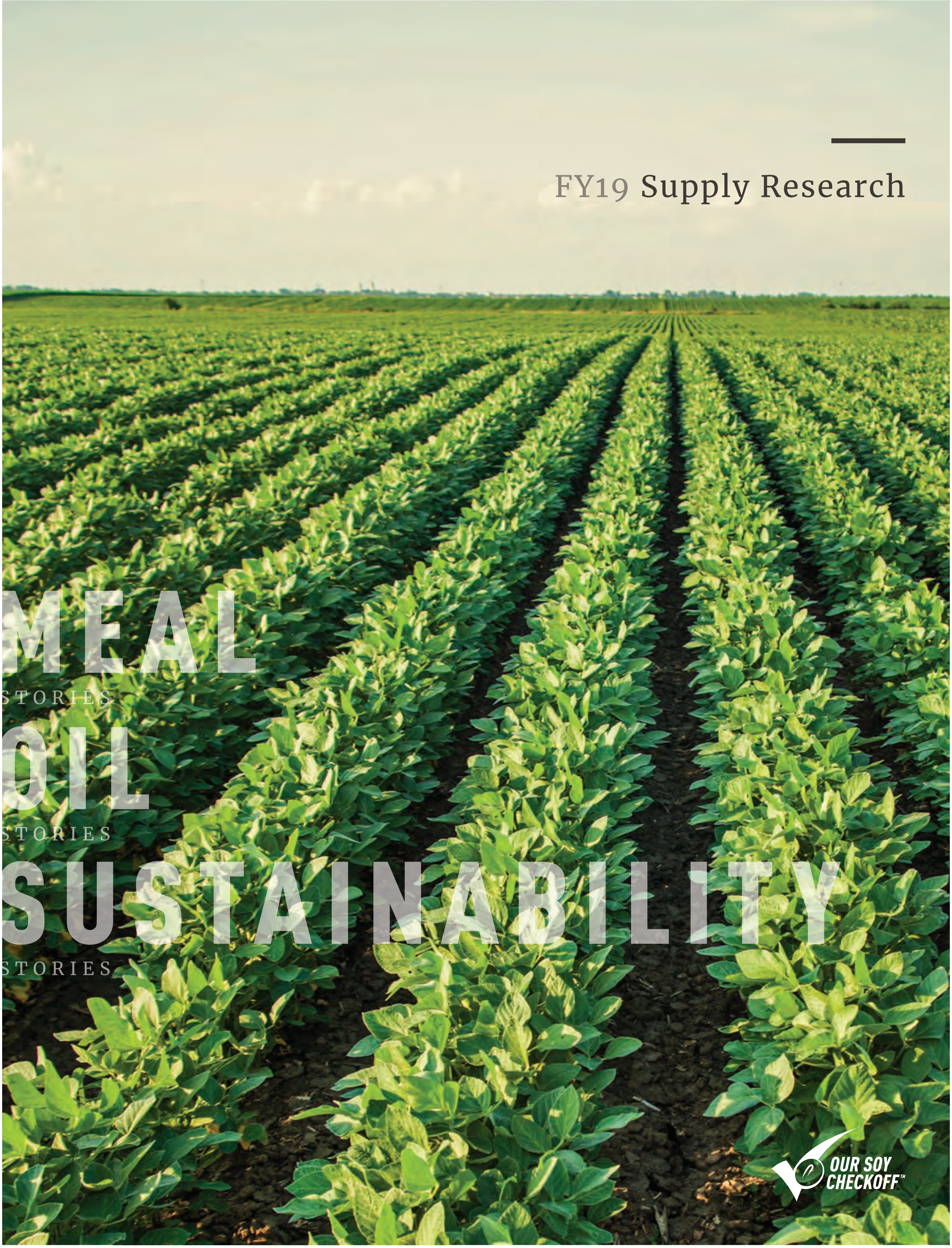

# FY19 Supply Research

MEAL

STORIES

OIL

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SUSTAINABILITY

STORIES

OUR SOY CHECKOFF™

# SUPPLY ACTION TEAM

# Bridging the Gap

Each year, the United Soybean Board invests a significant amount of checkoff dollars in research to develop new tools that improve production techniques and increase profit opportunities for farmers. This year, USB's Supply Action Team commissioned the enclosed comprehensive research report to inform farmers of the results of their checkoff investments and celebrate successful research projects that provide guidance for future scientific discoveries.

This report covers the mission of USB's Supply Action Team and includes the group's investment strategy. It features a summary of each research project funded by USB in its 2019 fiscal year, including a description of the project's importance, progress to date and how the results benefit soybean farmers. It also allows QSSBs to assess research gaps and align their programs where appropriate. It's a win-win.

My personal desire for the Supply Action Team is to address production challenges faced by soybean farmers throughout the U.S. We are continually looking for opportunities to fulfill the needs of farmers and even bring forward prospects for production research that may not yet be on farmers' radars.

We want to grow beans better. We want higher yields. We want improved stress tolerance and to understand effective pest-management practices. Compiling stories from researchers working to impact these issues is an essential link in the mission of our team.

I hope this report proves useful and becomes an annual activity. In the future, it could include research related to new uses and animal nutrition on the demand side of the USB investment portfolio. As USB increases its emphasis on industry collaboration, results of those partnerships could also be informative in future reports.

While reading these stories, I encourage you to think of the big picture. Think about the production challenges you face and the research you'd like the checkoff to fund, to bridge those gaps and ultimately increase your profitability.

Thank you,
**Tom Oswald**
*Chair, Supply Action Team*

# Soybean Production Research Community:
## Supporting Collaboration and Involvement

THE UNITED SOYBEAN BOARD SUPPLY PROGRAM PLAYS AN IMPORTANT ROLE IN CONNECTING SOYBEAN GROWERS, THE CHECKOFF FAMILY AND SOYBEAN RESEARCHERS. PROGRAM MEMBERS COMMUNICATE GROWERS' NEEDS AND WORK TIRELESSLY TO ENGAGE THE BEST RESEARCHERS AND PROJECTS TO FURTHER SOYBEAN-CENTRIC PRODUCTION RESEARCH TO HELP IMPROVE FARMER PROFITABILITY.

Photography credit: Jennifer Jones.

**Figure 1.** Gene Stoel, Ron Ohlde and others at NCSRP field day in Ohio in August 2019. Stoel is looking at a stink bug collection card.

USB directors and staff attend scientific meetings in agriculture, agronomy, genomics and breeding to keep up to date on the latest discoveries and results. Because of this, one of the great legacies of the soybean checkoff production research program is the community of soybean researchers. This community is the envy of many other crop research groups. USB-funded supply programs, at the direction of the Supply Action Team directors, encourage researchers from different disciplines to join forces and collaborate in new ways, sharing ideas and results.

Action team directors and staff engage with the U.S. Department of Agriculture, National Science Foundation, National Institute for Food and Agriculture, and the Foundation for Food and Agricultural Research. USB representatives sit on scientific boards such as the Council for Agricultural Science and Technology, the Honeybee Health Coalition and the Cross Commodity Board. This helps keep soybeans top of mind with funding agencies and researchers. It also allows USB to communicate the research needs of the soybean community and soybean growers at a broader level.

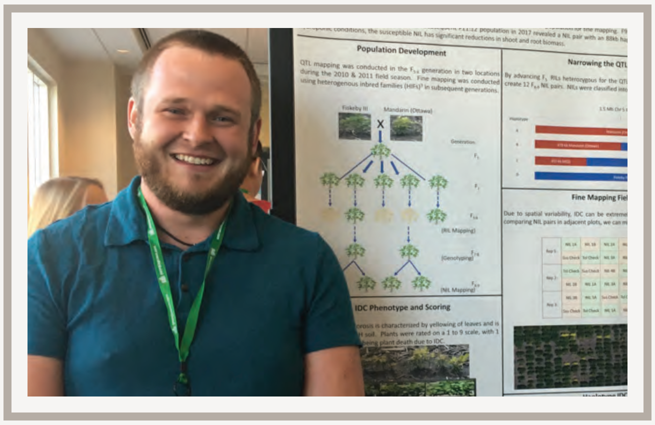
In 2019, the USB supply program provided support for the Southern Soybean Breeders' Tour, the Soybean Breeders Workshop and the Soybean Composition Workshop. USB directors and staff frequently collaborate to develop agendas and suggest presenters for these and other soybean research meetings. These USB-funded activities allow soybean researchers the opportunity to come together to develop proposals and share research results.

The USB supply program supports production research programs in regional and state soybean boards. Administrative support was provided in 2019 for each of the four regional programs. For the last several years, USB has funded and worked with the North Central Soybean Regional Program to develop and maintain the National Soybean Checkoff Research Database (https://www.soybeanresearchdata.com/) and the Soybean Research and Information Network (https://soybeanresearchinfo.com/). These public resources offer a one-stop shop to discover information about checkoff-funded research. It also increases transparency of checkoff-funded programs at state, regional and national levels, allowing the checkoff to leverage their funding in a more knowledgeable way.

Collaboration and leveraging resources is key to achieving scientific success in the twenty-first century. In 2019, the USB supply program leveraged approximately $19.3 million in actual and in-kind resources. This means that for each dollar invested in production research by USB, 85 cents matched investment. Furthermore, according to the Global Harvest Initiative 2106 Gap Report (https://www.globalharvestinitiative.org/GAP/Invest%20in%20Public%20Agricultural%20Research,%20Development%20and%20Extension.pdf), every dollar invested in agricultural research in the U.S. provides at least $10 in economic benefits to society. Thus, the reach of FY19 USB investments in public research plus the leveraged dollars exceeded $400 million in benefits to farmers and society.

Photography credit: Jennifer Jones.

**Figure 2.** USB sponsorship of a scientific meeting.



Photography credit: Jennifer Jones.

**Figure 3.** USB Ph.D. Fellow Ryan Merry, a student in Bob Stupar's lab at the University of Minnesota, displaying his award-winning poster at the 17th Biennial Conference on the Molecular and Cellular Biology of the Soybean.

# USB Strategic Plan FY17-21

**USB Core Value:** The United Soybean Board works with honesty and integrity to achieve maximum value for the U.S. soybean farmer's checkoff investments.

**MISSION**
MAXIMIZE PROFIT OPPORTUNITIES FOR U.S. SOYBEAN FARMERS BY INVESTING AND LEVERAGING SOYBEAN CHECKOFF RESOURCES.

**VISION**
U.S. SOY DRIVES SOYBEAN INNOVATION BEYOND THE BUSHEL.

**STRATEGY**
CREATE AND ENHANCE PARTNERSHIPS THAT INCREASE THE VALUE AND PREFERENCE FOR U.S. SOY.

## USB SUPPLY ACTION TEAM FY19 ALLOCATION $22,679,054

## Supply Goals

**Sustainable Production:** Soybean producers use improved seed varieties and the latest production techniques to maximize profit opportunities while meeting the standards of the U.S. Soybean Sustainability Assurance Protocol. **FY19 Allocation $18,410,097**

**Technology:** Farmers use big data and technological advances to maximize their profit opportunities. **FY19 Allocation $4,268,957**

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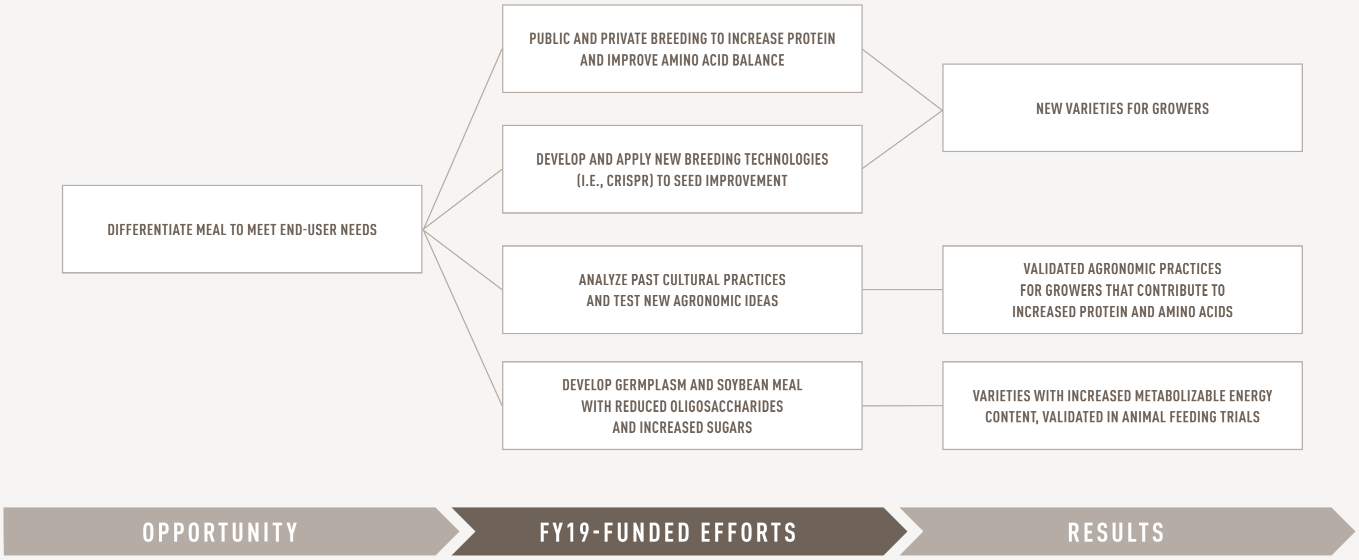
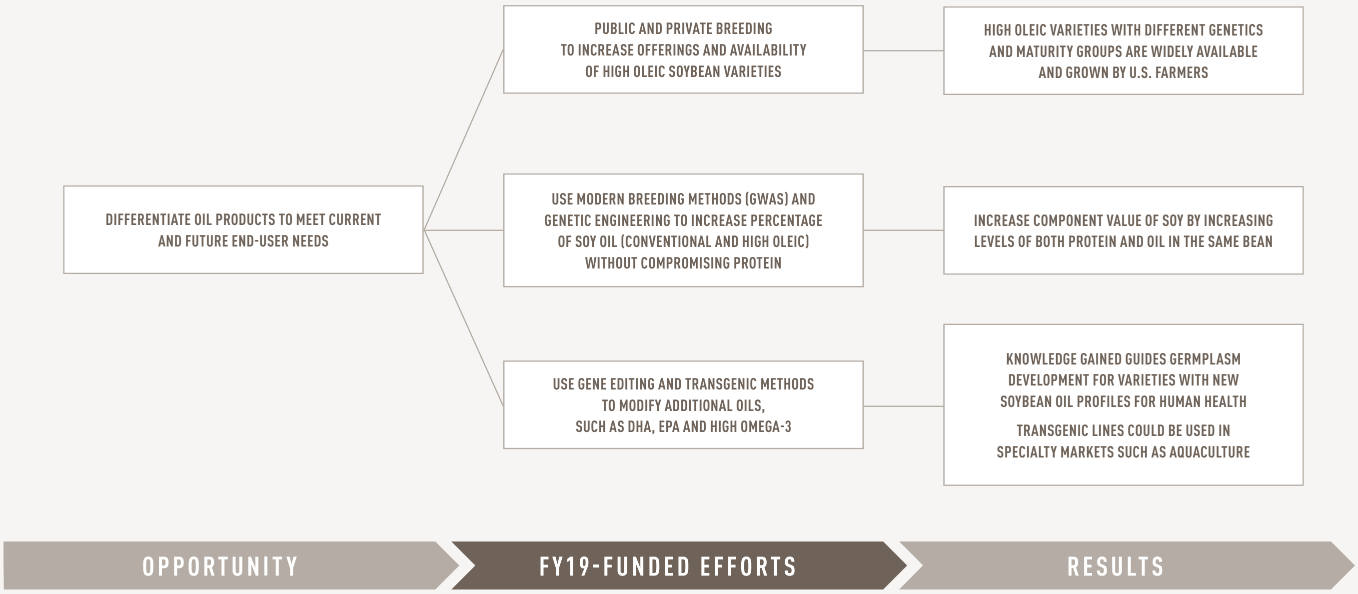
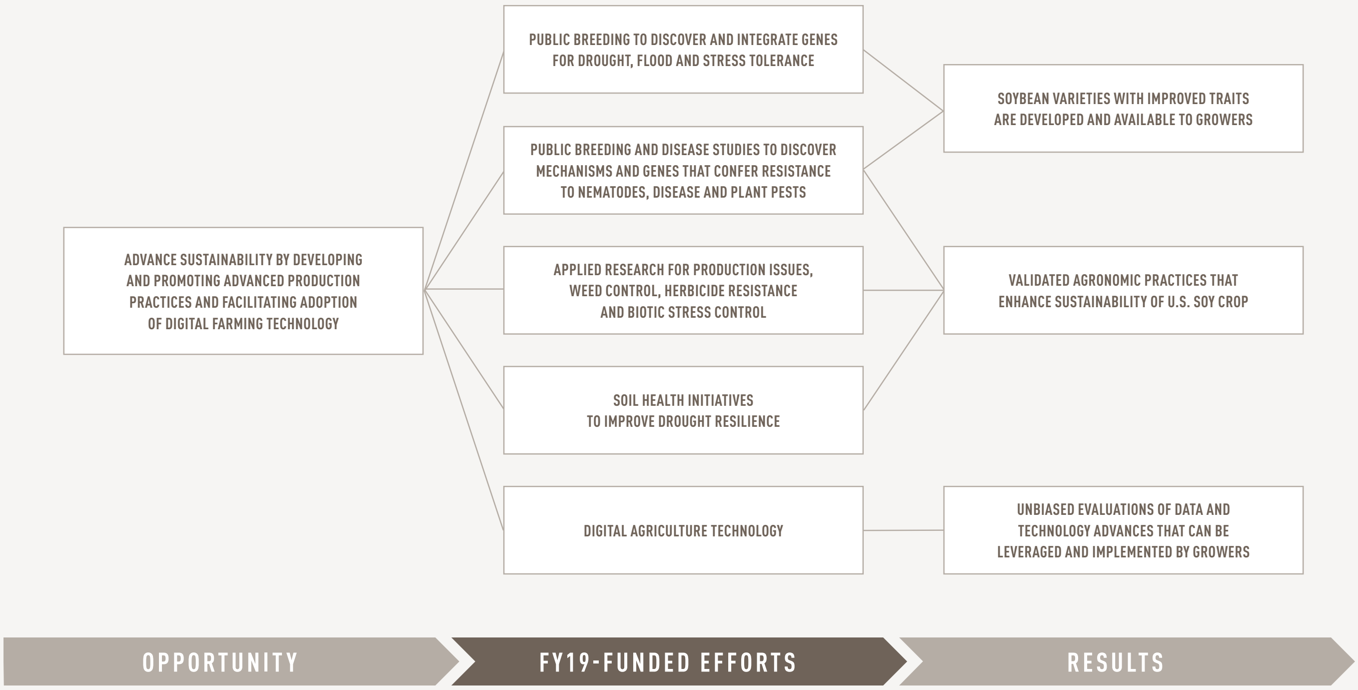
**Meal Supply Program:** Improved nutritional bundle in U.S. soybean meal supply. **FY19 Allocation $8,588,349**



**Oil Supply Program:** Increased soybean oil concentration and quality, increased supply of high oleic. **FY19 Allocation $3,881,624**



**Sustainability Supply Program:** Improved sustainability performance of U.S. soy. **FY19 Allocation $10,209,081**



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FY19 SUPPLY RESEARCH

# MEAL

STORIES

| FY19 SUPPLY RESEARCH | Proposal # | Target Area | Principal Investigator | Contractor | Funding |
|---|---|---|---|---|---|
| | 1920-152-0101 | Meal | Rainey, Katy | Purdue University | $688,206 |

# Modifying Soluble Carbohydrates in Soybean Seed for Enhanced Nutritional Energy Meal

By Lais Andrade Pereira, Purdue University

Most of the soybeans produced in the U.S. go to animal agriculture. Therefore, growing soybeans that produce a more valuable meal for animal feed would benefit both soybean farmers and the poultry and swine industries.

