnology, provide new strategies and technologies for breeding new resistance varieties of soybean.

Keywords: Wild soybean, superior germplasm, *Pythium*, molecular breeding

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Construction of a haplotype database for soybean based on gene region variations

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Soybean is a globally significant crop, serving both as a source of food and feed. Its genome sequence has provided researchers with valuable insights into its genetic diversity. However, to advance crop yield and biotechnology development, a comprehensive understanding of the molecular basis of this diversity is crucial. To address the knowledge gap, this study aims to construct a gene region-based haplotype database for soybean using variant call format (VCF) files from resequencing projects. The hypothesis is that the database will provide a valuable resource for the advancement of soybean genetics and breeding research, with the potential to enhance our understanding of soybean genetic diversity and accelerate the development of improved soybean varieties. To construct the haplotype database, variant call format (VCF) files were obtained from soybean resequencing projects. The database includes tables containing information on allele, GFF version, locus, reference ID, allele label, haplotype, and species ID. In addition, a user-friendly search user interface (UI) was developed using the React framework to provide easy access to a broad range of gene region variation data in a structured format. The haplotype database enables researchers to investigate the genetic structure differences of soybean cultivars and varieties in a more comprehensive manner. The gene region-based haplotype database represents a valuable resource for the advancement of soybean genetics and breeding research, with the potential to enhance our understanding of soybean genetic diversity and accelerate the development of improved soybean varieties. In conclusion, the construction of the gene region-based haplotype database for soybean has provided a valuable resource for the advancement of soybean genetics and breeding research. The database enables the investigation of the genetic structure differences of soybean cultivars and varieties in a more comprehensive manner.

Keywords: Soybean, genetic diversity, haplotype, gene region variations, cultivars

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Variation of stomatal density within and between leaves of soybean cultivars

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Stomata regulate carbon and water vapor exchange between leaves and the atmosphere, indicating climate change resilience. Stomatal density and patterns in mature leaves vary in different environmental conditions. This study aimed to understand the variation of stomatal density within and between different leaves of soybean. A total of ninety epidermal sections were evaluated at the basal, middle, and tip positions of leaflets at the unifoliate of Williams 82. Stomatal density showed significance among the positions in leaflets on the unifoliate of soybean. The optimal number of observations from each section was at least five observations of stomatal density. In this study, we identified the difference in stomatal density based on the leaflets on unifoliate and four different trifoliate. However, there was not a significant difference between the two leaflets of unifoliolate and among leaflets on the 1st, 2nd, 3rd, and 4th trifoliate in the soybean plants. The result showed that the lowest stomatal density was leaflets on the unifoliate and the highest one was leaflets on the 4th trifoliate. Our findings would provide effective information for evaluating stomatal density in soybeans. It would assist us in analyzing physiological differences under adverse climatic conditions, and phenotyping a large-scale population to identify the genetic factors responsible for stomatal density variations in soybean genotypes.

Combining a mutant allele of FAD2-1A with HD improves the ω-6/ω-3 ratio in soybeans

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The intake of an unbalanced ratio of ω-6 to ω-3 causes various health problems. Soybean oil generally has a ω-6/ω-3 ratio of 6–7:1. The recommended ratio in terms of health benefits is <4:1. This study aimed to identify the appropriate combination of mutant alleles that can reduce the ω-6/ω-3 ratio using three segregating soybean populations. F_2 individuals from each population were genotyped for microsomal delta-12 fatty acid desaturase 2 enzyme (FAD2-1A) and homeodomain-like transcriptional regulator (HD) genes, and their fatty acid profiles were assessed. The ω-6/ω-3 ratio of F_2 seeds carrying a mutant allele of both FAD2-1A and HD varied slightly according to the FAD2-1A mutant alleles. When combined with an hd allele, fad2-1aDEL, fad2-1aS117N, and fad2-1aW293STOP alleles ranged a ω-6/ω-3 ratio of 1.9–2.7:1, 2.7–3.9:1, and 2.6–3.6:1 in soybean seeds, respectively. This study revealed that the induction of mutations in FAD2-1ADEL and HD is the most efficient strategy to improve the ω-6/ω-3 ratio and elevate the concentrations of ω-3 fatty acids in soybean seeds. These results provide useful information for the development of novel soybean cultivars with a lower ω-6/ω-3 ratio and elevated ω-3 fatty acid concentrations, which may enhance human health in populations consuming soybean-based foods.

Genetic differentiation and evolutionary mechanisms of geographical populations of *Leguminivora glycinivorella* (Lepidoptera *Olethreutidae*) in China

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Leguminivora glycinivorella (Matsumura) is one of the principal pod borers of soybean (*Glycine max*) in China. This study investigates the genetic differentiation and evolutionary mechanisms of various geographic populations of *L. glycinivorella*. Genotyping by sequencing was used to develop genome-wide single nucleotide polymorphism markers and analyze the genetic characteristics of mature larvae from 13 geographic populations of *L. glycinivorella* to elucidate the genetic variation and differentiation across regions. To explore the evolution of populations and the environmental adaptability of *L. glycinivorella*, Q-type cluster analysis was applied to morphological, environmental, and host (soybean) cultivation characteristics. The phylogenetic tree demonstrated distinct clustering of populations from the Du'an and Guiyang regions. STRUCTURE analysis showed that the Du'an population differed genetically from the other regions. Clustering analysis showed that the Du'an and Guiyang populations were independent of each other. This significant genetic differentiation may have evolved into a new taxon in the Du'an population. The differentiation and evolution of the various populations correlate with the regional differentiation of host soybean cultivation characteristics, which is itself an adaptation to the selection pressures of regional climate changes which affect the development and survival of *L. glycinivorella*. Based on the above, we proposed concerning the co-evolution of agricultural pests and their crop hosts.

Keywords: Leguminivora glycinivorella, genotyping by sequencing (GBS), genetic differentiation, evolutionary mechanisms

Gene-allele constitutions, evolutionary motivators and breeding potentials of growth periods of global soybeans

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In soybeans (*Glycine max* (L.) Merr.), DSF (days of sowing-to-flowering) and DFM (days of flowering-to-maturity) are traits related to its adaptation to geographic and sowing seasonal environments. A sample of 354 soybean varieties from five world eco-regions was tested in four experiments in Nanjing, China. The novel restricted two-stage multi-locus model genome-wide association study based on gene-allele sequence as markers (GASM-RTM-GWAS) was performed. (i) In DSF (ranging in 20.6–95.5d), 141 genes with 406 alleles and in DFM (ranging in 45.8–114.6d), 135 genes with 384 alleles were detected, with six genes shared between DSF and DFM. All the gene-allele effects of the population including each accession were summarized in a matrix for each trait. (ii) Comparisons between the eco-region gene-allele submatrices indicated that the genetic adaptation from the center of origin to the four geographic sub-regions was characterized by new allele-emergence (mutation, ~10.4%/11.5% alleles for DSF/DFM) but without old allele exclusion (selection, ~0% alleles for DSF/DFM) in addition to inheritance (migration, ~88.6%/89.6% for DSF/DFM). While genetic expansion from primary maturity groups (primary MG-set) to early/late MG-sets (MG000–MG0/MGVIII–MGX) featured allele exclusion (~6.6%/7.0%) without allele-emergence (~0% alleles) in addition to inheritance (~99.7%/100.0%). (iii) Numerous recombination potentials (transgressive segregations) at both directions were predicted for geographic regions and the world, indicating breeding for further early/late soybeans is possible and allele recombination is an important evolutionary motivator. (iv) Genes of the two traits were mostly trait-specific involved in a same set of four categories of 10 groups of biological functions. (v) A set of key genes with their alleles of DSF and DFM were identified for further molecular function study. (vi) GASM-RTM-GWAS showed potential in detecting directly causal genes with their alleles and identifying differential evolutionary motivators between traits, therefore, capable of facilitating breeding by design in the improvement of soybean.

Keywords: DSF, DFM, GASM-RTM-GWAS, evolutionary motivation, global recombination potential

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Zinc-finger protein DISS1 from a quantitative trait locus contributes to salt tolerance in soybean

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High salinity largely hampers the growth and yield of crops like soybean. Seeking for major genes are the primary concern for solving this problem. In this study, in combination of QTL mapping, extreme group, expression profiling and hairy root transgenic analyses, we discovered a salt tolerance gene DISS1 (Dramatically Induced by Salt stress in Soybean 1). DISS1 functions as a transcriptional repressor to suppress the expression of multiple salt-sensitive genes including DIRG1, XTH32 and DIRB1. DIRG1 was found to be a transrepressor that constrains a series of stress genes such as PODs, OXSs, TPSs, ASNs and so on. In non-stress condition, DISS1 is barely expressed. Under high salinity, DISS1 is rapidly and strongly induced, leading to great suppression of DIRG1 and releasing those confined stress genes; ROS scavenging and osmoregulation are then enhanced for promotion of salt tolerance. Taken together, DISS1 acts as a switch to timely start the machine fighting for better survival under high salinity. Haplotype analysis revealed that pII allele of DISS1, with higher promoter activity under salinity, has been selected in cultivated soybean. Some rare alleles with highest promoter activities were also discovered and may be applied for further breeding of salt-tolerant cultivars.

Keywords: Soybean, salt stress, QTL, zinc-finger protein, transcriptional repressor

Soybean phenotyping: ideotypes for organic breeding

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The creation of focused breeding programs for organic farming systems was encouraged by the expanding market for organic farming. Breeding efforts are being stimulated by the rising demand for soybean varieties that are appropriate for organic farming in order to better meet the unique needs of those involved in the organic value chain. Within the ECOBREED project, extensive phenotyping on 200 soybean genotypes is carried out across contrasting environments in three countries, i.e. Austria, Romania and Serbia, which will enable the identification of useful traits (variation) and the level of local adaptation of genotypes. The following traits are assessed: yield and yield supportive traits, crop growth related traits, grain quality traits, plant architecture. Multi-trait genotype-ideotype distance index (MGIDI) implemented for genotypes selection, based on multiple traits. Classical linear multi-trait selection indices are available, but multi-collinearity and arbitrary weighting coefficient selection may erode genetic gains while. MGIDI provide genotype selection based on multiple traits easy to interpret. An ideotype is a genotype that contains a set of favorable traits that enables high performance under organic and low input production. Multi-location data were analyesd by linear-mixed model, and BLUP values were used for calculation of the multivariate genotype-ideotype distance index. Strength and weakness analysis performed, which are accounted for the proportion of each factor to the genotypes' MGIDI index. Factors clearly grouped similar traits and represent main performances (eg. yield supportive, seed quality, plant architecture). MGIDI and affiliated analysis, clearly identified best performance genotypes, with advantages and disadvantages of each genotype, allowing selection of soybean for organic and low input production and future breeding work. The promising usage of MGIDI index is interpretation of genotype performances in context of GxE and factors (trait groups) contribution across different environments.

Keywords: Glycine max, multi-trait genotype-ideotype distance index (MGIDI), organic production

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D38-M3, new soybean variety resistant to *Heterodera glycines* for northwestern Argentina

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Soybean cyst nematode (SCN), *Heterodera glycines*, is the most important soybean phytonematode world-wide. Cultural practices and resistant cultivars are the most effective tools for managing this pest. Most soybean cultivars in northwestern Argentina are susceptible to this nematode. The objective of this research was to evaluate resistance in advanced breeding lines to *H. glycines*. The experiments were conducted under greenhouse conditions in a completely randomized design with seven replicates. Eleven soybean genotypes from the Estación Experimental Agroindustrial Obispo Colombres Soybean Breeding Program were evaluated to SCN HG Types 2.5.7(race 5) and 5.7 (race 6), respectively. Seedlings of each genotype were inoculated with 4,000 eggs and juveniles from each HG type. Evaluation was done 30 days after inoculation by counting the number of females on each root-system. The classification of the lines as resistant or susceptible was based on the Female Index (FI) according to Schmitt and Shannon (1992). Most of the lines tested were susceptible to both HG types. Only EEAOC Exp-13/182 showed resistant to both HG types. A second trial was performed to confirm the resistance of this line and FI and the Reproduction Factor (RF) were calculated. The line EEAOC Exp-13/182 was resistant to SCN HG Type 2.5.7 (FI=2.5% - RF<1). This genotype also showed moderate resistance to SCN HG Type 5.7 based on the FI value (12.3%) but was resistant based on RF (<1). This breeding line, released on 2021 as “D38-M3”, also showed good agronomic and yield potential on *H. glycines* affected fields.

Keywords: Soybean cyst nematode, resistant cultivar

Pyramiding resistance to three fungal diseases by molecular assisted breeding in soybean

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The diseases Sudden Death Syndrome (SDS), Soybean Stem Canker (SSC) and Frogeye leaf spot (FLS) significantly affect crop yields of soybean [*Glycine max* (L) Merr.] in the Northwest of Argentina. Despite the existence of genetic resistance, there are no commercial genotypes that have resistance to the three diseases simultaneously. Although they can be controlled with fungicides, genetic resistance would constitute a more efficient, safe, sustainable and economical control alternative, for which it has been proposed to stack the resistance in a single genotype by Molecular Marker-assisted Selection (MAS). Microsatellite (SSR) molecular markers linked to R genes of interest can be used as a tool to accurately identify genotypes carrying these genes. In turn, through MAS, these genes can be introgressed into a single elite genotype by stacking. The objective of this work was to stack R genes through MAS using SSRs highly linked to QTLsms, Rdm4 and Rsc3, of resistance to SDS, SSC and FLS respectively, to obtain genetically improved germplasm resistant to the three diseases. For this, a genotyping of the EEAOC (Estación Experimental agroindustrial Obispo Colombres, https://www.eeaoc.gob.ar/) soybean germplasm bank was carried out and the parents were identified and selected. Then, cycles of directed crosses and backcrosses were performed until the lines carrying the QTLs/R genes were obtained. Finally, nine lines with different combinations of QTLs/R genes were obtained, of which two have all the MM linked to the QTLs/R genes for the three diseases, and in a genetic background of interest. The results obtained will allow progress in a more efficient, rapid and sustainable way in the development of genotypes with improved agronomic characteristics.

Keywords: Molecular breeding, MAS, pyramiding resistance

Validation of accelerating the advancement of generations in Korean soybean varieties [*Glycine max* (L.) Merr.] using speed-breeding platform

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The utilization of Light Emitting Diodes (LEDs) as a light source is a significant research topic due to its potential to advance generations rapidly. Historically, breeders relied on glasshouses during winter and spring months, requiring approximately four to five months to advance a single generation. However, the implementation of LED growth systems has significantly reduced the required generation advancement time by approximately 70 ~ 80 days. However, there is currently no information available regarding the use of speed breeding systems for Korean soybean varieties. This study aimed to verify the generation advancement period using a speed-breeding platform for 28 Korean soybean varieties that made up of different maturity groups. The study evaluated various agronomic traits such as plant height, number of nodes and branches, period to R1 and R6 stages, harvest, and the number of pods per plant, as well as the germination rate of the next generation seed. The LED module used in the study was manufactured by Wooreebio, which included blue (440 nm), green (530 nm), and two types of red light (630 nm, 660 nm). Photosynthetic photo flex density (PPFD) was measured at 506.0 ± 7.4 µmol/(m^2·s) at 30 cm above the ground. As a result, the period to R1 and R6 were measured at 29.1 ± 1.4 days, 52.5 ± 4.6 days. And the number of seeds per pod was measured at 4.5 ± 1.9. The use of the speed-breeding platform enabled the measurement of agronomic traits of soybean varieties and allowed for the development of one generation within around 73 days regardless of maturity group. Moreover, it was confirmed that this approach is suitable for Single Seed Descent (SSD) and these results could have significant implications for soybean breeding programs aimed at reducing breeding periods.

Global transcriptomics shows differential gene expression between two genotypes with contrasting response to *Macrophomina phaseolina* (Tassi)

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Charcoal rot, caused by *Macrophomina phaseolina* (Tassi), is a very destructive disease in Argentina and a large part of MERCOSUR. Large economic losses have also been reported in the USA, Canada, and other tropical and subtropical regions of the world. Seeds of high health, rotation and seed treatment fungicides are used to control charcoal root. Although the most effective method would be genetic resistance, it is conferred by QTLs and so far they have not been identified in commercial germplasm adapted to the MERCOSUR region. However, a resistant commercial genotype (Munasqa RR) has recently been identified in Northwest Argentina. In this context, it was proposed to identify genes that are differentially expressed in this variety compared to another susceptible (DM6.2iRR) in response to the pathogen. For this, a protocol was optimized under controlled conditions and seedlings of both genotypes were infected, and from mRNA extracted 72 h after the start of the treatments (T) the transcriptomes were sequenced using MACE (Massive Analysis of cDNA Ends). An average of 14 million readings per sample were obtained, 70% representing mRNA and 3% rRNA, the rest were not assigned. 98% of the assigned reads were mapped to the soybean reference genome. The quality of the reads and nucleotides per fragment was optimal. Principal component analysis and global heat map demonstrated a clear difference between T and genotypes (PC1 47%, PC2 23%). Volcano and scatter plots represented the change in gene expression between each T vs. its control and the varieties R (resistant) vs. S (susceptible). Of a total of approximately 46,000 genes mapped, 1,953 differentially expressed (GED) genes were identified in MunasqaRR and 1,472 in DM6.2iRR (p value < 0.01).

Keywords: Charcoal rot, resistance, DEGs, transcriptomics, soybean

Speed breeding in a soybean commercial breeding program

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Soybean breeding is a time-consuming process. Most of the traditional breeding programs take 10-15 years to release an improved cultivar. In the breeder's equation, a model of the expected change in a trait in response to selection, cycle time is the most powerful parameter for increasing genetic gain. Identifying of a system to accelerate generations by shortening each cycle is crucial in breeding programs. Double haploids and *in vitro* culture are some of the methodologies that have been developed; however, they have not been able to be implemented efficiently in the breeding programs for soybean. In this context, speed breeding emerges as a technology that allows to increase efficiency of the programs, reducing costs and the work required. The technique uses optimal light quality, light intensity, day length and temperature control to accelerate photosynthesis and flowering, coupled with early seed harvest. The objective of this work is to develop a speed breeding system that can be adapted to GDM commercial breeding program. A system in a growth chamber was evaluated, with a photoperiod of 10 hours of light provided by led lights, in 270 cm^3 tubes with coconut fiber as a substrate, fertilization and early harvest (R6). Materials from different maturity groups (from 000 to 8) were evaluated, obtaining days to flowering between 25-30 days and cycles to harvest between 56-63 days. The size of the plant at harvest was reduced to values between 22 and 31 cm, with at least 3 pods per plant (enough for SSD breeding system). These results show that it is possible to develop a simple, economical and efficient speed breeding system that can be incorporated into a commercial breeding program. Furthermore, this system could be complemented with other breeding tools such as genetic editing and/or genomic selection.

Keywords: Glycine max, genetic gain, speed breeding, growth chamber

The germplasm as a key factor in the changes in the soybean production systems and yield increase in South America

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The increase in soybean yields has been achieved through genetic gain, management and the interaction between them. From 1978 to 2022, average on-farm soybean yields have increased at a rate of 33.9, 46.4 and 21.5 kg hectare^{-1} year^{-1} in US, Brazil and Argentina respectively. Previous studies suggested that 50% of the yield increases are the result of improved genetics. In the decades of the 70s the soybeans in LATAM were introduced with the ‘full-season’ concept used mainly in USA Mid-West, assuming maximum frost-free period would maximize biomass and therefore determine maximum yields and better stress tolerance. However, in the mid 90´s in Argentina, the presence of biotic and abiotic problems related to this model leaded the industry to release early mg varieties capable of exploring better environments during the critical period. Early mg and indeterminate growth habit, combined with new available technologies (no-till and GMO traits among others), provided both higher yield potential and stability. From 2006 and on, Southern Brazil suffered a big change in the use of genetic resources, moving rapidly from determinate cultivars of longer mg to indeterminate earlier cultivars, leading to a big increase in the yields and a wider window of opportunity for planting. In several areas indeterminate and early maturity genotypes were key to the ‘safrinha’ system (soybean-corn double crop) which rapidly grew in area. In the last few years (2015 and on), Brazil´s Cerrados area also experienced a rapid change of germoplasm planted, also moving from late mg and determinate cultivars to earlier and indeterminate ones, maximizing opportunities to plant corn and cotton after soybean in the same season (safrinha). Through a deep genomic approach, the aim of this work is to demonstrate how changes and diversity in genetic offer throughout the last decades influenced deep changes in the production systems in LATAM.

Environmental characterisation to tackle genotype by environment interactions for soybean crop expansion

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In the context of European protein deficit, soybean (*Glycine max* L. Merr.) is of major interest for European farmers and breeders. Expanding the crop to new cultivation areas requires to understand and control genotype by environment interactions (GEI) that impede the genetic gain. New envirotyping methods, including target population of environments (TPE) characterisation, are key to develop efficient breeding programs for specific or broad adaptation. In this study, 602 environments from France to Russia were clustered in five Environments Types (ETs) using twelve eco-climatic factors, i.e. environmental variables calculated over specific phenological periods. These factors were previously identified as main drivers of GEI for yield in early maturity soybean (000 and 00). The GEI effect accounted for 38% of the soybean yield variation, and the environmental clustering explained 41% of the GEI effect. Five ETs composed the TPE and contrasted mainly by the intensity and timing of stresses related to temperature (cold stress during the vegetative growth and heat stress during the reproductive growth) and water availability (precipitation amount, evapotranspiration and drought throughout the crop cycle). Interestingly, we observed geographical and temporal variations in the ETs distribution across the TPE as well as in the ETs repeatability. These findings prevent from choosing one adaptation type in particular. However, specific adaptation to ET-3 and ET-4 seemed to be clearly advantageous in eastern Europe. Our results also demonstrated the need to evaluate the adequacy between yearly multi-environmental trials and the TPE allowing weighted selection strategy that overcome possible selection bias. This work will contribute to improve the existing soybean germplasm by considering risks linked weather variations and unpredictability, and more globally, to expand leguminous crops in Europe and to meet the increasing protein demand.

Keywords: Abiotic stresses, envirotyping, multi-environment trial, soybean, target population of environments

Agriplex 1K soy community SNP panel: trait marker

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The Agriplex 1K Soy Community SNP Panel incorporates two separate groups of markers: genomic and trait. The trait markers in this SNP panel are comprised of 88 publicly created and shared markers from participating soybean researchers. This includes twelve agronomic markers, twenty-four seed composition markers, nine maturity gene markers, thirty-two pathology markers, and eleven physiology markers. Specifically, this includes traits such as pod shatter, pubescence color, stem termination, seed coat color and luster, high oleic oil, Rag1 aphid resistance gene, *Pythium* spp. partial resistance, and iron deficiency chlorosis tolerance. You can learn how to decipher the SNP results into potential phenotypes. These trait markers were necessary to add to the 1K Soy Community SNP Panel as many researchers must find new associated markers of their traits of interest when a new marker panel is created. These traits can also serve as an exploratory tool to discover traits in germplasm collections that may have been undiscovered.

Identification of new resistance sources to soybean cyst nematode from the Korean soybean germplasm collection

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Soybean cyst nematode (SCN) is one of the most damaging pests, affecting 93.5% of the world's soybean growing area, including the United States, China, and Korea. SCN inhibits rhizobium, leads to nitrogen deficiency, and significantly interferes with soybean growth and yield, costing the US more than 1.2 billion dollars in yield losses every year. Rhg1 and Rhg4 genes are responsible for the SCN resistance in two representative genotypes known as the Peking-type and PI88788-type. Nearly all the SCN-resistant varieties contain either of these two resistance sources, and there is an increasing demand for new SCN resistance sources. To identify new resistance sources to SCN, we used whole-genome resequencing data of more than 700 accessions from the Korean soybean germplasm collection to identify single nucleotide polymorphisms (SNPs) located at Rhg1 and Rhg4. SNPs located at Rhg1 were selected to classify the types of SCN resistance *in silico*. Moreover, copy number variations of Rhg1 and Rhg4 were determined using either whole-genome resequencing data or droplet digital PCR to predict SCN resistance.

Genetic gains in the North Dakota State University soybean breeding program

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Soybean is a relatively new crop in North Dakota; however, it has become the number one crop for the state for acres planted and production value. Public breeding efforts began in 1986 through North Dakota State University and during this time 40 varieties in maturity groups 00 and 0 have been released. Although yields have increased during this time, the genetic gains and genetic diversity of the program have not been studied. It is important to understand these components of a breeding program to ensure yields do not become stagnant. We would like to quantify the change in gains over time in the released varieties from the North Dakota State University soybean germplasm collection. In addition, we would like to determine the amount of genetic diversity in this program to improve yields further. This knowledge will allow breeders in the maturity group 00 and 0 environment to continue to improve yields.

Keywords: Germplasm, genetic gain, diversity

Loss of function of GmSNAP02 on chromosome 2 confers resistance to soybean cyst nematode

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Fine mapping of a major novel quantitative trait locus on chromosome 2 in PI 90763 identified GmSNAP02, an α soluble NSF attachment protein gene paralogous with GmSNAP18 (rhg1) and GmSNAP11 (rhg2), as the best candidate for resistance to soybean cyst nematode (SCN) HG type 1.2.5.7 (Race 2). An insertion in GmSNAP02 in PI 90763 was confirmed, but how the function of GmSNAP02 contributes to resistance is unclear. Here we investigated the functional consequence of this insertion on the transcription of GmSNAP02. For this, PI 90763 (resistant) and PI 548402 (Peking, susceptible) differing for the insertion at GmSNAP02 were inoculated with SCN HG type 1.2.5.7. Root tissues of mock-inoculated and infected roots were harvested at three days post-inoculation for RNA isolation followed by qRT-PCR analysis using GmSNAP02-specific primers. GmSNAP02 expression was significantly upregulated in Peking, but not PI 90763, in response to SCN infection. In addition, we were unable to amplify GmSNAP02 full-length transcripts from PI 90763. CRISPR-Cas9 was used to delete portions of GmSNAP02 in Peking and PI 90763. Peking-edited composite plants exhibited reduced susceptibility to SCN HG type 1.2.5.7 whereas resistance in PI 90763-edited composite plants remained unchanged. Our results demonstrate that the insertion in GmSNAP02 in PI 90763 leads to a loss of function of GmSNAP02 conferring resistance to SCN HG 1.2.5.7.

Studying iron deficiency chlorosis: using soybean to turn the model right side up

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Iron deficiency chlorosis (IDC) negatively affects crop quality and yield. Studies from model species demonstrate shoots control or influence of iron uptake in roots. In this study, grafting of near-isogenic soybean lines Clark and IsoClark (iron stress tolerant and susceptible, respectively), demonstrated the Clark rootstock drives phenotypic responses in IsoClark leaves two weeks after iron stress. RNA-seq analyses from homo- and hetero-grafted plants 30 and 120 minutes after iron stress identified 518 and 846 differentially expressed genes (DEGs) in leaves and roots, respectively. Grafts with a Clark rootstock induced genes involved in iron uptake and utilization at 30 minutes in the root and by 120 minutes in the leaves, regardless of the leaf genotype. This suggests a mobile signal, initiated in Clark roots, regulates iron stress responses in soybean leaves. Better understanding of the complex differences between crop and model species will aid in the development of crops with improved IDC tolerance.

Investigating novel QTL to improve iron deficiency tolerance

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Early quantitative trait loci (QTL) mapping studies identified major regions of the genome controlling traits of interest in agronomically important lines. Continued crop improvement requires the identification and utilization of alternative genomic regions, likely in novel germplasm. Fiskeby III is a soybean line with high tolerance to abiotic stresses, including iron deficiency, a perennial problem in the upper Midwest of the United States. A previous study identified a novel iron deficiency tolerance QTL on chromosome Gm05 in Fiskeby III. Using virus induced gene silencing (VIGS), we targeted candidate genes in the Williams 82 genome sequence associated with the QTL. A single gene resulted in phenotypic changes under iron deficient and sufficient conditions. Using RNAseq we have examined gene expression patterns which have revealed Fiskeby III induces transcriptional reprogramming within 24 hours of iron stress, similar to other tolerant soybean varieties. While Fiskeby III induces all the canonical soybean iron deficiency responses, the individual genes and timing of these responses differs from other iron deficiency tolerant lines. Identifying the genes and understanding the pathways and timing utilized by Fiskeby III provides novel targets for improving abiotic stress tolerance in elite soybean lines.

Keywords: Abiotic Stress, RNA-seq, QTL, VIGS, iron

Breeding rust-resistant soybean varieties by pyramiding resistance genes using marker assisted selection in Mexico

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Soybean is one of the most important oilseeds in the world. In 2021, Mexico produced 288,202 tons, of which Tamaulipas contributed 13.8% (39,710 ton). However, this state also has the longest period with favorable climatic conditions for crop infection by the fungus (*Phakopsora pachyrhizi*), which causes Asian Soybean Rust (ASR), and can cause losses of 25 to 80%. Since all soybean varieties grown in Mexico are susceptible to this phytopathogen, the control method is through agrochemicals applications, which increases crop production costs. The planting of soybean varieties resistant to ASR is considered the most profitable strategy for its management. Using biotechnology tools, it is possible to generate ASR-resistant soybean varieties efficiently. Thus, Experimental Field Las Huastecas-INIFAP, Mexico in collaboration with JIRCAS, Japan began a breeding program through Marker-Assisted Selection (MAS) to develop ASR-resistant soybean varieties in 2016. The original soybean variety 'Huasteca 700' developed by INIFAP has good agronomic characteristics, however, it is susceptible to ASR. The experimental soybean line 'No6-12-1' developed by JIRCAS has the resistance genes Rpp2, Rpp4 and Rpp5 to ASR. These genetic materials were crossed resulting F_1 was backcrossed four times with 'Huasteca 700' as the recurrent parent. In each backcross, a total of six SSR markers sandwiching each locus were used for selection to obtain individual carrying the three target Rpp genes. The four backcrosses were performed to recover the genome of the recurrent parent ('Huasteca 700'), the selection of plants positive for the three genes in each backcross was by the markers: Satt620, SSR16_0908, Satt288, SSR18_1580, Sat_275 and Sat_280. The six markers allowed us to discriminate between plants that have the three pyramided genes from those that do not. There are four BC_4F_1 plants (theoretically, 96.9% of the genome is identical to the recurrent parent) with the characteristics of 'Huasteca 700' and the genes Rpp2, Rpp4 and Rpp5 of No6-12-1 (donor parent). MAS is being an efficient tool that is allowing to speed up the time to obtain ASR-resistant soybean varieties.

Keywords: Breeding, *Phakopsora pachyrhizi*, Asian soybean rust, phytopathogen, backcrosses

Application of DNA markers for variety protection and identification in a real case. Argentina experience in soybean

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Soybean is a very important crop in Argentina, and every year breeders presents in the National Seed Institute about 250 new applications. The number of varieties already in the national cultivar list is large (currently 1188 registered varieties). This makes very difficult to achieve distinctness of new varieties, because the number of varieties to be compared with the new ones is big. To address this problem, a project between public and private sector was carried out, with the aims of a) reduce the list of cultivars that should be compared in the field (based on UPOV model 2, that combines phenotypic and molecular distances), and of b) guarantee the traceability of varieties identity. For aim a), we select a set of 4004 SNPs markers distributed in the genome that represent the genetic background and yield an efficient discrimination power. Also, we determine the molecular distance threshold and the minimum phenotypical distance threshold, selected based on a 3 year field trials. The combination of both threshold, allow to select varieties that require comparison in the field. The results show that with 3 as minimum phenotypical distance threshold, and with 0.36 as molecular distance threshold, we reduce the pairs to be evaluated in the field by 55%. This is the fourth year that we used this method in the distinctness process. In relation to aim b), based on the 858 soybean varieties, that were genotyped, a set of 32 SNPs markers composed by the minimum number of markers that generates a unique DNA profile for each variety, were selected. The allelic profile of this small marker set is available for third parties, and currently is used by the National Seed Institute. The results allowed to reduce costs and duration of the distinctness process, and guarantee the traceability of varieties identity.

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Soybeans as a food and energy source

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High protein content with lower carbohydrate content, highlights soy as a unique vegetable protein source and they are good milk substitutes. Soy contains isoflavones, which are polyphenols with estrogenic properties highly. Many studies show that individuals consuming foods high in vegetable proteins have lower risk of cardiovascular disease and other metabolic disorders. Soybean is also an important source of bioenergy. As a source of bioenergy, preference is given to harvest residues that contain more cellulose and oil, for example soybean straw. In order to obtain greater energy benefits from soybeans, breeding is aimed at the production of varieties with higher biomass and increased oil yield per hectare, as well as the creation of varieties that would be more suitable for industrial processing for the production of technical oils. In this study, productive parameters of soybeans were examined in 2021 and 2022 in Dolovo, Serbia, on chernozem type soil. The subject of research was the soybean genotype Favorit. Average soybean yields varied from 2.8 t ha^{-1} (2022), to 2.92 t ha^{-1} (2022). The total soybean biomass yield was 4.15 t ha^{-1}, while the biogas yield was 372 m^3 ha^{-1}. The year 2021 was more favorable for soybean production, where higher grain yields, biomass and biogas yields were achieved.

Keywords: Soybean, food, energy genotype, grain yield, biomass and biogas yield

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Enhancing soybean breeding efficiency: a combined approach of genomic and phenotypic selection

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Genomic selection (GS) has shown great potential for accelerating the rate of genetic gain in plant breeding. The aim of this study was to implement GS in GDM North America soy breeding programs and to validate the potential effectiveness of within-family selection. In 2021, early-generation multiple-environment trials (MET) were carried out using a sparse testing experimental design. Families were represented in all locations with subsets of each full-sib (FS) following a balanced incomplete block design (BIBD). A total of 11,495 genotypes from four breeding programs were used in this study. Cross-validation within and between families was used to develop genomic predictive models for the training set. Each FS family contributed to a calibration set (CS) and a predicted set (PS) at an approximately 30/70 ratio for the target families, respectively. The phenotypic selections were performed only in the CS. Predictive models were developed from the CS (genotyped & phenotyped) using yield records pre-adjusted by location and experimental design effects. Genomic selections were performed for genotyped-only FS individuals in the PS. In 2022, validations were carried out to assess the performance of selected individuals from the CS and PS groups in advanced trials. Results showed that, 1) phenotypic means of genomic-based selected individuals for each family were not significantly different from those selected based on phenotypic data, 2) the genomic and phenotypic selection was capable to select top-yield genotypes in the advanced trials and, 3) Selecting the best genotypes from the CS and PS sets of each family resulted in an improved realized yield difference of up to 2.21% when compared to selecting the best phenotyped-only individuals. Taken together, our results indicate that GS for MET trials using sparse testing is a valuable tool to do between and within-family selection when testing resources are limited.

Keywords: Genomic selection, multiple-environment trials, balanced incomplete block design, soybean

Genetics and validation of molecular markers linked to rust resistance in soybean (*Glycine max* (L.) Merrill)

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Soybean owes worldwide reputation by virtue of its high quality protein (40%) and low cholesterol edible oil (20%). The important constraints for cultivation of soybean in India are outbreak of diseases and insect pests. Diseases play a major role in yield reduction. Among the diseases of soybean, rust caused by *Phakospora pachyrhizi* Syd. is one of the devastating foliar disease of soybean, another problem in breeding for rust resistance in soybean is lack of information regarding the genetic mechanisms controlling the inheritance of rust resistance. Keeping these things in view inheritance of rust resistance in soybean was studied in three crosses involving two resistant and one susceptible genotype, viz., DSb 21 x EC 241778 (Resistant x Resistant), DSb 21 x JS 335 (Resistant x Susceptible) and JS 335 x DSb 21 (Susceptible x Resistant). Seeds of all the generations viz., P_1, P_2, F_1, F_2 and F_3 were used in field screening. The F_2 segregation analysis of two crosses EC 241780 x JS 335 and JS 335 x EC 241780 cross combinations revealed that rust resistance is governed by a single dominant gene. In the cross combination, DSb 21 x EC 241780 it was observed that two dominant genes impart resistance. In all these crosses the F_2 results were confirmed by studying the F_3 progenies. Validation of molecular markers linked to rust resistance in F_2 population of cross JS 335 x EC 241780 was carried out using already reported 25 SSR markers. Out of the twentyfive SSR markers used for screening the parents, only three markers Satt 361, Satt 275 and Satt 215 exhibiting polymorphism between the parents were taken as candidate markers for analysis of 350 F_2 individuals. The polymorphic markers were further analysed by single marker analysis which showed a significant association with rust resistance, explaining the highest phenotypic variance of 8.62 per cent for the marker Satt 361 followed by 3.61 per cent for the marker Satt 275 and 3.19 per cent for the marker Satt 215 at 5 per cent level of significance.

QNE1 functions as a key flowering regulator determining the vegetative length of soybean cultivars

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The E1 gene is the major flowering time regulator and plays an important role in flowering time and maturity. However, the reason why cultivars carrying dominant E1 allele can adapt to a higher latitudinal area of the northern China still remains unclear. A novel QTL QNE1 (QTL near the E1) for flowering time was mapped to the proximal region of the E1 on chromosome 6 in two mapping populations. Fine mapping and positional cloning revealed Glyma.06G204300 encoding a TCP transcription factor is the strong candidate gene for QNE1. Association analysis further confirmed that functional SNPs at 686 and 1063 in the coding region of Glyma.06G204300 were significantly associated with flowering time. Glyma.06G204300 is primarily distributed in the nucleus. Furthermore, either soybean or rapeseed (*Brassica napus* L.) plants overexpressing dominant or recessive QNE1 could promote flowering. Although having similar impact on flowering time, QNE1 and E4 may control flowering time through different regulatory mechanism as revealed by expression studies and WGCNA analysis of flowering time related genes. Deciphering the molecular basis of QNE1 for flowering time enriches flowering gene networks in soybean and enables to breed soybean cultivars with broader latitudinal adaptation.

Exploration of selective genotyping and selective phenotyping for optimization of soybean genomic prediction models

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The accuracy of genomic selection can be affected by several factors including trait architecture and heritability, marker density, linkage disequilibrium between markers and trait loci, statistical models, training population size, composition, and population structure. The selection of a minimal and optimal marker set with high prediction accuracy as an alternative to reduce genotyping costs, computational time, and multicollinearity for the genomic selection is a challenging task. Furthermore, optimal training population size is mostly determined empirically, by random sampling a whole set of genotypes, which may not reflect the true relationships in the population and may lead to the loss of rare genotypes and alleles. Selective phenotyping could reduce the number of genotypes tested in the field while preserving the genetic diversity of the initial population. This study aimed to evaluate different methods of selective genotyping and phenotyping on the accuracy of genomic prediction for soybean yield. The evaluation was performed on three different populations: recombinant inbred lines, multifamily diverse lines, and germplasm collection. Strategies adopted for marker selection were: SNP pruning, approaches with and without re-estimation of marker effects, randomly selected markers, and genome-wide association study-based strategy. Reduction of the number of genotypes is performed by selecting a core set from the initial population based on marker data. In 10-fold cross-validation and external validation, the average prediction ability using all markers was different among examined populations. Generally, all datasets followed a similar pattern of prediction ability for different marker reduction strategies. The selective phenotyping procedure based on maker data in all cases had higher values compared to minimal values of random sample selection. Overall, obtained results indicate that selective genotyping and phenotyping can be integrated as useful tools that can improve or retain selection accuracy by reducing genotyping or phenotyping costs for genomic selection.

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Cloning and CRISPR/Cas9-mediated gene editing of male sterility gene Ms1 in soybean (*Glycine max* L.)

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The application of heterosis is a promising approach for greatly increasing yield in soybean (*Glycine max* L.). Nuclear male sterility plays an important role in crop heterosis utilization. Here, we report the map-based cloning and characterization of the Ms1 gene in soybean. We identified a male sterile gene from the ms1 recurrent selection population, investigated the genetic mechanism underlying the mutant's sterility, and fine mapped the locus to identify the male sterility gene using transcriptome, third-generation, and metabolome sequencing technology. Compared with the same region in fertile plants, the mutant region lacks a sequence of approximately 38.7 kb containing five protein-coding genes, including an ortholog of the kinesin-like protein gene NACK2, named GmMs1. CRIPSR/Cas9 was used to knock out the gene encoding microtubule motor driver protein NACK2, and the cultivated soybean variety ZP661 produced the same sterile phenotype as the ms1 sterile line, which proved that the gene was a functional gene controlling the nuclear fertility of soybean ms1. Metabolomics studies further revealed that the disorder of various metabolic pathways such as phenylpropane, plant hormones, starch and sucrose is the main cause of soybean nuclear male sterility. It is speculated that this mutation may cause gene differential expression and the key to male sterility. Our findings provide a strong foundation for elucidating the molecular mechanism of male sterility and creating novel male-sterile lines for the utilization of heterosis in soybean.

Keywords: Soybean, nuclear male sterility, CRISPR/Cas9, GmMs1

Molecular mechanism of GmGLP1 involved in the regulation of soybean (*Glycine max* L.) root composition under low nitrogen

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Plants can improve the efficiency of nutrient absorption and utilization by regulating themselves, especially the physiological and morphological structure of the root system, and enhance their survival chances under stress conditions. Among them, nitrogen plays an important role in the root development of soybean plants, especially under the condition of low nitrogen (LN), the molecular mechanism of regulating root development is still unclear. In this study, we identifed GmGLP1, a germin-like protein (ubiquitous plant glycoproteins belonging to the Cupin superfamily) crucial for lateral root development and highly induced by LN stress in lateral roots of soybean. GmGLP1 overexpression increased root biomass through development of an improved root system in soybean under LN, whereas a signifcant decrease in root biomass was observed in the gmglp1knockout mutant. Overexpression of GmGLP1 improved plant growth and root architecture in transgenic tobacco (*Nicotiana tabacum*) under LN. Natural variation of the GT-1 cis-element in the promoter (T to A) of GmGLP1 was strongly associated with its expression level under LN, and signifcantly increased LN-sensitive variation (type A) was observed in wild soybean compared to that in elite cultivars. Thus, type A variation in the promoter of GmGLP1 may have been a site of artifcial selection during domestication. suggesting that this site might be an important molecular target for regulating the composition of soybean roots and play an important role in the process of soybean domestication. It laid a theoretical foundation for further revealing the molecular mechanism of GmGLP1 participating in soybean root architecture and nutrient efficient utilization through regulating nitrogen utilization and root development signal pathway.

Keywords: Soybean, GmGLP1, low nitrogen, root architecture

GmWRI1c increases palmitic acid content to regulate seed oil content and nodulation in soybean (*Glycine max*)

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Soybean (*Glycine max*) is one of the vital oil crops that can provide abundant oil resources for humans, but the regulatory mechanisms underlying seed oil accumulation remain unclear. We identified a member of the GmWRI1s transcription factor family, GmWRI1c, which is involved in regulating soybean oil content and nodulation. Allelic variation analysis showed that GmWRI1c shows abundant sequence diversity, and has likely undergone artificial selection during domestication. An association analysis revealed a correlation between seed oil content and five linked natural variations (Hap1/Hap2) in the GmWRI1c promoter region. In the natural population, the relative soybean seed oil content of GmWRI1cHap1 increased by 3.73% and 4.09% in the two environments compared to GmWRI1cHap2. The transgenic and qRT-PCR experiment proved that the activity of the GmWRI1cHap1 promoter was significantly higher than that of GmWRI1cHap2, so it was speculated that the differential expression of GmWRI1c was the reason for the different oil content in soybean seeds. Overexpression of GmWRI1c in soybean hairy roots increased the expression of genes involved in glycolysis and de novo lipogenesis, the proportion of palmitic acid (16:0), and the number of root nodules. Using the soybean recombinant inbred line (RIL) population, it was further confirmed that GmWRI1c promoted soybean nodulation by regulating the proportion of palmitic acid. This study identified a favourable GmWRI1c allele that can be used to breed new varieties with increased seed oil content and nodulation.

Keywords: GmWRI1c, natural variation, nodule number, palmitic acid (C16:0), seed oil content

Mapping of distichous alternate phyllotaxis gene in soybean (*Glycine max* L.)

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Plant type is an important agronomic trait in crops, but little is known about the physiological and molecular mechanism underlying plant type in crops. Soybean (*Glycine max* L.) is a typical oil and cash crop. An ethyl methyl sulfonate (EMS)-induced soybean mutant with distichous alternate phyllotaxis phenotype was identified in the background of the soybean cultivar Zhongpin 661 (Zp661). We constructed recombinant inbred lines (RILS) population from a cross between Zp661 mutant and ZH13 in order to investigate the genomic locus associated with the trait. Using bulk segregant analysis (BSA) combined with the RNA-seq method, the Euclidean distance (ED) correlation algorithm detected 4 candidate genomic regions with 216 genes. The expression patterns of distichous alternate phyllotaxis and decussate alternate phyllotaxis related genes were analyzed based on RNA-seq analysis. The result showed that 15 genes might control distichous alternate phyllotaxis. Interestingly, there were four genes responding to cytokinin (Glyma.15G003200, Glyma.15G007400, Glyma.15G019500, Glyma.15G013300). Four genes responding to ethylene (Glyma. 15G004200, Glyma. 15G008600, Glyma. 15G018400, Glyma. 15G019400). One gene responding to gibberellin (Glyma. 15G012100), and eight genes responding to auxin (Glyma. 15G006100, Glyma. 15G010400, Glyma. 15G012800, Glyma. 15G017500, Glyma. 15G012700, Glyma. 15G020100, Glyma. 15G014200, Glyma. 15G020300). Therefore, we analysed the differences between plant hormones in our material, The IAA content of the distichous alternate phyllotaxis SAM was significantly lower than that of decussate alternate phyllotaxis, while the CTK content of distichous alternate phyllotaxis SAM and leaf nodes was significantly higher than that of the distichous alternate phyllotaxis. The difference in the distribution of phytohormone may be an important reason for the formation of distichous alternate phyllotaxis. This study provides a basis for soybean ideotype, with prospects for soybean breeding targeting grain yield enhancement.

Keywords: Soybean, distichous alternate phyllotaxis, bulk segregant analysis, RNA-seq, phytohormone

Genomic regions governing synthesis of phospholipids in soybean as revealed in F_2 biparental populations

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Soybean (*Glycine max* (L.) Merrill) is the major source of phospholipids, which are vital to human and animal nutrition and have many pharmaceutical and industrial applications. Identification of quantitative trait loci (QTLs) is the first prerequisite for the development of high-phospholipid soybean genotypes through molecular breeding. In the present study, two F_2 populations were developed from crosses I (JS20-34×AVKS215) and II (JS20-98×AVKS215) comprising 233 and 254 individuals, respectively. A total of 208 simple sequence repeat (SSR) markers found polymorphic in the F_2 populations were used for linkage map construction. QTLs associated with four phospholipids, namely phosphatidylethanolamine (PE), phosphatidic acid (PA), phosphatidylinositol (PI), and phosphatidylcholine (PC), were analysed via the inclusive composite interval mapping of additive QTLs (ICIM-ADD) method using QTL IciMapping software. A total of 7 and 6 QTLs for PE, 7 and 9 QTLs for PA, and 9 and 8 QTLs for PC were identified in F_2 populations I and II, respectively. Six QTLs were found to be significantly associated with PI in each of the F_2 populations. Five QTLs for each of PA and PC, three QTLs for PE, and four QTLs for PI were found common to both F_2 populations. The results showed some genomic regions, such as Sat_341-Satt331 on chromosome 10, and Satt325-Sat_387 and Sat_229-Satt510 on chromosome 13, were associated with all four phospholipids in both F_2 populations. QTLs associated with phospholipids were also found to be annotated with genes involved in phospholipid biosynthesis. The QTLs identified may be useful for breeding soybean genotypes with high levels of phospholipids and for identifying functional genes involved in phospholipid biosynthesis.

Genetic analysis for resistance to rust in soybean [*Glycine max* (L.) Merrill]

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Soybean [*Glycine max* (L.) Merrill] owes worldwide reputation by virtue of its high quality protein and low cholesterol edible oil. It has an average protein content of 40 per cent and is protein rich source than any of the common vegetable or animal food sources. The important constraints for cultivation of soybean in India are outbreak of diseases and insect pests. Diseases play a major role in yield reduction. Among the diseases of soybean, rust caused by *Phakospora pachyrhizi* Syd. is one of the devastating foliar disease of soybean. The present investigation was carried out during 2015-17 at the MARS, UAS, Dharwad and R & D Farm, Ugar Sugar Works, Ugarkhurd with different experiments. Exotic germplasm lines consisting of 144 including highly susceptible check JS335 and resistant checks viz., DSb21, EC241780 and EC241778 were evaluated during kharif 2015 at Dharwad for identification of new sources for resistance to rust and genetic diversity. Among 144 exotic germplasm lines evaluated, only one line EC242104 and resistant checks recorded disease grade 1 with highly resistant reaction. The k-means cluster analysis for yield related traits in 144 germplasm lines were grouped into eight clusters. Three genotypes viz., DSb21, JS 335 and EC241780 were utilized for hybridization during summer 2015 to study the inheritance pattern for rust resistance and variability. Subsequently, F_2 and F_3 populations were raised during kharif 2016 & 2017 respectively. Inheritance study revealed that rust resistance is controlled by single dominant gene in all the crosses. The F_3 families of three crosses, viz., JS335 x EC241780, EC241780 x JS335 and DSb21 x EC241780 have generated sufficient variability and also nine superior segregants. In addition to this, validation of molecular markers linked to rust resistance in F_2 population of cross JS335xEC241780 was carried out, only three markers Satt 361, Satt 275 and Satt 215 exhibited polymorphism. The polymorphic markers were further analysed by single marker analysis which showed a significant association with rust resistance.

Keywords: Soybean rust, inheritance, variability, markers

Linkage mapping of mungbean yellow mosaic India virus (MYMIV) resistance gene in *Glycine max* and *Glycine soja*

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Mungbean yellow mosaic India virus (MYMIV) is one of the most prevalent pathogen that limits soybean production in India. RILs derived from JS335 MYMIV susceptible variety × PI171443 (donor of MYMIV resistance gene) and F_2 population derived from MYMIV resistant variety SL525 × susceptible genotype NRC101 was used to study the inheritance and map the gene responsible for MYMIV resistance. F_1s were found to be completely susceptible. $F_{2:3}$ and RILs population segregated to fit a ratio of 1:2:1 and 1:1 indicating that single recessive gene-controlled resistance to MYMIV. Bulked segregant analysis (BSA) using 144 polymorphic SSR markers mapped MYMIV resistance gene on chr 6 (LG C2) within a 3.5-cM genome region between SSR markers GMAC7L and Satt322. Further, three F_2 populations derived from crosses of MYMIV susceptible *Glycine max* cultivars ‘Ankur’, ‘Sawarn Vasundhra’, and ‘JS335’ with MYMIV resistance doner PI 393551 (*Glycine soja* Siebold & Zucc), were phenotyped for reaction to MYMIV to determine inheritance of MYMIV resistance in PI 393551. All three F_2 populations exhibited a 15:1 ratio of individuals resistant and susceptible to MYMIV, indicating duplicate dominant inheritance of MYMIV resistance genes. Further, 1520 F_2 individuals reconstructed from JS335 × PI 393551 were subjected to BSA which showed genomic regions chr 8 and 14 associated with MYMIV resistance. A total of 78 plants with 100% MYMIV infection, which were expected to be homozygous for recessive genes, were genotyped using polymorphic simple sequence repeat (SSR) markers near linked markers on chr 8 and 14. Genetic analyses revealed tight linkages of MYMIV resistance with SSR marker BARCSOYSSR_08_0867 on chr 8, and with BARCSOYSSR_14_1416 and BARCSOYSSR_14_1417 on chr 14. The identified SSR markers that are tightly linked to MYMIV resistance genes will be useful breeding varieties for resistance against MYMIV and for transferring MYMIV resistance from *Glycine soja* into *Glycine max*.

Differential SW16.1 allelic effects and genetic backgrounds contributed to increased seed weight after soybean domestication

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Although seed weight has increased following domestication from wild soybean *Glycine soja* to cultivated soybean *Glycine max*, the genetic basis underlying this change is unclear. Using mapping populations derived from chromosome segment substitution lines of wild soybean, we identified SW16.1 as the causative gene underlying a major quantitative trait locus controlling seed weight. SW16.1 encodes a nucleus-localized LIM-domain containing protein: importantly, the GsSW16.1 allele from wild soybean accession N24852 had a negative effect on seed weight, whereas the GmSW16.1 allele from cultivar NN1138-2 had a positive effect. Gene expression network analysis, RT-qPCR, and promoter-luciferase reporter transient expression assays suggest that, SW16.1 regulates the transcription of MT4, a positive regulator of seed weight. The natural variations in SW16.1 and other known seed weight genes were analyzed in soybean germplasm. The SW16.1 polymorphism was associated with seed weight in 247 soybean accessions, showing much higher frequency of positive-effect alleles in cultivated soybean than in wild soybean. Interestingly, gene allele-matrix analysis of the known seed weight genes revealed that *G. max* has lost 38.5% of the *G. soja* alleles, and that most of the lost alleles had negative effects on seed weight. Our results suggest that eliminating negative alleles from *G. soja* led to a higher frequency of positive alleles and changed genetic backgrounds in *G. max*, which contributed to larger seeds in cultivated soybean after domestication from wild soybean. Our findings provide new insights regarding soybean domestication and should assist current soybean breeding programs.

Development of specialty soybean varieties through marker assisted selection and genome editing in India

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Under flagship plant breeding programme of Indian Council of Agricultural Research, development of specialty soybean was undertaken to cater the needs of different segments of soybean industries, namely, soy food, feed, oil, nutraceuticals and to fetch premium on the produce for the farmers. Given the importance of genetic elimination of protein digestibility-affecting Kunitz trypsin inhibitor (KTI) for both soy food and soyfeed industry, NRC127 was the first KTI free soybean developed by introgressing null alllele of KTI in climate smart variety JS97-52 from PI542044 using gene specific and linked markers Satt228, Satt409 linked to Ti locus on chr 8. Subsequently, MACSNRC1677 and NRC181 KTI free soybean varieties were also developed with varying maturity period and increased seed size. Indian consumers are not accustomed to the off-flavor associated with the soy products. Lipoxygenase-2 (Lox2) is the principal contributor to off-flavour generated in soy products. NRC132 was the first lipoxygenase -2 free variety developed using PI596540 by deploying gene specific marker and Lx2 linked marker Satt656 on chr 13. Subsequently, lox2 free NRC150 and NRC109 were also developed. More importantly, double null varieties NRC142 and NRC152 which are free from both KTI and Lox2 were developed deploying both Ti specific and Lx 2 specific marker, and linked markers for these traits. To obviate the need partial hydrogenation for improving shelf life of soybean oil and to meet the trans fat limits, high oleic acid soybean variety NRC147 (oleic acid 42 %) and genotypes NRC140 and NRC141 (oleic acid approximately 60%) were developed. All these special soybean varieties/genotypes yielded at par with or higher than the dominant varieties of the growing regions for which they were released for cultivation. Development of genome edited lines with enhanced level of isoflavones, the nutraceutical molecule and triple null lipoxygenase using *Agrobacterium tumefaciens*-mediated transformation are underway.

Identification of loci associated with protein and amino acid content in soybean germplasm suitable for growing in Europe

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Although soybeans have not traditionally been a staple crop in Europe, they have become increasingly important in recent years due to their nutritional value, providing an important source of plant-based protein for both human and animal consumption. However, the nutritional quality of soybeans can vary widely depending on the specific variety and environmental factors that can affect the protein and amino acid content of the crop. Soybean meal contains relatively low levels of sulfur-containing amino acids methionine and cysteine which is therefore often supplemented with additional sources, especially in animal feed. By identifying genetic markers associated with these traits, breeders might develop soybean varieties with increased content of proteins and specific amino acids, which may help improve the nutritional value of this crop. To investigate the genetic basis of variation in protein and amino acid content in soybean seeds, a genetically diverse panel of 192 European soybean varieties (MG000 – MGII) was grown in the field experiment at two locations over three consecutive years. The mapping panel was phenotyped using near-infrared spectroscopy and genotyped by the Illumina SoySNP50K iSelect BeadChip. Using principal components and the Bayesian-information and Linkage-disequilibrium Iteratively Nested Keyway (BLINK), positive signals were detected for protein levels on chromosomes 12 and 17. Signals for methionine, an insufficient amino acid in soybean proteins, were detected on chromosomes 7 and 14. Loci for threonine content were detected on chromosomes 2, 15, and 20; for valine on chromosomes 2 and 11; for serine on chromosomes 4, 8, and 14; and for isoleucine on chromosomes 2 and 16. The identified QTLs and the desired germplasm (donors) could be used to further improve the seed quality of soybean through breeding programs.

Characterization of the population structure and genetic diversity of a Chinese soybean diversity panel

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Soybean is a major commodity crop in Uruguay, and genetic diversity is essential for crop breeding programs to achieve genetic gain, adaptation, and stability. In this study, the genetic diversity and population structure of a soybean diversity panel from China were characterized by the Soybean Breeding Program of the National Institute of Agricultural Research of Uruguay (ISBP) in order to assess its potential for use in the program. A total of 230 lines derived from 49 original accessions (28 landraces and 21 cultivars) of soybean from eight Chinese provinces were genotyped using 5636 single nucleotide polymorphism (SNP) markers. The genetic diversity, population structure, and kinship were analyzed using principal component analysis (PCA), hierarchical clustering, and STRUCTURE analysis. The results showed that the Chinese soybean diversity panel exhibited a high level of genetic diversity, with an average expected heterozygosity of 0.35 and a polymorphism information content (PIC) average value of 0.34. The panel was structured into three major clusters, with no clear correspondence to the origin of the accessions. The kinship analysis revealed a low level of relatedness among the original accessions. We conclude that the Chinese soybean diversity panel is a valuable genetic resource for soybean breeding programs in Uruguay, as it exhibits high levels of genetic diversity and low levels of relatedness. The lines derived from the original accessions can be used to select for specific traits of interest, such as grain quality, disease resistance or yield potential to introduce novel genetic variation into the Uruguayan soybean germplasm. Overall, our results highlight the importance of having access to diverse germplasm through international collaboration, as it provides the material necessary for improving new breeding targets and for adaptation to changing environments.

Keywords: Germplasm, genetic variability, molecular markers, China, Uruguay

Assessment of grain quality traits in a Chinese soybean diversity panel

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Soybean grain quality with high protein and oil content is in high demand by domestic and international markets. The National Agricultural Research Institute of Uruguay (INIA) soybean breeding program aims to develop varieties that can meet those requirements. However, most locally available germplasm has middle to low protein content. An agreement between INIA and the Chinese Academy of Agricultural Sciences (CAAS) provided access to valuable genetic resources such as a diversity panel with high reported values of grain protein content. With the aim of phenotypically assess this panel for grain quality traits and yield, two field trials with an incomplete block design and rows as experimental units were conducted in La Estanzuela, Uruguay (S 34.34, W 57.70) in 2022 and grain yield, protein and oil content was measured. No significant genotype by trial interaction was found for any of the studied traits. The heritability was 0.68 for protein content, 0.84 for oil content, and 0.44 for grain yield. Phenotypic adjusted means for protein content ranged from 39.9% to 48.3%, for oil content from 15.7% to 20.7% and for yield from 371 to 4994 kg ha-1. Strong and negative correlations were found between protein and oil content and between protein and yield. Five accessions were identified with superior quality traits and yield. These results suggest that the diversity panel provides suitable additive genetic variance for obtaining genetic gains in grain quality and yield. The observed negative correlations will demand the use of strategies such as genomic selection and mapping of quantitative trait loci to select for high protein content without compromising acceptable levels of grain yield and oil content. To that end, the development of populations of recombinant inbred lines derived from the diversity panel and local germplasm is currently in progress.

Keywords: Nutritional quality, phenotyping, genetic diversity, soybean breeding programs, Uruguay

Coordinate inheritance of seed isoflavone and protein in soybean

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Soybean (*Glycine max* (L.) Merr.) is an important nutritional crop, rich in protein, oil, and isoflavones. However, breeding soybean cultivars that consistently produce high yields of protein, oil, and isoflavones requires a clear understanding of the genetic relationship among these traits. To investigate this relationship, two soybean genotypes with contrasting protein, oil, and isoflavone content and their 185 F8:10 recombinant inbred lines were studied in field trials over two years. The results showed that protein, but not oil, was significantly correlated with isoflavone content. Using genotyping-by-sequencing technology, a high-density genetic linkage map containing 3943 SNP markers was constructed, and 25 integrated isoflavone loci were identified. Of these loci, qISO1, qISO6.1, qISO6.3, and qISO6.4 were newly identified QTLs. Two major QTLs, qISO5 and qISO6.2, were finely mapped and shown to be associated with production of daidzein and genistein derivatives, as well as glycitein derivatives, respectively explaining 9.3-20.4% and 7.8-24.8% of the phenotypic variation in these traits. The combination of qISO5 and qISO6.2 had an additive impact on isoflavone production. Among the 13 QTLs linked with seed protein content, three also collocated with QTLs for isoflavone, indicating that seed isoflavone and protein content may be coordinately inherited. These results provide valuable insights into the genetic relationships among isoflavone, protein, and oil production in soybean, and could help inform soybean breeding programs aimed at producing cultivars with consistently high isoflavone production along with high protein or oil content.

Keywords: Seed isoflavone, seed quality, genetic basis, QTL, breeding, correlation analysis

Pigment identification and gene mapping of red seed coat of soybean (*Glycine max*)

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Soybean (*Glycine max* (L.) Merr.). The color of the grain is determined by the pigment in the seed coat, mainly anthocyanins. The biosynthesis of anthocyanins is affected by many genes, and the relationship between them is complex, which has not been fully studied. Identifying the pigment composition in the seed coat and locating the key genes affecting pigment synthesis will help to solve this problem. In this study, the RIL population was constructed by crossing yellow seed coat soybean SN14 and red seed coat soybean TXAJH. Firstly, we detected the composition and content of seed coat pigment in parents at different developmental stages. A total of 12 anthocyanins were found, and their contents in SN14 and TXAJH seed coat were significantly different. We believe that three anthocyanins, cyanidin-3-O-glucoside, peonidin-3-O-glucoside and petunidin-3-O-glucoside are the main reasons for the red color of TXAJH seed coat, which contribute 94% of the anthocyanin content in TXAJH mature seed coat. BSA-seq analysis of RIL population showed that the red seed coat gene was initially located in a region of length 8.66 Mb on chromosome 8, and then we fine mapped this region with 27 polymorphic SSR markers, narrowing the candidate interval to between 223 and 259 markers, with a length of about 702 kb. There are 37 non-synonymous variants in SN14 and TXAJH genes in this region. Through gene functional annotation and expression analysis, we predicted that four of them were candidate genes controlling the red seed coat. This study laid a foundation for further improving the regulation network of soybean seed coat pigment metabolism and analyzing the mechanism of seed coat color formation.

Keywords: Testa color, anthocyanin, BSA-seq, fine mapping

Mining the salt-tolerant genes from the wild side of the major crop soybean: The perennial cousin *Glycine tabacina* in China

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The subgenus *Glycine*, which contains around 30 perennial wild soybeans, is originated from Australia and reported to be more tolerant to salt stress than *Glycine soja* Sieb. & Zucc. and *Glycine max* (L.) Merr. However, limited by unabundant accessions and no high-quality reference genome, the relevant studies of perennial species confined to measure the physiological indicators of few accessions. As a member of perennial species, *Glycine tabacina* (Labill.) Benth. is also districted in China, and may have great potential for salt tolerance, which can be used for salt-tolerant soybean breeding. Here we de-novo sequenced one *G. tabacina* accession in China and identified about 60 accessions' salt tolerance at seedling stage by substrate culture. The chlorophyll fluorescence parameter, Fv/Fm was measured for fast detection. Salt-induced RNA-seq (from 0 h, 1 h, 6 h, 12 h, 24 h to 48 h) was conducted for mining the salt-tolerant genes. We found that under 500 mM NaCl treatment, 2.5 times that of salt-tolerant soybean standard, *G. max* was stressed after one day, and all seedlings died on the fifth day. While, *G. tabacina* began to damage on the third day and no seedlings died after half month. Some accessions can endure the salt tolerance for one month and podding after transferred to normal condition. More than 300 all-stage DEGs (differentially expressed genes) were detected by RNA-seq, and less than 30 genes were continuous upregulated. The study provides elite germplasm and gene resources for salt-tolerant soybean breeding.

Keywords: Glycine tabacina, salt tolerance, seedling stage, RNA-seq, Fv/Fm

Functional analysis of lodging related genes in soybean

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Lodging is common in the process of crop production, which not only has a negative impact on the high and stable yield of soybean, but also brings difficulties to mechanized harvesting. Lodging is a complex trait produced by multiple factors, which is closely related to environment, cultivation measures and its own genetic factors. Selecting lodging resistant varieties during sowing is the most fundamental measure to reduce the occurrence of lodging. Therefore, exploring the genes related to lodging is of great significance for breeding and application of lodging resistant soybean varieties. This study is based on F_2 and $F_{2:5}$ lodging population. The population took Fengshou 24 as the female parent and the semi-wild line Tongjiao 83-611 as the male parent. By constructing a high-density genetic linkage map of soybean, it accurately located the location of lodging and its related traits. The candidate genes within the localization range belong to the DUF domain family. The DUF domain protein family can interact with the pectin structure of the cell wall, participate in the modification of the physical and chemical properties of the plant cell wall, and respond to plant development or environmental signals. These proteins not only play a role in plant growth and development, but also respond to plant stress, such as drought, salt damage and aluminum toxicity. In order to further explore and identify the soybean stress-resistant genes for production, this study used the method of bioinformatics to identify the members of the soybean genome DUF domain family, and systematically analyzed its evolutionary relationship, gene structure, protein structure, chromosome location, and promoter cis elements.

Haplotype analysis of soybean leaf type regulator gene Ln and its homologous genes

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Soybean leaf type is an important component of ideal plant type, which is of great significance for the breeding of soybean varieties. The study of soybean leaf type is of great significance for the breeding of soybean varieties. In this study, we analyzed the haplotype of Ln and its homologous gene (Glyma.10G273800) in 598 materials from different regions of China. It was found that the Ln gene (Glyma.20G116200) and homologous gene (Glyma.10G273800) contained three haplotypes (Hap1–Hap3), respectively. There was no significant difference in leaf length between the three haplotypes of Ln gene, and Hap2 was significantly different from Hap1/Hap3 in leaf width, leaf area and leaf length width ratio. There were significant differences between Hap3 and Hap2 of Ln homologous gene in different leaf types. There was no difference in the ratio of leaf length to width between Hap1 and Hap2. There was no significant difference in leaf width phenotype at the 4th leaf site, and Hap1 at the 5th leaf site was significantly lower than that of Hap2. Leaf length, leaf area and leaf circumference Hap1 were significantly lower than those of Hap2. In terms of geographical distribution, Hap2 of Ln gene was mainly distributed in high latitude and high altitude regions, and no obvious regional bias was found in the distribution of the three haplotypes of its homologous genes. Genetic analysis showed that only D9H of Hap2 in Ln gene had artificial selection signal, while the other two mutation sites in Ln gene and two mutation sites in homologous gene had no artificial selection signal. This study provides theoretical basis for soybean leaf type breeding and has positive significance for increasing soybean yield.

Keywords: Soybean, leaf type, haplotype, Ln gene

Mapping yellowing leaf genes in soybean

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Soybean is an important food crop in China, and the selection and breeding of high-yield and high-quality soybeans is the main goal of soybean breeding. Leaf photosynthesis is an important process of plant accumulation of dry matter. The accumulation of photosynthetic products after flowering of soybean directly affects the grain weight of soybean, so it delays the aging of leaves, improves physiological functions, and is conducive to increasing yield. In this study, a soybean late leaf yellowing senescence mutant was identified from the EMS mutant library of soybean cultivars ZP661. Bying BSA-Seq, the gene controlled phenotype of yellowing leaf was mapped on chromosome 19 with more than 1.75Mb region with 219 genes in the interval. The results will helpful for map based cloning and marker assisted selection for delaying aging leaves in the late stage of soybean seed filling stage and increasing yield.

Keywords: Soybean, mutant, BSA-Seq, RNA-Seq, graph cloning

Establishment of genetic transformation system and accelerated evolution of gene editing in wild soybean

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Wild soybean is not only the wild ancestor of cultivated soybean, but also the excellent germplasm resources of improved cultivated soybean. In order to solve the problem of stem morphology, grain size and seed coat color of wild soybean, genetic transformation and gene editing were carried out to accelerate the evolution of wild soybean. How to use the existing wild plant resources to improve cultivated crops without producing invasive plants is a hot issue and effective method in botany and agronomy. Agrobacterium-mediated method had excellent stability, and a higher percentage of regenerated plants were determined to be successfully transformed. Because the target gene is integrated into the plant genome through the Ti plasmid of *Agrobacterium tumefaciens*, this means that the target gene can be accurately and completely transferred into the plant genome and participate in expression, and can also be moved selectively according to the purpose and demand of the experiment. Positive plants have been obtained by introducing the gene to improve soybean yield into wild soybean, so we can use agrobacterium-mediated gene editing in wild soybean.

Regulation of soybean seed weight by an underutilized component in GmBIN2-GmSW5-GmSW1 module

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Seed weight affects both seed yield and seed quality in soybeans. Cloning the inportent genes controlling seed weight and pyramiding elite alleles will facilitate yield improvement. However, the genetic basis underlying seed weight remains largely unclear. Here, we conducted a genome-wide association study (GWAS) and identified two genes, GmSW5 and GmSW1, that regulate seed weight in soybean. Both genes exhibit seed-specific expression, with GmSW5 enhancing seed weight and GmSW1 reducing seed weight. GmSW5 encodes a nucleus-localized bZIP-domain containing protein, and the two alleles of GmSW5 are both functional, but with different functions that GmSW5A had a stronger repressive ability than GmSW5C for 100-seed weight. We also found that the BIN2, a GSK3-like kinase, interacts with GmSW5, which directly suppresses GmSW1 transcription level, resulting in the formation of a new seed weight regulatory module. Moreover, a single nucleotide polymorphism can alter GmSW5 protein stability and affinity towards BIN2. Furthermore, the heavy-seed allele of GmSW5 has not been selected during soybean domestication, but is being utilized in the current improvement process. Overall, the module provides novel insights into understanding seed weight improvement process and also important clues for further yield improvement in soybean.

Keywords: GWAS, GmSW5, GmSW1, seed weight, soybean

Fine mapping of qPD05-1 related to pod shattering resistance gene in soybean

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The loss of soybean yield caused by pod shattering seriously restricts the development of soybean industry. Although there are abundant regulatory networks for pod shattering resistance in some Arabidopsis and cereal crops, our research on the molecular mechanism of soybean shattering resistance is relatively lacking. So far, only two genes PHD1 and SHAT1-5 were cloned. In the previous study, a high-density genetic linkage map was constructed using 260 recombinant inbred lines (RHL) of Heihe 43 (pod shattering resistance) and Heihe 18 (pod shattering). Three stable QTLs (qPD01, qPD05-1 and qPD08-1) for pod shattering resistance were mapped on chromosomes 1,5 and 8, respectively. In this study, based on this genetic population, a residual heterozygous line (RHL) population of 3526 individuals was developed. SSR markers 05-1306 and 05-1318 at both ends of qPD05-1 interval were used, 35 individuals were selected to recombinant plants between the two markers. Seven SSR markers with polymorphism between parents were used in the interval, and one Indel marker was developed according to the resequencing data of the parents. Then the mapping region was narrowed into an interval with physical distance of 127.813 Kb, containing 17 genes. None gene related pod shattering resistance is identified yet in this region. The Phytozome v13.1 database (http://www.phytozome.net/soybean) was used to analyze the expression levels of 17 genes in 9 tissues, and two candidate genes were obtained, which were involved in cell metabolism and oxidation-reduction process, respectively. The results of this experiment provide a theoretical basis for the cloning of pod shattering resistance genes and the study of molecular mechanism of regulating pod shattering resistance, and provide materials and technical support for molecular marker breeding of soybean pod shattering resistance.

Keywords: Soybean, pod shattering resistance, fine mapping, molecular marker, QTL

Identification of tissue-specific genes based on RNA-seq data and promoter activity analysis in soybean (*Glycine max* L.)

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Constitutive promoters were widely applied in genetic engineering and transgenic breeding by driving foreign gene to express in host plant. However, due to the high activity in almost every tissue, some supervenient negative effects produced in the transgenic generations, such as energy excessive consumption, gene silencing, and disease symptoms. By manipulating target gene expression pattern, tissue-specific and inducible promoters attracted researcher's attentions gradually. In this study, A totally of 17 tissue-specific genes in seven tissues were obtained from RNA-seq data and verified by qRT-PCR, including leaf, stem, root, flower, seed, shoot and vegetable tissue with a number of two, one, two, one, five, five and two, respectively. We cloned their promoters and sequence analysis revealed that there were undiscovered cis-elements controlling tissue-specific expression. The activity of three seed-specific promoters and two green-tissue specific promoters was detected by embryo tip transient transformation. Results showed that all five promoters had the ability to drive GUS expression, and four of them showed no significant difference compared with CaMV 35S. These results suggest that tissue-specific gene and promoter obtained from high-throughput RNA-seq data can provide prospective promoter resources for gene function study and agronomic traits improvement.

Keywords: Soybean, tissue-specific promoter, RNA-seq, GUS

Mining lesion-like genes in soybean based on BSA-Seq technology

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Lesion mimic is a phenomenon that necrotic spots occur spontaneously in leaves and leaf sheaths of plants without the influence of external pathogenic factors and adverse factors. Lesion-like mutants have been found in many plants. Lesion mimicking mutation (LLM) is similar to plant hypersensitive reaction, and cell necrosis can occur at the site of LLM, which belongs to programmed cell death. Many mottled leaf mutants show certain resistance to some plant pathogens. A lesion-like mutant was identified from the mutant library of soybean cultivar Zhongpin 661.Leaf phenotypes of F_2 and $F_{2:3}$ plants were investigated in Beijing and Hainan, respectively, by crossing the mutant with Jidou 12. The results showed that the lesion-like trait was controlled by a pair of recessive genes. BSA-seq method was used to locate the position, and F_2 normal leaves and diseased leaves were used to construct mixed pools respectively, and the sequencing depth more than 30×. Association analysis using the SNP-index method identified a region on chromosome 13 with a total length of 3.84 Mb containing 393 genes at a confidence level of 0.99. The results of this study laid a foundation for map-based cloning of soybean lesion like genes.

Keywords: Soybean, mutant, mixed pool sequencing, BSA-seq

Using CRISPR/CAS9 technique to knock out GmBADH1 gene to reduce salt tolerance of soybean

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Soil salinization seriously affects crop growth and development, resulting in crop yield reduction. Under salt stress, crops will synthesize and accumulate betaine dehydrogenase as an osmotic protection agent, thus reducing the impact of salt damage on crops. BADH gene encodes betaine dehydrogenase, which has been proved in many crop studies to regulate plant response to adversity stress. However, the regulatory mechanism in soybean is not clear, so as to explore its function. In this study, GmBADH1 gene was cloned, and fluorescence quantitative results showed that the gene was expressed in roots, stems and leaves, with the highest expression abundance in root species. The CRISPR/CAS9 vector was transferred into soybean by *Agrobacterium tumefaciens* mediated genetic transformation technology, and three targeted mutations were generated. The salt tolerance of the homozygous mutant was identified. The results showed that salt tolerance of the three mutant plants decreased to different degrees. The results of qRT-PCR showed that the expression of this gene increased after salt treatment, which further indicated that this gene was regulating the salt-tolerance traits in soybean. This provided an important theoretical basis for us to further explore the regulation mechanism of salt-tolerance genes, and also had important significance for the improvement of soybean varieties.