One major factor that affects soybean meal quality is the composition of soluble carbohydrates. Carbohydrates make up 35% of soybean meal, and of that fraction, 10% are the soluble free sugars: sucrose, stachyose, raffinose and galactinol. Sucrose is a nutritionally useful component for both human and animal diets, but the remaining soluble sugars are classified as raffinose family oligosaccharides (RFOs). In fact, RFOs are known to reduce metabolizable energy (ME) for animals and are considered anti-nutritional. Earlier poultry feeding studies conducted as part of this project indicated that the feed conversion ratio in chickens could be improved by reducing RFOs and increasing sucrose content in soybean seeds. Therefore, the meal derived from these soybeans is termed enhanced nutritional energy meal (ENEM). In addition, these studies showed the soybeans with lower RFO and higher sucrose had no negative agronomic impacts from these modifications. Therefore, modifying the soluble carbohydrate composition of soybean seeds continues to be one of the most promising soybean meal improvement targets, and the United Soybean Board has invested $688,206 in this project in 2019.

Specifically, this project involves a multi-institutional team with broad expertise, including plant breeders, molecular geneticists and nutritionists, among other specialties. The main goal is to develop soybean varieties with more than 7% sucrose and lower levels of RFOs (1-2%), which will result in an increase of ME up to of 100 kcal per pound of meal. The team is developing soybeans and conducting research to support the use of this value-enhanced soybean that can produce ENEM meal.

One of the first steps taken in this project was to understand the genes that are involved in controlling the production of sucrose and RFO in soybean seeds. It was determined that sucrose content is multigenic — that is, it is an inherited trait controlled by a number of different genes, and can be increased quantitatively through selection for sucrose levels. This team has discovered new gene variants (alleles) that encode soybean seeds with ultra-low RFOs and at least 7% sucrose. Several varieties with altered carbohydrate profiles are being characterized that may be the result of new genes. This ongoing project has also developed soybean lines that differ only in their RFO genes. These lines are called near-isogenic lines, or NILs, and are important controls needed to understand the effect of the RFO genes conferring higher sucrose in agronomic and feeding studies, with greater precision and accuracy.

All the breeders and geneticists on this project are working to incorporate these valuable new genes into high-yielding lines, to provide farmers with soybean varieties that are both high yielding and contain desirable levels of both sucrose and RFOs. In addition, they are stacking the higher sucrose soybeans producing ENEM with high oleic/low linolenic acid (HOLL) oil traits in order to obtain NILs with contrasting low and ultra-low raffinose/stachyose in a high oleic/low linolenic background.

There are several teams, each of which focuses on its own environmental factors, such as temperature, light, soil and rain, to investigate how those affect composition of soybean seeds with different genomic compositions. In addition, each team is developing soybean seed varieties that can be adapted to grow successfully in a range of environments while especially focusing on early and later maturity groups. Some of their specific goals are to understand how the sucrose and RFO content respond to different environments, and to identify germplasm that produces valuable meal in a wide range of environments. To date, they have identified genetic combinations that are responsible for a stable, increased energy meal soybean that produces at least 7% seed sucrose and has either low or ultra-low RFO across multiple environments, and studies are ongoing.

| LINE | SUCROSE (%) |
|---|---|
| CR13-395 | 5.99 |
| CR13-396 | 3.37 |
| CR13-397 | 6.30 |
| KB10-2201568b | 6.37 |
| KB10-22016000 | 6.42 |
| KB10-22016080 | 6.61 |
| KB10-221548b | 5.72 |
| KB10-2316810 | 7.00 |
| LD06-7620 | 5.22 |
| R07-2000 | 10.63 |
| R07-2001 | 10.87 |
| R08-1830 | 7.99 |
| R08-6039 | 8.94 |
| R13-10658 | 10.55 |
| R13-10676 | 7.62 |

**Figure 1.** Sucrose content (%) averaged across three years and four locations.

Future work will continue to identify and characterize new alternatives and innovative technologies and partners in order to reach even more exciting results. One new direction is gene editing, which has recently been incorporated into this project, to help more directly guide the evolution of new lines. In addition, the team is working with animal nutritionists at the USB Animal Nutrition Working Group, and talking to farmers and other stakeholders in order to understand and address their needs and goals. By continuing to communicate results to stakeholders and incorporate the needs and priorities of stakeholders in future plans, this project continues to produce new seed lines that can meet the current needs of soybean growers and ultimately the animal end users.

In summary, modified carbohydrate lines developed by breeders on this project have met both RFO and sucrose composition goals and are high yielding. A total of 23 lines were selected for the USDA Uniform Trials and were evaluated in the Preliminary Traited Material Test for 2019; results will be reported in early 2020. This is the first year of evaluations that will lead to the release of soybean lines in the next few years capable of producing ENEM. In the longer term, this project will provide valuable information for USB's strategic objective to capture the full value of U.S. soybean meal.

| | | | N | GALACTINOL (%) | | SUCROSE (%) | | RAFFINOSE (%) | | STACHYOSE (%) | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| GENOTYPE | RS2 | RS3 | 12 | 0.1 | c | 5.8 | c | 1.4 | a | 4.6 | a |
| | RS2 | rs3 (G75E) | 3 | 0.1 | c | 5.2 | c | 0.9 | b | 4.4 | a |
| | rs2 (W331-) | RS3 | 4 | 0.3 | b | 6.9 | b | 0.2 | c | 1.5 | b |
| | rs2 (W331-) | rs3snp6 | 7 | 0.4 | a | 8.3 | a | 0.1 | d | 0.3 | c |
| | rs2 (W331-) | rs3 (G75E) | 19 | 0.4 | a | 8.0 | a | 0.0 | d | 0.1 | c |
| | | $LSD_{0.05}$ | | | | | | | | | |

**Figure 2.** Mutant rs3G75E alleles combine with rs2W331- for high-energy meal. Data from 2018 field experiment in Columbia, Missouri.

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| Proposal # | Target Area | Principal Investigator | Contractor | Funding |
|---|---|---|---|---|
| 1920-152-0102 | Meal | Gillen, Anne | USDA | $57,530 |

# National USDA Uniform Soybean Tests/Yield, Disease Resistance and Quality Traits Evaluation of Public Breeding Lines

By Dr. Anne Gillen, USDA Agricultural Research Service

The availability of objective and uniform field trial data is crucial for the development of new soybean varieties. Since 1936, the Uniform Soybean Tests have been one of the main mechanisms supporting the release of new soybean lines and germplasm in the United States.

This program was founded by the U.S. Regional Soybean Laboratory in Urbana, Illinois, and initially covered only the northern growing region. Through this program, public soybean breeders were able to obtain objective multistate field data on new cultivars. Due to an increased demand for vegetable oils (caused by wartime shortages), in 1943, the program was expanded to cover the southern states as well. The USDA Agricultural Research Service (ARS) in Stoneville, Mississippi, has always managed the southern region test. Management of the northern test moved from the U.S. Regional Soybean Laboratory to the ARS at Purdue University in 1974, and has remained there ever since.

Over the decades, the Uniform Soybean Tests have been funded by ARS, universities and the United Soybean Board because they provided value to farmers through the lines that have been commercially released. In 2019, USB provided $57,530 to support this program.

The results of the Uniform Tests are publicly available and posted online at https://www.ars.usda.gov/southeast-area/stoneville-ms/crop-genetics-research/docs/uniform-soybean-tests/ (southern region) and https://www.ars.usda.gov/midwest-area/west-lafayette-in/crop-production-and-pest-control-research/docs/uniform-soybean-tests-northern-region/ (northern region). This unbiased evaluation and detailed analysis of new breeding lines allows public and private breeders to make informed decisions about future directions and potential release of new cultivars, as well as provides a valuable historical archive.

Recently, the focus of public breeding programs in the United States has shifted to include increasing protein content while maintaining oil and yield, and improving seed quality traits, in order to meet increasing competition from foreign soybean production and other oilseed crops. Funding provided by USB has allowed the Uniform Tests to analyze yield, seed composition, disease resistance and pest reaction relative to other currently available commercial cultivars, in addition to seed fatty acid, sugar and protein amino acid composition data. These data will show the value of non-GMO high oleic acid lines and will assist selection of modified carbohydrate lines to meet the current demands of the international soybean market, resulting in new, improved commercial seed varieties for farmers that will produce beans with an enhanced "nutritional bundle."

A very important aspect of the tests is that they foster the exchange of lines, enabling public breeders to increase the genetic diversity of their programs, which is key to success. Public soybean breeders are willing and able to produce high-yielding cultivars that meet current production goals in all growing regions of the U.S., but they need objective multi-field data. The Uniform Soybean Tests provide that data, and the proof is in the products. Between 2013 and 2017, over 61 public cultivars — lines licensed to companies for sale to farmers, or germplasm lines — were released based on data from the southern test. Between 2013 and 2018, 131 public cultivars — lines licensed to companies and germplasm lines — were released based on data from the northern region.

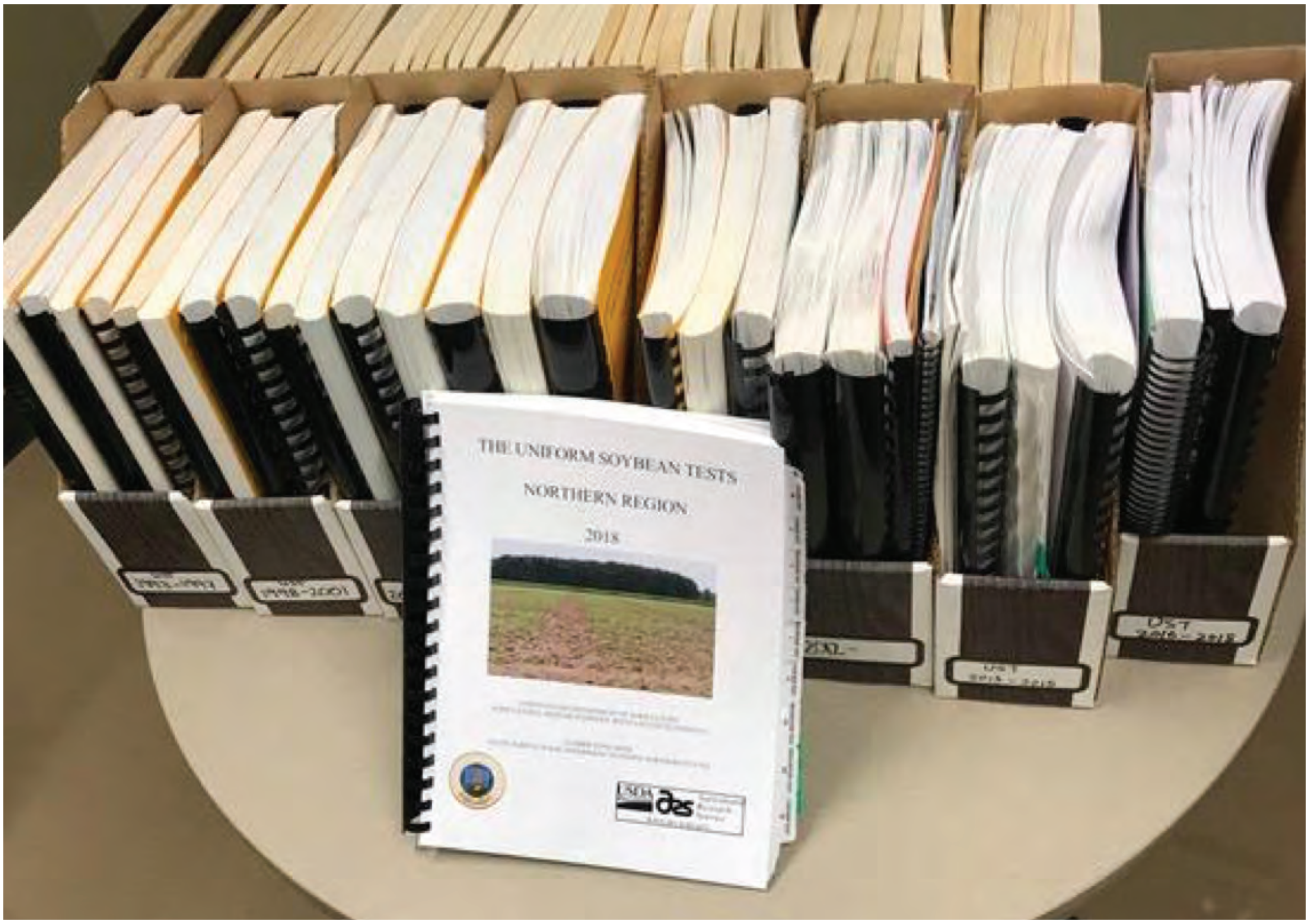

**Figure 1.** Annual reports of the northern test (1941-2018).

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| Proposal # | Target Area | Principal Investigator | Contractor | Funding |
|---|---|---|---|---|
| 1920-152-0103-A | Meal | Mian, Rouf | USDA | $1,512,400 |

# Discover Sources and Genes and Develop U.S.-Adapted Germplasm with Improved Protein Quantity and Quality

## Utilizing Diversity in Cultivated and Wild Soybeans

By Rouf Mian, USDA Agricultural Research Service in Raleigh, North Carolina

Soybean meal accounts for 60-70% of the total value of soybean crops. Recently, the meal-protein contents of commercial soybean cultivars in the U.S. have dipped below the minimum needed in the global marketplace. This situation is well-described in the 2015 USB Value Task Force Report: "U.S. soybean protein levels are on a path to decline from 34.5% in 2015 to 33.7% by 2030. There is broad agreement that doing nothing to address quality improvement in soybeans is not an option." The lower protein content of their soybean products puts U.S. growers at a significant disadvantage in the global marketplace. However, development of high-protein soybean is a difficult task, as historically seed protein content has been negatively correlated with both seed yield and seed oil content.

This decline in protein content was caused by decades of private sector breeding in the United States that focused on increasing yield, with little attention paid to protein content. In order to reverse this decline, dedicated public sector research over a significant period of time will be required. Although an increased yield is often associated with reduced seed protein, the negative correlation between the two is not absolute. Research has shown that soybean breeders can bring about meaningful (2-3%) increases in seed protein without an accompanying loss of yield. This is due in large part to the treasure trove of genetic diversity in seed protein expression that exists in the 20,000+ exotic soybean accessions preserved in the USDA Global Soybean Germplasm Collection. The majority of the current "on farm" soybean varieties trace their ancestry to only 17 of those 20,000 ancestors, meaning that U.S. varieties trace their ancestry to a very narrow genetic base. There are many protein genes that have never been used in commercial breeding, with a wide variety of traits. This global library of diversity can be used to identify new germplasms with desired characteristics, to discover and use genes that control seed protein synthesis and storage, develop innovative breeding technologies, and for the application of new genetic and genomic tools. This is the largest project currently in the meal supply portfolio and was funded by the United Soybean Board for $1,512,400 in 2019.

Earlier work identified the major high-protein gene (allele) on soybean chromosome 20 and led to the development of DNA markers that breeders can use for marker-assisted selection. In fact, high-yielding soybean cultivars/germplasm with more than 48% meal protein and balanced amino acid profiles are being developed using the latest breeding, genetic and genomic technologies.

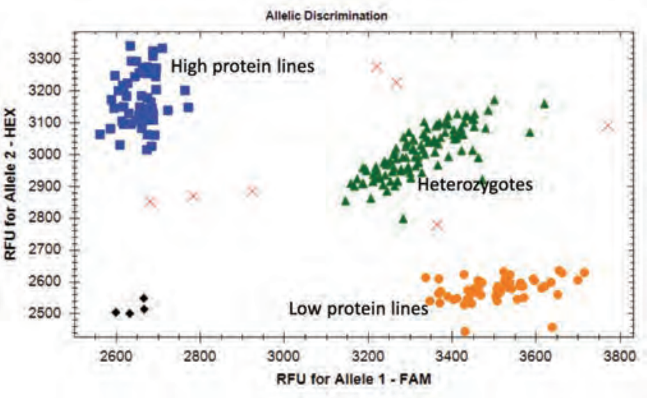

**Figure 1.** Selection of high protein lines with DNA markers developed at University of Georgia.

In a parallel line of research, thousands of breeding lines were screened for protein, oil and amino acid contents. More than 100,000 lines were screened for in FY19 alone, using a high-throughput NIR platform.

Many new genes that control both protein and amino acid content were identified from the germplasm collection. These sources were then developed and used as DNA markers

| LINE NAME | MG | YIELD | PROTEIN | OIL | MEAL PROTEIN | EXOTIC DIVERSITY | RELEASE YEAR/BREEDER/STATE |
|---|---|---|---|---|---|---|---|
| | | **% Check** | **13% M** | **%** | **%** | **%** | |
| **M10-2017102** | 0 | 97 | 37.7 | 16.2 | 49 | - | 2019, Lorenz, MN |
| **E11128T** | II | 95 | 39 | 16.1 | 50.5 | - | 2019, Wang, MI |
| **S09-13185** | VE | 100 | 37.9 | 18.5 | 50.5 | - | 2019, Chen, MO |
| **G11-7103** | VII | 98 | 38 | 19 | 50 | 50 | 2019, Li, GA |
| **TN15-5007** | V | 100 | 37 | 20 | 50 | - | 2019, Pantalone, TN |
| **USDA-N6004** | VI | 95 | 36 | 20 | 48 | 50 | 2019, Carter, NC |
| **USDA-N7004** | VII | 95 | 36.6 | 18.7 | 49 | 25 | 2019, Mian, NC |

**Figure 2.** 2019 releases of high-protein, high-yielding germplasm/cultivars.

for marker-assisted breeding programs, leading to many novel sources of high-protein soybean products. Several hundred lines have been screened with SoySNP50K, and several dozen lines have been sequenced, to fine-map the genes. Seven new high-protein, high-yielding germplasm/cultivars were released in 2019, some with high genetic diversity, as shown in Figure 2.

These cultivars will allow the development of higher protein soybeans with up to 48% protein meal. They will benefit not only U.S. soybean growers by making their soybeans more competitive in the global marketplace, but will also benefit private seed companies who can use these high-protein soybeans as parents in their breeding programs as well as poultry and livestock farmers who will be able to use the higher-protein soybean meal as feed.

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**FY19 SUPPLY RESEARCH**

| Proposal # | Target Area | Principal Investigator | Contractor | Funding |
|---|---|---|---|---|
| 1920-152-0103-B | Meal | An, Charles | USDA | $85,000 |

# Investigation of Molecular Mechanism Underlying Negative Impact of a High-Protein Gene/Allele on Yield for Soybean Meal Quality Improvement

By Charles An, Donald Danforth Plant Science Center

The global demand for soybean is driven by its highly valued oil, which has both dietary and industrial uses and by its high quality protein used primarily as animal feed. Approximately 60% of the value of soybean comes from meal, and about 40% from oil. Higher seed protein content is often negatively correlated with seed oil and yield. A century of soybean breeding in the U.S. has focused primarily on improving both yield and oil content, which is believed to have caused the seed protein content to decline significantly. In recent years, the protein content decease in some commercial soybean cultivars has become so severe that meal processors cannot meet the minimum protein content required for high-protein meal designation. Lower protein levels also put U.S. growers at a significant disadvantage in the international marketplace.

This project aims to reverse this trend using the tools of molecular breeding and biotechnology. The long-term goals are to discover the genes that contribute to seed protein content decrease in soybean, to understand their underlying molecular and genetic basis, and to develop new breeding and biotech strategies, including gene editing, which will enable reversing the decreasing protein trend with no or minimal impact on yield and oil content. For the past several decades, soybean researchers have shown that there are more than 100 QTL (quantitative trait loci) that contribute

**Figure 1.** Functions of POWR1 gene in controlling seed protein, oil and yield and its uses in soybean protein improvement are being tested by engineering the gene in soybean.

to the protein content change in the soybean natural population. This project uses a big-data driven technology platform, integrating computer technology with soybean genetics, molecular biology and genomic approaches to identify genes associated with these QTLs.

An interdisciplinary technology platform, analyzing 1,500 soybean accessions and 15 terabytes of genetic data, enabled discovery of QTL gene candidates on both chromosome 20 and chromosome 15. A gene associated with high protein on chromosome 20 was identified as coding for a transcription factor — a protein that regulates many other genes. The chromosome 15 gene has been tentatively identified as well. Both genes need to be validated. In addition, a closely related gene to this transcriptional factor is on chromosome 10.

The functions of the gene on chromosome 20 in controlling seed oil, protein content and eventually seed yield are being investigated, using traditional and genetic engineering strategies to produce new high-protein soybean cultivars. Over 100 soybean plants containing modified genes were screened and analyzed. In addition, an in silico (computer-based) genotyping approach and a wet lab perfect DNA assay for the high protein allele/version of the gene was developed. This assay was used to screen 1,261 wild and cultivated soybean lines for the high protein allele. From this screen, 217 soybean accessions were identified as having the high-protein allele.

The detailed knowledge being collected by this project about the protein QTL gene (candidate), the high-protein allele assay and the collection of high-protein soybean accessions will enable breeders to effectively integrate the high-protein trait into elite soybean cultivars, thus increasing the value of their soybean and making them competitive in the global marketplace.

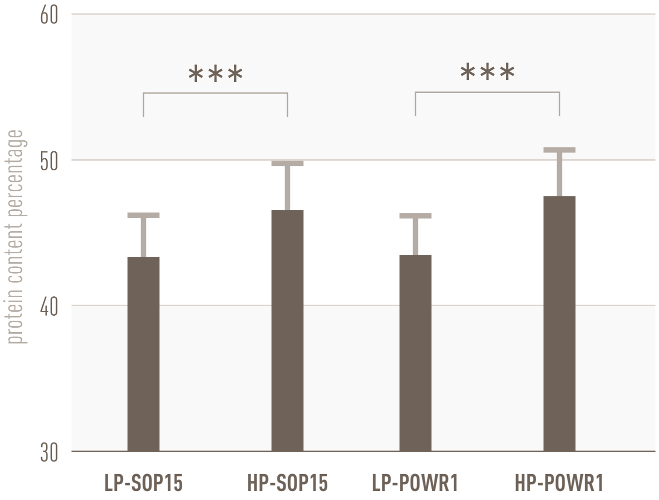

**Figure 2.** Average protein content in soybean accessions containing low protein (LP) and high protein (HP) versions of SOP15 and POWR1 genes respectively.

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| Proposal # | Target Area | Principal Investigator | Contractor | Funding |
|---|---|---|---|---|
| 1920-152-0105 | Meal | Krasheninnik, Nadia | Corteva | $762,000 |

# Improvement of Soybean Protein in Upper Midwest Germplasm

By Nadia Krasheninnik, Corteva Agriscience™

Over the past several decades, the protein levels in soybeans have shown a small, but significant, decline. The decline in soybean protein was the result of commercial varieties being selected for their yield overall, rather than protein content. A contributing factor was the significant expansion of soybean production in the U.S. into the Upper Midwest region, since the environmental conditions in that region often result in slightly lower total crude protein content.

This project allowed Corteva Agriscience™ to use their proprietary methodologies to improve the protein content in maturity groups suitable for growing in the Upper Midwest region, including Minnesota, North Dakota, South Dakota, and parts of Iowa and Nebraska — mainly maturity groups 0, I and II. This was accomplished through selective breeding of higher-protein, earlier-maturity and high-yielding soybean lines.

The first crosses between elite, high-yielding soybeans and high-protein parental lines were made in the spring of 2016. This work officially became a USB-funded project in the fall of 2017, after the initial crosses showed potential. Each season, the most promising lines that had good yield and were capable of producing a calculated meal with at least 47.5% protein were advanced to the next phase of testing.

Testing for protein, oil and other components lags behind initial harvest and yield analysis. Samples taken in the fall of 2018, prior to the 2019 field trials, were analyzed using Near Infrared (NIR) spectroscopy. Predicted protein content for over 11,000 samples averaged 34.5%, and ranged from 28.9% to 40.2% (all reported on a 13% moisture basis). Compositional predictions and spectral analysis were used to identify a subset of samples that were submitted for reference chemistry. Data from the reference chemistry was used to validate and extend predictive models. To enable studies into the relationship between protein and essential amino acid content, a further subset of samples was identified and submitted to the University of Missouri for reference analysis.

While the compositional analysis was underway, decisions were made regarding which lines would be advanced to elite, wide-area yield-testing trials across North Dakota, Minnesota, South Dakota and Eastern Canada for the spring and summer of 2019. This material was expected to provide the most comprehensive dataset for this project, since it encompassed multiple locations. Unfortunately, extremely challenging harvest conditions in the fall of 2019 did not allow the harvest and data collection from all locations planted in the Upper Midwest. Nevertheless, sufficient material was harvested to provide a robust dataset, from which final conclusions will be drawn.

December 2019 was the end of the official collaboration between Corteva Agriscience and USB on this project. The protein and oil content of all seed samples that could be harvested, along with the appropriate checks, have been determined by NIR. This data is currently being analyzed, and conclusions will be available in the final report.

This project was a test case for Corteva Agriscience's proprietary breeding tools, as applied to soybean protein composition. It provided additional information on the stability of the protein trait in the Upper Midwest environment. These learnings will inform future innovative products that will benefit growers and other producers.

| Proposal # | Target Area | Principal Investigator | Contractor | Funding |
|---|---|---|---|---|
| 1920-152-0106 | Meal | Fritschi, Felix | University of Missouri | $152,358 |

# Development of High-Yielding, High-Protein Germplasm by Enhancing Nitrogen Acquisition and Its Transport to Seed

By Felix Fritschi, University of Missouri

Soybean seeds are an important source of protein. However, although the protein concentration in soybean seeds is relatively high when compared to other legumes, the steady decline in protein content over recent years has made the production of high-protein seed meal from soybeans increasingly challenging for processors. In light of this decline, a research focus on seed protein quantity and quality is critical. This research will ensure that processors can continue to produce high-quality soybean meal and not only maintain, but enhance, the competitive edge of U.S. soybean meal in the global marketplace.

Soybean's relatively high seed protein content and its ability to access nitrogen from the atmosphere (also known as "nitrogen fixation") have resulted in its development as a very important crop in the United States. In soybean, nitrogen uptake and assimilation take place in different tissues than nitrogen utilization. Following its acquisition from the soil or atmosphere, nitrogen is reduced into ureides and amino acids in roots or nodules, and these organic nitrogen-containing compounds are exported to a variety of sink tissues (such as vegetative tissues and seeds) for plant growth and yield formation.

The focus of this research project is to understand and manipulate both uptake and export of organic nitrogen and sulfur throughout the soybean plant, with the goal of understanding how transport proteins function in the delivery of nitrogen metabolites to seeds, and on their role in seed development. This includes transport of nitrogen and sulfur from source organs (i.e., nodules and leaves), import of amino acids into seeds, and increasing both the number of seeds and the protein levels in those seeds. In 2019, this project was funded by the United Soybean Board for $152,358 to develop soybean lines with enhanced nitrogen acquisition properties and nitrogen and sulfur allocation, and therefore increased soybean seed and protein yield as well as seed nutritional quality.

To date, soybean plants have been grown that overexpress a transporter gene for the products of nitrogen fixation (ureides) under field conditions, and these plants were used to assess impacts on both yield and soybean seed protein concentration. In the first year, one of the transgenic lines produced seeds with an increased protein concentration, and a second year of field studies was conducted. Seed analyses from the second year are ongoing.

In addition, a number of transgenic soybean plants overexpressing amino acid transporters in the phloem and seed cotyledons have been developed, including transgenic lines which overexpress a single transporter or contain a combination of two transporters. These transgenic plants are currently being advanced to the next

**Figure 1.** Soybean transformation: shoot induction, shoot elongation and rooting.

generation, and the impact of overexpression on soybean protein composition and concentration, as well as yield, will be determined.

Enhancing soybean nitrogen acquisition, nitrogen allocation to seeds, and seed sulfur-containing amino acid concentrations will in turn increase soybean nutritional quality and thus the competitiveness of U.S. soybean. If verified under field conditions, the soybean lines generated by this project may translate into improved seed varieties, bring added value to soybean meal and subsequently even enhance demand. The continued investment into this research will ultimately result in improved soybean cultivars that will benefit soybean producers and their customers worldwide.

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| Proposal # | Target Area | Principal Investigator | Contractor | Funding |
|---|---|---|---|---|
| 1920-152-0107 | Meal | Locke, Anna | USDA | $130,728 |

# Improving Seed Protein Through Management and Understanding Nitrogen Metabolism

By Anna Locke, USDA-ARS

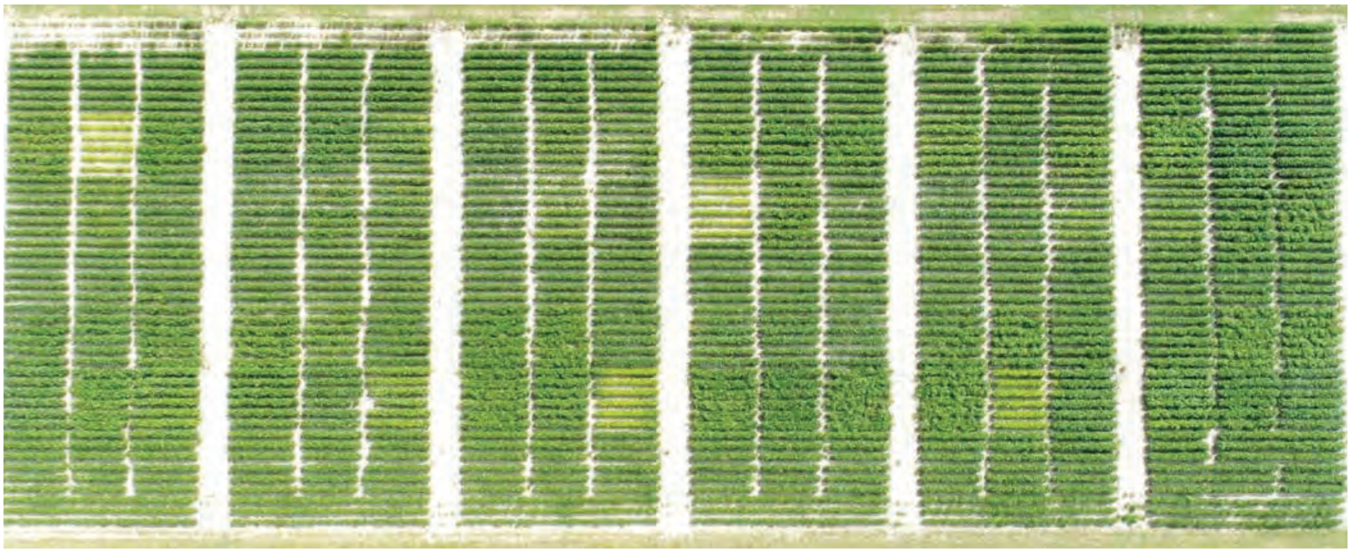
Protein-rich soybean meal is a premier feed source for poultry and livestock around the world. However, the concentration of protein in soybean seed has declined over the past few decades, and U.S. soybean has fallen below South American soybean in seed protein concentration. For U.S. soybean to maintain its value in the global marketplace, this trend of declining seed protein must be reversed. Seed protein concentration depends on both genetics and environment. Soybean breeders are working to genetically improve seed protein concentration, but breeding is a long-term process, and a more immediate response would be valuable. This project was funded for $130,728 in 2019 to investigate whether or not growers can improve seed protein concentration through environmental manipulations — specifically, by optimizing their farm management practices.

The objectives were to determine how farm management decisions, such as fertilizer application and tillage, impact soybean seed protein concentration in the final crop. Seed protein concentration is closely linked to nitrogen metabolism, so the impact of management practices on nitrogen metabolism in diverse soybean genotypes was investigated. Developing a better understanding of these factors, and the relationship between them, will give growers better information on how they can optimize seed composition on their own farms, and will help breeders develop new soybean varieties with higher seed protein.

The project began with a meta-analysis of peer-reviewed scientific data. Information was aggregated from over 70 studies that examined the impact of management on soybean seed protein content. The benefit of this strategy was that results based on data from multiple studies, conducted under a wide variety of conditions, are more likely to be useful for a wide range of growing environments and conditions.

In addition to the literature analysis, field studies were conducted to more closely examine the relationships between environment and seed protein concentration. In 2018, testing began on the impact of nitrogen application, sulfur application, tillage and irrigation on seed protein concentration in soybean. These tests were conducted at multiple locations in both North Carolina and South Carolina. Physiological and agronomic data, such as pod number, yield and quality (protein and oil), were collected in six soybean genotypes (Benning, Benning Hi-Pro, Nitrasoy, AG59X, NLM09-77, N7003CN) at four sites in the two states. In 2019, a plant density test was added to the project, and again data was collected for six genotypes at six sites representing diverse soil types throughout the Carolinas. Samples from the 2019 soybean harvest are currently being processed. Data from both years across multiple sites will be analyzed to provide new insights into seed protein concentration.

This project will have both short-term and long-term impacts for growers. Upon completion, the data from this project will identify management factors that influence soybean protein concentration, which growers can immediately use to make decisions on their own farms. In the longer term, information this project uncovers about nitrogen metabolism and differences in physiological and agronomic responses among soybean genotypes will improve efforts to increase soybean seed composition through conventional breeding and biotechnology-based improvements.



Photographer credit: Jeremy Martin (jeremy_martin@ncsu.edu)

**Figure 1.** Drone-captured image of six soybean genotypes under three different fertilization treatments at Sandhills Research Station in Jackson Springs, North Carolina, in August 2018.

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| FY19 SUPPLY RESEARCH | Proposal # | Target Area | Principal Investigator | Contractor | Funding |
|---|---|---|---|---|---|
| | 1920-152-0108 | Meal | Ciampitti, Ignacio | Kansas State University | $431,344 |

# Effect of Cultural Practices on Soybean Seed Quality:
## A Review and Research Studies

By Dan Davidson, Illinois Soybean Association

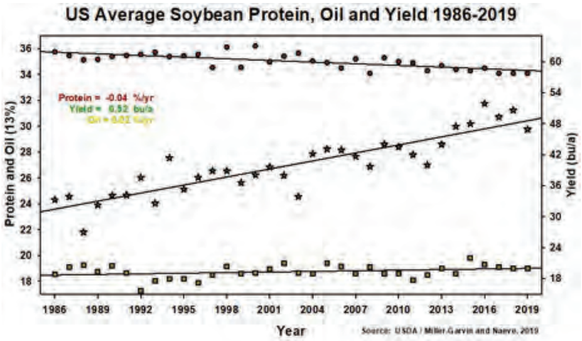
A team of researchers across the Midwest is exploring how today's management practices influence oil, protein and amino acid composition in soybeans. The goal is to identify practices growers can adopt that will improve the compositional quality of the soybeans they sell, and to share that information with farmers. This project was funded for $431,344 in 2019. It is in the second year of a three-year program.


Photo Courtesy of Peter Kovacs, South Dakota State University.

Figure 1.

Soybean is the second-most-popular crop grown in the U.S. (after corn), with over 80 million acres planted annually. Producers grow and market soybeans as a commodity, delivering directly to elevators or crushers. The soybean oil and protein serve as feedstock for a range of items including both animal and human protein products, edible and industrial oils, and other commercial products. Unfortunately, crude protein levels in U.S. soybean crops have declined below the 36% standard in recent years, as yields have increased while oil content has remained relatively stable.

Management practices are known to affect soybean yield. When growers adopt the best practices, and when weather cooperates, they can sometimes break the 100-bushel-per-acre ceiling on yield. However, it is not well-understood how these improved practices impact the protein and amino acid composition in soybean seed. For comparison, in bread wheat, it is well-known that timely applications of nitrogen and sulfur can improve seed protein content. For protein levels and amino acid content in soybean, it has long been known that both weather and genetics play a role, but how management practices impact these two components has not been well-established.



Source: USDA / Miller,Garvin and Naeve, 2019

Figure 2.

This project has three main goals: to use data from past field studies to evaluate the impact of cultural practices on protein content; to conduct field studies to identify best management practices that improve protein and essential amino acids; and to conduct a survey to assess grower knowledge and attitudes about seed quality and what practices could impact soybean seed composition at harvest.

A meta-analysis of previously collected data was undertaken to study and identify management practices that could affect composition. The goal was to identify practices that growers could adopt to sustain the recent gains in soybean yield, while at the same time improving or maintaining high levels of protein in the harvested seed. In U.S. soybean crops, it is known that protein levels can vary with geography. However, changes in amino acid composition across locations had not previously been studied. By analyzing the results of studies conducted across 14 states from 2012 to 2016, a trend was identified, with greater amino acid concentrations in southern than in northern latitudes. This work was published and shared widely with growers through extension activities.

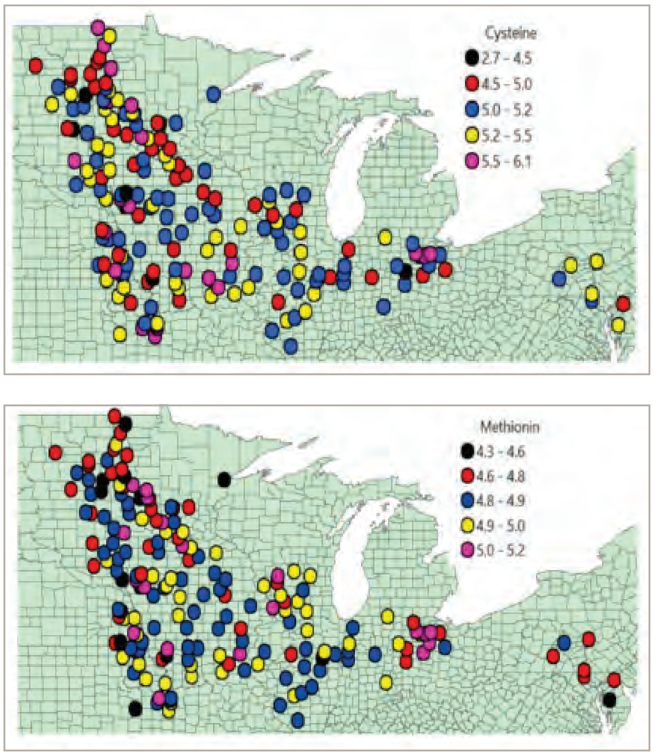


**Figure 3.** Spatial variation of essential amino acids cysteine and methionine (two of the amino acids that make up protein) in U.S. soybeans.

The investigation also focused on determining how management practices influence protein levels. While environment remains the dominant factor determining protein concentrations from year to year, management practices do have an impact. Some of the findings are listed below.

- Diverse crop rotations can improve protein levels.
- Application of small amounts of nitrogen (< 50 lb./acre) improved both seed protein content and amino acid composition.
- No effects on protein concentration were evident from today's most popular practices, including no-till, early planting, lower populations and narrower rows, seed treatment, foliar protection and foliar feeding.
- Maturity group (MG) selection did not influence amino acid composition in the northern states, but it did in the southern states, with longer MGs showing a decline in oil and an increase in protein.