Keywords: Soybean, GmBADH1, CRISPR/Cas9, salt tolerance

Mapping of new quantitative locus associated seed protein content in soybean (*Glycine max* L. Merr.)

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With the improvement of living standards, people's demand for plant protein is gradually increasing. Soybean protein is one of the main sources of plant protein, and it's a complex quantitative trait susceptible to environmental influence. Therefore, the main purpose of this study is to identified genes related to protein for directional developing high-protein soybean varieties. In this study, the offspring genetic population was constructed by crossing low protein content HN84 and high protein content JH4. By using SSR molecular marker technology to identify the genotype of F_2, seed protein content was measured using a Fourier transform near-infrared spectrometer made by Bruker, Germany. Through the complete interval mapping (ICIM-ADD) of QTL IciMapping4.2 for protein content QTL localization, located to two protein content QTL in chromosome 11 and 14, qP11 and qP14. Epistatic analysis found no positivity between the two intervals, indicating that the two intervals independently controlled protein content. Both loci overlap with the published QTLs in different genetic backgrounds, explains the protein content QTL in this study is a main effect QTL. This study provides materials and molecular marker for assisted selection high protein breeding and gene mining in soybean.

Keywords: Soybean, protein content, QTL

Locating QTLs related to 100-seed weight of soybean based on high-density genetic map

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The 100-grain weight is the key factor to determine the yield of soybean. Identifying the QTL and candidate genes related to the 100-grain weight, and then improving the grain size using modern molecular design breeding technology, is an important way to cultivate high-yield varieties with large grain. In this study, the recombinant inbred line population with 192 lines constructed by crossing Zhonghuang 35 and Zhonghuang 13 with significant difference in 100-grain weight was used as the test material. Through the weight sequencing of the two parents and RIL group, a high-density genetic map containing 4879 bin markers was constructed, with a total genetic distance of 3760.71 cM and a genetic distance of 0.77 cM between adjacent markers. The RIL population and its parents were planted in Shunyi, Beijing and Puyang, Henan, respectively. A total of 15 QTL loci related to 100-grain weight were detected in the two environments, distributed on chromosomes 3, 4 and 5, with a contribution rate of 5.48%~12.32%. This study is helpful for fine mapping and map-based cloning of soybean 100-grain weight related genes, and provides gene resources for further breeding of high-yield soybean varieties.

QTL mapping of salt tolerance gene in soybean at emergence stage

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Salt stress is one of the main abiotic stress factors affect soybean growth and yield. Previous studies have shown that the mechanisms of salt tolerance are different at different developing stages. Previously we established screening methods for salt-tolerance evaluation at both emergence and seedling stages of soybean. And cloned the key salt-tolerance associated gene GmSALT3 at seedling stage. A soybean cultivar ZH39 showed improved emergence than wild soybean 2738 under saline condition. To identify quantitative trait locus (QTL) for salt tolerance in soybean at emergence stage, a population of recombinant inbred lines (RIL) was developed by crossing a salt-tolerant soybean ZH39 and salt-sensitive wild soybean 2738. The ratio of the emergence index under saline condition to that under control condition was used as salt tolerance indices. Using single nucleotide polymorphisms (SNPs) makers, two major QTLs were mapped on chromosomes 3 and 6. The QTL located on chromosome 6 is selected as the target for fine mapping in our future work.

Keywords: Soybean, salt tolerance, emergence, QTL mapping

Time series canopy phenotyping enables the identification of genetic variants controlling dynamic phenotypes in soybean

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Plant phenotyping technologies have become more advanced, making it easier to collect phenotypic measurements at various time points. However, most statistical practices and approaches used to link genetic and phenotypic variation were developed during an era of single-time-point. In this study, time-series phenotypic data was collected using an unmanned aircraft system for a large panel of soybean (*Glycine max* (L.) Merr.) varieties to identify previously uncharacterized loci, focusing on the canopy coverage variation. The speed of canopy closure was also inferred from the time-series data, as it may be an important trait for weed control. Genome-wide association study (GWAS) identified 35 loci that exhibited dynamic associations with canopy coverage across developmental stages, including 10 known flowering time and plant height quantitative trait loci (QTL) detected in previous studies of adult plants, and novel QTL influencing canopy coverage. The time-series data enabled the identification of novel QTL that were disproportionately likely to act earlier in development, which may explain why they were missed in previous single-time-point studies. Additionally, the time-series data set contributed to the high accuracy of the GWASs, which was evaluated by permutation tests, as evidenced by the repeated identification of loci across multiple time points. Two novel loci showed evidence of adaptive selection during domestication, with different genotypes/haplotypes favored in different geographic region. In summary, this study shows that time-series data can improve the accuracy and statistical power to dissect the genetic basis of traits and offers a promising opportunity for crop breeding with quantitative growth curves, using soybean canopy coverage as an example.

Keywords: Canopy coverage, dynamic regulation, GWAS, unmanned aircraft system

Environmental stability of major QTL qSW20-1 for 100-seed weight and its effects on yield and quality in soybean

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Increasing yield is the top priority of breeding work. As one of the important yield traits of soybean, it is of great significance to excavate and clone the main quantitative trait loci (QTLs) related to 100-seed weight. In this study, a high-density genetic linkage map including 6,297 SLAF tags was constructed using 325 RILs derived from Qihuang 34 × Dongsheng 16. Based on the high-density map, combined with three environmental 100-seed weight phenotypes, a total of 11 QTLs and 1 pairs of epistatic QTLs were detected. Among them, 8 reported intervals and 3 newly discovered intervals were included, and an environmentally stable QTL qSW20-1 was mapped, which could explain 9.73% of the phenotypic variation. There was no epistatic interaction in the QTL, and the allele from Qihuang 34 significantly increased the seed number per plant and seed weight per plant without affecting protein content and fat content. Five candidate genes were predicted by gene annotation and expression pattern analysis. The results of this study laid a solid foundation for soybean yield-increasing gene mining and molecular design breeding.

Keywords: Soybean, 100-seed weight, SLAF map, QTL mapping

Using gene editing technology to create soybean haploid inducible line

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Compared with the traditional breeding technology, which even requires eight generations to achieve genetic homozygous, Double Haploid (DH) breeding technology can obtain pure lines in only two generations, which has important technical advantages and practical breeding value. At present, double haploid technology has been widely used at home and abroad to accelerate the breeding life of plants, but there is no advanced technology development and research application in soybean, an important economic and oil crop. Recently, a new gene ECS1/2 specifically expressed in egg cells has been shown to regulate the molecular mechanism of preventing multiple pollen tubes from entering the embryo sac. As a new maternal haploid inducer gene, it has been used in *Arabidopsis thaliana* and rice. The study of this new haploid inducible line gene has not been extended to other plants. Jack, an excellent soybean variety, was selected as the research material in this study, two GmECS1/2 genes in soybean were knocked out by the mature CRISPR/Cas9 gene editing technique and the tumefaciens-mediated method, and the soybean haploid induction line was created by genetic transformation technology. Meanwhile, in order to obtain pure and diploid plants, Wild-type plants were treated with colchicine of different concentration gradients to determine the effective concentration of soybean haploid chromosome doubling.

Keywords: Soybean, gene editing, genetic transformation haploid breeding

Identification of a novel salt tolerance related locus in wild soybean (*Glycine soja* Sieb. & Zucc.)

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As the main food and economic crop, soybean (*Glycine max* [L.] Merr.) production is seriously affected by salt stress. The wild ancestor of cultivated soybean, *Glycine soja* has higher salt tolerance and may contain novel stress resistance genes. In this study, we selected salt-tolerant wild soybean NY36-87 to hybridize with salt-sensitive cultivated soybeans Zhonghuang 39 and Peking to create two $F_{2:3}$ populations, and explore salt tolerance genes/loci. The phenotypic segregation ratios of the two populations were consistent with 1(tolerant): 2(heterozygous): 1(sensitive), indicating that salt tolerance in NY36-87 was controlled by a single dominant gene. Identified phenotypes of $F_{2:3}$ families to construct two extreme pools with tolerant and sensitive to discover loci associated with salt-tolerant using bulk segregant analysis (BSA) approach. The BSA results of the Zhonghuang39 × NY36-87 population showed that a salt tolerance locus was located in the 98kb interval on Chr.03, where the salt tolerance gene GmSALT3 was located in. In the Peking × NY36-87 population, the salt-tolerant gene was identified on Chr.18, we named it GmSALT18 and the fine mapping interval was narrowed to 241kb. These results indicate that NY36-87 contains a new locus related to salt tolerance in addition to GmSALT3, which provides a gene basis for soybean salt tolerance breeding.

Speeding propagation technology of soybean in Hainan

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The long generation cycle is one of the key factors limiting the developing of soybean varieties. By taking full advantage of the short day time and the natural environment of low latitude and high temperature, the allogeneic generation of Hainan was carried out. The purpose of this study is to establish a multi-generation and multi-generation scheme in Hainan, and to provide a theoretical basis for the rapid multiplication of soybean in southern China. Correlation analysis was carried out on 9 morphological and chemical traits data of ≥10°C effective accumulated temperature. It was found that effective accumulated temperature ≥10°C was significantly positively correlated with all agronomic traits. When the effective accumulated temperature ≥10°C was higher than 1500°C·d, the germination potential and germination rate decreased si gnificantly. Combined with fresh pod picking, the dried seeds can be picked in R6 period, which can save more than 20 days per generation compared with fully mature seeds. For fresh grain livestreaming, well-grown plants could be obtained at R6.5. The germination rate of Northeast and Huang-Huai-hai soybeans was greatly affected by high temperature in Hainan from May to July, so it is suggested to add generation in Huang-Huai-hai area during this period. It is estimated that the annual addition times of will be about 4 times a year.

Keywords: Soybean speeding propagation, winter nursing, sowing date, pod picking

Mapping of an incomplete dominant gene controlling multifoliolate leaf by BSA-Seq in soybean (*Glycine max* L.)

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The leaves of cultivated soybean (*Glycine max* L.) are comprising of three leaflets in general, but there are also individual varieties or mutants which have a high frequency of compound leaves with 4–7 leaflets, named multifoliolate leaves. Compound leaf formation enhances the plant's ability to adapt to the external environment. Study of related genes to multifoliolate leaves might contribute to the improvement yield level of and soybean agronomic traits. In this study, a multifoliolate leaf mutant Zhonghuang 622 was identified from the mutant library of soybean cultivar Zhongpin 661, which had 4–9 leaflets in each compound leaf. The compound leaf phenotypes of F_2 and $F_{2:3}$ populations from a cross between Zhongpin 661 and Zhonghuang622 were investigated in Beijing and Hainan, respectively. Analysis of phenotypic data from F_2 and $F_{2:3}$ populations revealed that the multifoliolate leaf trait was controlled by an incomplete dominant gene. BSA-seq method was used for gene mapping. The two bulks of normal trifoliate and multifoliolate individuals in F_2 population were constructed and sequenced for more than 35x average depth, which covered more than 99% genome compared to the reference genome. Through correlation analysis of mixed pool sequencing results by ED method, two regions were located on chromosome 11. Three regions were identified on chromosome 11 at confidence of 0.99 by the association analysis of SNP-index method. There were 690 genes located simultaneously and six SNP genes between parents by the two association analysis methods. These results lay the foundation for map-based cloning of the genes related to compound leaf development.

Breeding to improve drought tolerance in soybean

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Overcoming the devastation of drought is a cornerstone of sustainable soybean production. The increase in the frequency and intensity of droughts stress the need for building better long-term drought risk migration strategies. Since irrigation is not a viable option for many soybean growing regions, the alternative solution is the development of drought-resilient soybean cultivars. However, the development of drought-tolerant soybean has been difficult due to the narrow genetic base of North America soybean breeding stocks. From screening the USDA-ARS germplasm collection, multiple drought-tolerant PIs have been identified. Two of the most notable and widely used drought-tolerate PIs are PI 416937 and PI 471938, both of which have been shown to be slow wilting under water stress. While the mechanism behind the drought tolerance trait of PI 416937 has been well documented, the mechanism behind PI 471938 is largely unknown. To identify the specific biological processes and the gene(s) responsible for the drought tolerance trait of PI 471938 RNA-Seq analysis was conducted. A total of 44,934 differentially expressed genes (DEGs) were identified between NC-Roy (drought susceptible) and PI 471938 under the two treatments of well-watered and drought-stressed. Among these DEG’s there were several identified related to response to water, respiratory electron transport, aerobic respiration, and heat shock. Breeding efforts were also made to transfer the slow wilting trait from these and other PIs to develop elite adapted cultivars, which resulted in two releases. USDA-N8002 is the first North American release derived from PI 416937 and PI 471938. USDA-N7006 is the first North American soybean release derived from PI 407859-2 and also traces its parentage to PI 416937. The genes identified and drought-tolerant breeding stock developed from this research has the potential to lead to future advancements and successes for soybean breeding programs in improving drought tolerance.

GmSG induces stay-green by regulating chlorophyll degradation in soybean

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The significant sign of senescence and maturity in plants is the degradation of chlorophyll. The phenomenon that leaves or plants remain green in the late stage of senescence is called "stay-green". Stay-green is divided into functional and cosmetic. Functional stay-green increases crop yield. Cosmetic stay-green is an ideal material for analyzing chlorophyll degradation. Stay-green has been studied thoroughly in other crops, but less in soybean. We identified GmSG that involved in stay-green of soybean, using linkage map and genome-wide association study. The seed coat and leaves of the over-expressed plants remained stay-green at maturity, and the net photosynthetic rate of the leaves was stronger. The ultrastructure showed that there were relatively complete thylakoids even at the late stage of senescence. Chlorophyll a degraded slowly in the senescent leaves, and chlorophyll b was not degraded basically. Functional studies showed that there was an interaction between GmSG and chlorophyll catabolic genes, which led to stay-green. Our data collectively shows that GmSG regulates the degradation of chlorophyll during senescence, and these results further contributes to clarify the close relationship between chlorophyll degradation and stay-green.

Keywords: Stay-green, chlorophyll degradation, senescence, soybean

QTL mining of high protein content from high-protein germplasm ZY1106

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Heilongjiang province, as an important soybean commodity base in our country, lacks the high protein soybean varieties. But protein content, which is susceptible to environmental conditions, is a quantitative trait controlled by multiple genes. Therefore, the exploration of high protein genes/QTLs is an important means to promote molecular-assisted selection and improve breeding efficiency. In this study, a backcross population consisting of 384 lines was derived from a cross of elite cultivar HH50 as recurrent parent and high protein accession ZY1106 as donor parent. Through multi-generation protein content identification and SSR analysis, the main QTL/gene was mined to provide a method for the development of high protein molecular markers and variety breeding. The protein content of BC1 progeny in multiple generations was detected by near infrared spectrometer, and the correlation of protein content among generations was analyzed by biostatistics. The BC1 genotypes were identified by polyacrylamide gel electrophoresis, and QTL was mined for protein content by complete interval mapping using QTL ICIMapping4.2. The protein content of multiple generations of BC1 population was significantly positively correlated, indicating that the protein content was heritable and stable. A total of three QTLs (qPC-5, qPC-6, qPC-8) related to protein content on different chromosomes were identified. qPC-8 was stable between generations and was a new QTL. Allelic analysis showed that the protein content of different genotypes in this region was significantly different. A new high-protein QTL was identified, and its closely linked molecular markers could be used to assist in the identification of protein content. The results of this study provided a basis for mapping and cloning of high protein main QTL in soybean, and provided technical support for marker-assisted selection in high protein soybean breeding in the northern alpine region of Heilongjiang Province.

Keywords: High-protein germplasm, near-infrared spectrometer, inclusive composite interval mapping, QTL mining

Gene editing technology creates new soybean germplasm of early maturity

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Soybean is a typical short-day plant, which originated in the middle and low latitudes. However, since modern times, the high-latitude long-day region has gradually become the main soybean production area in the northern hemisphere. Photoperiod will affect the flowering period of soybean, which seriously limits the planting range of high-yield soybean varieties. In order to improve the high-latitude adaptability of soybean, it is necessary to use the method of gene editing to adjust the flowering time of soybean. Previous studies have shown that the reduction of leaf photoperiod sensitivity caused by mutations in flowering inhibition genes such as E1-E4 is an important reason for the shortening of soybean growth period and the continuous shift of planting area to high latitude areas. This study uses CRISPR/Cas9 gene editing technology to simultaneously knock out E1, E2, E3 and E4 in transgenic high-oleic soybean. By detecting the expression of E1, E2, E3, E4 genes and their downstream related genes in the transformed plants, the growth period of different genotype plants was determined to obtain new soybean germplasm with early maturity and high oleic acid. About 60 T0 plants were obtained through agrobacterium-mediated genetic transformation technology. The results of field experiments showed that the loss of function of single E1 or E2 gene could significantly shorten the growth period of soybean. However, E3 or E4 gene mutations did not significantly promote plant flowering, which is consistent with the known conclusion that two phyA proteins have a certain degree of functional redundancy in light signal transduction.

Keywords: Soybean, flowering stage, gene editing

A small open reading frame encodes a small protein that inhibits soybean symbiotic nodulation

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Legumes acquire fixed nitrogen from the soil and through endosymbiotic association with rhizobia. Symbiotic nitrogen fixation (SNF) in legumes was once the primary source of N for agricultural systems, and is a clean and sustainable source of nitrogen. In this study, we identified a functional gene YDX1 induced by rhizobia infection. CRISPR-Cas9 technology was used to obtain gene knockout mutants to confirm the function of YDX1. Gmydx1 mutants initiated as reduced plant height, yellow leaves and an increase in the number of nodules but showing white nodules without nitrogenase activity. At the same time, a small open reading frame was found in the 5'UTR of YDX1 that encodes a small peptide that inhibits soybean symbiotic nodulation. However, the inhibitory effect of this small protein on symbiotic nodulation of soybean is not caused by regulation of YDX1. The specific working mechanism remains to be studied.

Keywords: YDX1, small peptide, nitrogen fixation, nodulation, soybean

Identification of symbiotic nitrogen fixation genes in soybean by genome-wide association

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The biological nitrogen fixation of legumes plays an important role in the nitrogen cycle and agricultural production, among which soybean is dominant. Therefore, discovering new nitrogen fixation genes and clarifying molecular mechanism will provide insights for the nitrogen fixation efficiency of soybean. To understand molecular mechanisms between rhizobia with soybean, we established a phenotypic database of nodule of soybean for GWAS. This database contains 582 soybean accessions including 325 landraces and 257 improved cultivars. We identified three candidate genes by genome-wide association for nodule number. Meanwhile, we screened 12 nitrogen-sensitive materials and 7 nitrogen-insensitive materials. These results improve our understanding of the genetic mechanisms of symbiotic nitrogen fixation.

Keywords: Nodule symbiosis, nitrogen fixation, GWAS, soybean

Assessing soybean seed vigor: Utilizing hyperspectral imaging technology for non-invasive identification and gene discovery

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The vigor of soybean seeds has always been a concern of people, and the high vigor seeds often have better quality. Therefore, it is very important to test the seed vigor. Through rapid and high precision measurement, the germination state of seeds can be known in advance, and the seeds that do not germinate can be screened out, so as to improve the yield. Traditional seed detection methods include tetrazole determination, red ink staining, etc. These methods are not only time-consuming but also easy to cause damage to seeds. As a new nondestructive testing technology, hyperspectral imaging has the advantages of complete spatial information and wide detection range. In addition, hyperspectral imaging technology can be used to analyze the vigor phenotype of soybean seeds and apply the data to GWAS. In this study, we hope to build a deep learning model for nondestructive identification of seeds by hyperspectral imaging technology, and at the same time to mine genes related to vigor traits through GWAS, so as to provide a new means for phenotypic analysis of soybean vitality and other key traits.

Keywords: Hyperspectral imaging technique, soybean seed, vigor identification, GWAS

Protein and tofu quality: introgressing gy4 null allele into lipoxygenase free early Swiss line (000)

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In the western human diet, soybean is consumed mainly in the form of soya 'milk' and tofu. The quantity and the quality of the tofu produced closely depend on the protein content of the bean. High protein varieties are therefore needed for processing. However, a strong negative relationship between protein content and field yield is regularly observed. As a result, food varieties are often less productive and attractive for farmers. Composition of the main globulins also plays a major role in tofu production. It has been observed that the absence of subunit 11SA4 (glycinin) has a significant effect on the overall protein composition of the seed and increase tofu quality. The typical taste of soybeans due to the presence of lipoxygenases in the seed is generally considered unfavourable in Europe. Varieties free of these three enzymes are also preferred. A backcross program with an Australian line (gy4 and lox free) was therefore launched at Changins in 2013 in order to introgress these traits into our early genetic material. Swiss recurrent parents without lipoxygenase (also lox free) and adapted to our climatic conditions were used to accumulate the different traits required for food processing. Twenty-four genetically similar lines were obtained after six backcrosses. All this material was then processed into tofu in our quality laboratory. The protein content of the mutated lines is on average 1% higher than the wild type. The mass of tofu produced is significantly increased by 7% on average with a lower firmness trend. The field performance of this material still needs to be evaluated. This new protein composition inserted in genetic material adapted to our climatic conditions constitutes an interesting first step in the creation of high-performance food varieties for the Swiss soybean industry.

Keywords: Protein, tofu, taste, glycinin, backcrossing

Acknowledgements: We thank Andrew James (CSIRO) for providing us the donor line (gy4 null and lox free).

Deciphering the genetic basis of resistance to soybean cyst nematode combining IBD and association mapping

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In soybean (*Glycine max*), soybean cyst nematode (SCN, *Heterodera glycines* Ichinohe) is one of the most devastating pathogens worldwide. In current study, the re-sequenced pedigree population of Zhongpin03-5373 (ZP) and a diversity of 478 soybean accessions were used to explore the genetic basis of resistance to SCN. The cultivar Zhongpin03-5373 (ZP), derived from SCN-resistant progenitor parents, Peking, PI 437654 and Huipizhi Heidou, is an elite line with high resistance to SCN race3. A pedigree variation map was generated for ZP and its ten progenitors using 3,025,264 high-quality SNPs identified from an average of 16.2× re-sequencing for each genome. Through identity by decent (IBD) tracking, we showed the dynamic change of genome and detected important IBD fragments including a large number of cloned genes controlling important agronomic traits, such as determinate habit (Dt1), seed oil content (GmSWEET39), seed weight (GmPP2C) and nodule number (GmNNL1) et al. Further, by comparing genetic variation among resistant and susceptible cultivars in the pedigree, a total of 2,353 IBD fragments related to SCN resistance including SCN resistant genes rhg1, rhg4 and NSFRAN07 were identified. Moreover, 23 genomic regions underlying resistance to SCN race3 were identified by genome-wide association study (GWAS) in 481 re-sequenced cultivated soybeans. Ten common loci were found by both IBD tracking and GWAS analysis. The promising candidate genes in common genomic regions of IBD and GWAS analysis were identified including stress responsive proteins, cell wall modification related protein, maltose excess protein, cytochrome P450s, E3 ubiquitin ligase, so on. Our results more thoroughly elucidated the dynamics of genomic fragments during ZP pedigree breeding and the genetic basis of SCN resistance, which will provide useful information for gene cloning and the development of resistant soybean cultivars using a marker-assisted selection approach.

Keywords: Soybean, whole genome re-sequencing, soybean cyst nematode (SCN), identity by decent (IBD), genome-wide association study (GWAS)

Constitutive expression of FAD3 improves salt tolerance in soybean

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As one of the most important economic crops in the world, soybean is the main source of vegetable oil and protein. However, the increasingly serious soil salinization inhibits the normal growth and development of most crops, and eventually leads to yield reduction, which poses a serious threat to the world's crop production. The endoplasmic reticulum-type ω-3 fatty acid desaturase encoded by FAD3 is a key enzyme in the synthesis of α-linolenic acid (ALA) in plants, and it can introduce 3rd double bond to α-linoleic acid (18:2Δ9, 12), generating α-linolenic acid (18:3Δ9, 12, 15). In this study, the FAD3 gene was isolated and constitutively expressed in soybean. The mutant of overexpressing FAD3 in soybean enhanced the salt tolerance of transgenic plants. Gas chromatography analysis showed that the ALA content was significantly increased, and it could enhance the PSII photochemical efficiency and the activity of antioxidant enzymes. These results suggest that constitutive expression of FAD3 in soybean is an important method to increase the salt tolerance for soybean.

Keywords: Soybean, ω-3 fatty acid desaturase, salt stress

Excellent soybean germplasm creation with high oleic acid and low lipoxygenase by using multi-gene edited

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Improving soybean quality has been the focus of researches. Soybean flavor induced by three lipoxygenases (LOXs, including LOX1, LOX2 and LOX3) has limited human consumption of soybean. In addition, soybean oil is rich in polyunsaturated fats, when hydrogenated, produce trans fatty acids, posing a health threat. Moreover, the oxidation stability of soybean oil with higher oleic acid content is enhanced, which opens the soybean application market. In soybean, GmFAD2-1 encodes fatty acid desaturase which regulates the conversion of oleic acid (C18:1) to linoleic acid (C18:2). Beany flavor induced by three lipoxygenases (LOXs, including LOX1, LOX2, and LOX3) restricts human consumption of soybean. In order to improve the edible quality of soybean oil and protein products, it is necessary to cultivate a new mutant line without lipoxygenase and high oleic acid content. GmFAD2-1 and GmLOXs were edited using CRISPR/Cas9. The main variety Z602 was transformed by agrobacterium-mediated method. Target site sequencing was conducted on the gene edited plants, and editing efficiency and genotype of five sites were analyzed. Lipoxygenase activity and isozyme electrophoresis were determined on the homozygous edited materials, and the contents of five main fatty acids were determined by gas chromatography. There were seven homozygous mutants with different editing types have been obtained. Compared with its wild type Z602, lipoxygenase activity of the mutants was significantly decreased, and oleic acid content was increased as high as more than 80%. The mutants with favoriate traits enrich soybean germplasm resources, and provide and quality material as soybean food.

Keywords: Gene editing, lipoxygenase, fatty acid desaturase, soybean quality

A major novel quantitative trait locus QTL02 in PI 90763 contributes to SCN resistance

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Soybean cyst nematode (SCN, *Heterodera glycine* Ichinohe) is the most damaging soybean pest worldwide. Management of this pest remains challenging because nematodes are adapting and reproducing on SCN resistant soybean cultivars that are derived from PI 88788. Growing soybean cultivars with a source of SCN resistance other than PI 88788, such as PI 90763, can increase yield as a result of better nematode control. Recently, research with rhg1-a (GmSNAP18), rhg2 (GmSNAP11), and Rhg4 (GmSHMT8) in PI 90763 revealed epistatic interactions responsible for broad resistance to multiple HG types, however these three genes alone did not explain differences in female index between PI 90763 and Peking to SCN HG type 1.2- (Race 2). The objectives of this research were to identify genomic regions associated with resistance to SCN HG type 1.2-. In a population developed from a bi-parental cross between PI 90763 and Peking, a novel and major QTL on chromosome 2 (QTL02) was identified. A detailed analysis of different gene/allele combinations revealed that the QTL02 reduces female index to three SCN populations in combination with rhg1-a and rhg2. This QTL02 was fine-mapped to a 218 Kbp region containing 34 genes and GmSNAP02, a paralogous gene of GmSNAP18 and GmSNAP11, was identified as the best candidate. Aligning raw whole genome resequencing reads to GmSNAP02 revealed a pattern of mismatched read ends in PI 90763, which was absent from Peking, suggesting an insertion in GmSNAP02 in PI 90763. The presence of this insertion was confirmed by PCR and a TaqMan assay. Current work is focused on functional characterization of the candidate gene GmSNAP02.

Genetic variability of Bowman-Birk inhibitor in soybean in relation to total trypsin inhibitor activity and elimination of Kunitz trypsin inhibitor

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Bowman-Birk inhibitor (BBI) is a soybean seed serine protease inhibitor whose nutraceutical properties far exceed its anti-nutritional property arising from its trypsin and chymotrypsin inhibitor activity. High BBI soybean genotypes are sought for the commercial preparation of this nutraceutical biomolecule while low BBI content in Kunitz trypsin inhibitor (KTI)-free genetic background is desirable in soymeal manufacturing. In the present investigation, monoclonal antibody assay of 95 soybean genotypes revealed 11-fold genetic variation for BBI concentration. The study led to the identification of 3 very high (>20 mg/g defatted soy flour) and 5 very low BBI (< 4 mg/g defatted soy flour) soybean genotypes. Contribution of BBI to total trypsin inhibition activity ranged from 2.2 to 53.5% with average value of 11.6%. Genotypes with varying level of BBI raised consecutively for two years exhibited non-significant ($p>0.05$) effect of growing year on the trait. BBI content in BC_3F_2 derived KTI-free lines was at par with the recurrent parent. Low and high BBI content genotypes with diverse genetic background identified in the study may be exploited to develop mapping population to identify genomic regions underlying BBI in soybean.

Keywords: Bowman-Birk inhibitor, genetic variability, Kunitz trypsin inhibitor, total trypsin inhibitor

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Physiological responses of Mexican soybean genotypes to water stress

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Drought is the most important abiotic threat to soybean production. Plants can modify their physiology to cope with drought. This work aimed to investigate the effect of water stress on the physiological characteristics of three Mexican soybean genotypes. One early (H02-2309) and two intermediate (H98-1240 and Huasteca 700) genotypes were evaluated in a randomized block design with at least three replicates under well-watered and water-stressed conditions. Water stress was applied at the R2 stage of the plants by reducing irrigation gradually for 17 days. After that, recovery irrigation was applied. Well-watered treatments were maintained under normal irrigation. Physiological traits related to gas exchange and wilting rate were measured at 0, 7, 13, and 17 days of water stress and eight days after recovery irrigation. Plants and soil water status, and the number of branches and pods, were also recorded. Results indicated a significant difference in the soil water content after seven days of water stress. All physiological measures decreased at 13 and 17 days under stress conditions, except water use efficiency. Early and intermediate genotypes showed different responses to severe water stress. The water potential, CO_2 assimilation, and wilting rate were drastically reduced in the H02-2309 genotype, while the reduction in stomatal conductance and transpiration was less, resulting in a low water use efficiency under water stress and a reduced number of pods after recovery irrigation. In contrast, intermediate genotypes almost abolished the gas exchange under the same conditions. But they maintained a higher water potential and wilting rate. Interestingly, Huasteca 700, with the highest values of water use efficiency, showed the highest number of branches and pods when irrigation was restored. This genotype could be considered tolerant to water stress since it had the best water use efficiency, and its agronomic performance was not drastically affected by stress conditions.

Keywords: Glycine max, gas exchange, drought stress

MiniMax: an arabidopsis-like model plant for soybean functional genomics

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The development of newly improved soybean germplasm is essential to meet the rising consumer demand for higher quality and more nutritious food, as well as to swiftly respond to the emerging threats of new pathogens. To achieve this goal, improvements to transformation and gene editing reagent technologies will enable researchers to utilize traits more effectively from wild and un-adapted germplasm. This presentation will demonstrate transformation of various soybean germplasm using embryonic axis (EA), developmental regulator (DR) and tissue-culture-free mobile editing (ME) protocols. Transformation parameters will be established using a diminutive soybean genotype, MiniMax. MiniMax was developed by the USDA more than a decade ago. It is a short-statured plant with a lifecycle comparable to *Arabidopsis thaliana*. With the genomic and physiological characterization of MiniMax, we discuss its potential to be a useful legume model plant for generating mutants to functionally validate candidate genes of interest.

Identification of loci associated with leaf gas exchange traits in soybean

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Leaf gas exchange is associated with tradeoffs between carbon assimilation and water loss. Phenotyping of populations for leaf gas exchange can provide insights about the natural diversity for individual traits as well as the relationships between CO_2 assimilation and water loss. Here we report on results from a bi-parental soybean mapping population and a soybean diversity panel which were grown in multiple field environments and phenotyped for numerous traits including net photosynthetic rate (An), ratio of internal and atmospheric CO_2 concentrations (Ci/Ca), stomatal conductance (gs), intrinsic water use efficiency (WUEi), and specific leaf area (SLA). Leaves were collected pre-dawn, transferred to a growth chamber and maintained in the dark until light adaptation and measurements. Significant genotypic variation was observed in both populations, and preliminary analyses revealed a total 13 loci in the bi-parental population and 23 loci in the diversity panel. Some QTL were associated with multiple traits and QTL × Environment interactions were observed for some traits. Several QTL coincided with QTL that were associated with carbon isotope discrimination, a surrogate measure of water use efficiency. These results and candidate genes identified in the vicinity of several loci will be discussed with respect to prospects to enhance soybean yield and yield stability, particularly under water limited environments.

Determination of isoflavones contents in soybean cotyledons, using near-infrared spectroscopy and chemometrics

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Owing to their estrogenic properties, isoflavones from soybean seeds are of great interest for human health. However, secondary effects are ambiguous and concerns among French consumers rise because of their unwanted exposure. In the seed, cotyledon and embryo axis have unconnectedly determined isoflavones contents and composition. Cotyledons are separately processed in food industry. To create adapted varieties, breeders need high throughput phenotyping methods focused on cotyledon. Near infrared spectroscopy is routinely used as a fast and non-destructive tool to predict protein and fatty acid contents in soybeans. Infrared spectroscopy can enable the quantification of isoflavone contents in cotyledons through the scanning of whole soybean seeds. Replicated (n=3) near-infrared spectra (reflectance mode; 800 - 2800 nm) were collected i) on whole seeds on 360 samples of varieties collected on multiple environments (locations x years) in France; ii) on grinded or ungrinded cotyledons for 150 out of these samples. The reference analyses were made with HPLC. Data analysis was performed through chemometrics: PCA and PLS regression models were developed on the mathematically preprocessed spectra (1st, 2nd, and 3rd Stavisky Golay derivative, Standard Normal Variate associated with detrend transformation, Multiplicative Scatter Correction and no preprocessing). For the PLS models, the validation was externally made on 20% left samples. Promising prediction results with PLS were obtained (R^2 between 0.7 and 0.8), especially without preprocessing or with SNV correction. The error of prediction (RMSEP) was insufficient for quantitative application, but it allowed efficient qualitative screening for the extreme isoflavone contents. The spectra obtained from cotyledons didn't provide better predictions than those determined on whole seeds. Our model would be improved by using a larger number of samples exhibiting a wider variation in isoflavones contents. This would allow an optimal use of ANN algorithms to get more powerful models.

Explaining environmental influence on isoflavone accumulation in soybean cotyledon and embryo axis

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Background: Isoflavones accumulated in soybeans seeds are nutraceutical thanks to their estrogenic effect. Controlling these contents is a great stake for breeders, as for increasing content for pharmaceutical market than reducing for food industries. Genetic determinism is widely described, with a clear independent regulation between cotyledon and embryo axis. Nevertheless, the environment such as precipitations or low temperature during seed development highly influences isoflavone accumulation. Understanding and quantifying the environmental influence on isoflavones from each of these seed compartment is mandatory to draw standardized varietal evaluation despite maturity group diversity and climatic years fluctuation.

Hypothesis: Water input and minimal temperature at key development stages can explain most of the environmental impact on isoflavone content variability in cotyledons and embryo axis.

Methods: Cotyledon and embryo axis isoflavones were analyzed for 4 genotypes (2 early and 2 late maturity) cultivated in randomized complete block design with 2 sowing dates, 2 water regimes and 4 blocks. This trial was repeated two years at INRAE Auzeville (France) in 2017 and 2018. Agroclimatic, phenological data and genotype were used to explain isoflavones variability in each seed compartment.

Results: Significant variations of isoflavone content in cotyledons were found between genotypes. Irrigation increased cotyledon content in 2017 but not in 2018. Early sowing significatively impact cotyledons and embryo axis isoflavone content in an opposite way between early and late genotypes and with different intensity between 2017 and 2018. Agroclimatic parameters, especially between the R5 stage and harvest, explained the majority of the environmental variations in cotyledon and embryo axis isoflavone content.

Conclusion: Environmental influence can be efficiently modelized with a few agroclimatic indicators during seeds development and maturity for isoflavone content in the seed fractions, making possible genetic estimations among trials from diverse cultural contexts.

Effects of increasing seed per pod on yield and yield components of field grown soybean sibling lines

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Improvements in crop yield is the main goal of breeding programs. Yield is a multi-factorial trait mainly determined by the number of seeds per unit land area (SN) and seed weight (SW). Variations in soybean yield are often associated with changes in SN and pod number per unit land area (PN). This assumption is currently based on experiments performed with genotypes with little or null variability in the potential seed per pod (PSPP). So, the effect of increasing PSPP on yield is still largely unexplored. Field experiments were conducted at FCA, UNR (Zavalla, Santa Fe, Argentina) during 2019/20 and 2020/21 growing seasons in order to evaluated the effect of increasing PSPP on yield and yield components using sibling lines with variability in PSPP and SW. Twenty-nine genotypes were grown in six environments in randomized block design with three replications. Yield, SN, SW, PN, PSPP, seed abortion (SA) and developed SPP (DSPP) were determined at maturity. Data was analyzed using linear mixed-effect models, partial least square regression (PLSR) and dominance analysis. The increase in PSPP produced a direct increase in SN, independently of PN variations. Yield in high or low yielding genotypes was the result of different yield components combination. Higher yields (>366 g.m-2) were observed in genotypes with high SN (>2392 no.m-2), mainly because of high PN (>1166 no.m-2) or high PSPP (>2.9 no.pod-1). Conversely, lower yields (<333 g.m-2) were observed in genotypes with higher SW (>177 mg. seed-1). These results agreed with the dominance and PLSR analysis, which showed that PN and PSPP had the largest contribution to yield variation (64% and 17%, respectively). Thus, increasing PSPP have a positive effect in SN and yield and provide evidence for unexploited opportunities for enhancing soybean yield through the improvement of PSPP.

Keywords: Soybean, breeding, seed per pod, yield

Phenomic selection in soybean breeding

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Phenomic selection is a promising complement and alternative to genomic selection for improving breeding efficiency. The major advantage of using near-infrared spectroscopy (NIRS) to indirectly capture phenotypic variants and predict complex traits is its high-throughput and low cost. Using NIR spectra to predict individual performances in the context of breeding for yield remains relatively novel. Certain wavelengths of NIR light are absorbed by specific chemical bonds that constitute the components of tissues. The relative proportion of each of these bonds within the tissue quantitatively influence the nature of the absorbance or reflection of light at different wavenumbers. Phenomic selection was tested on 206 soybean genotypes, collecting yield and NIRS data. Spectra were obtained from different tissues, grains and dried, milled leaves, measuring absorbance in range 4000 – 10000 cm^{-1}. RR-BLUP model was used for phenomic predictions, considering NIRS data instead of molecular. Differences between collected plant and seed NIR spectra were observed, causing variation in prediction ability of RR-BLUP models, ranging between 0,6 and 0,7 that was at the level of previously determined genomic prediction. For selection of optimal phenomic prediction model, it was important to elucidate contribution of chemical bonds i.e. macromolecules to the model's prediction power, in order to avoid that the model itself and not selection for yield, affects other traits. In the seed, spectra with the highest prediction values were mostly located at the positions of protein and oil peaks. Therefore, developed prediction model basically predicts protein and oil content in seeds. Due to existing genetic correlations between protein and oil content with the yield, by employing this model one may take the risk of selecting genotypes based on altered chemical composition rather than yield. In terms of specific breeding goals, it is necessary to focus carefully on selection of optimal phenomic prediction model.

Keywords: NIR spectroscopy, phenomic selection, yield, macromolecules

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Determining genetic mechanisms of soybean maturity in North Dakota: expanding the molecular model for early maturity groups 00 and 0.

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Production areas of soybean (*Glycine max* (L.) Merr.) have grown in North Dakota to make it the number one crop in the state, however, state yield averages are among the lowest in the Midwest. Maturity is one of the most important agronomic traits impacting yield potential. North Dakota is characterized by having short season length due to frost risk and is necessary to have early-maturing soybean cultivars. Predominate maturity groups grown in North Dakota are MG 00 and 0. It is possible to "fine tune" maturity to an environment to maximize yield. The major genetic mechanisms of soybean maturity are well characterized. Genes E1, E2, and E3 have the largest effect on soybean maturity, where functional allele of these genes condition for late maturity and the null or semi-functional alleles for early maturity. It has been determined that variations of the non-functional or semi-functional alleles of these three genes create the MG 00 or 0 phenotype. However, it is not understood which combination of alleles is most favored for breeding purposes which can potentially affect yield. In addition, since these major genes are not fully functional it can be hypothesized that there are other genes that play major effect in this environment, which may be minor in other maturity groups with functional E genes. The goal of this research is to determine maturity alleles present in North Dakota. This will be accomplished by exploring known maturity gene alleles in the North Dakota State University soybean breeding program to determine which alleles are found. In addition, these alleles will be determined over time in historical cultivars to determine if allelic frequency changed over time. These results will enrich knowledge of maturity molecular model necessary to create an MG 00 and 0 cultivar and could identify new genetic mechanisms for yield gains.

Exploring multi-omics data for soybean research in the post-genomic era

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The past decade of -omics research has generated a massive amount of biological data, providing an unprecedented opportunity for soybean biological discovery and improvement. We consolidated and analyzed whole-genome sequencing data of over 5,000 soybean accessions, 8,000 soybean transcriptome sequences from a wide range of biological treatments, and millions of other genotypic and phenotypic data points, which are available in the public domain or generated by our laboratory. We have developed or adapted a range of data-mining strategies and pipelines to exploring the data for a variety of soybean studies, such as evaluating soybean germplasm, discovering causative genes and alleles of trait QTLs, and in-silico genotyping. In the presentation, we will describe the data-driven technology platform as well as several proof-of-concept studies that demonstrate its versatile uses in both basic and applied soybean research. Challenges and opportunities of developing and using the data-driven technology platform will also be discussed.

Molecular basis of seed protein and oil in soybean domestication and improvement

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The current demand for soybean is largely driven by its highly valuable seed protein and oil, which are used for human consumption, animal feed, and other industrial purposes. Protein and oil content are closely correlated with each other, as well as with various other important seed quality and yield traits in natural populations. Furthermore, they are associated with soybean domestication and improvement. This presentation will discuss our recently research results on the causal genes and alleles underlying two high-effect seed protein and oil QTLs (SWEET39 on Chr15 and POWR1 on Chr20), their roles in soybean domestication and improvement, and the molecular basis underlying the intricate correlation of seed protein, oil, and other traits (Zhang et al., PloS Genetics, 2020; Goettel et al., Nature Communications, 2022).

Genomic analysis and prediction within the Northern Uniform Soybean Tests

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The Uniform Soybean Tests - Northern Region (NUST) is a regional variety testing network coordinated by the USDA to evaluate experimental soybean strains developed by public institutions in the United States and Canada for their potential release as new varieties. Historical data from the NUST comprises a valuable multi-environment trial (MET) dataset including relevant elite soybean germplasm from maturity groups 00 to IV evaluated over 28 years in 199 locations, totaling 1,652 environments. Our aim was to characterize the genetic structure of the NUST experimental strains, perform genome-wide association studies using historical phenotypic data, and assess the usefulness of the historical data for creating genomic prediction training sets. SNP data was collected on 2,544 unique NUST experimental strains using the BARCSoySNP6K assay. High fixation index (FST) values between populations of early and late maturity groups indicated genetic differentiation between these populations. High FST values were observed in a region on chromosome 10 nearby the known soybean maturity gene, E2. Population relatedness and patterns of gene flow between breeding populations were determined using a TreeMix analysis on the marker data. The germplasm sharing among the public programs to maximize genetic diversity and provide well-adapted and stable genotypes was confirmed by the lack of clear clustering in the PCA, grouping of all public breeding populations in a single branch in the tree of relatedness Association studies on important agronomic traits identified significant QTL that overlap with known QTL previously reported in the literature. Moderate to high predictive abilities for most traits indicated that genomic prediction models based on data from the NUST historical data set could be used by breeders to improve their early-stage line selections. Findings from this study demonstrate the usefulness of this historical MET for future breeding efforts in trait mapping and training of genomic selection models.

Taming the wild soybean: discovery, validation and utilization of a non-pleiotropic seed protein QTL

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The well documented negative correlation and pleiotropic effect of major soybean seed protein genes with seed oil and grain yield has hindered breeders' ability to increase protein in elite cultivars. Therefore, the discovery and utilization of novel genes responsible for increasing protein without adversely affecting oil and yield is of major interest. A residual heterozygote derived near isogenic line (RHD-NIL) population developed from a cross between a maturity group (MG) V *G. max* line ('Osage') and a *G. soja* accession (PI 593983) was used to fine-map a protein-associated region on chromosome (Chr) 14 from ~16.5 to ~4.6 Mbp. The *G. soja* allele in this region significantly increased protein 0.65-0.72%, without significantly decreasing oil. Comparative genomics with the RHD-NIL WGRS data set narrowed the candidate gene list from 223 to 13 genes, and we developed an $F_{3:4}$ confirmation mapping population and a $BC_3F_{2:3}$ population for validation in an elite MG III background. For confirmation QTL mapping, lines were genotyped with 3K SNPs, hill plots were grown in one location with two replications, and near infrared spectroscopy (NIR) was used on ground seed samples to predict seed protein and oil content. Mapping results confirmed the *G. soja* allele at the peak Chr 14 QTL increased protein by ~0.63%, without significantly decreasing oil. Concurrently, we developed a KASP marker and an introgression $BC_3F_{2:3}$ population by selecting BC_3F_2 plants homozygous for the *G. max* or *G. soja* allele. Single plant rows were grown in 2022 in Columbia, MO and NIR was used to predict protein and oil. A mean separation of lines in both allelic classes revealed that the *G. soja* allele significantly increased protein ~0.79%, without significantly decreasing oil. Our results provide compelling confirmation and validation evidence for a non-pleiotropic protein QTL, which can be utilized in breeding programs to enhance protein content.

Keywords: Glycine soja, protein, oil, introgression

Identification of genetic loci conferring seed coat color based on a high-density map in soybean

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Seed coat color is a typical evolutionary trait. Identification of the genetic loci that control seed coat color during the domestication of wild soybean could clarify the genetic variations between cultivated and wild soybean. We used 276 F_{10} recombinant inbred lines (RILs) from the cross between a cultivated soybean (JY47) and a wild soybean (ZYD00321) as the materials to identify the quantitative trait loci (QTLs) for seed coat color. We constructed a high-density genetic map using re-sequencing technology. The average distance between adjacent markers was 0.31 cM on this map, comprising 9,083 bin markers. We identified two stable QTLs (qSC08 and qSC11) for seed coat color using this map, which, respectively, explained 21.933 and 26.934% of the phenotypic variation. Two candidate genes (CHS3C and CHS4A) in qSC08 were identified according to the parental re-sequencing data and gene function annotations. Five genes (LOC100786658, LOC100801691, LOC100806824, LOC100795475, and LOC100787559) were predicted in the novel QTL qSC11, which, according to gene function annotations, might control seed coat color. This result could facilitate the identification of beneficial genes from wild soybean and provide useful information to clarify the genetic variations for seed coat color in cultivated and wild soybean.

Research progress in soybean recurrent selection breeding based on male sterile gene ms1

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Narrow genetic basis is an important limiting factor for soybean variety improvement at present. The discovery of male sterility controlled by the recessive genic male sterile gene (ms) in soybean solved the problems of the difficulty in emasculation and pollination, and provided an effective way for the population improvement. Recurrent selection, is a breeding system that increases the required frequency of elite genes through cyclic selection, hybridization, re-selection, and re-hybridization. It omits the process of artificial emasculation and pollination of soybean, and changes the closed hybridization into open hybridization. The large number of donor parents overcomes the genetic simplicity and narrowness of the breeding base population, improves the heterogeneity of the base population, continuously gathers the excellent genes in the population, maintains the genetic diversity of the population and enriches the genetic base of breeding varieties. In the early 1990s, the Institute of Cereal and Oil Crops, of the HAAFS introduced the ms1 male sterile basic material from the National Soybean Improvement Center of Nanjing Agricultural University, and constructed the ms1 male sterile basic population containing 151 varieties from different ecological regions and micro-core germplasm material in China, and established the recurrent selection basic population suitable for the ecological conditions of the Huang-Huai-Hai region. Three improved subpopulations of high yield, high protein and oil content as well as different types of special selection groups for specific target traits were further selected and established. The recurrent population selection technology was innovated, and six varieties with high yield, high quality and wide adaptability (JD19, JD20, JD21, JD22, JD24 and JD1258) have been bred. A batch of elite germplasm with high protein/oil content and disease resistance has been cultivated, resulting in significant improvements in soybean breeding.

Changes in the m6A RNA methylome accompany the promotion of soybean root growth by rhizobia under cadmium stress

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Cadmium is the most widely distributed heavy metal pollutant in soil and has significant negative effects on crop yields and human health. Rhizobia can enhance soybean growth in the presence of heavy metals, and the legume-rhizobia symbiosis has been used to promote heavy-metal phytoremediation, but much remains to be learned about the molecular networks that underlie these effects. We demonstrated that soybean root growth was strongly suppressed after seven days of Cd exposure but that the presence of rhizobia largely eliminated this effect, even prior to nodule development. Moreover, rhizobia did not appear to promote root growth by limiting plant Cd uptake: seedlings with and without rhizobia had similar root Cd concentrations. We performed transcriptome-wide m6A methylation profiling to investigate changes in the soybean RNA methylome in response to Cd with and without rhizobia. Here, we provide some of the first data on transcriptome-wide m6a RNA methylation patterns in soybean; m6A modifications were concentrated at the 3'UTR of transcripts and showed a positive relationship with transcript abundance. Transcriptome-wide m6A RNA methylation peaks increased in the presence of Cd, and the integration of m6A methylome and transcriptome results enabled us to identify 154 genes whose transcripts were both differentially methylated and differentially expressed in response to Cd stress. These genes were associated with Ca2+homeostasis, ROS pathways, polyamine metabolism, MAPK signaling, hormones, and biotic stress responses. There were 176 differentially methylated and expressed transcripts under Cd stress in the presence of rhizobia. In contrast to the Cd-only gene set, they were also enriched in genes related to auxin, jasmonic acid, and brassinosteroids, as well as abiotic stress tolerance. They contained fewer genes related to Ca2+ homeostasis and also included candidates with known functions in the legume–rhizobia symbiosis. These findings offer new insights into how rhizobia promote soybean root growth under Cd stress.

(The article has been published.)

Morphological diversity of soybean in Maize Research Institute collection

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Maize Research Institute Zemun Polje soybean collection is a small, local collection consisted of approximately 500 accessions from different regions of the world. Until now, there has been no systematic and precise evaluation of samples in the collection, as well as the assessment of genetic diversity. Genotype's information is mainly limited to passport data, a description of several morphological traits and yield data. The aim of this study was to evaluate the level of phenotypic variation in collection on the sample of 90 accessions of different geographical origin. Morphological description was performed by screening of 16 traits according to the UPOV technical guideline for conducting of DUS tests in soybean. Diversity of morphological traits was estimated by Shannon diversity index. Morphological similarity of genotypes was estimated by the application of multivariate statistics models (analysis of homogeneity - HOMALS and cluster analysis). HOMALS analysis revealed the descriptors of high discriminatory power, with the greatest importance for genotype's distinction: colour of the seed coat, intensity of pod colour, pubescence colour, and to a lesser extent the growth type. Genotypes were divided into six homogeneous and moderately separated groups with similar morphological profiles. Geographical component of morphological variability, as well as pedigree-based grouping could not be observed in pattern obtained by HOMALS. Cluster analysis showed a slightly different grouping model compared to the HOMALS analysis. All genotypes were grouped according to the colour of flower into two main clusters, and further into subgroups according to the type of growth and pubescence colour. Genotype's positioning in subclusters did not correspond to their geographical origin. Consistency of clustering according to pedigree could be observed for individual pairs of genotypes or a small number of groups within subclusters. Despite the satisfactory level of morphological diversity of the collection revealed in the study, it is necessary to enrich the collection with new sources of variation to ensure continued success in breeding.

Keywords: Soybean, diversity, morphological descriptors

Genome-wide characterization and expression in analysis of PRR gene family in *Glycine max* and *Glycine soja*

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The genes in the PRR family are key components of the transcription-translation circadian network in plants, and comprise the core genes in the central oscillator translation feedback loop of *Arabidopsis* sp. They play important roles in several physiological processes and environmental adaptation. In this study, we identified a total 12 PRR genes of *G. max* (GmPRRs) and 14 PRR genes of *G. soja* (GsPRRs) and categorized into three groups. All 14 GsPRR genes of *G. soja* were distributed on chromosomes 3-7, 10-13, 16, 17, 19, while two less GmPRR genes of *G. max* than *G. soja* were absent on chromosomes 11 and 12 which were identified key circadian clock genes of soybean. The results of collinearity analysis demonstrated that of the genes in the PRR family, 20 and 24 pairs of homologous genes in *G. max* and *G. soja*, respectively, had been produced by fragment repetition. The findings revealed that the expansion of the PRR gene family in soybean is primarily attributed to fragment duplication. Moreover, the analysis of the expression profiles of GmPRRs and GsPRRs gene pairs with evolutionary relationships suggested that the GmPRRs and GsPRRs gene family may have changed at the transcriptional regulation level during polyploidization. The genome-wide identification and comprehensive gene expression profiling of homeobox gene family members in *G. max* and *G. soja* will provide opportunities for functional analysis to unravel their exact role in plant growth and development.

Phenotyping root system: dynamics of below ground architecture for productivity of soybean under low soil moisture

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Soybean is a high nutritional value leguminous crop with high oil (20%) and protein (40%) contents rich in health promoting secondary metabolites, such as, isoflavones, phyto-sterols, lecithins and saponins. Climate change scenario affecting plant growth is crucial to soybean crop. Low soil-moisture stress of 20-25 days particularly during seed fill stage depending on the intensity of drought reduce productivity of the crop from 40 to 80%, even with normal rainfall pattern throughout the cropping season. Root architecture is an important component of plant architecture which governs the water and nutrient uptake efficiency and thereby ensures plant growth and seed quality. In two MAGIC populations derived panel of 23 advance lines and tolerant NRC 136, EC 538828 and sensitive NRC 37 and JS 20-34 checks evaluated in rainout-shelter controlled soil-moisture stress (9.8%) condition, for yield associated drought tolerance trait – drought resistance index (DRI), and in PVC tubes for root traits at reproductive-stage. Tolerant check NRC 136 responded well (DRI=1.04) to the rainout-shelter condition. For lines viz. A(M/L)-II-92, A(E)-30, B(E)-21 and A(M/L)-II-16 were found high yielder under drought stress condition. The dynamics of root development in these lines involved lateral roots with narrow angle to soil surface, followed by penetrating traits with high number of tips, forks and crossings ensuring deeper roots and finally, water extracting traits in deeper root zone with finer roots having more surface area. It is proposed that auxin plays a crucial role during its synthesis in lateral root primordium and its translocation to fruiting bodies where it helps in continuous development of ovule during plant water stress, besides proper root development. The study connects developing deeper root system with promoting carbon sequestration in soil layers below 1 meter, a tool to combat changing climate scenario.

Multi-omics techniques for soybean molecular breeding

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Soybean is a major crop providing essential protein and oil for humans and animals. Recently, rapid progress in plant omics is ushering in a new era of precision design breeding. It facilitated advances in soybean transcriptomics, proteomics, metabolomics, and phenomics, all of which paved the way for an integrated approach to molecular breeding in soybean. Here, we summarize the latest progress in omics research, highlight novel findings made possible by omics techniques, note current drawbacks and areas for further research. We propose a conceptual workflow for the use of multiple omics datasets to identify key factors that regulate soybean yield, seed quality, stress biology, and other factors. Multiple omics approaches show promise for the efficient improvement of soybean breeding in future research. The genome is the basic foundation of soybean germplasm, whereas the transcriptome, proteome, metabolome, and phenome are the upper layers. In soybean breeding practices, genomics, transcriptomics, proteomics, metabolomics, and high-throughput phenotyping will need to be better integrated to construct the regulatory networks of complex traits and efficiently identify hub genes. Genome editing of specific hub genes will also help with their functional validation. After marker development for the hub gene(s), important or specific hub genes can be integrated to design a new soybean variety. Further development of integrated multi-omics resources can promote the efficient and accurate discovery of excellent alleles, providing more possibilities for soybean breeding. This review will be of interest not only to soybean breeders but also to researchers interested in the use of cutting-edge omics technologies for crop research and improvement.

Keywords: Soybean, multi-omics, molecular breeding

GmMLRK1 encoding a malectin-like receptor kinase provides resistance to soybean mosaic virus in soybean

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Soybean mosaic virus (SMV) severely damages soybean [*Glycine max* (L.) Merr.] yield and seed quality. Moreover, the underlying genetic determinants of resistance to SMV remain largely unknown. Here, we performed a genome-wide association study (GWAS) of SMV resistance in a 219 panel across four environments and identified a new resistance-related gene, GmMLRK1, at the major resistance locus Rsv4 on chromosome 2. GmMLRK1 encodes a malectin-like receptor kinase (RK) that was induced earlier and to a greater degree in leaves of the SMV-resistant cultivar Kefeng No. 1 than in those of the susceptible cultivar Nannong 1138-2 after inoculation. We demonstrated that soybean plants overexpressing GmMLRK1 show broad-spectrum resistance to both strains SC7 and SC3 on the basis of the reduced viral accumulation and increased reactive oxygen species (ROS) production and local cell death associated with the hypersensitive response. In contrast, GmMLRK1 knockout (KO) mutants were more susceptible to both pathotypes. Haplotype analysis revealed the presence of five haplotypes (H1-H5) within the soybean population, and only H1 provided SMV resistance, which was independent of its tightly linked SMV resistance gene RNase-H at the same locus. These results highlight a novel gene that helps improve the understanding of SMV resistance and could be used for breeding resistant soybean accession.

Keywords: Gemome-wide association study, GmMLRK1, malectin-like receptor kinase, soybean, soybean mosaic virus

Transcription factors GmERF1 and GmWRKY6 synergistically regulate low phosphorus tolerance in soybean

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Soybean (*Glycine max*) is a major grain and oil crop worldwide, but low phosphorus (LP) in soil severely limits the development of soybean production. Dissecting the regulatory mechanism of the P response is crucial for improving the P use efficiency of soybean. Here, we identified a transcription factor, GmERF1 (ethylene response factor 1), that is mainly expressed in soybean root and localized in the nucleus. Its expression is induced by LP stress and differs substantially in extreme genotypes. The genomic sequences of 559 soybean accessions suggested that the allelic variation of GmERF1 has undergone artificial selection, and its haplotype is significantly related to LP tolerance. GmERF1 knockout or RNA interference resulted in significant increases in root and P uptake efficiency traits, while the overexpression of GmERF1 produced an LP-sensitive phenotype and affected the expression of six LP stress-related genes. In addition, GmERF1 directly interacted with GmWRKY6 to inhibit transcription of GmPT5 (phosphate transporter 5), GmPT7 and GmPT8, which affects plant P uptake and use efficiency under LP stress. Taken together, our results show that GmERF1 can affect root development by regulating hormone levels, thus promoting P absorption in soybean, and provide a better understanding of the role of GmERF1 in soybean P signal transduction. The favorable haplotypes from wild soybean will be conducive to the molecular breeding of high P use efficiency in soybean.

Keywords: Low phosphorus tolerance, GmERF1, GmWRKY6, GmPTs, root development

GmEIL4 enhances soybean (*Glycine max*) phosphorus efficiency by improving root system development

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Phosphorus (P) deficiency seriously affects plant growth and development and ultimately limits the quality and yield of crops. Here, a new P efficiency-related major quantitative trait locus gene, GmEIL4 (encoding an ethylene-insensitive 3-like 1 protein), was cloned at qP2, which was identified by linkage analysis and genome-wide association study across four environments. Overexpressing GmEIL4 significantly improved the P uptake efficiency by increasing the number, length and surface area of lateral roots of hairy roots in transgenic soybeans, while interfering with GmEIL4 resulted in poor root phenotypic characteristics compared with the control plants under low P conditions. Interestingly, we found that GmEIL4 interacted with EIN3-binding F box protein 1 (GmEBF1), which may regulate the root response to low P stress. We conclude that the expression of GmEIL4 was induced by low-P stress and that overexpressing GmEIL4 improved P accumulation by regulating root elongation and architecture. Analysis of allele variation of GmEIL4 in 894 soybean accessions suggested that GmEIL4 is undergoing artificial selection during soybean evolution, which will benefit soybean production. Together, this study further elucidates how plants respond to low P stress by modifying root structure and provides insight into the great potential of GmEIL4 in crop P-efficient breeding.

Keywords: GmEIL4, GWAS, linkage mapping, low-phosphorus

Soybean natural tolerance to off-target dicamba

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Since the commercialization and widespread adoption of dicamba-tolerant (DT) soybean cultivars across the United States, numerous cases of off-target damage to non-DT soybean have been reported. Soybean is sensitive to dicamba, a synthetic auxin herbicide. Previous studies have focused on understanding the impact of growth stage, dosage, frequency, and duration of dicamba exposure on the severity of symptomology and yield loss. To date, limited research has investigated the effect of genetic components on the observed responses. Therefore, this study was conducted to estimate yield losses caused by prolonged off-target dicamba exposure across diverse genotypes and identify genomic regions associated with the differential response to off-target dicamba. A total of 553 soybean genotypes derived from 239 unique bi-parental populations were evaluated in nine environments over three years. A yield penalty of 8.8% was observed for every increment in damage score on a 1-4 scale with losses as high as 40%. A total of 382 genetically diverse accessions were used to identify genomic regions associated with soybean response to off-target dicamba exposure. Two models were implemented to detect significant marker-trait associations: the Bayesian-information and Linkage-disequilibrium Iteratively Nested Keyway (BLINK) and a model that allows the inclusion of population structure in interaction with the environment (G×E) to account for variable patterns of genotype responses in different environments. Both models identified significant associations in regions of chromosomes 10 and 19. The significant SNPs are located within candidate genes possessing annotated functions involving different phases of herbicide detoxification in plants. These results entertain the possibility of developing non-GM soybean cultivars with improved tolerance to off-target dicamba exposure. Identifying genetic sources of tolerance and genomic regions conferring higher tolerance to off-target dicamba may sustain and improve the production of other non-DT herbicide soybean production systems, including the growing niche markets of organic and conventional soybean.

Keywords: Breeding, dicamba, herbicide tolerance, genetics, genome-wide association studies

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Membrane proteomic profiling of soybean leaf and root tissues uncovers salt-stress-responsive membrane proteins

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Cultivated soybean (*Glycine max* L.), the world’s most important legume crop, has high-to-moderate salt sensitivity. Being the frontier for sensing and controlling solute transport, membrane proteins could be involved in cell signaling, osmoregulation, and stress-sensing mechanisms, but their roles in abiotic stresses are still largely unknown. By analyzing salt-induced membrane proteomic changes in the roots and leaves of salt-sensitive soybean cultivar (C08) seedlings germinated under NaCl, we detected 972 membrane proteins, with those present in both leaves and roots annotated as receptor kinases, calcium-sensing proteins, abscisic acid receptors, cation and anion channel proteins, proton pumps, amide and peptide transporters, and vesicle transport-related proteins etc. Endocytosis, linoleic acid metabolism, and fatty acid biosynthesis pathway-related proteins were enriched in roots whereas phagosome, spliceosome and soluble NSF attachment protein receptor (SNARE) interaction-related proteins were enriched in leaves. Using label-free quantitation, 129 differentially expressed membrane proteins were found in both tissues upon NaCl treatment. Additionally, the 140 NaCl-induced proteins identified in roots and 57 in leaves are vesicle-, mitochondrial-, and chloroplast-associated membrane proteins and those with functions related to ion transport, protein transport, ATP hydrolysis, protein folding, and receptor kinases, etc. Our proteomic results were verified against corresponding gene expression patterns from published C08 RNA-seq data, demonstrating the importance of solute transport and sensing in salt stress responses.

Development of the 1K SNP panel for genetics and breeding applications for the Brazilian soybean germplasm

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Brazil leads world soybean production, accounting for ~37% of it. Latest tools in molecular breeding have been applied to improve soybean yield, stability and other agronomic traits using genome-wide marker information. Successful medium and high-resolution SNP arrays for soybean have been developed to be highly informative across diverse germplasm, however they have not been evaluated for the small diversity pool of Brazilian genotypes. We propose a 1k SNP set highly informative to the Brazilian soybean genetic pool. We intend this to be a cheap, quick and accurate genotyping tool that can facilitate molecular breeding. 370 Brazilian cultivars, including historical and modern cultivars, were genotyped with SoySNP50K BeadChip. After filtering SNP data and identifying LD blocks, we selected around 1k SNPs based on MAF distribution and coincidence with BARCSoySNP6K. UPGMA dendrograms of the 1k set were compared with the whole set by Baker's Gamma association index and distance matrices were evaluated by Mantel's test. Finally, we tested the 1k set with genome-wide association mapping (GWAS) of traits previously mapped, such as the resistance to *Meloidogyne javanica*, presence of the EPSPS gene (tolerant to herbicide glyphosate) and stem termination type (STT). A total of 1,087 SNPs with an average MAF of 0.35, distributed in 1,753 haplotype blocks, were selected. The SNP set presents 33% of coincidence with the BARCSoySNP6K. The similarity between the two dendrograms was 0.81 and the correlation coefficient of the two distance matrices among all accessions was significant (0.89 at $P<0.01$). GWAS analysis identified a significantly association in the chromosome 2, 13 and 19 for the presence of EPSPS gene, and for the resistance to M. javanica and STT, respectively, as expected. This 1k SNP set is suited to explore genetic polymorphism throughout the soybean genome of the Brazilian genetic pool, providing a useful tool for molecular breeding applications.

Genome-wide scan for oil quality reveals a coregulation mechanism of tocopherols and fatty acids in soybean seeds

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Tocopherols (vitamin E) play essential roles in human health due to their antioxidant activity, and plant-derived oils are the richest sources of tocopherols in the human diet. Although soybean (*Glycine max*) is one of the main sources of plant-derived oil and tocopherol in the world, the relationship between tocopherol and oil in soybean seeds remains unclear. Here we focus on dissecting tocopherol metabolism, with the long-term goal of increasing α-tocopherol content and soybean oil quality. We first collected the tocopherol and fatty acid profiles in the soybean population (>800 soybean accessions) and found that tocopherol content increased during soybean domestication. Meanwhile, a strong positive correlation between tocopherol and oil content was detected. Five tocopherol pathway-related loci were identified by using the metabolite genome-wide association study (mGWAS) strategy. Genetic variations of three tocopherol pathway genes are responsible for total-tocopherol and composition in soybean population, at either enzymatic activity level, mainly caused by nonconserved amino acid replacement or gene transcriptional level. Moreover, the fatty acid regulatory transcription factor GmZF351 directly activated tocopherol pathway gene expression and then increased both fatty acids and tocopherol in soybean seeds. Our study not only elucidates the functional differentiation of tocopherol pathway genes in soybean populations but also provides a feasible framework for developing new soybean varieties with high content of α-T and oil quality in seeds.

Seed protein genetics linked with nitrogen and phosphorus translocation efficiency in soybean

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Soybean is a vital nutritional crop with high seed protein content, necessitating adequate nitrogen (N) and phosphorus (P) supplies for optimal protein concentrations. Despite extensive research on seed quality traits, limited information exists on genetic links between seed quality and nutrient supplies in soybean. This study generated a recombinant inbred line (RIL) population of 179 progeny using HC6 and JD17 as parents, which exhibit contrasting seed quality and N and P translocation efficiencies. Seed protein content and N and P translocation efficiencies were higher in HC6 than in JD17. Positive correlations were observed between seed protein content and translocation efficiency of N and P in RILs, indicating that high N and P translocation efficiencies may contribute to protein accumulation. A genetic map was constructed using 5,250 SNP markers, covering 3,154.83 cM. Six loci for quality and 13 loci for N and P translocation efficiency were identified. Two segments on chromosomes 6 and 20 contained significant markers for both quality and N and P translocation efficiencies, with LOD values ranging from 2.98 to 11.91 and PVE values ranging from 8.2% to 28.0%. A significant locus on chromosome 20 resulted from a transposable element (TE) InDel in Glyma.20G085100. Progeny lacking the TE exhibited higher N and P translocation efficiencies and increased seed protein contents. These findings provide genetic evidence that enhanced N and P translocation efficiencies can increase protein content in soybean seeds. Additionally, a TE InDel could serve as a genetic marker for breeding elite soybean cultivars with high protein content and efficient N and P translocation.

Mapping SCN resistance QTL in interspecific soybean crosses between *Glycine max* and *Glycine soja*

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Soybean cyst nematode (SCN; *Heterodera glycines* Ichinohe) remains the most economically important soybean pest worldwide. The objective of this study was to identify unique quantitative trait loci (QTLs) for resistance to SCN by utilizing a total of 165 $F_{2:3}$ lines derived from the cross LD10-10198 X PI 507623 (Population_1) and LD10-10198 X PI 507613 (Population _2). The parents, $F_{2:3}$ lines, differentials, and a susceptible check were inoculated with a HG type 2.5.7 population of SCN and grown in the greenhouse in 2022. The phenotypic data were collected by counting the total number of cysts on each plant after 30 days. Analysis of variance revealed significant differences among the lines for the cyst number, and the phenotypic data from these tests showed broad sense heritabilities of 0.63 and 0.56. QTL analysis was conducted by using QTL IciMapping (Version 4.2) software by implementing inclusive composite interval mapping of additive QTLs. One QTL (LOD>3) on chromosome 18 was identified for Population_1, and a total of four QTLs (LOD>3) on chromosomes 7, 18 (two QTLs) and 20 were identified in Population_2. The individual QTL found in Population_1 explained 34.9% of the phenotypic variation for the resistance, while the individual QTLs found in Population_2 explained 8.4-33.2% of phenotypic variation for the resistance to SCN. The findings may be useful to improve our understanding of the molecular genetic basis of resistance to SCN in soybean.

Keywords: Soybean, *Heterodera glycines*, QTL mapping

Circular RNAs: Potential key actors in soybean floral transition

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Soybean (*Glycine max*), a major crop in many countries, requires a short-day photoperiod for floral induction. Though key transcription factors controlling flowering have been identified, it is still unclear how the non-coding portion of the genome contributes to the floral transition process. This is especially true for single-stranded circular RNAs (circRNAs), which may be crucial for plant development and growth. We investigated the expression and potential function of circRNAs in floral fate acquisition by soybean shoot apical meristem in response to short-day treatment. Deep sequencing and in-silico analysis allowed us to identify 384 circRNAs, of which 129 showed expression patterns in the soybean shoot apical meristems that were unique to the short-day treatment. Also, we discovered 38 circRNAs with predicted miRNA binding sites that may affect the expression of many downstream genes via the circRNAs-miRNA-mRNA network. Interestingly, four distinct circRNAs were shown to have potential binding sites for miR156 and miR172, two essential microRNA modules controlling developmental phase transition in plants. Also, we discovered circRNAs originating from genes in the hormonal signalling pathways, particularly those for auxin and abscisic acid, which suggests a complex network underlying the floral transition. Thus, our study highlights the gene regulatory complexity during the vegetative to reproductive transition and paves the way to unlock floral transition in a crop plant.

Genome-wide association analyses for Brazilian soybean breeding

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Soybean [*Glycine max* (L.) Merrill] is one of the main Brazilian crops and exploring the width of the soybean genome and better understanding its characteristics has the potential to facilitate the development of improved varieties. In this study, we attempted to identify quantitative trait loci associated with composition of fatty acids in soybean oil, total protein and agronomic traits using genome-wide association analyses (GWAS). Two genomic libraries were constructed according to the Genotype-by-Sequencing double digestion using the restriction enzymes NsiI (NEB, New England) and MseI (NEB). Each resulting library was paired-end sequencing on a HiSeq 2500 (Illumina) in rapid mode, producing 150 bp reads per end. In total, 13002 SNP loci were genotyped in 96 soybean diverse accessions related to oil and protein content in their seeds. In soybean lines derived from multiparental crosses (LQs), 12468 loci were genotyped in 288 individuals. After filtering the results and applying p-value correction for multiple hypothesis tests, were identified a total of 171 associations between SNPs loci and soybean accessions related with seeds oil content (6), palmitic acid (13), stearic acid (12), oleic acid (55), linoleic acid (51), linolenic acid (3) and total protein (31). For LQs, 15 SNPs loci were associated with plant height (3), seeds yield (5) and number of days to mature (7). The genotyping of each individual as well as their phenotyping were uploaded to our assisted selection database for future studies. Ultimately, confirmation of these putative loci will provide new sources of variation for use in soybean breeding programs.

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Identifying soybean pod borer (*Leguminivora glycinivorella*) resistance QTLs and the mechanism of induced defense using linkage mapping and RNA-Seq analysis

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The soybean pod borer (*Leguminivora glycinivorella*) (SPB) is a major cause of soybean (*Glycine max* L.) yield losses in northeast Asia, thus it is desirable to elucidate the resistance mechanisms involved in soybean response to the SPB. However, few studies have mapped SPB-resistantquantitative trait loci (QTLs) and deciphered the response mechanism in soybean. Here, we selected two soybean varieties, JY93 (SPB-resistant) and K6 (SPB-sensitive), to construct F_2 and $F_{2:3}$ populations for QTL mapping and collected pod shells before and after SPB larvae chewed on the twoparents to perform RNA-Seq, which can identify stable QTLs and explore the response mechanism ofsoybean to the SPB. The results show that four QTLs underlying SPB damage to seeds were detectedon chromosomes 4, 9, 13, and 15. Among them, qESP-9-1 was scanned in all environments, henceit can be considered a stable QTL. All QTLs explained 0.79 to 6.09% of the phenotypic variation. Meanwhile, 2298 and 3509 DEGs were identified for JY93 and K6, respectively, after the SPB attack, and most of these genes were upregulated. Gene Ontology enrichment results indicated that the SPB-induced and differently expressed genes in both parents are involved in biological processessuch as wound response, signal transduction, immune response, and phytohormone pathways. Interestingly, secondary metabolic processes such as flavonoid synthesis were only significantlyenriched in the upregulated genes of JY93 after SPB chewing compared with K6. Finally, we identified18 candidate genes related to soybean pod borer resistance through the integration of QTL mappingand RNA-Seq analysis. Seven of these genes had similar expression patterns to the mapping parentsin four additional soybean germplasm after feeding by the SPB. These results provide additional knowledge of the early response and induced defense mechanisms against the SPB in soybean, which could help in breeding SPB-resistant soybean accessions.

Keywords: Biological stress, soybean pod borer resistance, linkage mapping, transcriptomics, soybean pod shells

A pivot to proteins in Australian soybean breeding

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The Australian soybean breeding program has long worked on targets of broad adaptation to latitude and planting window, disease resistances, yield and culinary quality. Soybean cropping in Australia extends from temperate regions (MG II) through to the low-latitude tropics (MG IX). All regions target the production of human-consumption quality beans, but some environments are more supportive of particular niche qualities. The program is now working on targets to enhance protein content and functionality and utility for food processing applications. So far, varieties consistently producing extra-high seed protein (c. 44%) with firmer gelling properties have been produced without loss of agronomic yield potential. In response to the need for high protein content ingredients combined with a preference to avoid extremely highly processed fractions with high food miles, the program now extends that work to target even higher seed protein, with diverse functional and flavour characteristics that can be used in food applications with relatively simple processing.