One of the strategies that many growers practice is treating soybean seed with a rhizobia inoculant to improve nodulation and nitrogen fixation and yield, which might be expected to improve protein and amino acid quality. A comparison was made between no inoculation, seed inoculation, seed and soil inoculation at V4, and seed and soil inoculation at R1, and no effect was found under any conditions. The conclusion was that in fields where soybeans were previously grown, and under ideal conditions with no stress, there was no benefit from inoculation or co-inoculation on seed quality.

**Figure 4.**

Data analysis continues, and new results will be published and reported as they are obtained.

Improving the protein content and oil quality has long been a mission of the soybean checkoff, in order to increase the value of U.S. soybeans in a competitive market. Growers can currently buy varieties that have greater protein levels, and soon they may be able to choose better management practices that can further improve the quality of their seed products.

"We still have a lot to learn about improving quality, but this is a great first step," said Dr. Ignacio Ciampitti, farming systems professor in the department of agronomy at Kansas State University and principal investigator on this project. "Quality is the result of multiple factors. We need more knowledge to understand the complexity involved in choosing both the right variety and selecting and selecting the right management practices to optimize protein under current environmental conditions."

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| **FY19** SUPPLY RESEARCH | Proposal # | Target Area | Principal Investigator | Contractor | Funding |
|---|---|---|---|---|---|
| | 1920-152-0112 | Meal | Yin, Frank | University of Tennessee | $33,961 |

# Impact of Sulfur Fertilization
## on Soybean Seed Protein and Amino Acids and Yield

By Sangeeta Bansal, Xinhua Yin and Angela McClure, The University of Tennessee

Sulfur is a component of the amino acids methionine and cysteine, and is required for protein synthesis. Protein is one of the most valuable components of soybean seeds, but in recent years the protein content of U.S. soybeans has been declining, significantly devaluing the soybean products. In addition, a decrease in sulfur content has been recently noted in crops in Tennessee and nationwide, likely due to the increased use of sulfur-free fertilizers, less sulfur deposition from the atmosphere to the soil, greater sulfur removal from soil by crops and decreased use of sulfur-containing pesticides. However, there is little information available about the impact of sulfur fertilization on soybean seed protein and amino acid content.

In 2018, two soybean trials were conducted on low-sulfur fertility fields at Jackson and Milan, Tennessee, with the support of the Tennessee Soybean Promotion Board. In 2019, two trials were carried out on low- and medium-sulfur soils at the Agricenter International in Memphis, Tennessee, which were funded by the United Soybean Board at the $33,961 level. All trials had the following treatments:

1) 0 lb. sulfur/acre (control)
2) 10 lb. sulfur/acre soil applied at planting by soil application
3) 20 lb. sulfur/acre soil applied at planting by soil application
4) 30 lb. sulfur/acre soil applied at planting by soil application
5) 20 lb. sulfur/acre applied with sprinkler irrigation (fertigation) at the fourth leaf growth stage (V4)
6) 20 lb. sulfur/acre applied with sprinkler irrigation (fertigation) at the beginning of the pod development stage (R3).

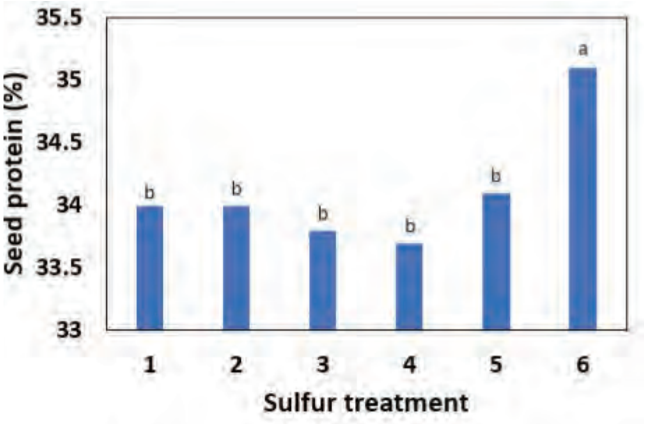

**Figure 1.** Effect of sulfur application on seed protein content at harvest at Milan, KY in 2018. Statistically significant difference is indicated by letters above the bars. Bars that are statistically similar are marked with the same letter; bars with statistical differences are marked with different letters.

The 2018 results generally showed that irrigation in the early growing season had a negative effect on leaf color and plant growth, possibly due to lower soil temperatures caused by irrigated water. Delaying application to the pod development stage caused light leaf color during the early growing season, but increased the use efficiency of the applied sulfur fertilizer. Seed yields were lower under the fertigated sulfur treatments (treatments 5 and 6). All five test conditions resulted in increased seed sulfur concentration on fields with low sulfur fertility. Residual soil sulfur level was higher under higher sulfur application rates (20 and 30 lb./acre) relative to zero sulfur after harvest.

In terms of seed quality, 20 pounds sulfur/acre applied via fertigation at R3 (treatment 6) resulted in higher protein levels than other treatments at Milan, which suggests that only sulfur application in late growing season is beneficial for protein. At Jackson, protein levels did not change.

**Figure 2.** Soybean 14 days after the fourth leaf growth stage on a medium sulfur soil in Memphis, TN in 2019.

The differential responses between locations could be due to the lower initial soil sulfur content at Milan. Application of 20 lb. sulfur/acre via fertigation at R3 resulted in the lowest linolenic but highest oleic level at Milan in 2018, which suggests that only sulfur application at late growing season is beneficial for oleic.

The results of the 2019 tests revealed that all five test conditions caused small, statistically insignificant increases in leaf chlorophyll content, plant growth, plant sulfur nutrition and seed yield on both low-sulfur and medium-sulfur soils. The seed samples from these trials are currently undergoing quality analyses.

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**FY19 SUPPLY RESEARCH**

| Proposal # | Target Area | Principal Investigator | Contractor | Funding |
|---|---|---|---|---|
| 1920-152-0113 | Meal | Graef, George | University of Nebraska | $524,867 |

# Increasing Genetic Diversity, Yield and Protein of U.S. Commercial Soybean Germplasm

By George Graef, University of Nebraska-Lincoln

The ability to improve soybean qualities by breeding new varieties depends upon sufficient genetic diversity. The U.S. soybean commercial germplasm currently contains just 17 accessions that contribute nearly 90% of the genes. With 45,000 soybean accessions available in world collections, and more than 22,000 in the USDA Soybean Germplasm Collection, making intelligent choices about which soybean plant introductions (PIs) will best contribute to enhancing yield and compositional quality is crucial. In addition, strategies and germplasm that will complement and enhance commercial breeding efforts for the long term must both be developed. Besides limitations on increasing yield, the narrow genetic base of soybean affects potential improvements in seed composition and other traits.

The general negative correlation between yield and seed protein concentration resulted in a protein decrease of about 2 percentage points over the last 85 years of breeding, with associated increases in seed oil concentration and yield. Unless breeders refocus efforts on protein concentration and overall balance of soybean seed composition, this decrease in seed protein concentration will likely continue, to the point where U.S. soybean is at a disadvantage in the global marketplace.

It is not sufficient to increase seed protein concentration alone without considering the correlated changes that occur to seed oil, soluble carbohydrates and other traits. Soybean processors expect 10 to 12 pounds of oil per bushel of soybean, and need to produce a soybean meal with 48% protein (47.5% to 48.5% to allow for measurement error). However, there is a wider range of soybean seed protein and oil concentrations that will result in the desired products. Therefore, estimates of seed protein, oil and carbohydrate concentrations must be considered, as well as processed yield of seed oil and 48% protein meal per bushel.

This comprehensive and coordinated $524,867 program includes applied development efforts to transfer genetically diverse, high-yielding lines with an improved nutritional bundle to commercial partners. It includes basic research to identify genes and gene functions related to seed composition, and especially to high protein.

### PROTEIN AND OIL COMBINATIONS

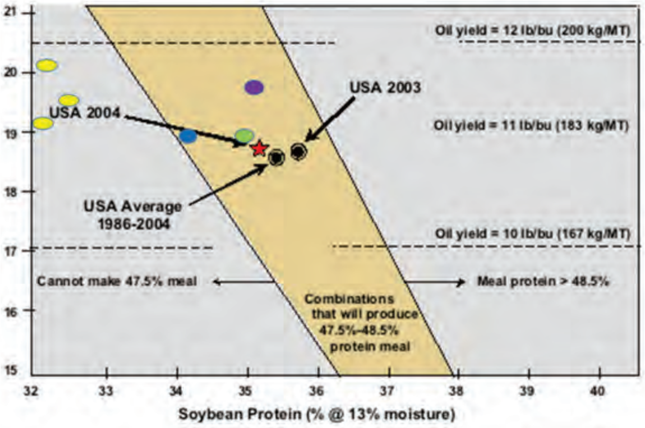

**Figure 1.** Seed protein and oil concentration values that will produce a 48% protein meal (shaded tan area). Processed oil yields of 10 and 12 pounds per bushel are shown by the dashed horizontal lines (From Brum, et al., 2005). Historical USA averages are shown in black circles for 1986-2004. The red star shows the USA 2004 average. Filled circles show Diversity Protein Tests averages for 2016 (green), 2017 (blue) and 2018 (purple). In 2017, a low-protein year overall, the high-yield checks in the USDA URT MGII, III and IV are shown in yellow circles — none made a 48% protein meal. The average of entries in the 2018 Diversity Protein Tests (purple circle) have greater value compared with the USA 2003, 2004 and long-term averages, producing at least a 48% protein meal, with higher yield and higher oil percentage. That's progress!

This project had four major objectives.

1) Use novel PI accessions from the USDA Soybean Germplasm Collection for yield improvement to develop and release soybean lines with significantly increased genetic diversity,

greater productivity, higher seed protein concentration and improved nutrition bundle. Industry partners are involved for wide-area cooperative evaluation throughout Maturity Groups 0, I, II, III, IV and V.

2) Identify and characterize candidate genes for the large-effect quantitative trait loci (QTLs) on Chr 15 and Chr 20, to evaluate the effects of gene candidates in different genetic backgrounds in greenhouse and field environments. Analyze the genetically diverse germplasm developed by all cooperators for allele status at the Chr 15 and Chr 20 QTLs.
3) Characterize protein composition, amino acid profiles and soluble carbohydrate composition of seeds from developed high-protein, high-yield lines from diverse G. max accessions and of selected experimental extreme high-protein lines from long-term selection programs.
4) Increase genetic diversity, seed composition and seed quality in the early-planting soybean production system (ESPS) in southern U.S. production areas.

This project will broaden the genetic base of U.S. soybean to allow sustained, long-term improvements in both yield and quality by encouraging the active introgression of novel soybean germplasm into soybean variety development programs. Because many of these diverse soybean accessions held by the Collection are not adapted to the U.S., and have not been improved upon by modern breeding, a large portion of the material resulting from these crosses is currently not suitable for modern production. Through screening and selection, breeding lines with high value in both yield and quality can be identified. After these germplasm enhancement activities, a critical action will be to share this germplasm with the seed industry through germplasm exchange and cooperative testing programs. This public-private sector partnership will advance long-term diversification of the breeding germplasm of U.S. soybean, and ultimately help deliver ever higher-yielding varieties with enhanced seed composition profiles.

**Figure 2.** Seed quality of experimental line DS25-1 compared with a commercial check.

The project is well on its way to success. The 2019 Diversity Cooperative Tests included 340 candidate varieties tested at 20 unique sites spread across seven states. Through cooperative testing with the university, USDA and industry partners, several new breeding lines in all maturity groups were identified that are superior both in terms of yield and expected meal protein relative. Some of these breeding lines are entered into USDA Uniform Soybean Tests, and have shown superior yield and composition compared with the high-yield checks in those tests. Significant progress has been made for improved yield and seed quality in the South, with high-yielding lines similar to commercial checks and a 75% reduction in monetary losses from seed quality issues.

This work has already led to new high-yielding, high-protein breeding lines that collectively include 72 new soybean accessions in their pedigrees, representing a four-fold increase in the number of accessions underlying the U.S. soybean genetic base. These new lines are being transferred to commercial companies with material transfer agreements. In the long term, these efforts are crucial and will continue to expand the genetic base of elite U.S. soybean, allowing for sustained, long-term success and profitability for U.S. soybean producers far into the future. Continued partnership with the seed industry through cooperative testing and germplasm exchange will remain a cornerstone of this project.

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| Proposal # | Target Area | Principal Investigator | Contractor | Funding |
|---|---|---|---|---|
| 1920-152-0114 | Meal | Miller, Amy | SGI/Benson Hill | $577,512 |

# Genetic Improvement of Seed Protein: Development of High Protein and High Sucrose Soybean Varieties with Highly Competitive Yields

By Amy Miller, Kevin Cook and Bill Rhodes, Benson Hill Seeds

The development of a high-performance soybean variety with high-protein, ultra-low raffinose-stachyose (two indigestible sugars) and high sucrose (digestible sugar) would be a great bonanza for the entire soybean industry. If these soybean varieties could also have desirable agronomics including yield, disease and nematode resistance, they would undoubtedly become the preferred soybean for animal feed and other uses. For over 18 years, this aggressive cross-breeding program has been working to develop such a line of germplasms, and incorporate them into commercial varieties.

It is well-documented that protein levels in soybeans have been decreasing since the 1980s, so developing lines with high protein content is now a priority. By testing for protein levels in early generations, the number of experimental lines that contain better-than-average protein (40% on a dry weight basis [DB]) has been increased.

Breeding for protein content is often a challenge because increasing the protein content almost always means lowering the crop yield. Through careful breeding, yields have been maintained at acceptable levels while incremental improvements to protein levels were being made. Each summer in the fields, and in winter nurseries, new combinations of distinctly different parents are crossed to ideally create enhanced offspring. Each new cycle uses a building block approach, combining new protein lines with each other to advance the higher protein materials while simultaneously making other improvements. Once a population reaches the F3 generation, a protein content analysis is run on each individual plant in that population using near-infrared (NIR) analysis.

Soyl G2014

| | |
|---|---|
| Protein_DB | 45.13% |
| Oil_DB | 18.65% |
| Protein_13% | 39.27% |
| Oil_13% | 16.23% |
| Moisture | 11.76% |
| Fiber_DB | 5.64% |
| Ash_DB | 5.33% |

**Figure 1.** NIR instrument used to measure protein and oil.

Plants that have lower than the desired protein levels are discarded, and not considered as future breeding stock, ensuring that the nursery and testing program is highly efficient. The table below shows one key protein variety, N35D950S, as compared to the currently available commercial checks.

In 2019, this program was funded by USB at the $577,512 level, and provided crucial support for this small company, allowing them to advance and increase varieties with winter productions. In 2019, about 40% of the seedstock production contained these higher protein varieties. Additional new varieties will be products in 2020 and 2021, helping Benson Hill Seeds maintain a leadership in the industry for high protein varieties.

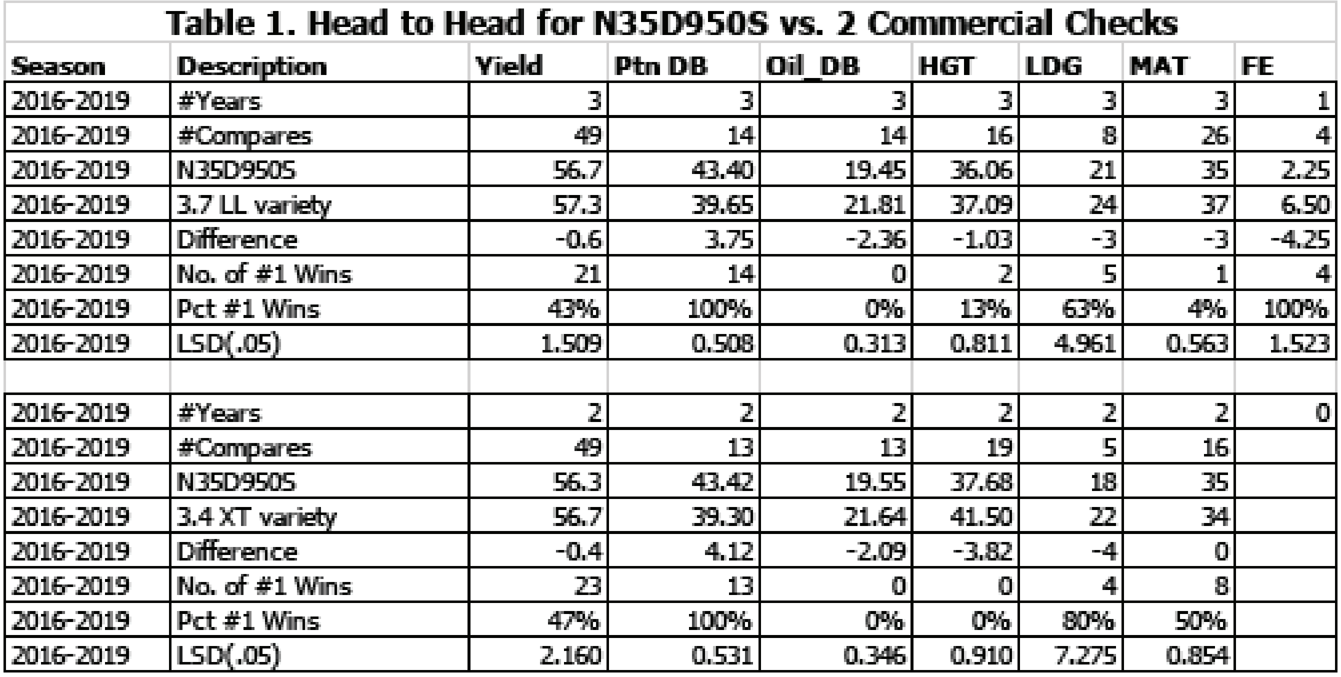

**Table 1. Head to Head for N35D950S vs. 2 Commercial Checks**

| Season | Description | Yield | Ptn DB | Oil_DB | HGT | LDG | MAT | FE |
|---|---|---|---|---|---|---|---|---|
| 2016-2019 | #Years | 3 | 3 | 3 | 3 | 3 | 3 | 1 |
| 2016-2019 | #Compares | 49 | 14 | 14 | 16 | 8 | 26 | 4 |
| 2016-2019 | N35D950S | 56.7 | 43.40 | 19.45 | 36.06 | 21 | 35 | 2.25 |
| 2016-2019 | 3.7 LL variety | 57.3 | 39.65 | 21.81 | 37.09 | 24 | 37 | 6.50 |
| 2016-2019 | Difference | -0.6 | 3.75 | -2.36 | -1.03 | -3 | -3 | -4.25 |
| 2016-2019 | No. of #1 Wins | 21 | 14 | 0 | 2 | 5 | 1 | 4 |
| 2016-2019 | Pct #1 Wins | 43% | 100% | 0% | 13% | 63% | 4% | 100% |
| 2016-2019 | LSD(.05) | 1.509 | 0.508 | 0.313 | 0.811 | 4.961 | 0.563 | 1.523 |
| | | | | | | | | |
| 2016-2019 | #Years | 2 | 2 | 2 | 2 | 2 | 2 | 0 |
| 2016-2019 | #Compares | 49 | 13 | 13 | 19 | 5 | 16 | |
| 2016-2019 | N35D950S | 56.3 | 43.42 | 19.55 | 37.68 | 18 | 35 | |
| 2016-2019 | 3.4 XT variety | 56.7 | 39.30 | 21.64 | 41.50 | 22 | 34 | |
| 2016-2019 | Difference | -0.4 | 4.12 | -2.09 | -3.82 | -4 | 0 | |
| 2016-2019 | No. of #1 Wins | 23 | 13 | 0 | 0 | 4 | 8 | |
| 2016-2019 | Pct #1 Wins | 47% | 100% | 0% | 0% | 80% | 50% | |
| 2016-2019 | LSD(.05) | 2.160 | 0.531 | 0.346 | 0.910 | 7.275 | 0.854 | |

**Figure 1.** N35D950S head-to-head comparison. Ptn — protein; HGT — height; LDG — lodging; MAT — maturity.

In the near future, this work will benefit growers by allowing them to produce soybean varieties with a premium demand by both crushers and end users, rather than commodity varieties. More importantly, higher protein levels in U.S.-produced soybeans will help to increase the demand for the U.S. product on the worldwide market, leading to higher prices for U.S. growers. Ultimately, this will allow the U.S. to maintain its reputation of higher-quality soybean quality in a very competitive global market.

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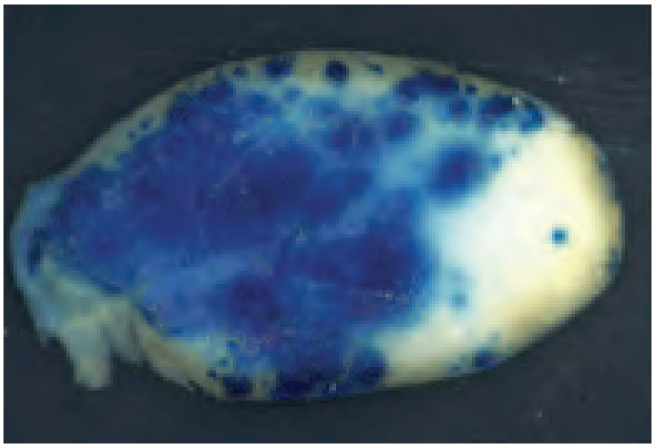
| Proposal # | Target Area | Principal Investigator | Contractor | Funding |
|---|---|---|---|---|
| 1920-152-0120-A | Meal | Parrott, Wayne | University of Georgia | $440,078 |

# New Breeding Technologies Applied to Meal

By Wayne Parrott, University of Georgia

## ENABLING TECHNOLOGY STARTS WITH USB-SPONSORED RESEARCH

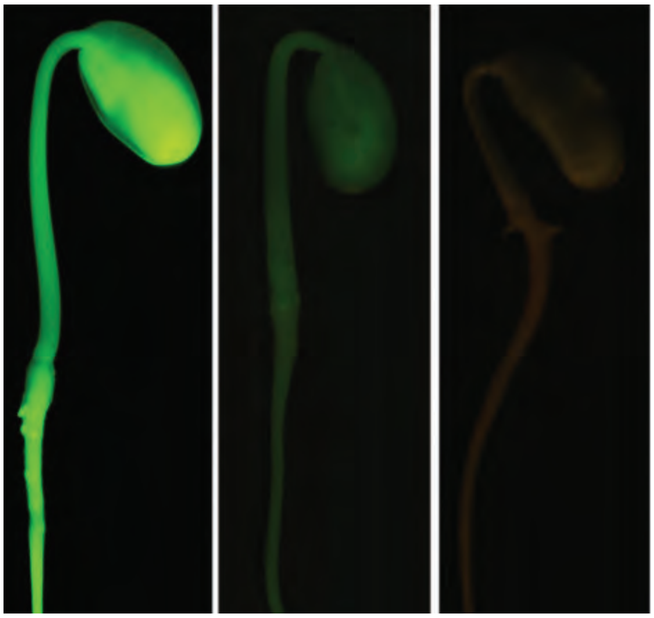
The advent of herbicide-tolerant soybeans contributed over $13 billion to US soybean farmers between 2006 and 2016. While these varieties are released and marketed by major seed companies, much of the necessary technology used to develop them comes out of university labs. So the next time you buy a bag of biotech beans, remember, USB-funded projects under the rubric of the Center for Soybean Tissue Culture and Genetic Engineering Center helped make it possible. A total of $440,078 of that funding helped support this project.



Getting DNA into a cell is just the first hurdle. Technology called "sonication-assisted Agrobacterium-mediated transformation" has been licensed to major companies, who then pay royalties to USB. In this photo, the color blue is an indication that DNA successfully entered the cells of an immature seed.

Once DNA has been inserted into a cell, the next challenge is to get a whole plant back. USB-sponsored research developed protocols to get whole plants back from cultured cells. The photo shows the process as a cell becomes a sphere of cells that grows into something more recognizable.

Once engineered into a plant, a transgene will not function without the proper promoter, which is what the gene's control switch is called. The photo shows green fluorescent protein expression with the industry standard promoter (CaMV 35S) compared with the GmubiXL promoter, which was discovered by USB-sponsored research and is now being licensed to companies.

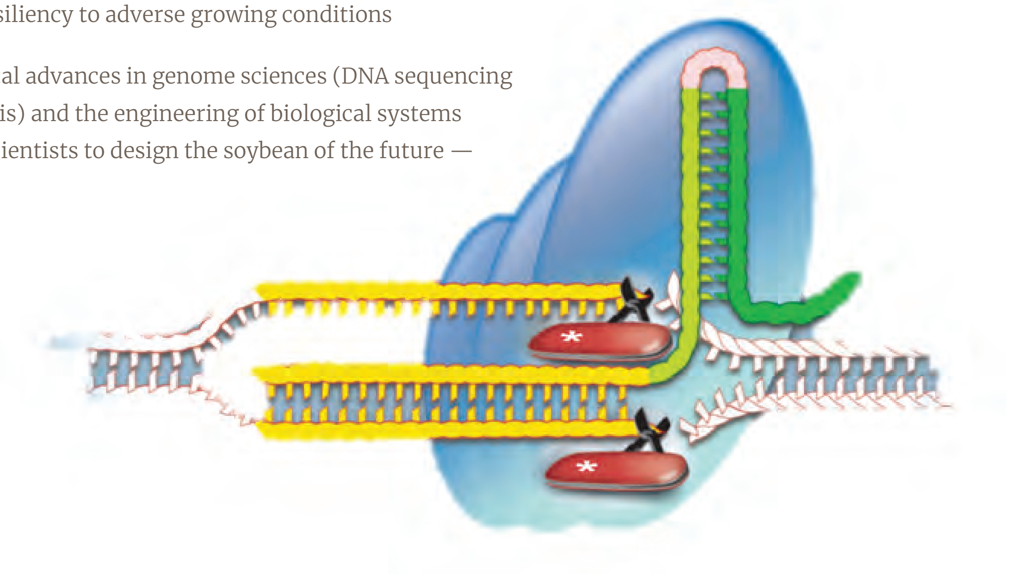


## MOVING FORWARD

All the technology previously used for engineering sets the stage for CRISPR genome editing. In turn, editing enables new breeding objectives that have been impossible or very difficult to obtain. Toward that objective, a toolbox of multiple Cas types to use with CRISPR is available for soybean. This is the main tool needed to:

- Alter protein/oil ratios
- Improve the quality of soybean protein
- Improve photosynthesis and thus yield
- Improve resiliency to adverse growing conditions

Technological advances in genome sciences (DNA sequencing and synthesis) and the engineering of biological systems will allow scientists to design the soybean of the future — with improved biological efficiency and more resilience — and will provide the raw materials needed by industries to provide sustainable alternatives to plastics. Every part of the plant will be designed with optimal qualities for targeted use and applications. There is a recognition that "the world needs plants that can be used fully, to the last molecule," and in the process deliver added value to farmers. Such designer crops would enable biomanufacturing to thrive in rural areas and fuel the bioeconomy in what has been referred to as a "bio-belt." The soybean may turn out to be the best crop to power the bioeconomy.



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**FY19** SUPPLY RESEARCH

| Proposal # | Target Area | Principal Investigator | Contractor | Funding |
|---|---|---|---|---|
| 1920-152-0120-B | Meal | Stacey, Bing | University of Missouri | $113,532 |

# Genome-Guided Modification of Soybean Meal Composition

By By Minviluz G. Stacey, University of Missouri

Of the 20 amino acids, there are a smaller number that humans and animals cannot synthesize, and thus must be obtained from their diet. These are called essential amino acids. All amino acids accumulate in seed storage proteins such as 7S and 11S, whose main function is to sequester nitrogen, carbon and sulfur in the form of proteins, or chains of amino acids. The seed storage proteins found in soybean meal are the major source of amino acids for livestock consumption worldwide, including both essential and non-essential amino acids. Compared to other common plant-based protein feedstocks, soybean meal is superior in terms of both crude protein content and the availability of total and digestible amino acids. However, the levels of the sulfur-containing amino acids, namely methionine and cysteine, in soybean seeds are sub-optimal. Recognizing the need to improve the quality of soybean meal, one of the goals of this project is to increase the content of sulfur-containing amino acids in soybean seeds by at least 1.5-fold.

To achieve this goal, $113,532 was allocated in 2019, and a potentially promising “push-pull” approach was developed. The end goal is to modify the genetic structure in order to alter the seed metabolism to favor the production of seed storage proteins with higher levels of sulfur-containing amino acids. There are three parts of this strategy: production of high levels of free methionine; production of reduced amounts of methionine-poor 7S soybean seed storage protein; and increased amounts of methionine-rich 11S storage protein, along with production of modified 11S (i.e., GY1) storage proteins with an increased number of methionine codons.

**Figure 1.** CRISPR/Cas9-edited soybean growing under greenhouse (A) and field (B) conditions

To increase free methionine levels in soybean seeds (trait 1, high free-met), the production of enzymes that degrade methionine was knocked out, thereby increasing methionine availability in seeds for use in protein biosynthesis. CRISPR-edited plants were grown in the field, and the free methionine in their seeds was measured. The high free-met seeds indeed contained about a six-fold higher free methionine when compared to wild-type unmodified seeds. Free methionine levels are very low, so a six-fold increase is not enough by itself to reach the goal.

To obtain genotypes with the second desired trait (reduced 7S and increased 11S production), the six genes encoding the 7S protein subunits (α, α' and β) were knocked out using CRISPR/Cas9 mutagenesis. By knocking out the methionine-poor 7S proteins, the production of methionine-rich 11S proteins were hypothesized to be increased through a process called protein rebalancing. The CRISPR-edited plants with 7S subunit knockouts were successfully developed (Figure 2A) and were planted in the field. Amino acid analysis showed, on average, 0.50-fold or 0.34-fold increase in total methionine content in seeds lacking α+α'+β or α+β 7S subunit proteins, respectively. Overall protein and oil content did not change in the 7S knockouts. This helps us to reach the goal of a 1.5-fold increase in total methionine, but by itself is probably not enough to reliably reach the target methionine levels.

To generate soybean with the third desired trait (increase in methionine-rich storage protein), three versions of Gly1 gene with increased met codons were designed. While the wild-type GLY1 protein in conventional soybean contains 1.4% methionine, all three modified versions were designed to have higher methionine levels: GLY1 (Ins) with 4.3% methionine, GLY1 (Mod1) with 7.2% methionine and GLY1 (Mod2) with 2.5% methionine. The transgenic plants expressing GLY1 (Mod1) in soybean seeds were obtained and planted in the field. Amino acid analysis of seeds showed up to a 50% increase in total methionine content.

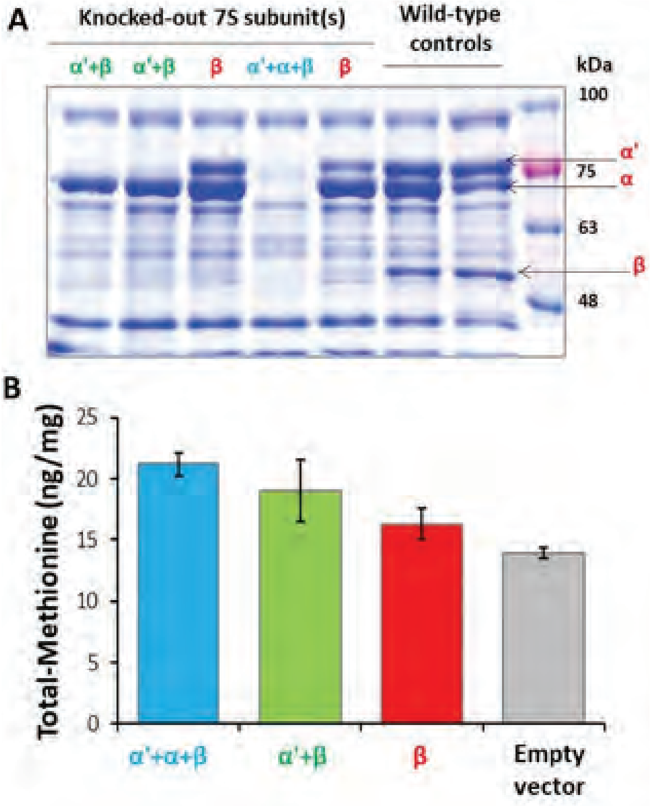

**Figure 2.** Generation of soybean genotypes lacking 7S proteins.
A. SDS-PAGE of seed proteins. B. Methionine content of 7S knock-out mutants.

In this project, techniques and reagents for high-efficiency CRISPR/Cas9-mediated gene knockout in soybean were developed, and in two years, eight genes were successfully and simultaneously knocked out, creating plants homozygous for all eight genes. The soybean genotypes that have been generated are all single-trait plants having one of the three characteristics identified as desirable. In the next phase, the three traits will be stacked to obtain further increases in seed methionine content, with the goal of achieving a 1.5-fold increase in seed methionine.

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| **FY19** SUPPLY RESEARCH | Proposal # | Target Area | Principal Investigator | Contractor | Funding |
|---|---|---|---|---|---|
| | 1920-152-0120-C | Meal | Lassner, Michael | Amfora, Inc. | $349,874 |

# Gene Editing to Increase Soy Protein Content

By Michael Lassner, Amfora, Inc.

Over the past 40 years, the average protein content of U.S. soybeans has decreased significantly as the major seed companies breed for yield at the expense of protein. This has reduced the competitiveness of U.S. soybean meal as a feed ingredient compared to alternative feed components.

This research is developing and commercializing gene-editing technology to increase the protein content of soybean in pursuit of two major products.

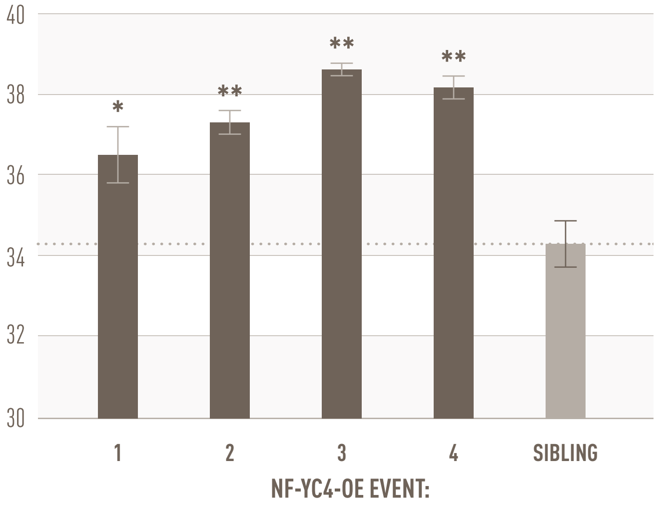
Amfora's first soybean product will be high-protein beans, with protein increased by at least 10% but with minimal impact on oil or yield. These will be broadly licensed to U.S. soybean seed companies to improve the value of commodity beans. This will maintain the status of U.S.-grown soybean as a preferred source of high-protein meal for use in animal feed, increase value across the supply chain and reverse the trend of decreasing protein content of U.S.-grown soybeans. They will also reduce the risk of lower prices and reduced inclusion rate of soybean meal in animal feed.

A second product, ultra-high-protein beans, will increase protein content by approximately 25%, to be an ingredient for plant-based meats and aquaculture feed. For these higher values, specialty markets, growers and crushers will be contracted to vertically integrate them as a high-value meal supplier to end users. The premiums should significantly increase growers' and crushers' profit margins.

This technology was licensed from Iowa State University (ISU), and the inventors continue to be involved. The original ISU work, funded by the United Soybean Board, demonstrated that GMO overexpression of QQS or NF-YC4, a genetic switch, could increase the protein content of soybeans by up to 18%. After several seasons of field growth, it was concluded that the increased protein could be accomplished with little decrease in oil or yield.

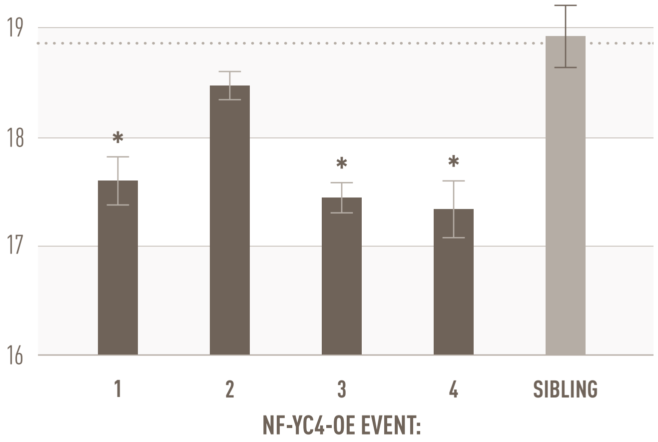
*Increased 6~12%*

**PROTEIN**



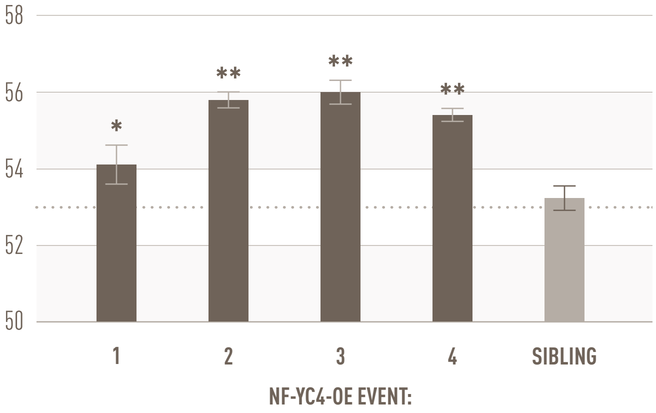
*Similar or Decreased*

**OIL**



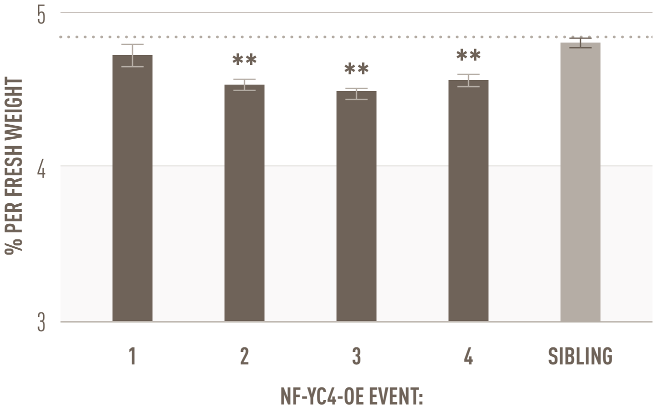
*Increased 5~7%*

**PROTEIN + OIL**



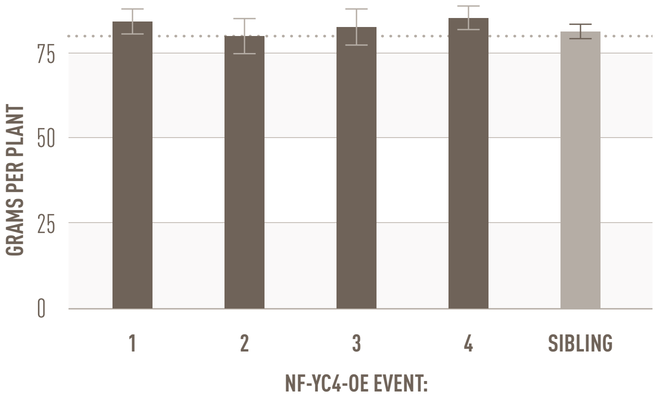
*Decreased 3.4 – 5.6%*

**SEED FIBER**



*Similar*

**SEED WEIGHT PER PLANT**



Transgenic overexpression of NF-YC4 increases seed protein content at the expense of fiber. The results above are from greenhouse-grown plants. NF-YC4 events exhibit no differences from controls in plant habit, flowering time or seed yield.