Rdm3 – a major QTL underlying resistance to southern stem canker in elite soybean germplasm

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Soybean southern stem canker (SSC) caused by a fungal pathogen, *Diaporthe aspalathi*, is an economically important disease in the southern region of the United States. Five loci, Rdm1 through 5, have been named based on segregation analysis and reactions with different isolates. The Rdm3 locus carried by the SSC-resistant cultivar Crockett provides good comparable resistance to SSC when compared to the cultivars possessing multiple Rdm loci. However, the genomic location of this locus is unknown and sources of resistance to SSC used in the breeding programs are undermined. This study aimed to map the Rdm3 locus from Crockett and determine the key sources of resistance to SSC in the Georgia Soybean Breeding Program. Using a recombinant inbred line (RIL) population derived from a cross of G81-2057 (susceptible) x Crockett (resistant), genetic mapping identified the Rdm3 locus on chromosome 14 that explained 55% of phenotypic variation. GSM975, an SNP marker tightly linked with the Rdm3 locus, could distinguish soybean lines based on their SSC resistance provided by the Rdm3 locus with high accuracy. To determine the key sources of resistance to SSC in elite germplasm, a panel consisting of 485 experimental lines from Georgia Soybean Breeding Program was selected to perform a genome-wide association analysis. The results revealed that the prevalence of the Rdm3 resistance allele in the elite soybean germplasm and the resistant allele at the Rdm3 locus provides a major source of resistance to SSC in this elite germplasm pool. Thirteen annotated gene models relevant to defense mechanisms against plant diseases were identified at the Rdm3 locus. The QTL and flanking marker information will provide useful information and tools to assist breeders in developing the SSC-resistant cultivars.

Keywords: Southern stem canker, Rdm3 locus, genetic mapping, genome-wide association analysis, marker-assisted selection.

Identification of high yielding and high quality soybean promising lines in different seasons

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Soybean is a highly photoperiod and temperature sensitive crop and therefore, its cultivation faces many challenges. In the current study, it has analyzed that soybean is facing several challenges such as lack of diverse germplasm with specific maturity groups adaptable to various environmental conditions, unavailability of climate-resilient, high yielding and high quality genotypes. Moreover, the absence of area-specific production technology and lack of coherent policy are the major bottlenecks for the cultivation of soybean in Pakistan. Soybean crop is generally produced annually but Pakistan is among the few countries with the opportunity to harvest two crops (spring and autumn) in one year. There is a need to identify best soybean promising lines having high yield and good quality. To identify promising lines, 102 diverse accessions of different maturity groups were collected from United States Department of Agriculture (USDA), USA, and five local varieties were collected from Mingora, Swat and NARC Islamabad, Pakistan. These genotypes were screened in natural field conditions of Faisalabad, Pakistan in spring and autumn seasons. These accessions were grown in Augmented Block Design along with 5 check cultivars. Data related to various morphological and quality related traits were collected in spring and autumn seasons and subjected to statistical analysis for ascertaining the significance of traits. It can helps in the selection of potential parents for desired traits introgression. High yielding and high quality soybean genotypes were identified in spring as well as in autumn season as compared to local check and other competitive exotic soybean genotypes. This study has proposed potential solutions for enhancing the cultivation of this very important oilseed legume for improved yield and quality.

Keywords: USDA, soybean, spring, autumn, check varieties

CRISPR/Cas9 mediated genome editing in *Glycine max* to induce soybean mosaic virus resistance

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Soybean is a leguminous as well as an oilseed crop. It is a rich source of protein and oil, and a major component of poultry and animal feed. It is one of the largest crops and accounts for 35% of the harvested area of oilseed crops worldwide. Its demand is increasing in the solvent extraction industry and is used to produce over 400 value-added products. Soybean is recently introduced in Pakistan and there is widespread risk of SMV-vector aphids. Soybean mosaic virus (SMV) is one of the major constraints in soybean production as it can destroy about 94% of field. CRISPR/Cas9-mediated knockout of negative regulators can help to develop SMV-resistant soybean lines in a short duration that offers widespread resistance. To achieve this, 100 accessions of soybean were imported from USDA and screened against various SMV strains. Some lines were susceptible at a specific developmental stage, in contrast, there were lines that were susceptible at multiple developmental stages. Two SMV multiple developmental stages susceptible lines were selected. Using bioinformatics, four negative regulators (A/B/C/D) and their homeologs that promote SMV attack were identified and guide-RNAs were designed. Line-specific tissue culture protocol was optimized, and vectors in various combinations of target genes were constructed. gRNAs+Cas9 complex was transformed using Agrobacterium-mediated transformation in both lines. a, b, and ab-edited lines exhibited SMV patterns but were reduced by up to 40%. On the other hand, cd-edited line showed 70-80% resistance but either c or d-edited lines remained susceptible. T0 plants are under various molecular, biochemical, and field tests. Selected GE lines showed resistance against SMV and are expected to demonstrate a significant increase in yield. In this study, a significant pool of germplasm possessing SMV resistance has been developed which can be used in future breeding programs targeting induction of SMV resistance in soybean.

Keywords: SMV resistance, soybean tissue culture, soybean mosaic virus, soybean transformation, susceptible genes

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CRISPR/Cas9 mediated genome editing for development of climate smart photoperiod insensitive soybean (*Glycine max* L.)

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Soybean is considered as an important member of legume family. Soybean was domesticated in northeastern China approximately 30-50°N and it is now grown all across the world in latitudes ranging from 53°N to 35°S. In Pakistan Soybean is regarded as non-conventional oilseed crop which was introduced in 1960s. As the net temperature of earth is being increased leading to climate change effects. Different abiotic stresses affect plant growth and development adversely resulting in yield losses. Photoperiod sensitivity is the major issue in adaptability of soybean and due to this factor soybean cultivation is restricted to only narrow ranges of latitudes. Understanding the genetic aspects of soybean photoperiodic regulation of flowering can be proved as a foundation to resolve paradox of early maturity and increased productivity. Flowering is inhibited by dominant alleles of E1 E, E3, E4, E7, E8, and E10, whereas flowering is promoted by dominant alleles of E6, E9, E11, and Photoperiod sensitivity, particularly to various light qualities under experimentally generated LDs, is mediated by E1, E3, E4, E7, and E8. CRISPR/Cas System provides the site-specific knock-out mechanism of photoperiod sensitive genes i.e. E1trascription factor for maturity to increase photoperiod insensitivity. Optimization of tissue culture protocol of soybean is a key step in this regard. Designing of gRNA and target sites (oligos) of the genes to be knocked out by using different bioinformatics tools like CHOP CHOP, CRISPR P2.0, and CRISPOR and Target design, vector construction and transformation are involved for the development of photoperiod insensitive mutant plant. The sustainable agriculture and challenges of climate change can be achieved through the development of cultivars with improved photoperiod insensitivity with stable yield. In this scenario the improvement of soybean by this research can contribute and can be proved as a best approach to meet zero hunger and hidden hunger goals.

Keywords: CRISPR/Cas, photoperiod insensitivity, transcription factor, bioinformatics, gRNA

Genetic response of domestic soybean genotypes towards abiotic stress tolerance

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Soybean is the most important crop belonging to the family Leguminosae. It is a rich source of protein (35-42%), and oil (18-20%) and contains low amounts of carbohydrates and other minerals, especially iron and calcium. In the Agricultural system, soybean is suitable as a catch crop, cover crop, and restorative crop. Soybean seeds as well as plants are used for food, oil, poultry feed, biofuel, green manure, forage, hay, and silage. Soybean has been cultivated in a wide range of environments from temperate to tropical, but its growth is severely affected due to climatic changes, abiotic stresses, and other environmental fluctuations. An experiment was conducted to investigate the soybean morphological and genetic response against these stresses. This experiment was conducted at Soybean Lab, Center for Advanced Studies in Agriculture and Food Security, University of Agriculture Faisalabad. The results showed that these stresses inhibited the increment of plant height and shoot length. Mostly, these stresses cause the reduction of biomass content in green parts of plants. This inhibition became more significant as the level, duration, and frequency of these stresses increased. While at higher concentrations of PEG-6000 treatment (15% and 17% PEG), some varieties have a higher length for finding the water to escape the drought but cause a huge reduction in shoot length. Similarly, under temperature salinity stress, higher levels of salinity cause the reduction of both roots and shoots, some varieties don’t tolerate the higher levels of salinity. While relative expression of GmSALT3, GmWRKY 15, and GmWRKY 17 transcriptional factors (TF) was also measured using real-time PCR. Expression of GmSALT3 TF was highest in 2018 at 150mM, while GmWRKY 15 in Faisal Soy at 150mM. In the case of temperature stress, heat shock factor (GmHSF-34 and GmHSF-38) causes the survival of some varieties at temperature and drought stress. Both factors GmHSF-34 and 38 have maximum expression in William-82 at 30 and 400C. The main objective of this study was to identify those lines which have tolerance against abiotic stresses at the seedling stage.

Keywords: Leguminosae, restorative crop, abiotic stress, nutritional crop

Genetic dissection of advanced soybean (*Glycine max* L.) germplasm for spring season cultivation in Pakistan

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Improvement in genetic gains of crops could be achieved by phenomics characterization, agronomic, physiological and stress related traits. The molecular and strategic breeding program require broad range of foreground and background phenotypic information for crop improvement. In this study, evaluation of 123 advance soybean genotypes (*Glycine max* L.) including seven local lines belongs to 000-lV maturity groups to specify the potential endogenous variation using morphological and yield related traits in two cropping seasons. The four traits out of six, yield per-plant (YPP), number of seeds per plant, number of pods per plant and plant height (PH) showed maximum variation (CV%) that directly correlate with variability in the subjected population. Plant height, number of pods, 100 seed weight and yield per-plant showed strong positive correlation in both years. Among the principal components, factor 1 and factor 2 showed maximum contribution in phenotypic variability ranges from 19-48.5% and 26-47.7% in first and second year respectively. Number of pods showed significant positive correlation with genotypes in both years. Dendrogram separated the genotypes into diverse groups, specified the presence of eminent variation. The presence of eminent variation and association among the accessions is indispensable for effectual conservation and utilization of germplasm. Principal component analysis (PCA) based scoring, the genotypes marked with highest score and desirable character can be selected for future breeding strategies and to identify heat stress tolerance quantitative trait loci (QTLs).

Keywords: Principal component analysis (PCA), *Glycine max*, correlation, cluster analysis, genetic diversity, multivariate

Vegetable soybean (Edamame): challenges and opportunities

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Awareness about the health and nutritional benefits of vegetable soybean and demand from both domestic and international markets has caused an increase in acreage of vegetable soybean in different parts of the world. Constraints include the narrow base of current varieties, lack of concerted efforts to breed varieties for disease and pest resistance and for local adaptation, poor crop management practices, lack of access to good quality seed and not well integrated value chains. The use of core collections, including the one recently developed by the World Vegetable Center, for screening for both biotic and abiotic stresses will help in developing robust varieties. It is vital for the vegetable soybean breeding programs to have well defined product profiles, developed in consultation with the major stakeholders so that the improved varieties are well accepted by the consumers and the adoption rate will be enhanced. The promotion efforts of vegetable soybean production should be well aligned with a sustainable seed production system. It is critical to have both the formal and informal systems of seed production encouraged to meet the demands of the growers. Owing to its highly perishable nature, maintaining the post-harvest shelf-life and minimizing the physicochemical and microbial deterioration that causes degradation of its color, texture and flavor are critical. Greater awareness about the nutritional importance of vegetable soybean and its difference from grain soybean, particularly the way it is consumed is paramount. Promotion activities should include preparation of recipes acceptable to the local communities.

SSR marker aided parental polymorphism survey for development of anti-nutritional factor free soybean with resistance to *Macrophomina phaseolina*

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BACKGROUND

Soybean [*Glycine max* L. Merr.] is a miracle crop as rich in protein and oil content. However, the existence of anti-nutritional factor in soybean seeds, namely Kunitz Trypsin Inhibitor (KTI), restricts its potential as functional food.

HYPOTHESIS

Industrially, heat treatment is used for eliminating KTI but residual activity still remains in seeds. Hence, KTI elimination at genetic level is crucial. For eliminating KTI within less time and greater efficiency, Marker Assisted Backcross Breeding will be effective approach and can be a better alternative for conventional breeding also.

METHODOLOGY

To eliminate KTI genetically, a dysfunctional null allele (titi) of the seed-specific KTI-3 gene was introgressed from NRC-101 and NRC-127 to charcoal rot-resistant two genotypes Suvarna soya and AMS-MB-5-19. Prerequisite for introgressing desirable trait into variety from donor parent is the Parental Polymorphism Survey (PPS). To conduct PPS, >379 SSR markers were chosen, covering each linkage group possessing less than 4kb distance. F_1 was developed by four parental combinations. Similarly, BC_1F_1 was derived by backcrossing confirmed hybrids with recurrent parents.

RESULTS

PPS had been conducted by using 379 SSR markers. Out of them, 55 were found to be highly polymorphic for all parental combinations. The PIC value for markers was ranged 0.40 to 0.91 with an average of 0.55. Foreground screening of F_1 and BC_1F_1 progenies was done by employing null KTI allele specific marker. Background screening was done by employing selected polymorphic markers for estimating recovery of recurrent parent genome with observing 60-70% recovery in recombinant BC_1F_1 progenies.

CONCLUSION

On the basis of above experimental data, it is feasible to select hybrids that possess the target null KTI allele with recurrent parent genome and these hybrids are then forwarded for Marker Assisted Backcross Breeding (MABB) toward development of a soybean genotype free from KTI along with resistant to charcoal rot disease.

Keywords: Soybean, KTI, parental polymorphism survey, marker assisted backcross breeding

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Development of efficient *in vitro* florigenesis system in popular Indian soybean variety JS-335 amenable to genome editing

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BACKGROUND

Soybean is among the most important source of oil and protein across the globe. There are certain limitations in developing new varieties of soybean by conventional breeding due to narrow genetic base. Recently genome editing by CRISPR/Cas9 is becoming very popular to induce targeted mutations resulting in crop improvement which can be utilized for soybean improvement.

HYPOTHESIS

In soybean transformation efficiency is highly relied on the regeneration as not all the cells transformed lead to recovery of viable plant regeneration. Consequently, efficient *in vitro* regeneration is directly associated with recovery of transformants. Therefore, an efficient *in vitro* florigenesis could be effective by avoiding time consuming vegetative phase and getting T_1 seeds.

METHODOLOGY

In the present study we have used cotyledonary node with axillary bud as explants *for in vitro* florigenesis of popular Indian soybean genotype JS-335.

RESULTS

We sought to develop a simple, efficient and a specific regeneration system in which there is *in vitro* florigenesis using cotyledonary node with axillary bud as an explant in popular Indian variety JS-335. Murashige and Skoog (MS) medium augmented with thidiazuron (TDZ) and α naphthalene acetic acid (NAA) resulted in development of flower buds from the proximal end of the explant. TDZ acts as a potential growth regulator to induce in vitro florigenesis. After the flower is developed there is *in vitro* fertilization and pods development. The pods formed were matured within 40-45 days on hormone-free medium. There is no difference between the pods developed under *in vitro* and *in vivo* conditions.

CONCLUSION

This pathway of *in vitro* florigenesis have great potential for *in vitro* flowering and *in vitro* fertilization, which further can be explored to induce targeted mutations using CRISPR/Cas9 in popular Indian soybean genotype JS-335.

Keywords: Soybean, *in vitro* flowering, thidiazuron

Acknowledgements: We sincerely thank the Department of Biotechnology, Ministry of Science and Technology, Government of India, New Delhi and Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola, Maharashtra State, India.

Targeting GmBADH2 gene specific mutations in soybean for development of fragrant allele specific marker

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BACKGROUND

Soybean is rich source of vegan protein but its consumer acceptance is less due to its beany flavor. Nowadays, Vegetable soybean is getting popular due to its sweeter taste, bold seeds, extra-large pods and high nutrient content along with pleasant aroma which fetch higher price and demand than those of normal soybean genotypes.

HYPOTHESIS

The aroma in soybean is due to SNP in BADH2 gene which results in accumulation of 2AP. If vegetable soybean is added with fragrance, then it will be formidable than grain type soybean. So, identifying diversity among aromatic and non-aromatic soybean genotypes and development of allele specific marker for aroma trait is effective approach for introgression of aroma trait in Soybean genotypes.

METHODOLOGY

Characterization of vegetable and grain type soybean genotypes was carried out using SCoT and EST-SSR markers. Also, to find out SNP in BADH2 gene, whole gene specific primers was designed by considering entire exonic and intronic region, for avoiding exclusion of single base pair mutation in BADH2 gene.

RESULTS

Characterization of aromatic and non-aromatic soybean genotypes was carried out using 20 SCoT primers. In that 40 amplicons were found polymorphic and average no of alleles were 2.9 per primer with percent polymorphism of 58.9%. In Molecular analysis using EST-SSR marker, PIC values ranged from 0.334 to 0.837 with an average of 0.559. Also, fragrance determination protocol was designed with desirable KOH concentration. Designed allele specific primer E10 (GmBADH2-G2) showed polymorphic banding pattern comprising with 200bp new allele in vegetable soybean, AGS-464. The sequence alignment revealed single nucleotide polymorphism in triplet codon AAA (Swarna soya) in place of CAA (AGS-464) distinctly in exon 10 region.

CONCLUSION

The PCR primer(s) targeted to mutation showing distinctness among aromatic and non-aromatic genotypes could further be exploited in MAS for introgression of fragrant allele in soybean.

Keywords: Soybean, BADH2, 2AP, SNP, MAS, EST-SSR

Acknowledgements: We sincerely thank the Asian vegetable research and development center, Taiwan for providing experimental material and Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola, Maharashtra State, India to conduct investigation and furnished facility.

CRISPR/Cas9 based genome editing for development of food-grade Indian soybean

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BACKGROUND

Malnutrition is a major problem to be addressed in India. Since soybean has a high source of protein, folic acid and minerals, it is the ideal choice for a protein-rich diet. However, there are few constraints like anti-nutritional factor (KTI), beany flavor and non-aromatic nature of soybean seeds hinder the preference of soybean as eatable food. Therefore, it is imperative to raise the standard of soybean.

HYPOTHESIS

Non-aromatic nature of soybean is due to the functional BADH2 gene. The nonfunctional BADH2 mutants convert GABald to 2AP, which release aroma. Similarly, the absence of KTI gene results in easy protein digestion. Therefore, editing BADH2 and KTI genes will make it non-functional leading to a pleasant aroma and easy protein digestion.

METHODOLOGY

In the present investigation, the sequences of target genes BADH2 and KTI gene have been downloaded from phytozome v9.1 and target sites have been identified using CHOPCHOP web tool. Construct for each gene have been developed and cloned in pDirect 22. After validation, *Agrobacterium tumefaciens* strain AT0711 with edited construct was used for the transformation of genotypes viz., AMS 100-39, AMS MB 5-18, AMS-1001 and Swarna-Vasundhara using the edited Agrobacterium. Transformation has been attempted with two strategies. Sonication assisted and agro-infection with shaking. Co-cultivated cultures will be sub-cultured for shooting and rooting. The T0 plants will be screened with a gene-specific primer.

RESULTS

In-vitro regeneration protocol has been standardized for genotypes prior to Agrobacterium infection. The selection of developed shoots will be made with positive selection and plants will be grown for the next generation, the edited gene will again be screened.

CONCLUSION

The investigation will result in the development of good quality soybean with pleasant flavor and free from anti-nutritional factor which can directly be consumed by user and may become an asset for the soy industry.

Keywords: GABald, CRISPR, CHOPCHOP, agro-infection, genome editing

Acknowledgements: We sincerely thank the Department of Biotechnology, Ministry of Science and Technology, Government of India and Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola, Maharashtra State, India.

Marker assisted introgression of null KTI allele into charcoal rot resistant soybean genotypes

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Background

Since soybean seeds contain Kunitz trypsin inhibitor (KTI), soy flour must first be heated to inactivate it before use in food and feed products. The heat treatment affects the quality and solubility of seed proteins and increases processing costs of soy-meals and feeds. Genetic eradication of KTI is a crucial and useful strategy. To genetically eliminate KTI from two well-known charcoal rot-resistant soybean genotypes, SuvarnSoya and AMS-MB-5-19, the molecular marker-assisted backcross breeding (MABB) strategy was used.

Hypothesis

Marker-assisted backcross breeding accelerates the introgression of a desirable trait(s) through foreground and background selection. To produce soybean varieties resistant to charcoal rot and devoid of kunitz trypsin inhibitor, it would be advantageous to genetically eliminate the inhibitor from these two soybean genotypes.

Methodology

In the present investigation, a null KTI allele (kti) was introgressed from donor to recipient cultivars using MABB method. Foreground selection was performed in BC_1F_1 and BC_2F_1 using null allele-specific marker and 'Ti' gene tightly linked marker Satt228 located on linkage group A2. Selected polymorphic markers distributed throughout all the linkage groups were utilized for background selection in plants carrying 'kti' allele.

Results

The foreground selection for the kti allele was performed using two SSR markers while 56 polymorphic SSRs were used for background selection. It was shown that 24 introgressed plants in BC_1F_1 recovered 60% to 75% of the recurrent parent genome in all four cross combinations. Similar to this, four plants in BC_2F_1 were found to have the KTI null allele by introgression. After biochemical analysis of the seeds using native PAGE, KTI peptides were not discovered in the seeds of the selected plants (ktikti). The chosen plants exhibited the same level of infection resistance against charcoal rot as the recipient cultivar.

Conclusion

The MABB approach accelerated the development of soybean lines resistant to charcoal rot without KTI. For the production of soy-based foods and feed that might gain more acceptance, these lines will be perfect for growers, consumers and soybean-based industry.

Keywords: Soybean, Kunitz trypsin inhibitor, marker-assisted backcross breeding, Satt 228, KTI null allele

Acknowledgements: We sincerely thank the Department of Biotechnology, Ministry of Science and Technology, Government of India, New Delhi and Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola, Maharashtra State, India.

Comparative cDNA profiling of susceptible and resistant soybean genotypes and effect of silicon in response to charcoal rot disease (*Macrophomina phaseolina*)

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BACKGROUND

Charcoal rot (*Macrophomina phaseolina*) being one of the ten most yield-suppressing soybean diseases 0f soybean worldwide, it affects up to 77% in Indian weather conditions. This fungus becomes aggressive in hot-dry weather. The *M. phaseolina* can survive in soil for 3-4 years. Hence controlling the infestation of *M. phaseolina* is challenging. The Dr. P.D.K.V., Akola University has developed the Suvarn Soy (AMS-MB-5-18) genotype possessing resistance to *M. phaseolina*.

HYPOTHESIS

A little information is available on *M. phaseolina* in context of its molecular response towards genotypes. Deciphering the molecular mechanism, it is needed to through track down gene(s) responsible for resistance. The identification of TDFs leads to speed up the breeding programmes to develop more genotypes resistant to *M. phaseolina*.

METHODOLOGY

To better understand the fundamental mechanisms for resistance the study performed morphological and molecular analysis. The cDNA profiling was done on resistant (AMS-MB-05-18) and susceptible (TAMS-38) genotype to elucidate differential gene expression of soybean against charcoal rot disease with different silicon treatments including calcium and potassium silicate.

RESULTS

Macrophomina phaseolina was molecularly characterized via sequencing, and the data was uploaded to NCBI (GenBank accession number: MZ823608). A considerable difference in potassium silicate absorption in disease resistivity was observed in a screening experiment employing scanning electron microscopy. The 1.7mM potassium silicate showed clear deposition of silicate in soybean leaf trichomes. Potassium silicate was observed to prevent the infestation of charcoal rot while also increasing plant height and biomass. Leaf tissue was used to examine transcriptional changes at two distinct times following germination. Differential gene expression was studied in 60 samples of cDNA profiling to decode TDFs (transcript-derived fragments).

CONCLUSION

In the use of silicon (Si) supplementation presents a promising potential. The uptake of potassium silicate 1.7mM, on the other hand, indicated considerable disease tolerance in the susceptible genotype TAMS-38. The efficiency with which plants collect silicon shows an active silicon system against CR disease resistance.

Keywords: C-DNA profiling, charcoal rot, *Macrophomina phaseolina*, potassium silicate, soybean

Acknowledgment: We sincerely thank to Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola, India for providing facility to conduct experiment.

Soybean transcriptome atlas provides gene-level insights into the genetic basis and dissection of traits

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While genome-wide association studies (GWAS) can identify loci associated with a trait, determining the causal gene from those loci remains a bottleneck. Transcriptome-wide association studies (TWAS) offer a potential solution by identifying gene expression-phenotype associations or integrating expression quantitative trait loci (eQTL) with GWAS results. Here, we present the first soybean (*Glycine max* [L.] Merr.) diversity panel RNA-Seq on seedling shoots providing genome-wide insights into genetic regulation of gene expression while additionally enhanced the capturing of expression variation resulting from structural variations and alternative splicing. Our study demonstrates the robustness of TWAS, using seedling shoot expression data, in successfully identifying known genes associated with traits across different tissues and developmental stages. In addition to identifying the known genes associated with qualitative and quantitative traits, we further validated a novel gene named pod color L2 using gene editing, which highlights the power of TWAS in identifying novel trait-associated genes. Moreover, we discovered several known and novel genes associated with flowering time, which complements the results from GWAS and provides additional genetic targets for further investigation. The genes identified by our improved TWAS had diverse causal variations, including SNP, InDel, gene fusion, copy number variation, and alternative splicing. Overall, our study provides transcriptome information to aid in the prioritization of candidate genes from soybean GWAS results and presents a broadly applicable methodology for other species.

Keywords: RNA-Seq, eQTL, GWAS, TWAS, flowering time

Theme B:

Food, feed, and nutrition

	Sessions and workshop *(chair)*
B1	Chemistry and nutrition of soybeans and soy products *(Keshun Liu, Carl M. Parsons)*
B2	Feed and aquaculture developments *(Mian N. Riaz, Iani Adrian Chihaia)*
B3	The future of soy is food *(Matthias Krön)*
B4	Soy protein functionality and processing *(Leonard Sagis)*
B5	Soy foods, soy bioactives, and improvement for human health *(Keshun Liu, Istvan Rajcan)*
B6	Assessing sustainability of soybean supply chains *(Thomas Nemecek, Werner Zollitsch)*
W6	Soybean processing technologies *(Werner Sitzmann)*
	Posters B: Food, feed, and nutrition

The untapped potential of soybeans for human nutrition in sub-Saharan Africa

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B1: Chemistry and nutrition of soybeans and soy products

Over 800 million people faced hunger in 2021 – 150 million more people since 2019, before the COVID-19 pandemic. Moreover, close to 3 billion people are unable to afford a healthy diet and poor-quality diets are linked to 11 million deaths per year. In sub-Saharan Africa (SSA), the only region where the rate of stunting continues to rise, efforts have included bold investments in youth and women in agriculture, especially in nutrient-dense cash crops. Soybeans fix nitrogen and offer a complete protein to populations, with a significantly lower impact on the environment than many other crops. Plant-based sources of quality protein, oil, and fiber like soybean are less expensive but require transformation. Efficiencies in processing at scale may help alleviate the burden of food insecurity, bringing nutrition at a lower cost without jeopardizing cultural norms and evolving appetites. With the goal of enhancing the utilization of soybeans, and thus, improving smallholder livelihoods in SSA, the Soybean Innovation Lab (SIL) has shown the potential for processors to bring the nutrient density of soybeans into human nutrition applications. Vegetable oil is a valuable commodity in the SSA region, and in the case of soybeans, it is often the result of mechanical expellers rather than solvent extraction. This process renders a leftover cake containing oxidizable fats with limited shelf life and applications beyond feed. SIL has piloted a low-cost, washing method for cake, which accumulates protein and removes oxidized oil and other anti-nutrients such as phytic acid, offering processors a more functional ingredient for a diversity of food applications. The growth of the food processing sector in SSA clearly shows an intention to meet consumer demands, all the while offering sustainable income-generating opportunities for smallholder farmers through higher-value products that appeal to changing markets and tastes.

Novel and simple method to determine contents of Kunitz trypsin inhibitor and Bowman-Birk inhibitor in soybeans

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B1: Chemistry and nutrition of soybeans and soy products

Soybeans are the number one source of plant proteins for food and feed, but the natural presence of proteinase inhibitors (PI), namely Kunitz trypsin inhibitor (KTI) and Bowman-Birk inhibitors (BBI), can induce undesirable or desirable health effects. Since both inhibitors have inhibition toward trypsin, measurement of trypsin inhibitor activity cannot differentiate them. Over the years, three major types of methods have been developed to measure KTI or BBI concentration in soy products, including gel electrophoresis, immunoassays, and chromatography. However, these methods suffer from several inherent problems, including complex procedures, requirements of specific and expensive instruments, limitation to only KTI or BBI, time- and labor-intensity, inaccuracy, and high cost. They are more suitable for qualitative than quantitative measurements. For the past few years, we have developed and optimized methods to accurately measure trypsin inhibitor activity and chymotrypsin inhibitor activity, respectively. Using the two new methods as a basis, we have recently developed a simple and cost-effective method to simultaneously determine contents of KTI and BBI in soybeans. We validated the new method by measuring two KTI-null and two wildtype (common) soybean cultivars for PI composition. On average, wildtype soybeans contained 14.8 mg/g KTI and 6.2 mg/g BBI, totaling 21.0 mg/g, but KTI-null soybeans had about half amounts of total PI, resulting from several-fold reduction in KTI content. By developing the novel and simple method that can differentiate the two inhibitors in terms of mass composition, we can easily identify which inhibitor has more effect on animal nutrition and human health, and at the same time facilitate plant scientists to screen and develop soybeans with altered PI composition. All these efforts will lead to the development of better soybeans for improved feed or food with optimal nutrition.

Keywords: Method, soybeans, trypsin inhibitors, Kunitz, Bowman-Birk

Effect of digestible essential to non-essential amino acids ratio on soybean meal productive energy (Arkansas Net Energy) in broilers: Implications beyond metabolizable and classic net energy

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B1: Chemistry and nutrition of soybeans and soy products

Energy is a major cost in feed formulation. Arkansas Net Energy (Ark-NE) indicates soybean meal (SBM) contains more usable energy than determined with other energy systems. The objective was to develop a model applicable to predict Ark NE of SBM based on its content of digestible nutrients compared to apparent metabolizable (AME, AMEn) and classic NE (CNE). Four 56-d experiments (EXP1 to EXP4) with pelleted diets following Cobb500 guidelines included SBM dilutions with a filler (EXP1) or corn (EXP2) and reformulating to include increasing oil (EXP3) or digestible balanced protein (EXP4). Performance, body composition (Dual Energy X-Ray Absorptiometry), and heat production (calorimetry chambers) were assessed, and digestible nutrients in 60 diets were determined. Data were analyzed in JMP using mixed models. A model for Ark-NE (kcal/kg) was developed with the coefficients for the intercept and digestible contents of crude protein (dCP%), fat (dFat%), starch (dStarch%), age (d), and age2, being –134.7, +81.09, +70.65, +36.7, –13.76, and –0.45, respectively ($adjR^2=0.62$; every predictor $P<0.0001$). A derivated model was obtained, including an adjustment factor for the essential to non-essential digestible amino acids ratio (ENEDAR; $\text{Ark-NE}_{\text{ENEDAR}}$): $\text{Ark-NE}_{\text{ENEDAR}}$ (kcal/kg) = Ark-NE (0.95 $\text{ENEDAR}_\text{T}/\text{ENEDAR}_\text{R}$ + 0.05) (accuracy >92%), being ENEDAR_T and ENEDAR_R the ENEDAR of the assessed and reference (ENEDAR_R = 0.82) SBM, respectively. The Ark-NE model and the ENEDAR adjustment factor were validated with a stratified random data-splitting with a refitting approach. Digestible amino acids for SBM accounted for more than 90% of the Ark-NE value. Models for AME and AMEn were not influenced by ENEDAR ($P>0.39$), and CNE was less influenced by dCP% than Ark-NE. FCR and feed intake were correlated more with Ark-NE than AME or CNE (< P value; $>adjR^2$). In conclusion, Ark-NE can be predicted based on the digestible nutrient content of SBM, credits the ENEDAR, and is a better predictor of performance.

Keywords: Arkansas Net Energy, productive energy, net energy, heat production, broilers

Aqua feed extrusion with soy based ingredients

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B2: Feed and aquaculture developments

Aquaculture feed production worldwide is increasing rapidly. This relatively new, and highly profitable market is the fastest growing of all animal feed markets. Traditionally, animal protein supplements were the foundation of any aquaculture feed formulation. However, given the limited world supplies and the increasing price of these animal protein supplements, aquatic nutritionists are considering alternative protein sources. Plant protein supplements are generally cheaper per unit of nutrient as compared to the animal protein supplements. The use of soy in diets provides a sustainable feed solution for aquaculture and is the most used protein source in aquaculture feeds worldwide. Soy has a high protein density, is highly digestible for most cultured fish and shrimp species, can replace high-cost animal proteins, and yields rapid fish growth. Soy is an environmentally sound solution to the growing global demand for nutritious seafood and is critical for aquaculture because it is an internationally traded product that is available year-round. A large majority of aquaculture diets now contain 25-30% soy as a key part of the formulation, and there are a variety of applications in which soy products can be used in aquaculture diets, including, soybean meal, soybean oil, soy protein concentrate, full fat soybean meal, fermented soy, soy lecithin, soy hulls, and soy isolates, among others. This change in protein some time become a challenge to make high quality aqua feed, it soy proteins are not processed correctly. Since protein play several roles other than nutrition such as proving elasticity and binding and expansion of feed pellet during extrusion. If soy protein is not processed correctly, it will not make good quality pellet. The use of soy-based aquafeeds supports pond water quality by reducing nitrogenous wastes in water that impact fish health. Soybean meal costs significantly less than fish meal. Reducing feed cost is critical to improve efficiency and maintain sustainability in aquaculture business.

Fermented soybean meal and quality control for aqua feeds

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B2: Feed and aquaculture developments

Soy products - especially soybean meal (SBM) - are extensively used in aqua diets to partially or completely replace fish meal and animal proteins. In specific diets, high-level replacement of FM by SBM has been successful but generally requires special treatment of the SBM and/or the addition of specific amino acids including taurine (an amino acid absent from SBM).

Much of the potential to replace FM or animal proteins is related to the variation in digestibility and utilization of SBM. This depends greatly on the type and degree of heat treatment and the resulting concentration of the residual anti-nutritional factors (ANF). Quality parameters to estimate SBM digestibility and level of residual anti-nutritional factors (ANF) have been developed for monogastric animals but not for aquatic spp. and extrapolation to aquatic species is at best doubtful.

Partial removal of the ANF is achieved through toasting or expansion/extrusion or by extraction (as in the case of SPC or ISP). An alternative, efficient and economical method for ANF reduction has proven to be the fermentation of SBM. Fermentation - most notably the solid-state fermentation of SBM (FSBM) - are rapidly gaining interest allowing the inclusion – or increase in inclusion – of soy in aqua diets. This is especially important for carnivorous fish and shrimp where inclusion of FSBM allowed successful replacement of fish meal and improved performance. However, methods of production for FSBM may vary considerably and this complicates the development of consistent, applicable quality parameters.

For a more extensive and efficient use of FSBM in aqua or in specialty specific quality parameters need to be developed. The classical quality parameters for SBM do not apply directly for FSBM. Specific QC parameters need to be developed for FSBMs. Production parameters and end-product nutrient-concentrations (i.e., pH, specific fermentation products such as organic acids) were reviewed and found applicable as QC parameters according to the type of fermentation.

The case of novel ingredients in the feed and food industry: how to approach novelty

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B2: Feed and aquaculture developments

Sustainability is currently a strong driver for replacing animal proteins in the feed and food industries and some activists claim large-scale animal protein production is an unproductive use of natural resources when compared to the environmental impact of plant-based protein production. Indeed, feed production for animal consumption and food preferences in humans are changing as there is greater moral and nutritional interest in the vegan lifestyle. The complexity of animal products and the physicochemical principles you need in order to assemble novel plant-based ingredients, each with their own physical, functional, nutritional, and sensory attributes is a major challenge. In addition, sustainability must also include the entire ecosystem from clean energy sources to feedstock. On the other hand, fermentation has enormous potential to positively transform the food system, but feedstocks are the major cost driver for most fermentation processes. Fermentation processes can take advantage of a variety of host organisms, feedstock sources and cultivation processes, offering an unparalleled opportunity to decouple protein production from limited resources such as land, freshwater and other inputs. Traditionally used to improve food functionalities such as preservation, taste, and texture, novel fermentation solutions are enjoying a resurgence with new, innovative technologies being developed across various segments of the feed & food industries. New generation genetic modification technologies can be applied to increase the functionality of microorganisms including novel ones for fermentation and benefits. The use of precision fermentation for the production of major food components (proteins, lipids, and carbohydrates) is emerging as an attractive option for the transformation of food systems. In this presentation, we will highlight a leading-edge fungal framework being used for precision fermentation, and we will briefly overview our groundbreaking technology to create novel ingredients that can accelerate the growth of the feed and food industries.

Keywords: Plant proteins, precision fermentation, fungi, food

Soy industry 4.0 is here. a bold rethinking of innovation in value-added soy products

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B2: Feed and aquaculture developments

When it comes to choosing plant protein, global consumers and end users have a host of choices. Soy protein's composition of essential amino acids sets it apart as the gold standard among plant proteins. Protein yield per acre from soybeans is unparalleled, making it a preferred, economically advantageous source of plant protein for animal feed. Whole beans are processed into a multitude of value-added ingredients – meal, oil, flour, isolates, concentrates, and fermented products, each of which has many variations. Meanwhile, it is well known that protein quality can be influenced by several factors including the choice of raw material, the use of additives, and the processing techniques.

The history of industrial soy processing goes back to 1922 when the first commercial soy processing plant was established in Decatur, IL, USA. Since then, three generations of value-added soy products were developed (mechanically and solvent-extracted SBM, soy protein concentrates and isolates, and fermented SBM) by using different processing methods: mechanical and solvent extraction, aqueous alcohol leaching, enzymatic treatment, and fermentation. The shortage of high-quality protein ingredients for aqua species, for early rearing and young animal nutrition during the past decade, has favored the development of more suitable digestible plant protein ingredients, to serve as a substitute for the animal-based and/or be a solution for scarce plant protein sources needed for optimal animal performance, health, and profitability. In this context, the value-added soy ingredients become a significant part of the production process in aquafeeds, it is, therefore, natural to focus this review on the driving forces that are expected to lead to greater value-added soy ingredients utilization over the next decade: breeding technologies, adoption of new traits, smart farming, intelligent solution for storage and processing, including fermentation technology.

The use of ultra high protein, low oligosaccharides soybean meal varieties in aquaculture

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B2: Feed and aquaculture developments

By leveraging the natural genetic diversity of plants, it is possible to develop ingredients that are better for animals, people, and the planet. Benson Hill has used traditional breeding techniques to develop a soybean that is uniquely suited for the needs of the aquaculture industry. By prioritizing quality traits such as protein density and low antinutrients in the breeding process, costly and unsustainable processing steps can be avoided. The resulting ingredient has demonstrated high performance in Atlantic salmon diets when compared to SPC, and has a favorable carbon and water footprint when compared to SPC. This is an example of how soy genetics can be used to unlock value in specific end-markets.

Effects of molten-globule state and renaturation treatment on the structure and functional properties of soybean 11S globulin

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B4: Soy protein functionality and processing

At present, as good functional characteristics, soybean protein has been widely used in food field, but it is easy to cause denaturation and aggregation during the preparation of soybean protein, thus reducing the functional characteristics of soybean protein. However, due to soy protein can cause degeneration and aggregation during preparation process, the functional characteristics have been reduced. Molten-globule (MG) state is a structural state between the nature state and t extended state of globulin, which has good functional characteristics; in addition, in processing applications, whether removing denaturation conditions can lead to protein renaturation and restore its good functional properties needs to be systematically studied. In this study, soybean 11S globulin was used as raw material to prepare fused spherical soybean 11S globulin through heat treatment and acid treatment, and the denatured protein treated by acid treatment, alkali treatment, heat treatment and guanidine hydrochloride was refolded. The structural changes of soybean 11S globulin treated above were characterized by fluorescence spectrum, circular dichroism and other spectroscopic methods, and the functional characteristics changes such as foaming and emulsification were systematically investigated. The results showed that soybean 11S globulin had large hydrophobic area, extended tertiary structure and retained some secondary structure (87.18%) under the conditions of pH 1.0 and 12 h; and the soybean 11S globulin reached the typical MG state after being treated at 100°C for 30 minutes. In addition, the functional characteristics of MG 11S globulin and renaissance protein have been improved. This study provides a certain theory for the further development of soybean protein.

Keywords: Soybean 11S globulin, denaturation, renaturation, molten globule state

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Tackling challenges in alternative protein food formulation with a focus on improving functionality and sensory appeal

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B4: Soy protein functionality and processing

Taste, texture, and nutrition remain the primary hurdles for the alternative protein arena. In fact, many consumers agree that taste and nutrition are equally important in plant-based products. Food scientist are constantly searching for new quality and functional protein ingredients that can help them formulate tasty and appealing products. Soy remains fundamental to the alternative protein space for its functionality and versatility, in addition to being highly recognizable by consumers. Further, it's one of the most sourced plant proteins, is supported by a robust body of research and is one of the few non-animal proteins that is a 'complete protein' with a protein digestibility corrected amino acid score (PDCAAS) of 1. Soy has paved the way for other plant proteins by demonstrating that processing soybeans, in the right way, leads to quality soy protein solutions and, ultimately, some of the most appealing, on-trend consumer products. In this presentation, we will discover some of the main challenges that plant-protein food formulators face and how recent research in plant-based ingredients has led to the discovery of new functionalities, as well as improved sensorial appeal of novel plant-based foods.

Keywords: Plant-protein processing, functionality, future foods, industry challenges

Connecting soybean fractionation to novel food applications

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B4: Soy protein functionality and processing

Soybean is a versatile and much demanded crop worldwide. It can be used to make traditional soy-based foods, which have been consumed for more than two thousand years. In the past decades, soy has gained additional importance, because it serves as a source for a broad range of ingredients, which are applied in modern products like meat and dairy analogues. Unfortunately, the production of soy-based ingredients for those products requires extensive processing, making the processes used for those products often less efficient than processes used to create the traditional soy-based food products. To improve the efficiency of soy-based processing, we propose to better connect fractionation of soy to the desired application. To do so, novel fractionation routes should be developed to deliver ideal ingredients for next generation soy-based foods. Main focus is then on designing the required functional properties of the ingredients, instead of current focus on requirements for high purity and protein solubility. In addition, this new approach probably leads to a broader diversity in soy-based ingredients with specific functionality and composition. In this presentation, we show how different fractionation routes lead to different functionalities and how the fractionation process for soybeans can be optimized to make next generation ingredients for meat analogues.

Traditional fermented soybean products: emphasis on processing, microbial contribution, nutritional and biological activities

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B4: Soy protein functionality and processing

Traditional Chinese fermented soybean products have been widely consumed in Asia for its favorable aroma and taste. During fermentation, the nutrients in soybean undergo a series of biochemical reactions catalyzed naturally by microorganism secreted enzymes. Therefore, many bioactive substances such as bioactive peptides, isoflavones, and unsaturated fatty acids are produced, making fermented soy products more advantageous in nutrition and health. In this work, historical evolution and traditional fermentation processing of four traditional fermented soybean foods including furu, douchi, soybean paste and soy sauce will be discussed. The microorganisms involved in fermentation and biological functions have also been investigated. The changes of bacterial diversity during furu fermentation have been studied, and a total of 213 phyla, 378 classes, 722 orders, 1137 families, 1834 genera, and 2757 species were identified using high throughput sequencing. Our study revealed that *Acinetobacter* was the major microflora during white furu ripening stage, yet *Lactobacillus* and *Enterococcus* was the dominate lactic acid bacteria from red and stinky furu, respectively. We found that the bacterial diversity varied depending on the types of fermented products, and the bacterial communities altered among different stages of fermentation. Furthermore, major challenges such as high salt content and formation of biogenic amines will be introduced in this study. We conclude that the establishment of scientific standard and innovated fermentation processing is the potential solutions to improve the safety of traditional fermented soybean products.

Keywords: Fermented soybean products, nutrition and functionality, microbial diversity, traditional fermentation

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Influence of kori-tofu on lipid and glucose metabolism and its resistant protein content

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B5: Soy foods, soy bioactives, and improvement for human health

Kori-tofu is a frozen and dried soybean curd, and a traditional food in Japan. We aimed to assess the potential of kori-tofu to improve cardiometabolic health outcomes in humans. In a double-blind randomized controlled cross-over trial, 45 subjects aged 40-70 years with (mildly) elevated cholesterol levels received a four week kori-tofu intervention or whey protein control intervention with a four week wash-out period in between. Cardiometabolic biomarkers were measured before and after both interventions. A significant decrease in total cholesterol, LDL cholesterol, HbA1c, fructosamine and systolic blood pressure was observed within the kori-tofu intervention. However, many of these findings were also observed in the control intervention. Improvements in cardiometabolic markers within the kori-tofu intervention point toward potential beneficial health effects. Due to the lack of significant effects as compared to control, however, currently there is no substantiating evidence to claim that kori-tofu has beneficial effects on cardiometabolic health. The potential of a 4-week food intervention to improve the cardiometabolic marker profile may be considered promising, but should be studied further to draw firm conclusions.

It is reported that repetitive soy protein consumption has lipid metabolism improvement effects, involved in cholesterol reduction. One of the factors that might cause these effects is undigestive protein named ‘resistant protein (RP)’. Therefore, we investigated the RP content in total protein of various intermediate and derivatives obtained during the production process of kori-tofu. In soymilk, the RP content was the same as soybean even if the soymilk solid content was varied. In fresh tofu, however, the RP content was not increased by mere coagulation of soymilk, but was increased considerably by gain of press strength. Slow freezing and low-temperature aging increased the RP slightly. We concluded that the primary reason for abundant RP content of kori-tofu is caused by the strong pressing in the fresh tofu making process. Slow freezing and low-temperature aging processes also contribute to increasing the RP.

Keywords: Kori-tofu, resistant protein, lipid metabolism, glucose metabolism

Multifunctional bioactive peptides derived from soy protein hydrolysates: antioxidant activity and inhibitory activity on α-glucosidase and α-amylase

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B5: Soy foods, soy bioactives, and improvement for human health

In recent years, researchers have explored food-derived peptides to circumvent the side effects of synthetic drugs. Soy protein hydrolyzed peptides are of interest because of their multiple biological activities. This study therefore used seven enzymes (alcalase, neutrase, trypsin, bromelain, pepsin, papain and flavorase) to hydrolyze the soy protein and obtained seven enzymatic polypeptides, examined the structure, subunit composition, and *in vitro* antioxidant activities, α-glucosidase and α-amylase inhibition kinetics of protein hydrolysate obtained from the soy protein. The degree of hydrolysis (DH) of the seven methods were between 7.18 and 28.95%. The average particle size and zeta-potential of the seven protease hydrolysates were significantly decreased ($P < 0.05$) compared with soy protein. The enzymatic hydrolysis method had a significant effect on the surface morphology of the enzymatic residues. The enzymatic polypeptide from alkaline protease method had better thermal stability and stronger infrared absorption. By 5-axe cobweb chart method, the enzymatic polypeptide from alkaline protease method was proved to have the strongest comprehensive antioxidant activity. The enzymatic polypeptides of alkaline protease and papain had stronger inhibition activity of α-glucosidase. Kinetic analysis showed that α-glucosidase-inhibition and α-amylase-inhibition by different concentrations of hydrolysates is mixed-type inhibited and un-competitively inhibited respectively. Next, the enzymatic polypeptides of alkaline protease peptides will be purified and identified, analyzed by peptidomics. In conclusion, it can be deduced that soy protein alcalase protein hydrolysates may play critical and indispensable role as bio-tools in diabetes treatment.

Keywords: Soy protein, enzymatic polypeptide, antioxidant activity, α-glucosidase inhibition, α-amylase inhibition

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Structure, properties and potential prebiotic activity of high-purity insoluble fiber from soybean residue (okara)

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B5: Soy foods, soy bioactives, and improvement for human health

Okara is a major residue produced during the manufacture of soy milk and soybean curd (tofu), which has a low utilization rate and is a cheap and easily available resource of insoluble dietary fiber (IDF). Large prospective cohort studies demonstrate that IDF has a potential prebiotic effect, but the mechanism of its prebiotic function remains unclear. In this study, the structure and physicochemical properties of high purity insoluble dietary fiber (90.5%, HPIDF) extracted from okara were characterized, the structure and physicochemical properties changes of HPIDF at different stages of digestion were analyzed through *in vitro* simulation digestion, and the effects of HPIDF on obesity and colon inflammation were investigated *in vivo*. The results showed that the main monosaccharides of HPIDF identified were galactose, arabinose, xylose, rhamnose, and glucose. HPIDF had a loose and porous structure, polysaccharide functional groups, and a typical crystalline cellulose I structure. Furthermore, HPIDF had ideal oil-adsorption capacity, cholesterol adsorption capacity, cation exchange capacity, and heavy metals adsorption capacity. The dissociation degree of some functional groups resulted from different digestive environments, which revealed the reason for the differences in the physicochemical properties of HPSIDF in different digestion stages. *In vivo*, HPSIDF supplementation reduced ameliorated dyslipidemia, impaired glucose homeostasis, systemic inflammation, and fat accumulation in HFD-fed mice. Moreover, HFD-induced gut microbiota dysbiosis and decreased SCFA production were recovered by HPSIDF supplementation. Additionally, intake of HPIDF affected the proliferation of the key bacteria in the feces of mice with acute ulcerative colitis, and then affected the synthesis of SCFAs, providing a positive role for colon health. This is the first systematic study on the structure, physicochemical properties, and prebiotic activity of HPIDF. Our findings indicated that HPIDF could be used as an ideal functional ingredient, which plays a positive role in promoting the comprehensive utilization of okara.

Spermidine in soybean: from genetic variation to health-promoting food products

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B5: Soy foods, soy bioactives, and improvement for human health

Soybean is one of the richest food sources of the polyamine spermidine. Recent findings have identified spermidine as a major health protecting component with strong anti-aging effects. Spermidine-mediated health effects might be due to its strong autophagy inducing property as well as due to anti-oxidant and anti-inflammatory characteristics. Thus, spermidine concentration of soybean could partly explain the long-term health benefits associated with soy-food consumption and might therefore become increasingly relevant for food-grade soybeans in future. A set of early maturity genotypes was grown in replicated field experiments near Vienna, Austria for three seasons, and spermidine and other polyamine concentrations were determined from seed samples using ultra-high performance liquid chromatography (UHPLC). In addition, a mass spectrometry imaging approach was implemented to generate high-resolution chemical images to visualize the spatial distribution of spermidine and other phytochemicals in cross sections of soybean seeds. Spermidine concentration was in the range of 167-291 mg/kg dry seed, whereas putrescine and cadaverine were between 3 and 29, and spermine between 31 and 179 mg/kg, respectively. Statistically significant genetic as well as environmental variation was found for all polyamines analysed. Thus, the results confirm earlier reports on soybean polyamine levels and the high spermidine concentration in particular. Mass spectrometry imaging revealed higher concentrations of spermidine in the germ rather than in cotyledon tissues with highest concentrations found in shoot and root meristem regions. In contrast to spermidine, isoflavones were found in root parenchyma tissue exclusively. The results demonstrate the presence of genetic variation in soybean spermidine concentration, which could be exploited in future breeding programs. Differential distribution of spermidine within the seed could be helpful in food processing for either enriching or reducing spermidine during processing. This would support the development of specialty soy-food products with an additional health benefit.

Keywords: Spermidine, ultra-high performance liquid chromatography, mass spectrometry imaging, soybean seed composition, soy-food products

Identification and validation of candidate genes controlling tocopherol synthesize pathway in soybean

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B5: Soy foods, soy bioactives, and improvement for human health

Soybean is one of the most important crop sources of tocopherol (vitamin E) for human. Tocopherol has four isoforms, namely α-tocopherol (α-Toc), β-tocopherol (β-Toc), γ-tocopherol (γ-Toc), and δ-tocopherol (δ-Toc). The ratio of α-Toc, an isoform with the highest vitamin E activity in human, is lower in soybean in comparison with other oil crops. To identify quantitative trait locus (QTL) and their candidate genes controlling tocopherol synthesize pathway, a recombinant inbred line population (RIL) developed from a cross between soybean cultivars 'K099' and 'Fendou 16' was used. The RIL population was cultivated in field conditions in three years (2017, 2020, 2021) and the seeds harvested from each RIL were subjected to tocopherol analyses by HPLC. A genetic map constructed with 1,448 GRAS-Di (genotyping by random amplicon sequencing-direct) and 176 SSR markers was employed for QTL analysis. As a result, a total of 40 22 QTLs for seed tocopherol contents and ratios were identified. Of these, two QTL clusters in chromosomes (Chr) 9 and 14 produced consistent large effects on of tocopherol composition across the three years. The QTL cluster localized on Chr 9 might correspond to the γ-TMT3 gene, which controls the conversion of γ-Toc to α-Toc. The QTL cluster localized on Chr 14 was novel, which might control the conversion of MPBQ (a precursor of δ-Toc) to DMPBQ (the precursor of γ-Toc). The effect of the QTL cluster on Chr 14 was validated in a pair of near isogenic lines and its candidate gene, Glyma.14G132800, was mined. The identified QTLs and candidate genes might be used in the breeding programs aiming to improve α-Toc content in soybean seeds.

Keywords: Soybean, quantitative trait locus (QTL), tocopherol, candidate gene

Soybean isoflavone molecular breeding in China

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B5: Soy foods, soy bioactives, and improvement for human health

Soybean isoflavones are secondary metabolites of great interest owing to their beneficial impact on human health. Therefore, it is more important to develop soybean cultivars with high isoflavone content by molecular marker assisted breeding. Firstly, we established the HPLC/UPLC determination method to analyze isoflavone content in over 2000 soybean accessions in multiple environments, and elucidated the geographical distribution of isoflavone components in China, and selected some soybean accessions with high isoflavone content. Secondly, we also constructed the RIL populations using high/low isoflavone parents, and assembled the high-density genetic linkage map based on SLAF-seq method. Furthermore, we identified the QTL underlying isoflavone content using linkage analysis, BSA-seq, GWAS methods. Thirdly, we determined a C2H2 zinc-finger TF gene, GmZFP7 contributes to isoflavone accumulation by regulating the expression of the gateway enzymes (GmIFS2 and GmF3H1) of competing phenylpropanoid pathway branches to direct the metabolic flux into isoflavone using map-based cloning method. Based on BSA-seq and WGCNA methods, we found a hub gene GmIE3-1, which significant influence on isoflavone accumulation using VIGS and RNAi hairy root transformation. Moreover, according to GWAS and WGCNA methods, we also identified four hub genes, encoding basic-leucine zipper (bZIP) transcription factor, MYB4 transcription factor, early responsive to dehydration, and PLATZ transcription factor, respectively, which significantly influenced isoflavone accumulation in soybean. Finally, we developed four soybean cultivars with high isoflavone content and high-yielding, including ZD27, ZH68, ZH102 and ZH203, using molecular assisted breeding.

Keywords: Soybean, isoflavone, HPLC, GWAS, BSA-seq, WGCNA, cultivar

Sustainability assessment of soybean supply chains: concepts, methods and insights

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B6: Assessing sustainability of soybean supply chains

Soybeans provide manifold services and benefits to the global food system. As the most widely grown legume crop worldwide with a very high protein yield per area, it strongly contributes to the protein supply for animals and humans. Being a legume crop, it can produce large amounts of high quality protein without needing nitrogen fertilisers, which mitigates several environmental impacts related to N fertilisers and N emissions. Legume-based cropping systems lead e.g. to reduced climate change, fossil energy use, eutrophication and acidification impacts. However, soybean is often grown in previously deforested areas e.g. in South America, which is associated to very high emissions of greenhouse gases and loss of biodiversity. To ensure that the production and use of soybeans lead to an overall improved sustainability, a comprehensive assessment is crucial. This should encompass all phases from soybean production including trade and processing, to its use as animal feed and food for humans. Hereby, all dimensions of sustainability need to be addressed, including the environmental, economic, and societal dimensions. An overview of the main concepts and methods will be given. The life cycle assessment (LCA) methodology is well established to assess the environmental impacts of products and supply chains. Numerous LCA studies have shown the benefits of legumes in animal feed rations and human diets. However, soybeans should not be considered in isolation, but the changes in the whole diet or production system have to be included. It should not be overlooked that the use of soybeans in animal diets competes with human nutrition, which can be quantified by specific indicators for feed-food competition. In the last years, there is growing interest in alternatives to animal-sourced food and soya-based alternatives generally rank among the best choices from the environmental and nutritional perspective.

Environmental impact of soybean products in the GFLI database

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B6: Assessing sustainability of soybean supply chains

The Global Feed LCA Institute (GFLI) is an independent animal nutrition and food industry institute with the purpose of developing a publicly available Animal Nutrition Life Cycle Analysis (LCA) database to support the meaningful environmental assessment of animal nutrition products and stimulate continuous improvement. The GFLI database allows feed, livestock and aquaculture sectors to use LCA data based on a harmonized methodology that is PEFCR Feed compliant to calculate the environmental footprint of animal products in a transparent and trustworthy manner and make meaningful comparisons.

The database currently contains more than 1500 secondary feed datasets and will be regularly expanded in coverage and geography when new projects have been finalized. The current database contains soybean cultivation from 29 different countries as well as soybean processing into meals and oils for 14 different countries. All of the current soybean and soybean derived products in the GFLI database are based on statistical approach.

The environmental impact of soybean cultivation and soybean derived products varies considerably around the world. This is due to statistical differences in yield of the soybean cultivation (kg/ha), energy use for machinery, energy use for irrigation and synthetic fertilizer use. Another important impact is the impact of land use change that is related to soybean cultivation. This is especially high for South American countries, where soybean cultivation has expanded over the last 20 years, partly at the expense of forests.

The environmental impact data of the GFLI secondary data can be used to determine the environmental impact of animal feeds, but also sets a benchmark for company specific data. The GFLI Institute is exploring the possibility of accepting branded (i.e. company/product-specific) data. Hereby GFLI hopes to bring the best data available for various sectors and the LCA community in general.

Disclosure of certified Donau Soja/Europe Soja soybean cultivation datasets

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B6: Assessing sustainability of soybean supply chains

Blonk Sustainability has supported Donau Soja to disclose life cycle inventory (LCI) datasets for soybean cultivation representing key European producing countries. The creation of high-quality LCI datasets is key for robust environmental footprinting studies and is attracting growing interest by private and governmental actors. Under the context of the European Green Deal and the single market for green products, the European Commission is working on implementing the Product Environmental Footprint (PEF) guidance, where such data is needed. Moreover, initiatives like the Global Feed LCA Institute (GFLI) are built to increase LCI data availability for the sector. The soybean datasets have been created in compliancy with the PEF guidance and the requirements of the LCI databases Agri-Footprint and GFLI. Blonk Sustainability conducted a Life Cycle Assessment of Donau Soja / Europe Soya certified soybean farms in four different countries: Serbia, Croatia, Ukraine, Romania. Primary data collection originated from a defined set of soybean farms that are representative of the four countries. Data was collected for all relevant activity data such as: fertilizers application, pesticides application, irrigation, fuel use, land occupation, and land use change. In the results interpretation, a major focus was given to climate change, where all countries score quite similarly, between 0.3 and 0.4 kg CO_2 eq./kg soybean. The most impactful activities within cultivation are related to the direct emissions occurring on farm, and in a minor fashion the production of agrochemical products and the burning of fuel used in mechanical operations. Through sharing primary data, stakeholders and other interested parties can identity the hotspots within the supply chain and understand how the geographical location of cultivation activities might influence the environmental performance of soybean cultivation. It can also help to support policy creation and decision-making for the stakeholders within the value chain.

Keywords: LCI, data, environmental footprint

The role of soybean for re-designing European cropping systems

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B6: Assessing sustainability of soybean supply chains

Legume-supported cropping systems affect environmental, production and economic impacts. In Europe, legume production is still marginal with grain legumes covering less than 3% of arable land. A transition towards legume-supported systems could contribute to a higher level of protein self-sufficiency and lower environmental impacts of agriculture. Suitable approaches for designing legume-supported cropping systems are required that go beyond the production of prescriptive solutions. We implemented an interactive research process with scientists and advisors in 16 study areas in nine European countries to understand, explore and develop cropping systems with a focus on soybean and other grain legumes. The results of 24 rotation comparisons showed that grain legume integration decreased N fertilizer use and nitrous oxide emissions (N_2O) in more than 90% of the comparisons with reductions ranging from 6-142 kg N ha^{-1} and 1-6 kg N_2O ha^{-1}, respectively. In over 75% of the rotation comparisons, cropping systems with legumes had lower nitrate leaching. Protein yield was in over 80% of the legume-supported cropping systems higher than in the reference systems and highest protein gains were found in soybean-supported systems with up to 1,338 kg ha^{-1} crude protein. Energy yields were lower in legume-supported systems in more than 90% of all comparisons. Cropping systems with legumes had lower standard gross margins in 15 cases of all comparisons. Considering only soybean-supported rotations, 6 out of 11 comparisons showed higher standard gross margins than the reference systems considering the prices of 2015-2019. This outcome would change with higher prices for soybean grain and higher fertilizer costs in the reference systems. Here we demonstrate that re-designing legume-supported cropping systems through a process of close stakeholder interactions provides benefits compared to traditional methods and that a large-scale application in diverse study areas is feasible and needed to support the transition to legume-supported farming in Europe.

Keywords: Environment, economics, multi-criteria assessment, crop rotation, soybean

Overall solution approach of soy processing for feed

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W6: Soybean processing technologies

Soy is a valuable source of protein. At the same time, it contains a significant amount of antinutritional substances, especially protease inhibitors, which must be technologically reduced, this is done more often by heating the material. Soya is also an important source of oil (after oil palm, it is the largest source of vegetable oils for food, feed and technical purposes). Both chemical extraction and mechanical pressing are widely used to extract the oil. Extracting oil by mechanical means brings a number of benefits. Mechanical oil extraction process is based on separation oil from seed in screw press, therefore no chemicals (hexane) are used. Before pressing is oilseed usually extruded. During the process of extrusion, the flash steam is released. The amount of flash steam is up to 65 kg/t of soya bean. Recuperation of flash steam has great potential for energy savings of process of extrusion-pressing processing, which is from 20 kWh/t to 30 kWh/t. The extruder is very effective in reducing antinutritional substances and at the same time very gentle to nutritional valuable substances. It is thus an ideal heat treatment for soybeans.

Soy processing technologies – how to determine the nutritional value of soybean products?

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W6: Soybean processing technologies

Sufficient heat treatment of soybean products is necessary to inactivate antinutritonal factors, mainly trypsin inhibitors and lectins, which naturally occur in raw soybeans. Insufficienty heated soybean products are poorly digestible, cause digestive disorders and depress animal performance. At the same time, over-processing needs to be avoided as excessive heat treatment destroys valuable nutrients like heat-labile amino acids, reduces the amino acid digestibility and the energetic value. Thus, an optimal balance between under- and over-processing must be found to create a nutritionally valuable product. Near infrared reflectance spectroscopy (NIR) is a quick and reliable method to estimate the nutritional quality of soybean products with minimal efforts. NIR calibrations have been developed for proximate parameters like dry matter, curde protein and ether extract, for nutrients like amino acids and fatty acids, but also for trypsin inhibitor activity and parameters which indicate over-processing. This range of parameters extensively describes the nutritional value of soybean products, allowing for informed decisions regarding their production, trading and usage.

Food extrusion with soy-based materials

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W6: Soybean processing technologies

Extrusion processing plays a role at various stages of transforming soybeans from farm to fork. There are several forms of extrusion used for different applications, from expelling to single and twin-screw style extruders. Converting soybeans into foodstuff relies on technology, process, and final product applications. Applications range from pressing out oil and stabilizing oilcake, to production of textured vegetable proteins and everything in between such as snacks, nutritional beverages, and breakfast cereals. Food categories with soy-based ingredients continue to grow, especially meat analogue and high protein foods. Growth in these segments is highly influenced by the quality of the raw materials and their performance characteristics due to their refining. Soy varieties and processing history of soybeans affect the performance in the extrusion process. Fat and protein content of the whole beans and functionality and taste of the refined ingredients such as concentrates and isolates influence properties of finished products. Ongoing research is focused around understanding how properties of the raw materials influence texturization as well as finding ways to minimize processing steps. Soybean derived proteins have a protein score comparable to eggs and meat, making soy a key ingredient in a more sustainable food chain.

Evaluation of soybean meal substitution for fish meal on two different fish (goldfish and sturgeon)

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Production of aqua feed has to satisfy rapid growth for the food demand of aquaculture industry. Fish meal, the most limited, unpredictable and expensive constitutes in diets is a main source of protein in fish diets. Hence, the aquaculture industry is looking for some cheaper, more sustainable protein sources. Soybean meal is high in crude protein, more digestible, relatively well-balanced amino acid profile, lower price and more sustainable protein source than fish meal. So, it has been evaluated as a suitable ingredient for fish feed. Although it is known to be one of the most nutritious plant protein sources, the use of soybean meal has not been completely admitted throughout the aquaculture industry because there is concern about some biologically active compounds within the soybean-based diets that have influences on fish health, growth, and reproductive development. The main anti nutrient factor that effects on minerals availability is phytic acid. Majority of the phosphorus in soybeans is present in the form of phytate, which is not digested by fish. For investigating the potential using of soybean meal as a primary protein source, two experiments conducted on two different species with discriminate feeding habit. In the case of carnivorous and omnivorous species, Persian sturgeon and goldfish selected. The results showed that fish meal is more sufficient for Persian sturgeon, and soybean meal could be just partly an alternative protein source only if phosphorous supplied by incorporation with microbial phytase or phosphorous. Surprisingly, the results of soybean meal inclusion in goldfish diets showed better weight gain and specific growth rate. Although, the findings revealed no significant differences for condition factor and food conversion rate, an omnivorous species better growing-out through soybean inclusion in diet would be a promising achievement for designing a more efficient and cheaper diet in the future.

Keywords: Soybean, fishmeal, goldfish, Persian sturgeon, diet

Preparation of soy immunogens for producing soy-allergen specific antibodies used in immunological assays

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Soy is the most mused vegetarian protein provider and common ingredient of industrially produced foods. Beside several nutritional and health benefits, soy is classified as an allergenic source affecting 0.2-0.4% worldwide. Considered a paediatric phenomenon, soy allergy can be persistent and impacting life quality of allergic patients, even causing severe allergic reactions and anaphylaxis. Common "treatment" of food allergy is allergen avoidance based on food labelling. However, recent regulations do not indicate threshold levels but knowing the actual concentration of allergens in food is crucial for allergen avoidance and preventing allergic reactions. We develop immunoassays such as enzyme-linked immunosorbent assays (ELISA) or lateral flow devices (LFD) to detect soybean allergens qualitatively and quantitatively in food, based on the availability of soy-specific antibodies recognizing and binding to soy allergen(s), especially Gly m 4 – Gly m 6 and P34. Crucial step of antibody production is the preparation of well-defined immunogens containing high amounts of soy allergen, which are used for the immunization of Balb/c mice. Common methods to produce immunogens based on the allergen size include cut-off filtration, size-exclusion high performance liquid chromatography (SEC-HPLC) or recombinant allergens. We used these methods to produce well-defined immunogens from soy flour, verified by SDS-PAGE and Qtrap mass spectrometry, for generating soy allergen-specific monoclonal antibodies, as identified by ELISA and Western Blot screening. Those antibodies will be applied in highly sensitive and specific immunoassays for measuring soy allergen exposure in food samples by detecting the real molecular allergen amount, ideally in a personalized manner.

Innovative aspects

- Size-exclusion chromatography is a suitable method to produce well-defined soy-allergens and screening material for the production of soy-allergen specific antibodies.
- Immunoassays using those antibodies will help the food industry to perform a fast food allergen screening to identify hypoallergenic food.
- Improvement of allergen risk management of soy allergic patients.

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Consumer perception assists edamame (vegetable soybean) variety development

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Consumption of edamame has increased significantly in the U.S. over the last 20 years, but most edamame is still imported from Asian countries. There is an urgent need to establish the selection criteria in edamame breeding. The objectives of the study are to 1) identify edamame genotypes and sensory attributes preferred by consumers using sensory evaluations, and 2) explore the genetic basis of consumer desired traits using a genome-wide study. For the sensory study, untrained participants used a traditional 9-point acceptability (hedonic) scale to evaluate overall-liking, aroma, appearance, taste, and texture, and a 5-point scale to evaluate sweetness intensity of edamame varieties in multiple environments. Next, participants used a check-all-that-apply list of selected sensory terms to describe the sensory characteristics of each edamame sample. Overall acceptability of edamame genotypes was significantly different among all genotypes ($p < 0.05$). Samples described as "bitter," "sour" or "starchy" were associated with lower acceptability scores while "salty" and "sweet" were correlated with higher acceptability. The GWAS focused on sweetness (sucrose and Ala) due to its high correlation to consumer acceptability. We used an association mapping panel of 189 edamame accessions genotyped with a SoySNP50K BeadChip to identify single nucleotide polymorphisms (SNPs) related to sucrose and Ala levels in edamame beans from three locations across two years. A total of 43 and 25 SNPs was associated with sucrose content and Ala content in the edamame beans, respectively. In addition, three PI accessions (PI 532469, PI 243551, and PI 407748) were selected as the high sucrose and high Ala parental lines for the perspective breeding of sweet edamame varieties. The results of this study will help to develop edamame varieties with improved consumers' acceptance, and eventually promote edamame production as a specialty crop in the U.S.

Soybean extraction using 2-methyloxolane as a bio-based alternative to hexane – from lab to industry

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It is known that, even today, the production of soybean oil and protein is still largely dependent on hexane extraction, in spite of its known toxicity and environmental issues. Since 2012, a new bio-based solvent - 2-methyoxolane (2-MeOx) - appeared in the industry with several scientific publications showing its potential for the extraction of various oilseeds. Post graduate research identified 2-meOx as a non-toxic and economically feasible substitute for lipid and protein extraction. Therefore, the possibility to replace hexane with 2-MeOx was investigated with soybeans. First, the extraction products obtained were compared to those typically obtained with hexane. Then, the impact of the solvent switch on the extraction process was investigated, starting from lab to large-scale trials. At lab scale, analyses were focused on extraction yields, composition, and quality of the extraction products (oils & proteins). Experimentally, 2-MeOx gave higher crude extraction yields compared to hexane due to the co-extraction of more polar additional compounds such as phospholipids and isoflavones. Crude oils extracted with 2-MeOx showed enhanced antioxidant activities and oxidative stabilities. Defatted meals showed no significant impact on the % proteins or on the amino-acids profile. In 2021, based on these encouraging results, Pennakem Europa and 4 European partners (the EcoXtract project consortium) obtained funding from the European Union's Innovation Council to test 2-methyloxolane in a 10 t/day plant located in UK (New Holland Extraction) to support bringing 2-methyloxolane to the market. The plant successfully producing oils with less 1 ppm solvent and a well-defatted meal (< 1%) in trials in October 2021 and June 2022. The presentation will describe the main plant modifications and the results of the campaign. New Holland Extraction is now able to run with hexane or with the 2-methyloxolane to support the process optimization work and to serve as a demonstrator for the conversion of larger plants.

Tofu texture quality is affected by soybean proteins, storage and food processing.

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Soybean has been used for making tofu and related products for thousands of years and is a great source of plant proteins for human consumption. Proteins in soybean play a major role in the quality of tofu, particularly the firmness, which is used in commercial grading of tofu products for different cooking purposes. Both protein content and protein composition (mostly glycinin and beta-conglycinin) in the raw materials affect the tofu quality, and their quantities are primarily affected by genetics. However, postharvest storage conditions play an important role to influence tofu quality. Improper storage conditions may induce changes in protein structures and result in the failure of the proteins to form proper curd, and hence leading to the loss of economic value or total waste of soybean raw materials. Adverse conditions of storage humidity and temperature induce changes in seed cellular structures and make them hard to soak and result in less protein yield in the soymilk, which is an intermediate product during tofu making, even though soymilk is consumed as a beverage. In addition to changes in protein structures, lipids may be oxidized to produce high acidity and off-flavor during adverse storage. Aside from the raw material genetic effect and storage impact, food processing technology also plays an important role in determining the quality of tofu. Our recent studies show that protein A3 protein subunit is related to tofu firmness in addition to protein content and compositional patterns of glycinin and beta-conglycinin in soybean. This presentation will give an overview of the chemistry of proteins for tofu making and make recommendations for preservation of freshness of soybean and present a rapid method for proper use of coagulant for tofu making, and other measures for quality control of soybean for tofu making.

Aggregation and gelation of soymilk protein after alkaline heat treatment

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Soaking soybeans under alkaline conditions improves the extraction rate of protein and is therefore widely used in the production of soy products. However, the gel-forming ability of soymilk proteins is likely to change after heating under such conditions, which has an impact on tofu quality. This study aimed to clarify the effect of the thermal aggregation state of proteins after heat treatment under slightly alkaline conditions on the coagulation characteristics of soymilk. Results showed that the aggregation and binding pattern of proteins changed after slightly alkaline heat treatment (AHT). The ability of 7S and the acidic (A) subunit of 11S to bind to the basic (B) subunit of 11S weakened. These subunits converted into non-particulate proteins, which decreased particulate protein content and particle size. During gelation, the soymilk after AHT exhibited a slow intermolecular binding in the 'pre-aggregation' stage, which led to late gelation onset and a uniform gel network. With the increase in non-gelable protein content in the gel, the water holding capacity (WHC) enhanced and but the hardness decreased. Therefore, the processing technology of AHT of soymilk is suitable for the processing of unsqueezed gel products, such as silken/packed tofu. However, this way of processing soymilk results in the loss of non-gelatinizable protein through the excretion of whey when producing firm/pressed tofu. In order to improve the conversion efficiency of soymilk protein after AHT, this study also investigated the effect of different coagulants, including the addition sequence, on the aggregation behavior of soymilk protein and the quality of tofu. Results showed that introducing acid-coagulants (AC) or AC combined with salt-coagulants, each protein subunit to aggregate into particles indiscriminately to form a continuous, uniform network structure and the recovery rate of the protein increased by about 10%, while the textural qualities of the tofu are also improved.

Keywords: Alkaline heat treatment, coagulation, protein conversion rate, tofu

Textured soy protein (TSP) inclusion in selected local dishes from Nigeria: impact on the sensory attributes and acceptability

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The sensory qualities and general acceptance of textured soy protein (TSP) added to the southern Nigerian dish egusi (white seed melon, Cucumeropsis mannii) broth (TSP-egusi) and stew sauce (TSP-sauce) were examined in this study. Twenty trained panelists participated in the evaluations of the organoleptic quality and acceptability of thirty TSP samples from different producers (Harvest Innovation TSP crumbles and chunks (6), Wenger TSP from concentrate and flour (6), and ADM Textured Vegetable Protein (18)). The swelling ratio for samples BST (ADM Textured Vegetable Protein, PRODUCT CODE 165109) and BSP (WENGER TSP from Concentrate) ranged from 2.05 to 5.39; the mean value was 2.61 overall. There were significant differences in acceptability (P 0.05) between soups and sauces. The best samples of TSP-stew were made with OST and RST from Harvest Innovation Hisolate Texsoy Chunks. The worst sample was made with IST from ADM Textured Vegetable Protein TVP U–105. Sample RST was the best for TSP-soup, and the Harvest Innovation Hisolate Texsoy Crumbles (sample code ASP) and ADM Textured Vegetable Protein TVPU–814 (sample code GSP) were the best for TSP-stew. The TSP samples that rehydrated best had a good swelling ratio, which meant that a small amount could make enough protein for a poor household. Harvest Innovation Hisolate Texsoy Chunks were well-liked in TSP-stew. Harvest Innovation Hisolate Texsoy Crumbles and ADM Textured Vegetable Protein TVPU–814 CH 12 UNP PRODUCT CODE 165814 were both generally accepted for TSP-soup. TSP could be incorporated as a substitute for meat or fish in the researched dishes to obtain acceptable products and improve nutritional quality without affecting their taste. Soups and stew sauces made with TSP could help people in developing countries who can't afford meat to get enough protein in their diets.

Keywords: Textured soy protein, egusi soup, stew, sensory properties, acceptability

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Analysis of key factors and formation mechanism of Douchi flavor components

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In order to clarify the key factors affecting the production of the flavor components of Chinese traditional fermented Douchi and their formation mechanism, this study determined the influence of core functional microorganisms on the flavor of Douchi based on high-throughput sequencing, modern analytical instrument detection and biomimetic technology, and analyzed its functional role in the fermentation process. On this basis, Douchi strains with high yield of flavor substances were screened out, and the fingerprint of Douchi flavor components was established. The results showed that the main flavor compounds in Douchi were the characteristic alcohols and aldehydes in soybeans, mainly 1-hexanol, 1-octen-3-ol and nonanal, and 2-methoxyphenol, 3-octanone contribute to the characteristic aroma of pickled vegetables, mushrooms, earth and stinky feet to Douchi. *Aspergillus* and *Tetragenococcus* were the dominant strains in the pre-fermentation stage of soybean meal, and *Staphylococcus*, *Bacillus*, *Brevibacterium*, and *Millerozyma* were the dominant bacterial genera in the later stage of fermentation. There were 6 bacterial genera and 13 fungal genera that were highly correlated with the flavor components of Douchi during fermentation. RDA analysis showed that *Millerozyma* was positively correlated with reducing sugar concentration, alkali protease, and amino acid nitrogen, pH, acid protease and total sugar concentration were positively correlated with *Bacillus*, and total sugar concentration had the greatest impact, indicating that environmental factors have a good correlation between the core functional flora and the quality of samples. The research results provide some theoretical guidance for the analysis of the formation mechanism of flavor components in Douchi and the development and utilization of flavor components.

Keywords: Douchi, flavor components, dominant microorganisms, key factors, formation mechanism

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Soybean nutritional quality: introduction of the winter cover crops in soybean rotations

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Soybean (*Glycine max* L. Merill) is a high-value crop due to its high content of protein and oil, which contribute nutrients to both humans and animals. The objective of this study was to evaluate the effects of the winter cover crops on soybean nutritional quality. For this purpose, near-infrared spectroscopy was used, due it is an extremely potent tool in plant-related field of research for analysis. It is commonly used to estimate characteristics of grain including protein, starch, and oil content. Based on reliable calibration models, the spectral characteristics of the examined sample are related to the content of the component of interest. After mulching of cover crop biomass and conservation tillage, two soybean varieties were sown (NS Mercury, 00 maturity group and NS Altis, 0 maturity group). The field trial was grown under conventional production in agroecological conditions of Southeastern Europe at two sites (Rimski Šančevi and Čurug locations) in 2021. A pure rye crop (1) and mixture of peas and oats (2) were sown as a cover crop, while the control treatment (C) was an area without cover crops (period autumn – spring). Total protein and oil content of soybean, were analysed by Antaris II Thermo Scientific FT-NIR, while OMNICTM software was used for data processing and calibration. The relation between the seed quality parameters and use of cover crops was seen through obtained results. The average protein content of different genotypes was in the range of 35.67 to 44.30, while the oil content ranged between 18.78 and 23.22 % of dry matter. The obtained results indicate that there is a statistically significant difference between the average protein and oil content within the cultivars and within the locations.

Keywords: Glycine max, protein content, oil content

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A bean characteristic model for thermal processing of soybeans

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In thermal processing of soybeans, the product quality is primary influenced by processing conditions and bean characteristics. A change in the bean characteristics requires an adjustment of the process conditions to ensure consistent product quality. Currently, this adjustment is done subsequently after analysing different parameters of the already processed product. This means a retrospective way for adjustment where damage and faulty processing, up to 15 percent of the processed soybeans, may be the consequences. On the other hand, nutrient and energy losses by the fact of too intensive treatment are also possible adverse effects. In the present study, a prospective solution for process adjustment using a model based on bean characteristic is presented. Therefore, an initial model was developed using 60 soybean samples. The samples differed in defined bean characteristics, growing region and variety. The defined bean characteristics such as grain size, protein-, oil- and inhibitor content or thousand-corn-weight were analysed before the thermal treatment using a laboratory roaster. The bean characteristics were collected using chemical, physical and imaging techniques. Changes in processing quality expressed by protein solubility in potassium hydroxide (KOH) and trypsin inhibitor activity (TIA) due to bean characteristics are described by the use of support vector regression and multivariate linear regression. The applied methods for characterization, the relationships between bean characteristics and treatment as well as the accuracy and reliability of the best fitting model are presented. As result a Pearson correlation of 0.555 could be reached.

Keywords: Thermal processing, soybean characteristics, regression model

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Mechanism of the glucono-δ-lactone induced soymilk gelation: enthalpy and entropy transformation in the cross-linking of protein molecules

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This study aimed to explore new techniques to regulate the quality of soy products. The glucono-δ-lactone (GDL) induced soymilk gelation process and the gel network structure characteristic were compared as a matter of temperature, and the role of reaction kinetics was discussed. Results showed that there were similarities in the development of G′ curves under different temperatures, whereas the gel network structures and the energy requirements of cross-linking reactions were different. In the high-temperature region (70 °C–95 °C), the exposure and binding of reactive groups were promoted. The activation enthalpy (ΔH^*) required by protein aggregates decreased and the effect of entropy reduction ($-T\Delta S^*$) was enhanced, which led to shorten the preaggregation time (tg) and increase the gelation rate (k), resulting in the formation of rough, porous gel network with high stiffness. By contrast, in the low-temperature region (40°C – 70°C), high enthalpy contributions and low entropy changes were required, then a fine, soft, and tender gel network formed. Besides, a funnel-shaped model of the enthalpy–entropy energy transformation mechanism of soymilk gelation was proposed. The results of this study revealed that adjusting the enthalpy–entropy energy requirements of the protein cross-linking reaction could be utilized to the regulation of the network structure and quality of soymilk gels.

Keywords: Enthalpy, entropy, rheology, gel structure, soymilk gels

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Study on the effect of soy protein subunit composition on the tofu yield and quality

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In recent years, the market demand for high-quality raw materials soybeans for the processing and production of soy products has continued to grow rapidly. A large number of studies have confirmed that the storage protein content of raw soybean, 7S (β-conglycinin) and 11S (glycinin) globulin components have extremely important effects on the yield, texture properties, taste and nutrition of tofu. Although there are many studies on the relationship between soybean 7S and 11S globulin components and tofu processing quality, the results are not the same. In order to further analyze the effect of protein composition characteristics of raw soybean on the formation of tofu gel, this study focused on the effects of subunit composition of high protein soybean raw materials on the yield, hardness, protein composition change and protein gel microscopic network structure of tofu. The specific reasons for the differences in yield and quality of tofu made from different raw materials of soybeans were also clarified. The results showed that the ratio of 11S/7S globulin components in soybean seeds is a good indicator for evaluating the yield and texture properties of tofu. The difference of protein composition in soybean seeds will lead to the difference of pH value in soybean milk, and then affect the coagulation of soybean protein by coagulants and tofu yield. The soybean seed size is another important factor affecting the yield of tofu. In addition, the tofu made from soybean seeds with high content of 7S globulin and β subunit has soft texture and more pores in protein gel network structure. This study will provide a theoretical reference for improving the quality and yield of tofu.

Keywords: Soybean, tofu quality, storage protein component, 11S/7S ratio, tofu yield

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Multilinear regression for estimation of KOH-solubility and TIA in soybeans

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In order to optimally digest the protein of soybeans, soybeans have to be technologically treated (mostly by heat treatment) to destroy its antinutritional factors (especially trypsin inhibitors (TIA)) before consumption by monogastric animals is possible. Nevertheless, if the heat treatment is too intensive, protein digestibility can also be reduced (measured via KOH solubility). Thus, the art of thermal treatment of soybeans is to treat in as much as necessary but also not too intensively. Unfortunately, the optimal process conditions, which are controlled by temperature and duration, can vary between different soybean varieties or by other extrinsic factors (fertilizer status, drought stress). The aim of this study was to develop a plant model that describes the relationship between the process variables (temperature and duration) of a processing plant and the resulting quality (TIA/KOH) of the product using multilinear regression. In relation to the whole bean, the regression equation for KOH ($R^2 = 0.9089$ ($p<0.0001$)), in addition to the factor “multi” (= temperature x duration), the parameters dry matter, and the color parameters L and b were found. For TIA ($R^2 = 0.6944$ ($p<0.0001$)), in addition to “multi”, the parameters dry matter, crude protein, and the color parameters L and b were found. From this it can be deduced that the thermally treated bean can already be used to optimize these nutritionally relevant parameters. By transforming the multilinear regression equation, it should now be possible to calculate the minimum value of the factor "multi" (= duration x temperature) at which the nutritional-physiological factors (KOH and TIA) are still in a range at which no performance losses are to be feared. At the same time, the energy input required for toasting could thus be reduced to a minimum.

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Evaluation of off-flavor in soymilks made with Mexican soybean [*Glycine max* (L.) Merr.] cultivars

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There are two soybean breeding programs in Mexico that generate materials adapted to maturity groups VIII and IX. Little is known about the organoleptic attributes of Mexican soybean materials, therefore, we assume that soybeans of Mexican varieties present unpleasant flavors, such as beany flavor. It has been reported that low beany notes and a high milky attribute, as well as low rancidity, favor an acceptable flavor in soybean drinks. In this work, a descriptive sensory analysis was carried out to characterize the off-flavors present in 13 soybean drinks of 11 Mexican varieties and two varieties from the United States that were reported by the NIAS Japan Soybean Germplasm Bank to be free of lipoxygenases that cause a beany flavor, and one commercial control drink. The result of the descriptive sensory analysis indicated that the soybean drink of the Mexican variety Guayparime S-10 with the lowest value and the U.S. varieties JP-30790 and JP-28955 with low notes stood out in the beany flavor. In the milky attribute, the Huasteca 700 and Vernal varieties stood out with high values and the JP-30790, JP-28955 and Guayparime S-10 materials with intermediate values, and in the rancidity attribute, all the soybean drinks analyzed stood out with low scores. This study has shown that soybean varieties grown in northern Mexico present contrasting flavor characteristics that affect the palatability of the soybean drink. Soymilks of genotypes JP 30790 and JP 28955 were expected to show no significant difference with the control drink, because they were reported to be lipoxygenase free, however, they presented a higher beany flavor than the control soymilk and the one of Guyparime S-10 variety.

Keywords: Soymilk, off-flavor, beany flavor, lipoxygenases

The processing characteristics of soy protein isolate at subunit level: Exploring the impacts of α and α′ subunits on SPI gelation and foaming properties based on natural hybrid breeding varieties

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Soy protein isolate (SPI) is widely used as a gelling agent and foaming agent in the food industry due to its excellent processing characteristics, but little is known about its important protein structural characteristics. As a key in elucidating the structure-function relationships of SPI, subunit composition can explain many functional properties of soy protein. The gel and foams properties of SPI deficient in β-conglycinin subunits (α-lack, α′-lack, and (α+α′)-lack) were explored in this study. The results showed that the lack of α′ subunit significantly enhanced ($p < 0.05$) the gel strength and water-holding capacity of the resultant gels. The α′-lacking gel had a more uniform and denser network structure. Subsequently, the lack of α′ subunit increased the zeta potential, surface hydrophobicity ($H0$), and fluorescence intensity of the gel. These changes in the physicochemical properties of the gel affected their heat-induced gelation. In addition, gel solubility results signify that hydrophobic and disulfide bond interactions are the main forces in the gel formation process. The microstructure and foaming properties of α′-lack SPI were better than other SPI, which indicated that the α subunit in α′-lack SPI could generate fine bubbles with high stability. The interfacial shear rheological index of α′-lack SPI was also the highest, so subunits may be related to the firmness of the air-water interface, foam rheology was shown to correlate not only to interfacial tension but also to the protein solutions' interfacial elasticity. These results indicated that component of subunits determined the different characteristics, and the purposeful selection of special soybean variety with special protein subunits or components for protein processing research could provide important theoretical guidance for the development and application of soybean protein products and further promote the development of deep processing of soybean protein.

Keywords: Soy proteins isolate, subunit-deficiency, gelling property, air-liquid interface, foaming properties

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Analytical verification of the geographical origin of soybeans – unlocking traceable and sustainable crops towards trusted supply chains

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Sustainability is a key concern in the production of soybeans, as the crop has been linked to issues such as deforestation, habitat destruction, and greenhouse gas emissions. This has led to concerns about the environmental impact of soybean production and the need for more sustainable practices. One way to address these issues is through the use of traceability systems, which allow for the tracking of soybeans from their origin to the final product. Traceability can help to ensure that soybeans are produced using sustainable practices and that they are not associated with deforestation or other environmental harm. It can also help to prevent the use of illegal or unethical practices, such as child labor or exploitation of workers.

Testing soybeans to verify their geographical origin allows for the identification of crops that have been grown using sustainable and responsible practices. Further, it enables companies to ensure that they are complying with ESG regulations around the responsible sourcing of soybeans, particularly those related to labor and environmental standards. Finally, it provides consumers with greater transparency and confidence in the products they purchase, allowing them to make informed choices that align with their values and beliefs. In this presentation, the deployment of multi-isotope fingerprints (carbon, nitrogen, sulphur, oxygen, nitrogen) of soybeans, using Isotope Ratio Mass Spectrometry is described, aiming to use a global isotope database to control supply chains and verify traceability. Challenges, pros and cons as well as future perspectives are discussed towards the implementation of high-end lab analytical technologies to the common aim of traceable and sustainable soybeans.

Biogenic amines in Chinese fermented soybean curd: emphasis on formation mechanism, microbial contribution and control methods

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Furu, a traditional Chinese fermented soybean curd widely consumed in Asia for its favorable aroma and taste. Biogenic amines (BA) are low molecular weight nitrogenous organic bases which can cause toxic effects to consumers. During fermentation, high amounts of free amino acids hydrolyzed from soybean proteins can be catalyzed by bacterial amino acid decarboxylase to form BA. In this work, quantification of 8 types of BA in furu (white, red and stinky furu) showed that tyramine, putrescine and cadaverine are the main BA which exhibited a significant positive correlation ($p<0.05$) as fermentation proceeded, while spermidine disappeared after the fermentation was completed. The BA content in stinky furu (6389.0 mg/kg – 7255.5 mg/kg) was significantly higher than that in white (2020.5 mg/kg – 2667.8 mg/kg) and red furu (581.6 mg/kg – 702.2 mg/kg) in the ripening stage. Additionally, this study carried out to investigate the changes of bacterial diversity during fermentation. A total of 213 phyla, 378 classes, 722 orders, 1137 families, 1834 genera, and 2757 species were identified in rufu using high throughput sequencing. We found that the bacterial diversity varied depending on the types of furu, and the bacterial communities altered among different stages of fermentation. Our study revealed that *Acinetobacter* was the major microflora during white furu ripening stage, yet *Lactobacillus* and *Enterococcus* was the dominate lactic acid bacteria from red and stinky furu, respectively. This work provided an improved understanding of BA content and its microbial contribution associated with Chinese furu, which laid the groundwork for controlling BA in the production of traditional fermented soybean products.

Keywords: Chinese fermented soybean curd, biogenic amine, microbial diversity, high throughput sequencing

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Spatial distribution of on-farm soybean Argentine quality

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Exports from the agro-industrial sector of Argentina represents 70% of total exports, with the soybean complex accounting for 38%. Worldwide, Argentina is the third soybean producer. Due to Argentina´s extension, soybean composition shows a wide variability. Assessing grain quality parameters like protein (% db), oil (% db) and PROFAT (sum of protein and oil % db) is, therefore, relevant for international trade. The aim of this study was to define areas with higher composition values using geospatial statistics. Eight homogenous areas were defined by experts and an interpolation analysis was applied within each area for the mentioned parameters. Grain samples of soybean as a single season crop (n=313) were collected in the whole country, provided by farmers (2020-2021), while a subset data (10%) was used for validation and a kriging interpolation approach was applied under 4 models (Gaussian, exponential, circular, spherical). The circular model fitted best for protein with a mean squared error (MSE) of 1.90%. Areas with highest protein (37-39%) were Southeast of Salta and North of Cordoba (14% of the evaluated area). On the other hand, the exponential model fitted better for oil and PROFAT, with a MSE of 1.3% and 1.5% respectively. South of Santa Fe (15% of the evaluated area) was the area with the highest oil level (23.5- 26%). The highest PROFAT value (59.5-61 %) was identified in Southeast of Salta, North of Cordoba, South of Santa Fe and Northeast of Buenos Aires (37% of the evaluated area). In conclusion, different quality areas were defined using kriging interpolation and this is the first approach using farm harvested soybean to define the spatial distribution of grain composition.

Keywords: On-farm sample, quality parameters, geospatial statistic, interpolation

Acknowledgements: This work was conducted between Argentina Agricultural National Institute (INTA) and Argentine Soybean Chain Association (ACSoja). A special gratitude to Dr. Fernando Gimenez, leader of INTA Cereals and Oilseeds Program, M.Sc. Marcelo Tolchinsky and INTA Extension Group and farmers who provided the samples and Argentine Edible Oil Association (CIARA).

Soybean grain protein and oil differences between Argentine cropping areas: an on-farm sampling approach

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Soybean (*Glycine max* (L.) Merr.) is the most important crop in Argentina. It grows in different types of environments along the national area, considering an ample latitudinal range (-21°S to -55°) and different crop management. Argentinian soybean quality was previously studied in regional or partial approaches. In order to explore differences between environments and soybean quality along the country, 544 soybean on-farm samples (crop cycle 2020-2021) from eight homogenous areas, were studied: Core (n= 243), Santa Fe Centro (n= 78), Cordoba Sudoeste (n=58), Cordoba Norte (n=43), Buenos Aires Sur (n=37), NOA (n=35), Entre Ríos (n=26) and NEA (n= 24). Each area sample number was proportional to the sown surface. Seeds composition (protein (% db), oil (% db), PROFAT (sum of protein and oil % db)) was determined using a near infrared spectroscopy (NIR) device. As a result, the means of the analyzed samples were: 36.6 ± 1.5 (% db) for protein, 23.0 ± 1.6 (% db) for oil and 59.5 ± 1.4 (% db) for PROFAT. Among the studied areas, Santa Fe Centro showed the maximum oil mean value (24.7 % db) and NOA the maximum protein (38 % db) and PROFAT values (60.6 % db). On the other hand, Buenos Aires Sur showed the minimum oil mean (21% db) and PROFAT (57.9% db) values, and Santa Fe Centro the minimum protein (35.7% db). Remarkably, a geographical indication could be useful to enhance the commercialization of high quality seeds. This ongoing study is carried on by this research group in order to validate these grain quality differences observed among areas.

Keywords: Soybean protein, soybean oil, soybean PROFAT, genotype, environment

Acknowledgements: This work was conducted between Argentina Agricultural National Institute (INTA) and Argentine Soybean Chain Association (ACSoja). A special gratitude to Dr. Fernando Gimenez, leader of INTA Cereals and Oilseeds Program, M.Sc. Marcelo Tolchinsky and INTA Extension Group and farmers, who provided the samples, and Argentine Edible Oil Association (CIARA).

A label-free aptasensor for turn-on fluorescent detection of ochratoxin A based on SYBR Gold and single-walled carbon nanohorns

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Ochratoxin A (OTA) is primarily produced by the fungi *Aspergillus ochraceus* and *Penicillium verrucosum*. OTA frequently contaminates the foodstuffs, such as maize, wheat, grape, coffee, and derived products. OTA is toxic, causes serious damage to liver and kidney, increases teratogenicity, and has been considered as a potential carcinogen (group 2B). The conventional methods for OTA detection are both financial and time costly as they contain complex steps in the preparation and detection of the samples. The tests are usually carried out by professionally trained personnel. All those features limit the application of these methods and fail to meet the detection requirements nowadays. Due to OTA's toxicity and its threats to human beings, a rapid, sensitive and precise detection method is urgently demanded. Here in this study, we demonstrated a label-free and "turn-on" fluorescent aptasensor for OTA detection based on the interaction between single-walled carbon nanohorns (SWCNHs) and SYBR Gold dye. This new fluorometric method presents major advantages including low cost, high sensitivity, and simple performance. Furthermore, due to the good selectivity, this method shows great potential for a routine test for OTA contaminations in a wider range of food sources, such as cereals, coffee, and dried fruits. In this study, the integration of nanostructures such as SWCNHs with specific recognition molecules such as nucleic acids in the detection and analysis of samples obtained from complex matrix demonstrating a development potential in the field of toxins or pathogen tests for the safety of food.

Promoting the fiber formation for high moisture extrusion cooking on soy proteins

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Texture and sensory quality are important for meat analogue products. The addition of gums in the protein matrices may provide a strategy to improve the fibrous structure by high moisture extrusion, to well mimic the texture and sensory properties of real meat. Three food gums, iota carrageenan (ICGN), carboxymethylcellulose sodium (CMC) and sodium alginate (SA), were investigated for their effects on promoting the fiber formation of extrudates. Results obtained indicated that lamellar structures were formed at the cooling zone for samples containing 6% CMC and 6% SA. The addition of 6% SA enhanced the quality of extrudates, such as improved rehydration rate and digestion rate. Furthermore, interactions between protein molecules over the extrusion process were also analyzed, and the importance of the type of molecular bonds responsible for the formation of extrudates was determined to be: disulfide bonds ≥ hydrogen interactions > hydrophobic interactions. Results of this work would be of importance in the design of meat analogs using soy protein for improved texture and sensory quality, and more important, in the understanding of protein–protein interactions during extrusion for sustainable health and improved consumer acceptance.

Evaluation of different heat processing combinations on the protein quality of soybeans and rapeseed

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The requirement for rumen undegradable protein increases over the years to supply the nutritional requirements of high-yielding animals. To increase the content of rumen undegradable protein in feeds, processes with heat are often used (i.e. expander). Nevertheless, little is known on how different combinations of heat treatments during feed production may affect protein quality and how to determine it. A trial was conducted with different heat processing combinations using rapeseed meal, high-protein soybean meal (48%) and soybean extracted meal (44%). For each feedstuff, a sample was not processed, and another preconditioned at 85°C with steam addition. Further, the feedstuffs were expanded with three different specific mechanical energy (SME) inputs: 20, 40 and 60 kWh/t. For post-conditioning, each feedstuff was kept at 95°C either with oven or in 100 l mixer with direct steam injection. Each tempering method had three time-sets (10, 20 and 30 min), totalling 23 combinations per feedstuff. Heat effect was evaluated by measuring reactive lysine, protein solubility in KOH (0.2%), and crude protein fraction C. Statistical analyses were conducted using a multifactorial analysis of variance on SAS 9.4 program. The results showed that the contents of reactive lysine and fraction C as well as protein solubility differed significantly among feedstuffs. Regarding the influence of the expander, it is evident that for those samples which were only expanded and not further processed, the contents of reactive lysine ($P = 0.0350$) and fraction C ($P = 0.0031$) differed significantly among the different expander stages. However, no significant influence was observed on protein solubility for expanded feedstuffs without further post-conditioning. Processing with expander had the greatest effect on protein solubility and fraction C, whereby the expander with low energy input is recommended. For a post-conditioning, the indicators differ depending on the feedstuff, but the shortest time is recommended.

The geoeconomy of soy production chain in Brazil and its consolidation in the global market

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In the 2020/2021 harvest, soybean production in Brazil reached more than 139 million tons cultivated in thirty-nine million hectares, which represents 45.2% of the area used by agricultural plantations throughout the country. Thus, soybean exports totaled 86.1 million tonnes in the 2020/2021 agricultural year, with the equivalent of US$ 38.6 billion. The grain, oil and bran segments added up to US$ 48 billion, representing 17.1% of all Brazilian exports and 40% of Brazilian agribusiness exports. The central question of this work was to identify the geoeconomic combinations responsible for the new dynamics of this productive chain in the Brazilian territory, and its consolidation in the world market. Cholley's geographic combinations (1964a) were used as a category of analysis. Methodologically, this text was developed through bibliographical and documentary research. Operationally, it was possible to work with primary and secondary sources in a contextualized way. Information and data were also sought in the reports of the Ministry of Agriculture, Livestock and Supply (MAPA), in the Brazilian System of Agribusiness Foreign Trade Statistics (AGROSTAT). The increase in exports of agricultural commodities was not only due to the growth in international demand, but also to gains in market share in various market niches. Even so, it is worth mentioning that there was an increase in soy exports, but Brazil retreated in exports of industrialized products due to the tariffs practiced by the main buyers. The growth of Brazilian production and the international market for soybeans is based on three major interrelated drivers: 1) Consolidation of soy as an important source of vegetable protein, especially to meet the demand of the meat agribusiness. 2) The creation of a National Innovation System, and 3) the dynamic agro-industrial competitive advantages, which in turn are conditioned to geographic combinations.

Keywords: Geographic combinations, agro-industrial comparative advantages, exports

Enzymatic activity of lipoxygenases from Mexican soybean [*Glycine max* (L.) Merr.] varieties

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It is known that undesirable flavors in soybean products are the result of the enzymatic action of the three soybean lipoxygenases (lipoxygenase-1, lipoxygenase-2 and lipoxygenase-3) (LOX-1, LOX-2 and LOX-3), encoded by the genes Lox1, Lox2 and Lox3. These enzymes catalyze the oxidation of fatty acids present in soybeans, which produce hydroperoxides of fatty acids that continue through the metabolic pathway to generate some compounds such as alcohols, aldehydes and ketones, responsible for undesirable flavors (mainly the "beany" flavor). Mexican soybean varieties are presumed to have significant enzymatic activity because soybean breeding programs in Mexico have not targeted in flavor traits in the grain. To identify the presence of the three soybean lipoxygenases and quantify their enzymatic activity, a double biochemical assay of enzymatic activity (defatted soybean extracts and non-defatted soybean extracts) was carried out in a Cintra 10e spectrophotometer, the absorbance change was measured at 234 nm and recorded every 10 seconds for three minutes. The presence of LOX-1, -2 and -3 was identified in the soybean materials analyzed and variability of the enzymatic activity of LOX-1, LOX-2 and LOX-3 was observed. Defatted soybean extracts showed lower enzymatic activity than non-defatted extracts. LOX-1 showed higher enzyme activity at the population level. Guayparime S-10 of recent harvest, Huasteca 300 and JP 28955 were the varieties that presented lower enzymatic activity. The study also showed that JP 28955 and JP 30790 (reported free of lipoxygenases by the NIAS Japan Soybean Germplasm Bank) had LOX activity. Guayparime S-10 of recent harvest was presumed to have the lowest enzyme activity due to its short storage time.

Keywords: Soybean, enzymatic activity, lipoxygenase, beany flavor

The ability of productive energy (Arkansas Net Energy) to predict feed intake and feed conversion ratio for broilers as compared to metabolizable and classic net energy

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Predicting feed intake and performance is important for industry nutritionists. Arkansas Net Energy (Ark-NE) is productive energy and dependent upon performance. Objective was to develop models to predict feed intake (FI, g/bird/d) and feed conversion ratio (FCR) for broilers as a function of the dietary contents of Ark-NE, apparent metabolizable (AME, AMEn), and classic net energy (CNE). Broilers in four experiments (E1 to E4) were fed diets according to Cobb 500 guidelines for 56 d. A total of sixty pelleted diets were provided for five feeding phases. The diets included a partial replacement of SBM with cellulose (E1) or corn (E2) and increasing oil (E3) or total digestible amino acids (E4). Body composition (Dual Energy X-Ray Absorptiometry), heat production (respiratory chambers), and performance were determined. Mixed models in JMP were used to model the data, with the experiment a random factor. FI and FCR were transformed (Ln) for convenience to improve regression assumptions. Models based on Ark-NE were: $\text{Ln FI} = -2.77 - 0.000097\ \text{Ark-NE} + 0.038\ \text{age} - 0.00159\ \text{age}^2$ ($\text{adj}R^2 = 0.94$) and $\text{Ln FCR} = 0.6646 - 0.000225\ \text{Ark-NE} + 0.0112\ \text{age}$ ($\text{adj}R^2 = 0.81$). Only the models based on Ark-NE showed a consistent and inverse relationship between Ark-NE and FI or FCR. AME and AMEn were not able to predict FI ($P = 0.3768$ and 0.4271, respectively). The relationship between CNE and FI was inconsistent among studies. The FCR was not consistently responsive to AME, AMEn, and CNE. When Ark-NE, AME (or AMEn), and CNE were included simultaneously as predictors of Ln FCR, the model ($\text{adj}R^2 = 0.81$) showed no collinearity, a strong influence of the ArkNE ($P<0.0001$), and no significant ($P>0.66$) effect of AME, AMEn, or CNE on FCR. In conclusion, Ark-NE is consistently a better predictor of FI and FCR than AME, AMEn, and CNE.

Keywords: Arkansas Net Energy, productive energy, metabolizable energy, net energy, predicting feed intake, broilers

POWR1 is a domestication gene pleiotropically regulating seed quality and yield in soybean

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Seed protein, oil content and yield are highly correlated agronomically important traits that essentially account for the economic value of soybean. The underlying molecular mechanisms and selection of these correlated seed traits during soybean domestication are, however, less known. Here, we demonstrate that a CCT gene, POWR1, underlies a large-effect protein/oil QTL. A causative TE insertion truncates its CCT domain and substantially increases seed oil content, weight, and yield while decreasing protein content. POWR1 pleiotropically controls these traits likely through regulating seed nutrient transport and lipid metabolism genes. POWR1 is also a domestication gene. We hypothesize that the TE insertion allele is exclusively fixed in cultivated soybean due to selection for larger seeds during domestication, which significantly contributes to shaping soybean with increased yield/seed weight/oil but reduced protein content. This study provides insights into soybean domestication and is significant in improving seed quality and yield in soybean and other crop species.

Evaluation of new phosphorus sources for the northern region of Mato Grosso, Brazil

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The objective of this study was to compare traditional sources of phosphorus with new technologies in order to offer to farmer’s operational advantages, making the operation less costly, besides to allowing the replacement of S jointly. The trial was installed in an experimental area at Embrapa Agrossilvipastoral, in the municipality of Sinop, Mato Grosso, Brazil, in a Dystrophic clayey Haplustox soil. The Experiments began during the 2020/2021 soybean growing season, followed by second-crop of corn in the same season, for two consecutive years. The experimental design adopted in the experiments was a randomized block design, with six treatments and four replications. Treatments consisted of NPK fertilizers with the following compositions: 12:42:00 + 10%S (50% S0 + 50% SO4+); 11:42:00 + 10S (50% S0 + 50% SO4+); 0:40:00 + 12%S (100% SO4+); TSP 00:45:00 (without S); Mix (SSP + TSP) and a control, without fertilizers. The N, Ca, and S amounts were balanced in all treatments to not interfere with the evaluations. Complementary fertilization with nitrogen, potassium, and micronutrients was in the sowing line. Soybean sowing was in October, and corn second crop in February, following the region's sowing calendar. We evaluated soybean and corn grain yields and their respective yield components in two consecutive harvests. The results observed for soybean did not show differences for any of the treatments, except for the number of pods per plant, in the 2021/2022 season. Concerning maize, there were differences in height, number of grains per row, and grain yield. For the 2022 crop season assessment, treatments differed in height and grain yield. When used by farmers, the newly formulated fertilizers containing sulfur can guarantee the nutrient supply and reduce application costs, with no differences from traditional fertilizers.

Amino acid, protein, fat content and length of growing period of soybean genotypes grown in north-eastern Europe

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Soybean is not widely cultivated crop in Estonia (north-eastern Europe), but this may change in the future due to climate warming. Soybean is an important protein and oil crop for human consumption and animal feed. This crop is excellent source of essential amino acids. It's important to know the content of biologically active compounds and nutritional value of soy that have grown in local conditions. The aim of this study was to evaluate the amino acid (AA), protein (PR) and fat (FA) content as well as length of growing period (LGP) of soy genotypes grown in Estonian conditions. The field experiment with seven genotypes was conducted, using 5 m2 plot size in four replications at the Estonian Crop Research Institute in 2017 and 2018. The weather conditions were different in tested years. In 2017, the weather was mostly cool and humid, whereas the hot and dry conditions predominanted in 2018. The AA content was measured by LC-UV method, PR content by Kjeldahl and FA content by INFRATEC. Data were analysed with software package Agrobase 20 using ANOVA and Pearson's correlation analyses. The results revealed that there was no significant impact of genotype and year on PR and FA content of soy in tested years. Nevertheless, the significant ($p \leq 0.05$) differences occured in LGP and some AA content between years and genotypes. Growing period of soybean was longer (156 days) in 2017 compared to 112 days in 2018. AA content was higher in 2017. In addition, a clearly significant negative correlation ($r = -0.954$) was found between PR content and LGP in 2017. Furthermore, the correlation analyses showed that there were significant positive relationships between different amino acids in 2017, while in 2018, there were less correlantions found.

Keywords: Soybean, amino acid, protein, fat, length of growing period

Acknowledgement: This study was supported by Applied Research Program of the Ministry of Rural Affairs of Estonia.

Theme C:

Agronomy, physiology, and agrotechnology

Sessions and workshops *(chair)*

C1 Yield and seed composition response to environment *(Seth L. Naeve)*
C2 Using data-driven knowledge for profitable crop management *(Paul Esker, Seth L. Naeve)*
C3 Soil fertility and plant nutrition *(Hong Liao)*
C4 Soybean rhizosphere and nitrogen fixation *(Angela Sessitsch)*
C5 Seed production, vigour and technology *(Julia Buitink, Michael Gohn)*

W1 Soybean for Africa *(Brian Diers)*
W3 Knowledge exchange for European soybean agronomists *(Leopold Rittler)*
W4 Science For Success: A model program for applied research and extension outputs *(Rachel Vann)*

Posters C: Agronomy, physiology, and agrotechnology

Potential of soybean for relay cropping systems: what we know and what we don't?

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C1: Yield and seed composition response to environment

Relay cropping is a multiple cropping system that consists in planting a second crop (i.e. relay crop) into a standing first crop (i.e. primary crop) prior to the first crop harvest allowing two potential harvests per year. Relay cropping has potential to address food security and environmental sustainability via spatio-temporal diversification of cropping systems. This is especially the case for soybean that presents key characteristics required for a relay crop including the market availability of a wide panel of genotypes, capacity to resist to and recover following mechanical damages via ramification, good combination as a relay crop of small-grain cereals etc. Despite these potential advantages, soybean as relay crop is poorly adopted worldwide, especially in Europe. For instance, in France and Europe, there has been a steady increase in soybean acreage in the last years under conventional cropping (i.e. crop grown in pure stand with traditional sowing date) while no official record exists to date on soybean grown by farmers under relay cropping. This lack of adoption of the crop under relay cropping could be due to several technical locks characterizing relay cropping system in general. A better understanding of the factors affecting adoption of soybean under relay cropping is therefore a prerequisite to put in place key mitigation strategies. In light of this, and based on a review of grey and scientific literature as well as the authors' practical experience, here we report key knowledge gaps affecting the adoption of soybean under relay cropping with a particular emphasis on crop management complexities. Finally, we propose three research priorities to fill the current knowledge gaps in soybean relay cropping research and implementation, worldwide.

Keywords: Competition phase, food security, multiple cropping, primary crop, recovery phase

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Redefining soybean critical period

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C1: Yield and seed composition response to environment

The concept of critical period for yield determination is useful to design and manage crops in order to maximize resources use efficiency. Currently, a relatively long period of more than 40 days during the late reproductive stages is considered as the most critical for soybean yield determination. There is controversy on the developmental stages for the start and the end of this period. Furthermore, no attempts have been made to increase the temporal resolution of the effect of the stress within this wide critical period. Our objective was to define the critical period for soybean by assessing a high temporal-resolution dynamic of the effect of stress on yield and yield components. We imposed short and intense carbon deprivation stresses by shading (75%) during ten different nine-day periods from V2 (two leaves) to R8 (full maturity) plus an un-shaded control over a crop that was growing under potential conditions in St. Paul Campus (UMN) during 2022. The critical period for grain yield determination defined by a significant yield depression because of shading started at the R4 stage (full pod) and continued for 38 days until R6.5 stage (full seed). Within this wide period, the sensibility to the stress was not even, the maximum yield depression (23%) was registered when the stress was imposed between R5 (visible seed) and R5.5 (half seed) growing stages. The dynamic for the effect of the stress on kernel number and weight per kernel was also assessed. Results will be relevant for crop management adjustment and design, for fito-centric approach for pest management, and to predict or simulate the impact of weather on yield.

Keywords: Critical period, yield depression, kernel number, wheight per kernel, stress

Using on-farm research to identify highly productive regional soybean crop production systems

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C1: Yield and seed composition response to environment

Soybean (*Glycine max*) crops have been introduced into new producing areas of Argentina since 1990. The flooding Pampa was a traditional beef cattle raising area with little agriculture and agricultural research experience. For this reason, an on-farm research program was established by AACREA regional producers to identify the most productive and stable soybean crop system. We hypothesized that the variety maturity group (MG) in combination with the sowing date was crucial to obtain high and stable yields of the crop under the variety of environments prevailing within the region. On-farm experiments using large plots and conventional machinery and technologies were set and conducted by farmers to assess different soybean cultivars in a wide range of sowing dates and environments (climate and soil type) between 2010 and 2018. Yield and yield components were determined. A continuous learning-through-experience process helped to improve crop performance in the region; meanwhile, a wide database was conformed and used to test the hypothesis. In the analysis, the cultivars used were grouped into three maturity groups: MG-III, MG-IV, and late-maturing MG-IV together with early-maturing MG-V. Sowing dates included experiments grouped in three periods: Early: from 1st October to 5th November; Medium: from 6th November to 25th November; and Late: from the 25th November onwards. Data was analysed using RStudio and InfoStat software. Grain yield was highly dependent on crop grain number determination, but grain filling conditions significantly explained differences in the yields of the various experiments established by farmers. Maturity group IV sowed at early and medium sowing dates was the most productive and stable crop system. Data collected by farmers on their own farms proved to be useful to help them improve their soybean crop systems using local information. Confidence in large-scale plot results allowed a quick and effective technology transfer with significant regional impact.

High temperature responses vary among soybean genotypes in open-air field conditions

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C1: Yield and seed composition response to environment

High temperatures can reduce soybean productivity and seed quality, as demonstrated in moderate, long-term temperature elevation as well as short, severe heat stress experiments. However, high temperature response variation among genotypes had yet to be investigated in open-air field conditions. To test the hypothesis that soybean responses to elevated temperature will vary among genotypes, we measured the physiological and agronomic responses of ten soybean genotypes to a 4 °C above ambient air temperature increase during the seed fill period. Plants grew in the ground and in open air conditions, to realistically mimic an agronomic setting. We found that physiological and seed composition responses to elevated temperature varied among genotypes and among the three years of the field study. While daytime photosynthesis and nighttime respiration were increased by elevated temperature under some conditions, both respiration and the maximum velocity of carboxylation by Rubisco acclimated after the first week of exposure to elevated temperatures. Critically, the economically important seed oil concentration was usually reduced by elevated air temperature during the seed fill period, and seed protein responses to temperature were highly variable. This experiment demonstrated variability in soybean germplasm for high temperature responses in a controlled, open-air field experiment, and suggests that soybean seed composition responses to high temperature could be optimized through breeding.

Keywords: Heat, abiotic stress, physiology, seed composition

Cropping system effects on maize and soybean yield and yield stability

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C1: Yield and seed composition response to environment

The two-year rotation of maize (*Zea mays* L.) and soybean [*Glycine max* (L.) Merrill] developed rapidly as soybean replaced small grains and forages in animal-centered systems beginning in the mid-20th century; by 1980, these two crops together occupied more than 80 percent of the cropped area in the U.S. Corn Belt. Concerns remain that these two crops grown in sequence fail to produce the economic and environmental stability that a longer, more complex rotation might provide. Long-term (20-year) crop rotation studies in Illinois did not support the hypothesis that the cropping sequence of maize and soybean lacks inherent stability. Adding wheat (*Triticum aestivum* L.) into the maize-soybean rotation increased both maize and soybean yields by about five percent at one site, but not at a second site. The three-crop sequence, however, produced lower economic returns than the maize-soybean rotation. Tillage increased yields of maize and soybean at one of the two sites, but tillage costs neutralized this advantage. Soil samples taken 17 years after the start of this experiment showed only minimal differences in soil organic carbon and total nitrogen among rotations, including monocrop maize and soybean. In another set of experiments, soybean following two maize crops in a three-year sequence produced slightly higher yields (average of seven percent) than soybean following a single crop of maize at three sites, but not at another three sites. The three-year sequence did not produce higher returns than the two-year sequence, however. Economic considerations may change the proportion of maize and soybean grown by producers in the US Corn Belt in coming decades, but we consider it likely that crop sequences with maize and soybean will continue to dominate in this region.

Keywords: Cropping system, tillage, crop yield, economic returns

Using simple cultivar phenotyping and photothermal algorithm to explore the suitability of soybean crop in France

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C1: Yield and seed composition response to environment

To develop new cropping strategies for soybean (including early sowings for drought escape or late sowings as a double crop after a cereal, early harvest as green seeds), a good prediction of phenology under different photothermal conditions is required. For that purpose, a simple phenology algorithm (SPA) was developed and calibrated in controlled conditions then evaluated at field level (Schoving et al., 2020). The objective of this study is the evaluation of soybean crop suitability in France according to maturity groups and contrasting sowing dates. SPA simulates the duration of the main phenophases between VC and R7 stages as a function of temperature and photoperiod. It was calibrated for 10 cultivars in 2017 and 5 cultivars in 2022 (from MG 000 to II) using a simple phenotyping method to determine genotypic parameters. Historical series of daily temperature were retrieved from the SAFRAN historical reanalysis which covers France at 8 × 8 km resolution. The SPA model was applied on each of the 8602 grid cells on the 2001-2021 period. The crop duration (from sowing to harvest) was simulated for the 15 cultivars sown (i) as soon as possible from March 15 (early sowing, flexible date); (ii) on 5th May (conventional, fixed date) and (iii) on 1st July (double cropping). Climatic projections from 2023 to 2100 under the different RCP scenarios were downloaded from the DRIAS website (http://www.drias-climat.fr/). Climate change is leading to the expansion of the soybean growing area at France level. When choosing adapted cultivars, it is theoretically possible to grow soybean on a large proportion of the French territory when considering only photothermal requirements and realistic conditions for sowing (temperature) and harvesting (date). This methodology - simple cultivar phenotyping and photothermal algorithm - is currently evaluated for the prediction of other grain legumes phenology to design more diversified and agroecological cropping systems.

Keywords: Agroecology, grain legumes, maturity group, phenotyping, photoperiod sensitivity

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Critical phosphorus levels are required to optimise soybean's biological nitrogen fixation capacity

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C3: Soil fertility and plant nutrition

It is widely recognised that soil available phosphorus (P) plays a key role in soybean plant nutrition, with soybean requiring approximately 25 kg of P in the form of P2O5 to yield 1000 kg of soybean seeds (Bagale, 2021). Soybean biological nitrogen fixation (BNF) is strongly influenced by soil available phosphorus (P), with P having both direct and indirect effects on soybean's N2 fixing potential. The multistep process of successful nodule development, nodule function and nodule metabolism are all influenced by P. It is hypothesised that through increased plant P acquisition and allocation to below-ground organs, P addition will increase nodule formation and N2 fixation. Through controlled environment studies, we aim to improve the understanding of the role of P in improving plant's N2 fixing potential. We aim to define critical soil available P levels and P fertiliser application rates to optimise soybean BNF. Nitrogen fixation capacity is assessed through analysis of nodule morphology, the proportion of N derived from fixation (%Ndfa) through relative ureide analysis, and onwards yield determining characteristics under various P treatments. Work is currently ongoing and results will be presented at the symposium. Improved understanding of the role of P within BNF will allow for more accurate accounting of soil available P content and P fertiliser recommendation in computer-based models. Through improving soybean's N2 fixing potential and P nutrient efficiency, progress can be made to optimising production and closing soy yield gaps.

Keywords: Phosphorus, nodulation, BNF, relative ureide

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Influence of biological seed treatment on soybean grain yield in the U.S.

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C3: Soil fertility and plant nutrition

Biological seed treatment in soybean (*Glycine max* (L.) Merr.) is a growing market in the U.S., with multiple microbially active ingredients and several proposed benefits. Some of the claimed benefits include improving nitrogen fixation, stimulation of root growth, increasing phosphorus, sulfur, and other nutrient absorption, and control of diseases, with the aim to increase soybean grain yield. Farmers are often bombarded with marketing claims about biological seed treatments. In many cases, there is little or no third-party evidence of quantitative assessment regarding these biological seed treatments' ability to improve soybean yield. Therefore, this project's objective was to evaluate if biological seed treatments improved soybean yield across the U.S. Field experiments were established using a common protocol during the 2022 growing season at 49 locations across 17 U.S. states, examining the effectiveness of nine commercial biological seed treatments to increase soybean yield. The experimental design was a randomized complete block with six replications. Treatments included microbes from the genera *Bradyrhizobium*, *Bacillus*, *Azospirillum*, *Pseudomonas*, *Pantoea*, *Delftia*, *Trichoderma*, and *Glomus*. Some of the products had multiple active ingredients (microbes). Results showed that the effects of treatments were not significant ($p=0.4229$) nor varied among the examined locations ($p=0.0985$). Also, Bayesian analysis indicated that a high probability (>80%) of the yield difference (each treatment minus untreated control) being higher than zero was mainly found in the treatment products that contained *Trichoderma* only, *Bradyrhizobium* only, and the arbuscular mycorrhizal fungi *Glomus* mostly in Southern U.S. states. In these locations, the yield difference ranged between 80 to 155 kg/ha; however, none was significant (95% credible intervals included zero). Overall results suggest that the biological seed treatments tested in this study in a wide range of environments rarely increased soybean grain yield.

Keywords: Inoculant, seed treatment, microbe, symbiosis, yield

Acknowledgments: Science for Success group for the initiative and Soybean Checkoff funds through the Qualified State Soybean Board in each state for the research funding.

The soybean sugar transporter GmSWEET6 participates in sucrose transport towards fungi during arbuscular mycorrhizal symbiosis

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C3: Soil fertility and plant nutrition

In arbuscular mycorrhizal (AM) symbiosis, sugars in root cortical cells could be exported as glucose or sucrose into peri-arbuscular space for use by AM fungi. However, no sugar transporter has been identified to be involved in sucrose export. An AM-inducible SWEET transporter, GmSWEET6, was functionally characterized in soybean (*Glycine max*) and its role in AM symbiosis was investigated via soybean transgenic plants. The expression of GmSWEET6 was enhanced by inoculation with the cooperative fungal strain. Promoter analysis revealed that GmSWEET6 localized mainly to arbuscule-containing cells during symbiosis. Heterologous expression in a yeast mutant showed that GmSWEET6 mainly transported sucrose. Transgenic plants overexpressing GmSWEET6 increased sucrose concentration in root exudates. Overexpression or knockdown of GmSWEET6 decreased plant dry weight, P content and sugar concentrations in non-mycorrhizal plants, which were partly recovered in mycorrhizal plants. Intriguingly, overexpression of GmSWEET6 increased root P content and decreased the percentage of degraded arbuscules, while knockdown of GmSWEET6 increased sugar concentrations in RNAi2 plants compared with wild-type plants when inoculated with AM fungi. These results in combination with subcellular localization of GmSWEET6 to peri-arbuscular membranes indicate that GmSWEET6 is required for AM symbiosis by mediating sucrose export for use by fungi.

Keywords: Arbuscular mycorrhizal fungi, soybean, sucrose export, SWEET, symbiosis

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From root-associated microbes to nutrients acquisition efficiency in soybean

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C3: Soil fertility and plant nutrition

Root-associated microbes are critical for plant growth and nutrient acquisition. However, scant information exists on optimizing communities of beneficial root-associated microbes or the mechanisms underlying their interactions with host plants. In this report, we demonstrate that root-associated microbes influence host plant growth and nutrient acquisition, and provide a strategy to harness beneficial microbes with different function through SynCom technology to improve soybean nutrient acquisition efficiency and yield. We isolated 1893 strains from soybean root-associated compartments. Three synthetic communities (SynComs) were constructed after potential beneficial function and plant growth promotion verification. Functionally assemblage of SynComs promoted significant plant growth and nutrient acquisition in pot experiments under different nutrient conditions. Field trials further revealed that application of SynComs stably and significantly promoted plant growth, facilitated N and P acquisition, and subsequently increased soybean yield. Among the tested communities, SynCom1 exhibited the greatest promotion effect, with yield increases of up to 36.1% observed in two field sites. RNA-seq implied that SynCom1 application also regulates N and P signaling networks at the transcriptional level, and genes related to auxin responses. Further study showed the better performance of SynCom1 is consistent with nodulation and nitrogen fixation on soybean. Detail analysis suggest two strains assigned to Pantoea in SynCom1 could significantly enhanced biofilm formation in *Bradyrhizobium* and thus its promoting colonization on soybean roots. In addition, two *Pantoea* strains could also induced genes expression in the flavone/isoflavone metabolism pathway. Therefore, we concluded that optimized the interaction among SynCom would be a promising strategy to maximize the potential beneficial functions of the whole community.

Ecology, functions and application of soybean seed endophytes

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C4: Soybean rhizosphere and nitrogen fixation

The plant microbiome is considered as an accessory genome for plant providing complementary functions to their host such as nutrient mobilization and acquisition or functions to better cope with biotic and abiotic stresses. Different plant compartments from the rhizosphere to seeds host diverse microbiomes, which are shaped by soil, host and environment. Furthermore, a number of microorganisms has been identified and selected for application as biofertilizer, plant strengthener or biopesticide and benefits seen in lab and greenhouse trials are highly promising. In particular, seeds host microorganisms, which are of key importance for early plant development, and seeds include highly interesting strains for microbial applications. Furthermore, seeds can be efficiently used as vector to deliver microbial inoculant strains and thereby improve field success of microbial applications.

How additional inoculation can improve N_2 fixation and yield in soybean under field conditions in Denmark

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C4: Soybean rhizosphere and nitrogen fixation

Soybean has grown in Nordic countries to increase local protein sources, however, it faces considerable questions regarding crop management strategies. Since soybean is a new crop on Danish fields, there is a lack of the nitrogen-fixing bacteria genus *Bradyrhizobium* in cultivated soils, which compromise soybean biological nitrogen fixation (BNF) capacity. To improve this mechanism, the research hypothesis assumes that the use of inoculant with *B. japonicum* will ensure efficient nodulation to sustain high yields with BNF, instead of using mineral N fertilizer. The aim of this study was to evaluate BNF driven nitrogen acquisition and effect on soybean productivity of seed inoculation and additional inoculation at the V_3 (third node) phenological stage with *B. japonicum*. Field experiments were carried out in the 2022 growing season at the Foulumgaard Experimental Station (central Denmark) in plots that had never been cultivated with soybean. Results showed that no nodules were found in roots of the non-inoculated soybean (control treatment) and in the mineral N fertilizer treatment. Promising results were observed with additional inoculation in V_3 which increased the number of nodules by 44% and nodule dry weight by 48% when compared to the seed inoculation treatment. The number of pods per plant and pods fresh weight at full flowering stage increased by about 34% with seed inoculation and by 60% with additional inoculation compared to the mineral N fertilizer treatment. Yield varied from 951 to 1,878 kg ha^{-1}depending on crop management strategy, with the greatest performance for the additional inoculation in V_3 treatment. Thus, results showed that it is possible to grow soybean under Danish field conditions with *B. japonicum*, providing a sustainable method, furthermore, a positive response with an additional inoculation in V_3 was observed. Highlighting the importance of studying regions without soybean cropping history.

Keywords: Bradyrhizobium japonicum, nodulation, isotope dilution method, *Glycine max*, sustainable agriculture

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Genetic diversity and symbiotic effectiveness of indigenous root nodulating bacteria associated with soybean in Benin (West Africa)

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C4: Soybean rhizosphere and nitrogen fixation

Nitrogen is one of the deficient nutrients for soybean [*Glycine max* (L.) Merrill] cropping and can be fully supplied by the biological nitrogen fixation process. Assessment of soybean indigenous rhizobia is very important for identifying strains potentially useful as bio-fertilizer. This study was carried out to assess the genetic diversity, symbiotic effectiveness (SE) and tolerance to abiotic stresses of soybean rhizobia strains from three agroecological zones in Benin. Isolation was performed following standard procedures for Rhizobium study. Symbiotic effectiveness of strains was assessed by inoculating them to soybean seedling in greenhouse and ability of nineteen nodulated strains to promote tolerance to pH, salinity and temperature was tested on specific medium. Nodule number (NN), shoot, root and nodule dry weight (SDW, RDW and NDW respectively), shoot nitrogen content (SNC) and percent of nitrogen derived from the atmosphere (%Nfda) were recorded. Most of the strains were found to be sensitive to extreme pH (3 and 11), high temperature (45°C) and elevated salt concentration (3%), with the presence of some outliers. Nodulation on soybean roots was achieved in 58% of isolated strains. The highest and lowest NN were recorded for the strains BANIL1b and TCHBA2b (66 and 13 nodules plant-1 respectively). Interestingly, the strains varied greatly for their SE relative to the un-inoculated N-fertilized control, with values ranging from 39.95 to 109.09%. Furthermore, significant positive correlation was found between NN and SDW ($r = 0.79$, $p< 0.001$). Finally, high variation was found for both SNC (9.4 - 115.7 mg plant-1) and %Ndfa (11 - 90%). Sequence analyses of 16S rRNA gene revealed five species: *Rhizobium* sp., *Bradyrhizobium yuanmingense*, *Enterobacter cloacae*, *Pseudomonas* sp., *Bacillus* sp. and *Burkholderia* sp. These results constitute the first report of genetic diversity of soybean nodulating bacteria in Benin and emphasized their great potential as promising candidate for field experiments.

Keywords: Soybean, root nodulating bacteria, abiotic stresses, symbiotic effectiveness, Benin

Core rhizosphere microbiota is associated with nutrient cycling in field-grown typical soybean varieties

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C4: Soybean rhizosphere and nitrogen fixation

Rhizosphere microbial communities play pivotal roles in soybean nutrient uptake. The diversity and composition of the soybean rhizosphere microbiome and their driving factors have been well studied. However, the core microbiota recruited by soybean rhizosphere and its function in nutrient cycling still need further investigation. We hypothesize that the core rhizosphere microbiota shared by different soybean varieties grown under varied field conditions would be closely involved in nutrient cycling. Five soybean varieties (one wild, two admixture, and two cultivated) were grown in the field in Shijiazhuang and Xuzhou, China. The bacterial and diazotrophic communities in rhzisphere and bulk soil were characterized by amplicon sequencing, the abundance of microbial genes involved in nitrogen cycling was quantified by qPCR, and the ecological function of operational taxonomic units (OTUs) was predicted by FARPROTAX. The rhizosphere effect (compartment) was the major determinant of variation in bacterial communities, while the soybean variety effect was significant but much smaller. The diazotrophic community composition significantly differed between rhizosphere and bulk soil, between study sites, and among soybean variety types in Shijiazhuang. Sinorhizobium was the major diazotroph genus in both sites and enriched in rhizosphere. The abundance of amoA, nifH, nosZ, and nirK genes was significantly higher in soybean rhizosphere than that in bulk soil in both sites. A core microbiota enriched in the rhizosphere of all the five varieties in both sites was identified, which was composed of 49 OTUs mainly belonged to the phylum Thaumarchaeota, Actinobacteria, and Proteobacteria. This core microbiota was enriched possibly due to increased total nitrogen, organic carbon, and pH in soybean rhizosphere soil, and was associated with multiple nutrient cycling, including carbon and nitrogen. These results demonstrate that soybean rhizosphere enriches specific bacteria involved in nutrient cycling, and facilitates the construction of artificial core root microbiota to promote soybean nutrient uptake.

Keywords: Soybean rhizosphere, core microbiota, diazotrophic community, nutrient cycling

Keeping Bradyrhizobia inoculant high quality standards: a challenge for French farmer organizations and French research bodies inside Europe

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C4: Soybean rhizosphere and nitrogen fixation

Based on the Australian experience, French bodies have promoted high quality inoculants since the middle of the sixties. Absence of contaminant, high concentration par seed (106 / soybean seed), selected strain maintained by INRAE, were the main dispositions. Downstream, the technical institute (former CETIOM and now named TERRES INOVIA), in connection with INRAE and private inoculants producers were used to conduct field trials in Bradyrhizobia free soils to evaluate and compare new commercial proposals. In recent years, several changes occurred. Farmers survey carried out in the past decade, show that Peat and microgranulars inoculations are in strong reduction at the farmers' level. Industrials are focused on liquid inoculants and ways to increase time interval between inoculation and sowing. Soybean pre-inoculated seeds have also been recently commercialized with more or less success, but this is clearly identified has the next important target for most of them. Another challenge for farmers organizations and public bodies is to keep high quality inoculants, with adaptation to new regulations at the European Union level which allow a mutual recognition procedure among UE states with heterogeneous policies for *Bradyrhizobium* inoculants market authorizations. The consequence for the French situation, where a unique selected strain of *Bradyrhizobium diazoefficiens* G49 has been selected and used for more than 40 years, is how to keep high quality standards for farmers, to be able to control it, including new strains and new technologies and to finance this all adaptation.

Molecular understanding of seed vigor acquisition during maturation

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C5: Seed production, vigour and technology

The desirable characteristics of seed vigor are the seed longevity in the dry state, high and synchronous germination and seedling establishment, even under suboptimal conditions. The production of high vigor seeds is a key leverage to achieve high and stable yield, especially with the anticipated reduction of pesticides. Seed vigor is acquired via a poorly understood genetic program during late maturation, which spans from the end of seed filling to the dry quiescent state. Here, we will focus on the importance of certain biochemical and molecular events that occur during late maturation for the acquisition of seed vigor. The main biochemical markers that coincide with the acquisition of longevity are an increase in raffinose family oligosaccharide sugars and the degradation of chlorophyll. Using a near-isogenic ultra-low RFO line (Jack *rs2 rs3*) containing natural variations that affect the function of the two raffinose synthase (RS) genes *RS2* and *RS3*, we demonstrate that over four consecutive years, *rs2 rs3* seeds showed a consistent reduction in longevity by 20-30% in the mutant line compared to WT. Seeds of the *rs2 rs3* mutant exhibited increased water uptake during imbibition and electrolyte leakage, both factors being detrimental to seed vigor. The importance of further maturation between stages R7 and R9 was further underscored by a network-based gene co-expression approach during maturation and imbibition of developing seeds. Network inference identified the existence of a gene module that represents a transcriptional program that is only activated in imbibing seeds of stage R9, with a strong overrepresentation of genes involved in meristem and organ development. We will show that this module is correlated with improved seedling development. Altogether, the processes occurring during late seed maturation should not be neglected when considering the production of high vigor seeds.

Exploiting genetic diversity of a diverse European soybean collection to improve seedling establishment in Europe

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C5: Seed production, vigour and technology

The selection and breeding of cultivars adapted to European growth conditions are urgently needed to overcome the European dependence for imported soybean. Europe-wide assessment of the agro-climatic suitability of soybean production areas under future climate shows that rising temperatures represent an opportunity to grow the crop further north. To increase yield potential in this region, these new 'European' cultivars must have a shorter growing cycle and be sown early to avoid unfavourable conditions during seed filling and harvest. However, early sowing, especially in the north-west of Europe implies that seeds must cope with cold temperatures of the seedbed. This is risky because cold severely reduces the speed of germination and seedling emergence and exacerbates the chances for imbibitional injury, which in turn, increase the exposure to pests and pathogens. Here, we evaluated the performance of a diverse collection of 359 soybean accessions relevant for breeding in Europe (the EUCLEG collection, MGI/II-MG000) for traits that are important for optimal seedling emergence in north-western Europe. Seed lots were produced in the north-western and eastern regions of Europe and phenotyped for seed hardedness (physical dormancy), seed germination and seedling emergence during imbibition at ambient and cold temperatures. Considerable genetic variation was discovered in cold tolerance during early imbibition, suggesting that genetic improvement for early sowing can be achieved. However, the production environment had a strong effect on both seed hardedness and seed(ling) vigor traits, with a significant effect between different maturation groups. Genome-wide association analyses identified significant loci for the different seed vigor traits, explaining between 10 to 35% of the phenotypic variation. The underlying genetic basis of the seed traits and their interaction between each other will be presented.

Improvement of soybean emergence by seed-coated-treatment with biostimulants

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C5: Seed production, vigour and technology

Increasing soybean productivity and extending its production areas is a major challenge for the supply of proteins for animal feed and human nutrition. In French North climate, soybean can be exposed to low temperatures during germination and emergence that strongly hampers the seed vigor and the efficiency of N_2 fixation, increases the risk of competition with weeds and finally reduces seed yield and/or protein quality. In order to improve the emergence under cold conditions, our goals were to test biostimulants as a promising strategy for (i) accelerating the seed vigor and seedling emergence and (ii) facilitating establishment of symbiosis for N_2 fixation in soybean. Biostimulants (Vv09 and Vv10 containing bio-available micronutrients, supplied by Via Végétale; AgxA, a yeast-based biostimulant provided by Agrauxine by Lesaffre) were uniformly coated with Bradyrhyzobium inoculum on seeds of cv. ES Comandor. Compared to the control seeds (coated with water), biostimulants significantly improves the germination rate at 12°C with a T50 (time corresponding to 50% germination) of 70 h (control), 53.5 h (Vv09 and 54.3 h (Vv10). Proteomics analysis carried out 48h after sowing reveals that biostimulants may act on the metabolic awakening of the seed in response to low temperature via the up-regulation of proteins involved in the amino acid synthesis (cysteine synthase, glutamine synthetase), in energy metabolism (ATP synthase), and in thermotolerance and response to oxidative stress (glutathione S-transferase, HSP70, dehydrin, peroxydase). Additional experiments under controlled and field conditions have shown that biostimulants significantly increase the number of nodules (Vv09) and the N status in leaves or shoots and tend to improve the seed yield and N content in seeds (Vv09, Vv010 and AgxA). These results highlight the beneficial impacts of biostimulants on low temperature tolerance during germination and emergence as well as the improvement of N_2 fixation efficiency leading to better agronomic performances.

Product profile aligned breeding in tropical soybean: a foundation for integrating quantifiable attributes into new varieties

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W1: Soybean for Africa

The soybean breeding program at International Institute of Tropical Agriculture (IITA) is the largest public breeding program in Africa. Developing soybean varieties that meet the needs of both farmers, national programs, seed companies and processors in sub-Saharan Africa remains the core goal of the IITA's breeding program. The needs of both farmers, national programs, seed companies and processors form the different market segments on which Product Profiles (PPs) are cast. A Product Profile formally describes a set of minimum quantifiable attributes or traits which a new variety should have compared to older varieties for it to be released onto a particular market segment. Formalised PPs also function as breeding commitments between breeders and value chain actors (farmers, national programs, processors and funders) and the traits must be quantifiable in absolute or relative terms with a target benchmark set for each trait to have the desired variety 'designed' during the breeding process. Developing and formalizing PPs requires in-depth cross-functional knowledge and expertise. Using its network of collaborators involving cross-functional teams, the IITA soybean breeding program formally developed and adopted PPs based on four market segments: (i) medium-to-late maturing soybean for the lowland savanna for central, western, east & southern Africa; (ii) medium-to-late maturing soybean for the mid-altitude savanna for central, western, east & southern Africa; (iii) early-maturing soybean for the lowland savanna for central, western, east & southern Africa; and (iv) early-maturing soybean for the mid-altitude savanna for west, east & southern Africa. Progress and variety advancement decisions based on the results from the 2019/20 to 2021/22 seasons, selected a total of 15 varieties for advancement from 150 varieties which were either in the second year or third year of testing using target benchmark set for each trait in the PPs and input from the cross-functional teams.

Keywords: Product profiles, market segment

The Pan African trial platform, a novel and new seed system approach that works

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W1: Soybean for Africa

There has been much written about the poor state of seed systems, from R&D through to utilization of improved high-quality seed by small holder farmers in Sub-Saharan Africa. A notable and recent effort, the African Seed Access Index (TASAI) current collects large quantities of data measuring the state of seed systems across key markets in Africa. Boring into the TASAI data, for example, shows the average of the two dominant soybeans varieties being 15 years in Malawi, with an annual release rate of .11 varieties per year between 2002 and 2019. Recently though Malawi has released 8 new varieties between 2020 and 2022, for an annual release rate of 2.66 varieties per year, a 24x improvement. This presentation will describe source of the improvement, the Pan African Trial platform, a new private sector led approach operating in 24 countries and 140 locations across west, southern, and east Africa. The talk will not only specifically show the impact the trials have on the SSA soybean system, but more importantly will explain why, as an effort to guide policymakers in new directions.

Genetic gain, achievements and challenges of the IITA soybean breeding program in Nigeria: implications for other west African countries

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W1: Soybean for Africa

The demand for soybean has been rapidly growing in Nigeria and several west African (WA) countries driven by the fast-growing demand for high protein foods and edible oil, and the soy meal as a supplementary animal feed. However, its productivity in the WA region has been one of the lowest (<1 t/ha) globally. Several production constraints have contributed to the low yields; some of which includes, limited availability of high yielding varieties, weak seed and extension systems. In the effort to increase the availability of improved varieties in the region, the IITA Soybean Breeding Program (ISBP) based at Ibadan, Nigeria has been developing new varieties for the region. Accordingly, the program has been the primary supplier of advanced breeding lines to the different national programs in the region. Every year, the breeding program has been developing 4,000 -5000 breeding lines from F4/F5 progeny populations. The genetic gain (GG) of the ISBP was estimated based on data obtained from 20 locations and six years and revealed highly significant and positive GG of 1.82 % for grain yield and 1.89% for plant height per year, while the GG for days to flowering was highly significant and negative (-1.11). In the multi-location variety trials used for GG estimation, 11 genotypes showed superior performance than both the IITA and commercial checks. In these multi-location yield trials, the top yielding genotype, TGx 1485-1DxTGx1989-19F-4 (3.15 t/ha across locations mean yield) showed yield increases of 18.9 and 23.5% over the IITA best check (TGx 1448-2E) and the commercial check (SC-Signa). The ISBP in collaboration with the national soybean breeding program released two early maturing varieties i.e., TGx 2020-4E and TGx 2024-7E for Nigeria in 2022.

Phylogenetic and seed composition stability analyses for cultivars in the USAID soybean innovation lab pan-African soybean variety trials

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W1: Soybean for Africa

With increasing utilization and demand for soybean in Africa, evaluating performance within and across important growing regions is critical for identifying elite cultivars. Many smallholder farmers in sub-Saharan Africa (SSA) grow soybean for livestock feed and as a protein source to ameliorate malnutrition, yet soybean production in these regions needs to be further increased through improving genetic gains for agronomic performance. The Pan-African Soybean Variety Trials (PATs), managed by the USAID Feed the Future Soybean Innovation Lab (SIL), directly address this need. African public and private soybean breeding programs submit lines for evaluation in the PATs, resulting in panels of soybean lines that were grown in 2021 at 113 locations within 24 SSA countries. With the phenotype data collected in these multi-environment trials (METs), genotype by environment interaction (GEI) effect on genotype performance can be determined using stability analyses. To utilize this MET data, we conducted stability analyses on seed protein and oil content of 19 lines grown in the PATs with two replications at 11 locations across Zimbabwe. Seed protein and oil content for samples from each plot were predicted by near-infrared spectroscopy (NIR) using a Perten model DA 7250 and the following stability analysis methods were used and compared: genotype plus genotype-by-environment (GGE) biplot analysis, additive main effects and multiplicative interaction (AMMI) analysis, and random regression modeling (RRM). With SoySNP3K Illumina array-based genotyping data obtained for these 19 PAT lines, we also performed phylogenetic analysis between the lines and a diversity panel subset of accessions in the USDA soybean germplasm collection. The best performing and most stable lines identified by the stability analyses will be recommended for utilization by breeding programs and farmers. Additionally, accessions identified by the phylogenetic analysis will be recommended for integration into breeding programs to increase genetic improvement and diversity.

Evaluation of soybean genotypes for response to *Coniothyrium glycines*, the cause of red leaf blotch

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W1: Soybean for Africa

Red leaf blotch (RLB) is an important fungal disease of soybean caused by the fungus *Coniothyrium glycines*. Currently, up to 50% loss in yield can be attributed to RLB infection in sub-Saharan Africa. Until now, there are no known varieties with resistance to RLB. In this study 230 soybean lines were screened under screen-house conditions to identify sources of resistance. Soybean line SB25 was used as the susceptible check. Red leaf blotch isolate ZS20B, previously isolated from infected soybean plants in Zambia, was the pathogen used in this study. Seed from each line were germinated in seedling trays filled with a sterile potting mixture (2:2:1, v/v) of red soil, sand, and manure. Seedlings were maintained in the screen-house (25-30°C) for two weeks prior to infection with the RLB. Infection with RLB was achieved by spraying a spore suspension (2.08×109 spores/ ml) on plant leaves using a hand-held spray bottle until run off. Plants were then transferred into a transparent plastic chamber at 25°C and moist paper towels were placed in the chamber to provide extra humidity. Disease severity was assessed at three weeks post infection based on a 1–5 scale with 1 representing no disease and 5 representing 66-100% severity in comparison to the level of disease on the SB25 susceptible check. Fifty-four lines had a disease severity ranging between 1-1.9, 103 in the range of 2-2.9, 54 in the range of 3-3.9, while 19 had a severity of 4 or above. Eighty-six lines that had a severity of between 1 and 2.5 were re-screened under similar conditions. Out of the 86 lines only 44 were re-screened due to poor germination. Thirty-eight of these had a severity ranging from 1-2. Four lines had a severity of 3, while two lines had a severity of 4. The 38 genotypes are regarded as potential sources of resistance and will be further evaluated to validate and genetically characterize the genetics of resistance.

Keywords: Resistance screening, *Coniothyrium glycines*

Seed Co's commercial soybean variety attributes, distribution and availability across Africa

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W1: Soybean for Africa

Seed Co international Limited has 80 years of experience in the production and supply of certified high-yielding seed varieties bred in Africa for African climatic and geographic conditions. Its vision is to dominate the Agro industry in Africa by breeding seed, feeding, and leading in Africa. It is involved in crop seed research, production, processing, distribution, and marketing of soya bean, among other crops. The company's customers range from small scale, emergent to commercial farmers. Being an African company, it is constantly adapting to 3rd world challenges by setting up strategic research stations across the continent. Using knowledge of Africa and its complex agro ecologies, and global benchmarking, SeedCo has been able to release up to 37 soybean seed varieties on the continent. Each of these is well tailored to meet the abiotic and biotic challenges facing specific regions of the continent. The soybean breeding program is driven by commercial demand. Commercial units provide information on: Emerging markets, trait requirements by stakeholders, changes in demand of commercial varieties and market share, potential future needs by growers and other value chain players. This information is used to set breeding objectives. Breeding then begins, to achieve commercial goals. The superior genetics are tested widely across target environments. Product Development gathers farmer's perceptions on the variety. The product is then launched and promoted. Forecasting of required volumes Production, processing and branding Marketing and distribution follows. SeedCo markets its products in countries where it operates. Licensing out may be considered for countries outside operational area if there is marked advantage over our current genetics. Collaborations with research institutions as IITA and now SIL are key to our agenda of wide testing.

Soybean in Italy: challenges faced and solutions adopted by farmers

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W3: Knowledge exchange for European soybean agronomists

From early 1980s soybean average in Italy increased reaching an area of about 500 Kha (10% of them organic). The pedo climatic conditions limit the majority acreage to north region (Po valley), where the rainfall and irrigation (on 50% of soybean area) sustain the yield of 3.0 to 4.0 t/ha. Currently 10% of total acreage is under contract with a specific use destination (mainly food and 25% high quality feed). Rainfed soybean is grown mostly as relay crop after winter cereals, using minimum or no till techniques. Relay crop offers a better control of summer weeds (*Chenopodium, Xanthium, Datura, Solanum, Amaranthus* and *Sorghum* ssp.). Other innovative techniques like Crimped cover crops are increasingly used especially by organic farmers. Weeds represent a challenge for conventional (herbicide resistant weeds) and organic production. Weed topping after flowering is a growing practice to control weed's dissemination and to ease harvest. *Nezara viridula* and mostly spider mite (*Tetranychus urticae*) are present leading to variable yield losses. Green stem disorder is sometimes present when strong water stresses affect the seed-setting phase (end of July) of May-sown soybean crops.

Soybean seedling emergence under current and future climate across Europe and the possibility for sowing date adaptations

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Soybean (*Glycine max* (L.) Merr.) has potential to improve the sustainability of agricultural systems due to several advantages it offers, including its capacity to fix atmospheric nitrogen (Watson et al., 2017). In Europe, this crop is much less reliant on conventional pesticides compared to its leguminous counterparts (e.g. pea). The introduction of this crop into European cropping systems not only offers their diversification but also helps increase the protein self-sufficiency of the continent thereby reducing import dependency (Guilpart et al., 2022). However, little is known to date about i) the areas suitable for soybean emergence and seedling establishment across Europe, ii) how ongoing climate change will affect the distribution of these suitable areas, and iii) the possibility of adaptation of soybean crop by shifting sowing dates. To address these questions, a simulation study was conducted by coupling climate change scenarios (historical: 1981-2010 and RCP 4.5: 2040-2069; (Webber et al., 2018)), machine learning algorithms, a soil-crop model (STICS), and a crop emergence model (SIMPLE). Six sowing dates (from early to late) were considered. Preliminary results showed a significant shift in seedbed climatic conditions across Europe under climate change, especially due to an increased seedbed temperature. For late sowing dates, previously unsuitable areas resulted suitable for soybean establishment, across boreal and northern continental regions, but the opposite was observed across southern Mediterranean areas. In contrast, early sowings show good potential for soybean seedling establishment in northern France, the southern Mediterranean, Alpine and southern continental regions.

Keywords: Agro-climatic indicators, sowing dates, seedling emergence, modeling chain

Acknowledgements: This work was supported by SusCrop ERA-Net LegumeGap project.

Projected future climate conditions for growing soybeans in Canada

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The soybean industry in Canada aimed to extensively expand soybean production to benefit the industry from new early-maturing varieties and the warming climate. However, setbacks in the soybean industry since 2017 demonstrated the impacts of climate risk and global market uncertainty, especially in the face of climate change. Therefore, developing a better understanding of future climate conditions that will impact soybean growth in Canada is needed for decision-making in the sector, such as prioritizing regions for expansion and developing climate change adaptation strategies through either agronomic management practices or breeding new cultivars. Based on climate projections from a set of global climate models, we analyzed climate conditions for growing soybeans including growing season start, crop heat units, precipitation, precipitation deficits, and climate extremes, in the near-term (2030s), the mid-term (2050s) and the distant future (2070s). We found that a future warmer climate would favour the expansion of soybean production further north and west but precipitation deficits could constrain growth on the semiarid Canadian prairies unless irrigation is introduced. Heat- and drought-tolerant cultivars should be developed to adapt soybean production to a changing climate, in addition to the adoption of late-maturing cultivars that will benefit more from the prolonging growing season and increasing crop heat units.

Keywords: Climate projection, climate change impacts, growing season, crop heat unit, soybean

How fast can we plant soybean using advanced planting technology?