The ISU inventors also devised a strategy to use gene editing of the NF-YC4 gene to achieve similar results. Gene editing has both lower regulatory costs and faster timelines than traditional GMO technology. The USDA has adopted a policy of exempting from regulation gene-edited crops such as the soybean lines that are being developed. Since 2011, the USDA has responded to more than 75 requests affirming that products meeting certain criteria are not regulated. Canada, Brazil, Argentina, Chile, Japan, USSR and Australia also have published policies that gene-edited crops would not be regulated as GMOs.

Preliminary analyses of soybean lines grown in summer 2019 suggest that gene editing can increase the protein content of beans similarly to the transgenic lines described above. These lines are being planted in a 2019/2020 winter nursery to make homozygous stabilized lines that can be accurately assessed for protein increase and editing results. The ultimate goal is to provide edited lines that can be incorporated into breeding programs and commercialized as soybean varieties with higher protein content. In addition, backcrosses with food-grade beans will initiate the development of the ultra-high-protein beans destined as high-value ingredients for aquaculture and plant-based meats.

For company information, visit https://www.amforainc.com/.

| Proposal # | Target Area | Principal Investigator | Contractor | Funding |
|---|---|---|---|---|
| 1920-152-0127 | Meal | Cortasa, Salmeron Montserrat | University of Kentucky | $197,780 |

# How Does Management Increase Soybean Seed Protein?

## A Mechanistic Approach to Identifying Limitations and Opportunities

By Montse Salmeron, University of Kentucky

Developing strategies that ensure U.S. soybean is competitive in global markets is essential for soybean producers. Annual U.S. soybean yields have increased by 0.49 bu/acre annually since 1986; seed protein concentration has fluctuated but declined slightly over the same period from about 35% in 1986 to about 34% in 2019, which could make it less competitive in the global marketplace. Seed composition (total protein and oil, amino acid and fatty acid profiles) is affected by genetic, environmental and management factors. Therefore, a combination of breeding efforts to address the genetic component and site-specific management practices are required for optimal productivity and quality of soybean seeds.

While numerous genetic solutions are being developed, the potential effects of management and cultural practices on seed composition has received relatively little attention. Soybean producers use a wide variety of management practices that can influence nitrogen availability (i.e., use of cover crops, fertilizer and inoculant applications). Combining phenotyping tools with process-based eco-physiological models provides new opportunities for site-specific recommendations.

This $197,780 project specifically evaluated management practices that increase nitrogen availability during the seed-filling phase, quantifying changes after fallow vs. a winter cover crop, which affect the crop's need for biological nitrogen fixation early in the season. Aerial images were also used to quantify crop nitrogen status. The dataset generated will help improve carbon- and nitrogen-cycling predictive tools in soybean.

Soybean field trials were conducted during 2019, and a second set of trials is planned for 2020. Preliminary results support the idea that management and cultural practices increase crop productivity through an indirect effect on nitrogen cycling. Growing irrigated soybean after a winter cereal rye cover crop increased soybean yields in two out of six cultivars in Kentucky and Arkansas. Additionally, late nitrogen fertilizer applications increased yields in Arkansas and Minnesota by as much as 5 bu/acre. Analysis of soybean seed composition is currently underway.

This project will benefit U.S. soybean producers by providing information on how different management practices affect seed composition and yield, allowing them to make choices that could increase the value of the soybean meal they produce. The dataset generated by this project will be used by crop modelers to improve predictive tools for soybean carbon and nitrogen cycling, biological nitrogen fixation and seed composition. The evaluation of information collected from aerial images as diagnostic tools of nitrogen status will inform future research, allowing for site-specific recommendations.

Given the negative correlation between soybean yield and seed protein concentration observed over the last decade, it has become imperative to identify management practices that will increase productivity of high-quality soybean crops without loss of yield, while ensuring long-term economic and environmental sustainability.

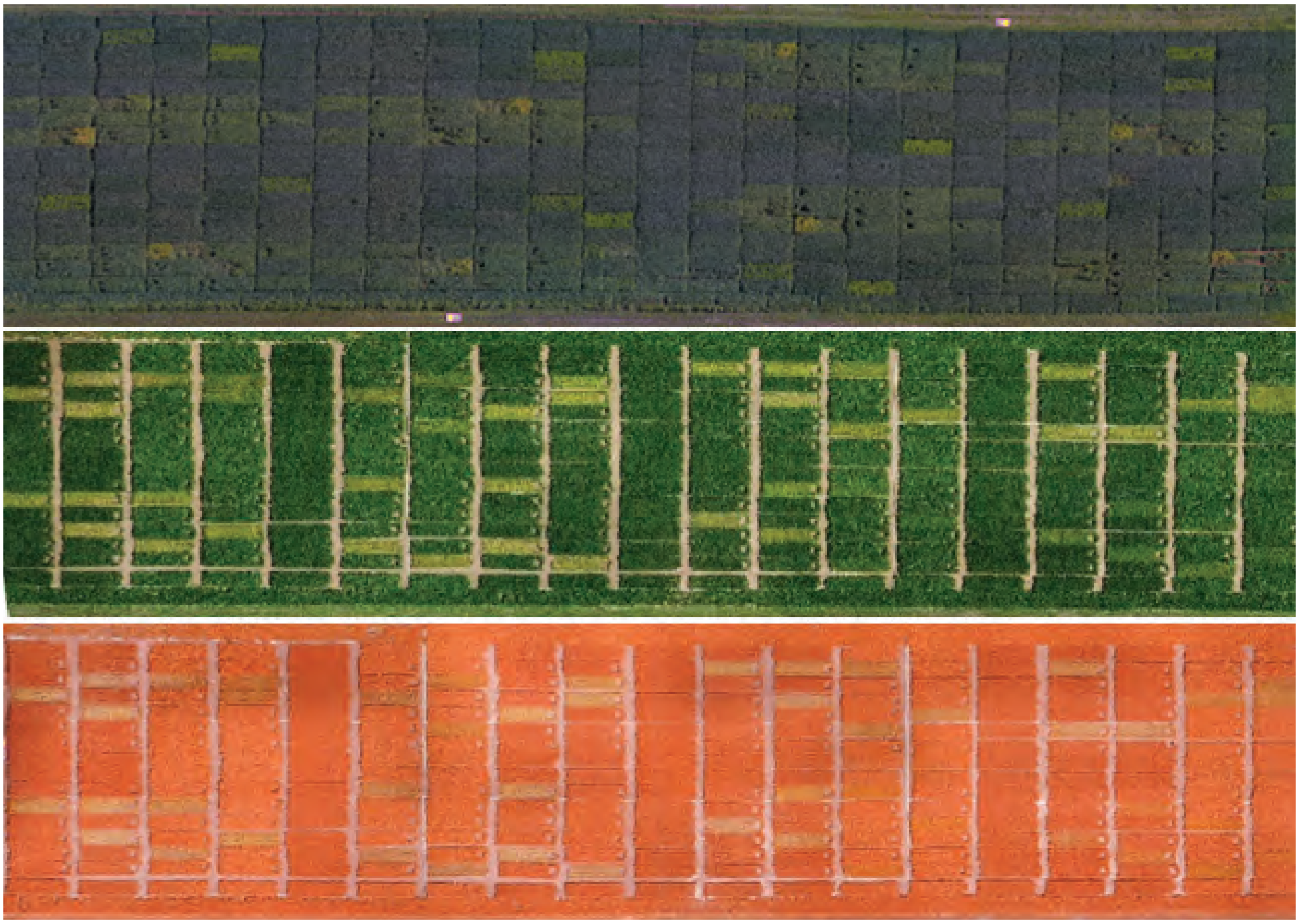


**Figure 1.** Aerial images of experimental plots collected in Kentucky (top) and in Arkansas (middle and bottom) during the soybean growing season, which were sampled for various laboratory analyses related to nitrogen status. The bottom panel is the normalized difference vegetation index (NDVI) using near infrared (NIR); the NDVI has arbitrarily been assigned an orange color in this image.

| Proposal # | Target Area | Principal Investigator | Contractor | Funding |
|---|---|---|---|---|
| 1920-152-0131-A | Meal | Kaeppler, Heidi | University of Wisconsin-Madison | $315,000 - 3 years |

# Genomic Tools to Enable Trait Discovery and Deployment - Genome Editing Pipeline Acceleration

By Heidi Kaeppler, University of Wisconsin

Two main methods can be used to modify traits in soybean seeds, genetic engineering (also referred to as "transformation" and genome editing.

Soybean transformation involves inserting gene sequences encoding a specific trait into a chromosome of a target cell and regenerating a plant in which all of the cells contain the gene insertion. The inserted genes can encode novel traits (for example, herbicide resistance), increase or alter expression of native traits (for example, grain quality), or reduce or eliminate expression of negative traits (such as trypsin inhibitor production).

In genome editing, gene sequences encoding the DNA-cutting enzyme, Cas9, and RNA "guide" sequences are transformed into soybean cells. As part of the cell's natural DNA repair processes, mutations in the target sequence can occur, resulting in shutting down expression of that gene, also called "knockout". More recently, genome editing has been used to cut DNA sequences in specific locations and insert new sequences into that location, which is useful for adding new genes/traits or for replacing the current gene sequence with an improved version.

Current public soybean transformation and editing systems are limited by genotype specificity, where only a few soybean lines or varieties can be successfully transformed and edited low efficiency and high cost, and the specialized expertise and precision required. This $105,000 project aims to develop and optimize novel soybean transformation and editing systems.

The initial focus was to develop a transformation system targeting transgene DNA delivery into soybean meristems (stem cell-containing growing points inside the shoot apex of the seedling within a soybean seed), and regenerating transgenic, clonal plants from the transformed cells. Using meristems allows regeneration of transgenic plants in almost any soybean variety and is much faster than traditional methods. The new system has been used to genetically engineer several soybean lines and varieties, as well as to deliver gene-editing components into soybean for editing applications. Initial results are positive, but further improvement in efficiency and breadth, to reduce both costs and complexity, are needed.

A second goal is investigating novel systems to make targeted DNA sequence changes, without integration of editing machinery into the soybean cell, by delivering the Cas9 protein and guide RNAs (and not their DNA sequences) into the target cells. The idea is to encapsulate the Cas9 protein and guide RNAs within extremely small, synthetic "nanoparticles" for delivery into cells. Work is underway to adapt existing nanoparticle-based systems from mammal to plant systems. If successful, this system would work with any soybean genotype, would provide efficient, rapid editing, and would be a "nonintegrating" method, meaning the resulting soybean germplasm would potentially have less regulatory and cost hurdles than traditional genetic engineering methods.

This project is part of the "Genomic Tools to Enable Trait Discovery" iPOP funded by USB in 2019. Overall, development and optimization of soybean transformation and editing systems should result in rapid advancement of soybean genetics and breeding efforts, through greater efficiency, access, and potential reduction of regulatory hurdles for the engineered/edited soybean lines. The Wisconsin Crop Innovation Center is a public fee for service transformation facility, and the knowledge gained will benefit all soybean researchers who use the facility.

**Figure 1.** Imbibed soybean seed, embryo with attached cotyledon, and isolated meristem-containing embryonic axis explants.

**Figure 2.** Transformed soybean plant with leaf discs removed for genetic analysis.

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| Proposal # | Target Area | Principal Investigator | Contractor | Funding |
|---|---|---|---|---|
| 1920-152-0131-B | Meal | Stupar, Robert | University of Minnesota | $314,998 - 3 years |

# Genomic Tools to Enable Trait Discovery and Deployment - Genome Editing Methods

By Robert Stupar, University of Minnesota

Genome editing methodologies offer great promise for trait enhancement in all crop species. However, each species faces unique challenges when adopting these methods. Even with CRISPR-Cas9, the most successful platform to date, efficient implementation and customized applications are required in order fulfill that promise. This project will develop enabling technologies specifically for soybean genome editing, that will optimize the development of new traits and make these tools more accessible to a wider range of soybean researchers.

There are several challenges that are particular to soybean. The first challenge is the efficiency of soybean regeneration. When a novel gene or a gene edit is introduced to a plant, it is generally introduced into a single cell. That cell is then treated with nutrients that allow it to divide and eventually become an entire new plant. Even in the best cases, this process of regeneration is not very efficient; so many attempts are required to get a new plant that expresses the desired genetic modification. In soybean, the process is complicated by the fact that only some genotypes (cultivars) are easily regenerated. Ideally, conducting the desired genetic modifications in modern, high yielding, elite commercial soybean would be preferable. However, because of this challenge, the modifications often have to be made in older lines (such as Williams 82) which are then crossed with elite soybeans to make a new commercial variety. This project will address this challenge by making plant regeneration more efficient and more general for a variety of different genotypes.

Another challenge is that the CRISPR-Cas9 system itself is not very efficient. The easiest modifications to make with the system are small cuts in the DNA that result in a non-functioning gene (also called a knock out). It is more difficult to make precise changes or to replace a small segment of undesired DNA with a segment of desired DNA. As with regeneration, if efficiency is low, more and more attempts need to be made in order to achieve the desired result. This increases the time and cost needed to introduce new traits. This project is experimenting with ways to increase this efficiency, particularly when precise changes that do not eliminate gene function, but instead change it, need to be made.

Lastly, entirely new ways of changing gene expression are being investigated. Building on past genomic research, some funded by USB, the researchers are looking beyond just DNA sequences to find methods that will turn up or turn down gene function and expression. One could envision this as turning on a faucet. You always get water coming out, but depending on how far you turn the knob you can get more or less water. A method to precisely determine the timing and/or amount of gene expression could be very powerful.

This project was funded by USB for $104,999 in 2019, and is a part of the iPOP (innovative project) called Genomic Tools to Enable Trait Discovery. Once developed, these tools will protected by patents where appropriate, and could then be licensed or otherwise made available to public and private researchers.

**Immature seeds (10-14 DAF)**

**24 hrs. after transformation**
(transformation freq. ~45%)

Protoplast isolation and transformation using green-fluorescent protein. In this project, the protoplast system is being optimized to improve transformation and regeneration.

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**FY19 SUPPLY RESEARCH**

| Proposal # | Target Area | Principal Investigator | Contractor | Funding |
|---|---|---|---|---|
| 1920-152-0131-C | Meal | Joshi, Trupti | University of Missouri | $187,191 - 3 years |

# Applied Genomics to Improve Soybean Seed Protein

By Trupti Joshi, University of Missouri Columbia

USB and other organizations have funded sequencing of hundreds of soybean genomes, generating a large quantity of data. The research funded in his project aims to use the data to understand genetics controlling traits important to soybean production. This ambitious $62,397 project is assembling an “allele catalogue” of natural variants for every gene in the soybean genome (think of the human gene for hair color, where an individual might have the brown allele, the blonde allele, the black allele, etc.). This catalogue can be used to identify new genes controlling traits incorporated into elite germplasm develop new lines with improved genetic qualities.

The researchers began with the a test case of the recently discovered gene thought to be responsible for the high oil content found in many commercial soybean varieties. The allele of this gene found in most U.S. grower’s soybean is likely having a negative impact on the protein. It is possible that by changing this allele, the amount of protein can be increased. To identify new alleles of this gene, soybeans known to have higher protein, such as wild soybean and Japanese and Korean cultivars were investigated. The research identified least six distinct alleles of this gene among cultivated and wild soybean, of which Alleles #1 and #5 are the predominant versions. Allele #1 is the major allele responsible for high oil (and low protein) present in all of the sequenced Chinese cultivars and 98.6% of the North American cultivars (see figure). In the U.S. and China, Allele #5 is largely nonexistent. However, in Japanese and Korean cultivars, where protein concentration is substantially higher, the frequency of Allele #5 is 25% higher than it is in the Chinese and North American cultivars. Even more telling, in wild soybean, where protein concentration is highest, Allele #5 is the most prevalent at 55%.

The observed trend between protein content and frequency of Allele #5 suggests that this allele may be contributing to the higher protein concentrations observed in wild soybean, as well as Japanese and Korean cultivars. Thus, protein in U.S. soybean may be increased by incorporating this allele into the genome of new cultivars. Alleles #2, #3, #4 and #6 are all rare. Some are present only in wild soybean, and some are present only in groups of soybeans not depicted in the figure. Their roles are currently under investigation.

Further testing in the field will be needed to confirm that the incorporation of Allele #5 improves seed protein content under typical growing conditions. However, this example illustrates one way in which the allele catalogue can be exploited to identify genetic resources that can improve traits in that are important to soybean growers.

This project is one of the research projects funded by USB in 2019 as a part of the iPOP process (innovative proposal). The overarching project is called Genomic Tools to Enable Trait Discovery. The information and bioinformatics tools developed in this project will be made available to public and private researchers, accelerating trait discovery and integration of those traits into new soybean lines that farmers can grow.

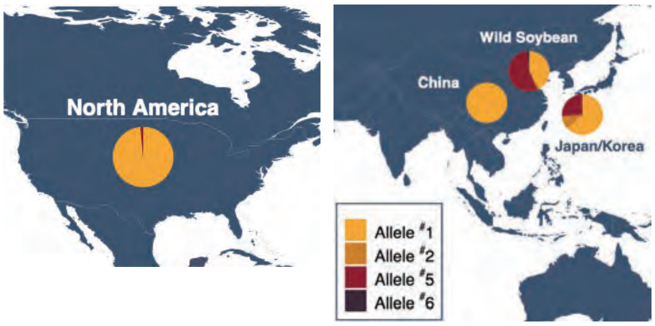

**Figure.** Allele #1-is thought to contribute to high oil (and low protein). It is prevalent in the US and China. Allele #5 correlates with high protein (and lower oil) and is less common in the US, but more common in Japan, Korea and wild soybeans. Alleles #2 and #6 are rare and their contributions to oil and protein level are unknown.

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| Proposal # | Target Area | Principal Investigator | Contractor | Funding |
|---|---|---|---|---|
| 1920-152-0131-D | Meal | Meyers, Blake | Danforth Plant Science Ceter | $180,000 - 3 years |

# Re-Engineering the Soybean Flower
## to Capture Hybrid Vigor

By Blake Meyers, Danforth Plant Science Center

Hybrid breeding has significantly elevated the productivity of maize and rice, and hybrid vigor, the phenomenon in which offspring outperform their parents, has been used for over a century to increase crop yields, improve abiotic and biotic stress tolerance, and enhance seed quality. Hybrid vigor increases crop yields by up to 15-50%, enabling growers to achieve “more yield with less field.” A full 65% of the total maize cultivation is now hybrid. Field trials using soybean that was hybridized by hand increased yields by 10-20% - a promising solution for future agricultural needs.

However, soybean is a self-fertilizing plant that does not readily hybridize with other plants and, therefore, lacks the benefits of hybrid vigor. Soybean makes small, inconspicuous flowers that fail to efficiently attract pollinators or disperse pollen. One current technique to promote hybrid breeding is to cross male-sterile flowers with male-fertile flowers. To generate male-sterile flowers, the anthers must be manually removed, which is inefficient and labor-intensive. Other strategies include using potentially hazardous chemicals or male-sterile genetic mutants that are not 100% effective, and may take years to integrate. This $60,000 project aims to transform soybean from an inbreeding to an outcrossing crop.