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Planting speed is a critical concern for Mississippi soybean producers because planting windows are narrow due to high soil moisture holding capacity. Slower planting speeds mean improved singulation and stands, but large acreage requires that planting be accomplished within a narrow timeframe during favorable weather windows, so that producers often plant at higher speeds than are optimal for stand establishment, which may affect yield and economics (e.g., tractor operator hours). Off-the-shelf precision planting technologies that claim to improve singulation, emergence, and stands are already available to growers, but involve significant capital expenditures for uncertain return. Our objective was to quantify soybean response at different planting speeds using mechanical vs. precision planting technology. Two planters were tested: a traditional ground-driven mechanical planter and a precision planter equipped with downforce, electronic metering, and high-speed seed tube planting technology from Ag Leader. The planters were tested at 7.9, 10.8, 13.6, and 15.5 km/h. The trial was repeated at five locations throughout Mississippi in 2022: Brooksville (clay with poor seedbed preparation), Starkville (loam with poor seedbed), Stoneville (clay with excellent seedbed), Stoneville (silt loam with excellent seedbed), and Verona (moderate seedbed). Plots were at least 46 m long and four rows wide, but harvest area was trimmed to 10.7 m by two rows wide. Data were collected on emergence, stand uniformity, yield, and return on investment. Stand generally decreased at faster planting speeds, but the decline was greater when using mechanical compared to precision planter components. Plant spacing variability increased with faster planting speeds and was more variable with the mechanical planter. Soybean yield was not affected by planting speed. Faster planting speeds appears feasible under Mississippi conditions, such that more acreage may be covered during favorable early planting windows.

Selection for high and stable yield in soybean breeding lines using digital phenotyping methods

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Soybeans have become a valuable alternative in crop rotation for Swiss farmers to sustainably produce protein for human consumption and animal feed. To achieve high yields, plants need to efficiently absorb sunlight energy, convert it into photochemical energy and finally into biomass. To ensure stable yields, soybean has been successfully adapted to the colder Swiss climate and must be robust to a range of environmental conditions. In a first field trial in Eschikon, the conversion efficiency of sunlight into photochemical energy in soybean elite lines was determined by photosynthesis measurements using chlorophyll fluorescence. In further field experiments, energy conversion into biomass was approximated by measurements of leaf area index and plant height using drones and 3D laser scanners. In this way, the growth dynamics during the vegetation period under different conditions could be determined. Linear modeling was used to determine the environmental variables that determine growth rate, such as vapor pressure deficit, light intensity, or temperature. Understanding the environmental constraints on soybean production will allow the selection of lines that are tolerant to specific environmental conditions and efficient in biomass production. This will facilitate the adaption of soybean lines to the future climate which ensures high yield and yield stability.

Comprehensive evaluation of soybean germplasm resources collected from China and Europe under cold conditions

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To identify elite soybean germplasm resources showing cold tolerance at the seedling stage in the northern part of China’s Heilongjiang Province, in this study 158 soybean germplasm resources from China and Europe were evaluated through a comprehensive analysis of seedling emergence rate, final seedling emergence rate, relative seedling emergence rate, and seedling emergence index under artificial low-temperature stress treatment conditions and early low-temperature stress treatment conditions in the field. With the preliminary results, the relative seedling emergence rate might be deployed as an index for evaluating artificial cold tolerance with an efficiency up to 60%. We suggested four grades with variations on cold tolerance: grade I (high cold tolerance) containing 10 accessions, with a relative seedling emergence rate of 75–100%; grade II (moderate cold tolerance) containing 22 accessions, with a relative seedling emergence rate of 45–75%; grade III (moderate cold sensitivity) containing 33 accessions, with a relative seedling emergence rate of 20–45%; and grade IV (high cold sensitivity) containing 93 accessions, with a relative seedling emergence rate of 0–20%. A highly significant linear positive correlation between the final emergence rate and relative emergence rate in artificial cold tolerance identification experiment was observed, and a significant positive correlation between relative emergence rate and emergence index (EI) in the Heihe experimental site was observed. The European germplasms showed cold tolerance better that of Chinese germplasms. Except one Chinese germplasm (C63, Heihe 5), in six cold-tolerant germplasms five were from Europe (A28, ES Senator; A54, Tajfun; A65, Augusta; A75, GL Hermine; A76, Josefine). These cold-tolerant soybean germplasms may provide a material basis for cold-tolerant soybean varieties breeding and mining the functional genes with cold tolerance in the early-maturing cultivation areas of China.

Keywords: Chinese and European soybeans, cold resistance, identification

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Screening of soybean genotypes based on root morphology and shoot traits using the semi-hydroponic phenotyping platform and Rhizobox technique

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Root-system architecture is vital for improving soybean (*Glycine max* L.) growth and nutrient uptake. We characterised root-system architecture and shoot traits of 30 soybean genotypes in a semi-hydroponic system 35 days after sowing (DAS) and validated eight genotypes with contrasting root-system architecture in 1.5 m-deep rhizoboxes at the flowering stage. Among them, two genotypes were selected for evaluation through to maturity. Abundant variation was observed in 11 of 13 measured roots and shoot traits during the early growth stage. After late growth stages, strong positive correlations were found between root traits and shoot traits, except for specific root length and diameter. Seed yield and yield traits at final harvest significantly differed between two contrasting soybean genotypes. The large-rooted genotype had a higher harvest index than the small-rooted genotype. Soybean genotypes with larger root systems had a long time to flower than those with smaller root systems. Genotypes with large-root systems had 106% more leaf area and 245% more shoot dry weight than those with small systems, presumably due to high canopy photosynthesis to supply the demand for carbon assimilates to roots. Total root length and root: shoot ratio-traits data collected in the rhizobox study strongly correlated with the same traits in the semi-hydroponic phenotyping system. We found genetic variation and phenotypic plasticity in other root and shoot traits such as taproot depth, root dry weight, specific root length, and average root diameter among the tested genotypes. Phenology, particularly time to flowering, was associated with root system size. Some root and shoot traits in the semi-hydroponic phenotyping system at the seedling stage produced similar rankings at the later phenological (flowering) stage when grown in the soil-filled rhizoboxes. The soybean genotypes characterised by vastly different root traits could be used for further glasshouse and field studies to improve adaptation to drought and other specific environments.

Keywords: root system architecture, semi-hydroponic platform, 1.5 m-deep rhizoboxes, root length, harvest index

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Relay-cropping of soybean cultivars into wheat for ecological intensification of agriculture

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Ecological intensification is a way to address key agricultural challenges of the 21st century including adaptation to climate change, feeding the growing population, and reducing depletion of natural resources. As an example, relay-cropping (RC) allows to harvest two crops within the same year thereby increasing land use efficiency. Compared to sequential double cropping, sowing soybean into standing wheat allows for earlier establishment in the growing season and drought escape (1, 2).

Twenty-one soybean cultivars (MGs 000 to II) were sown on 6 May 2021 into a very early and short-straw wheat cultivar (harvested on July 6) in south-west France (43.53°N, 1.48°E). Both crops were irrigated. Soybean biomass was sampled at R1, R5, R8 growth stages and canopy cover was monitored with hand-held and mobile sensors. The radiation available for soybean in RC was compared to a sole soybean crop. Soybean cultivars were harvested between 29 Sep (00-000) and 12 Oct 2021 (other MGs).

The interspecific competition in RC decreased soybean's number of vegetative nodes, total biomass and leaf area but increased specific leaf area. The best adapted cultivars to RC exhibited a faster recovery capacity, resulting in a higher aboveground biomass and leaf area at the end of vegetative growth. Grain yield ranged from 1.1 t/ha (MG 000) to 3.1 t/ha (MG II), consistently with the biomass production. In general, late-maturing cultivars resulted in highest yields due to longer crop duration and superior recovery capacities.

Keywords: ecological intensification, relay-cropping, shade tolerance, plant recovery, variety testing

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Supplemental pollination by an adequate honeybee population provides improved soybean yields

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Brazil is the leading global soybean producer, with a soybean area of ca. 43 Mha (2022/2023) and an estimated harvest of 160 Mt, representing 41% of the world's production. Due to its expansion, the crop approached apiaries' sites. Additionally, we observed a growing interest of beekeepers in migrating their beehives close to soybean fields, along with a similar willingness of farmers to have apiaries near soybean areas due to preliminary observations of improved soybean yield. Three experiments were set up in Londrina, Brazil, to assess the soybean yield when the flowers were either visited or not by *Apis mellifera* L. (Hymenoptera: *Apidae*). The study, developed in 2017/18, 2018/19, and 2019/2020 growing seasons, had three treatments: (1) open plots with free access to bees; (2) caged plots with a honeybee beehive inside; and (3) caged plots free of bees. The visitation of honeybees on soybean flowers was monitored on several dates during the blooming stage, starting at 9 am, 10 am, and 11 am. Results indicated consistent visits of honeybees to soybean flowers, in open and caged plots with beehives, with higher visit frequency observed at 11 am. During the three years of study, caged (639 kg.ha-1 - 12.97%) and open (274 kg.ha-1 - 5.58%) plots had soybean yield increments compared to caged plots with no beehive. The yield increments were associated with more pods with 3 or 4 seeds, a higher number of seeds per pod, and higher seed weight. A smaller number of empty or one-seeded pods in treatments with the presence of bees was also observed, compared to the caged plots without bees. These results indicate that supplemental honeybee pollination may increase soybean yield, thus reducing the need for expanding area, resulting in a more sustainable soybean production system and less GHG emissions.

Proximity with bee repositories increases soybean yield

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The Brazilian Forest Code imposes restrictions on land use on private properties, being the farmers obliged to preserve 20-80% of the native vegetation in the so-called Permanent Preservation Areas and Legal Reserve Areas. Accordingly, in soybean farms the landscape interlaces preserved areas and cropped fields. The preserved sites are repositories for pollinators (chiefly bee species), which can potentially increase soybean yield. Two experiments, at the sides of a native forest fragment in Campo Novo do Parecis (Brazil), were set up to understand the effect of pollinating bees on soybean yield. That fragment hosted both native bees and several colonies of honeybees (*Apis mellifera* L. (Hymenoptera: *Apidae*)). Treatments consisted of six plots located at different distances along six transects (replications) up to 600 m (experiment 1) or 800 m (experiment 2) starting from the forest border. During soybean blooming, we assessed the bee population three times a week. We harvested four meters of two consecutive rows of soybeans for yield evaluation and 1m for estimating yield components. In experiment 1, the bees' population decreased from 8.6 (12.5 m) to 0.95 m-2.5 min^{-1} (600 m); on experiment 2, 5.1 bees m-2.5 min^{-1} were observed at 25 m and 0.9 at 800 m from the forest border. For experiment 1, the yield for distances up to 100 m showed no statistical differences between them, averaging 3,880 kg ha^{-1}, being 16 % higher than the average yield (3,338 kg ha^{-1}) for distances of 200 m and over. For experiment 2, the soybean yield of up to 100 m from the border (4,201 kg ha^{-1}) was statistically higher than the ones located at 200 m and over (3,667 kg ha-1), representing a yield increment of 14.6%. These results indicated that soybeans cultivated up to 100 m from a bee repository have greater yields than those cultivated beyond that distance.

Application of collecting trough method to identify the drought resistance of soybean

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Drought resistance is a complex quantitative trait in soybean. However, the accurate drought resistance of plant is difficult to obtain because the water content of soil is difficult to control. In the present study, to control the water content of soil, collecting trough with different diameters were placed on the field to collect rain, and its effects on soybean growth and field environment were evaluated, and then the feasibility of this method to identify the drought resistance of soybean was tested. The results showed that the plant height, number of nodes per plant, grain number per plant and seed weight per plant were decreased significantly as the diameters of collecting trough increased, but differences in the number of branches per plant, pod height was not significant. The diameters of collecting trough also had influence on the soil temperature and moisture, particular on the soil temperature at 5 cm depth at 14:00 and soil moisture between 0~10 cm depth. Above results indicated that placing collecting trough between soybean rows can reduce soil moisture; there are significant differences among different diameters of collecting trough in drought resistance related traits, e.g. plant height, number of nodes per plant, number of seed and seed weight per plant. Thus, treated the normal filed management as control, placement of collecting trough with diameter of 25 cm on field can be used to evaluate the drought resistance of soybean.

Keywords: Soybean, drought resistance, identify method, collecting trough method

Effects of temperature and light conditions during the late growth stage on delayed stem senescence and cytokinin levels in the xylem exudate of soybean

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Delayed stem senescence (DSS) in soybean plants is defined as retarded leaf and stem maturity, even if pods mature normally. This phenomenon is a serious problem for the combine harvesting of soybean in Japan. Our previous research showed that DSS occurred more frequently in June sowing than in July sowing, and the plants sown in June were exposed to higher temperature and higher solar radiation intensity during reproductive growth stage as compared to those sown in July. We additionally observed higher levels of cytokinins, plant hormones with senescence inhibition activity, in xylem exudate after pod-setting stage (R4) under the DSS-induced condition. Thus, this study investigated the effects of the respective environmental factors, temperature and light intensity, after R4 on cytokinin levels in the xylem exudate and the occurrence of DSS. Pot experiments were conducted in the experimental field of Nihon University (Fujisawa-city, Kanagawa, Japan). In temperature treatments, soybean plants grown in the field environment were moved to an air-conditioned room at R4 stage, whereas control of light intensity was achieved by shading using cheese clothes under the field condition. The temperature control experiments revealed that high temperature after R4 promoted the DSS symptom, while low temperature after R4 inhibited the occurrence of DSS. On the other hand, decreased light intensity after R4 did not affect the severity of DSS. The cytokinin levels in the xylem exudate at R5 correlated with the severity of DSS in the temperature treatments, but the change in light intensity did not influence the cytokinin levels. These results indicated that high temperature after pod-setting stage have a profound effect on the occurrence of DSS, and this effect is mediated by the increased cytokinin levels in the xylem exudate.

Soybean in 1,000 gardens: catching rhizobia to introduce high protein containing soybean for a sustainable agriculture in Flanders

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The currently available soybean inoculants in Belgium contain non-native rhizobia that are insufficiently adapted to the local environmental conditions of Flanders. In the field, this often leads to an unsuccessful inoculation of soybean, and results in a volatile yield and protein content. In this 'Citizen Science' project we engaged over 1000 Flemish locals and farmers to sow 1 m² of uninoculated soybean in their garden, with the goal to "trap" local rhizobia in order to increase yield and protein content. In over 20% of harvested plants, root nodules were found. Ghent University and VIB identified various bacterial genera, including *Rhizobium* and *Bradyrhizobium* sp. The nodulation capacity of these bacteria was determined in both greenhouse experiments and in field trials. In the greenhouse, this was analyzed in five soybean variaties under controlled conditions in a soil/sand mixture, and compared to a commercial control strain. Important parameters were number and dry we ight of nodules per plant, and the chlorofyl content. In two field trials, with an identical set-up, 20 strains were tested in two soybean varieties and in three replicates. Strains were compared to the same control as in the greenhouse. Of the over 30 strains tested so far, two were able to nodulate and fixate nitrogen in both greenhouse experiments and in the field, and a noticeable variability in nodulation capacity was observed between soybean varieties. Moreover, both strains resulted in a statistically equal or higher (+ 7,0 and 14,2%) yield than the control, and a significantly higher number of nodules per plant (+116,4 and 97,8%). One additional strain developed nodules in the greenhouse, but was not yet tested in the field. We intend to continue this search further in 2023 in both greenhouse experiments and in additional field trials.

Keywords: Citizen science, rhizobium, nodulation, inoculation

Transcriptomic and ecophysiological analyses revealed contrasted soybean mineral nutrition under individual or combined heat and water stresses

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In a context of climate change, with more frequent drought events and heatwaves, it is predicted that soybean yields will drastically decrease in the near future. Soybean being the most widely grown legume crop in the world, there is an urgent need to improve its ability to sustain its growth under such conditions in order to guarantee high levels of productivity. The aim of this study was to explore the influence of heat and/or water stress on soybean growth and its water and mineral nutritions. Two soybean genotypes, displaying contrasted root architectures during their vegetative stage were grown under controlled conditions in the 4PMI high-throughput phenotyping platform where either optimal conditions, or heatwaves, or water stress, or both heatwaves and water stress were applied. Plants were characterized for their morphology, their water uptake, the mineral composition of their tissues and their root transcriptome (RNA-seq analyses). An ecophysiological structure-function framework, enabled us to link structural variables (leaf area, root architecture, biomass, etc.) to functional variables (water use efficiency, element uptake and use efficiencies…) in order to understand the interactions between water and element fluxes at the whole plant level, and to quantify the overall tolerance of plants to each stress. Under combined stress conditions, one genotype appeared more susceptible. The genotypic difference was mainly due to functional changes, particularly for water uptake and to differences in the content of certain elements in the roots (magnesium and calcium with their role in osmoregulation, and nickel, sulfur or sodium). This cross-analysis of the plant ionome, root architectural traits and root transcriptome under different stresses, is offering us new insights into soybean adaptation to climate change.

Keywords: Ionome, *Glycine max*, climate change, root architecture, water uptake

Genetic variability of nodulated root system architecture in relation to biomass allocation and biological nitrogen fixation in a collection of european soybean genotypes.

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Soybean [*Glycine max* (L.) Merr] is the legume with the largest cultivated area worldwide and its yield depends largely on symbiotic nitrogen fixation and root architecture. This study aimed to explore the genetic variability of root architectural traits and di-nitrogen fixing activity in a small collection of nine European cultivars belonging to the same maturity group during their early stages. Cultivars were grown in rhizotubes in the 4PMI high-throughput phenotyping platform and allowed us to capture images of the whole nodulated root system over time. New image analysis approaches were implemented to characterize root architecture and nodulation at high throughput. Significant genetic variability was identified for the width of the root system, the root density, and for the number of lateral roots. Differences in symbiotic fixation among the different genotypes were observed, while inoculating the same N_2-fixing bacterial strain. Differences were also observed in the location of the nodules on the roots, the number of nodules, the specific N2-fixing activity of the nodules or the time when the highest rate of nodulation was reached. This study allowed us to highlight trade-offs among root and nodule traits, and structural and functional traits. Some more productive genotypes were characterized by a good balance between efficiency in producing efficient nodules and maintaining a large number of roots at the early developmental stages, that could result in a higher uptake of nutrients and water in these genotypes. Finally, both the image analysis approach and the results could be used for breeding programs of soybean, that could explicitly consider the root system architecture, when the plant interacts in symbiosis with N_2-fixing bacteria.

Keywords: N2-fixation, root system architecture, nodules, *Glycine max*, root phenotyping

Can upper midwestern US farmers profitably intercrop soybean and soft red winter wheat?

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Intercropping (IC) soybean with soft red winter wheat (SRWW) can potentially increase economic returns for farmers in the upper mid-western US. However, intercropping has limitations when compared to traditional soybean and SRWW production such as reduced soybean growth from crop competition and damage from wheel traffic during wheat harvest and soybean planting. This research aims to answer important questions for farmers wondering if intercropping soybean into SRWW is a viable option and special management practices that might be used. An experiment was conducted in 2022 in Wisconsin, USA to better understand these systems. A split-split plot design with four replications was used. Whole plots were intercropped soybean and non-intercropped soybean. The split plot was strip-tilled soybean and no-till soybean planted on three dates. The split-split plot treatment of a foliar fungicide was only applied to the SRWW. All winter wheat was planted in 19 cm rows in the previous fall and soybean was planted in 76 cm rows. No intercrop treatments affected wheat yield, which averaged 5780 kg ha-1. Strip-till and late planting produced the greatest IC soybean yield. No yield differences were observed for non-IC soybean regardless of planting date or tillage. The non-intercropped soybean treatments provided the highest net system returns at a range of market prices. As a result of our experience in 2022, we will add a wheat skip-row treatment to reduce IC soybean/wheat competition. Also, we will add row pushers to the combine to reduce the amount of soybean leaf clipping during wheat harvest.

Keywords: Soybean, intercropping, wheat

Wisconsin soybean variety performance trials: providing information to help producers meet their goals

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Soybean variety selection is one of the most important decisions that producers make each season. The Wisconsin Soybean Variety Performance Trials are conducted with the objective to give producers unbiased information needed to select varieties that satisfy their specific goals and are most likely to perform best in their geographical location. Seed companies, breeders, and University research specialists voluntarily submit commercially available or experimental varieties. Varieties are grouped into four different regions (south, central, north-central, north) of the state by maturity group. Within each region, the variety trial is replicated into three different micro-climates to capture any genetic by environment interactions that may take place. Tests are conducted using a randomized complete block design with four replicates. Management practices are established to represent common practice in the surrounding region. All varieties are evaluated for grain yield, moisture, lodging, protein, oil, and maturity. Disease ratings are assessed when applicable. In the southern region, the past 15 years of glyphosate tolerant trials have shown an average difference from the lowest to highest yielding variety of 1,305 kg ha^{-1}. Those same trials reveal that variety selection can alter protein content by up to 3.8% and oil content by 2.7%. Data from the variety performance trials has also been utilized to update the optimum maturity group zones across Wisconsin as well as develop artificial intelligence algorithms to predict field specific management decisions. This poster will overview data that soybean variety trials can offer producers to help them meet their goals.

A large network of soybean fields analyzed to determine yield-limiting factors and improve soybean crop in France

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With the increase of soybean crop areas in France, especially in new production areas, it is necessary to continue to increase the competitiveness and sustainability of soybean. The objective was to set up a large multi-year network of fields to acquire references and identify main yield-limiting factors. A better knowledge will permit agronomic diagnosis, limit crop failures, and enable development of technical innovations for successful crop.

The network was 180 fields monitored in 2021 and 130 fields in 2022. These fields were in different areas with a large variability of: pedoclimates, cropping systems, earliness group, cropping practices. Each field was visited thrice to carry out measurements: plants density, biomass, nitrogen nutrition index (NNI), nodulation, weeds, diseases, pests, phenology, yield and components.

A large yield variability was obtained between new and historic production areas: 12-39 q/ha (mean by production areas). The low water availability was a main limiting factor since the rainfed fields suffered of yields 60% lower than those obtained in irrigated fields in 2022 (dry year) and 27% lower in 2021 (wet year). Weed pressure and poor regularity of emergence also limited yield since negative relationships were found. An average yield difference of 40% was noted between fields identified as "no weed" and "high weed pressure"; and up to 72% of yield difference between fields with regularity classified as “very homogenous” and “very heterogenous”. The year with the highest water availability induced higher NNI at flowering than the driest year (0.85 > 0.71, in average) which seems to show that water availability is important in nitrogen nutrition as it was also observed for other legume crops. The difference between the field with NNI< 0.5 and those with NNI>0.95 was 30% of yield decrease probably due to nitrogen stress.

This large database is still being analyzed to complement the yield-limiting factors. This network revealed a certain complexity in the follow-up and the analysis of results obtained by various experimenters. It will be renewed in 2023 with a reframing of the field number and measurements.

The use of biostimulants in soybean cultivation and their impact on yield and quality

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Biostimulants belong to the group of formulations which primary purpose is to protect the agricultural environment as well as to improve the biochemical, morphological and physiological processes of a crop under exposure to biotic and abiotic stresses. The implementation of biostimulants as a modern branch of agricultural technology was an impulse to start research to examine the impact of commercially available products in soyabean cultivation. The purpose of the study was to determine the effect of the application of applied biostimulants on biometric traits, yield and chemical composition of soybean seeds. The field experiment was conducted at the Swojec Experimental Station of the Wrocław University of Environmental and Life Sciences in 2020. The experiment was established using the randomized sub-block method with two variable factors. The factors of the experiment were, in order: I - soybean varieties (Lissabon - medium-early, Merlin - medium-early) and II - four biostimulants (Optisil, Aminoprim, Naturamin, Plonvit). Biostimulants were applied three times during the growing season, i.e. at the stage of the first trifoliate leaves, at the beginning of flower bud development and at the beginning of the green pod according to the specified doses for soybeans. After harvesting, the content of total protein, crude fat and macronutrients (N, P, K, Mg, Ca) in the seeds was determined. The applied biostimulants had no significant effect on soybean morphological traits as well as seed yield. The interaction of the factors studied resulted in significant differences in TSW and germination capacity. The highest TSW and germination capacity were found in the Merlin variety when Aminoprim was applied. The chemical composition of the seeds in terms of protein and fat content differed significantly between the soybean varieties tested in favor of the Lissabon variety. Optisil caused significant increase in protein and fat content in the seeds.

Keywords: Soybean, biostimulants, yield, cultivation, variety

Sowing date as a factor that affects the yield and quality of soybean seeds

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Sustainable agriculture and care for nature, as well as food safety and the health of people and animals, are the main topics of the European Green Deal. One of the main goals of this strategy is to improve the yield of crops, which should be achieved by reducing the use of fertilizers and plant protection products in favor of non-chemical methods to promote yield. Therefore, the following work hypothesis was formulated: How does the date of sowing contribute to the quantity and quality of soybean seed yield in southwest Poland? In the years 2016-2019, field experiments were carried out in a split-plot design with two variables. The sowing dates were the first factor: 1. half of the second week of April, 2. 10 days later than the first date, 3. 20 days later than the first date. And soybean cultivars: Lissabon and Merlin were the second factor. Among the traits tested, such as TSW, seed yield, protein and fat content, and protein and fat yield, the most important characteristic that describes the quantity and quality of seeds is protein yield. All the factors examined affected this trait. The highest protein yield was obtained both on the earliest date of sowing and on the date delayed by 10 days. Lissabon cv. had the highest protein yield compared to Merlin. The 2016 year was the most favourable for soybean yielding. A strong impact of the weather course in the years of research on this trait was also shown in the interactions of factors. In southwest Poland, it is recommended to sow soybeans in the second-third week of April and in the beginning of May; however, this problem needs more investigation, especially in countries at higher latitudes, so that soybean production could be profitable and does not involve risk.

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Effect of struvite (Cristal Green) fertilization on biometric traits and changes in soil elements content under soyabean cultivation

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Faced with declining phosphorus resources and increasing demand for this fertilizer caused increased interest in looking for alternative sources of phosphorus. This problem particularly concerns the agriculture, since this sector relies mainly on phosphorus. One source of phosphorus is struvite recovered from sewage sludge. Available fertilizers produced on the base of waste, have not yet been thoroughly tested or registered as a mineral fertilizer in the EU's general regulations on the EU fertilizer market. In 2022, a pot experiment was established at Wroclaw University of Environmental and Life Sciences using fertilizer produced from sewage sludge in soybean cultivation. The first factor was the differential placement of phosphorus fertilizer: band and broadcast placement of granules at a height of about 5 cm below the sown soybean seeds. The second was two types of phosphorus fertilizers. Fertilizer doses in the experiment were based on the optimum for growing soybeans under field conditions, i.e. 70 kg ha^{-1} P_2O_5, 120 kg ha$_{-1}$ K_2O and nitrogen 30 kg ha^{-1} N. Nitrogen fertilizer (ammonium nitrate 36%) and potassium salt (60% K_20) at the same dose were applied to all pots. Abellina soybean inoculated seeds were sown in the second decade of May 2022. Phosphorus fertilizer placement significantly affected two biometric parameters of soybeans, i.e. first pod set height and stem weight after harvest. Significantly higher pod height as well as stem weight was observed when phosphorus fertilizer was placed band. The method of fertilization of soybeans significantly affected the phosphorus content of the soil. Significantly more of this element was found with broacast fertilization of soybean. The application of struvite significantly contributed to an increase in soil magnesium content, as struvite contains this element in its composition, which may be important when fertilizing Polish soils, which are characterized by low abundance of this element.

Keywords: Struvite, seeds, phosphorus, magnesium, potassium

Investigation of expression characteristics of flowering genes in leaves according to soybean growth stage and solar response

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In soybean, flowering is not only an important period of transition from vegetative stage to reproductive stage, but also a factor that affects yield. In this study, in order to find out whether the day length and temperature affect the flowering of soybeans, the correlation between meteorological data and flowering days in 6 regions for 4 years from 2019 to 2022 was checked, and the expression of GmFT2a and GmFT5a in short day condition quantity was confirmed. Flowering days increased with higher latitude and decreased with higher temperature. Flowering days and temperature showed a negative correlation, and integrated temperature and daily precipitation showed a positive correlation. GmFT2a and GmFT5a, which are flowering genes under short day condition, were most expressed in the second trifoliate, resulting in the leaf having the most effect on flowering under short day condition. In addition, since the expression of flowering genes tends to increase significantly from 12 days after emergence, flowering management is required from 12 days after emergence when grown under short day condition. As a result of this study, it was confirmed that pre-flowering treatment was carried out at the time of the second trifoliate, 12 days after emergence, when grown under short day condition. In the future, experiments under long-day conditions and experiments by temperature conditions will be conducted to find out the timing of pre-flowering treatment by day length and temperature.

Factor productivity and effect of individual input of production on growth and yield of soybean [*Glycine max* (L.) Merrill]

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Soybean [*Glycine max* (L.) Merrill] is a highly accepted-important oilseed and legume crop. Higher acceptability across the world resulted in an increase in the area, production and productivity of this crop. In Indian context area under soybean is steadily growing, while production and productivity is low compared to the world and leading countries. To bridge this gap, factors of production determining the per-unit productivity plays a crucial role. In order to identify and quantify the factors of soybean production and their contribution, field experiments were conducted during 2019 and 2020. Management practices viz., recommended seed treatment, seed inoculation, recommended dose of fertilizer, weed management, insecticide application, sowing on ridges and furrow were evaluated as separate seven treatments comprising full practice and omission of each practice from it. Soybean grown with full practice (3,221 kg ha^{-1}) gave significantly higher seed yield over omission of RDF (2,489 kg ha^{-1}) and weed management (2,560 kg ha^{-1}). Increase in seed yield with full practice over omission of RDF was higher by 22.72% and over omission of weed management it was 20.52% high. Yield gap was maximum with omission of RDF (732 kg ha^{-1}) and omission of weed management (661 kg ha^{-1}). Economic gain due to full practice was maximum over the omission of the individual practice. Least break-even cost was incurred with full practice (₹ 12.93 kg^{-1}), while higher was with omission of weed management (₹ 14.81 kg^{-1}). Crop output efficiency in terms of partial factor productivity and agronomic efficiency was higher with full practice than rest of the management practices. Results of the study revealed that soybean crop grown with optimum management practices gives higher seed yield. An omission of the factors of production significantly affects the growth, yield attributes, seed yield, and the economic benefits from the soybean. Supply of nutrition through recommended dose of fertilizer and weed management are the major factors contributing to soybean yield loss and attributed to maximum yield gaps compared to full practice comprising all management practices and omission of the other factors of production.

Keywords: Factor productivity, inputs of production, management practices, soybean, yield gap

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Soybean deficit irrigation in central-north Italy: first results

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An experiment was conducted on soybean to determine whether deficit irrigation could be an effectively applicable practice. The study was conducted from May to October 2022 in Italy in two different sites (Veneto Region and Tuscany Region). In each site, an open field experiment with 4 plots (40 m x 40 m) was implemented to compare two different irrigation regimes: complete restoration of crop water requirements (100% CWR) and regulated deficit irrigation (70% CWR during the entire cycle with restoration to 100% CWR during flowering).

The same cultivation practices were carried out for all plots and the same irrigation system was used (micro-irrigation), which was managed through continuous monitoring of the soil water balance using capacitive probes and tensiometers. The cumulative water received by soybean (irrigation + rain) was approximately 443 mm (70% CWR) and 498 mm (100% CWR) in Veneto region and 456 mm (70% CWR) and 458 mm (100% CWR) in Tuscany region.

No significant effect was exerted by the two irrigations management on soybean pods and seeds number and dry weight. Significantly higher pods number (+31.5%) and seeds dry weight (+49.2%) were obtained in the Tuscany site than Veneto site (50.8 pods plant-1 and 18.9 g plant^{-1}). The number of seeds per plant was not significantly different between the two experimental sites with an average value of 131 seeds plant^{-1} whereas the 1,000 seed weight was higher in the Tuscany site than Veneto site (204 g vs. 153 g).

Although the obtained results need to be confirmed in the next growing seasons, they suggest that the proposed deficit irrigation management for soybean is able to save water without negative impact on the crop yield.

Influence of fertilization on yield and protein content of selected soybean varieties in Austria

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The influence of targeted nitrogen fertilization of cereals on yield and crude protein content is well known. In the case of legumes, nitrogen fertilization is generally not applied, as this only impairs the activity of the rhizobia and reduces nitrogen efficiency. The aim of selected field trials conducted by Josephinum Research is to investigate the influence of nitrogen and phosphorus fertilization on selected soybean varieties. All experiments are repeated four times, randomised and repeated over several years. The variants with nitrogen fertilization were also carried out at two points in time, as trials from previous years pointed to reduced rhizobia growth and lower yields when nitrogen fertilizers are applied before sowing. In the case of variant one, fertilization was carried out before sowing and in the case of variant two, at the time of flowering, with the aim of administering appropriate nutrients at the time of the highest nitrogen requirement. The variants with phosphorus were only fertilized before sowing. In the trial year 2022, all varieties show an influence of fertilization on yield and crude protein yield; yield differences are also significant in some cases. On average across all varieties, the crude protein yield could be increased by up to 5 % at 30 kg N/ha and up to 14% at 60 kg N/ha by a targeted N application at flowering. Fertilization with 30 kg P_2O_5 before sowing shows only a minimal increase in yield on average for all varieties, although a yield increase of around 8% is observed for the variety Adelfia with a similar crude protein content. It remains to be said that targeted fertilisation can increase both yield and crude protein content of soybeans, although there are sometimes large differences between varieties.

Keywords: Fertilisation, nitrogen, crude protein yield

Regulation of pod setting by photoperiod after flowering in soybean (*Glycine max* (L.) Merr.)

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Soybean yield is determined by the pod number, seed number, and individual seed weight; it correlates more strongly with the number of pods than with the other two components (Schou et al., 1978). Therefore, analysis of the pod setting mechanism would contribute to increasing pod number and the possibly yield. The pod setting of bambara groundnut (*Vigna subterranea* (L.) Verdec.) was promoted by short-day conditions (Nishitani et al., 1988). Onions (*Allium cepa* L.) develop bulbs in response to long-day photoperiods (Garner & Allard, 1920). Potato (*Solanum tuberosum ssp. andigena*) requires a short day for tuber formation, and grafting experiments have confirmed that the photoperiod for tuberization is perceived in the leaves (Martínez-García et al., 2001). Therefore, in this study, we investigated the effect of short-day treatment duration on pod setting time, the organs that sense photoperiod associated with pod setting, and the mobility of the signal related to pod setting in soybean. As a result, short-day treatment of leaves decreased the number of days from flowering to pod setting. The duration of short-day treatment and the number of days from flowering to pod setting were negatively correlated. On the other hand, short-day treatment of flowers after flowering did not promote pod setting. In Y-shaped plants, pod setting on one side was not induced by short-day treatment of leaves on the other side, and vascular tissue of the two stems was not connected at the junction. Our results suggest that a signal produced in leaves under short-day condition moves to the nodes and promotes pod setting after flowering.

Keywords: Photoperiod, pod setting, soybean

Western hemisphere quality and production capacity of soybean protein

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Soybean [*Glycine max* (L.) Merr.] is the main protein source for monogastric livestock. Following the increased global demand for animal protein, the Americas' soybean production doubled in the last 20 years and currently it is responsible for more than 80% of the global soybean production. Future demand for soybean production will depend on grain protein concentration and on the amino acid profile (protein quality). The main objective of this study was to assess spatial patterns in soybean protein concentration and the proportion of the most critical amino acids in protein. On-farm soybean grain samples totaling 1,840 were collected to match actual production statistics across each of the main productive regions: North America, Brazil and the Southern Cone (Argentina, Uruguay and Paraguay) during three cropping seasons (2012-2013). A consistent spatial pattern for protein concentration and quality was detected across regions. Brazil produced 2.0 and 5.4% more protein per tonne of grain than did the North America and the Southern cone, respectively. Relevant patterns in protein concentration among sub regions within regions were also detected. We also detect differences in the amino acid profile across regions. The abundance of the most critical amino acids (lys, thr, try, met, cys) in protein was diluted as protein concentration was increased. This dilution process was less than proportional compared with the increase in grain protein concentration. Thus, in general, the amount of critical amino acids per ton of grain was increased with protein concentration. Results from this paper provide benchmarking values of regional expected protein concentration and protein composition across the Americas. After integrating these results with public production statistics, we were able to estimate the amount of protein and amino acids produced by the western hemisphere.

Keywords: Soybean, protein, amino acids, Americas, patterns.

Effect of different nitrogen sources on soybean growth: from nodule efficiency to yield

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Soybean production has multiplied by 15 since the 1950s. As an increasing number of people are reducing their meat consumption, the need for protein-rich plants is rising and more applications for soybeans are emerging as food alternatives to replace animal proteins. Other than their great nutritional qualities, soybeans are able to form nodules and thus fix atmospheric nitrogen, reducing the need for high quantities of fertilizers. Adding inorganic nitrogen fertilizer is known to reduce nodule formation and activity, limiting the efficiency of nitrogen fixation. To optimize soybean production while limiting the use of inorganic fertilizers, we tested the use of newly developed organic nitrogen-containing biostimulants on soybean growth. We compared the effect of inorganic nitrogen (classical fertilizer), organic nitrogen (new biostimulants), and no nitrogen addition on soybean growth and development. We could show that the different nitrogen sources had a major influence on soybean growth at different levels: while the application of inorganic nitrogen reduced the average nodule size and the respective dry weight, no difference in nodule size and weight was observed when the organic biostimulant was used instead. Additionally, we saw an increase in plant biomass only when the organic nitrogen-containing biostimulant was used. The supply of nitrogen sources and their influence on soybean growth has so far been studied mainly for inorganic nitrogen. Further research into the influence of organic nitrogen could hold great promise for improving soybean yield and quality.

Keywords: Nitrogen sources, nodule development, soybean growth

Transcriptome mapping key genes encoding PR1 protein involving in necrosis responding to SMV infection basing on bulked segregant analysis

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Necrosis caused by soybean mosaic virus has not been specifically distinguished from susceptible symptoms and the molecular mechanism for occurrence of necrosis is largely overlooked in soybean genetic researches. To expand molecular mechanism behind necrosis reactions, transcriptomic data derived from asymptomatic, mosaic and necrosis pool were assessed. Field evaluation reveals that SMV disease have a serious influence on soybean production as indicated by decreasing 22.4%~77.0% and 8.8%~17.0% of yield and quality production, respectively. Compared to asymptomatic and mosaic plants, 1689 and 1752 up and down regulated DEGs were specifically found in necrosis plants. Interestingly, the top 5 enriched pathways with up regulated DEGs are highly related to the process of stress response, whereas, the top 3 enriched pathways with down regulated DEGs are highly related to the process of photosynthesis, demonstrating that defense systems are extensively activated, however, the photosynthesis systems were severely destroyed. Further, results of phylogenetic tree basing on genes expression pattern and amino acid sequence, as well as validation experiments discovered three PR1 genes, Glyma.15G062400, Glyma.15G062500 and Glyma.15G062700, are specially responded in necrosis leaves. Meanwhile, exogenous SA but not MeJA could obviously induce the three PR1 genes expression on heathy leaves. Contrastingly, exogenous SA obviously decreased the expression level of Glyma.15G062400, Glyma.15G062500 and concentration of SMV, but increased Glyma.15G062700 expression in necrosis leaves. The results together suggest that Glyma.15G062400, Glyma.15G062500 and Glyma.15G062700 are special response genes in necrosis symptom which will greatly facilitate us better understand mechanism behind necrosis caused by SMV disease.

Linkage analysis and genome-wide association analysis of seed coat -traits in soybean

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Soybean seed coat color is a typical morphological marker and evolutionary feature. Therefore, study of soybean seed coat color-related traits is of great significance to both evolutionary theory and breeding practice. In this study, we used 185 F8:10 recombinant inbred lines (RILs) from the cross between a cultivated soybean Jidou 12 (JD12) and a wild soybeanY9 (ZYD02739) as the materials to identify the quantitative trait loci (QTLs) for seed coat color and seed navel color. We used three models, Single markers Analysis (SMA), Interval Mapping (IM)， Inclusive composite interval mapping (ICIM)， identified 23 QTLs for seed coat color and 13 QTLs for seed navel color. The three models identified stable QTLs were located on chromosomes 2 and 8 (qSC02 and qSC08), and located on chromosomes 8 and 14 (qSC08 and qSC14), which, respectively, for seed coat color and seed navel color. Meanwhile, Genome-wide association analysis (GWAS) was used to verify and analyze 250 GBS sequencing populations for seed coat color and seed navel color. The results show that both populations colocalized to two stable QTLs (qSC02 and qSC08). Two candidate genes (CHS3C and CHS4A) in qSC08 were identified, according to Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis. Three genes (Glyma.02G024600, Glyma.02G024700 and Glyma.02G024800) were predicted in the novel QTL qSC02. These genes belongs to Glutathione transferase family members (GSTs) are enzymes that help to control plant development and stress responses, and are also involved in anthocyanin accumulation. These results contribute to established foundation for studying genetic mechanism and evolutionary relationship of seed coat color in soybean between wild and cultivated soybean.

The effects of different row spacings on yield and yield components of soybean cultivars from different maturity groups

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The aim of this research was to determine the effects of three different row spacings (0.25, 0.50, 0.75 m) on seed yield and yield components of soybean cultivars from maturity groups III and IV (Nova and Umut). The cultivars were placed in the main plots and row spacings in sub-plots. The cultivars of the main plots were in maturity group III (Nova) and maturity group IV (Umut-2002). The split plots had the following row spacings; narrow, medium and wide-row spacings of 0.25, 0.50 and 0.75 m, respectively. In the trials, the intra row spacing was fixed 5 cm on every row spacing. According to two year results, differences between row spacings were statistically significant on seed yield and all of the examined components except for harvest index and the first pod height. The difference between years was found to be significant for all characteristics observed except for plant height and first pod height. The cultivar x row spacing interaction was significant for only 100 seed weight. It was revealed that when the row spacing increased, the seed yield increased. In the study, while the highest seed yield (2239 kg ha^{-1}) was obtained from 0.75 m row spacing, the lowest seed yield was obtained from 0.25 m (1,895 kg ha^{-1}) row spacing. Also, significant differences were found between cultivars in terms of seed yield and all of the examined components. The cultivar Umut-2002 gave higher seed yield (2,147 kg ha^{-1}) than Nova cultivar (2002 kg ha^{-1}).

Carbon footprint of soybean crops associated with different agronomic management in the province of Tucumán (Argentina) between 2018 and 2021

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Carbon footprint is an environmental indicator that quantifies the total amount of greenhouse gas (GHG) emissions caused directly or indirectly by a system. Agricultural inputs or stages that generate the highest GHG emissions constitute hotspots in the process and are susceptible of improvement. The objective of this study is to determine the equivalent carbon dioxide (kg CO_2eq) emissions of soybean production in Tucumán (Argentina) taking into account four agronomic crop sequences. The sequences were BSTMBS; TSTMBS; BSBMBS; BSBSBS (fallow= B, soybean= S, wheat= T, corn= M). The evaluated system included from fallow to harvest for each crop. For the inventory, data recorded in field activities were preferably used, supplemented by specialized publications. The data used are representative of Tucumán, obtained from applied technology surveys carried out between 2018 and 2021. The study was carried out using the life cycle assessment (LCA) methodology. The IPCC 2013 GWP100a impact evaluation model was used to obtain the carbon footprint. SimaPro® and a calculator developed by INTA and INTI were utilized as a software tool to carry out life cycle modeling and calculations. The results showed a variation of the soybean crop carbon footprint between 184 and 211 kg CO_2eq/t. The main factors are crop residues (38-43%), seeds (24-25%), fuel burned in various tasks (14-15%), herbicides (10-13%) and fertilizer (7%). Another result of this study was the carbon footprint (kg CO_2eq/t grain) for each sequence: TSTMBS 157.5; BSBMBS 158.6; BSTMBS 158.9; and BSBSBS 207.2. The TSTMBS sequence revealed the lowest carbon footprint, between 1% and 24%, compared to other sequences. In addition, an inverse relationship between carbon footprint and soybean yield was observed. In Tucumán, soybean planted after corn, yields an average of 20% more than soybean in monoculture. This crop rotation has an impact on the soybean carbon footprint, by reducing the amount of CO_2eq/t emitted.

Keywords: Crop rotation, greenhouse gas, crop residues, grain crops

Effect of winter cover crops and tillage systems on soybean production

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The adoption of conservation practices such as no-tillage (NT) and cover crops (CC) is critical to promoting long-term soil health and agricultural sustainability. This study evaluated wheat, and hairy vetch as winter CCs, along with control, on soybean growth under NT and conventional tillage (CT). The field trials were conducted in Louisiana, USA in two soil-types with very fine sandy loam (VFSL) and silt loam (SL) soil textures. The biomass yield of CCs was significantly higher for wheat under CT than NT, while similar for vetch in both soil types. In VFSL soil, CT had 29% higher plant height compared to NT, while CCs had no significant impact on plant height under both tillage systems. In SL soil, similar to VFSL, NT had 30% smaller plant height than CT, while CC either slightly improved or had no effect on plant height in both tillage systems. In VFSL soil, the grain yield averaged 3.24 Mg ha^{-1} across years in the CT system and 3.02 Mg ha^{-1} in the NT system, with vetch CC tending to produce a higher grain yield compared to wheat and the control. In SL soil, NT had a significantly lower grain yield than CT, ranging between -10% and -55% depending on the CC. In SL soil, CC, especially vetch, significantly improved grain yield under NT, while having a minimal, but mostly negative effect under CT. The major causes for the lower yield under NT in both soil types were higher soil bulk density, which significantly suppressed crop growth, and the higher weed pressure. Overall, CCs could improve soil quality and increase crop yield in humid sub-tropical regions, but such effects vary with CC type and tillage system. Grain yields under NT may be improved through incorporation of CC during fallow periods and improved weed management.

Streptomyces spp. treatment increases soybean yield and seed quality under field conditions

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Nowadays the development of sustainable and environmentally-friendly strategies using soil beneficial microbes as plant growth-promoting rhizobacteria (PGPR) and biocontrol agents (BCA) is gaining great consideration to reduce the use of agrochemicals in crops production. Considering the above, 78 actinobacteria from the genus *Streptomyces* were isolated from the rhizosphere and endosphere of soybean plants from the Argentinean core production zone. Among these, two strains (N2A and N9) were selected based on their *in vitro* compatibility with *Bradyrhizobium japonicum* (B), their ability to produce phytohormones, siderophores, phosphate solubilization and their antagonism to fungal pathogens. These strains were evaluated during 2021/22 growing season to analyze their effect as PGPR under field conditions. The treatments were N2A+B, N9+B, fungicide (F+B) and control (B). The assay was performed following a completely randomized block design at the experimental field (Agronomy Faculty-Zavalla, Santa Fe, Argentina). As results, *Streptomyces* spp. treatments significantly promoted seedlings emergence compared to B and F+B. At harvest, both Streptomyces strains significantly increased seed yield (g/m2) compare to the conventional agronomical practice (F+B), and N2A+B also showed higher yields than B ($P<0.05$). These increases were related to rises in seed number/m2 and pod number in the main stem ($P<0.10$). Finally, a significant increase in vigor and health status was observed for seeds obtained from plants of both Streptomyces treatments in comparison to seeds obtained from B and F+B treatments, while no differences were observed in protein and lipids content. Altogether, we demonstrated that the seed treatment with *Streptomyces* spp. promotes growth and development of soybean crop, possibly due to a better nutritional and health status of the plants, increasing yield and the physiological and sanitary quality of the seeds in field conditions. Thus, we propose the replacement or complementation of traditional agrochemicals with more efficient and environmentally friendly technologies based on these soil bacteria.

Keywords: Actinobacteria, biostimulants, biocontrol agents

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Science for success: a model program for applied research and extension outputs

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The Science for Success project has evolved into a high functioning engine delivering data-driven best management practices (BMP’s) to U.S. soybean farmers. The Science for Success team is composed of soybean Extension personnel from across the U.S. who serve more than 90% of U.S. soybean acres that collaboratively investigate BMP’s and subsequently disseminate gleaned information using diverse outreach strategies including partners such as SRIN, CPN, and the U.S. Soybean Research Collaborative (USSRC). This team leverages QSSB funding and other funding sources (ie USDA-NIFA) to support common-theme localized research efforts needed to generate data-derived BMP’s. We seek USB funding through the proposed project to support subsequent collaborative Extension efforts. This project then generates Extension publications, social media campaigns, videos, and webinars that will deliver BMP’s to U.S. soybean farmers. In this workshop, we will discuss the key components that have made Science for Success effective and dive into lessons learned. Core topics of focus will include conducting effective collaborative research, delivering recommendations using diverse outreach strategies, and the mentorship value of this initiative for junior faculty and graduate students.

Research progress of green and low-carbon nutrient management technologies for soybean

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The soybean is one of the most important food plants of China. Efficient nutrient management in soybean production is a major demand for the development of green and low-carbon agriculture. With the application of intensive management and traditional production technology, the problem that extensive soybean nutrient management technology does not match the "carbon peaking and carbon neutrality" goals is increasingly prominent. At present, it is necessary to implement carbon emission reduction and carbon fixation technologies to achieve soil carbon surplus. How to balance the carbon source and sink of soybean soil through nutrient management has become the key issue of green and low-carbon agriculture development. This paper reviewed the research status and development of green and low-carbon nutrient management technologies of soybean. The carbon sequestration and emission reduction effects of soybean-soil nutrient cycle fixation, intelligent fertilizer application and healthy planting system were summarized. Suggestions were put forward for the improvement of soybean nutrient management technology in China.

Keywords: Soybean, green and low-carbon, nutrient cycling, green intelligent fertilizer, rotation

Responses of leaf expansion and plant transpiration of different soybean genotypes to soil water deficit

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The responses of ecophysiological processes such as leaf expansion and plant transpiration to soil water deficit have been reported to be genotype-dependent. To study such responses in soybean, a two-year (2017 and 2021) outdoor pot experiment was carried out on the Heliaphen automated phenotyping platform at INRAE in Toulouse (France). Six varieties (Sultana-000, ES Pallador-I, Isidor-I, Santana-I/II, Blancas-II, and Ecudor-II) belonging to four maturity groups were subjected to progressive water deficit from the reproductive stage R1 for 17 and 15 days in 2017 and in 2021, respectively. The fraction of transpirable soil water (FTSW) was used as an indicator of soil water deficit. Non-linear regression was used to calculate FTSWt, i.e., the FTSW threshold for which the rate of the ecophysiological process in stressed plants starts to diverge from a reference value. Among six varieties, regarding leaf expansion, cv. Santana was more tolerant (FTSWt = 0.36 in 2017, FTSWt = 0.48 in 2021) to soil drying while cv. Isidor (FTSWt = 0.69 in 2017, FTSWt = 0.71 in 2021) and cv. ES Pallador (FTSWt = 0.69 in 2017, FTSWt = 0.70 in 2021) were the most sensitive. However, there was no such difference between their transpiration responses (for Santana, FTWSt = 0.22 in 2017, FTSWt = 0.30 in 2021; for Isidor, FTSWt = 0.42 in 2017, FTSWt = 0.33 in 2021; for ES Pallador, FTSWt = 0.30 in both 2017 and 2021 experiments). Such a non-destructive phenotyping method could bring new information to variety testing process and provide paths for integrating genotypic variability into crop growth models used for simulating soybean responses to water deficit at a plant, field, or regional level.

Keywords: Soybean, genotype, water deficit, leaf expansion, plant transpiration

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Economic analysis of different crop rotations that include soybean in Tucumán, Argentina in the period 2013/2014-2021/2022

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Since the 2013/2014 season, the EEAOC conducts in Tucumán, Argentina, a long-term trial to study the effects of different grain production systems on the sustainability of the system (considering crop productivity, physical-chemical properties and carbon balance). The trial consists of 18 treatments that include: soybean monoculture with winter fallow; with wheat, with chickpeas and/or winter cover; and a soybean-corn rotation system, in frequency 1:1 and 2:1, also with winter fallow, wheat and/or chickpea as a commercial crop; and winter covers (legume before corn and grass before soybean). The objective of this work is to evaluate economic aspects of the treatments in a period of nine growing seasons (2013/2014 through 2021/2022). The agricultural gross margin per hectare (GM) was used, the difference between income and expenses, a useful indicator to decide between productive alternatives. The GM1 accumulated in the period was calculated for each plot, without considering the extraction of phosphorus from income crops, neither the contribution of nitrogen from the covers (10.6 kg N/t in grasses, 20 kg N/t in legumes). GM2 was determined by valuing the nitrogen of the covers in terms of kg of urea, the extraction of phosphorus from the income crops in kg of triple superphosphate. GM1 (USD/ha) varied, in monoculture between 451 and 1780; in the 1:1 rotation between 1070 and 1919; and in the rotation 2:1 between 1058 and 2123. In these systems, the least profitable treatments presented coverage in winter. In GM2 the treatments with covers, in rotation, were more profitable than those with fallow. The contribution of the coverage compensated from 22% and more than 100% the cost of the exported phosphorus. The differences between GM1 and GM2 reflect the importance of measuring and economically valuing the largest number of inputs and outputs, to analyze the potential and sustainability of each system.

Keywords: Gross margin, rotation, cover crops, corn

Organic and low input soybean production: role of winter cover crops in production systems and its effect on yield parameters

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Cover crops are being used as a tool to reflect agro-ecosystem services. Cover crops differ in the traits by which they capture resources and influence the local agro-ecosystem. The aim of the research was to examine the effects of the winter cover crops in soybean production through the analysis of yield in the two sustainable cultivation systems (organic and low input) during 2021. The experiments were set up according to the block system method with random plots in four replications at two locations Čurug and Rimski šančevi, Serbia. Winter cover crops were following: pure rye crop (R) and a mixture of peas and oats (P+O) (85:15), while the control treatment (C) was an area without cover crops. It was assumed that soybean will respond positively to the selected treatments, which resulted in an increase in grain yield. Yield and yield parameters positively reacted on mixture (P+O) as a pre crop. The beneficial effect of P+O cover crop is mainly associated with rapid growth during autumn and the ability to scavenge large amounts of residual N from deep soil layers with its large tap root and through process nitrogen fixation. Yield was in the range 2.8 – 3.6t/ha. The lowest yield 2.1 t/ha were recorded at the control plot for NS Mercury variety. Average height of NS Mercury variety was 73cm in low input production (Rimski šančevi), whereas NS Mercury in organic production (Čurug) was 92.5 cm. Also, differences in the number of soybean pods and 1,000 grain mass were noted. Agronomic practices which include the introduction of cover crops in soybean production, could significantly contribute to increasing the sustainability of agricultural production systems and to offer the practical solutions that are profitable long term investments in crop production.

Keywords: Glycine max, sustainable cropping systems

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ECOBREED participatory trials as valuable tool for farmer involvement in soybean breeding process

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As a part of the ECOBREED project, farmer participatory trials for organic soybean production were set up in Serbia, Germany, Romania, Austria and Slovenia (seasons 2021 and 2022). By setting up trials direct with organic producers, it was enabled that farmers assess the adaptability of the soybean varieties for organic production in specific agro-ecological conditions at their farm locations. This is one practical tool that enables organic producers to contribute and to be involved in the process of creating new soybean varieties, especially in crops composite population (CCPs) observations together with breeders. ECOBREED farmer participatory trials for organic soybean comprised field testings of soybean varieties from three breeding institutions from Serbia, Austria and Romania (Serbia-Institute of Field and Vegetable Crops, Austria-Saatzucht Gleisdorf, Romania-NARDI-Funduela). The network of organic participatory farms was established in 2021 at 17 locations in 5 European countries. Soybean varieties were selected according to agro-ecological conditions in each country. On all farms same trials observations and assessments were performed: emergence, canopy closure, growth development, height, lodging and yield were evaluated as well as occurrence of diseases and pests. Trial results have big variations among countries, within countries and specific locations (different variety responses). After two years of setting up participatory trials for organic production the farmers got a solid foundation and a direction for selecting a soybean variety for specific agro-ecological conditions that is in line with the farmers' requirements and the established production goal. Farmer participatory trials are a crucial milestone to define local criteria for variety selection and for increased adoption of new and improved soybean varieties to low input and organic production.

Keywords: Trial network, organic production, soybean producers

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Dynamics of tocopherol concentrations accumulation in soybean exposed to brief episodes of heat and drought stress during grain filling

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Soybean oil contains tocopherols, antioxidant compounds that have nutraceutical value for humans. Tocopherol isoforms are alpha, beta, gamma, and delta-tocopherol (α-, β-, γ-, and δ-toc, respectively). The objective was to determine tocopherol concentrations of soybean exposed to heat stress (HS) and drought stress (DS) during the grain filling period (GFP). We hypothesized that HS and DS modified tocopherols accumulation pattern during GFP (R5-R7), negatively impacting total tocopherols (T-toc) at harvest. We conducted a greenhouse experiment using a non-transgenic soybean genotype. Four treatments were evaluated: control, plants under irrigation, at 80% of soil field capacity (FC); HS, plants under irrigation exposed to temperatures >32°C for 6h around noon; DS, plants maintained at 40% FC, and plants under HS×DS interaction. At stages R5, R5.5, R6, R6.5 and R8, previously marked pods were collected for tocopherols determination. The T-toc resulted from summing α-, β-, γ-, and δ-toc. At R5.5, non-significant changes ($p>0.05$) were observed for α-toc, β-toc, and T-toc, while γ-toc decreased 8% on HS and increased on average 17% under DS and HS×DS. δ-toc decreased on average 15% among stress treatments. At R6, α-toc increased markedly under the three stresses, reaching 181% on HS×DS; in contrast, δ-toc decreased, although to a lesser extent, by 28% on average. β-toc increased by 33% under HS and 59% under DS; whereas γ-toc decreased 16% on average under HS. At R6.5 a similar trend to R6 was observed. At R8, α-toc increased 24% under HS and 64% under DS, β-toc increased 50% under DS, whereas δ-toc decreased under both stresses (15 and 8%, respectively), with non-significant changes of γ-toc and T-toc by any stress. We conclude that, although the tocopherol isoforms varied during GFP, this did not negatively impact on T-toc at harvest. Furthermore, α-toc stood out as the most sensitive isoform, always increasing under stress conditions.

Keywords: Glycine max, health-beneficial compounds, tocopherols, high-temperature stress, water deficit

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Early detection of bacterial pustule on soybean through hyperspectral imaging

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Over the past few decades, bacterial infections have expanded widely across soybean fields. The bacterial pustule (*Xanthomonas axonopodis pv. glycines*) is one of the most severe diseases affecting soybean seed size and yield. Advanced technology for plant stress detection has become popular in the present scenario. A non-destructive method of disease detection based on image analysis has been developed for the early detection of diseases with high accuracy, ultimately saving time and labor. While plenty of technologies were developed, hyperspectral imaging (HSI), a non-destructive technology, is widely used in the agricultural sector. Realizing the importance of disease detection, we propose an approach to detect the soybean bacterial pustule using the hyperspectral camera at the early growth stage. This experiment aims to identify the specific wavelength for disease detection of bacterial pustule infection. The experiment was conducted under controlled environmental conditions in the plant growth chambers. The experiment consisted of i) control (non-inoculated) and ii) treatment (bacterial pustule inoculated) with two soybean varieties, Cheongja 3-ho and Daechan. The bacterial inoculation was done 12 days after planting (DAP) and the hyperspectral imagery data were collected 48 hours after inoculation. The Python 3.9 package algorithm analyzed the data obtained from the ENVI V5.5.3 software. Our results showed that the bacterial pustule revealed few symptomatic regions after inoculation on both varieties at the early growth stage. Among the two varieties, Daechan revealed fewer symptoms than Cheongja 3-ho. A significant difference in the leaf reflectance of the diseased and healthy leaves was present in the green region of spectral reflectance. The bacterial pustule was classified on the third day after inoculation with accuracy of 97.5% and 100% in Cheongja 3-ho and Daechan based on the analysis algorithm's two-way ANOVA results. These results showed that the HSI is feasible to pre-detect the bacterial pustule and may provide guidance for a further related research topic.

Keywords: Hyperspectral imaging, leaf reflectance, soybean, disease detection

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Preserving soybean seed quality with Insuter Bag Bio® vacuum technology

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Soybean (*Glycine max* (L.) Merr.) is one of the cultivated species with the shortest seed longevity, rapidly losing seed quality during storage after a year of harvest. Thus, it is non-recommendable to store a carry-over of seeds for the next seed production season. The Bag Bio® technology developed in Argentina by Insuter SRL company preserve food and seeds quality for long periods of time by storing them under a modified atmosphere (vacuum or N2 gaseous) in hermetically closed containers which can be placed in warehouses without thermal control. We evaluated: i- if the Bag Bio® technology was suitable for maintaining soybean seeds quality, and ii- if the seeds stored for 12 months under vacuum performed similar to those harvested in the current production season. Seven month after harvesting, soybean seeds, cv. DM 52R19 were stored for 12 months (Acopio Arequito SA, Argentina) under vacuum conditions in 1,000 kg containers (Bag Bio®). Soybean seeds of the same cv. currently harvested were used as control. Both seeds, storaged and currently harvested were sowed in field during the growing season 2019-2020. No differences in the seeds germination´s test were found between before and after the seed storage period (96 % vs. 94 %) respectively. Crop field yield was 5,029 kg ha^{-1} for stored seeds while the control ones yielded 5,800 kg ha^{-1}. According to our results, vacuum seed storage becomes a simple and safe way to preserve soybean seed quality until the next production season, avoiding quality losses and warranting seed availability in case of scarcity because of season production problems. The Bag Bio® technology provides the seed industry an interesting, sustainable and accessible avenue to produce carry-over soybean seed.

Keywords: Soybean seeds, seed quality, carry-over, storage

The effects of different tillage practices on the growth, yield component, and economic efficiency on soybean (*Glycine max* L.) under irrigated conditions

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Soybean (*Glycine max* L.) is one of the most important protein crops in Hungary, due to the production of meat, dairy and high-quality oil. While the biological, physical, and chemical characteristics of the soil can be impacted by tillage intensity and tillage system, proper soil management can have a significant impact on the yield. The purpose of this research was to determine the effects of the different tillage methods on yield components and soil properties. The field experiment was conducted at the Szárítópuszta Experimental and Training Farm of Hungarian University of Agriculture and Life Sciences in the Gödöllő Hills, during the period of April to November 2022, under the agro-ecological zone: aez-3 (northern Hungarian uplands), where climatic conditions is moderately dry continental. One soybean variety has been released in the experimental field, where the soil type is rust-brown forest soil (Luvic Calcic Phaenozem), formed on bedrock typed sand mixed loess. Four different tillage treatments have been applied systematically in a randomized split-plot design in four replicates: mouldboard ploughing with levelling (28-32 cm), disking (16-20 cm), deep loosening (40-45 cm), and no-tillage (direct-drilling). Soil properties were measured monthly to examine the effects of tillage systems. Tillage systems had significant ($P < 0.05$) effects on harvest index and yield. In our short-term tillage experiment, deeper soil tillage provided significantly higher values on yield components. The results of this study highlight the importance of finding the possible sustainable tillage methods under agroecological conditions. By choosing the optimal tillage system in ever-changing agroecosystem that can produce high-quality and stable yield, suitable soil management is obvious for achieving sustainable agricultural production.

Keywords: Tillage methods, soybean, irrigated conditions, economic efficiency, sustainability

The mechanism of green stem disorder (GSD) through functional linkage between above- and below-ground part in soybean (*Glycine max* (L.) Merr.)

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Green stem disorder (GSD) in soybean is that the stem and leaves remain green and keep high moisture content after the pods have matured, and GSD lowers the seed quality in harvesting with machine. Disturbances in the sink-source ratio induces GSD and depodding in soybean at R5 increased the amount of cytokinin content in xylem sap and induced GSD (Larry et al. 1990). In plant development, cytokinins have key roles like delaying senescence. They are mainly synthesized in the roots and transferred to shoots through xylem. Additionally, in *Arabidopsis thaliana*, nitrate fertilization induced expression of cytokinin synthesis gene (Takei et al, 2004). Therefore, we investigated the interaction with nitrogen and cytokinins in shoot and root for comprehension of GSD in soybean. In soybean plants depodding at R5, the nitrogen content in the root and cytokinin content in xylem sap significantly increased, inducing GSD. When high nitrogen fertilization was applied to plants, cytokinin content in xylem sap significantly increased, inducing GSD. These results indicated that transport of nitrogen from shoot to root was the signal of GSD. When the stem on one side of Y-shaped plants, which the vascular tissue of the two stems was not connected at the junction, was depodded at R5, the plants of the other side also induced GSD. These results indicated that nitrogen moved shoot to root, and GSD was consequently induced by cytokinin biosynthesis at root in soybean.

Keywords: Green stem disorder, cytokinin, nitrogen

Corn insertion into a long-term continuous soybean cropping system accelerates the changes of soil bacterial functional structure in promoting soil nutrient cycling

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Long-term continuous cropping of soybean will cause the accumulation of pathogenic microorganisms, causing continuous cropping obstacles, and finally resulting in a decline in crop yields. Corn insertion into a long-term continuous soybean cropping system occurs frequently in the corn-soybean rotations system, which is common practice in improving soil fertility and crop yield. It is mainly believed that the soil microbial community may play a central role in driving a variety of ecosystem functions and maintaining plant productivity in the ecological process. To clarify the soil micro-ecological mechanism of crop rotation to promote crop yield. The effect of corn insertions (Sm: no corn insertion, CS: 3 cycles of corn-soybean rotations, and CCS: 2 cycles of corn-corn-soybean rotations) on bacterial functional structures and microbial activity were investigated in a long-term field experiment. Our result showed that the bacterial functional structure was altered by corn insertion ($p < 0.01$). The dominant functional groups in the cycling of N were nitrification then followed by aerobic ammonia oxidation, nitrogen fixation, and nitrogen respiration. The abundance of the top three functional groups (nitrification, aerobic ammonia oxidation, and nitrogen fixation) was significantly higher under the corn-soybean rotation system. Using random forest modeling, we identified the PC1 of principal coordinate analysis of bacterial functional groups, which was the main predictor of soil potential nitrogen mineralization (PMN). In summary, corn insertions mainly regulate soil fertility indirectly by changing the soil's bacterial functional structure. In the case of crop rotation without external nutrient input, the increase in crop yield and above-ground biomass were largely due to the positive role of bacterial functional groups in soil nutrient cycling.

Keywords: Corn insertion, rotation, soybean, bacterial functional structure

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Zoning of some soybean varieties, under the conditions of climatic changes in south-eastern Romania

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The paper presents results of the research undertaken at the Braila Agricultural Development Research Station, for the zoning of some soybean varieties, cultivated both for green beans and for dry beans, in the pedoclimatic conditions of South-Eastern Romania. There are presented dynamics of regional climate changes over the last 20 years, the importance of protein crops in Romania and the production and quality results following the testing of 8 soybean varieties for dry beans and 3 varieties for pods, in the year 2022, including in the successive crop and with different densities and fertilizations.

The choice of soybean varieties resistant to heat is the best in the conditions of South-Eastern Romania, because pollination can only be successful in conditions of sufficient relative humidity of the air, in order to form the pods. Also, the culture must be kept clean of weeds, in order to obtain good productions. A classification of the yields of varieties was made, with specific recommendations for each variety tested, both regarding the sowing season and the fertilization applied, inclusive in double crops.

Keywords: Soybean, zoning, climatic change, technologies

Influence of foliar application of thiourea on growth and yield attributes of soybean

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Soybean, the miracle crop of 21st century is a leguminous crop being cultivated intensively in different agro climatic regions in India which has diverse food and industrial uses with promising source of vegetable oil, neutraceuticals and protein. In the quest of higher and stable yields, the crop has received immense attention by the researchers. In spite of its high yield potentiality, soybean productivity is much lower than developed countries mainly due to sub optimal application of fertilizers. There is a need to improve the nutrient management involving the use of new fertilizers formulations. Thiourea is a compound of carbon, nitrogen, sulfur and hydrogen and is a versatile reagent in organic synthesis. The properties of urea and thiourea differ significantly because of the relative electro-negativities of sulfur and oxygen. The present study on influence of foliar application of thiourea on growth and yield attributes of soybean was carried out at the University of Agricultural Sciences, Dharwad-India during 2022-23. The response of two soybean varieties viz JS-93-05 and MACS 1188 was investigated to different levels of thiourea applied at two intervals after the crop is sown. Among various thiourea levels, foliar application of thiourea at 750 ppm at 20-25 DAS and at 50-55 DAS recorded significantly higher growth parameter like plant height (68.17 cm), branches per plant (6.08), pods per plant (73.61) and seed index (15.24 g) followed by the application of same fertilizer at 500 ppm at the same intervals. The interaction effect showed that foliar application of thiourea at 750 ppm in both the varieties recorded significantly higher seed and biological yield compared to other treatment combinations. Thus, foliar application of thiourea at 750 ppm proved most productive and remunerative compared to other levels, which can be exploited for enhancing the soybean productivity.

Keywords: Soybean, thiourea, foliar application, growth parameters, yield

Preliminary results of underground drip irrigation on soybean and other crops in Tucumán, Argentina

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Soybean is the most planted crop in Argentina, and contributes significantly to generate income to the country by grains and by-products exports. Thus, research in order to improve its productivity or expand its agricultural frontier is constant. Irrigation systems at a commercial level constitute a novel alternative but research is still incipient. Among irrigation systems in use, underground drip irrigation (URI) has the lower costs as an advantage, which would allow its use in extensive crops. Besides, soybean is incorporated in a sequence of grain crops, and implementation of irrigation will benefit them all. The objective of this research was to determine the influence of URI on yield of four grain crops in Tucuman (soybean, corn, wheat and chickpea), as well as their components. In a first experience carried out in the 2018/2019 season, summer crops (soybean and corn) could not be evaluated, due to the fact that rainfall was sufficient and irrigation threshold was never reached. In the next summer season (2019/2020), corn hybrid was strongly affected by diseases, thus data were not considered, while the soybean trial showed a yield increase of about 13% in the irrigated plots, which meant an increase of 2.77 kg per irrigated mm. These results were mostly explained by a larger size of grains. In both winter crops tested, due to normally dry winters in Tucuman, irrigation was needed on the 2019 winter trials. It increased wheat yield by almost 50% and chickpea yield by about 25% (mainly explained by a greater number of grains per area and not by grain size). Also, yield differences between two irrigation tapes spacing (ITS) (105 cm and 130 cm) were evaluated, and no significant differences were found on winter crops nor on soybean, which indicate that ITS in these crops can be increased and thus reduce the cost of the initial investment.

Keywords: Soybean, corn, wheat, chickpea, grain crops, drip irrigation, underground irrigation, irrigation tapes spacing

Analysis of soybean maturity groups in north-western Argentina

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A trend to shorten the maturity groups (MG) in soybean crops has been noticed in northwestern Argentina (NWA) in the last 10 years, due to several reasons like productive intensification, sanitary aspects, operability, among others. In order to acknowledge the performance of MG soybean in NWA, data from the last 22 seasons of the NWA Soybean Evaluation Network were analyzed by regression tests and descriptive statistics. Scatter graphs were used first to compare yields of the short (V and VI) and long MG (VII and VIII) varieties in every location, which indicated that most locations did not show differences greater than 5%. The combination of location and season as tested environment also showed that long-cycle varieties obtained the highest yields in most of them; but when the quality of the environment improved, short MG varieties generated higher yields than the long MG varieties. To understand these results, the 22 evaluated seasons were divided into three sub-periods according to their crop cycle accumulated rainfall: a central sub-period (of very prolonged drought), and the before and after the drought sub-periods. The first period presented accumulated rainfall similar to the reference values, and here long-cycle varieties obtained the highest yields. In the following sub-period, characterized by severe water deficits and lowest yields, the long-cycle varieties continued to present the highest yield values. Finally, last sub-period was characterized by accumulated rainfall higher than normal, showing that in this case short MG varieties obtained the highest yield values. Comparing the contrasting situations observed, long MG varieties showed a more defensive performance, which allowed them to excel in this situation. As the environment quality improved, short cycles showed higher yield potentials than the long ones.

Keywords: Soybean, maturity groups, environment, drought

Varietal differences in branching plasticity of soybean cultivars in relation to planting density

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Background

It is generally known that there are varietal differences in the planting density response in soybean cultivars. This is largely due to the variation in the degree of branch development (branching plasticity in relation to planting density) between cultivars from sparse to dense plantings.

Materials and methods

This branching plasticity with respect to planting density can be calculated and expressed as the ratio of the number of branch nodes at the most sparsely planting to the most densely planting. However, this method requires a lot of labour and cannot be used to examine a large number of cultivars, so we examined a simplified evaluation and found that branching plasticity can be explained by the number of branch nodes after the main stem is pinched at V2~V3. We examined the branching plasticity of more than 40 cultivars including old indeterminate cultivars from the northern U.S., Canada, and determinate cultivars from northern Japan by the number of branch nodes after pinching.

Results

We found that the higher the maturity group, the higher the branching plasticity in indeterminate cultivars compared to determinate cultivars. Among indeterminate cultivars, there was a high correlation between yield and branching plasticity, and high-yielding cultivars had higher branching plasticity. Among determinate cultivars, there were high-yielding cultivars with low branching plasticity among cultivars with large hundred-grain weights and cultivars with a high number of pod per node, but excluding these cultivars, there was a correlation between yield and branching plasticity, and the high-yielding cultivars had high branching plasticity as well as indeterminate cultivars.

Conclusions

Most of the high-yielding indeterminate cultivars had higher branching plasticity. Even among determinate cultivars high-yielding cultivars tended to have higher branching plasticity excluding cultivars with large hundred-grain weights or with a high number of pod per node.

Keywords: Branching plasticity, branch nod, determinate cultivars, indeterminate cultivars, planting density

An approach using ultrasound technology for zinc supplementation in soybean seeds

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Background: The kinetics of nutrient uptake by seeds can be favored by ultrasound technology, as there are changes in the mechanical properties of the cell wall.

Hypothesis: Ultrasonic waves accelerate the uptake of zinc by soybean seeds, contributing to the initial development of seedlings.

Methodology: We analyzed artificially aged soybean seeds of two genotypes (57H0123 TP IPRO and M5838 IPRO cultivar). First, seeds treated with ultrasound were examined by fluorescence microscopy and hydration curve to better understand the structural changes that occur in the seed coat and cotyledon regions, and to determine the kinetics of water absorption. Next, zinc uptake was monitored by X-ray micro-fluorescence spectrometry (µ-XRF) to verify the concentration of zinc adhered to the seed surface or translocated to the internal tissues. Finally, multispectral images of the generated seedlings were captured for analysis of pigment content and maximum quantum efficiency of photosystem II.

Results: The acoustic cavitation caused by the ultrasonic waves induced a loosening in the cell wall of the soybean seed coat from both cultivars, which accelerated the speed of seed hydration. The µ-XRF technique showed that this occurred concurrently with an increase in zinc uptake by seed tissues. Seedlings from seeds treated with zinc in combination with ultrasound had higher levels of chlorophylls and anthocyanins, which positively affected the photochemistry of photosystem II.
Conclusions: The use of ultrasound technology is a potential tool for more effective treatment of soybean seeds with zinc, and efficiently contributes to better seedling establishment.

Keywords: Glycine max, photochemistry of photosystem II, X-ray spectroscopy

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Fast-K: a prompt determination method to foliar K concentrations in soybean under field conditions

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Brazil imports more than 90% of potassium fertilizer (K) mainly used in soybean, a specie that exports 20-22 kg/ha of K_2O through grains. Neglecting the concept of replacing K removed from the soil can lead to K depletion, resulting in a significant reduction in crop yield due to K deficiency. To address this issue, a fast and reliable method for evaluating K in soybean leaves in field conditions was developed. The methodology, called Fast-K, provides faster, reproducible, and calibrated results, evaluating K in three nutritional status: deficient, "hidden hunger," and adequate. With this diagnosis, nutritional management strategies for crops can be defined within the same growing season. Fast-K reduces the time between sample collection, preparation, and result reporting. The method is based on the use of a portable ion-specific meter, such as the Horiba Laquatwin®, for the determination of K concentrations in aqueous extract (water-soluble K). To perform the analysis, five to six trifoliate leaves without petioles should be collected at the third node from the top of the plant, representing the average condition of the plot or contrasting conditions/plants with and without symptoms. The recommended growth stage for sampling is R2/R3 (full bloom/beginning of pod formation), but the methodology has also been calibrated for growth stages R4 (pod formation) and R5.1 to R5.3 (grain filling) to increase the sampling range. The required materials are a portable balance with precision of 0.1 g, a container for manual macerating or a portable blender, a graduated falcon-type tube, and a ion K meter. So far, Fast-K has proven to be an efficient option for determining K nutritional status in soybean plants, with high correlations with levels determined by traditional methods. This makes Fast-K a reliable tool that can expedite decision-making in cases where K deficiency can compromise the profitability of soybean systems.

Keywords: Portable meter, nutritional deficiency, tissue analysis, soluble potassium

An investigation of soil properties in soybean fields and their correlations in the Paranapanema Valley, Brazil

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The Paranapanema River, located in southern Brazil, is 929 km long and forms the border between the states of Paraná and São Paulo. The main species cultivated in the region are soybean, corn, and sugar cane, with median yield levels in relation to Brazilian means. However, there is still untapped potential in these subtropical production environments to be unlocked. One of the steps that can help in this process is more assertive limestone and gypsum recommendations. We hypothesize that gypsum recommendation can be improved in the Paranapanema Valley by building a model containing more chemical and physical attributes besides sulfur (S) and clay, which are commonly used in conventional recommendation calculations. For this, 3,300 soil samples were collected from 26 municipalities along the Paranapanema River. Twenty soil attributes were measured (pH, CEC, OM, base saturation, Al, H+Al, Ca, Mg, K, S, P, Prem, Cu, Zn, Fe, Mn, B, sand, clay and silt). As for S, highest correlations were found with sand (-0.74), clay (0.76) and remaining phosphorus (Prem) (-0.72). The Prem is a measure of the soil P fixation capacity, with low values indicating high fixation. Therefore, in areas with high S contents, soils tended to be more clayey and with higher capacity to retain P. Moreover, organic matter (OM) was 0.85 correlated with clay (0.85) and was therefore included in the model, composed of S, clay, sand, Prem and MO. As a recommendation parameter, we used the average gypsum requirement obtained by three most commonly used formulas in Brazil for calculating the need for this amendment. So far, of the several deep learning models tested, one of them was able to predict the need for gypsum with 92% certainty. However, mathematical adjustments are still being made before the model is released, as well as field and greenhouse tests.

Protein extraction protocol for the early proteomic profile of soybean germination

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Seed germination is the expansion process defining the embryonic axis (Ax) growth without cell division. This expansion is induced by the water turgor pressure on the cell walls of the elongation zone (EZ) of Ax. Thus, cell walls are weakened to allow water entries, its plastic deformation and the Ax growth. Expansin proteins (EXP) primarily promote cell wall remodelling. In soybean (*Glycine max* L. Merr), Ax germination begins after 8 h of water incubation and Ax is visibly elongated at 12 h. We previously identified the EXP1 transcript involved in germination. Also, we demonstrated that Abscisic acid (ABA) inhibits germination, with a repressive effect associated with EXP1 expression. This work aimed to identify an extraction protocol for proteins with initial action during soybean Ax germination. Soybean Ax, cv. Williams 82, were incubated for 9 h in distilled water at 27 ± 1 ºC. EZ were then dissected and powdered in liquid N2. Two protein extraction protocols (EP1 and EP2) were evaluated for cell wall proteins and one for total proteins (EP3). Protein identification was performed by Mass Spectrometry using Uniprot and Phytozome databases and a functional and metabolic analyses was carried out using JVenn, BUSCA, Target P2.0 and ShinyGO softwares. From 1,098, 2,984 and 1,699 total proteins identified in EP1, 2 and 3, respectively, 132, 1314 and 147 were unique to each EP and 778 were shared among the three EPs. Seven Expansins were identified in EP2 (EXP1 exclusively), while only one was found in EP1 and two in EP3. EP2 allowed extracting a significant amount of unique proteins, other cell wall remodelling proteins, and proteins involved in early germination processes. Therefore, the protocol used for obtaining EP2 would be suitable to study the proteomic profile of early events during soybean Ax germination and its possible control by ABA.

Keywords: ABA, early proteomic profile, expansin, germination, *Glycine max*

Developing soil-test-based phosphorus and potassium fertilizer recommendations for soybean

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Most land-grant universities in the United States have developed fertilizer-P and K recommendations based on soil-test P and K concentrations from 0-10, 0-15, or 0-18 cm soil depth. Recent soybean [*Glycine max* (L.) Merr.] research in Virginia showed that deeper soil sample (0-30-cm) is better for fertilizer recommendations than shallower sample (0-6-cm) due to nutrient leaching down to the soil profile. We evaluated soybean yield response to five different fertilizer-P (0, 20, 39, 59, and 78 kg P ha^{-1}) and K (0, 37, 74, 112, and 148 kg K ha^{-1}) rates and Mehlich-3 soil-P and K concentrations from 0-15 and 0-30-cm depths across 56 sites in Louisiana from 2020 to 2022. Out of 56 sites, fertilizer-P and K significantly increased soybean yield at 19 and 25 sites, respectively. Soil-P concentration explained 46% of relative soybean yield variability for 0-15-cm and 23% for 0-30-cm depths. However, soil-K concentration explained 81% of relative yield variability for both depths. Since soil-P and K concentrations at 0-30-cm depth did not improve the predictability of relative yield variation over 0-15-cm depth, we developed critical concentrations for 0-15-cm depth only, which were 23 mg P kg^{-1} and 122 mg K kg^{-1}. Below these critical concentrations, 60 and 93% of the sites were responded positively to fertilizer-P and K, respectively and above these critical concentrations, only 13 and 3% of the sites were responded positively. Our multiple regression model among relative yield, soil-test concentrations, and fertilizer rates showed that for maximum yield, soybean requires 39, 29, and 20 kg P ha^{-1} for fields with ≤10, 11-20, and 21-35 mg soil-P kg-1 and 112, 84, and 56 kg K ha-1 for fields with ≤60, 61-90, and 91-130 mg soil-K kg^{-1}, respectively. No fertilizer-P or K is needed for fields with >36 mg soil-P kg^{-1} and >131 mg soil-K kg^{-1}.

Germination response of vegetable soybean (Edamame) to temperature

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Demand in the United States for edamame has increased over the past two decades. Edamame has become the second-largest soyfood consumed in the US (25,000-30,000 tonnes annually). While domestic edamame production is slowly increasing, at least 90% of edamame consumed in the US is imported. Poor crop establishment is a major problem limiting edamame production. Seedling emergence ranges from 50-85%, which is lower than that of grain-type soybean (>85%). The seedling can be improved by growing under the optimal temperature. Up to now, edamame is planted still based on grain-type soybean recommendations. The objective of this study was to determine the minimum, optimum, and maximum emergence temperatures of edamame. It will provide a scientific basis for deciding the best planting time of edamame, ultimately improving its seedling stand in the field. In this study, two edamame cultivars (VT Sweet and UA Kirksey) were tested for their seedling emergence percentage and emergence rate (i.e., emergence speed) at 5, 7, 10, 13, 16, 19, 21, 23, 25, 27, 29, 32, 35, 38, 41, 45°C (soil temperature) using the LabField Simulation Table. Two grain-type soybean cultivars (MFS-48P1 and USF5618v) were checks. The results showed that the optimal temperature range for edamame seedling emergence is 25-32°C, which is lower than that of grain-type soybean (29-36 °C). The soybean did not emerge at around 4°C, however, edamame seeds may have a higher tolerance to low temperatures compared with grain-type soybean. The maximum temperature varied little among soybean types, with an average of 41.08°C. In addition, edamame needs more thermal time to emerge. The results indicate that edamame has a different emergence response to temperature compared with grain-type soybean. Edamame should be planted earlier than grain-type soybean and later planting may reduce edamame seedling emergence.

Keywords: Vegetable soybean, emergence, temperature

Nitrogen fertilizer application of commercial soybean farms and its impact on soybean yield and nutritional composition in Hungary

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Soybean, due to its biological nitrogen (N) fixation via nodule bacteria, can meet its N requirements from the air and the soil. Several studies report that excessive N fertilization may negatively affect plant productivity and soil condition. The amount of N applied by Hungarian soybean farmers varies widely and sometimes exceeds the recommendations significantly. This study evaluates the effect of increased N application on soybean yields and nutritional quality. Unlike similar studies, the assessment relies on data collection from commercial soybean farms instead of field experiments. Soybean samples along with the applied agrotechnological information were collected from 58 Hungarian farm enterprises in 2021 and 2022. The total number of samples was 526. The nutrient composition of grains was determined by Near Infrared (NIR) Spectroscopy. Soybean yield and nutritional composition were assessed at different N fertilizer ranges (<20, 20-50, >50 kg/ha) by taking soil quality (week, medium, good) conditions and growing season length (120-140, 140-150, >150 days) into account. Yield average value was highest (2.893 t/ha) at 20-50 kg/ha N-level, 10.2% above the yield average at <20 kg/ha and 15.1% above the yield average at >50 kg/ha N-levels. The coefficient of variation (CV) of yields in each N categories were extremely high (CV>32%). The average values of crude protein (CP), digestible lysine (SID Lys) and crude ash (CA) varied significantly among the N-ranges, in contrast to crude fibre (CFi) and crude fat (CF). The variability of nutrient parameter values was low (CV <10%) in each N-levels, except for CFi where the CV value was 12-14%. Soil quality and the length of growing season showed moderate effect on all parameters. Understanding the practice of N application and studying its effects helps farmers to identify the factors that contribute to cost-effective, ecologically sustainable, and high-quality soybean production.

Keywords: Commercial soybean farms, grain nutritional component, nitrogen fertilizer application, NIRS analytics, yield

Acknowledgements: We thank for the Association of Hungarian Soybean and Protein Crop Producers for conducting the collection of samples and farm data, as well as for providing NIRS analytics.

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Construction and application of soybean nodule development and senescence gene resource bank

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Delaying the nodule senescence and extending the nitrogen-fixation time are the key measures to solve the problem of de-fertilization in the later period of soybean cultivation, and the effective ways to bring nitrogen fixation into full play to promote the sustainable development of agriculture. In our studies, notable different nodulation phenotypes in the soybean roots inoculated with *Bradyrhizobium japonicum* strain 113-2 at five important developmental stages (branching stage, flowering stage, fruiting stage, pod stage and harvest stage) were shown, and the gene resource bank related to soybean nodule development and senescence (including soybean and rhizobium genes) was constructed. Combined with the genome-wide analysis of gene family proteins, lots of the candidate gene resources that regulate the soybean development and senescence were screened. Then we obtained dozens candidate high-nitrogen-fixing transgenic soybeans that could delay the nodule senescence by soybean stable genetic transformation and gene editing. The results of greenhouse and field experiments showed that the candidate high-nitrogen-fixing transgenic soybeans can promote plant growth, delay the nodule senescence and the maturity, and so on. Our results provided a basis for the establishment of technologies, such as delaying the nodule senescence, prolonging the effective nitrogen fixation time of nodules, and enhancing the symbiotic nitrogen fixation efficiency, and supported theoretical guidance for the cultivation of high nitrogen fixation soybean varieties.

Keywords: Soybean, nitrogen fixation efficiency, nodule senescence, gene resource bank

The function of nitrogen nutrient on the monocarpic senescence in soybean

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Monocarpic senescence is a unique phenomenon in soybean (*Glycine max* [L.] Merrill), making the plant death and dry at harvest time. However, the function controlling this monocarpic senescence are not well known. Sinclair and de Wit (1975) insisted that nitrogen shortage during seed filling could cause the plant early senesced therefore to monocarpic senescence. The objective of this study was to investigate how does nitrogen nutrient controls the plant senescence. Soybean variety Fukuyutaka was sown on a 4 l pot in a vinyl house in Saga University, Japan. The plants were sufficiently watered with the solution containing 100 mg l^{-1} nitrogen and other necessary elements during growth period. Then nitrogen concentration was changed between 5–800 mg l^{-1} from R6 (full-seed) to R7 (physiological maturity) stages. Parallel to the progress of plant senescence after R6 stage, the leaf-SPAD value, leaf nitrogen and soluble protein contents decreased gradually in control plants, however, these parameters remained the level in higher nitrogen concentration treatments, whereas decreased quickly in lower nitrogen treatments. Therefore, the leaves and stems in higher nitrogen treatments remained green even at harvest time when the whole plant senesced in control and lower nitrogen treatments. The effect of nitrogen treatments only appeared after R6 stage, indicating R6 is the critical stage for nitrogen shortage. The relative expression of an autophagy gene, GmATG8c, which is associated with nitrogen remobilization, showed the lower the nitrogen availability the earlier the up-regulation. The present results revealed that the shortage of nitrogen could stimulate the leaf senescence, while increasing soil nitrogen availability at R6 stage could delay even stop the leaf senescence, indicating that the nitrogen availability is a key regulating factor for the monocarpic senescence in soybean.

Keywords: Monocarpic senescence, nitrogen, soybean

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Chilling tolerance and early sowing of soybeans in northern Germany

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Soybeans are a major source of protein for food and feed worldwide. Besides - as member of the legume family - they have a range of positive effects in crop rotations. Thus, growing soybeans in Germany is interesting, especially for organic farming where the use of imported cheap GMO soybean cake is not possible. With their origin in subtropical regions, soybeans demand relatively high temperatures during vegetation period. Climate change will shift cultivation limits to the north, nevertheless enhanced chilling tolerance will improve adaptation to cooler climate and help to extend cultivation period in northern regions as a contribution to overcome negative correlation between earliness and yield potential. In this study, 22 soybean cultivars were investigated for their cold germination ability in climate chambers. Besides, those genotypes were grown in the field with an early and an optimum sowing date (soil temperature >11°C) at a location in northern Germany in 2 years 2021 and 2022. Results of tests under controlled conditions showed significant variation between genotypes in their cold germination ability. In contrast, germination under early sowing conditions was prolonged in time, but less in quantity so that plant density was only marginal affected and no significant correlation to cold germination determined in climate chamber could be found. Main effects of early sowing in the field were: earlier emergence, prolongation of the period between emergence and end of flowering, higher seed set, shorter period between end of flowering and maturity and lower seed weight (mainly in case of drought stress 2022), lower protein content as well as earlier harvest of later 000 maturity types (mainly in 2021). Besides, early sowing led to an increase in yield on average of the genotypes. All effects were dependent on the weather conditions of the year and on the genotype with considerable variation amongst them, indicating more or less suitability of single genotypes for early sowing and potential for improvement by breeding.

Keywords: Chilling tolerance, cold germination rate, early sowing, harvest, yield parameter

Technological quality of soybean food-grade varieties and lines in northeast Italy

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During the 1980s, soybean acreage in Italy grew from experimental dimensions to almost one-half million hectares; in early 2010s Italy ranked at the 15th place among soybean producing countries. Organic soybean production accounts for 4.5 % of the EU acreage, but only for 2.3% of Italy. In the last two decades, soybean breeding targeting non-GM, low antinutritive factors and adaption to northern Italy conditions was in place in Friuli Venezia Giulia (FVG) region. Indeed, new food-grade varieties need integrated knowledge across breeding, crop management and food science. Grain samples from three soybean varieties and five lines, tentatively suited for food-grade production (large seeds, pale hilum, high seed protein) were collected in two Friuli Venezia Giulia locations in the fall of 2021. Crop management, planting times, genealogy and MG of soybean materials are presented. Tofu was prepared according to Mullin et al. (2001). In order to obtain a firm tofu, Ca sulphate as coagulant was used. Soymilk and tofu colour was measured by portable spectrophotometer. As for soymilk colour, the range of CIELAB parameters (L*, a*, B*) was limited, as expected. As for tofu colour, both the parameters L* and a* showed significant differences across soybean materials, with the latter showing differences for the two locations. Texture Profile Analysis (TPA) of tofu was performed according to Gunasekaran and Ak (2003), using a Lloyd LFP plus dynamometer. Eight tofu cubes (20x20 mm) per sample were tested; two compression cycles at 100 mm/min speed were performed. Among TPA parameters, i.e. hardness, adhesiveness and cohesiveness, no significant effect of varieties and/or lines was detected. Indeed, adhesiveness was different for tofu made out of seeds collected from the two locations.

Keywords: Organic soybean, food-grade varieties, soymilk, tofu, texture profile analysis

Management as a driver of yield improvement: analysis of the main soybean-producing regions in Argentina

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Globally, an increase in demand for grains is expected due to population growth, economic growth, and changes in diets. To meet this growing demand in a sustainable way, it is necessary to increase the achievable yield of crops, which involves reducing current yield gaps. As Argentina is one of the main producers of soybeans, the study analyzes the climatic and technological conditions that explain soybean yields in three key production areas of the country: NEA East (II), North Center (VI), and La Pampa North- Buenos Aires West (IX). The study aims to identify which practices contribute to reducing the gap and therefore should be encouraged. In years with adverse weather conditions, the hypothesis is maintained that the use of inputs and management practices are essential. Using a panel regression, it was found that management and technology are more important in Zone II than in VI and IX, regardless of weather conditions. When moving from a low Technological Level (TL) to a high one (use of inputs + management practices), Zone II shows a 45% more positive effect on yield than Zone IX and 52% compared to Zone VI. The variables that best define soybeans's TL are the use of herbicides, phosphorous fertilizers, and the adoption of direct sowing as a management practice. As conclusion, it can be mentioned that Region II, being an area with less production history and environmentally fragile, could be a key area where the transfer from Low to High TL would increase the harvested volume of the oilseed without changing the surface area destined for this crop.

Keywords: Management, yield, Argentina, technological level

Monitoring of the quality of French soybean production

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In France, a soybean quality survey is published annually by Terres Univia, the French oil seed and rich protein crop interbranch organization and the technical institute, Terres Inovia. This survey is mainly based on samples of the yearly harvest submitted by the elevators. The quality parameters included are water content, impurities, oil, and protein. Since 2014, more than 800 samples have been analysed showing that French survey can reach a very good global quality. For each parameter, the results are compared according to the production region (South, East, or West), the technical itinerary (rainfed or irrigated conditions), and the estimated outlet when it is claimed for the seed sample (feed or food). The results are analysed taking into account the yearly agronomic and climatic events in order to understand their value and the observed interannual variations. This observatory is considered as a useful tool for operators of the soy value chain. It allows to range local production in the framework of the national average and of other worldwide suppliers. It also gives to processors useful indications on the expected quality of the annual seed supply. It is improved depending on the needs of the operators and market changes. For example, the comparison between outlets was only introduced in 2018. New parameters can also be added according to the concerns expressed by the operators, such as forthcoming observations on the proportion of green beans in the samples.

QTL mapping of oil content in soybean

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Oil content is a crucial quality trait of soybean, which is controlled by multiple genes. It is of great significance to locate soybean oil content-related loci and mine candidate genes for directional breeding of soybean varieties with high oil content. In this study, an F_2 population consisting of 3097 individual plants was constructed by crossing the excellent variety Qixiaoli No.1 as the female parent with the high-protein germplasm Suinong 76 as the male parent. The genotypes of F_2 population were identified by using high-density SNP chip of “ZDX1” and the physical map was constructed. Combined with the oil content phenotypic data, the initial mapping interval of two oil QTLs was located on chromosome 15 using the IciMapping 4.2 software. Using 18 polymorphic SSR markers screened out, the mapping range was narrowed from 49.77 Mb to less than 1Mb. Combining “ZDX1” with SSR markers, there are also two oil QTLs was located on chromosome 15 using the IciMapping 4.2 software. One is around 400 kb. The other one is less than 10kb, containing only 1 gene. These results provide a theoretical basis for soybean oil content gene cloning and oil regulation mechanism research, as well as elite material and molecular marker for breeding high oil soybean.

Keywords: Soybean, oil content, QTL mapping

Soybean productivity in delayed and normal onset of monsoon in transitional tract of Karnataka India

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Soybean (*Glycine max* L. Merrill) is an introduced oil seed crop with multiple uses in India. An analysis was made to know the impact of normal/delayed onset of onset of monsoon conditions on sowing dates of soybean and its effect on yield behavior. The trials were conducted on soybean at the MARS, UAS, Dharwad (India). The data was compiled on soybean grain yield grown under normal as well as under delayed onset of monsoon conditions from the trials conducted by the Post Graduate students of The Department of Agronomy, College of Agriculture, Dharwad and also from the All India Coordinated Research Project on Soybean, Main Agricultural Research Station, University of Agricultural Sciences, Dharwad-580 005 (Karnataka, India). Results of the trials conducted over the years (15 seasons) from 2006 to 2020 during kharif season indicated that out of 15 seasons, hardly in three seasons the soybean was sown with the normal onset of monsoon conditions during June on 22nd 2006, 24th 2007 and 27th 2013 against 12 seasons under delayed onset of monsoon conditions during July on 25th 2008, 4th 2009, 21st 2010, 28th 2011, 20th 2012, 18th 2014, 2nd 2015, 2nd 2016, 17th 2017, 9th 2018, 5th 2019 and 9th 2020. A yield variation in soybean was noticed under normal as well as under delayed onset of monsoon conditions. Soybean seed yield reduced to the extent of 28 percent when sown in the month of July (under delayed onset of monsoon conditions with an average seed yield of 2,0144 kg/ha) compared to the recommended sowing period (2,588 kg/ha) Ist fortnight of June. The results clearly indicated that under delayed onset of monsoon, performance of photo and thermo sensitive soybean was affected in terms of seed yield. Hence, there is a need to develop the agro-techniques for genotypes under climate resilience.

Estimation of soybean yield parameters under lodging conditions using RGB information from unmanned aerial vehicles

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The estimation of yield parameters based on early data is helpful for agricultural policymakers and food security. Developments in unmanned aerial vehicle (UAV) platforms and sensor technology help to estimate yields efficiency. Previous studies have been based on less cultivars (<10) and ideal experimental environments, it is not available in practical production. Therefore, the objective of this study was to estimate the yield parameters of soybean (*Glycine max* (L.) Merr.) under lodging conditions using RGB information. In this study, 17 time point data throughout the soybean growing season in Nanchang, Jiangxi Province, China, were collected, and the vegetation index, texture information, canopy cover, and crop height were obtained by UAV-image processing. After that, partial least squares regression (PLSR), logistic regression (Logistic), random forest regression (RFR), support vector machine regression (SVM), and deep learning neural network (DNN) were used to estimate the yield parameters. The results can be summarized as follows: (1) The most suitable time point to estimate the yield was flowering stage (48 days), which was when most of the soybean cultivars flowered. (2) The multiple data fusion improved the accuracy of estimating the yield parameters, and the texture information has a high potential to contribute to the estimation of yields, and (3) The DNN model showed the best accuracy of training (R^2=0.66 rRMSE=32.62%) and validation (R^2=0.50, rRMSE=43.71%) datasets. In conclusion, these results provide insights into both best estimate period selection and early yield estimation under lodging condition when using remote sensing.

Keywords: UAV, yield, soybean, lodging, machine learning

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Dissecting the genetic basis of soybean seed germination through a combination of phenomics, genomics, and transcriptomics

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Seed quality is a crucial factor affecting soybean (*Glycine max* (L.) Merr.) yield, but our current understanding of the genetic regulation of seed germination is limited. In this study, we utilized a multi-omics approach to dissect the genetic basis of seed germination-related traits in a globally derived population that had both genotype and expression data. To high-throughput collect seed germination-related traits, we developed an automatic high-throughput phenotyping platform using a cost-effective Raspberry Pi device. We trained a deep learning YOLO model with a 98.4% mean average precision (mAP) and 99.3% recall rate to identify and track germinating seeds. Time-series of germinating data were recorded. We then applied a combination of genome-wide association studies (GWAS) and transcriptome-wide association studies (TWAS) to identify the quantitative trait loci (QTL) and genes related to seed germination. Our findings not only shed light on the genetic basis of seed germination in soybean, but also demonstrate the feasibility of applying deep learning in phenotype identification.

Keywords: Soybean, seed germinate, YOLO, GWAS, TWAS

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Agrobacterium-mediated genetic transformation of soybean and creation of new germplasm

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One of the most significant oil crops in the world is soybeans. Breeders' primary objective is to create new, high-yielding soybean cultivars. Therefore, this study intends to carry out genetic transformation experiments on the samples of newly developed high yield and excellent soybean varieties by using different gene carrier systems with independent intellectual property rights, From these samples, we can screen for good recipients with high genetic transformation efficiency; At the same time, obtain the glyphosate-resistant transgenic soybean materials based on the excellent soybean varieties, and then explore the excellent germplasm. One receptor genotype with a high genetic transformation efficiency was examined. The experiment of transforming Bar+snap18 gene was carried out on seven soybean varieties, and HN71 high-quality receptor was initially screened out. The average bud induction rate was 33.5%, elongation rate was 44.4%, and average transformation rate was 1.38%. And two positive plants were obtained. Four new glyphosate-resistant transgenic soybean materials edited by vector genes were obtained. Six gene editing events of three T0 generation vectors with Jack as the receptor were discovered by test strip and DNA target sequence testing of T0 generation regenerated seedlings using Jack receptor material for genetic transformation.

Discovery and utilization of genes involved in soybean cyst nematode resistance

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The objective of this study is to identify new genes that confer resistance to Soybean Cyst Nematode (SCN) using Chinese soybean germplasm resources, evaluate the breeding value of these new genes and establish the genetic basis of important resistance sources. Here, through a genome-wide association study (GWAS) for SCN, we cloned qSCN3-1, one of the proteins belongs to the SNARE membrane fusion system, and all currently cloned resistance genes are related to the SNARE membrane fusion system. Overexpression of qSCN3-1 increased SCN resistance. Then, we elucidate qSCN3-1 and other resistance genes function, the interaction between this protein and the cloned main effect genes SNAP18 and SHMT08, as well as the minor effect gene SNAP11 against soybean cyst nematodes, was validated by LUC and BIFC. This work provides genetic resources and breeding materials for excellent gene aggregation and provides genes/markers and parents for molecular breeding of new varieties with persistent and stable resistance.

Keywords: Soybean, GWAS, cyst nematode, functional verification, resistance gene discovery

Gm01 controls flowering time in soybean

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Flowering time affects adaptability and yield of soybean. However, the genetic basis of flowering time is still unclear. Here, we selected 1490 soybean accessions and measured their flowering time in three different environments in 2017. A total of 6 million single-nucleotide polymorphisms (SNPs) from our previously resequenced accessions were used to perform GWAS for flowering time. We identified 81 SNP markers associated with flowering time on 19 chromosomes in soybean. We detected strong signals associated with flowering time in a genomic region containing Gm01. Further analyses suggested that we detect one non-synonymous SNP (T/A) resided in the fourth exon, with the T materials flowering earlier than A materials. The TT genotype was gradually selected through domestication and selection, while the AA genotype was eliminated and was mostly dispersed in low latitude regions. In addition, knockout of Gm01 using CRISPR/Cas9 technology promote flowering in LD conditions, indicating that Gm01 could be involved in flowering regulation in soybean. In summary, this study provides valuable insights into molecular mechanisms of flowering regulation in soybean and may help in the screening for superior germplasm.

Keywords: GWAS, flowering time, soybean, Gm01

Natural variation of LMYG leads to leaf variegation and high 100-seed weight

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Leaf, a crucial organ for photosynthetic carbon assimilation in soybean, and its color is not only related to the trapping power and conversion efficiency of light energy, but also closely related to the yield of soybean. Here, we identified a natural variant mutant, LMYG (leaf mutant with yellow-green), locating in the phytochrome biosynthetic pathway, as a major gene involved in soybean photosynthesis, using fine mapping study. Compared with the green leaf wild type (WT), the 100-seed weight, and protein content increased significantly. The LMYG mutant phenotype is caused by an early termination of protein translation. Knocking out GmLMYG resulted in more grana thylakoid stacks in chloroplasts and increased photosynthetic efficiency and starch content, while over-expressing GmLMYG produced the opposite phenotypes. GmLMYG interacted with two genes in the phytochrome biosynthetic pathway, and this interaction may contribute to the observed enhanced chlorophyll synthesis. GmLMYG knockout lines had superior yield traits, such as 100-seed weight, compared to the wild type and over-expression lines. Additionally, we identified an elite haplotype of GmLMYG in 100-seed weight, which appears to have been selected during soybean domestication. Our study sheds light on the molecular mechanism by which GmLMYG modulates photosynthesis and provides a promising strategy for improving the yields of soybean and other crops.

Keywords: Soybean, leaf mutant, fine mapping, 100-seed weight

Role of fungicides and bioagents as seed treatment components on seed yield and quality in soybean

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The study was conducted to know the influence of fungicides and bio agents on yield and quality parameters of soybean var. DSb 21. The field experiment was carried out at ‘H’ Block. Main Agricultural Research Farms and laboratory experiment was carried out in Seed Quality Testing Laboratory, Seed Unit, University of Agricultural Sciences, Dharwad. Among the treatments seed treatment of *Bacillus subtilis* @ 10g/kg of seed recorded the higher plant height (23.67, 54.27 and 57.64 cm), number of branches per plant (3.33, 4.73 and 5.93) and number of leaves per plant (32.47, 61.13 and 96.33) at 30, 60 and 90 DAS and higher number of pods per plant (94.93), seed yield per plot (3.75 kg) and seed yield per hectare (2168 kg) over other treatments under the study. Higher seed germination (96.33%), seedling vigour index (3766) and lower seed infection (3.67%) was recorded in Carboxin + Thiram @ 3g/kg of seed. The results can be recommended for improvement of quality in soybean in India.

Biofertilizers inoculation to amplifyseed yield and quality in soybean

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The research was carried out at Main Agricultural Research Station, Dharwad during kharif 2021 to study the effect of biofertilizer inoculation in soybean. Amongst 8 treatments, T8: 100%RDF+ PPFM (1%) foliar spray recorded significantly higher plant height (78.00 cm), number of pods per plant (65.80), seed yield per plant (12.59 g) and seed yield per hectare (2702 kg). Laboratory analysis of resultant seeds from field experiment revealed that significantly higher quality parameters viz., germination (96.33%) and seedling vigour index-I (3587.32) were reported in seed treatment with 75%RDF+Rhizobium+MDSR14+12c.

Influence of foliar application of PGRs and nutrients for enhancing the productivity of soybean under rainfed condition

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Soybean (*Glycine max*) is a predominant edible plant and a major supply of plant protein and oil world wide. Global demand for soybean keeps increasing as its seeds provide essential proteins oil and nutraceuticals. In a quest to meet high tend demands for soybean it has become essential to introduce agro technical methods that promote adoptability to complex environments, improve soybean resistance to a biotic stress and increase productivity. Plant growth regulators are mainly exploited to achieve this due to their curvical role in plant growth and development. The present study was conducted at Agricultural Research Station, Dharwad Farm. University of Agricultural Sciences, Dharwad. India during the year 2019-20, 2020-21 to investigate the influence of foliar application of PGR's and nutrients for enhancing the productivity of soybean under rainfed condition. The experiments consist of nine treatments viz., NAA 20 PPM, NAA 30 PPM, GA 30 PPM, GA 40 PPM, GA 50 PPM urea 2%, KNo3 1% pulse magic 1% and control. Among all the treatments foliar application of GA 40PPm at flowering and pod formation stage recorded higher yield (2158 kg/ha) compare to control (1595 kg/ha) and also recorded higher photosynthetic rate (29.35 µ mol co2 m2 s-1), proline content (3.05 µ mol g), oil contents (18.93%), Protein contents (39.10%), chlorophyll content (3.21 mg/g.fr.wt) and also recorded higher relative water content in GA 40 PPM (82%) compared to control 73%.

Identification of soybean GmBAS1 gene and its effect on soybean plant type structure

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There are two main aspects on improving in soybean yield, one is to increase the yield of soybean single plant, and the other is to increase the planting density to increase the population yield. The increase of planting density will affect the light reception of soybeans, so it is necessary to improve the soybean plant structure to improve the light reception of soybeans under dense planting. Plant height, petiole angle and internode length are important components of soybean plant type. As the sixth largest plant hormone, brassinolide (BR) is commonly used to improve crop stress tolerance, and BR has been shown to change the plant type structure of many crops such as rice and Arabidopsis. In soybean, more people apply brassinolide to improve its stress resistance, yield and other traits, while the effect of endogenous BR on soybean plant type is less studied. Therefore, in this study, plant type mutants were created by changing the content of endogenous BR in the hope of finding a soybean plant type suitable for dense planting. In this study, one metabolic gene GmBAS1 of soybean BR were obtained. The gene was identified by bioinformation analysis, overexpression and gene editing vector was constructed, and the mutants were obtained by *Agrobacterium tumefaciens* mediated soybean cotyledon node method. The results showed that the plant height and the petiole angle of overexpressed soybean plants were decreased, all of which were conducive to improve the lodging resistance of soybean and realizing the dense planting of soybean.

Keywords: Brassinolide, soybean, plant type, genetic transformation

Accurate estimations of the growth phase of soybeans by utilizing a large phenotypically dynamic dataset

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Soybean (*Glycine max* (L.) Merr.) is a crucial leguminous seed crop with significant economic value. Yield is closely associated with the flowering and maturity stages, which are the critical phenological stages. Manual assessment of phenological stages is currently a limiting factor in soybean breeding innovation. The recent advancements in an unmanned aircraft systems phenotyping platform have presented an efficient means for crop phenotyping research. In order to quantify seasonal changes, this study extensively examined the distinctive physiological responses to environmental conditions by utilizing high-throughput phenomics in various soybean cultivars. The objectives of this study are: 1) to develop accurate growth curves for soybean cultivars by utilizing photothermal accumulation area based on RGB images captured by unmanned aircraft systems. 2) to improve the models' performance by combining with a merit-based integrated filter and a field spatial-correction model. 3) to evaluate the models' performance and environmental portability, and explore exceptional germplasm resources. The proposed merit-based integrated filter was effective in reducing noise and improving the accuracy of crop vegetation estimations. Furthermore, the field spatial-correction model was successful in eliminating the impact of spatial heterogeneity and climate effects, resulting in more accurate estimations. The rescaled shape models in different locations are consistent with the growth curve of soybean cultivars, indicating that the proposed methodology provides accurate estimates of the growth phase of crop genotypes in different environments. Overall, the proposed methodology can effectively quantify the seasonal changes in soybean cultivars and provide valuable insights into their dynamic physiological responses to environmental conditions.

Keywords: Unmanned aircraft systems, soybean cultivars, photothermal accumulation area, spatial heterogeneity, phenological phase

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Physiological roles of magnesium on rubisco activity, biological nitrogen fixation, yield and seed quality of soybean plants

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Magnesium (Mg) is considered a forgotten element in plant nutrition due to few studies. Magnesium deficiency is a limiting factor in the photosynthetic process, because is a structural part of chlorophyll molecule, and an activator of rubisco enzyme. Potassium (K) presents antagonism with Mg and, depending on the fertilization management, it can promote Mg deficiency in plants affecting photosynthetic machinery and crop yield. This study aimed to evaluate foliar Mg application on photosynthetic and rubisco activity, biological nitrogen fixation (BNF) of soybean plants and interactions with yield. To test our hypotheses, 3 experiments were carried out: Experiment I: Foliar application of 1 kg of Mg (magnesium sulfate) in 10 soybean genotypes significantly increased photosynthetic pigments, net photosynthesis rate, rubisco activity, flow of sugars from leaves to roots and nodules, which increased soybean nodulation. Plants fertilized with Mg showed increased antioxidant metabolism and greater flow of sugars resulted in a higher concentration of ureides (allantoin and allantoic acid) in the leaves, promoting greater yield. The main goal of Experiment II was to investigate the antagonist effect of K and Mg on soybean yield. Higher K doses decrease Mg uptake by plants, affecting ureide metabolism and soybean yield. Higher K doses should be balanced with Mg for greater productivity and sustainability in soybean production. Experiment III aimed to evaluate foliar Mg applications on the photosynthetic process and rubisco activity under field conditions. The application of 500 g of Mg was crucial for greater photosynthesis, rubisco activity and soybean yield. Mg fertilization is important to enhance rubisco activity, sugar metabolism and ureides metabolism to increase yield and protein of soybean seeds.

Keywords: Rubisco, magnesium, photosynthesis, yield

Can vetch as a previous cover crop counteract N limitation in high-yielding soybean?

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Seed yield and protein content in soybean depend on nitrogen nutrition. Around 60% of seed N comes from remobilization, and N uptake during seed-filling (soil or biological N fixation, BNF) is especially critical for high-yielding soybean. Evidence suggests an N limitation at high yields with a subsequent existence of an N-gap in soybean. Since N fertilization has not been successful in increasing seed yield because of the negative relationship between soil N and BNF, we hypothesized that residues from a legume cover crop planted before soybean will slowly release N and raise N supply for a high-yielding soybean. The objective of this work was to assess the effects of including vetch as a cover crop to counteract a possible N limitation in high-yielding soybeans. Two treatments, vetch cover crop/soybean and fallow/soybean, were evaluated in 13 high-yielding commercial fields located in the Pampas region of Argentina in the 2019-20 and 2020-21 cropping seasons. The total aboveground biomass of vetch ranged from 1748 to 4937 kg ha^{-1}. Aboveground biomass at R5 averaged 9486 kg ha^{-1} when vetch was the previous crop, a 12% increase compared to fallow. Seed yield ranged from 4132 to 7034 after fallow (5234 kg ha^{-1}) and from 4191 to 6630 after vetch cover crop (5198 kg ha^{-1}). In four out of the 13 fields, vetch cover crop/soybean outyielded fallow/soybean (5-7%), whereas in four fields fallow/soybean increased 5-11% over vetch/soybean ($P<0.05$). Including vetch as a cover crop before a high-yielding soybean showed different impacts depending on the site. In sites where fallow outyielded vetch, N may have not been the main limiting factor, suggesting that water availability or the particular soil indigenous N conditions in each field may have affected the performance of the following soybean crop.

Effect of post emergence application of Imazethapyr 10 SL on soybean ecosystem and its residual toxicity on succeeding crops

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Soybean (*Glycine max* L. Merill) is an introduced and commercially exploited oilseed crop in India. On account of multiple uses soybean is regarded as 'golden bean' or 'miracle crop' of the 21st century. The crop is photo-and-thermo sensitive crop hence, mainly cultivated under rainfed situation (kharif) which favors infestation of various grassy, sedge and broad leaved weeds and exert competition for various growth resources. Pre emergent herbicides have been recommended, however farmers are approaching for post emergent herbicides for effective control of weeds in soybean. Research was initiated under All India Coordinated Research Project on soybean from 2012 and continued as part of Post Graduate studies upto 2019-20 at Main Agricultural Research Station, University of Agricultural Sciences, Dharwad (Karnataka state). The experimental results under AICRP on soybean indicated that application of Imazethapyr 10 SL @ 100 g a.i. ha^{-1} as early post emergent proved effective against weeds in soybean (Cv. JS-335) ecosystem to achieve higher seed yield (25.00 q ha^{-1}) without any phytotoxic effect on soybean. On the basis of these results, the research was continued as part of post-graduate studies in order to assess the residual effect of Imazethapyr 10 SL which was used against weeds in soybean during kharif 2016-17 season and after harvest of soybean, its residual effect was assessed on performance of succeeding crops like rabi sorghum, wheat, chickpea and safflower during rabi 2016-17season. On succeeding crops like wheat and sorghum, Imazethapyr 10 SL exerted mild residual toxicity with lower germination percentage (80%) for two seasons (2016-2017). The research results indicated that Imazethapyr 10 SL exerted phytotoxic effect (phytotoxicity rating 7 to 9) on wheat and rabi sorghum germination, however, such adverse effect (phytotoxicity rating 1 to 2) was not observed with chickpea and safflower during 2016-17.

Influence of non-edible oil-cakes on growth and rhizosphere of soybean: An analytical investigation using python algorithm

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A field and pot experiment were conducted at Department of Agronomy, VIT School of Agricultural Innovations and Advanced Learning, Vellore Institute of Technology, Tamil Nadu, India during Kharif 2022 and Rabi 2022 to study the influence of non-edible oil-cakes on growth and rhizosphere of soybean. The experiment was laid out in a randomized block design (RBD) (field experiment) and completely randomized block design (pot experiment) replicated thrice with fifteen treatments. The data were analysed using python programming. The treatment consists of T1- 100% neem cake (NC), T2-, 100% castor cake (CC), T3- 100% pungam cake (PC), T4-75% NC + 25% CC, T5 - 50% NC + 50% CC, T6-25% NC + 75% CC, T7-75% NC + 25% PC, T8-50% NC + 50% PC, T9 -25% NC + 75% PC, T10-75% CC + 25% PC, T11-50% CC + 50% PC, T12-25% CC + 75% PC, T13-100% recommended dose of NPK, T14-100% rhizobium and T15 – absolute control. Observations on plant height, leaf area index (LAI), dry matter production (DMP), root length, grain and haulm yield were recorded in both the experiment. The results revealed that, the maximum plant height, LAI, DMP and root length were recorded in 50% Cake + 50% pungam cake (T11) and 50% NC + 50% PC (T8). However, the grain and halum were recorded maximum in 100% recommended dose of NPK (T13), The results of these studies showed that composted non-edible oil cakes might be utilised as a soil amendment in place of agrochemicals to boost productivity, control diseases that are transmitted through the soil, and enhance soil health through increasing intensity of soil nutrient supply by rhizosphere development.

Increasing soybean production in Europe: impact on cropping systems and environmental impacts

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The introduction of soybean in agricultural land is a promising strategy for crop rotation diversification and protein self-sufficiency in Europe. Soybean cultivation can produce plant proteins for human or animal consumption, while reducing the need for chemical fertilizers through nitrogen symbiotic fixation. Replacing maize with soybean can also have positive effects on the environment by reducing nitrate leaching and greenhouse gas emissions. To investigate the impact of replacing maize with soybean, researchers used the crop model STICS to simulate cropping systems across 100 sites in Europe. The simulations were run under actual and near-future scenarios over 25 years, using a decision model to adapt sowing, tillage, and fertilization dates to annual weather and to choose an appropriate maturity group for each crop. The study found that the introduction of soybean reduced nitrate leaching and N2O emissions related to fertilizer inputs. However, it was also found that storing carbon in the soil was reduced when soybean was introduced. Overall, the study highlights the potential benefits of introducing soybean in crop rotations, particularly for reducing the environmental impact of agriculture. While soybean cultivation may not be without its drawbacks, such as reduced carbon storage in the soil, these findings suggest that the benefits may outweigh the costs. Further research is needed to fully understand the impact of soybean cultivation on soil carbon storage and to optimize management practices for its cultivation.

Keywords: Crop modelling, STICS, GHG emissions, C storage, nitrate leaching

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AFERE: soil fertility assessment and fertilizer recommendation webtool to agricultural systems

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Brazil is one of the pillars of global food security, standing out as the largest producer of soybeans, projected to yield ~153 million tons in 2023. However, negative nutrient balances (lower inputs than exports through grains) are common in several soybean production areas due to monitoring failures and to inadequate fertilizer management. Our proposal is that AFERE will be a useful digital platform for farmers and technicians seeking assertive recommendations and efficient crop management. AFERE has been developed by the Brazilian Agricultural Research Corporation (EMBRAPA). The main objective is to enable users to monitoring soil fertility and plant nutrition conditions in their fields. In addition to helping with data organization (soil and plant tissue analysis results), AFERE offers fertilizer and soil correction recommendations, interpretation of plants nutritional status through tools such as DRIS (Diagnosis and Recommendation Integrated System) and CND (Compositional Nutrient Diagnosis), sufficiency ranges, and the creation of nutrient balance tables for specific field areas. As a basis for the generation of fertilization indexes and calculations, AFERE relies on a database with over 10,000 soil and plant analysis results, as well as a wide list of cultivars and fertilizers. This information in cross-referenced with the data the users enter into the system, enabling more assertive decisions. The system has two types of profiles, one for farmers, in which they can keep track of the nutrient balances on their farms, and another for technicians, who can register each of their clients and control the information on their production areas separately. AFERE can be accessed at http://afere.cnpso.embrapa.br/balanco/site/index.php. Currently AFERE already has more than 2000 accounts, and new efforts are being made to develop an English version of the platform, allowing its application to grain crop production areas throughout the world.

Keywords: Nutrient balance, digital platform, nutritional interpretation, agronomic recommendations

Keeping rhizobia inoculant high quality standards: a challenge for French organizations, inside Europe

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Based on the Australian experience, French bodies have promoted high quality inoculants since the middle of the sixties. Absence of contaminant, high concentration par seed (106 / soybean seed), selected strain maintained by INRAE, were the main dispositions. Downstream, the technical institute (former CETIOM and now named TERRES INOVIA), in connection with INRAE and private inoculants producers were used to conduct field trials in Bradyrhizobia free soils to evaluate and compare new commercial proposals. In recent years, several changes occurred. Farmers survey carried out in the past decade, show that Peat and microgranulars inoculations are in strong reduction at the farmers' level. Industrials are focused on liquid inoculants and ways to increase time interval between inoculation and sowing. Soybean pre-inoculated seeds have also been recently commercialized with more or less success, but this is clearly identified has the next important target for most of them. Another challenge for farmers organizations and public bodies is to keep high quality inoculants, with adaptation to new regulations at the European Union level which allow a mutual recognition procedure among UE states with heterogeneous policies for *Bradyrhizobium* inoculants market authorizations. The consequence for the French situation, where a unique selected strain of *Bradyrhizobium diazoefficiens* G49 has been selected and used for more than 40 years, is how to keep high quality standards for farmers, to be able to control it, including new strains and new technologies and to finance this all adaptation.

Keywords: Inoculation, quality controls, evaluation, regulation

Functional anatomy of *Glycine max* L. cv. MiniMax leaves

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An anatomical and histochemical analysis was carried out on the miniature soybean (*G. max*) genotype MiniMax. The protocols for anatomical sections were refined although known features such as the paravenal parenchyma can be easily identified in longitudinal sections and in cross sections of the leaf blade. In larger vascular bundles the paravenal parenchyma cells are extended to reach the epidermis. This structural observation may play a role in water and nutrient resource distribution, like the bundle sheath extension. Petiole, petiolule and leaf blade sections allowed the observation of single-layered palisade parenchyma and mesophyll with 5 to 7 layers of spongy parenchyma. The organization of the petiolule is like the petiole, although with a less developed vasculature. The paravenal parenchyma is located between the chlorenchyma and the leaf is amphistomatic with a single layer epidermis. There is a lignified-walled cell layer and a starch sheath in the same position circumscribing a pith parenchyma. This lignified-walled cell layer is almost aligned with primary phloem fiber and procambial initials. Calcium oxalate crystals were observed surrounding this layer and in the phloem region while starch was observed in this this layer and in the outer pith cells. Detailed anatomical and histochemical analysis has contributed to characterization soybean leaf using the MiniMax genotype as a model plant. This data will help with the characterization and comparative analysis commercial soybean cultivars and further insight in to paravenal parenchyma function.

Late season nutrient application on soybeans for yield and grain quality

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Focus on fertilizer application (especially S and N) in soybean production increased in the United States to maximize grain yield and increase grain protein concentration. It is partially due lower atmospheric deposition from stricter environmental regulation, and to explore the possibility of late-season nutrient application to complement biological N-fixation during the grain fill period. Objectives of the study was to determine if nutrient application during the grain filling period increases grain yield or grain protein concentration in South Dakota. Field studies were conducted in 2020 and 2021 at two locations (near Brookings and Beresford) in eastern South Dakota. Two varieties (MG1.1 and M2.4) and 7 fertilizer treatments (untreated control, 22 kg ha^{-1} S as ammonium sulfate (21-0-0-24S) applied either at planting, R3, R5, or R6 growth stages, additional inoculate applied at V4 growth stage, and the combination of inoculate at V4 and fertilizer at R5) were arranged in complete randomized block design with 4 replications. Year x location interaction impacted the grain yield, grain protein and oil concentration. The late-season nutrient application did not increase grain yield and grain protein concentration compared to the untreated control.

Keywords: Nitrogen, sulfur, grain protein, late-season, grain fill

Planting date effects on soybean response to sulfur

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Reductions in atmospheric deposition of sulfur (S) coupled with increases in yields of *Glycine max* (L.) Merr. (soybean) has led to S deficiencies in Indiana. Poor nodulation due to limited S, and thus a decrease in nitrogen (N) supply, restricts the yield and quality of soybean grain (i.e., protein). Sulfur is a key component of methionine and cysteine, which are important amino acids in the nutrition of foodstuffs. Traditionally, S deficiencies tend to be present in coarse textured soils that are low in organic matter. Situations have developed where soybean response to S has been substantial in high fertility soil even with organic matter greater than 3%. The objective was to determine if planting date has created situational S deficiency of soybean and if it can be overcome and even enhanced with supplemental S. A two-way factorial (Planting Date x N+S Fertility) has been evaluated in West Lafayette, IN from 2018 through 2022. Timely (early May) vs. late plantings (early June) were established in each year, which were crossed with N+S Fertility regimes (e.g., ammonium sulfate, ammonium thiosulfate, pelletized gypsum, urea). Soybean yield increased (7 to 18 bu/ac or 470 to 1200 kg/ha) with N+S Fertility regimes in every timely planting in 2018, 2020, 2021, and 2022. Whereas, soybean yield did not differ with any N+S Fertility when planted late. Mineralization of soil organic matter in the timely plantings were limited due to cool and wet conditions, and thus, soybean positive response to applications of S. The late plantings experienced warmer soils and thus, mineralization was not limited. Seed size and protein also increased with N+S fertility regimes within the timely plantings. Field conditions that limit mineralization of organic matter (e.g., early plantings, no-till, high residue) may be more responsive to supplemental S.

Keywords: Sulfur, planting date, mineralization, nodulation, fixation

Lodging and photomorphogenic light signals responses in two sibling lines of late maturity group with differences in plant height

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Soybean lodging can limit crop yield potential and increase harvest losses. An excessive height and vegetative growth in response to different environmental factors is the main cause of plant lodging. Two sibling lines with short and long stems were obtained in our Lab (FV-N9.30 and FV-N9.114, respectively). Siblings were grown in low (20 plants/m^2) and high (40 plants/m^2) density in the field (complete randomized block design- 3 replications) and exposed to supplemental red (R, 660 nm) and blue radiation (B, 450 nm) in a greenhouse experiment (complete randomized design- 3 repetitions). In the field, plant high, internode length and lodging were higher in FV-N9.114 ($P<0.05$); while yield, seed number, seed per pod and pod number were higher FV-N9.30 ($P<0.05$). In the greenhouse, internode length in FV-N9.30 was shorter for R and B radiations (15 % and 22 %, $P<0.0001$) compared to FV-N9.114. These results demonstrate that FV-N9.30 reduced the stem elongation when expose to different inductive enviromental factors suggesting a genetic regulation of plant height in this line. Thus, FV-N9.30 could be a potential donor of the short stems trait to reduce lodging and increase yield in late maturity group genotypes.

Enhancing soybean adaptation to winter sowing under rainfed conditions in mild Mediterranean climates

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Soybean (*Glycine max* L. Merr.) is an important legume with high dietary protein and oil content, as well as a good source of animal feed [1]. The intensive livestock production system contributes about 14.5% to global greenhouse gas emissions [2], hence soybean milk and 'meat' (tofu) could serve as an eco-friendly protein alternative to animal protein. Soybean is a good soil amendment option since the synergy between rhizobium and root nodules can fix atmospheric nitrogen [3]. Soybean is mostly considered to be a 'tropical' crop, making it suitable for summer seasons in temperate climates. In Spain, soybean is predominantly an irrigated crop during summer, but recent climate change dynamics and declining water resources for irrigation threaten production. Therefore, the objective of the IAS-CSIC breeding program has been the improvement of the genetic base and adaptation to very early sowings under rainfed conditions through classical breeding. The selection of photoperiod-insensitive and drought-tolerant lines during late winter sowing can be an important strategy for breeding rain-fed varieties in the temperate zone. The IAS-CSIC soybean breeding program assembled 500 gene-bank materials which were evaluated for photoperiod adaptation at a pre-breeding phase. The resulting 3% adapted lines were then selected as parents and hybridized with advanced breeding lines in a pedigree breeding program. The best-selected 20 cross combinations are currently planted in progeny rows of six single plant selections per cross at the F4-5 stage for further selections. These are expected to be screened after the F6 stage for stresses associated with the Mediterranean zone and advanced genomic tools deployed for further improvement and variety release.

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Understanding short season soybean adaptation, yield, and its components in western Canada

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The rising global demand for plant-based sources high in protein and oil has been one of the main drivers in soybean (*Glycine max* L. Merr.) expansion to Northern latitudes. In western Canada, cool temperatures, dry periods, and short frost-free window during the growing season present challenges for soybean production. However, new soybean cultivars in maturity groups (MG) 000 and 00 have been developed and now available for cool arid western cropping regions. These cultivars must be able to fit their life cycle to the lowest requirement of accumulated heat units (<2500°C) and often face water shortage. In this sense, several traits, and responses to the interactions with abiotic and biotic factors can affect soybean growth and final yield. Indeed, more information about soybean adaptation and response to higher latitudes is needed. For two summer seasons (2021, 2022), 24 cultivars ranging in the maturity groups MG 000 and 00 were evaluated in Saskatchewan (latitude 52°N) at 4 locations under dryland conditions. Our main goals were to access soybean growth curve progression and its relationship to days of the year (DOY), Crop Heat Units (CHU), the Growing Degree Day model GDD and water availability. As well as to determine yield and its components in order to identify and characterize the best physiological traits for soybean improvement in SK. Our results showed that germination was compromised when observed temperature <10°C. Growth and development were drastically depleted in locations with less than 100 mm of rainfall. Among the cultivars tested, those in MG 000 were the best adapted to SK in dry years. They matured earlier (CHU<2500°C), yielded more, and had positive yield component results. Overall, our findings provide insights into critical traits affecting soybean yield and its components.

Keywords: Cool regions, physiological traits

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Phenotyping of soybean phenology to temperature and photoperiod

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Improving soybean production in Europe under climate change needs a good prediction of cultivar phenology under different temperature and photoperiod conditions. For that purpose, a simple phenology algorithm (SPA) was developed (Schoving et al., 2020). Before being applied, SPA requires the calibration of 7 genotypic parameters; therefore, a simple phenotyping method was designed and set up under natural and controlled conditions to determine these parameters. Two experiments were performed with 10 cultivars in 2017 and 5 cultivars in 2022 (from MG 000 to II): 1- A pot experiment on the Heliaphen platform (INRAE Toulouse, SW France) under natural climatic conditions with 4 to 6 planting dates (from March to September). The main phenological stages were recorded (BBCH and Fehr & Caviness scales), once or twice a week. 2- An experiment on germination response to temperature (from 4 to 43°C) under controlled conditions (cold room and incubator). Mean cardinal temperatures (minimum, optimum, and maximum) for germination were ca. 2, 30, and 40°C, respectively with significant differences among cultivars. For most stages, ANOVA analysis showed a highly significant effect of the interaction between cultivar and planting date on thermal time accumulation. A photothermal time formalism was used to evaluate by optimization the sensitivity of each cultivar to photoperiod and the optimal physiological development days of the cultivar to complete a given phenological phase (PDD). The optimization of the PDD plant parameter from hourly temperature values instead of daily values improved the prediction of phenology by SPA. This approach - simple cultivar phenotyping and photothermal algorithm - is currently evaluated on several grain legumes to design more diversified and agroecological cropping systems.

Keywords: Agroecology, grain legumes, maturity group, phenotyping, photoperiod sensitivity

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Catching rhizobia to introduce high protein containing soybean for a sustainable agriculture in Europe

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To develop sustainable protein products, Europe would strongly benefit from soybean production at northern latitudes. However, soybean is not adapted to these environmental conditions and therefore the cultivation of protein-rich soybean is challenging. While several soybean varieties have been bred for optimal growth, to guarantee consistent high-protein beans, plants also need effective interaction with suitable soil bacteria that can fix nitrogen in root nodules. These nitrogen-fixing bacteria allow legumes to act as natural nitrogen fertilizers and green indicators of soil nutrition, and as such improve crop yield without the damaging effects of chemical nitrogen fertilization. Likewise, legumes contribute to solving major environmental challenges such as nitrogen pollution and declining soil quality. The current commercial inoculants are not adapted to cultivation under the local soil and environmental conditions of North-Western Europe. This hampers the interaction and leads to insufficient bean protein content for human food consumption. Local strains, adapted to our conditions may be more competitive and have a superior positive effect on soybean production. We set up a citizen science project to trap endogenous nitrogen-fixing bacteria that nodulate locally grown soybean. To have access to a large geographical gradient and different soil types in Flanders, over one thousand citizens across Flanders were recruited to grow and monitor soy plants, frequently entering data regarding plant phenotype into a platform specifically designed for the project. The outcome of the project in terms of microbial discovery but also correlations between soil parameters such as nutrient levels, soil texture, and nodulation performance will be discussed.

Manipulating of GmPTF1a/b improved nutrient efficiency through root architecture modifications and nodulation in soybean

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Improving crop nutrient efficiency becomes an essential consideration for environmentally friendly and sustainable agriculture. Nitrogen (N) and phosphorus (P) are the most frequently required mineral macronutrients, and are primarily taken up from soils by roots. Therefore, root architecture and morphology are of great importance for improving crop nutrient efficiency. Moreover, the symbiotic nitrogen fixation (SNF) is a prominent natural source of N in agroecosystems, which are additional important strategies to enhance nutrient acquisition. However, the molecular and mechanism of root growth and SNF associated with N and P efficiency are still largely unknown. In this study, a bHLH transcription factor (TF), GmPTF1a, was found here, together with its homolog GmPTF1b, to be critical for soybean root architecture modification (RAM) and nodulation. Manipulation of GmPTF1a/b severely influenced root growth, rhizobial infection and nodule formation. Further genetic and biochemical analysis demonstrated that the GmPTF1a product directly binds to E-box motifs in the GmPL5;1(nodulation pectate lyase gene) and GmEXPB2 (cell wall expansin) promoters to activate them transcription, and thereby facilitate RAM and rhizobial infection. Thus, P and N content were significantly enhanced by overexpressing GmPTF1a. Taken together, our findings implicate a bHLH TF as a key regulator of RAM and nodulation that participates through the novel molecular mechanism of activating genes associated with cell wall loosening and localized degradation.

Keywords: GmPTF1a/b, nodulation, root architecture, E-box, cell wall modification, soybean

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Decipher rhizosphere bacterial communities of soybean in soybean-maize intercropping system

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Rhizosphere microorganisms provide multiple beneficial functions to host plants. Although soybean-maize intercropping has been widely applied in agriculture production, and beneficial functions have been achieved, rhizosphere microbial community of soybean in the soybean-maize intercropping system remains elusive. Here, we focus on the effects of maize intercropping and rhizobium inoculation to the bacterial communities in the rhizosphere of soybean by the llumina Miseq platform. Our result suggested that maize intercropping and rhizobium inoculation increased the diversity of rhizosphere bacterial communities in soybean compared with soybean monoculture treatments at various degree. The PCoA results indicated that the structure of rhizosphere bacterial communities in soybean was shifted between monoculture and maize intercropping. Bacterial composition analysis showed Proteobacteria was the dominate bacteria in the rhizosphere of soybean. Compared with monoculture treatments, the relative abundance of Firmicutes was significantly increased in intercropping treatments, while the relative abundance of Bacteroidetes was decreased. In addition, rhizobium inoculation had little effect on the composition of bacterial in soybean rhizosphere. At the genus taxonomic level, the relative abundance of most microorganisms belonging to proteobacteria was lower in intercropping than monoculture. Function prediction revealed that functions relative to nitrate reduction were higher in intercropping system than monoculture, while functions relative to nitrogen fixation were higher monoculture treatment than intercropping. Results suggested rhizobium inoculation had no obvious effect on functions relative to nitrate reduction and nitrogen fixation. Moreover, our results also suggested the structure of rhizosphere bacterial communities were correlated with shoot biomass, nitrogen uptake, phosphorus uptake and potassium uptake in soybean. Over all, these findings indicate potential strategies for the intercropping management of soybean rhizosphere microbiomes.

Keywords: Soybean, intercropping, rhizobium inoculation, rhizosphere, 16S rRNA

Capturing the phosphorylation of NARK, the central kinase controlling nodulation in soybean

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To meet the global food demand whilst reducing the environmental impact, local legume cultivation, with soybean considered a highly important one, is essential. Legumes establish a symbiotic relationship with nitrogen-fixing rhizobia via nodulation in which atmospheric nitrogen is converted into assimilable plant nutrients. As nodulation is energetically costly, the nodule number is tightly controlled through the process of systemic autoregulation of nodulation (AON). Additionally, nodulation is inhibited locally in the root by nitrate present in the rhizosphere. The key component of both regulation pathways is the protein kinase, NARK. NARK acts in the shoots to regulate nodulation in response to rhizobia and in the roots to restrict this process if nitrate is available. Despite long-lasting research efforts, the signaling pathway downstream of NARK remains elusive. My project aims to reveal how NARK regulates nodulation, possibly by interacting with distinct protein partners in response to rhizobia and nitrate. I am currently doing phosphoproteomics and shotgun proteomics to explore NARK-dependent phosphorylation and protein abundance changes in roots and shoots, identifying putative NARK targets. To functionally validate the role of novel signaling components I have recently established virus-induced gene silencing for use in nodulation research.

Keywords: Nodulation, NARK kinase, proteomics, AON

Effect of row spacing, seeding rate and nitrogen fertilization on yield and yield components of soybean

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Soybean crop management has not been studied much in central Europe as compared with cereals. We assessed the effect of variety, row spacing, seeding rate and nitrogen (N) fertilization on yields and yield components of soybean in a two-year experiment in Gleisdorf, Austria. The varieties Lenka, Naya and Xonia were tested in row spacings of 13 cm, 38 cm and 76 cm with 30 germinable seeds m^{-2}. Additionally, 60 seeds m^{-2} were tested at 13 cm row spacing, and 38 cm row spacing was additionally established with N fertilization. Faster soil coverage was obtained with a high seeding rate or narrower row spacings. First pod height differed between varieties and increased with higher seeding rate. Grain yield was not affected by treatments but yield components differed. The widest row spacing resulted in a lower plant density but more pods $plant^{-1}$, grains $plant^{-1}$, grains pod^{-1} (in one year) and a higher thousand kernel weight (TKW). The higher seeding rate resulted in a higher plant density but less pods $plant^{-1}$ whereas grains pod^{-1} and TKW did not differ. N fertilization did not affect the grain yield. Correlation analysis showed a high adaptability of soybean to different seeding rates and row spacings through modulation of yield components.

Keywords: Soybean, yield components, row spacing, soil coverage, first pod height

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Agricultural benefits of herbal leaving mulching in soybean production

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New eco-friendly agricultural practices for sustainable soybean production are needed, since year by year pest outbreak in canopy layer is observed. The aim of the study was to compare different types of herbal leaving mulches in order to verify soybean health and productivity. The study was conducted at the Prusy Experimental Station of the University of Agriculture in Krakow, located near Krakow, Poland (50°07’28’’ N, 20°05’34’’ E), during two growing seasons. Over two years (2021-2022), one factorial experiment was conducted in three replications. The study evaluated three different types of herbal leaving mulches (*Foeniculum vulgare*, *Borago officinalis*, *Calendula officinalis*) sowing at the beginning of sobean development. Yield and health of the soybean plants were assessed. Foliar diseases were identified based on visual symptoms as described in the literature and by microscopic observations of causal pathogens. We proved that yields varied from 3.11 t ha^{-1} to 3.44 t ha^{-1}. Soybean cultivated with *Foeniculum vulgare* as a herbal leaving mulch yielded an average of 0.4 t ha^{-1} higher than control. *Cercospora kikuchii*, *Fusarium oxysporum* and *Ascochyta* spp. were recorded in the years of research. Various effects of herbal species on the occurrence of diseases were observed. It was shown that the cultivation of soybean with *Foeniculum vulgare* reduced infection by *Cercospora kikuchii* and *Ascochyta* spp., and increased infection by *F. oxysporum*.

Keywords: Yield, herbs, leaving mulch, diseases

Climate change in Ukraine, its impact on agriculture and the need to find adaptive solutions in this context

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Over the past 30 years, Ukraine's climate has been changing intensively: since 1991, each subsequent decade has been warmer than the previous one. In 10 regions of Ukraine precipitation in 2014-2018 was 7-12% less than average annual rate. There is a tendency of expanding the area with insufficient precipitation (less than 400 mm) in the warm season. The climate has already become drier across the country. At the same time, some regions of Ukraine have seen positive changes: the length of the active growing season has increased by 10 days on average. All of these factors created unpredictable conditions for farming, with each year becoming unforeseeable and extreme for some or even all crops. It is important for producers to take climate change into account as one of the main risks in their production activities and profits. Scientists both in Ukraine and globally are paying attention to the issue of the climate change and the threat it poses to humanity. In fact, there are many different forecasts, and they are all disappointing for agricultural producers, as in any of the scenarios, from the most optimistic to the most pessimistic, changes will lead to lower yields or partial or complete crop losses. Producers need to understand the changes in their region and correctly assess the future impacts. Donau Soja Organisation is acting on the European protein transition, including risks of climate change for the agriculture. Donau Soja in Ukraine united the experts from various fields, including agronomists, agrochemists, soil scientists, ecologists, plant breeders, experts from the Ukrainian Hydrometeorological Centre, and scientists of the Institute of Water Problems and Land Reclamation, on the discussion of adaptation of agricultural producers to climate change. As the result the Atlas 'Climate change and adaptation of Ukrainian soybean producers' was published by Donau Soja. It includes visual content (maps, cartograms, graphs, charts) and data that are otherwise not widely available to Ukrainian farmers, such as results of research conducted by the Ukrainian Hydrometeorological Centre and the Institute of Water Problems and Land Reclamation of the National Academy of Agrarian Sciences of Ukraine, recommendations from the Food and Agriculture Organisation (FAO), etc.

Keywords: Climate change, farming, agriculture, yield, soybeans

Soybean cultivation in Ukraine: current state, challenges and practical solutions

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Soybean, as an important legume, has became popular in Ukraine for the last 15 years. Additionally, interest in this crop has been increasing over the last 10 seasons. The main soybean cultivation areas are concentrated in the forest-steppe zone. The peak of crop cultivation was in 2015-2017 when the maximum area was 2.1 million hectares. The average yield of soybeans in the country is about 2.5 t/ha. The main problems during soybean cultivation in Ukraine include air drought, non-compliance with cultivation technologies, and climate change. Over the last 30 years, climatic conditions in Ukraine have significantly changed. The seasons of 2020 and 2021 were particularly atypical, for example, the dry season of 2020 with a lack of rainfall led to a loss of crops of approximately 14%, while the extremely rainy season of 2021 was better for average soybean yields in Ukraine but led to partial losses for individual farmers. In Ukraine, the traditional soil treatment system with a combined protection system is most commonly used for soybean cultivation, but no-till, strip-till, and verti-till technologies are also widespread. The best predecessors for soybeans are winter and spring wheat, and good ones are corn, sugar beets, potatoes, and perennial cereal grasses. Meanwhile, legume crops, sunflowers, and perennial legume grasses are unsatisfactory predecessors. For the plant protection products, registered for soybeans in Ukraine-2023, 99 active substances are used, two of which are included in the WHO list of highly hazardous substances, and 34 are not approved in the EU. About 70% of soybean areas are grown using nitrogen-fixing bacteria-based preparations, and the growing trend is their combined use with PGP bacteria, which prevents premature aging and death of nodules and provides an additional nitrogen yield of 30 to 50 kg/ha.

Keywords: Soybean, PGP-bacteria, nitrogen-fixing bacteria, growing technology

Theme D:

Weeds, diseases, pests, and their management

	Sessions *(chair)*
D1	New and emerging pests and diseases *(Tomislav Duvnjak, Tessie Wilkerson)*
D2	Strategies for disease, pest and weed control *(Yuanchao Wang)*
D3	Advanced tools and sensors for soybean protection *(Asheesh K. Singh)*
D4	Managing disease, pest and weed resistance *(Daniel Ploper)*

Posters D: Weeds, diseases, pests, and their management

Integrating transgenic and native resistance for nematode management

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D1: New and emerging pests and diseases

Plant-parasitic nematodes are a major threat to global crop production accounting for over $80 billion USD annually in losses. Soybean is no exception, with the major pest species being the soybean cyst nematode, *Heterodera glycines*, lesion nematode, *Pratylenchus brachyurus*, root-knot nematode, *Meloidogyne incognita* and *M. javanica*, and reniform nematode, *Rotylenchulus reniformis*. Nematologists and plant breeders have expended a great amount of time and resources to incorporate native resistance genes for these nematode pests into commercial soybean cultivars. Resistant cultivars became a cornerstone of effective nematode management in soybean. Unfortunately, nematode problems persist in soybean due to several factors including a lack of native resistance genes for all pest species (root lesion nematode), and pest adaptation to resistance genes (soybean cyst nematode). BASF has developed the first transgenic trait that will be commercially available for nematode control. BASF's nematode resistant soybean (NRS) trait expresses a Cry14Ab Bt-toxin that provides control of both root lesion and soybean cyst nematode. In this presentation we will cover the collaborative work between BASF's trait development and soybean breeding programs to develop and pyramid transgenic and native resistance traits. This collaboration aims to provide effective and durable protection against the full suite of nematode pests attacking soybean in the United States, Canada, and Brazil.

Keywords: Heterodera, *Pratylenchus*, plant resistance, resistance management

A method to obtaining novel sources of resistance in soybean against SCN

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D1: New and emerging pests and diseases

Soybean (*Glycine max* (L.) Merr.) is an economically important crop for a variety of reasons including being a partner in sustainable agricultural management practices, as well as an important source of food, feed, and fuel. Soybean growth in many areas of the world is being threatened by a plant parasitic nematode called soybean cyst nematode, *Heterodera glycines* Ichinohe, (SCN) which causes yield losses of up to 80%. Once the pest is present in the soil, irradication is almost impossible. Currently, only two resistant genes are being widely rotated in SCN infested fields, rhg1 and Rhg4. Because of this, SCN populations have begun to learn the resistance mechanism and overcome it. Hence, the identification of novel resistant genes in soybean is necessary to save this crop. A state-of-the-art pipeline has been developed through applying functional genomics and systems biology practices; this involved investigating various computational databases, including single nucleotide polymorphisms, loss of function, TAIR10 and BLASTP, in addition to two leading sequence-based PPI prediction tools: Protein-protein Interaction Prediction Engine (PIPE4), and Scoring PRotein INTeractions (SPRINT). These two computational engines were used to search the entire soybean and SCN proteomes for proteins interacting with already known resistance proteins, rhg1 and Rhg4. Using this pipeline on entire soybean genome, a short-list of 56 candidate genes was attained. Of these candidates, 4 were published about recently, one SCN resistance, and 3 in resistance to other pathogens. In addition, 16 of the 56 genes contained annotations related to pathogen resistance in plants. Further investigation of these potential candidate genes is currently in progress through an RNA-seq experiment, with the aim of developing allele-specific markers to assist breeding programs developing new resistant cultivars to SCN.

Keywords: Soybean cyst nematode, host-pathogen interaction, pathogen resistance, soybean, bioinformatics

GWAS and RNA-seq analyses to identify SNPs and candidate genes for target spot (*Corynespora cassiicola*) resistance in soybean (*Glycine max*)

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D1: New and emerging pests and diseases

The genetic mechanism for resistance to target spot (*Corynespora cassiicola*) in soybean is unknown therefore a genome wide association study (GWAS) and RNA seq were conducted. A diversity panel consisting of 246 soybean genotypes for target spot resistance against two *C. cassiicola* isolates were phenotyped for disease severity in a controlled environment. High-density SNP data from the SoySNP50K array was used as genotypic data for the analysis. The fixed and random model circulating probability unification (FarmCPU) GWAS model was implemented using GAPIT v3 package in an R environment. A total of 14 and 33 loci were significantly associated with resistance to LIM01 and SSTA, *C. cassiicola* isolates, respectively. Of these, six loci had significant associations with resistance for both isolates. The genes lying 100 Kb upstream and downstream for each significant marker identified 993 candidate genes. To screen the candidate genes more efficiently, an integrated RNA-seq analysis data with GWAS, reduced the number to 238 differentially expressed genes in disease infection tissue compared to control in either resistant or susceptible genotypes. The major genes were involved were associated with defense response to pathogen, innate immune response, chitinase activity, histone H3-K9 methylation, and salicylic acid mediated signaling. The future implications of this study will provide the potential to develop molecular markers for breeding target spot resistant varieties.

Taproot decline of soybean, caused by *Xylaria necrophora*, an emerging threat to profitable soybean production in the southern United States

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D1: New and emerging pests and diseases

In 2007, observational reports of what was deemed a “new” root disease of soybean were made from Arkansas, Louisiana, and Mississippi. The group working on the disease decided on the name taproot decline (TRD) and the causal organism was initially placed in the genus *Xylaria* based on colony morphology as well as the production of fungal structures in culture and in field situations. In most cases, dead man’s fingers, which are a sign produced by members of the genus *Xylaria* were observed in soybean fields most notably by dead plants. Since that time an increasing number of counties/parishes have been observed to contain infected plant material. Moreover, Alabama, Georgia, Missouri, and Tennessee also reported observations of taproot decline. The key symptoms associated with TRD include mild to severe interveinal chlorosis depending on soybean plant growth stages. One of the main diagnostic attributes to help distinguish TRD from some of the other soybean root diseases that produce interveinal chlorosis is by observing the degraded taproot. Soybean roots of plants exhibiting interveinal chlorosis associated with TRD generally are brittle and covered with black stroma that oftentimes can be observed on the taproot as well as infected lateral roots. Initial research worked to recover the causal organism from plant material, conduct Koch’s postulates in the greenhouse to confirm pathogenicity, and subsequently identify the causal organism which is now identified as *Xylaria necrophora*. Since the initial identification of the disease, research has aimed to determine the potential yield losses associated with the disease and continue to determine the distribution throughout the midsouthern U.S. Several research projects continue across the region to consider whether commercial cultivars are resistant to the fungus, effect of seed-applied and in-furrow fungicides and consider the overall yield losses that can be attributed to the disease.

Ground versus aerial herbicide applications: Spray drift, soybean impacts, and regulatory implications

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D3: Advanced tools and sensors for soybean protection

Ground and aerial applications of auxin mimic herbicides (HRAC/WSSA Group 4) are common internationally for managing weeds. However, substantial injury to susceptible species, particularly soybean [*Glycine max* (L.) Merr.], has occurred from spray drift. Therefore, this research investigated three null hypotheses: 1. Downwind spray drift would be equivalent from commercial ground and aerial application equipment; 2. Spray drift of a synthetic auxin herbicide, florpyrauxifen-benzyl, would not impact soybean growth and reproductive structures; 3. Predicted drift from United States Environmental Protection Agency (US EPA) models (AgDISP and AgDrift) would not fit measured drift. A field spray drift experiment was conducted near Stuttgart, Arkansas, USA, using florpyrauxifen-benzyl and PTSA dye. Nine downwind collection stations (from 1- to 61-m) containing Mylar plastic cards, water sensitive cards, and potted soybean plants (V3-V4 growth stage) were used for data collection from full-scale, commercial ground and aerial application equipment. Application parameters were input into US EPA drift models, and predictions were compared to measured drift. Collected field data were consistent with US EPA model predictions. Generally, with both systems applying a Coarse spray in a 13 kph average wind speed, the aerial application had a 5.0- to 8.6-fold increase in drift compared to the ground application, and subsequently, a 1.7- to 3.6-fold increase in soybean injury. An approximate 25% reduction of soybean reproductive structures up to 30.5 m downwind and nearly 100% reduction at 61-m downwind were observed for ground and aerial applications, respectively. In summary, all null hypotheses were rejected. Aerial applications require three to five upwind swath adjustments to reduce drift potential similar to ground applications. Regardless of application method, florpyrauxifen-benzyl drift was detrimental to soybean which may not only affect yield, but also pollinator foraging sources. As US EPA models accurately predicted spray drift, these models can be confidently used for directing future regulatory action.