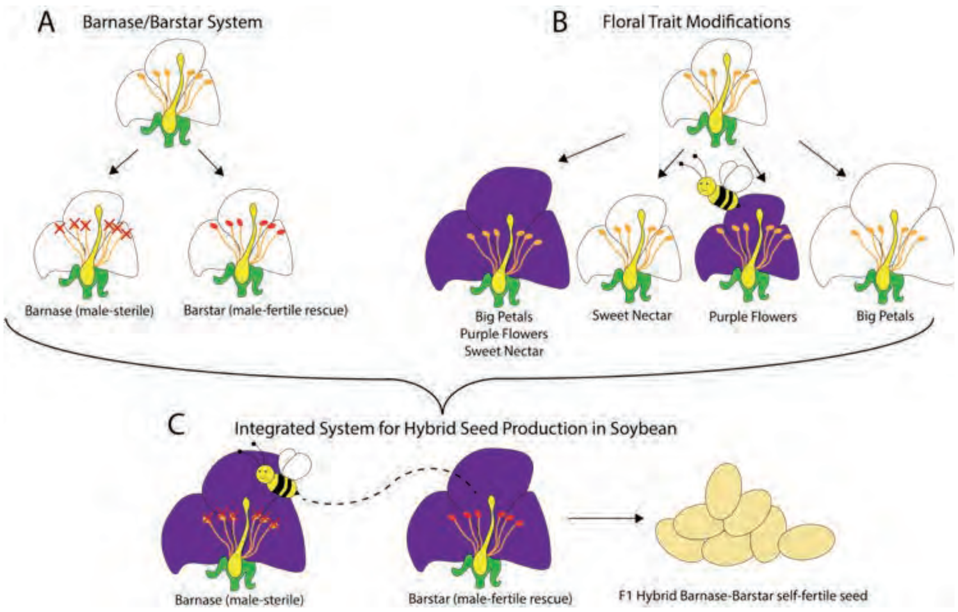
Biotechnology offers an environmentally sustainable, cost-effective, and time-efficient approach for producing hybrid soybeans that have been re-engineered to attract pollinators. Honeybees are the most likely insect pollinator for soybean, and they prefer sweeter, larger purple flowers over smaller white flowers. The ideal soybean for hybrid breeding would contain large, purple flowers that are visible to pollinators and have sweet, sucrose-rich nectar.

To generate this ideal plant, the genes that control flower color, growth size, and sucrose concentration in the nectar have been identified. Using dead Cas9 (dCas9, a variant of CRISPR), the activation of the purple petal gene, the petal size gene, and the sucrose concentration gene have been increased.

Large purple flowers may increase pollinator visits, but more is required. Self-fertilization will be blocked by engineering single-generation male-sterility into soybean using Barnase/Barstar, a two-component system that is currently used by Bayer to successfully promote hybrid breeding in canola, another self-fertilizing crop. The Barnase/Barstar system involves the production of male-sterile “Barnase” plants that express a ribonuclease that blocks pollen maturation and cannot self-fertilize, and male-fertile “Barstar” plants that express a ribonuclease inhibitor that is able to rescue male fertility, and therefore self-fertilization, in the Barnase-Barstar hybrid offspring.

The integration of floral trait modifications and the Barnase/Barstar system will result in a complete hybrid seed germination system for soybean that can directly increase overall yield. Future field trials will examine pollinator visitation and overall performance of these new hybrids.

This project is one of the most innovative research projects funded by USB in 2019, and is a part Genomic Tools to Enable Trait Discovery iPOP. Once developed, these tools will protected by patents where appropriate and then made available to public and private researchers. This program also has the potential to receive royalties if the discoveries are significant enough for companies to license.



Integration of (A) Barnase/Barstar system for male-sterile lines and (B) floral trait modifications to create (C) hybrid soybean seed.

| Proposal # | Target Area | Principal Investigator | Contractor | Funding |
|---|---|---|---|---|
| 1910-153-0236 | Meal | Naeve, Seth | University of Minnesota | $402,000 |

# US Soy Quality Initiative
## to Improve International Competitiveness

By Jill Miller-Garvin and Seth Naeve, University of Minnesota

As a commodity, soybean has a highly variable composition, and its chemical makeup directly affects the quality of products produced from it, which in turn drives the price buyers are willing to pay. Understanding how soybean composition is affected by genetics, environment, and management practices allows some control over the chemical composition. Benchmarking soybean composition and changes across producers, states, regions, and so on allows the industry to determine whether soybean quality is improving, holding steady, or declining over time. An assessment of soybean quality directly after harvest and before the import season allows purchasers to make decisions that are more informed. The primary aim of this $402,000 project is to evaluate the composition of US soybeans directly from the farmer, in order to provide new crop quality data to aid international purchasers.

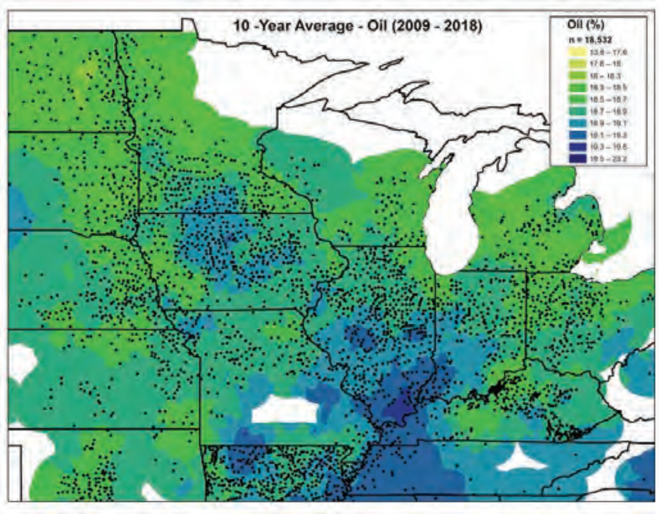
Through the 34-year history of this project, the average protein concentration of US produced soybean has declined at a rate of 0.04 % per year. This represents a real decrease from about 35.8% protein in 1986 to about 34.4% in recent years. Oil has increased in turn, but at only at half the rate. This is one of the biggest challenges facing US producers today.

There are secondary constituents that help buffer the economic impact of reduced protein level. For instance, lower protein soybeans are enriched in the most important amino acids for animal growth. This means that lower protein soybeans have a greater value than simply protein concentration might indicate. In addition, some of the decrease in protein may be offset by increased sucrose. While sucrose may not have the same value to a monogastric animal as protein, it does provide value to the end-user.

Each October, samples to be analyzed project are received from farmers, tested for protein, key amino acids, and oil content by NIR. Both written and PowerPoint reports are generated. These reports capture the quality of the new crop and compare the results to previous years. The reports are presented and distributed at Soybean Outlook conferences organized by USSEC in Japan, Korea, Taiwan, and China in mid-November. Primary purchasers, processors, manufacturers, feed-millers, and end-users participate in these conferences to learn about the quality of the new crop in the US. A final report is generated in December after all samples have been received. This report is circulated throughout the global soybean community including producers, trade professionals, media, and academia (the full report is available on request; summary of the report can be found at this link: https://www.soymeal.org/soy-meal-articles/u-s-soybean-quality-report-2019/).

Soybeans being analyzed by NIR (near –infrared reflectance)

This project is important to US soybean producers. It provides an educational service with far-reaching impact, strengthening communication about US soybean quality to both domestic and international partners. In addition, it provides technical assistance to the US soy community (including producers, academics, and industry) about changes in quality resulting from the environment, management, and variety choice, enabling future changes targeted to improve quality. Finally, it provides thousands of US producers with soybean quality results contextualized within state, region, US, and historical values, and, perhaps most importantly, that comprehensive soybean quality data helps to improve the perception of the quality of US soybeans that will help grow demand and preference for US soybeans.



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FY19 SUPPLY RESEARCH

# OIL

STORIES

| Proposal # | Target Area | Principal Investigator | Contractor | Funding |
|---|---|---|---|---|
| 1920-162-0109 | Oil | Bilyeu, Kristin | USDA | $ 1,063,337 |

# High Oleic/Low Linolenic Acid Soybean Deployment

By Dr. Kristin Bilyeu, USDA Agricultural Research Service

Cooking and vegetable oils are nearly invisible components of the human diet, but they provide many essential nutrients. Since 2005, there has been a drastic decline in the use of soybean oil in foods, mainly due to federal regulations concerning unhealthy trans fats. One way to recapture this market share for soybean oil is through the High Oleic/Low Linolenic acid (HOLL) seed oil trait. HOLL soybean oil provides full functionality for cooking and vegetable oils, but does not produce trans fats. In addition, HOLL soybean oil is eligible for an FDA Qualified Health Claim that indicates, in part, there is supportive evidence that consumption may reduce the risk of coronary heart disease. The United Soybean Board-funded HOLL Soybean Breeding (2013-2015), Development (2015-1019) and Deployment (2019-present) projects have been working to address this issue, with research and development of soybean germplasm and varieties with the HOLL seed oil trait. In 2019, it was funded at the $1,063,337 level.

The main goal was to identify an environmentally stable oil trait that will lead to oleic acid greater than 75% in combination with less than 3% linolenic acid. A validated molecular breeding strategy resulted, which captured the four genes necessary for stable expression of the trait across all U.S. maturity groups. A dedicated backcrossing program provided a means to rapidly incorporate the four-gene trait into existing elite genetics for this complex breeding objective. Concurrently, each program leveraged resources to enhance soybean yield potential and actualize their selection strategies to accelerate HOLL variety development across the eight breeding programs.

HOLL soybean germplasm and competitive yielding varieties across all U.S. maturity groups are being realized, as demonstrated by results of the Uniform Trials (UT) yield testing program in 2018 and 2019. The UT 2018 test had 33 HO or HOLL entries, and 12 HOLL soybean varieties with greater than 89% of the relevant mean check yields as well as six HO soybean varieties with 90-104% of the mean check yields. A total of 58 HOLL lines were tested in 2019, and results are expected shortly. These numbers will continue to increase as the project matures.

Commercialization of HOLL soybean varieties from this project will be subject to licensing agreements, but there has already been significant progress in that direction. There were 23 material transfer agreements (MTAs) used by both public and private breeders which helped create regional and state demand for HOLL soybean varieties. The non-GMO High Oleic (HO) trait used in this project has been awarded a trademark name: SOYLEIC™. During 2019, planting agreements were established with interested commercial groups across all soybean maturity groups for 17,000 acres of SOYLEIC soybean. The HO SOYLEIC third-party commercial licenses were finalized December 2019, and 20 commercial soybean varieties with the SOYLEIC trait will potentially be marketed by 2023.

The HOLL project has achieved a true base. The research and variety development program has led to the establishment of a foundation for new HOLL varieties. The SOYLEIC trait can remain non-GMO or accept existing or novel herbicide traits. Commercialization efforts are underway, and entities that have interest or possess lines with the SOYLEIC trait have begun reviewing the commercial license. The vast maturity group spread, competitive yields and premium potential will enable HOLL soybean varieties to help reclaim the market share for soybean oil.

**Figure 1.** HOLL soybean plots — Fisher Delta Research Center Field Day, August 2019. (Photo: Jennifer Jones)

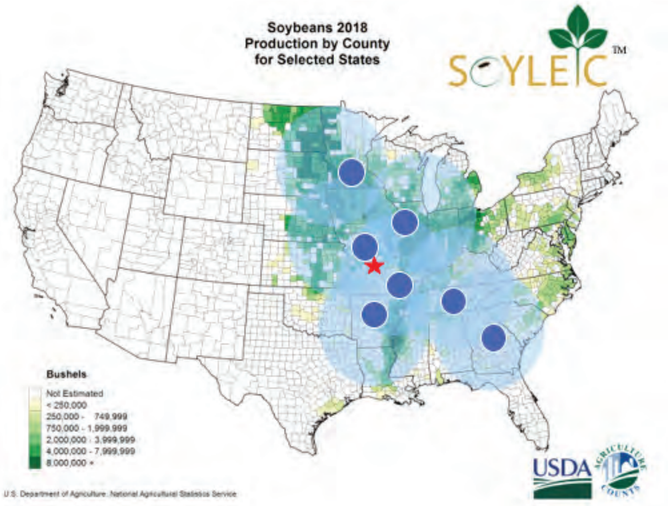

**Figure 2.** High oleic breeding program includes 8 locations and maturity groups from 0-VII. Within each of these locations breeders have achieved 100% yield parity, >75% oleic acid and <3% linolenic acid.

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| Proposal # | Target Area | Principal Investigator | Contractor | Funding |
|---|---|---|---|---|
| 1920-162-0111 | Oil | McHale, Leah | The Ohio State University | $ 483,451 |

# Developing and Characterizing Soybean Germplasm with Increased Oil While Maintaining Protein and Yield

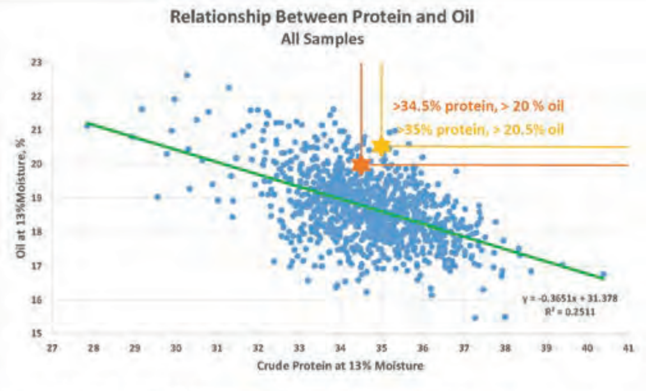
By Kyujung Van, The Ohio State University

The recent U.S. FDA ban on partially hydrogenated oil (PHO) as a food additive, and other factors, has significantly reduced the market share for soybean in the oilseed market. Within this challenge is the opportunity to develop new soybean lines with specific oil characteristics and to restore and even expand the market for soybean oils. For example, omega-3 fatty acids for human dietary needs are currently supplied mainly by fish oil, but providing this and other essential fatty acids in a vegetable oil such as soybean would significantly expand the market for this land-based product.

The soybean market has shown interest in high oleic soybean oil and potentially other high-value oil compositions. One way to maximize the value per acre is to develop new cultivars that have high oil content without decreasing either protein content or overall yield. The ultimate goals of this project are to develop soybean germplasm with ≥ 20.5% seed oil concentration (at 13% moisture) while maintaining protein concentration (≥ 35%) and yield. Another goal is the development of transgenic soybeans with modified oil components to develop a high-quality soybean for use in aquaculture and human food applications. To this end, the project was funded for $483,451 in 2019.

The negative correlation between protein concentration and oil concentration in soybean has made it difficult to develop soybean cultivars with increased concentrations of both components. In order to break this connection, effort was made to identify the genomic regions that control both seed protein and oil concentrations through "trait-specific" alleles — those that contribute to one trait without impacting the other trait. Over 600 plant introductions (PIs) were selected from the USDA Soybean Germplasm Collection for their agronomic characteristics, as well as their protein and oil contents. A genome-wide association approach was used for the identification of alleles for both protein and oil. A genetic region on chromosome 5 was identified which increased seed oil with no effect on protein content as well as a second genetic region on chromosome 10 which increased seed protein content with little effect on oil content.

The ambitious metric for this program was to develop soybean germplasm with ≥ 20.5% seed oil concentration (at 13% moisture) while maintaining protein concentration (≥ 35%) and yield. (See yellow quadrant in Figure 1.) The researchers continually came very close and recommend that slightly lower levels are more practical at this time (20% oil; 34.5% protein). (See orange quadrant in Figure 1.) Even at this level, a 47.5% meal will be produced.



First and NASS samples 2014 (https://www.unitedsoybean.org/article/average-soybean-protein-and-oil-at-13-percent-moisture-crop-year-2014)

**Figure 1.** Relationship between protein and oil.

The efforts in this program have already led to the development of cultivars, varieties and germplasm lines with elevated seed oil concentration without a reduction in protein or yield. The lines range in maturity from Maturity Groups II-VI. Included are six released cultivars, one marketed by the Missouri Foundation Seed program and five licensed by private companies for commercialization. The cultivars have 19–21.1% oil, 33.2–34.6% protein and high yields (≥ 100% o check cultivars). Adoption of varieties that meet these goals by private breeders and growers would greatly increase the quantity of meal and oil produced.

Transgenic strategies are being explored for the development of value-added essential fatty acids in soybean seeds. These include the synthesis of very long chain fatty acids, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), coupled with production of a high-value carotenoid, astaxanthin and tocotrienol. These modified soybeans hold potential as ingredients for aquaculture feed, human food and nutraceutical applications.

**Figure 2.** Soybean seed embryos overexpressing genes targeting the co-synthesis of EPA and DHA, astaxanthin and tocotrienol.l.

There is an immediate opportunity from this program in ensuring lines developed are commercialized so the value to the farmer is realized. Long-term opportunity is to continue to push the envelope toward increased protein and oil in the same bean. In addition, the research will advance understanding of fatty acid pathways in soybean and ultimately will facilitate the development of soybean with innovative oil composition. These enhancements will enable soybean to expand into new markets and new uses.

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**FY19 SUPPLY RESEARCH**

| Proposal # | Target Area | Principal Investigator | Contractor | Funding |
|---|---|---|---|---|
| 1920-162-0115-A | Oil | Thelen, Jay | University of Missouri | $95,286 |

# Increasing Soybean Oil Yield
## Through Targeted Gene Silencing and Overexpression

By Jay Thelen, Ph.D., University of Missouri

Consumer demand for vegetable oils has steadily increased worldwide for the past four decades, mainly due to their versatility. Soybeans are of the major sources of vegetable oils in the U.S., but with a lower oil content than most oilseed crops (16-22% of dry weight). Increasing oil content without losing protein content would increase overall seed yield and value. To do this, the first rate-limiting step in oil biosynthesis has been targeted. It is catalyzed by acetyl-CoA carboxylase (ACCase), which can be manipulated to push more of the plant's carbon into oil biosynthesis.

Two populations of soybeans have been developed that contain increased levels of ACCase. The first has a repressed negative regulator of ACCase called biotin/lipoyl attachment domain containing protein (BADC), and the second has overexpressed a limiting subunit of ACCase called alpha-carboxyltransferase (-CT). These same variants produced seeds with higher oil content in both Arabidopsis thaliana and Camelina sativa, an oilseed cover crop. In Arabidopsis, oil content increased between 10% and 30%.

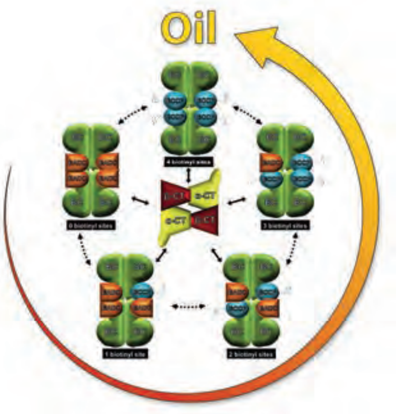

**Figure 1.** Reducing the level BADC protein in developing soybean seeds is hypothesized to increase ACCase activity, resulting in more seed oil production. BADC usually competes with BCCP, a required ACCase subunit, limiting activity of the enzyme.

The next step was to develop soybean seed for enhanced ACCase, in order to increase oil production in seeds without doing so elsewhere in the plant, which may have negative effects on growth. Soybeans are currently being propagated to trait stability and highest oil content, and promising lines are being identified. Field trials and full phenotypic characterization are planned as the next steps in this

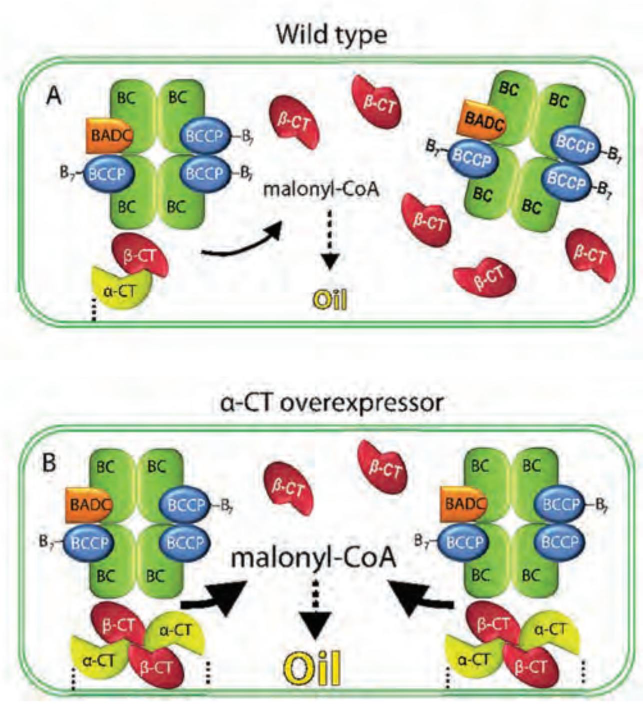

**Figure 2.** Increasing expression of α-CT (the limiting ACCase subunity) is hypothesized to increase assembly of functional ACCase, resulting in higher seed oil production.