Keywords: United States Environmental Protection Agency, droplet size, models, off-target movement, pollinator foraging sources

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Listening to insects: using microphones and deep learning algorithms to monitor soybean pests

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D3: Advanced tools and sensors for soybean protection

Insect pest data is critical for agricultural decision-making. Acquiring accurate information on an insect pest's presence, identification, and location is labor-intensive and costly, resulting in increased inequity. Larval boring insects are especially difficult to detect without destroying a crop, as they are located within the plant. Yet, larvae are often the primary economically damaging life stage. To address this discrepancy, we developed and tested a novel cost-effective method to determine 1) the presence of boring larval insect(s) and 2) identify the species of boring larvae. Specifically, we hypothesized that microphones paired with bespoke deep-learning algorithms could be interpreted as insect presence and species identification data. We tested the hypothesis in a laboratory and controlled environment setting across several insect pests and crop species, including soybean. Early results are highly promising. The next research stage is to field test the method in soybean fields under both observational and experimental conditions. Knowing the species and density of boring larval pests increases the capability to precisely time and space agricultural interventions, such as pesticides and beneficial insects. Specifically, this data has the potential to dial in the timing of insecticide interventions, increasing the efficacy of our tools. Overall, an accessible digital tool should decrease the economic cost of managing insect pests in soybean systems.

Keywords: Pests, digital entomology, boring insects

Acknowledgments: Thanks to the University of Wisconsin-Madison’s teams of engineering design students who assisted in a rugged prototype design.

UAS longitudinal phenotypes to measure and select soybean stress resilience

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D3: Advanced tools and sensors for soybean protection

Unoccupied aerial systems (UAS) allow plant breeders to measure phenotypic variability on the same genotype multiple times during a season, variables defined as longitudinal traits. Historically, soybean breeders have relied on measurements of yield and what we can observe visually to select superior lines. There is a need to develop methods to combine UAS metrics over time to phenotype newly enabled longitudinal and physiological traits and explore their use in predictions of yield potential and environmental resilience in a changing climate. UAS metrics describing early-season, vegetative canopy development can define and differentiate crop resilience to the environment and other stressors. Since 2013, we have been collecting UAS RGB imagery of soybean yield trials and breeding populations in multiple locations ad from images of green canopies, we can quantify canopy coverage, canopy height, canopy color, above-ground biomass, herbicide resilience, multi-spectral vegetation indices, longitudinal combinations of these for maturity and stress detection, stand counts, and row length, and we are working on canopy temperature with a thermal camera. Herbicides have been the first focus due to the economic importance of weed control, and the fact that herbicide application is a relatively easily-controlled stress treatment to the crop. UAS-acquired RGB methods can measure field herbicide resilience, and we have classified resilience to PPO inhibitor pre-emergent herbicides in the field for hundreds of diverse lines using UAS data only.

Method for detecting datura in high-resolution UAV images of soybean fields

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D3: Advanced tools and sensors for soybean protection

The common datura (*Datura stramonium*) is an important agricultural weed and also contains toxic secondary plant constituents, namely the tropane alkaloids scopolamine and atropine. These can lead to acute symptoms of poisoning in humans and animals. Seeds and plant parts of the datura are harvested and unintentionally mixed with the harvested crop. Unmanned areal vehicles (UAVs) are used - after conventional weed control measures - to detect datura that has escaped the weed control measures or later emerges on agricultural fields so that it can then be targeted. These UAVs are equipped with a high resolution camera and are flown at medium height. The images are split into training and test sets. The training set consists of 377 images cropped from the original images with a resolution of 1024 × 683 pixel and the test set consists of 9 images in full resolution. Two different Convolutional Neural Network (CNN) based methods were trained on these images, one CNN based method for object detection and a method for semantic segmentation. The model using semantic segmentation achieved an Intersection Over Union (IoU) score of 0.68 and a F1 score of 0.80. These results show that the model performs well and has a low number of false positives as well as false negatives. In contrast, the evaluation results for the object detection model achieved an IoU score of 0.13 and F1 score of 0.27. This model has difficulty accurately detecting the target object. The results show that the tested CNN-based method for semantic segmentation is a valid choice for accurately detecting datura plants in soybean fields.

Citizen science: Shaping the future of AI applications in agriculture

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D3: Advanced tools and sensors for soybean protection

The future of AI applications in agriculture is being shaped by citizen science involvement in data collection. iNaturalist, a citizen science dataset collected by people worldwide, includes around 70 million images of various kingdoms such as *Animalia, Plantae, Fungi, Protozoa*, and *Chromista* etc. Within the Animalia kingdom, the phylum *Arthropoda* includes the class Insecta, which contains a large number of insect-pest species. The Insecta class dataset available on iNaturalist is highly accurate, diverse, taxonomically, biologically, ecologically, and agriculturally relevant. It consists of over 13 million insect images belonging to around 100,000 distinct insect species. However, this large and diverse dataset presents significant challenges for AI models to solve, including intra-class dissimilarity, inter-class similarity, morphological differences across different stages in insect life cycles, sexual dimorphism, and camouflaging backgrounds. We have developed a deep-learning model called InsectNet, which effectively addresses the challenges associated with identifying insect-pests in agriculture. Our model integrates four key concepts, including a large dataset of insect-pests' images collected through citizen science, hierarchical self-supervised learning of large models, supervised fine-tuning that accounts for data imbalance, and uncertainty quantification to enable abstaining from making uncertain predictions and making conformal predictions. By leveraging these concepts, our approach allows for accurate identification of over 2,500 agriculturally relevant insect-pest species classes, including pollinators, predators, parasitoids, decomposers, and pest species, with an accuracy rate of over 95%. The InsectNet model is readily available through a web-based portal and an easily reusable software stack.

Cyber-Agricultural Systems (CAS)

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D3: Advanced tools and sensors for soybean protection

The recent progress in pervasive sensors, artificial intelligence, intelligent actuators, and scalable digital infrastructure has given rise to Cyber-Agricultural Systems (CAS). CAS has three core elements - sensing, modeling, and actuation, and encompasses concepts of smart agriculture, precision agriculture and digital agriculture in the context of weed, disease and pest identification and management. CAS is also enabling the framework of digital twins that fuses AI and bio-physical models for more informed exploration of “what if” scenarios, and facilitating the incorporation of ideotype designs of crops. CAS is poised to transform crop breeding and production through improved efficiency, productivity, sustainability, and adaptability to shifting climates.

Documenting fungicide resistance in the southern United States: a continuing series of surveys from Mississippi

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D4: Managing disease, pest and weed resistance

Following the initial identification of isolates of the frogeye leaf spot fungus, *Cercospora sojina* with confirmed QoI fungicide resistance in 2010, additional surveys of soybean pathogens were conducted throughout the United States. Soybean production in Mississippi presents an interesting situation, since automatic fungicide applications in the absence of disease, historically with QoI-containing fungicide products, have been commonplace for the past two decades. The initial isolates of *C. sojina* with documented resistance to the QoI fungicides from Mississippi were identified in 2012. Following the initial identification, a statewide survey determined that the G143A amino acid substitution was the dominant genotype with > 93% of *C. sojina* isolates containing the substitution. In addition to *C. sojina*, several additional soybean plant pathogens have been confirmed to contain the G143A amino acid substitution that confers resistance. During 2016, isolates of *Corynespora cassiicola*, which causes target spot were confirmed to be resistant to the QoI fungicides in Mississippi. A subsequent statewide survey between 2019 and 2021 determined that isolates of *C. cassiicola* containing the G143A substitution were the dominant genotype with greater than 85% of the isolates containing the amino acid substitution. In all, between *C. sojina* and *Corynespora cassiicola*, 91% and 89% of the counties in Mississippi were confirmed to contain isolates with the G143A genotype, respectively. More recently, isolates of the fungi that cause Cercospora leaf blight (CLB) as well as Septoria brown spot have also been confirmed to contain the G143A substitution. A multiyear project considered isolates of *Cercospora* spp. responsible for CLB from Stoneville in west MS and Verona in east MS. A greater proportion of the isolates from Stoneville, 96%, as compared to Verona, 51%, were determined to contain the G143A substitution. Lastly, a limited survey has determined that isolates of *Septoria glycines* also contain the G143A substitution.

Herbicide resistant weeds in South America

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D4: Managing disease, pest and weed resistance

South America is responsible for more than half of the soybean production in the world. Among the producing countries in this region, Brazil, Argentina and Paraguay stand out. In South America one of the main problems in the production systems is the weed control, especially due to the high presence of herbicide resistant populations, which makes their control more difficult and costly. The number of resistant species have been increasing year after year, especially those of glyphosate resistant weeds. In Brazil, there are already reports of 53 cases of weeds resistant to herbicides, 19 of which are resistant to glyphosate, covering eleven different species. In Argentina, 48 cases of resistance were reported, 32 of which to glyphosate, in 26 different species. In Paraguay, there are six reported cases of herbicide-resistant weeds, five of which are related to glyphosate, in different species. In addition to the problem of simple resistance to glyphosate, there has been an increase in cases of multiple resistance, that is, to glyphosate and other herbicide with a different mechanism of action, which restricts even more the chemical control options. Cases of multiple resistance already total nine in Brazil, 14 in Argentina and three in Paraguay. Among the most important resistant weeds present in the region are: *Amaranthus hybridus*, *Amaranthus palmeri*, *Conyza* spp., *Digitaria insularis*, *Eleusine indica*, *Lolium multiflorum*, *Sorghum halepense*, among others. There is no single or isolated solution to combat resistance, which is why the main recommendations have been based on integrated weed management (IWM), which is defined as the selection and integration of control methods (such as preventive, cultural, mechanical, chemical, etc.) and the set of criteria for its use, with favorable results from the agronomic, economic, ecological, and social points of view.

Keywords: Resistance, herbicides, glyphosate, integrated management

In vitro sensitivity of *Corynespora cassiicola* isolated from soybean to different chemical fungicides in northwestern Argentina

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D4: Managing disease, pest and weed resistance

Target spot of soybean, caused by *Corynespora cassiicola*, has become an important disease in northwestern Argentina (NWA). The Fungicide Resistance Action Committee (FRAC) classifies *C. cassiicola* as a high-risk pathogen for the development of fungicide resistance. The objective of this study was to determine the fungicide sensitivity of six *C. cassiicola* isolates to different commercial fungicides. These isolates were collected during the 2014/2015 growing season from soybean fields located in the provinces of Tucumán and Salta, in NWA. The 50% effective concentration (EC50) values were estimated by the relative mycelium growth reduction on PDA medium added with fungicides doses of 0, 0.1, 1, 10, 100 and 1000 ppm of active ingredient. The fungicides used contained: azoxystrobin 25%, trifloxystrobin 50%, pyraclostrobin 25%, difenoconazole 25%, pydiflumetofen 20%, thiophanate-methyl 50%, mixtures containing epoxiconazole 5.0% + pyraclostrobin 13.3%, trifloxystrobin 15.0% + prothioconazole 17.5% + bixafen 12.5%, difenoconazole 12.5% + pydiflumetofen 7.5%, and fluxapyroxad 5.0% + pyraclostrobin 8.1% + epoxiconazole 5.0%. At 7 days, CE50 was estimated for each isolate and chemical product using the methodology by Kataria and Grover (1978). Azoxystrobin and trifloxystrobin were inefficient to control all isolates (EC50 >1,891.3 ppm and >3,103.2 ppm, respectively). Pyraclostrobin was inefficient for four isolates (EC50 >148.8 ppm) and slightly efficient for two isolates (EC50 19.6 to 49.8 ppm). Difenoconazole was moderately efficient for 5 isolates (EC50 1.6 to 6.2 ppm) and slightly efficient for one isolate (EC50 44.2), pydiflumetofen was moderately efficient (EC50 1.7 to 5.8 ppm), the mixture of epoxiconazole + pyraclostrobin was also moderately efficient (EC50 1.8 to 8.1 ppm) and so was trifloxystrobin + prothioconazole + bixafen (EC50 1.1 to 4.1 ppm). Thiophanate-methyl was inefficient for one isolate (EC50 10,471.3 ppm), moderately efficient for another (EC50 3.6 ppm), and highly efficient for four isolates (EC50 <0.3 ppm). The mixtures difenoconazole + pydiflumetofen and fluxapyroxad + pyraclostrobin + epoxiconazole were highly efficient for all isolates (EC50 <4.9x10-1 ppm and <5.7 x10-2 ppm, respectively). Since *C. cassiicola* showed variability in sensitivity to the fungicides evaluated, there is a high risk of selection for resistance. Further work needs to be carried out to detect possible mutations, increasing the number of isolates tested.

Keywords: Glycine max, target spot, fungicide resistance

Fungicide resistance in *Cercospora sojina*, causal agent of frogeye leaf spot: detection and management in the U.S.A.

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D4: Managing disease, pest and weed resistance

Frogeye leaf spot, caused by *Cercospora sojina*, is one of the most damaging foliar diseases of soybean in the U.S.A. Since 2010, isolates of *C. sojina* with resistance to quinone outside inhibitor (QoI) fungicides have been detected in the U.S.A. The initial detections of QoI-resistant *C. sojina* from three states in 2010 have now grown to detection in over 20 states. In light of QoI-resistance, management of frogeye leaf spot with fungicides can only be accomplished when active ingredients from fungicide classes other than QoIs are included. Research to determine if sensitivity of *C. sojina* populations to demethylation inhibitor (DMIs) and methyl benzimidazole carbamate (MBC) (SDHI) fungicides is ongoing and has shown that differences in sensitivity to these fungicide classes can be observed from year to year. In light of these findings, soybean growers in the U.S.A. are encouraged to implement non-chemical frogeye leaf spot management strategies, and if needed based upon field scouting and disease risk, apply fungicide products that include active ingredients from different fungicide classes that are effective against frogeye leaf spot.

Cost of weed resistance to herbicides in soybean crop in Brazil

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The first Brazilian case of weed resistance to herbicide occurred in 1993. So far, there are 54 cases reported, with resistance to glyphosate as the most important. The increase in weed control costs is one of the main consequences of that resistance, which is not usually addressed in scientific publications but is of great importance to the productive sector. The objective of this work was to estimate the economic impact caused by weed resistance in the leading Brazilian agricultural production system: the soybean crop. The methodology had two steps. We first determined the size of the areas with weed resistance in distinct Brazilian regions using five methods: questionnaires for technical assistance and producers, seed collection and greenhouse tests, experiments conducted directly in the field, visits to areas with suspected resistance, and surveys by specialized companies. The second step was to quantify the costs of the relevant control alternatives, especially the chemical ones. The choice of herbicides, doses, and the number of applications depended on the density of the infestation and whether or not there was a mixed weed population. The area with resistant weed populations was 24.4 million ha, being the three main species: *Digitaria insularis*, *Conyza* spp., and *Lolium multiflorum*. For isolated weed populations, the average annual increase in control costs per ha was US$ 15.63 for *Lolium multiflorum*, US$ 16.12 for *Conyza* spp., and US$ 42.68 for *Digitaria insularis*, which represented an average increase of 46.2%, 44.5%, and 102.3%, respectively. By analyzing the total area of soybean infested with resistant weeds, the control cost increased by an average of US$ 1.34 billion per year.

Keywords: Glyphosate, *Conyza* spp., *Digitaria insularis*, *Lolium multiflorum*, multiple resistance

Soybean weed control in the mid-south United States

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Soybean production in the Mississippi Delta region of the United States is often conducted on fields that are largely prone to soil erosion. This erosion is predicated on the three factors of highly erodible silt-based soils, rolling topography, and the frequent occurrence of high intensity rainfall events soon after soybean planting in the spring of each year. For this reason, essentially all soybeans are produced under some form of reduced till or no-till production system. This results in an absolute or near absolute reliance on herbicides for weed control. Gone are the days of simple weed control relying on several applications of glyphosate in-season on Roundup Ready crops to provide perfect weed control with no crop injury. Multiple weed species have developed resistance to glyphosate and farmers have incorporated additional modes of action to control weeds. Some of the alternate chemistries are naturally safe across soybean cultivars and others rely on transgenic crop varieties to impart herbicide selectivity. In recent years many soybean fields in the Midsouth United States have utilized dicamba resistant soybean cultivars to control problematic dicot weeds such as *Conyza canadensis* and *Amaranthus palmeri*. While initial control was good with these auxin herbicides, recent findings have shown that resistance has started to occur in these and other populations. Additionally, the co-application of auxin herbicides with glyphosate or other modes of action has decreased grass weed control in some situations.

Utility and effectiveness of an integrated seed destruction system for Palmer amaranth escapes in soybean

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Herbicide resistance continues to increase throughout the US. Populations of Palmer amaranth (*Amaranthus palmeri*) resistant to up to 6 herbicide modes of action have been identified. With resistant populations of Palmer amaranth spreading at a rapid pace, integrated weed management techniques such as cultural and mechanical practices for control have become increasingly important. The concept of harvest weed seed control has been adopted by many growers in Australia due to the lack of herbicide options for resistant rigid ryegrass (*Lolium rigidum*). Initial trials indicate that the design of the single mill Harrington seed destructor may not suffice in its current form, for Southern US soybean harvest. Results from first harvest runs with the destructor in 2018 revealed that weed-free soybean can be harvested at normal speeds with no issues. Unfortunately, any moisture from green plant tissue parts in weedy areas of the field end up in the lower chaff fraction and result in the rapid clogging of the seed destructor mill. In 2020, a Redekop twin-mill seed control unit (SCU) was purchased and installed on a Class 8 John Deere S680 combine. Plots were arranged in a randomized complete block design to evaluate soybean harvest aids, effects on Palmer amaranth seed shatter, and Redekop SCU efficiency. Data were subjected to ANOVA and means were separated utilizing Fisher's protected LSD. Results indicate that harvest aids will likely be necessary for the Redekop SCU when harvesting soybean prior to a killing frost. Green material or harvest chaff with high moisture content significantly reduced airflow and thus chaff flow through the twin-mill Redekop SCU system. Additionally, some seeds escaped destruction via loss at the combine head and through the chaff separation process. Future research will include the addition of a conveyer that will physically move the lower chaff fraction from the screens into the mill system.

The use of rhizobacteria *Bacillus subtilis* for biotic stress management in soybean plants

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The current study looks at the possibility of using plant growth-promoting rhizobacteria (PGPR) *Bacillus subtilis* as biocontrol agent to enhance plant's tolerance to insect attack. Under stress conditions, the metabolism of a plant cell is generally characterized by elevated formation of reactive oxygen species (ROS). However, plants have developed antioxidant protective mechanisms against oxidative stress. The aim of this paper was to determine the activities of antioxidant enzymes present in roots and leaves of inoculated and non-inoculated soybean seedlings (with and without exposure to mites, *Tetranychus urticae*), as well as the level of lipid peroxidation (LP). Results have shown higher LP intensity in non-inoculated plants treated with mites compared to the control group. During biotic stress caused by mite attack, inoculation successfully reduced oxidative stress. Thus, it seems that inoculation with *B. subtilis* itself produced mild stress, which was beneficial to soybean plants. Superoxide dismutase (SOD) activity was in positive correlation with LP intensity ($r = 0.99$), while pyrogalol- and guaiacol-peroxidases (PPX, GPX) were especially active in plant roots after the inoculation. Catalase (CAT) activity was minor both in leaves and roots. Due to PGPR properties of *B. subtilis* influence on plant morphology was examined as well. Characterization of two isolates, denoted as B3 and B44 (*Bacillus* spp.), was carried out. Both isolates have shown ability to produce HCN, enzymes: lipase, urease, gelatinase, but not cellulase. However, isolate B3 was found to be positive for production of siderophores, while B44 was not. The obtained results indicate that inoculation of soybean seeds with *B. subtilis* improves plant tolerance to mite attacks.

HPLC analysis of phenolic acids and flavonoids in soybean seedlings inoculated with *Trichoderma asperellum*

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The need for high and quality agricultural production has led to an excessive use of chemical fertilizers, causing serious environmental pollution. Bioferti¬lizers and biopesticides are used as an alternative for maintaining high production with low ecological impact. *Trichoderma* species are free-living fungi in soil and root ecosystems and one of the most commonly used biological control agents against plant pathogens. Plant phenolics are known as effective natural antioxidants which enable plants to counteract negative abiotic and biotic types of stresses. Phenolic acids and flavonoids are the most potent antioxidants among phenolics, and thus, we investigated their presence and content in leaves and roots of soybean plants inoculated with *Trichoderma asperellum*. The mobile phase in the HPLC analysis was MeCN with 2.0% acetic acid (A) and Milli-Q water with 2.0% acetic acid in gradient mode (92% A at 0 min, 80% A at 18 min, 60% A at 25 min, 55% A at 30 min, 35% A at 40 min, 20% A at 50 min, constant 20 % A for 4 min, 90% A at 57 min following 3 min constant 90%), with the flow rate 1.0 ml/min. This was equipped with a ZORBAX SB-Aq column. The analysis showed the presence of ferulic, protocatehine, epicatehine, p-hydroxy benzoic, gallic, caffeic, p-coumaric and vanilin acids, and flavonoids naringenin, kaempferol and rutin. The presence of catehine, resveratol, quercetine and miricetine has not been established. The content of the phenolics was significantly higher in soybean leaves compared to roots. The most abundant compounds were ferulic (1.17-2.29 µg kg^{-1}) and p-coumaric (0.80-1.97 µg kg^{-1}) acids. There were no significant changes in the phenolics contents before and after the inoculation with *T. asperellum*. We may conclude that this biocontrol agent does not provoke oxidative stress in soybean plants and it may be safely used in agricultural production.

Weed reduction in soybean: a comparison between chemical and mechanical weed reduction taking into account plant height and yield effects

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A successful control of weeds in soybean production is an important factor, because losses due weeds have been one of the major limiting factors for soybean production in Belgium. They compete with soybean for sunlight, soil moisture and nutrients. Early in the season this competition is the most critical period. Integrated weed control in the cultivation of soybean is therefore crucial. Under field conditions this can consist of chemical pre-emergence weed control followed by chemical and/or mechanical post-emergence weed control. In Belgium the number of chemical post-emergence solutions are scarce, due to lack of authorised broad spectrum active substances. Farmers and policy makers reevalute chemical weed control because of the environmental risks of herbicides and the influence on farmers and public health. Causing new opportunities in chemical weed control are not feasible anymore. It's therefore necessary to chose for a system taking into account the possibilities for mechanical weed control in pre- and post-emergence. During a two years study Inagro compared three weed reduction techniques: mechanical, chemical and a combination of both on 2 different row spacings: 13 cm and 39 cm. The objective of this study is to find the optimal combination with minimal yield losses.

Based on this two year study a row distance of 13 cm is interesting in weed reduction if only one chemical herbicide is applied. Once mechanical solutions are added next to a herbicide or only mechanical weed reduction is applied a row distance of 39 cm is recommended. On yield no impact has noticed due to the herbicides or mechanical passages. However, in plant height, there was a negative trend for mechanical weed reduction. Those plants showed lower stems.

Floral bud distortion: insights of peculiar floral malady prevailing in Indian soybean (*Glycine max* L.)

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Siphonogamy is a key event in the reproductive course of plants extensively studied in past. However, a floral malady is one of the major biotic stresses adversely affecting soybean productivity in India. However, an insight of a peculiar and often harmful disorder prevailing in Indian soybean is needed to understand. The structural abnormalities may lead to dysfunction of floral organs. Investigation on the abnormalities at structural, cytological, biochemical and molecular level help to understand complexity of the malady. Similarly, diagnosis of symptomatic plants could help to identify the causal organism associated with the malady. RNA-Seq can be used for knowledge discovery of malady by morpho-physiological key pathways. The comparative studies were conducted for understanding of cellular targets, their biochemical and molecular behavior in symptomatic and asymptomatic soybean plants. The diagnosis of causal agent(s) was carriedout using PCR and ELISA. The RNA-Seq analysis was done using three sets of symptomatic and asymptomatic plant tissues at R5 stage. The extensive random survey revealed severity of the malady was ranging from 2.0 to 90.0% under field condition IN Central India. Diagnosis of symptomatic plants revealed presence of phytoplasma and found Pollen dysfunction causes 'Floral Bud Distortion' in soybean. The significant increase in carbohydrate, protein along with chlorophyll content index was recorded in symptomatic plant. RNA Seq using Illumina HiSeq NGS data revealing 17,454 differentially expressed genes, 5561 transcription factors, 139 pathways and 176,029 genic region putative markers single sequence repeats, single nucleotide polymorphism and Insertion Deletion. Roles of PmbA, Zn-dependent protease, SAP family and auxin responsive system are revealing mechanism of the malady having abnormalities in pollen development. This is the first report of transcriptomic signature of the soybean malady possessing morphological and metabolic changes which attracts insect for spread of disease. All the genic region putative markers may be used as genomic resource for varietal improvement and new agro-chemical development for disease control to enhance soybean productivity.

Keywords: Soybean, floral malady, floral bud distortion, pollen dysfunction, transcriptomics, RNA-Seq, karyotyping

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Diaporthe caulivora has both mating type genes but *D. longicolla* has either MAT1-1-1 or MAT1-2-1 making it heterothallic

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Species of genus Diaporthe cause important soybean diseases including seed decay, pod and stem blight and stem canker. In central Europe *D. longicolla* is the predominant species, along with *D. caulivora*, *D. novem* and *D. eres*. Morphologies and sequences of these species vary and sometimes overlap. Part of this variation might be explained by different mating types of the isolates. Also, mating type analysis might help to distinguish isolates. The Diaporthe mating type locus (MAT) has been characterized and Santos et al. (2010) designed primers based on the MAT locus for mating type diagnosis in most Diaporthe species. Here, 30 European Diaporthe isolates were tested for their mating type using these primers named MAT1-1-1FW/RV and MAT1-2-1FW/RV. This way we found MAT1-1-1 in eight *D. longicolla* isolates, in two *D. eres*, and in two *D. novem* isolates. MAT1-2-1 was found in 13 *D. longicolla* isolates, one *D. caulivora* isolate, three *D. eres* isolates and one *D. novem* isolate. Interestingly all *D. longicolla* isolates had either the one or the other mating type. On the other hand for the self fertile *D. caulivora* MAT1-1-1 could not be amplified, probably due to insufficient primer specificity. Using genomic data for *D. caulivora* and *D. longicolla*, that recently became available, we identified the MAT1-1-1 gene for *D. caulivora* and we found both loci in different genomes of *D. longicolla*. We corroborated both findings by using new primers and sequencing in our own isolates. Therefore, we are first to identify the MAT1-1-1 gene in *D. caulivora* and to provide evidence that *D. longicolla* is a heterothallic species, which is in contrast to earlier findings and could explain the difficulties in finding the sexual form of *D. longicolla*. Mating experiments with different *D. longicolla* isolates and experiments to identify physiological differences between the mating types are ongoing.

Keywords: Diaporthe, mating type analysis

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Mechanical weed control interacts with soybean yields in organic farming in Luxembourg

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The European Soybean Declaration (2017) initiates Luxembourg to increase regional cultivation of soybeans and protein crops. Soybean is not yet an established crop in Luxembourg, mainly due to knowledge gaps in mechanical weed control (Zimmer, 2016). The performance of different weed control methods was tested to maximize soybean grain yield. Field trials were set up at three organically managed sites in 2018 and 2019. Five different weed control treatments, a negative and a positive control were tested in a randomized complete block with four replicates. Soybean yield parameters, weed cover and weed control efficiency were analyzed. Soybean yields showed significant differences between the treatments, however water stress was defined as the main yield limiting factor. Higher yields, comparable to the positive control, were observed with hoeing compared to harrowing (-34 %). Significantly higher weed cover with harrowing was negatively correlated with yield (r = -0.84). The lower selectivity of the harrow led to reduction of -28 % of soybean density, compared to -11 % for the hoe. Summarizing, hoeing, while no difference whether finger weeder is applied or not, seems to be the best option for weed control and yield optimization in organic soybean cultivation in Luxembourg. However, if there continues to be more and more frequent summer droughts, yields will be significantly impacted and crop profitability will be reduced.

Keywords: Soybean, mechanical weed control, field trial, Luxembourg, organic farming

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Assessing quinone outside inhibitor (azoxystrobin) fungicide sensitivity of off-target species of *Diaporthe* in the United States soybean

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Species of *Diaporthe*, associated with pod and stem blight, Phomopsis seed decay, and stem canker in soybean (*Glycine max*), have caused total estimated yield losses ranging between 0.1 and 5.6 million tonnes between 2015 and 2019 in the United States. Among the fungicide products, those containing quinone outside inhibitor (QoI) are labelled against foliar pathogens, which includes Diaporthe species causing pod and stem blight. These fungicides can inhibit the fungal mitochondrial respiration by binding to the quinine oxidizing site in the cytochrome b (cytb) complex III. Point mutations in cytb are associated with resistance to QoI fungicides, which have been designated as having a high risk for resistance development. The objective of this study was to determine the sensitivity of isolates of *Diaporthe aspalathi*, *D. caulivora* and *D. longicolla* to the QoI fungicide, azoxystrobin. Sensitivity of 43 isolates obtained from multiple U.S. states was evaluated with radial growth assays on 2% water agar amended with azoxystrobin at 0, 0.001, 0.01, 0.1, 1.0, and 10.0 µg/ml. Salicylhydroxamic acid (20 mg/ml) was added to all plates to block the alternative respiration pathways of the fungi. The experiment was conducted twice in a completely randomized design and each of the isolates were placed on three replicate media plates with a 6-mm-diameter mycelial plug. Plates were incubated for five days at 22°C in the dark. The effective fungicide concentration (EC50) that reduces fungal growth by 50% relative to the non-amended media was determined for each isolate. Significant differences in EC50 values were observed among the isolates of *D. aspalathi* (0.14 to 13.25 µg/ml; $P<0.0001$), *D. caulivora* (0.019 to 35.20 µg/ml; $P<0.0319$) and *D. longicolla* (0.078 to 8.53 µg/ml; $P<0.001$). Future experiments need to be performed to confirm *in vitro* sensitivities and field efficacy of azoxystrobin against species of Diaporthe.

Keywords: Diaporthe, soybean, fungicide resistance

Testing soybean varieties for their tolerance against *Rhizoctonia solani* anastomosis group 2-2IIIB

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An important part of sustainable agriculture is growing tolerant varieties of soybean against Rhizoctonia, an important fungal pathogen found in Flemish fields. Previously, several varieties were already tested for their tolerance against Rhizoctonia under controlled conditions where significant differences were observed. However, the market of varieties is constantly growing leading to better adapted varieties to North-Western European conditions. Using a bioassay, we tested twelve early maturing soybean varieties (MG000) recently registered to the European variety catalogue. Four replicates and 12 soybean plants per replicate were tested in a randomized complete block design. Each design was tested four times. Two different isolates of *Rhizoctonia solani* anastomosis group (AG) 2-2IIIB on sterile wheat kernels were used to perform the bioassays, also a control treatment is taken into account for each isolate. Sprouted soybean seeds were sown in plastic trays and inoculated after 5 days by sowing the infested wheat kernels in a row of 40 kernels between lines of both 6 soybean plants. Two weeks after inoculation, plants were evaluated for appearance of disease symptoms following a severity scale (0 = no symptoms, 5 = seedling dead, complete constriction). Furthermore, the fresh aboveground biomass was measured of inoculated and non-inoculated plants. To calculate the relative biomass, fresh biomass of inoculated plants was divided by the fresh biomass of non-inoculated plants. There was a small significant difference in disease severity among the varieties. The variety Ceres PZO is the most tolerant, while Aurelina is considered the most susceptible in these assays. The relative fresh biomass of inoculated plants was significant negatively correlated with the disease severity. We can show that disease development on the roots has a negative effect on aboveground biomass development.

Diversity and prevalence of plant-parasitic nematodes associated with soybean in Kenya

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Soybean yield is largely affected by pests and diseases, including plant-parasitic nematodes (PPN). So far, data on the diversity and distribution of the PPNs associated with soybean in Kenya is lacking. Therefore, the main objective of this research was to study the prevalence, diversity and density of PPN associated with soybean in Kenya. Field surveys were conducted in select fields in major soybean growing areas including Siaya, Kakamega, Busia, Bungoma and Nakuru counties in November 2019. Plant roots and soil samples were taken using a sampling shovel to an approximate depth of 15-20 cm in a zig-zag pattern across the area sampled. One bulk soil sample was collected per field, regardless of its size. A total of 50 samples (25 root and 25 soil), each weighing at least 1000 g, were collected. Nematode extraction was conducted in 300 ml of each soil sample collected using a modified Baermann funnel technique and nematodes were collected after 24-72 hours. The roots from each sample were carefully washed under running tap water and gently blotted dry with a kitchen towel; clean roots were then chopped into small pieces followed by weighing 15 g of the chopped roots. The nematodes were extracted, counted under a stereoscopic microscope at ×40 magnification and identified to the genus level. Six PPN genera were identified across the five counties. Busia and Bungoma had the most taxa with 6 and 5 taxa, respectively while Nakuru had the lowest. *Rotylenchus* was present in all the counties followed by *Meloidogyne*, and *Helicotylenchus*. Given the importance of soybean, further studies are needed to identify suitable management for control of these nematodes.

Keywords: Soybean yield, distribution, management

Twenty-one years of free soybean cyst nematode testing and education in Wisconsin

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Soybean cyst nematode (SCN) is the most yield-limiting disease of soybean in the United States. Growers lose 3.5 million metric tons of soybean worth USD$1.5 billion in revenue annually. SCN is a major pest of soybean in the upper midwestern region of the US. SCN is present in 80% of Wisconsin's counties and has potential to spread statewide. Because visual symptoms of SCN are not specific or always notable, soil testing is used to accurately diagnose SCN. The Free SCN Testing Program started in 2002 and is managed by the Agronomy Department at the University of Wisconsin-Madison (UW). Our goal is identifying SCN presence on Wisconsin farms. Growers request testing kits via email, phone, or website (www.coolbean.info). Kits include a welcome letter, soil sampling instructions, a soil sample information form, a sample collection bag, and a mailing envelope. Growers or crop consultants take soil samples and mail them to our partner testing lab. Growers can submit up to four free samples yearly. Samples are analyzed for SCN and other plant-parasitic nematodes. The lab emails results to growers with recommendations for adjusting current management practices. Lab data is shared with UW researchers for further data analysis. Since 2015, almost 12,000 kits were sent to 62 of Wisconsin's 72 counties. Nearly 7000 unique samples were tested since the program's inception. The number of kits requested decreased in recent years, however, an increasing number of new growers request kits every year. The Soybean Checkoff funds this project, and vital education on the importance of SCN and management practices for mitigation is ongoing. Soil samples collected for this program are leveraged by testing for soil-borne pathogens such as *Fusarium* spp. and *Phytophthora* spp. A complete analysis of all grower and kit data over the lifespan of the project will be presented at the conference.

Using cultivar traits and spatial arrangement as strategies to improve weed suppression in soybean

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The need to increase local production of soybean is increasingly becoming important in Europe. Soybean and soybean meal demand remains high in the animal feed industry where the deficit in supply is largely met through imports from outside of the EU. Other than environmental limitations such as temperature and day length, competition from weeds remain a major constraint to soybean production, most especially if grown organically. We hypothesised that cultivars with high trait values for above-ground canopy traits (leaf area index (LAI) and specific leaf area (SLA)) and low red: far-red light ratio are more competitive against weeds than cultivars with low trait value or possess high red: far-red ratio. In addition, we hypothesized that reducing inter-row spacing coupled with high seed density offer optimum conditions for yield. Field grown soybean cultivars were evaluated for two years in three locations with different pedoclimatic zones in Sweden. The soybean cultivars were grown under weedy and weed-free conditions and each treatment was separated by a fallow plot. Crop trait data as well as crop and weed biomass were sampled twice, at crop flowering and maturity. Soybean cultivars with larger LAI values were more competitive and registered lower relative weed biomass (R^2=0.42) while no clear trends were shown for SLA and red: far-red ratio. Ambella and Bilyavka cultivars particularly had more than 50% less weed biomass in the crop field as compared to the fallow plot. For optimum crop performance, a lower seed density (64 seed m^{-2}) and intermediate row spacing (25 cm) offered optimum conditions for crop yield. Using intrinsic crop competitive ability through focusing on competitive traits, combined with creating a conducive micro-environment through spatial arrangement has potential for increasing soybean competitiveness against weeds and yield.

Keywords: Weed suppression, spatial arrangement, above-ground traits, cultivars

Incidence and severity of mustard leaf spot of soybean in Africa

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Soybean production has intensified in Africa due to the growing demand for its use in livestock and human consumption. Diseases and insect pests are a major threat to soybean production. With the expansion of soybean production in Africa, diseases and insect pests are likely to increase and new ones are likely to appear. Here we report the occurrence of an unknown diseases or a disorder that was observed in the Pan African Soybean Variety Trials conducted across Africa. The disease/disorder causes yellowish lesions on leaves. Fourteen trials were established in six countries in the 2019 to 2020 growing season with the number of entries varying between 30 and 40. Disease evaluation in the plots was conducted in the mid to late pod filling stages using a visual severity rating based on a pre-transformed scale from 0 to 5, where 0 = no visible symptoms and 5 = 91 to 100% of the canopy affected. Disease incidence was scored 0 to 1 based on absence or presence, respectively, of the disease in the plot. Data analysis of the ratings for each of the seven locations was determined with JMP Pro Version 13. Mustard leaf spot was observed at eight locations with the incidence and severity ranging from 9 to 76% and 0.1 to 2.2, respectively, on the soybean entries. These symptoms were found at high incidence levels but not at high severity levels. The cause of these symptoms has yet to be identified. This is the first report of these symptoms in Africa, and based on these symptoms, the disorder has been described and named “mustard leaf spot”. There is need to need to conduct further studies to identify the causal organism and how to manage it.

Keywords: Diseases, soybean production, yield

Soybean floral display, pollen production, and yield are impacted by simulated drift rates of auxin herbicides

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Pollinators are imperative for global agricultural production and sustainable ecosystems. Soybean [*Glycine max* (L.) Merr.] flowers have been identified as a source of nectar and pollen for various pollinators. However, soybean is sensitive to auxin mimic herbicide drift (HRAC/WSSA Group 4) that may negatively impact these potential foraging sources. The null hypothesis of this research was that soybean floral characteristics, pollen production, and grain yield would not be impacted by multiple auxin mimic herbicides, simulated herbicide drift rate, nor exposure timing. Greenhouse and field experiments were conducted near Lonoke, Arkansas, USA, using an auxin-susceptible soybean variety. The greenhouse experiment consisted of four auxin mimic herbicides (florpyrauxifen-benzyl, 2,4-D, dicamba, and quinclorac) at two soybean growth stage exposure timings (V4 and R1) with two simulated drift rates (1/100th and 1/1,000th of the label rate). The field experiment consisted of a single exposure timing (V4) with the same auxin mimic herbicides and simulated drift rates as in the aforementioned greenhouse experiment (except only a 1/100th rate of quinclorac was utilized). Nontreated controls were also included in both experiments. Data collection comprised soybean reproductive structure (flowers and pods) counts, floral display size, pollen production, and grain yield. Results showed that dicamba and florpyrauxifen-benzyl were more damaging to soybean than 2,4-D and quinclorac. Soybean floral display size (petal and stamen length) was reduced following exposure to dicamba and florpyrauxifen-benzyl. Following V4 exposure, dicamba and florpyrauxifen-benzyl at 1/100th of their labeled rate reduced reproductive organs by 35 and 39%, pollen grains per anther by 28 and 18%, and grain yield by 38 and 24%, respectively, compared to the nontreated control. In summary, the null hypothesis was rejected. This research illustrates that in addition to yield reduction, simulated drift rates of auxin mimic herbicides can negatively impact pollinator foraging sources by severely diminishing floral and pollen development.

Ectopic expression of a rice triketone dioxygenase gene confers mesotrione tolerance in soybean

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Herbicide-resistant weeds pose a challenge to agriculture and food production. New herbicide tolerance traits in crops will provide farmers with more options to effectively manage weeds. Mesotrione, a selective pre- and post-emergent triketone herbicide used in corn production, controls broadleaf and some annual grass weeds via hydroxyphenylpyruvate dioxygenase (HPPD) inhibition. Recently, the rice HIS1 gene, responsible for native tolerance to the selective triketone herbicide benzobicyclon, was identified. Expression of HIS1 also confers a modest level of mesotrione resistance in rice. Here we report the use of the HIS1 gene to develop a mesotrione tolerance trait in soybean. Conventional soybean is highly sensitive to mesotrione. Ectopic expression of a codon-optimized version of the rice HIS1 gene (TDO) in soybean confers a commercial level of mesotrione tolerance. In TDO transgenic soybean plants, mesotrione is rapidly and locally oxidized into noninhibitory metabolites in leaf tissues directly exposed to the herbicide. These metabolites are further converted into compounds similar to known classes of plant secondary metabolites. This rapid metabolism prevents movement of mesotrione from treated leaves into vulnerable emerging leaves. Minimizing the accumulation of the herbicide in vulnerable emerging leaves protects the function of HPPD and carotenoid biosynthesis more generally while providing tolerance to mesotrione. Mesotrione has a favorable environmental and toxicological profile. The TDO-mediated soybean mesotrione tolerance trait described here provides farmers with a new option to effectively manage difficult-to-control weeds using familiar herbicide chemistry. This trait can also be adapted to other mesotrione-sensitive crops (e.g. cotton) for effective weed management.

Keywords: Soybean, mesotrione, herbicide tolerance trait, HPPD

Biological control of charcoal rot of soybean in Tucumán, Argentina

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Macrophomina phaseolina is the causal agent of charcoal rot on soybean, an economically serious and potentially destructive disease. One of the strategies to manage this pathogen is the use of chemical fungicides as seed treatment. However, chemical control sometimes has limited effects on disease severity and may pose a threat to the environment, so the use of biological products could be an alternative for charcoal rot management. Different species of *Trichoderma* have been recognized as potential biocontrol agents of plant diseases. The objective of this research was to isolate and characterize native *Trichoderma* spp. from different commercial fields in northwestern Argentina and study their antagonistic effect against *M. phaseolina* on soybean. Isolations were performed from soil samples followed by cultural and microbiological characterization of the different native *Trichoderma* spp. isolated. Then, the antagonist effect of these isolates against *M. phaseolina* was studied *in vitro* (dual-culture technique), under greenhouse conditions, and finally under field conditions with pathogen artificial inoculations at planting time during two soybean crop seasons (2019/2020 and 2020/2021). The results demonstrated that isolate Tr009 had a biocontrol effect against *M. phaseolina* on soybean. This control was evidenced *in vitro* by competition for the substrate (percent inhibition of radial growth= 71.3%) and mycoparasitism, as well as by a higher emergency of plants compared to the *M. phaseolina* inoculated control, both under controlled and field conditions. Also, under field conditions, treatments that included *Trichoderma* presented lower disease severity and colony-forming unit index values than the pathogen inoculated control. Moreover, Tr009 improved weight and length of soybean plants under greenhouse conditions. By means of molecular methods, isolate Tr009 was identified as *Trichoderma koningiopsis*. These results indicate that this biological tool can be used against *M. phaseolina* and thus favor a sustainable management of soybean charcoal rot. Further studies should be carried out to evaluate the effects of this biological agent against other economic important crop pathogens.

Keywords: Macrophomina phaseolina, *Trichoderma* spp., disease management, sustainability

Biological control of *Sclerotinia sclerotiorum* on soybean in Catamarca, Argentina

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Sclerotinia stem rot (also known as white mold), caused by *Sclerotinia sclerotiorum*, is an important soybean disease in Argentina, where yield losses of up to 55% have been reported. This soilborne fungus affects numerous plant species and survives as sclerotia, mainly in the soil. The objective of this study was to evaluate different biological products to reduce the viability of sclerotia of *S. sclerotiorum*. A total of five treatments were evaluated during the 2020/2021 soybean season in a field trial located in Los Altos, Catamarca province, Argentina. These included an untreated control and four biocontrol products (based on *Trichoderma koningiopsis*, *Trichoderma harzianum*, *Bacillus subtilis*, and a mixture of *Bacillus subtilis*, *Bacillus amyloliquefaciens* and *Bacillus pumilus*) applied in water with a CO_2 backpack sprayer, equipped with three hollow cone nozzles (TeeJet model TXA 8001VX) on a handheld boom, calibrated to deliver 150 L ha^{-1} at a pressure of 294 kPa at the V8 growth stage. Treatments were arranged in a randomized complete block design with four replications. Plots consisted of four 6-m rows, spaced 0.5 m apart (12 m^2) with plastic trays (25x17x4 cm) placed between rows. Each plastic tray contained 2 kg of sand in which 25 sclerotia with 99% of viability were placed on the surface. The plastic trays were removed 20 days after the application and the viability of the sclerotia analyzed in the laboratory on potato-dextrose-agar medium. The viability of the sclerotia ranged from 19% (*Trichoderma koningiopsis* treatment) to 25% (*Bacillus subtilis* treatment), whereas the untreated control had 99% viability. Sclerotia were colonized by the biological products with values from 69% (*Bacillus subtilis*) to 80% (*Trichoderma koningiopsis*). These results indicate that biological products are effective in reducing the viability of sclerotia and thus could be an effective management strategy to reduce the impact of the initial inoculum of *S. sclerotiorum*.

Keywords: Sclerotinia stem rot, biological products, *Bacillus* spp., *Trichoderma* spp.

Efficacy of single-site and multi-site fungicides mixtures for management of soybean foliar diseases in northwestern Argentina

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Target spot, caused by *Corynespora cassiicola*, and frogeye leaf spot, caused by *Cercospora sojina*, are fungal diseases that reduce soybean yield in Tucumán and other provinces in northwestern Argentina. The objective of this research was to evaluate under field conditions the efficacy of different chemical fungicides for the management of target spot and frogeye leaf spot. A total of 16 treatments were evaluated during the 2020/2021 crop season in a trial located in La Cruz, Tucumán province, Argentina. These included an untreated control, three commercial mixture of single-site fungicides from different chemical groups [strobilurins (QoI) and triazoles (DMI)], three multi-site fungicides (mancozeb, chlorothalonil and cuprous oxide) and nine combinations of them applied at the R3 growth stage. Treatments were arranged in a randomized complete block design with four replications. Plots consisted of four 6-m rows, spaced 0.5 m apart (12 m^2). Severity of target spot and frogeye leaf spot at the R6 stage and yield (kg/ha) at maturity were determined. Environmental conditions during the season were favorable for both crop growth and disease development. Efficacy for target spot control ranged from 21.9% to 61.1% for multi-site fungicides, from 43.8% to 54.9% for mixture of single-site fungicides, and from 34.7% to 80.9% for multi-site + single-site fungicides mixtures. Efficacy for frogeye leaf spot control ranged from 22.9% to 59.3% when multi-site fungicides were evaluated, from 73.8% to 83.6% for mixture of single-site fungicides, and from 68.0% to 89.1% for multi-site + single-site fungicides mixtures. Compared to the untreated control, yield increases for all treatments ranged from 13.9 to 585.4 kg/ha (0.6 to 26.6%). For multi-site fungicides the range was from 13.9 to 133.1 kg/ha (0.6 to 6.1%); for single-site fungicides 344.2 to 404.6 kg/ha (15.7 to 18.4%); and for multi-site + single-site fungicides mixtures 217.2 to 585.4 kg/ha (9.9 to 26.6%).

Keywords: Chemical control, target spot, frogeye leaf spot

Weather factors associated with stress in soybean in northwestern Argentina during the 2021/20022 growing season

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Disease surveys on soybean are carried out every year in nothwestern Argentina (NWA), a region, located between latitudes 22º and 29º South and longitudes 63º and 68º West that includes the subtropical provinces of Tucumán, Salta, Jujuy, Santiago del Estero, and Catamarca. During the 2021/2022 season, these surveys revealed the presence of soybean plants affected by heat canker and sunscald. In December 2021 and January 2022, when soybean plants were in the early vegetative stages, NWA experienced unusually high temperatures combined with scarce precipitations. These conditions induced the occurrence of symptoms such as heat canker, visible in plants at the V1-V3 growth stage, and sunscald, observed at the V2-V8 growth stage. Heat canker was observed as a darken area where the hypocotyl was in contact with the soil surface, indicating cell death due to high temperatures. The root system remained white and looked normal but affected plants often died. Leaves affected by sunburn presented a brown to rusty appearance. Incidence of heat canker and sunburn was recorded in different commercial soybean fields in Tucumán province. In San Agustín, Cruz Alta department, crops presented 10% of plants with heat canker at the V1-V2 growth stage. In Villa Benjamín Araóz, Burruyacu department, heat canker was present in 5% of the soybean plants at the V1-V2 stage. With respect to sunburn, a 70% incidence was determined at V3-V4 in San Agustín, whereas in La Cruz, Burruyacu department, incidence was 5% at V5. Finally, 5% of sunburn was registered on a soybean commercial field in Villa Benjamín Araóz at the R1-R2 stage. Differences in the incidence of heat canker and sunburn could be explained by the environmental conditions recorded in both departments. From 1 December 2021 to 15 January 2022, Cruz Alta presented 148.8 h and Burruyacu 115.8 h of temperature above 35°C, compared to the 10-year average 2011-2021 (91.2 h and 80.2 h, respectively). Also, Cruz Alta presented 85.8 mm of rainfall and Burruyacu 114.8 mm compared to 210.9 mm and 215.3 mm, respectively, for the same 10-year average.

Keywords: Heat canker, sunburn, high temperatures, abiotic disease

XEG1: A case study of microbial attack and plant immunity in the apoplast

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The apoplast constitutes a major interaction niche in plant-microbe interactions. During infection, microbial pathogens secrete a large repertoire of effectors that act in the apoplast to modulate host conditions for infection. Plants respond to microbial attack via secretion of hydrolytic enzymes or perception of conserved molecular patterns or apoplastic effectors using cell surface immune receptors to mount defense. In the arms race with plants for survival, pathogenic microbes evolved sophisticated strategies to modulate plant immunity to favor successful infection. The apoplastic effector XEG1 is a glycoside hydrolase 12 protein secreted by the soybean root rot pathogen *Phytophthora sojae*. XEG1 displays hydrolase activity toward the cell wall component xyloglucan and essential for *Phytophthora* infection. As a countermeasure, soybean secretes the inhibitor GmGIP1, which binds directly to XEG1 and inhibits its hydrolase activity, to increase soybean resistance. *P. sojae* secretes a paralogous PsXEG1-like protein, PsXLP1, with lost enzyme activity. XLP1 binds GmGIP1 more tightly than XEG1, and acts as a decoy protecting XEG1 from the inhibitor GmGIP1. Further analyses revealed that XEG1 can be degraded by host aspartic protease GmAP5 in the apoplast. However, XEG1 undergoes N-glycosylation, which protects XEG1 from GmAP5 degradation. Although XEG1 is a crucial virulence factor, overexpression of XEG1 significantly compromises *P. sojae* infection due to increased plant defense during infection. XEG1 can be recognized by a plant membrane-localized receptor-like protein RXEG1 to mount defense. Moreover, structural basis analyses showed that RXEG1 also acts as an inhibitor towards XEG1 and plays a dual immunogenic role in plant defense. Together, these studies revealed that co-evolutionary arms race tailored the multi-layered defense and counter-defense in plant-microbe interactions.

Response of soybean advanced lines to *Macrophomina phaseolina* in northwestern Argentina

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Charcoal rot, caused by the fungus *Macrophomina phaseolina*, is an economically important disease of soybean worldwide. The use of resistant cultivars provides an effective approach for disease management and can reduce crop losses while supporting a sustainable production system. The objective of this work was to evaluate the response of soybean germplasm lines to *M. phaseolina*. A field test was conducted at San Agustín, Tucumán province, Argentina during the 2020/2021 soybean season. The trial was planted by hand at a density of 23 seeds/m and included five soybean advanced lines from the EEAOC Soybean Breeding Project and the cultivar Munasqa RR as the charcoal rot resistant control. Treatments were arranged in a randomized complete block design with four replications. Plots consisted of four 3-m rows, spaced 0.5 m apart (6 m2). A *M. phaseolina* isolate from Tucumán was used to prepare the inoculum. When soybean seeds were planted, 10 g of rice colonized with *M. phaseolina* was applied by hand per meter of row. At the R7 growth stage, disease severity and colony forming units index (CFUI) were estimated on 10 plants per plot using the methodology by Paris et al. (2006) and Mengistu et al. (2007). The resistant control (Munasqa RR) presented a severity value of 1.45 and a CFUI of 2. The line 57Bs54x19/257 had a severity value of 2.8 (moderately susceptible) and a CFUI value of 92 (susceptible). The line 587Pr*-18/133 presented a severity of 1.9 (moderately resistant) and a CFUI of 37 (moderately susceptible). Line 587Pr@-18/049 presented a severity of 1.6 and a CFUI of 18 (moderately resistant). Lines MD 6210 Mte@18/053 and MD Mte 5825-01 had severity values of 1.3 and 1.9, respectively (moderately resistant), and CFUI values of 2 and 4, respectively (resistant). These results demonstrated that there are some local elite soybean lines that exhibit a good response to charcoal rot.

Keywords: Charcoal rot, susceptible germplasm, moderately resistant germplasm, moderately susceptible germplasm, disease management

Influence of two stink bug species (Heteroptera: *Pentatomidae*) on soybean morphological traits

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Abundance of *Halyomorpha halys* (Stål), the brown marmorated stink bug (BMSB), and *Nezara viridula* (L.), the southern green stink bug (SGSB), after their introduction on the territory of Europe, has been constantly increasing. In order to assess potential damage, a set of trials was conducted in semi-controlled, field conditions in isolation cages, 1 m in diameter and 1.8 m in height, during 2020 and 2021. Testing was done on five different soybean varieties that belong to 00-III maturity groups (Fortuna, Romansa, Princeza, Sava and Senka). There were three types of treatments/cages, a control treatment without stink bugs (C), a treatment with BMSB specimens and a third treatment with SGSB specimens. In each isolation cage with stink bugs five specimens per plant were introduced. The results showed significant effects of stink bugs feeding on soybean plants. In 2020 the number of seeds per plant, average for all five varieties, was the highest in the C (118.9) while BMSB and SGSB treatments were with much less seeds (10.7 and 6.5 respectively). The number of completely damaged seeds was the lowest in the C (4), 11.1 in the SGSB and 26.8 in the BMSB treatment. The seed weight per plant was the highest in the C 27.6 g, in BMSB 3.5 g and the lowest in the SGSB treatment 1.7 g. In 2021 the average number of seeds per plant was the highest in the C (102,2), while 66.5 in BMSB and 58.22 in the SGSB treatment. The number of completely damaged seeds was the lowest in C (1.2), 4.6 in BMSB and 5.4 in the SGSB treatment. The seed weight per plant was the highest in C (19.7 g), in BMSB 11.1 g and the lowest in the SGSB treatment (9.6 g). Significant variations between soybean varieties and years have been observed.

Keywords: Nezara viridula, *Halyomorpha halys*, soybean yield decrease

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Soybean stem canker: molecular markers assisted stacking of Rdm/Rdc genes

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Soybean [*Glycine max* (L.) Merr.] crop is seriously affected worldwide by stem canker (SSC) disease, caused by *Diaporthe aspalathi* (*ex D. phaseolorum var. meridionalis*) (SSC-Da) and *D. caulivora* (SSC-Dc). Both *Diaporthe* spp. coexist in the core productive zone of Argentina and can infect stem and seed tissues. Furthermore, with the increased seed movement between northern and southern states in US and between US and South America, the development of resistant germplasm is determinant to reduce the impact of these pathogens on crop production and seed quality. Thus, the objectives of this work were (i) to analyze the resistance sources to SSC-Da (Rdm genes) and SSC-Dc (Rdc gene) present in the varieties currently sown in Argentina, as well as in germplasm from the University of Arkansas (US), by genotyping with molecular markers (MM) linked to Rdm3, Rdm4, Rdm5 and Rdc1 genes previously identified by our group; (ii) to stack Rdm3, Rdm4-5 and Rdc1 genes conferring resistance to SSC-Da and SSC-Dc, respectively, in order to confer a more durable and broader resistance to both causal agents. The results indicate that 55% of the genotypes have the Rdm4 gene incorporated, less than 5% have the Rdm3 gene, 21% have other alleles and the remaining do not present resistance alleles. Meanwhile, none of the tested genotypes have the MM linked to Rdc1 gene incorporated. These results suggest that most of currently cultivated soybean germplasm has the same resistance source to SSC-Da and does not present resistance to SSC-Dc. Finally, based on these results and assisted by the linked MM, the stacking of Rdm3, Rdm4 and Rdc1 genes in elite germplasm with good agronomic performance is in progress, to be used as donors in breeding programs aimed to incorporate a broader and more durable resistance to SSC.

Keywords: Diaporthe spp., genetic resistance, genotyping, markers-assisted selection (MAS), stacking

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Aggressiveness among isolates of *Diaporthe caulivora*, the main cause of stem canker of soybean in Uruguay

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Soybean is the most important crop in Uruguay. Stem canker caused by *Diaporthe caulivora* is one of the most yield limiting diseases, causing up to 24% yield losses. Management of this disease has been done mainly by breeding for resistance, as fungicides applications have been very erratic. Screening for resistance to *D. caulivora* is done by cutting a superficial slant on the stem, one cm above the cotyledons at stage V2-V3 and placing a 5 mm agar-disc of 5-day old colony. Evolution of lesion length is measured up to 14 days after inoculation. Knowledge of aggressiveness is needed to be able to select the correct isolate for this test. Thus, six isolates were inoculated onto six different cultivars using the above-described methodology. The experiment was carried out in a growth chamber a 21°C, using a complete block design, with four repetitions over time, and one plant per pot as experimental unit. Results indicate that there are statistical differences in aggressiveness between isolates and that there are also differences in susceptibility between cultivars. This type of knowledge is capitalized directly by improving the screening test for stem canker in our soybean breeding program.

Soybean resistance against Asian soybean rust potentiated by Optimus®, a novel-induced resistance booster

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Soybean (*Glycine max*) is one of the most important crops grown worldwide and the fungus *Phakopsora pachyrhizi*, the causal agent of Asian soybean rust (ASR), results in yield losses greater than 50%. Management of ASR has historically relied on intensive fungicide spraying and some cultural practices. Since resistance to most fungicide-active ingredients is a current issue, more environmentally friendly tools deserve research to ensure an increasing food demand. Due to the impact of ASR in decreasing soybean yields, this study hypothesized that an induced resistance booster such as Optimus®, a unique formulation consisting of peptides and phosphite, could enhance the resistance of soybean plants against *P. pachyrhizi* infection. Plants from a cultivar susceptible to ASR were evaluated for ASR severity, chlorophyll a fluorescence, photosynthetic pigments, some key metabolites, as well as differences in selected gene expression. In-vitro assay indicated that Optimus® did not affect the germination of urediniospores of *P. pachyrhizi*. On the other hand, reduced ASR severity, lower malondialdehyde concentration, and less colonization of leaf tissues by *P. pachyrhizi* were noticed in plants treated with Optimus®. Furthermore, higher phenolics and lignin production, as well as a stronger up-regulation of PAL genes, were a common pattern of Optimus® to boost soybean defense against ASR. In addition, plants sprayed with Optimus® showed less impairment of their photosynthetic apparatus and maintained higher concentrations of chlorophyll a+b and carotenoids. Therefore, these results demonstrate the potential of using Optimus® as an effective and defensive strategy in soybean plants to cope with *P. pachyrhizi* infection.

The effect of Gene Rhg5 on the reproduction of *Heterodera glycines* race 6

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Soybean holds great presence in global agriculture. Among several factors that negatively affect crop yield, soybean cyst nematode (*Heterodera glycines*) stands out due to its high damage potential, large dissemination and wide genetic variability. Among the controlling alternatives to this pathogen, the use of resistant cultivars has shown as of the best acceptance with farmers. However, obtaining them is a complex activity since it might involve recessive action genes which are difficult to select. Rhg1 and Rhg4 have been the most used genes. Other genes, such as Rhg2 and Rhg5, have gained attention as possible resistance sources to cover the broad spectrum of races. The objective of this work has been to verify the effect of the Rhg5 gene on the reproduction of *H. glycines*. For this purpose, four segregating populations were obtained from crossing commercial cultivars (resistant x susceptible), which were phenotyped according to their reaction to the soybean cyst race 6 nematode. Each population held 980 plants on average. Five tubes from each plant were sown in greenhouse to perform phenotyping in $F_{2:3}$, totalling a trial with 3,920 plants categorised into 707 resistant plants, 1422 moderately resistant plants and 1791 susceptible plants. Also in $F_{2:3}$ individual plants, plant tissue was collected, as well as DNA extraction was performed. Polymorphic SNPs will then be screened among parents, from the SoyChip 50K, and which are in regions linked to the Rhg5 gene. The genotyping will be done by target-GBS through new generation sequencing in order to correlate the selected marks with the phenotype obtained by grouping haplotypes according to the patterns observed in the parents.

Keywords: Heterodera glycines, genetic resistance, molecular markers

Harmonia axyridis immigration and oviposition in response to variable aphid density on soybean

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Predatory insects can make decisions about where to forage, and also their reproductive allocation based on the available density of prey. In this study we performed a field experiment to assess density-dependent immigration and egg-laying of the ladybeetle *Harmonia axyridis* in response to variable soybean aphid (*Aphis glycines*) densities on soybean, and a greenhouse experiment exposing gravid females to variable aphid densities to quantify oviposition over a short period of time. These experiments allowed us to assess egg laying decisions in response to the immediate environment. We found that greater prey density recruits more *H. axyridis* immigration and oviposition at a greater rate. In addition, gravid females will refrain from oviposition under conditions of low prey density but are willing to oviposit in the presence of higher prey density. These factors contribute to a density-dependent response of this predator which has been shown to help suppress soybean aphid outbreaks in agricultural production.

Physiological and biochemical responses of *Leguminivora glycinivorella* infected with *Beauveria bassiana*

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To clarify the physiological and biochemical responses of *Leguminivora glycinivorella* infected with *Beauveria bassiana*. In this study, *Leguminivora glycinivorella* larvae were infected with *Beauveria bassiana* using the spray method. The strain was TL-1, a highly pathogenic strain obtained from laboratory screening, and the concentration of the spore suspension was 108 spores/ml. The physiological and biochemical changes of the *Leguminivora glycinivorella* infected with *Beauveria bassiana globosa* were studied using uninfected larvae as the control group. The results showed that the water content, glycogen content and protein content of *Leguminivora glycinivorella* larvae in the control group were relatively stable (slightly decreased). The water content of the infected larvae decreased from 49.68% in 3 d to 27.45% in 9 d. The glycogen content decreased from 78.16 µg/mg in 3 d to 48.65 µg/mg in 9 d. The protein content decreased from 96.16 µg/mg in 3 d to 51.25 µg/mg in 9 d. The differences between the three indicators and the control group were significant. The activity of superoxide dismutase (SOD) and catalase (CAT) in the protective enzyme system was inhibited and peroxidase (POD) activity was enhanced after the infestation of *Leguminivora glycinivorella* larvae. The results of the above study can provide a scientific basis for further research on the biological control technology of *Leguminivora glycinivorella* using *Beauveria bassiana*.

Keywords: Leguminivora glycinivorella, *Beauveria bassiana*, protein, glycogen, protective enzymes

Pest control efficacy of insecticides with drone application in soybean against defoliators

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In India, soybean has been the number one oilseed crop in terms of both area and production and has shown unparallel growth over the last few decades in terms of area and production. However, its continuous cultivation with simultaneous increase in area has led to increase in insect pest incidence. Currently, soybean is severely attacked by about a dozen of insect pests. Unmanned Aerial Vehicles (Drone Technology) are a recently developed aerial spraying technology. However, the pesticides control efficacy is unknown. The effect of drone technology in terms of soybean insect pest control efficiency was assessed in the Main Agricultural Research Station, University of Agricultural Sciences, Dharwad (Karnataka-India) during Kharif 2021-22. As a traditional method Air Pressure Knapsack (APK) sprayer was used as a comparison. The insecticide combi product, chlorantraniliprole 9.3% w/w + Lambda-cyhalothrin 4.6% w/w ZC @ 200 ml/ha sprayed with UAV at a spray volume of 20 litres per ha has recorded maximum protection of crop from defoliator insect pests in soybean (100 per cent control of defoliators viz., *Spodoptera litura*, and *Thysanoplusia orichalcea* at 24 hours after spraying) as compared to spraying of same insecticides with same dose with APK sprayer at the spray volume of 500 litres per ha (62.50 per cent control of defoliators at 24 hours after spraying). During the year of investigation (Kharif 2021-22), there was a severe outbreak of *S. litura* in soybean which was successfully managed with the drone application of insecticides with two sprays at a spray interval of 15 days. Further, chlorantraniliprole 9.3% w/w + Lambda cyhalothrin 4.6% w/w ZC at 200ml/ha has recorded highest grain yield of soybean compared to the application of same insecticide at the same dose with APK sprayer. This study clearly suggests the integration of drone technology in the management of insect pests very effectively.

Keywords: Soybean, defoliators, drone, insecticides, management

Effects of temperature on the development and reproduction of *Maconellicoccus hirsutus* (Hemiptera: *Pseudococcidae*)

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Maconellicoccus hirsutus is a new quarantine pest on soybeans in southern China. Understanding the impact of temperature on the developmental period of *A. sinensis* is crucial to predict its population dynamics and likelihood of reaching a stable population. We investigated the influence of temperature on development, survival, and fecundity of *M. hirsutus* in the laboratory by rearing them under a series of temperatures (16, 19, 22, 25, 28, 31, and 34°C, ± 1°C) at relative humidity 70±5% and a photoperiod of 12 L : 12 D. The test results showed that the developmental period of each larval instar of *M. hirsutus* shortened with increasing temperature. At 16°C, the generation development period of female adults was longer than that of male adults, which was 116.00 d. The shortest generation development period was 40.80 d at 34°C. The developmental starting temperature of the female adult generation was 7.64°C, and the effective accumulated temperature was 976.77 degree days. The developmental starting temperature of the male adult generation is 11.48°C, and the effective accumulated temperature is 454.53 degree days. The pre-oviposition and oviposition period, fecundity, and adult lifespan of *M. hirsutus* shortened with the increasing temperature. The number of eggs laid by a single female was the highest at 19°C, reaching 452.45 eggs/female; the lowest at 34°C, only 28.00 eggs/female. The optimal values of net reproductive rate (R0 = 212.54 offspring) and population trend index (I = 92.91) were found at 22°C. Our findings indicate that *M. hirsutus* has the greatest rate of population growth at 22°C, which can provide a scientific basis for predicting the in-field population dynamics of *M. hirsutus*.

Keywords: Maconellicoccus hirsutus, temperature, life table

Exploring the potential of reduced herbicide doses in soybean cultivation with conventional and conservation soil tillage

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Reducing the use of pre-emergence herbicides can help to alleviate environmental concerns and reduce costs. An integrated weed management strategy that focuses on eliminating emerged weeds (POST-EM) instead of applying herbicides before weed emergence (PRE-EM) or using lower doses of PRE-EM herbicides can achieve effective weed control while maintaining crop yield. Field experiments were conducted in 2019 and 2020 to investigate the potential of using reduced doses of PRE-EM herbicides plus POST-EM herbicides or POST-EM herbicides alone for soybean cultivation under different soil tillage. The weed community was mainly composed of annual grass and broadleaved weeds, with *Echinochloa crus-galli* (L.) P. BEAUV being the most prevalent species. Results showed that conventionally tilled plots had significantly fewer weeds and weed biomass, higher herbicide efficacy, and yields. Using a 50% or 75% dose of PRE-EM herbicides, supplemented with POST-EM herbicides on conventionally tilled plots achieved 94-98% efficacy. However, conservation tillage required full doses of PRE-EM herbicides for similar efficacy. Weed biomass was smaller for conventionally tilled soils, with significant differences between control plots (35% difference) and 25% and 100% PRE-EM dose (57% and 90% difference). Grain yields did not differ between 25% and 100% doses of pre-emergence herbicides, regardless of tillage. Nevertheless, grain yield differed significantly between soil tillages, yielding on average 23% more with conventional tillage. Overall, the results suggest that reducing the use of pre-emergence herbicide doses by 50% on conventionally tilled fields can be achieved in some cases without risking weed bank enrichment, resistance emergence, or yield loss. An IWM approach that emphasizes POST-EM herbicides could also minimize environmental concerns associated with PRE-EM herbicides while maintaining crop productivity.

Keywords: Soybeans, herbicides, reduced doses, soil tillage

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A *Faboideae*-specific floral scent betrays seeds to an important pest

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Seed predation is important in mediating the population and community dynamics of plant in ecosystems. Foraging for pre-dispersal seeds is a challenge for granivores, because the reproduction of plant is normally in a short period of time. How seed feeders use a reliable cue to switch host plants has not been understood. Seeds of many legume species are of economic importance by providing highly nutritious plant proteins for humans and livestock. Insect granivore is one of most serious threats to the yield. Here, we found that the bean bug *Riptortus pedestris* invaded soybean and faba bean fields mostly during the flowering period, independent of the cultivars and locations, indicating that flowers may attract them. Chemical analysis revealed that 1-octen-3-ol was the leading component (accounting for about 55 %) of floral scents of five soybean cultivars. By the electrophysiological and behavioral tests in the laboratory or field, we had confirmed that the compound was attractive to *R. pedestris* nymphs and adults. Importantly, 1-octen-3-ol was as attractive as the aggregation pheromone of *R. pedestris* to adults and nymphs, supporting a high potency of the compound. By screening floral scents of a variety of legume species representing different subfamilies, we found that the compound was largely conserved in the *Faboideae* (*Papilionoideae*), in which 1-octen-3-ol was the major constituent in many species. The result suggests that it may help the bug find a range of host species including soybean and faba bean.

Keywords: Stink bugs, population dynamic, chemical ecology, soybean pests, kairomone

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Current research and outlook for soybean pest management in China

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Pest is one of the important factors affecting the yield and quality of cultivated soybean. We have summarised the progress of research on Chinese soybean pests and their management. To date, more than 400 pest species have been identified based on the literature review and the results of a large-scale survey since 2008. More than 20 major pests occur every year and cause serious damage. The pest occurrence and damage characteristics show a variety of regional characteristics in three eco-regions (northern eco-region, Huang-Huai-Hai eco-region and central-southern eco-region). The main factors affecting the occurrence of soybean pests include global climate change and cropping systems. Some new occurrence characteristics presents in cultivation areas across the country. Specifically, the occurrence of soybean pests from north to south was shown as: from large size to small size, from chewing pests to sucking pests, from low reproductive rate and less generation pests to high reproductive rate and more generation pests. The soybean pod borer, *Leguminivora glycinivorella*, and soybean aphid, *Aphis glycines*, have been the most important pests in Northern eco-region. *Riptortus pedestris* and the grubs is the secondary new pest in Huang-Huai-Hai eco-region. The root fly *Delia platura* and *Maconellicoccus hirsutus*, are newly discovered pests on soybean. Soybean pest management in China is mainly based on resistance breeding combined with monitoring, plant quarantine, cultural, biological, physical, mechanical and chemical controls. Although pesticides play an important role in practice, the lack of reasonably use has threatened the sustainability of China's soybean production. Integrated pest management in soybean is not the only strategy, and it still needs to be improved and developed, but it's the best alternative for pest control. IPM is expected to remain the main strategy for soybean pest management for the foreseeable future. Further innovation in green prevention and control technology will also be a focus of future research in view of the new pest problems brought about by crop restructuring, agricultural system change and global climate change. Integration of the above technologies will reduce pest damage and production costs and improve soybean production capacity. Finally, some suggestions have been made to adapt soybean pest management to the current situation.

Impact of cultivar, row spacing, and herbicide combinations on weed control efficacy and yields of soybean

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Narrow row spacings in central European soybean cultivation pose challenges for integrated weed management, relying heavily on herbicide use due to limited mechanical weeding options. To tackle this issue, field trials in 2018-2020 in Slovenia investigated the potential of using soybean genotypes that can increase row spacings and the impact of cultivar and row spacing on herbicide efficacy. Two soybean cultivars, ES Mentor and PR91M10, were tested at 25 cm and 50 cm row spacings, with pre- and post-emergence herbicides S. Pre-emergence (PRE-EM) herbicidal active ingredient S-metolachlor (0.96 l/ha a.i.) and post-emergence (POST-EM) a.i imazamox (28 g/ha a.i.) and bentazone (0.96 l/ha a.i.) were used either as a combination or only POST-EM. The weed community mainly consisted of annual grass and broadleaved weeds. The study observed a significant interaction only between cultivar and row spacing for the number of days to canopy closure. PR91M10 closed rows 6-7 days earlier than ES mentor at 50 cm row spacing, while ES mentor closed canopy 1-2 days earlier at 25 cm row spacing. The herbicide use was the only significant factor in weed control and weed biomass, while weed cover and grain yield differed also between cultivars. The combination of PRE-EM and POST-EM herbicides showed the highest level of weed control (97%), while the efficacy of only POST-EM herbicides was significantly lower (80-85%) due to smaller efficacy on grass weeds. The highest yields were observed with the combination of PRE-EM and POST-EM herbicides. Differences between cultivars in weed competitiveness in control plots were small, with PR91M10 showing slightly less weed biomass, suggesting, that there may not be enough phenotypic variability among early/mid early soybean cultivars to have significant differences. Further research is needed to explore the potential of other soybean cultivars and their interactions with different weed communities and herbicides.

Keywords: Soybeans, weeds, efficacy, herbicides, row spacing

Acknowledgements: The study was co-funded within the framework of the Public Service Program in Agriculture, financed by the Ministry of Agriculture, Forestry, and Food, and the project "Grain Legumes - Cultivation, Processing and Use", which was implemented under the European Innovation Partnership for Agricultural Productivity and Sustainability (EIP-AGRI) action "Cooperation" and funded by the Ministry of Agriculture, Forestry, and Food.

Research progress on green control techniques for soybean pod borer *Leguminivora glycinivorella* (Lepidoptera: *Tortricidae*) in China

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Leguminivora glycinivorella is a major borer pest of soybean in China, Japan, Korea and the Russian Far East. In northern China, the seed damage in the field usually ranges between 5% and 20% but may be greater than 30% for some susceptible cultivars if control measures are not implemented. In recent years, with the concept of sustainable agriculture and green plant protection becoming increasingly popular, relevant scientific and technological workers have carried out research on green control techniques for *L. glycinivorella* from the aspects of pheromones, natural enemies, microorganisms and biological pesticides and have made some new progress. ① Pheromone utilization technology: *L. glycinivorella* sex pheromone has achieved good results in the monitoring and control of the pest, which interferes with the balance of the population sex ratio by trapping and killing male adults and affects the normal reproduction of *L. glycinivorella*. ② Utilization of natural enemy insects: Research has shown that egg parasitoids such as *Trichogramma chilonis* have strong preference and adaptability to *L. glycinivorella* eggs, which have broad prospects for large-scale promotion and application. ③ Utilization of insecticidal fungi: Fungal preparations such as *Beauveria bassiana* can effectively infect the overwintering larvae of *L. glycinivorella*, thereby effectively reducing the overwintering source base. ④ Utilization of transgenic insect-resistant varieties: the promotion of transgenic *L. glycinivorella* resistant soybean varieties cultivated by molecular biological technology will also become a new strategy to control *L. glycinivorella*. According to the law of occurrence and damage of *L. glycinivorella*, combined with the scale of modern agriculture, mechanization, intelligent features, the organic integration of traditional agricultural control methods and modern green control technology, the establishment of production of *L. glycinivorella* integrated green control technology integration model is the inevitable trend of effective prevention and control of *L. glycinivorella* damage.

Keywords: Soybean pod borer, soybean, integrated control

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The influence of weather and climate changes on the occurrence of diseases

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It is known that for the development of a disease, it is necessary to meet three basic prerequisites: the presence of a pathogen, a sensitive host and favorable environmental conditions for pathogen development. One and at the same time the most environmentally acceptable way of fighting pathogens is the creation of resistant or less sensitive hosts (varieties or hybrids) through breeding programs and projects. Where the presence of pathogens has already been recorded and where breeding programs are aimed at suppressing these pathogens, it is to be expected that the problem of the susceptible host has been partially or even completely resolved. But, pathogens are living organisms and, searching for their living space, they necessarily change and adapt to new conditions. We can influence some of them, but what happens if environmental conditions change? That will cause changes in the population of pathogens (emergence of races) or even the appearance of new pathogens in a certain area. Some of these pathogens that were not previously present on certain crops or did not cause economic damage. We have witnessed how the weather and climate have changed significantly in the last few years, and it is increasingly difficult to forecast the weather conditions that producers have to face during the growing season. The intention is to show, on an admittedly small sample and space, the changes in weather conditions that have occurred in the last few years by comparing changes in temperatures and precipitation amounts through multi-year averages. These values have changed significantly and certainly have an impact on the emergence of new or greater economic significance of existing diseases.

Keywords: Pathogens, diseases, resistance, breeding, weather conditions

Efficacy of cyclobutrifluram to control charcoal rot on soybean in northwestern Argentina

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Charcoal rot of soybean, caused by *Macrophomina phaseolina* (Mp), is an economically important disease that affects roots and lower stems worldwide, especially when warm and dry conditions prevail during the growth cycle. The objective of this study was to evaluate in the field the efficacy of different seed treatments to control Mp on soybean in Tucumán, Argentina. The trial was conducted during the 2019/2020 crop season and included artificial inoculation of the pathogen to the soil at planting time (10 g of rice colonized with Mp per meter of row). Seven treatments were arranged in a randomized complete block design with four replications. Plots consisted of four 3-m rows, spaced 0.5 m apart (6 m2). Within the chemical seed treatments, three doses of Syngenta´s new molecule cyclobutrifluram (20, 40 and 80 mL of Victrato® 50 FS/100 kg of seed) were included in mixtures with 100 mL/100 kg of seed of Apron® Maxx RFC (2.5 g fludioxonil + 3.75 g metalaxil-M). Two chemical control treatments, Apron® Maxx RFC (100 ml/100 kg of seed) and Apron® Maxx Advanced (100 ml/100 kg of seed), were also included, along with a non-seed treatment (NST) control and a non-seed treatment Mp inoculated control (NST-Mp). The chemical treatments significantly increased plant emergence at 14, 21 and 28 days after planting compared to the NST-Mp control. Also, the chemical seed treatments resulted in higher canopy percent values than the NST-Mp control. The NST-Mp control presented 2.1 of charcoal rot severity whereas the chemical seed treatments presented values between 1.2 and 1.5 (Paris scale 1 to 5). The colony-forming units per gram were 11,100 in the NST-Mp control and ranged from 700 to 6,150 in the chemical seed treatments. These results indicate that the new molecule cyclobutrifluram, recommended against nematodes and *Fusarium* spp., has also the potential to control charcoal rot of soybean when used as a seed treatment.

Keywords: Macrophomina phaseolina, seed treatment, disease management, chemical treatment

Screening and management of pod borer, *Cydia ptychora* in soybean

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Soybean (*Glycine max* (L.) Merrill) is popularly referred as 'golden bean' or 'miracle crop' is a major oil seed crop with multiple uses as seed contain 20 percent oil and 40 percent protein. In India, it is spread out over a large area of 1.2 m.ha, 12.04 million t of output and productivity of 1,052 kg/ha. In Karnataka, the pod borer *Cydia ptychora* is a major insect pest of soybean that causes significant economic damage. It oviposits on the soybean pods and the larva after hatching bore into the seeds, producing yellowish dry frass and exits through the pods exit hole. It webs within the pods and production of frass will severely reduce food value and germination capacity. Studies on field screening of soybean genotypes against *C. ptychora* was conducted during kharif 2022 at MARS, University of Agricultural Sciences, Dharwad, Karnataka, India. Among 20 screened genotypes four genotypes DLSb 1, DSb 34, KDS 1096 and NR CSL 2 were found resistant (R), six genotypes DLSb 2, DSb 21, MACS 1460, NRC 138, VLS 99 and DSb 23 were categorized as moderately resistant (MR). The correlation studies revealed a significant negative correlation (r = -0.952) between *C. ptychora* incidence and pod wall thickness and positive correlation (r = 0.410) of *C. ptychora* incidence and pod trichome density. Among insecticide molecules, chlorantraniliprole 9.3%+lambda cyhalothrin 4.6% ZC @ 0.4 ml/l was found effective in controlling *C. ptychora* with significantly lower larval population during 3 and 7 days after spraying, which excelled over all other treatments and recorded seed yield of 27.68 q/ha.

Keywords: Screening, *Cydia ptychora*, management

In-vitro evaluation of combination of bio-pesticides against *Spodoptera litura* in soybean

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Soybean is in high demand in the market due to its high protein level and good quality oil, which contains vital fatty acids. It's mostly used for human consumption, cattle feed and industrial applications. This crop is now grown in over 50 countries and is the most widely produced and consumed oil seed crop in the world. Tobacco caterpillar, *Spodoptera litura* (Fab.) (Lepidoptera: *Noctuidae*), was reared under in-vitro condition and the second instar larvae were selected to conduct bio-assay. The larvae were treated with different combinations of microbial bio-pesticides under laboratory conditions. Spore suspensions of conidia were imposed on larvae with topical spray and leaf dip method. Results of the investigation revealed that *Metarhizium rileyi* (2×108 cfu/g) was most effective treatment against *Spodoptera litura* with highest larval mortality (100%) after a week of treatment imposition followed by the treatment *M. rileyi* + *Bacillus thuringiensis var. kurstaki* (Btk). The least pupation (8.34%) was observed in larvae treated with *Beauveria bassiana* (2x108cfu/g). A sequential follow up from this assay was done on the resulting pupae and adults, if any. Further treatment of the resultant pupae caused mortality and adult malformation. The healthy moth emergence and fecundity was least in *B. bassiana* (5%). Thus, the present investigation confirmed the efficacy of different combinations of microbial bio-pesticides against *S. litura* that can be exploited under field conditions while planning for pest control strategies in an eco-friendly manner.

Keywords: Tobacco caterpillar, in-vitro evaluation, microbial bio-pesticides

Predation function of *Episyrphus balteatus* De Geer at different instar larvae to *Aphis glycines* Matsumura

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To determine the predation function of the *Episyrphus balteatus* De Geer on *Aphis glycines* Matsumura, functional response of the of larval of the *E. balteatus* were carried out in the laboratory on all instar. The results show that the functional response of the *E. balteatus* to the predation of *A. glycines* in all instar larval was consistent with the Holling-II type functional response. The disk equations were Na=1.0293N/(1+0.0519N), Na=1.0307N/(1+0.0059N) and Na=1.0016N/(1+0.0028N) respectively. The instantaneous attack rate a' were 1.0293, 1.0307 and 1.0016, and the treatment times were 0.0504d, 0.0057d and 0.0028d respectively. The ability of *E. balteatus* larvae to find *A. glycines* decreases with increasing *A. glycines* densities. The model of the effect of the *E. balteatus* own density on search is consistent with the Hassell model. The results of the trial showed that the *E. balteatus* were more effective in controlling *A. glycines*. Therefore, in soybean production, we can through some operations, such as planting flowering plants around the fields, attracting more *E. balteatus*, a common hoverfly in nature, to the field, and then better to control of the soybean aphid by means of green prevention and control.

Keywords: Episyrphus balteatus, *Aphis glycines*, functional response, searching effects

GmST1-R participates in jasmonic acid mediated resistance to soybean mosaic virus

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Soybean mosaic virus (SMV) is one of the most widespread and devastating viral diseases worldwide. The genetic architecture of the qualitative resistance of soybeans to SMV remains unclear. Here, Rsvg2 locus was identified underlying soybean resistance to mosaic virus by genome wide association and linkage analysis. Fine mapping result showed that soybean resistance to SMV strains G2 and G3 was controlled by a single dominant gene, GmST1, which was located on chromosome 13 and encoded a sulfotransferase (SOT). A key variation at position 506 in the coding region of GmST1 changed the structure of the encoded SOT and changed SOT activity levels between the RSVG2-S and RSVG2-R alleles. In the RSVG2-S allele carrier Hefeng25, the overexpression of GmST1 carrying the RSVG2-R allele from the SMV-resistant line Dongnong93-046 conferred resistance to SMV strains G2 and G3. Compared to Hefeng25, the SMV protein coat gene was downregulated and the SMV load was decreased in transgenic plants carrying the RSVG2-R genotype. SMV infection differentiated both the accumulations of jasmonates and the expression patterns of genes involved in JA signaling, biosynthesis and catabolism in RSVG2-R and RSVG2-S genotype carriers. This characterization of GmST1 suggests a new scenario explaining soybean resistance to SMV.

Effects of different refrigeration temperatures on egg viability of soybean pod borer

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Soybean pod borer *Leguminivora glycinivorella* (Matsumura) is an obligate diapause insect. A large number of well-developed and instar-consistent larvae are needed as test insect sources in scientific experiments. However, normal feeding in the laboratory is difficult to meet the test requirements. In order to solve this problem, we tried to actively regulate the development progress of *L. glycinivorella* by low temperature refrigeration. In this experiment, the eggs of *L. glycinivorella* were stored at 1, 4, 7, 10 and 13 °C for 5, 10, 15, 20 and 25 days after 24, 48 and 72 h of development, respectively. The effects of egg refrigeration on egg hatching and egg growth and development were studied. The results showed that, when other conditions were constant, the hatching rate decreased with the extension of refrigerating time, increased with the increase of refrigerating temperature, and increased with the extension of egg development time. Egg preservation at 1°C and 4°C was only suitable for a short time, and should not be kept for more than 10 days. The hatching rate was higher than 90% at 13°C. When other conditions were constant, the development duration of eggs shortened with the increase of refrigerating temperature. There was no significant difference in the development duration of eggs stored at 1,4, and 7°C, while the development duration of eggs stored at 10 and 13°C decreased with the increase of refrigerating time. The results provide a reference for the refrigerating period, refrigerating temperature and refrigerating time of eggs to produce consistent development of tested *L. glycinivorella* larvae.

Keywords: Leguminivora glycinivorella (Matsumura), egg, low - temperature refrigeration, hatching rate, development

Lignin may decrease phytophthora root and stem rot occurrence on soybean seedlings as a physical barrier?

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Phytophthora root and stem rot (PRSR) caused by *Phytophthora sojae* is a major constraint in soybean (*Glycine max*) production worldwide. Wounding of soybean hypocotyl is the biggest promoting factors. In general, wound healing plays an important role in maintaining the physical barrier; however, little is known on the relationship between soybean and *P. sojae*. PRSR occurrence reduces as inoculation is delayed after wounding by accumulation of some causal substances, lignin or suberin, on the wounded surface. Soybean seedlings were inoculated with *P. sojae* at 0–24 h after mechanical wounding. Mortality rate (MR) was measured at 8 d post-inoculation. Additionally, the lignin and suberin accumulation time was measured to determine the causal substances. The wounding of soybean seedlings was followed by a flooding treatment. The wounded plant part was harvested at 0–192 h after flooding. Through observation of stained cross-sections accumulation ratio (AR) of lignin and suberin was measured. The MR decreased from 94 to 8% as the interval between *P. sojae* inoculation and wounding increased, and MR was not significantly different with that of unwounded at 20 h after wounding (HAW). The AR of lignin and suberin was approximately 0.9 at 48 and 120 HAW, respectively, and lignin was thought as the causal substances relating to the MR decrease. We found that a 20 h period between wounding and inoculation with *P. sojae* was sufficient to protect the seedlings. Lignin deposition could contribute to the decrease in the mortality rate.

Keywords: Lignin, phytophthora stem and root rot (PRSR), *Phytophthora sojae*, soybean, wound healing

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Volatile organic compounds for plant disease management

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Plant responses to pathogens are known to be mediated by volatile organic compounds (VOCs). Here, we investigated VOC emission from soybean roots infected by *Fusarium tucumaniae* (Ft). This pathogen is the main causal agent of sudden death syndrome (SDS) of soybean in Argentina. For plant infection, inoculum was prepared by culturing Ft on potato dextrose agar (PDA) for 1 week at 25°C in the dark. Five agar plugs (6 mm diameter) with mycelium were added to autoclaved sorghum grain and incubated at 25°C in the dark for 2 weeks. Then, a layer of infected sorghum was distributed in pots on top of a nursery substrate and covered with the same substrate. Five susceptible SDS genotype seeds were placed and covered with substrate again. Once foliar symptoms appear, VOCs were identified in soybean infected root segments and in the rhizosphere soil by head space solid phase microextraction coupled to gas chromatography-mass spectrometer and compared with VOC emission from PDA mycelium plugs. Identified VOCs were classified into acids, terpenes, aldehydes, ketones and alcohols. Some chemical compounds differ between pathogen growth conditions. Among these, benzaldehyde was only found in the rhizosphere and in infected roots, thus suggesting a role for this VOC in early plant defense signalling. We then analyzed changes in Ft growth by benzaldehyde. The experiment was performed by growing a Ft plug on PDA at 25°C in dark in the presence of an imbibed filter paper with benzaldehyde on the lid of the plate. Plates were joined using sealing tape and the radii of fungal colonies were measured at different times. We found that benzaldehyde could effectively inhibit fungal growth in a concentration dependent manner. In conclusion, Ft triggers changes in soybean root VOC production highlighting the biological relevance of VOCs as a new strategy for SDS management.

Keywords: Fusarium tucumaniae, sudden death syndrome, benzaldehyde, plant-pathogen interaction, phytopathogen fungi, SPME

Genome analysis of the pathogen causing cercospora leaf blight and purple seed stain of soybean

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Cercospora leaf blight (CLB) and purple seed stain (PSS) are serious concern for soybean cultivation in worldwide countries. One of the well-known CLB causal agents is *Cercospora kikuchii* (Tak. Matsumoto & Tomoy.) M. W. Gardner. Also, some other pathogenic fungi such as *Cercospora cf. sigesbeckiae* and *Cercospora cf. nicotianae* found to be cause of leaf blight in soybean. In addition, another species *Cercospora sojina* causes frogeye leaf spot disease in soybean leaves. To elucidate differences among the soybean pathogens that belong to Cercospora group, we compared genomes of the pathogens. First, chromosome-level genome assembly of *C. kikuchii* was produced. The assembly contains 9 contigs comprising 34.44 Mb. Eight of the nine contigs retain telomeric repeats on both ends. Several pathogenicity related genes such as effector genes, secondary metabolite gene clusters, and carbohydrate-active enzyme (CAZyme) genes were predicted from the assembly. Genome completeness estimated by BUSCO was 99.4% based on the coding proteins predicted from the assembly. Then the assembly was compared with another soybean pathogen *C. sojina*. *Cercospora sojina* has a larger genome (40.12 Mb) than *C. kikuchii*. As a result, these two genomes showed high similarity, but some of the predicted pathogenicity related genes were unique to *C. kikuchii*. Also, number of CAZyme genes in *C. kikuchii* genome was greater than in *C. sojina* genome. The functional validation of the predicted pathogenicity related genes of *C. kikuchii* genome is in progress.

Keywords: Cercospora leaf blight, purple seed stain, *Cercospora kikuchii*, genome

Diseases associated with edamame production in the mid-Atlantic region

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Demand in the United States for edamame or vegetable soybean (*Glycine max* (L.) Merr.) has increased over the past two decades and is the second largest soyfood consumed in the US (25,000-30,000 tons annually). While domestic edamame production is on the increase, at least 70% of edamame consumed in the US is imported from China. Fungal disease is one of the main factors limiting edamame quality and yield in the US. Although foliar, root, and seed soybean diseases have been studied widely, there is little information about diseases specific to edamame pods in the US. The objective of this study was to evaluate and identify edamame pod diseases for the purpose of potentially increasing edamame production in the US. Research trials were conducted at Virginia Tech's Eastern Shore Agricultural Research and Extension Center in Painter in 2018 and 2019 to evaluate advanced edamame breeding lines developed by Virginia Tech and the University of Arkansas. The results showed that despite minor levels of foliar diseases, significant pod disease was noted in certain cultivars, with pod incidence varying from 7% to 62%. Diseased pods exhibited circular, discolored, and sometimes sunken lesions. Fungi were isolated from the diseased pods while no bacteria were recovered and pathogenicity was confirmed by fulfilling Koch's postulates. The isolates were further morphologically and molecularly identified, using the ITS4/5 primer set and sequencing extracted DNA. A total of four causal agents were identified, including *Colletotrichum truncatum*, a number of *Fusarium* spp., *Phomopsis* spp., and *Cercospora kikuchii*. The results contribute towards a better understanding of the diseases on edamame and provide a scientific basis for effective disease management to further increase domestic edamame production.

Keywords: Vegetable soybean, pod disease, mid-Atlantic region

Gene regulation and expression dynamics of maternal effect and early zygotic transcripts in *Riptortus pedestris* (Fabricius, 1775)

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Riptortus pedestris, commonly referred to as bean bug, is an economically important agricultural pest in East Asia. They comprise a group of polyphagous insects that has a strong foraging preference for leguminous crops such as soybeans. Notwithstanding, its molecular and developmental biology is an interesting yet relatively unexplored aspect due in part to its being a non-model organism whose both early and late development starkly contrast that of *Drosophila*. Riptortus is blastokinetic that exhibits a short germband mode of development, while *Drosophila* is non-blastokinetic with a long germband. In the latter context, the onset of embryonic development is generally understood by the precise spatiotemporal expression dynamics of the maternal effect genes in a large syncytium. Furthermore, the anteroposterior and dorsoventral axes specification, and the corresponding segmental fates are committed before gastrulation. Meanwhile, embryonic segments in short germband are progressively committed, and in most cases, it is accompanied by extraembryonic tissue formation through blastokinesis. While maternal effect and zygotic genes have been well characterized in *Drosophila*, it is unclear how homologs especially in species with developmental nuances behave to set up distinct developmental trajectories. It is hypothesized that these nuances are facilitated by distinct gene expression dynamics of homologous maternal effect and early zygotic genes in *Riptortus*. Through comparative analysis, the expression dynamics of maternally and zygotically transcribed genes in Riptortus has been found to exhibit temporal trends different from Drosophila. Motif analysis reveals salient features that are associated with temporal trends of developmental gene expression between the two species of insects – reflecting a possible mechanism by which homologs are differently regulated. Overall, this study presents important findings of insect early development with emphasis on short germband and blastokinesis, features which are absent in Drosophila yet widespread in the Class Insecta.

Keywords: Germband, blastokinesis, maternal effect genes, zygotic genes

Identification and mapping of genetic resources conferring resistance to multiple plant viruses in soybean

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Soybean mosaic disease (SMD) can be caused by a variety of viruses, most of which have been largely overlooked in breeding programs. Effective mitigation of the adverse of SMD might result from breeding cultivars with broad-spectrum resistance. However, reports on broad-spectrum resistance to multiple viral strains have been limited. To catalog viral community members behind SMD, virus samples were collected from symptomatic field plots and pathogenicity of component strains was assessed. Preliminary ELISA and PCR detection revealed that 39.58% and 66.67% of samples contained two or more virus strains, respectively. Only three soybean accessions were completely asymptomatic, while 42% exhibited moderate or severe susceptibility, indicating that co-infection of multiple viral strains remains a significant threat in current soybean production systems. Further, a RIL population consisting of 150 F7:9 strains derived from two soybean genotypes with contrasting reactions to virus infection was constructed and explored for significant markers and resistance genes. QTL analysis returned a reliable locus, named GmRmv, on chromosome 13. Significance of GmRmv in imparting resistance to SMD was further confirmed in NIL lines and delimited into a 157-kb interval that contains 17 annotated genes. Among these genes, three, Glyma.13G190000, Glyma.13G190300 and Glyma.13G190400, each contained LRR domains, as well as, significant variation in coding sequences between resistant and susceptible parents. Hence, these three genes are considered strong candidate genes for explaining GmRmv significance. In summary, this research opens a new avenue for formulating strategies to breed soybean varieties with broad-spectrum resistance to multiple viral strains associated with SMD.

Keywords: Soybean mosaic disease, co-infection, genetic resource, QTL mapping, broad-spectrum resistance

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Are narrow-leaved soybean cultivars much less competitive than broad-leaved to weeds? A comparative analysis of two cultivars

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The commonly cultivated broad-leaved soybean cultivar Abelina in western Europe is deemed more competitive and productive in relation to the narrow-leaved soybean cultivar Antigua. However, the validity of this assumption has not yet been thoroughly researched. Abelina and Antigua cultivars were grown in 2022 in Lower-Saxony, Germany, in three levels of weed pressure: Low, High or No (control); determined by the number of weeds and species, ground cover, and biomass. Soybean growth parameters (height, chlorophyll content (SPAD), ground cover and biomass) and yields were assessed. Multispectral images of the experimental fields have been acquired per UAV flights and will be analyzed to spectrally characterize each cultivar. The study shows that the measured growth parameters for the cultivars were all negatively affected by increased levels of weed pressure, except SPAD values. Two trends were observable. On the one hand, Abelina's parameters were significantly reduced under Low and High weed pressure in comparison to the control, respectively. On the other hand, Antigua's parameters were only significantly reduced under High than under No or Low weed pressure. Compared to the control, Abelina's yield was reduced by 31% and 47% at Low and High weed pressures respectively, whereas Antigua's yield was reduced by 2% and 72%, respectively. Negative correlations between weed ground cover at flowering (BBCH 65) and soybean yield were observed (r = -0.84 for Abelina and r = -0.72 for Antigua). While cultivated under comparable weed pressures, Antigua's yield potential was only significantly reduced under High weed pressure. Under Low weed pressure, Abelina's yield and growth parameters were significantly more affected than Antigua's.

Keywords: Soybean, weed pressure, crop-weed interactions, multispectral data

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A new distinct geminivirus causes soybean stay-green disease

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Soybean is one of the most valuable legume crops in the world with high nutritional value. In recent years, the emergence of soybean stay-green syndrome has caused huge yield losses and become a prominent problem in soybean production in China. The soybean stay-green like disease is also increasing reported in other regions of the world, including North America, posing a serious threat to soybean production. However, the cause of the stay-green disease remains obscure. Here, we report a novel intergeneric recombinant geminivirus, named soybean stay-green associated geminivirus (SoSGV), as the causative agent of SGS by fulfilling Koch's postulate. The epidemiological assessment reveals SoSGV is prevalent in the fields, and it is undergoing geographical expansion and genetic differentiation. Moreover, the mode of transmission of the virus has been studied comprehensively. Our identification of SoSGV as the causal agent of soybean stay-green disease, along with our greater understanding of its epidemiology and transmission, will help to develop effective management strategies to control this prevalent disease.

Keywords: Soybean viral disease, stay-green syndrome

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MicroRNAs regulation in soybean and pest *Riptortus pedestris*

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Food security is a global concern under increase of population and climate change. Soybean is one of the most important crops with abundant nutrients and can serve as the potential candidate for sustainable agriculture. However, various environmental stresses challenge its growth and cause massive loss of yields from both abiotic and biotic perspectives. Non-coding RNAs such as microRNAs (miRNAs), are involved in gene regulation. In this study, miRNA regulation is investigated under salinity stress and its possible involvement in the interaction between soybean and its distinct pest, pod-sucking stinkbug *Riptortus pedestris*. Salinity stress was tested in two soybean accessions, cultivated soybean (C08) and wild soybean (W05) under time-dependent treatment, which aims to disclose miRNA regulation in soybean upon salt stress. In addition, genome of *R. pedestris* was sequenced and assembled, as well as messenger RNA and small RNA transcriptomes to provide resources of *R. pedestris*, followed by seed-feeding experiment to provide its small RNAs and gut microbiota information during feeding. Regarding salinity stress in soybean, small RNA analysis suggested a set of differentially expressed miRNAs and one miRNA arm-switching case, miR166m, in leaf and root tissues. Further investigation on the targets of both arms of miR166m implied the involvement of arm-switching in soybean in response to salinity stress. Moreover, two cases of miRNA:lncRNA interacting pairs (miR166i-5p and lncRNA Gmax_MSTRG.35921.1, and miR394a-3p and lncRNA Gmax_MSTRG.18616.1) were also identified under salinity stress. In addition, one miRNA candidate miR-281 was screened to potentially involve in the interaction between the stinkbug and soybean by targeting the same gene 3-hydroxy-3-methylglutaryl CoA synthase (HMGS) via opposite arms. All findings broaden the regulatory perspectives of non-coding RNAs in soybean under salinity stress, and interaction between soybean and *R. pedestris*, which will benefit soybean breeding in improvement of salt tolerance and agricultural applications in pest management.

Keywords: Soybean, microRNA, salinity stress, *Riptortus pedestris*

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Interaction of AUUB 209 (*Streptomyces enissocaesilis*) and AUDT 626-(*Streptomyces racemochromogenes*) rhizobacteria and *Sclerotium rolfsii* root rot pathogen on relative expression of defence related genes in soybean through qRT-PCR

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The present investigations were carried out to assess the interaction of rhizobacteria and target pathogen in soybean ecosystem at Molecular Genetics Lab, UAS, Dharwad to gain the insights into molecular basis of host-pathogen interaction during 2022. Defence related genes were analysed for their expression levels in response to pathogen (*Sclerotium rolfsii* Sacc.) and rhizobacterial isolates (AUUB 209- *Streptomyces enissocaesilis* and AUDT 626 (*Streptomyces racemochromogenes*) through quantitative real time polymerase chain reaction. A total of five defence related genes viz., Pathogenesis related protein 1 (PR 1), Pathogenesis related protein 2 (PR 2), Pathogenesis related protein 2 (PR 10), Polyphenol oxidase (PPO) and Chalcone synthase (CHS) were selected and analysed for their expression levels under different treatments where rhizobacteria were applied as seed treatment (10g/kg seeds) and drenching at 35-40 days after sowing. The results revealed that the highest expression levels of PR 1 (2.75 fold), PR 2 (7.88 fold), PR 10 (4.16 fold) and PPO (8.50 fold) observed in the treatment (Host + Pathogen + AUUB 209 + AUDT 626) and the highest (3.27 fold) CHS gene expression was recorded in the treatment (Host + AUDT 626). The positive check (Host + Pathogen + *Trichoderma harzianum*) recorded 2.42, 5.74, 4.15, 6.33 and 1.34 fold change of PR 1, PR 2, PR 10, PPO and CHS genes respectively. This is the first report on rhizobacteria and root rot pathogen interaction in soybean in India.

Keywords: Interaction, rhizobacteria, defense genes, tripartite, soybean, qRT-PCR

Soybean – weed competition according to the row distance and orientation

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Canopy structure design is important in integrated weed management programs, improving crop yield in the short-term and reducing weed populations in the long-term. Modifying the row orientation and interrow spacing to increase light interception by the crop, could reduce weed competition through greater shading of weeds in the interrow spaces. Our objective was to evaluate the effect of the interrow distance and row orientation on crop performance. In the experimental field of University of Buenos Aires (34° 35'S; 58° 29'W), a split plot completely randomized factorial experiment with five replicates was established. Factors were interrow distance (17.5 and 52cm), row orientation (North-South and East-West) and weeds (with and without). The cultivar DM40R16 (Don Mario ®) MG IV was planted on December 16th with an experimental drill at a density of 30 seeds/m^2 in a weed free 4 m^2 plot. Once soybean crop emerged, all plots were divided in two subplots: with and without weeds. At harvest, soybean plants were collected, dried, weighed and processed. Number of branches per plant, yield components per plant, main stem and branches, biomass and yield were estimated. The number of branches and pods per plant and pods per branch were higher in East-West than North-South orientation. Total number of grains, grain weight and yield were higher with interrow distance of 0.52 m than 0.17 m. Grain number per pod and crop biomass were not affected by treatments. Weed competition reduced the number of branches and pods per plant, nodes and pods in the main stem and pods in branches, as well as total number of grains, grain weight and yield. Although modifying the crop row orientation or the interrow distances increase the yield and yield components there were no advantages on weed - crop competition.

Keywords: Interrow spacing, canopy structure, integrated weed management

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Competition between different soybean varieties and selected broadleaf invasive weeds

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The goal of our research was to examine the competitive ability of selected soybean varieties and invasive weeds, and opportunity to select the most competitive soybean varieties based on the loss of crop grain yield. Field trials with competition between three soybean varieties (NS Apolo, Fortuna, NS Zmaj) and three invasive weeds (*Abutilon theophrasti*, *Ambrosia artemisiifolia* and *Xanthium strumarium*) were conducted in 2020 and 2021 at location Novi Sad. The additive design with four replications were used. Soybean was sown at a density of 450,000 seeds ha^{-1} and six rows per plot with inter-row spacing of 50 cm. Weeds were sown at the same time with densities of 0.5, 1, 5 and 10 weeds per m^{-1} of soybean row. The three central rows of the plot were used to calculate the soybean grain yield. Lower densities of *X. strumarium* (0.5 and 1 m^{-1}) in NS Apolo variety caused 35 and 69% yield losses, 33 and 68% in Fortuna and 36 and 72% in NS Zmaj. Higher densities (5 and 10 m-1) caused yield losses in NS Apolo 89 and 96%, in Fortuna 90 and 95% and in NS Zmaj variety 94%. At the lowest density of *A. theophrasti* (0.5 m^{-1}), the yield losses in all three varieties were 15, 26 and 16%, while at a density of 1 m^{-1}, yield losses were 53, 55 and 61%. In the treatments with densities of 5 and 10 m^{-1}, yield losses of all three varieties were about 90 and 95%. The lowest density of *A. artemisiifolia* caused yield losses of 17, 21 and 24%, while at a density of 1 m^{-1} a yield losses were 47, 46 and 54%. Densities of 5 and 10 m^{-1} caused similar yield losses of around 85 and 94% for NS Apolo and Fortuna, and 94 and 96% for NS Zmaj.

Keywords: Soybean, competition, *A. theophrasti*, *A. artemisiifolia*, *X. strumarium*

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The influence of different densities of invasive weeds on the dry plant biomass of soybean

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The goal of our research was to examine the competitive ability of selected soybean varieties and invasive weeds, and opportunity to select the most competitive soybean varieties based on their dry plant biomass. Field trials were conducted in 2020 and 2021 at location Novi Sad. Three varieties of soybeans with different morphological traits from the same maturity group (NS Apolo, Fortuna, NS Zmaj) and three very harmful and difficult to control invasive weeds (*Abutilon theophrasti*, *Ambrosia artemisiifolia* and *Xanthium strumarium*) were selected for the tests. The additive design with four replications were used. Soybean was sown at a density of 450,000 seeds ha-1 and six rows per plot with inter-row spacing of 50 cm. After soybean sowing, all weeds were sowed on the same time in four densities by hand (0.5, 1, 5 and 10 weeds per m^{-1}). Average dry biomass of aboveground part of plants was obtained 8 and 12 weeks after soybean emergence, from a sample of plants from 1m^{-1} of the fifth row after drying to constant weight and compared with weed free plots. In both years, the variety in which weeds at all densities had the least effect on the reduction of dry biomass was Fortuna. In 2020 and 12 weeks after Fortuna variety emergence, *A. theophrasti* caused a decrease of dry biomass by 7, 23, 50 and 62%, *A. artemisiifolia* decreased it by 18, 21, 49 and 56%, while *X. strumarium* affected a decrease of 15, 41, 70 and 74 %. In 2021 with dry and unfavorable weather for soybean production, *A. theophrasti* with four densities caused 9, 7, 33 and 52% reduction of Fortuna, while *A. artemisiifolia* contributed with 21, 24, 45 and 63%. In the same year, *X. strumarium* caused the least reduction of dry biomass in NS Zmaj variety (19, 39, 68 and 71%).

Keywords: Soybean, weeds, *A. theophrasti*, *A. artemisiifolia*, *X. strumarium*

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Evaluation of soybean germplasm and identification of resistance loci associated with *Phytophthora sojae* and *Pseudomonas amygdali pv. tabaci* in soybean

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The evaluation of soybean germplasm plays a pivotal role in breeding to enhance resistance to biotic stresses. Phytophthora root and stem rot by *Phytophthora sojae* (PRSR) and wildfire disease (WFD) by *Pseudomonas amygdali pv. tabaci* are widely occurring in soybean fields in South Korea and major soybean growing countries. In this study, 983 soybean germplasm was evaluated for resistance to *P. sojae* and 254, 21, and 708 genotypes were determined as resistant, intermediate, and susceptible, respectively, based on hypocotyl inoculation technique. Resistance to WFD was tested using spraying of bacterial suspension and, of 762 accessions, 81, 128, 360, 142 and 51 genotypes were scored as disease index of 1-5, respectively. Using these phenotypic data and 180K SNP data, genome-wide association analyses were conducted to identify genomic regions associated with resistance to for the two pathogens. Tens of SNPs were significantly associated with resistance to *P. sojae* on 6 chromosomes (Chrs.), including Chrs. 1, 3, 9, 10, 16, and 18. The most significant SNPs were detected on Chr. 3, which is a well-known Rps gene region. Genomic regions on 6 chromosomes were identified significantly associated with resistance to *P. amygdali pv. tabaci*. Detailed results will be presented in the poster. This will provide useful information for further research and breeding for resistance to *P. sojae* and *P. amygdali pv. tabaci* in soybean.

Keywords: Germplasm, phytophthora root and stem rot, wildfire disease, GWAS

Mapping quantitative disease resistance loci conferring resistance to multiple *Pythium* species in two soybean populations

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Pythium species, an oomycete pathogen, are associated with seedling diseases in a range of host crops including soybean. In a previous study, new sources of resistance to *Pythium sylvaticum*, *Pythium irregulare* and *Pythium torulosum* were identified. In this study, two advanced generation recombinant inbred line (RIL) populations of PI 408029 (resistant) x Williams (susceptible) and PI 408097 (resistant) x Williams (susceptible) with population sizes of 198 and 307 individuals, respectively, were used for fine mapping. These populations were screened using a modified seed rot assay. Genetic maps for these RIL populations were constructed and expanded using JoinMap 4.0 and over 2500 markers were mapped in each population. The population of PI 408029 x Williams had two sizable effect quantitative disease resistance loci (QDRL) conferring resistance to two different species and a minor QDRL conferring resistance. The population of PI 408097 x Williams identified one large effect QDRL conferring resistance to three different *Pythium* species. The results from this study should be useful in marker-assisted breeding and development of *Pythium*-resistant cultivars.

Keywords: Pythium, quantitative disease resistance loci, recombinant inbred line population, resistance

Functional divergence of a glycoside hydrolase and its decoy partner in *Phytophthora* evolutionary continuum

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Plants and pathogens constantly adapt to each other. In turn, the adaptation pressures impose on pathogens shape their variations and the fitness towards corresponding hosts. Our study shows that the XEG1/XLP1 gene pair originated from *Phytophthora*, and they had functioned differently through the evolution of *Phytophthora*, which is caused by the dependence of *Phytophthora* on its virulence function. Based on the distribution, phylogeny, collinearity, and gene arrangement, it appears that the XEG1/XLP1 gene pair originated with the *Phytophthora*. As well, different *Phytophthora* XEG1/XLP1 paralogs exhibit different abilities to induce cell death, induce the expression of defense genes, and produce reducing sugars. Actually, XEG1/XLP1 ancestor genes produced reducing sugars less efficiently and induced immunity less effectively. A further analysis revealed that XEG1/XLP1 displayed different selection pressures, and these pressure sites were crucial to its ability to produce reducing sugars and induce immune. Meanwhile, the replacement experiments with ancestral genes in *P. sojae* indicate XEG1/XLP1 virulence in soybean is closely related to its ability to produce reducing sugar, and as virulence increases, immune activation increases as well. Overall, the results show that XEG1 and its decoy partner XLP1 continuously adapted to their hosts through functional divergence during *Phytophthora* evolution.

Keywords: Phytophthora, soybean, divergence, selection pressure, ancestor genes

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Glufosinate-resistant Palmer amaranth: occurrence and resistance mechanism

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Palmer amaranth is the most troublesome weed of U.S. soybean production, partly because of its propensity to evolve resistance to herbicides. This research aimed to develop an understanding of the sensitivity of Palmer amaranth accessions to glufosinate across U.S. soybean production regions and report on the recently identified glufosinate resistance mechanism. Our hypothesis was that sensitivity of Palmer amaranth to glufosinate was decreasing over time. Each year since 2018, Palmer amaranth plants with reduced sensitivity to glufosinate from U.S. crop production fields, mainly soybean, have been identified. From these field collections, glufosinate-resistant Palmer amaranth was confirmed in Arkansas in 2020, making it the first broadleaf weed resistant to glufosinate. In the region where resistance was confirmed, glufosinate has been a commonly used option to control Palmer amaranth in soybean and cotton crops. The three identified resistant accessions varied in resistance levels to glufosinate, with LD50 values of 5.1 to 27.4 compared to two susceptible standards. The region where resistance was confirmed had historically been an early adopter of glufosinate-resistant soybean and cotton, with some fields receiving 10 consecutive years of multiple in-season glufosinate applications prior to resistance confirmation. No target-site mutations in glutamine synthetase were found in any of the resistant accessions, whereas amplification of the chloroplastic glutamine synthetase (GS2) enzyme is the mechanism in the most highly resistant accession. This accession had > 80 gene copies and 15- to 31-fold increased gene expression of GS2 compared to susceptible plants. Our group is the first to explain the resistance mechanism in glufosinate-resistant Palmer amaranth. Additionally, the use cytochrome P450 and glutathione-S-transferase inhibitors to improve glufosinate performance on the other resistant accessions is under evaluation. With glyphosate-resistant Palmer amaranth found in all major U.S. soybean-producing states and glufosinate enabled on an estimated 85% or more of the U.S. soybean acreage, efforts must focus on diversifying weed control programs if the later herbicide is to remain an effective option.

Keywords: Herbicide resistance, glufosinate, Palmer amaranth, resistance mechanism

Acknowledgements: Funding for this research was provided by the Arkansas Soybean Research and Promotion Board and BASF.

The green stem and foliar retention syndrome caused by *Aphelenchoides besseyi* in the Brazilian soybean crop

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The incidence of the aerial nematode *Aphelenchoides besseyi* in soybeans was discovered and described in 2017, causing soybean yield reductions of up to 60%. Its occurrence restricts to regions of hot and rainy climate in central-north Brazil, with an estimated area of 5.2 million ha. The initial symptoms are usually observed from the plant flowering on, causing leaf distortion and curling, deformations in the stems, and thickening of the nodes. Angular necrotic lesions appear on the leaves. Floral racemes become necrotic, reducing pod production. The pods remain green, deformed, and with necrotic spots. Grains produced from infected plants do not mature and often rot. The plants remain green after the end of the crop cycle, showing a green stem and leaf retention. Frequent rains favor the infection of plants by *A. besseyi*, which occurs in the vegetative stage. The nematode can penetrate the roots and move up internally or externally in the plant. The main sites of nematode multiplication are floral racemes and young leaves. The nematode can survive in the soil as a mycophagous or by anhydrobiosis housed in crop debris. Other important host crops are cotton (*Gossypium hirsutum*), common bean (*Phaseolus vulgaris*), cowpea (*Vigna unguiculata*), and chickpea (*Cicer arietinum*). Host weeds are *Commelina benghalensis*, *Amaranthus viridis*, *Synedrellopsis grisebachii*, and *Leonotis nepetifolia*. The most effective control measures known so far are regarding the cultural management of crops, such as early desiccation before soybean sowing and early control of host weeds, and the planning of succession of non-host crops in the soybean production system. Corn, as the second crop, after soybean, shows positive results in *A. besseyi* control. Sources of genetic resistance are not yet known. Chemical and biological control are feasible, and product efficiency is still under evaluation.

Keywords: Aerial nematode, soja louca II, *Glycine max*

Two decades of Asian soybean rust in Brazil

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From its first report in 2001, Asian soybean rust (ASR), caused by *Phakopsora pachyrhizi*, became one of the most important diseases of the crop in Brazil, with an annual mean control cost of USD 1.8 billion. Brazilian environmental conditions are conducive to the year-round survival of the fungus, mainly in volunteer plants during the intercropping seasons. Disease management bases itself on regulatory measures, chemical control, and genetic resistance of cultivars. Abiding by the regulatory laws, which establish a soybean-free period for each Brazilian state, is the most effective action to reduce the inoculum by eliminating volunteer plants between seasons and avoiding late-season and off-season soybean cropping. The escape strategy by sowing earlier maturity group cultivars at the beginning of the crop season is also effective. A national network of field trials monitors and compares the efficiency of widely used fungicides for managing ASR, in different producing regions, since 2004. Besides the fungicides' efficiency, the results allowed monitoring the changes of sensitivity of the pathogen to the different modes of action over the years, along with bioassays and molecular analyzes. Reduction in fungicide efficiency was reported for the demethylation inhibitors (DMIs) in 2007, for the quinone outside inhibitors (QoIs) in 2013, and for the succinate dehydrogenase inhibitors (SDHIs) in 2016. Due to the lower sensitivity to single-site fungicides, the use of multi-site fungicides (mancozeb, chlorothalonil, and copper) increased in Brazil. Resistant cultivars with Rpp genes have been developed but are unavailable in all producing regions. Fungicides are recommended in resistant cultivars to protect the Rpp genes and for other diseases control. All the available management strategies adopted together kept the disease under control in the past years and the severity has varied yearly according to environmental favorability and sowing date.

Theme E:
Business, legal, and corporate social responsibility (CSR)

Session *(chair)*

E1 Policy and the regulatory environment *(Donal Murphy-Bokern)*

Science and technology provides opportunities but the interaction between public and business policies are crucial to innovation. This is especially true of the soybean. The aim of this session is to foster dialogue between experts in the natural sciences, policy, and business to find ways forward in governance that advance the contribution of soybean to sustainable development.

WSRC11 is taking place at a time when there is great focus on links between land-use change and food production. Breaking these links is essential to the sustainable development of all food value chains, especially those that use soya. The session will focus on decoupling soya production from tropical and sub-tropical deforestation and conversion. This involves both public and private policy action. On the public side, the recently developed European Union’s Deforestation Regulation provides a very relevant policy backdrop to a discussion that is relevant across the world. On the private side, corporate social responsibility (CSR) in the food industry is an important driver. We have four presentations from four experts who have devoted much of their professional lives to decoupling our food production and consumption from deforestation and conversion of natural ecosystems, especially through the development of sustainable value chains. They will share their insights as background to a workshop-style discussion that all participants can contribute to (organized within the Eat4Change Project).

The European Union’s Deforestation Regulation and soya

Lieven Callewaert[1,2]

[1]*Collaborative Soy Initiative, Amsterdam, The Netherlands,* [2]*Round Table on Responsible Soy*

E1: Policy and the regulatory environment

This talk will reflect on the European Union’s Deforestation Regulation (EUDR) and its implications for the global soya industry. The EUDR will come into force in 2023. It seeks to prevent companies from placing commodities linked with deforestation and forest degradation onto the EU market. Soya is amongst the commodities covered by EUDR. This talk will discuss the impact this regulation will have on the global soya industry, and the potential implications for producers, importers, and exporters. It will also provide insights into the enforcement, including how authorities will track and monitor imports into the European Union. Finally, this talk will explore some critical aspects from a broader holistic perspective considering the risk of hindering sustainable development in particular.

How to stop deforestation properly

Ursula Bittner[1]

[1]*Greenpeace, Vienna, Austria*

E1: Policy and the regulatory environment

Europe's consumption of soya and palm oil links the European economy to deforestation in exporting countries. The European Union seeks to reduce its impact on deforestation and forest degradation. This presentation will elaborate on why the EU regulation on deforestation-free supply chains is a milestone on the way towards proper regulation of imports to protect forests. Why private certification schemes are an insufficient tool to protect our forests, ecosystems and human rights will be explained drawing on a report published by Greenpeace in 2021. Continued deforestation and conversion of other natural or semi-natural habitats is clear evidence that (mainly voluntary) certification schemes for land-based commodities alone are not adequate as instruments to address deforestation, forest degradation and associated human rights abuses, e.g. violations of indigenous rights. Some certification schemes have had a positive impact locally. However, certification has inherent flaws in its functionality and therefore is an insufficient tool to address global forest and ecosystem destruction. Regardless of the effectiveness of the certification schemes, checking each label, its promises, and whether or not it can be trusted requires a lot of personal knowledge and research on the part of consumers. Therefore, governments need to develop policies that more effectively and directly protect people and the planet. Governments and business owners have to take deforestation and human rights abuses seriously and implement effective tools to avoid them.

Deforestation and conversion-free soy supply chains

Guillaume Tessier[1]

[1] *Global Soy Lead - WWF Brazil, Brasilia, Brazil*

E1: Policy and the regulatory environment

Unsustainable soya supply chains continue to contribute to high carbon emissions, habitat destruction, biodiversity decline and human right violation in multiple biomes around the world. For instance, land conversion for soybean is responsible for 5 to 14% of food system land use change emissions. This WWF presentation will show through recent data analysis the impact of the rapid expansion of soybean production – due to high demand - on nature and people in Brazil. This will serve to highlight why deforestation and conversion-free soya supply chains need to be achieved by now. Then this talk will address how companies can implement the Accountability Framework guidance for setting an ethical supply chain and what are the existing solutions for a quick implementation, such as traceability systems combined with robust monitoring, reporting and verification systems.

Keywords: Deforestation and conversion of other ecosystems, greenhouse gas emissions, traceability and transparency, international regulations

Acknowledgements: This session is organized within the Eat4Change Project.

From shelf to field: why retailers are critical partners in the delivery of deforestation free soy

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E1: Policy and the regulatory environment

Deforestation from soy production is linked to a relatively small share of global demand. Despite the vast majority of soya is embedded in retail supply chains through its use in animal feed, a growing interest in action from shareholders and stakeholders has rapidly raised the profile of soya as the primary forest-risk commodity that requires an urgent response. However, its indirect role in our food system through feed creates unique challenges in a market where the trade in soya is dominated by a handful of companies further upstream. For the past five years, retailers have been working together to harmonise the demands that are transmitted up their supply chain to provide an aligned consistent requirement for livestock producers and feed mills in transitioning to a market where deforestation-free soya is the market norm. In April 2023 these efforts were bolstered through the adoption of the EU's new Deforestation Regulation, but this, on its own, will not deliver the change we need to see. This talk will highlight the progress being made in retail supply chains and review the components of policies that have been successful in shifting supply chains. It will cover the voluntary, and now minimum, requirements to illustrate what the roadmap of the next two years will look like.

Keywords: Deforestation, certification, supply chain, stakeholders

F: Other themes

Harnessing wild diversity and omics to meet global challenges

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Climate change poses critical challenges for natural diversity, agriculture and environmental sustainability, and food security. The global COVID-19 pandemic has revealed a crucial need for strategies to boost the human body's immune responses. Compared to crop cultivars, crop wild relatives harbor much higher genetic diversity. They can adapt to diverse habitats, and some even thrive in harsh environments. Thus, crop wild relatives exhibit unleashed potential to meet global challenges. In this talk, I will discuss our recent research on the applications of wild legume species to understand the genetic basis of broad resistance to pests and phytochemical diversity in natural populations by leveraging interdisciplinary omics tools.

Keywords: Climate change, human health, pest resistance, abiotic stress, crop wild relatives

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Using soybeans to help K-12 instructors teach environmental science and biology concepts

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Examples of STEM within agriculture are numerous and can range from concepts that relate to genetic expression and cellular biology to ecosystems and environmental science. Many modern farmers also rely heavily on engineering-based and statistical principles, such as using global positioning system data, developing variable rate application maps using statistical interpolation methods, and executing the prescribed map through variable rate technologies. Some efforts have been made to teach elementary and middle school students about agricultural systems, but one major limitation to further incorporation was a lack of instructional resources available. Another potential barrier for incorporating agricultural examples is the knowledge level of the instructor. Improving teacher knowledge of agricultural processes may be one mechanism to improve self-efficacy and increase adoption of novel materials in classrooms. Three professional development workshops addressing Next Generation Science Learning Standards were offered in 2021-2022 (virtual) and 2022-2023 (in-person) for teachers highlighting ways to use soybeans, and other agricultural crops, to teach environmental science, ecology and cellular biology concepts in classrooms. Each workshop presented two to three novel curriculum modules that teachers could use in their classrooms with IRB-approved surveys used to collect feedback. Sessions in 2022-2023 also included tours of research facilities. To date, 54 teachers have registered for these workshops and the material-hosting website garnered 167 users and >3,900 page views in 2022. Initial survey respondents reported teaching 185 students/year on average. Teacher scores on knowledge-based questions in the first year all increased from the pre-test (taken 0-24hr prior to the session) to the post-test (taken 7 days after the session). Teachers also expressed increased likelihood to incorporate agriculturally-related activities in their teaching in the future. Given 98% of students in the United States take biology, inclusion of curricula using soybeans may better expose students from non-farming communities to agricultural concepts.

Keywords: K-12 curriculum, instructional methods, professional development

Effect of plant growth regulator treatment on isoflavones in soybean

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The soybean (*Glycine max* (L.) Merrill), an important food crop in the world, is popular because of its high quality protein and oil content. Soybeans as a food have long been known for their beneficial effects on health and are well-recognized globally. Isoflavones, significant soybean secondary metabolic products, may be crucial in avoiding some cancers and lowering the risk of cardiovascular disorders. This study investigates the correlation between plant growth regulator and the effect on the isoflavone levels in soybean leaves. The study was carried out in the green-house of the southern crop department in miryang. Soybeans (Seonpung) were cultivated in 1/2000 of the Wagner pot. Ethephon (500, 1000, 2000 ppm) and ABA (100, 200, 400 ppm) were used as plant growth regulators, and they were each treated on R2, R5, and R7 stage. After treatment, leaves were sampled three times at intervals of 5 days, and the content of 6 isoflavones and coumestrol was analyzed. Soybean isoflavones were analyzed using ultra performance liquid chromatography (Acquity UPLC H-Class system, Waters). The isoflavones content showed an overall highly in the R5 stage, and the level was similar to that of no treatment in the R2 and R7 stage. The difference between the growth regulators was found to be higher than that of ethephon when ABA was treated. The coumestrol content was confirmed to be high in the order of R7, R5, and R2 on the treatment time, and it was found that the content increased as the treatment time was delayed. In the treatment with the growth regulator, the coumestrol content tended to be higher when ethephon was treated than ABA.

Keywords: Soybeans, cultivation, isoflavones

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1000 Gardens: citizen science to investigate adaptation of soybean across Germany

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Relative to the imports, soybean production in Germany is very low [1]. Due to favourable environmental conditions (e.g. temperature and rainfall patterns), the main cultivation areas in Germany lie in the two southern states Baden-Württemberg and Bavaria [2,3]. In order to expand soybean production, cultivars adapted to other regions are needed. In conventional multi-environment trials, the number of environments is limited and cannot possibly cover the whole country. A citizen science approach, however, can be utilised to greatly increase the number of environments in which the genotypes are tested. Citizen science experiments involve untrained citizens in the research process, especially data collection [4]. Our citizen science project '1000 gardens' can further be viewed as a tool to educate the public on scientific work in general and on soybean in particular [5]. We performed two cycles of '1,000 gardens' in 2016 and 2018, where ultimately 1731 and 912 citizen scientists, respectively, contributed data. In 2016, 1,736 genotypes, in 2018, 1,020 genotypes were evaluated for several traits, including flowering time and maturity. In this experiment several genotypes could successfully mature in many regions across the whole country in both years. A clear and consistent regional pattern could not be observed. However, in both years, maturity was reached slightly earlier in the South of Germany. Heritabilities were promising for flowering time in 2018 (0.60) and time to maturity in 2016 (0.56) and 2018 (0.70). Due to the unique structure of the experiment we were able to evaluate data for the majority of regions in Germany, covering a variety of growing conditions. We could observe that many regions at all latitudes show potential for soybean cultivation. Most importantly, supported by the high values for heritability, we found the data obtained by citizen scientists to be sufficient to evaluate the adaptation of soybean in Germany.

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Economic analysis of organic soybean cultivation in Luxembourg, based on a site-specific scenario analysis

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Soybean is one of the most important protein crops worldwide due to its high protein content and favorable composition of essential amino acids. In Luxembourg, the area of soybean cultivation is currently less than 20 ha per year, while at the same time an estimated 27,500 tonnes of soybean are imported (Zimmer et al., 2021). Growing indigenous soy is only an alternative if it is economically viable. A scenario analysis of 2019 to 2021 was conducted considering local conditions. Variable machinery costs were based on the rates of the Luxembourg Machinery Ring (MBR). The degree of own mechanisation corresponds to an average organic farm. The prices for drying and cleaning correspond to the local prices of 2021. The soybean selling price is based on the price paid in Luxembourg in 2021. At a selling price of 800 €/t, for soy for feed and the monetary crediting of the N subsequent delivery, the soy crop achieves a positive contribution margin from a yield of 1.5 t/ha. However, the crop only becomes interesting for cultivation from yields of 2.0 t/ha upwards. Assuming that the selling price of organic soybeans will remain at a high level in the future, increasing yields and stabilizing them is the first priority in order to increase profitability. Weed-free stands and adapted varieties are essential. The reduction of cleaning and drying costs as well as the establishment of a local processing structure are also key factors.

Keywords: Soybean cultivation, economic analysis, protein self-sufficiency, grain legumes

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Changes in nodule bacterial community may determine soybean symbiotic nitrogen fixation under elevated CO_2

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The global increase in atmospheric CO_2 concentration (eCO_2) is a source of great concern, not only because of its influence on climate but also its 'fertilisation effect' on the growth of C_3 plants in agriculture. Leguminous plants have a growth advantage under eCO_2 compared with other types of C_3 plants due to the mitigation of N deficiency through the stimulation of symbiotic N_2 fixation (SNF) under eCO_2. The potential link between elevated CO_2-induced stimulation of N_2 fixation of different soybean cultivars and nodule bacterial communities was investigated. A typical legume crop, soybean, was used as the test material in an open top chamber experiment conducted under ambient CO_2 levels (410 ppm) and elevated CO_2 levels (550 ppm). Locked nucleic acid oligonucleotide-PCR clamping with Illumina MiSeq sequencing targeting 16S rRNA genes was conducted. The results showed that eCO_2 significantly affected Sobs, Shannon and Chao indices, while Ace diversity was significantly influenced by cultivar. *Ensifer* and *Bradyrhizobium* were the most abundant genera in nodule bacterial communities across all soybean cultivars. *Propionibacterium* and *Enterococcus* were both present in lower abundance. Soybean nodule bacterial communities were affected more by soybean cultivar identity than by eCO_2. There were also significant correlations between the dominant operational taxonomic units (OTU) with SNF% (percentage of symbiotically fixed N content to total N), which partially explained the evident differences in symbiotic N_2 fixation under eCO_2 even within the same legume species. This study also underpins future efforts to improve the contribution of SNF by manipulating nodule bacterial communities.

Keywords: Climate change, N cycling, soybean, rhizobia

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Argentina's soybean meal: a threat or an opportunity in the sight?

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United States is steeply increasing its oilseed crushing capacity for its growing soybean oil needs, with an installed capacity expected to increase by 28% over the 2022-2026 period. Projected with consumption trends, which aspire to grow but to a lesser extent, United States could increase its exportable supply of soybean meal by close to 50% in the next five years, due to the joint production of soybean meal and oil. Projections of global consumption of soybean meal are also showing growth at a rate below the US production-increase plans. In this sense, a potential global oversupply of soybean meal could lead to falls in the international prices of this product. This situation will have a direct effect on Argentine foreign trade since soybean meal is its main exportation product. Within this framework, potential alternatives are developed for the use of soybean meal in Argentina. We quantify scenarios for the improvement of the foreign trade of bovine meat, poultry meat, dairy products and even pork meat, with the potential to reverse the Argentine pork trade deficit. We conclude that the negative effects of the situation at the US can be lessen. In fact, this situation represents a huge opportunity to diversify the Argentine export basket.

Keywords: Agribusiness, agricultural and food policy, soymeal exports, international trade

Advances in crop and weed management of edamame grown in the United States

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Edamame, the vegetable version of soybean, is a nutraceutical food with strong growth among U.S. consumers; however, until recent years nearly all edamame consumed within the U.S. was imported from East Asia. To capitalize on a growing market and ensure traceability, the U.S. vegetable industry wanted to grow edamame domestically, yet basic agronomic practices were undefined and weed interference was a major problem. Prior to 2010, a single herbicide was registered. Since then, USDA-ARS research 1) facilitated the registration of 11 herbicide active ingredients from eight site-of-action groups, 2) developed non-chemical weed management tactics, 3) identified cultivars most promising for Midwest production, and 4) developed contemporary planting guidelines suitable for machine-harvested edamame. Moreover, three additional herbicides are being considered for registration. Much of this work was coordinated with the U.S. Environmental Protection Agency, the IR-4 Project, and numerous academic and industry partners. Despite these advances, challenges remain to U.S. grown edamame. While edamame is being produced in several regions of the country, adoption of improved cultivars and refinement of weed management systems are likely to vary among and within regions. Consumer demand for organic products is rising; however, economical, efficacious weed management in organic production systems are poorly developed. Finally, risk of crop injury from dicamba use patterns in dicamba-tolerant soybean is of concern in the edamame supply chain. Nonetheless, rising consumer demand for edamame globally will facilitate additional innovation in crop and weed management.

Keywords: Edamame, immature seed, maodou, mukimame, vegetable soybean

Jacquin and Haberlandt: milestones of Austrian soybean history

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In Europe, soybean is known for roughly 300 years only when it was first described and depicted by the German physician and botanist Engelbert Kaempfer. In Austria, the Dutch-Austrian scientist Nikolaus Joseph Freiherr von Jacquin (1727-1817) delivered a first botanical description of the soybean plant in a publication dated 1786 which was accompanied by a book mainly consisting of plant illustrations (*Icones plantarum rarorum*, Vol. 1, dated 1781-1786). In that volume, soybean is termed "*Dolichos Soja*" [sic !]. A remarkably detailed full-page color illustration (plate no. 145; see front cover page of this publication) of a soybean plant is presented: Along a main stem, four tri-foliate leaves and purple flowers in axillary positions are shown, leaves and stem are pubescent. In addition, a brown, mature pod probably containing three seeds and one individual dark-brown seed are given as well. In comparison to most present-day soybeans, the stem appears rather thin and winding and has long internodes. Jacquin employed several artists to create his plant illustrations based on copper engravings which were colorized after the print. It is not known whether the soybean pictured in Jacquin's volume was grown at the Vienna botanical garden. However, the high level of detail in the illustration suggests that the artist had a live specimen of a soybean plant available when producing the picture. Moreover, catalogue records on seed exchange indicate that soybean was grown at the Vienna botanical garden by 1816 and very probably in the years before. The second and much better documented appearance of soybean in Austria was the Vienna World Exhibition of 1873, where soybean seeds and soy-food products had been presented. Following the expo, the Austrian agronomist Friedrich Haberlandt (1826-1878) initiated a series of agronomic soybean trials in a large number of locations across central Europe. Haberlandt summarized the results in his landmark publication of 1878 (title in German: *Die Sojabohne – Ergebnisse der Studien und Versuche über die Anbauwürdigkeit dieser neu einzuführenden Culturpflanze*) which contributed to the world-wide dissemination of soybean. Haberlandt was the first agronomist who recognized the considerable potential of the soybean plant for the European agriculture.

Keywords: Soybean history, Europe, botanical illustration, Vienna World Exhibition, field experiment

Soy and agro-food change (SoyChange): Austria from a multi-level perspective, 1870s-2020s

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Soy's journey around the world was a paradoxical development: the cultigen simultaneously acted as a protagonist of the 'western diet', rich in meat and dairy products, and its antagonist, endorsing alternative vegetarian and low-meat diets. Yet, the development of the 'soy paradox' was neither deterministic nor linear; on the contrary, soy's emergence as a global commodity was a rocky road. Before soy became part of the mainstream as a cash crop, it emerged in several niches as a novel solution to specific problems in different branches of the economy. Our research project investigates these developments from a historical perspective on the transitions of agro-food regimes. Through the lens of the particular commodity soy over a long-term period – the last 150 years – with a focus on a particular country – Austria – in its inter- and transnational setting we reconstruct the – partly successful, partly failed – transitions from niche to regime levels. The central question is how knowledge transfer, commodity chains and regulatory institutions interacted in soy's emergence from niche to mainstream in the context of agro-food transitions in Austria since the 1870s. In the last decades, Austria has emerged as a trader as well as producer in the global commodity web around soy. In order to address soy-related developments on multiple levels (from local to global) that bring about agro-food transitions, the project rests on an innovative combination of transition theory, food regime analysis, and commodity studies. We argue that the emergence of soy and its implementation in agriculture and nutrition involved suitable arrangements of knowledge, commodification, and institutions.

Keywords: agro-food transition, soy, niche, regime, multi-Level Perspective, Austria

Soya geographical origin investigation using inductively coupled plasma-mass spectrometry combined with elementomics

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Amazon rainforest destruction is a major issue that directly impacts ecological sustainability, and the main cause of this deforestation is agriculture. Soya cultivation has been expanding exponentially into the Amazon Basin, due to the world demand for this crop. In Europe, businesses may be linked through their supply chain to deforestation as there is the potential they are buying soya directly or indirectly through supply chains from ambiguous or even fraudulent sources. The purpose of this study was to develop a robust and reliable method to determine the geographic origin of soya, to support businesses in making important sourcing decisions. Soya (n=527) obtained from 13 different countries were used in the study. Inductively coupled plasma mass spectrometry (ICP-MS) was used to identify the elemental composition of soya samples obtained. At least 47 elements were used to build statistical models that could distinguish soya from the different countries. In combination with chemometric evaluation, both unsupervised and supervised pattern recognition techniques, principal component analysis and partial least squares algorithms, respectively, were determined in order to discriminate and classify soya samples according to their geographical origins. This method achieved a cross-validation classification accuracy of 97.5%. Thus, we have demonstrated that the geographical origin of soya can be correctly identified by using ICP-MS. This method holds exceptional promise to become a powerful tool for businesses to make informed decisions about the sourcing of soya and will serve to help protect the Amazon rainforest.

Keywords: Soya, deforestation, geographical origin, sustainability, food authentication

Integrated transcriptomic and proteomic analysis of a cytoplasmic male sterility line and associated maintainer line in soybean

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Heterosis is a critical phenomenon in crop improvement. Cytoplasmic male sterility (CMS) and Restorer gene (Rf) systems are essential components for heterosis-based breeding. However, the molecular mechanism underlying CMS remains largely unclear in soybean. We integrated a morphological investigation with comparative analyses of transcriptomic and proteomic changes in pollen from the CMS line W931A and its maintainer line, W931B, at the uninucleate microspore (UM) and binucleate pollen (BP) stages. Compared to W931B, which had healthy, oval pollen grains, W931A showed shrunken or degraded pollen grains with an irregularly thickened endothelium and decreased starch accumulation. Transcriptomic comparisons revealed a total of 865 differentially expressed genes (DEGs) in W931A over the two stages. These genes were primarily associated with pentose and glucuronate interconversions, sphingolipid metabolism, and glycerolipid metabolism. Proteomic analysis revealed 343 differentially expressed proteins (DEPs), which were mainly involved in carbon metabolism, glycolysis/gluconeogenesis, and nitrogen metabolism. Consistently, gene ontology (GO) biological process terms related to pollen development were enriched among DEGs at the UM and BP stages. Notably, four genes with demonstrated roles in pollen development were differentially expressed, including AGAMOUS-LIKE 104, PROTEIN-TYROSINE-PHOSPHATASE 1, and PHOSPHOLIPASE A2. A total of 53 genes and the corresponding proteins were differentially expressed in W931A at both the UM and BP stages, and many of these were pectinesterases, polygalacturonases, peroxidases, and ATPases. The results of this study suggest that pollen development in W931A is likely regulated through suppression of the identified DEGs and DEPs. These findings increase our understanding of the molecular mechanism underlying CMS in soybean, aiding future research into soybean fertility and promoting the efficient use of heterosis for soybean improvement.

Keywords: Soybean, cytoplasmic male sterility, uninucleate microspore, binucleate pollen, transcriptome, proteome

Acknowledgements: The Program on Industrial Technology System of National Soybean (CARS-04-PS07), the National Key Research and Development Program of China (Grant No. 2016YFD0101503).

Toward substitution of hexane in soybean oil extraction by methyloxolane, a plant-based solvent recently approved in Europe for food use: a semi-industrial scale study for key process parameters evaluation

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Commercial hexane is still the reference solvent for the soybean extraction industry. Alternatives to hexane are urgently needed as its main component, n-hexane, has recently been classified as neurotoxic to humans by ECHA (STOT RE1). Extraction with supercritical CO_2 and hot ethanol already offer alternatives to hexane. However, these solvents require a very different industrial set-up. Methyloxolane (EcoXtract®) is a bio-based solvent that was added to the list of permitted solvents for food and feed production in Europe on 26 January 2023. Methyloxolane offers a new, viable alternative to hexane, providing high quality oils and defatted, stable protein-rich ingredients. Furthermore, replacing hexane with methyloxolane does not require significant technical changes to extraction equipment.

A previous study investigated the potential of methyloxolane for the extraction of soybean oil and concluded that methyloxolane is an excellent candidate for replacing hexane in industrial extraction processes, as it yields a similar composition of oil and defatted meal. Based on these results, a 10 tonnes/day oilseeds hexane crushing plant in the UK (New Holland Extraction) was converted to operate with methyloxolane or hexane in September 2021. Two soybean extraction campaigns with methyloxolane took place in October 2021 and July 2022, producing well defatted meal and well desolventised oil. In order to optimise the extraction process at a further scale-up (300-600 tonnes/day plant), this study investigates in detail the main process parameters in the extraction of soybean oil with methyloxolane in a six-stage semi-industrial countercurrent extractor (Model IV Crown Iron Extractor). The influence of different variables (temperature, solvent to matrix ratio, water content of the solvent) was investigated through a comprehensive characterisation of the crude oils and the meals. This study confirmed the high extraction performance of methyloxolane and optimised the extraction parameters to obtain a completely defatted soybean meal and a high-quality crude oil.

Keywords: Methyloxolane, bio-based solvent, semi-industrial countercurrent extraction, green extraction, soybean oil

Amino acid profile evaluation on Argentinean on-farm sampled soybean

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Soybean (*Glycine max* (L.) Merr.) is the most important crop in Argentina, while soybean meal (SBM) represents the main exportation income of the country. SBM is a relevant source of protein and amino acids (AA) for feed worldwide. Chemical composition, protein quality and nutritive value of commercial SBM depends on numerous factors, including seed variety, environmental conditions, beans storage and oil extraction processing conditions. Particularly, SBM´s Key AA (%) value represents a relevant tool for feedstuffs international marketing, and is defined by the sum of methionine, cysteine, tryptophan, lysine and threonine, being lysine the most relevant AA for monogastrics. Grain on-farm samples (n=544) from eight homogenous Argentine cropping areas (North West, North East, North Cordoba, South West Cordoba, Core, Center of Santa Fe, Entre Rios, South Buenos Aires) were analyzed in order to assess their protein (% db), oil (% db), and PROFAT (sum of protein and oil % db). AAs profile was also determined from a subsample (n=100). Both a near infrared spectroscopy (NIR) device and HPLC Ms/tQ were used for the determinations. Quality mean values of total samples were 36.6 % (db) of protein, 23.0 % (db) of oil and 59.6 % (db) of PROFAT. Essential AA, non-essential AA and Key AA (%) mean values from the subsample were 41.18 %, 58.82 % and 14.36 %, respectively. Four from the eight evaluated areas showed a KAA (%) index upon the mean. Regarding lysine, its national mean value was 6.16 %, the highest one coming from Santa Fe Centro and the lowest from the North West. Remarkably, we found no relationship (p=0.95) between lysine (%) and protein (% db). In conclusion, this first approach indicates that Argentinean soybean composition, KAA and lysine mean values were relevant and varied between the different areas of Argentine soybean crops.

High throughput phenotyping and analysis of soybean seeds and pods based on image processing techniques

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Soybean (*Glycine max* (L.) Merr.) is highly versatile as they can be used for both food and oilseed feed. The shape, colour and number of grains in soybean seeds and pods are crucial indicators for selecting high-quality soybeans. However, visual inspection is time-consuming and error-prone, particularly in colour recognition due to the inspection environment. This study employed image recognition to accurately classify the colour of soybean seeds and pods and detect the number of seeds in the pods. A fusion of the watershed algorithm and the K-means clustering algorithm achieved effective segmentation of soybean seeds and pods, resulting in a high accuracy in colour classification. On the basis of the effective segmentation, vertical lines are made for each pod, the pixel values of the intersection of the vertical line and the pod are arranged in order, and the number of peaks is the number of pods. Single, double and triple pods can be effectively detected with high accuracy in the training set. The study demonstrated the strong generalization ability in the model of seed colour classification and pod number recognition in different scenarios, providing a reference for artificial intelligence breeding.

Soybean spacing detection in UAV images using image analysis

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Significant increase in agricultural production is the key to meet the global food security goals. Accurate seeding is a critical factor in achieving a successful and profitable soybean production since precise seeding can help control weed growth, optimize plant spacings and avoid uneven plant growth. So, there is a need to further explore the performance of seeders in the field because the movement of seeds can be better studied in the field compared with by simulation or indoor experiments. However, it’s time-consuming and laborious to manually measure the spacings in the real field although the research of this kind is essential for realization of more precise seeding. Therefore, this study proposes the method to estimate the soybean spacings in the large-scale field by UAV images. The field experiment was conducted under the circumstances where two different planters, the vacuum seeder (1 seed/hole) and the conventional planter (2 seeds/hole), seeded soybean in a large farmland. The UAV images were obtained by automated flight for 5 days, then analyzed to detect every individual and plant in several processing ways, including deep learning (YOLOv4) and image analysis. The author discovered that difficulty varies depending on how many seeds are planted in one hole (2 seeds/hole was more challenging in detection as a plant than 1 seed/hole) as well as how accurately the methods that are proposed in this study can detect each plant overall. Nevertheless, there is room for improvement in this pipeline to enhance the accuracy, especially when plants are too tiny in images to be detected and two individual plants that are supposed to be seeded in one hole did appear far away from each other because of the seeder’s performance. In conclusion, this study provides the method that can accurately detect soybean spacings in UAV images under two different situations.

Acknowledgements: This study was partially supported by Japan Science and Technology Agency (JST) AIP Acceleration Research JPMJCR21U3 and Sarabetsu SARABETSU SUPER VILLAGE PROJECT.

Applied research and development for French soy priorities contributing to France´s national protein strategy

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French agriculture relies on imported soy to feed its livestock and to fulfil a growing demand for food applications. Terres Inovia, the R&D organization of the French oilseeds and pulses industry board, has developed a research portfolio to increase domestic supply. Areas sown to soy in France started at 20,000 ha, rose to 150,000 ha, with an objective of 250,000 ha. The overarching hypothesis behind French R&D was to increase technical and economical competitiveness on 2 levels: domestic soybean meals compared to imports, and competitiveness compared to dominant crops in farmers' rotations. We illustrate priorities and results of the French R&D portfolio with a selection of scientific and grey literature from Terres Inovia and its partners. The first bottlenecks to develop French soy were to attain sufficient yields and protein levels at the crop level, and to develop a grain crushing concept that did not require massive volumes. Genetic progress has provided 0.2-0.36 t/ha yield and 0.5 % points protein increases every decade, while our grain crush concept now equips circa 50% of domestic crushing capacity. Different modelling and data analysis strategies are providing insight on where to further develop soy: specifically, phenological modelling, a GIS rule-based system, and economical analysis of benchmark crop rotations with new rotations involving soy show the potential and current limits to further expansion. Building on this, we identify R&D avenues to develop soy further. To gain areas in new regions, the main limitation is a lack of economical return for farmers compared to existing dominant crops. This requires a strong focus on increasing yield, by increasing genetic gain and focusing on specific aspects of crop husbandry in these new regions. R&D efforts to increasing value by addressing the specific needs of certain food markets are also being undertaken.

Keywords: Genetic gain, animal feed, crop diversification, climate change, phenology

Legume Hub: Europe's knowledge platform for legumes

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The Legume Hub was developed in the Legumes Translated project funded by the European Union's Horizon 2020 Programme for research, technological development and demonstration. The purpose of the Legume Hub is to provide access to validated knowledge for all those interested in the development of legume crop production and use. The Hub is a platform dedicated to sharing knowledge and successful practices across value chains, from plant breeding, on-farm activities, through to processing and consumption. It is a multi-lingual publishing platform featuring articles, videos, images and blogs. Each article is attributed to its authors and organisations. All articles published through the Hub are independently peer-reviewed and each is citable as a scientific or technical publication. The Legume Hub is community-owned and governed through the European Legume Hub Community. All users involved in the development of legume cultivation and use are invited to join the Legume Hub Community by registering as a member. Members have increased access to the Hub's resources and are able to connect with other members. Every member is invited to contribute to the Hub by creating a personal profile related to his or her legume work. By doing so, one is able to publish articles and provide information about legume projects. With the Legume Hub, we want to offer new or existing projects the opportunity to use an existing long-term platform for publication and dissemination rather than investing in short-term project sites. The Hub Community also gives its members a voice in the public debate on the development of legume-supported cropping as an association of innovators, scientists and technologists.

The soybean agribusiness system in Argentina: challenges to remain competitive

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The increase in world population and purchasing power has boosted demand for soy products of all types. In Argentina, soybean area grew from 37,000 hectares in 1970 to 19 million in 2022. The Argentine Soybean Agribusiness System (ABS), is the most important economic sector in the country, generating a portfolio of products valued at USD 23 billion. The purpose of this paper is to analyze the competitiveness of the Argentine Soybean ABS. Analyzing the degree of competitiveness of a system is related to how well it is able to adapt to changes in the context. Argentine leadership in world soybean and sub-products commerce is the result of adaptations to different disruptions, by innovations in the institutional, organizational and technological environments. Technologically, the need to produce in an even more environment-friendly way must be emphasized, particularly in nutrient reposition, while aspects related to transport and logistics must be addressed. Also, the dependence on few buyers is a threat when trying to market an increasing production. It is imperative that Argentine widen its portfolio of products to try and mitigate this risk. This scenario is framed in an organizational environment that has deeply changed to accompany the growth of the system. At farming level, organizational adaptation in a context of institutional uncertainty mostly explains why Argentina keeps growing its agricultural sector. The efficiency found at productive and industrial level, along with the evolution of the organizational and technological environments on which they are based are the key drivers that explain the continued positive evolution of the Argentine Soybean ABS. The negative points are found mainly at the institutional level and the lack of alignment between environments could cause loss of competitiveness in the future. Argentina must improve its institutional conditions in order to survive in this highly competitive global business.

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- Ensuring optimum and rapid utilisation and exploitation of project deliverables and innovations by relevant industry and other user/stakeholder groups.

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This year, United Soybean Board (USB) is investing $37 million in soybean research. Several researchers currently funded by USB are attending with you! Look for sessions by Robert Stupar, Carl Parsons, Seth Naeve, Henry Nguyen, Zenglu Li & Rachel Vann. Also, connect with Jack Cornell (USB staff) who plays a key role in the Farmers for Soil Health initiative.

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