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**FY19 SUPPLY RESEARCH**

| Proposal # | Target Area | Principal Investigator | Contractor | Funding |
|---|---|---|---|---|
| 1920-162-0115-B | Oil | Hildebrand, David-1 | University of Kentucky | $51,989 |

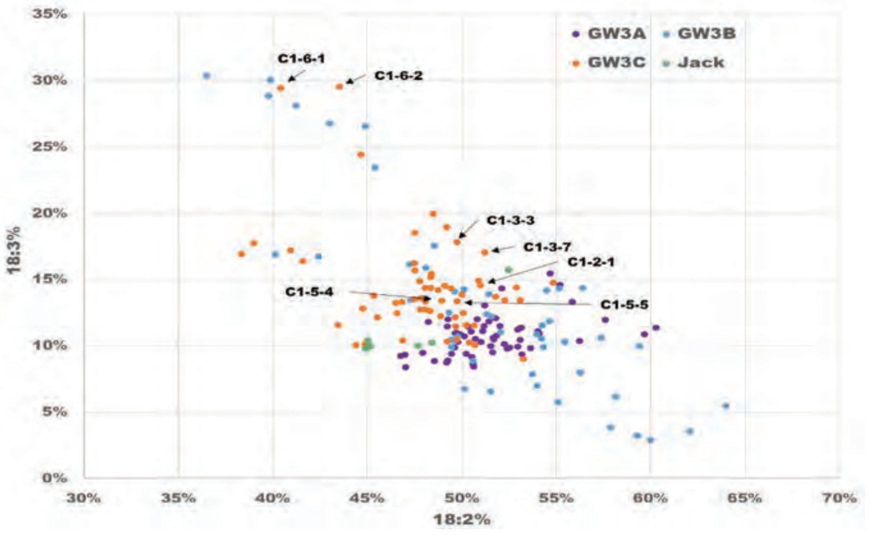
# Development of High ω-3 Soybean Germplasm

By David Hildebrand, University of Kentucky

This research is developing high ω3 (α-linolenic acid or ALA) soybean lines using a new, precision, non-GMO approach. Because ALA is an essential fatty acid which must be consumed by animals and humans, this germplasm will be useful as an animal feed. It will also facilitate developing soybeans with high levels of other essential ω3 acids in the future.

This $51,989 project has three main goals: 1) test soybean ω-3 desaturases in yeast; 2) combine the highest activity ω-3 desaturase with a soybean oil body oleosin promoter; and 3) insert the construct into soybeans, select the highest ω-3 somatic embryos and grow out plants. A yeast strain was transformed with the three soybean ω3 biosynthesis genes GmFAD3A, GmFAD3B and GmFAD3C. GmFAD3A and GmFAD3B have higher activities than GmFAD3C in yeast, especially at low temperatures. Seed oil from a wild soybean relative, Glycine soja, was determined to have higher ω3 levels than cultivated soybeans, Glycine max, and the former were grown to clone the relevant genes. Soybean tissues have already been produced that contain some of the new genes, and some show much higher ω3 levels. These were grown out to whole plants, and the resulting seeds will be tested for oil content.

Increasing the quantity and quality of oil in soybean crops will significantly increase their market value, supplying the growing demand for direct human consumption, high ω3 eggs and aquaculture ω3 feed.



Scattergram of alpha linolenic acid (18:3) and linoleic acid (18:2) levels in matured and desiccated somatic embryo lines with soybean ω3 desaturase (FAD3A, B or C) Different lines of show significantly higher and lower linolenic acid levels than the parental cultivar Jack. Relationship between protein and oil.

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| Proposal # | Target Area | Principal Investigator | Contractor | Funding |
|---|---|---|---|---|
| 1920-162-0121-A | Oil | Wang, Dechun | Michigan State University | $102,020 |

# Development and Commercialization of Advanced High Oleic Germplasm Lines with Natural Mutations

By Dechun Wang, Ph.D., Michigan State University

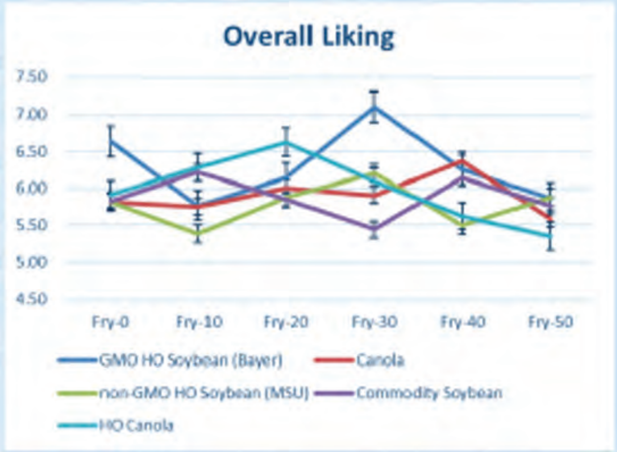
High oxidative stability and long shelf life are critical characteristics of soybean oil. Until recently, these qualities could be improved in soybean oil through partial hydrogenation, which produces some trans fat as a byproduct. However, in 2018, the FDA changed the status of partially hydrogenated oils due to the presence of those trans fats, making them no longer generally regarded as safe. This resulted in them being significantly less valuable in the marketplace, with a corresponding loss of market share for soybean oil as compared to other vegetable oils with lower levels of trans fats.

There are some varieties of soybean oil with higher levels of oleic acid which do not require partial hydrogenation in order to have a high oxidative stability and long shelf life, which means they do not contain trans fats. Demand for high oleic soybean oil is projected to increase rapidly in the next few years, and to recover the loss of market share of soybean oil. This $102,020 project involves developing those varieties.

The Soybean Breeding Program at Michigan State University uses conventional breeding with high oleic mutants to develop non-GMO high oleic soybean varieties. These varieties, named Olasoy, also have low levels of linolenic acid and saturated fats, thus improving their shelf life and nutritional content. Olasoy varieties are developed by crossing high-yielding soybean varieties with soybean FAD2 mutants, originally identified at the University of Missouri. Over 1,000 Olasoy breeding lines were developed and tested in the field at different scales. The maturity of these lines ranges from 1.6 to 3.0. Two Olasoy varieties were released in 2019. The two varieties were planted on 860 acres on 10 commercial farms in Michigan, and the seeds will be crushed for a pilot non-GMO high oleic oil extraction by Zeeland Farm Services.

USB funding leveraged with an additional grant to extract, process and test oil from our non-GMO, high oleic varieties. The grant was jointly funded by Michigan Translational Research and Commercialization, the Michigan Soybean Promotion Committee and Zeeland Farm Services. With the help of seed oil experts at MSU and elsewhere, non-GMO high oleic oils were compared with GMO high oleic soybean oil, commodity soybean oil, high oleic canola oil and regular canola oil in their ability to fry 50 batches of French fries. Oil samples were collected after every 10 batches, and the samples were analyzed for oxidation and other changes in oil quality. The French fries were tested for differences in color, taste, texture and overall flavor. The MSU non-GMO high oleic oil showed significantly lower levels of oxidation than commodity soybean oil or canola oil, as indicated by significantly lower values of peroxide and anisidine after 50 batches of frying. No significant differences were found in color, taste, texture or overall liking of the French fries fried with different oils.

These results offer much promise for the rapid development of high oleic, trans-fat-free varieties to help restore the global market demand for soybean oils.



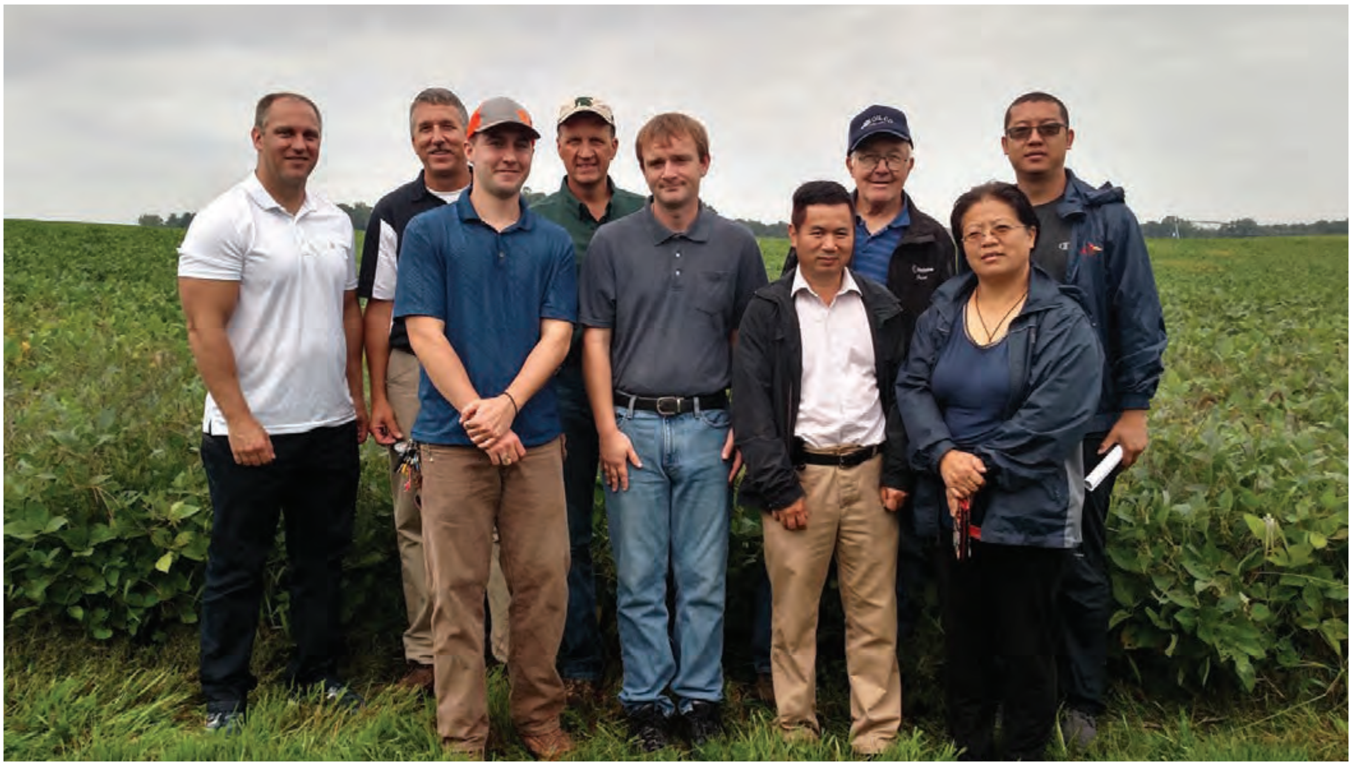


**Figure 2.** High oleic field in Michigan, September 2019. Front row (left to right): Dr. Paul Collins, MSU Soybean Breeding Team; Ben Glass, Zeeland Farm Services; Dr. Dechun Wang, MSU Soybean Breeder; and Cuihua Gu, MSU Soybean Breeding Team. Back row (left to right): Scott VanSingel, State Representative; Mark Seamon, Michigan Soybean Promotion Committee; Randy Laurenz, MSU Soybean Breeding Team; Fred Patin, soybean grower; and Dr. Zhaoming Qi, MSU Soybean Breeding Team..

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| Proposal # | Target Area | Principal Investigator | Contractor | Funding |
|---|---|---|---|---|
| 1920-162-0121-B | Oil | Miller, Amy | SGI/Benson Hill | $1,189,272 |

# High Oleic Soy Diversity: Development of High-Yield Competitively High Oleic/Low Linolenic Acid Soybean Varieties of Diverse U.S. Geographies

By Amy Miller, Kevin Cook and Bill Rhodes, Benson Hill Seeds

The demand for soybean oil has decreased in recent years because of health concerns about trans fats and other issues. Developing soybeans with healthier oil will increase demand and help to recapture a segment of the soybean market that has been lost. With the discovery of natural oleic genes in the soybean germplasm, it has become possible to develop soybean varieties with a high content of oleic oil. Combining those genes with the existing known genes for low linolenic oil will create a single line with both high oleic and low linolenic (HOLL) profiles, meaning a healthier oil that will increase the demand for soy oil in the global marketplace. This is the largest project in the oil category, funded for $1,189,272 in 2019.

This lab has been working on HOLL oil soybean lines since 2012. There are five genes involved, making it a complex task. In order to incorporate all five into the germplasm, a screening program was initiated that utilized the marker lab and gas chromatograph technology. In the past year, almost 200,000 data points related to the oil genes have been processed through the marker lab.

The ultimate goal is to develop new soybean varieties with the desired oil quality, while maintaining the high levels of agronomic and yield performance levels that producers expect and need. The early generation screening system was used to increase the number of experimental lines that could be evaluated in a timely manner. When combining all of these genes into a variety, there are many individuals in the segregating populations that do not have the needed genotypes. By screening for the desired genes in early generations, there are fewer experimental lines to evaluate, allowing work to focus on the genotypes in that population that are most likely to produce the products with the desired characteristics. By maintaining an aggressive crossing program, both in the U.S. and in winter nurseries, significant progress has been made over the last six years. There are currently varieties in production from early group 1 to early group 5, which will cover most of the production areas in the U.S. The winter production in the 2018-2019 season was highly successful, with five new varieties returning from Chile and Argentina for 2019 production in the U.S. From all the testing in 2019, four promising new candidates have been identified to advance in the next winter production system.

This type of soy oil, which is both non-GMO and HOLL, will be unique to the market. In addition to HOLL traits, enhanced meal traits (low raffinose-stachyose) have also been included in these germplasms, which should give a product that will produce a very unique oil but also an improved meal. One group 4 variety with this combination has already been produced in 2019. As the HOLL market expands, it will command a premium and thus benefit soy producers. In the long term, it will increase the demand for U.S. soy products in the global marketplace.

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FY19 SUPPLY RESEARCH

# SUSTAINABILITY

## STORIES

| Proposal # | Target Area | Principal Investigator | Contractor | Funding |
|---|---|---|---|---|
| 1920-172-0110 | Sustainability | Dorrance, Anne | The Ohio State University | $677,801 |

# Confirmation, Characterization and Deployment of the Perfect Markers and Developing Germplasm for Resistance to Phytophthora Sojae, Pythium Spp. and Fusarium Graminearum in Soybean

By Anne E. Dorrance, The Ohio State University

Seed is expensive for companies to produce and for growers to plant. Ultimately, growers would like to plant once (no replanting due to seedling diseases) and at reduced seeding rates, to maximize their profitability. They would also prefer not to have to worry about resistance to diseases and other pathogens. Reducing the impact of seedling diseases caused by soil-borne pathogens through variety development and selection would go a long way to achieving this goal.

In fact, soybean growers lose up to 90 million bushels of potential crop annually due to seedling pathogens and root rot, which are especially bad during wet springs and when seedlings are planted early. Understanding the causes of these diseases, and enhancing the seedlings' ability to resist them, would significantly improve early stand development and increase yields, not to mention avoid the necessity to re-plant.

One of the most effective management tools for minimizing losses to disease is the use of genetic resistance — developing and planting genetic varieties that are naturally resistant to these pathogens, thus allowing growers to maintain consistently high yields. However, resistance to these soil-borne pathogens is controlled by several to many genes that are scattered throughout the soybean genome. Today's soybean varieties are developed through marker-assisted selection. However, with today's expedited breeding platforms, knowing the region(s) associated with resistance is no longer sufficient. Identifying the specific gene or pathway responsible for a specific characteristic is really essential for developers and growers to ensure they have the right gene(s) for all of these pathogens. Very little was known about the genetics of resistance or susceptibility to the seedling and root rot diseases at the start of this project.

To make this identification possible, $677,801 was allocated in FY19 as a continuing effort to identify and map resistance to several pathogens, including those caused by Phytophthora sojae and more than eight species of Pythium and Fusarium graminearum. The first goal was to identify the candidate genes and defense pathways that are necessary for high levels of resistance. That information was then used to lead to the development of accurate molecular markers for rapid incorporation of this resistance into U.S. germplasm, and ultimately into commercially available varieties that growers can select for planting.

A second goal was to make germplasm and soybean lines that contain many of these new and novel gene(s) for resistance to more than one of the pathogens across numerous maturity groups. The germplasm was made available to public and private breeders to help expedite incorporation of this resistance into soybeans for U.S. growers. The more widely a variety can be planted, the more likely it is to be accepted by growers.

The third and final aim is to improve the process itself — the identification and analysis of candidate gene discovery and function in soybean. These mapping efforts have identified and proposed numerous genes that may be involved in disease resistance; however, proving they are the actual gene is a long and cumbersome process. As part of this project, new tools for candidate gene analysis through virus-induced gene silencing will be developed.

To date, more than 100 different sources of resistance combined to soil-borne diseases caused by Phytophthora sojae, more than eight species of Pythium, and Fusarium graminearum have been identified. Over the past five years, resistance has been mapped toward the soil-borne pathogens, Phytophthora sojae, six different Pythium spp. and Fusarium graminearum from populations derived from soybean cultivars and plant introductions. Additionally, RNA sequencing has been used to identify thousands of genes responding to pathogen infection. The results from this sequencing is being used to characterize the resistance mechanisms and facilitate rapid marker development and deployment of resistance in high-yielding cultivars. This work is advancing understanding of how resistance works, and could be applied more broadly to develop a general mechanism of disease resistance in soybean.

Results to date have been promising. Several germplasm lines with enhanced resistance were released, and more are in progress as resistance to multiple pathogens is being stacked in a single new germplasm for release. The project is well on its way toward its long-term goal of releasing multiple lines for different types of soybean use, with a diversity of resistance to several soybean pathogens.

**Figure 1.** Soybean seedling damping off caused by Phytophthora sojae. The field study received more than 3 inches of rain within 14 days after planting.

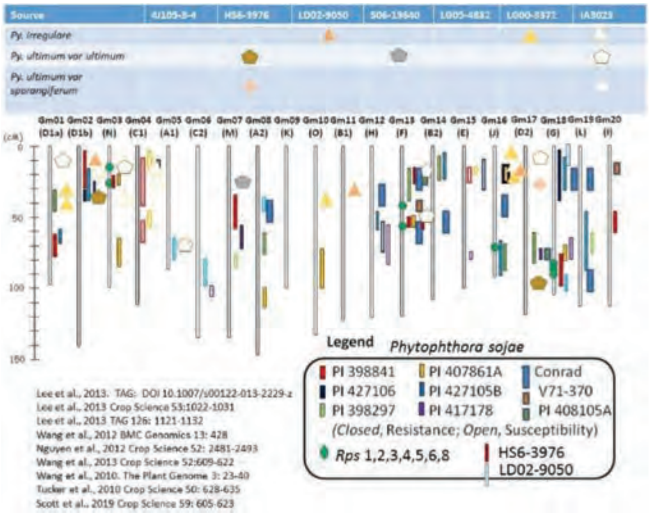

**Figure 2.** Regions of the soybean genome associated with resistance toward P. sojae or three different species of Pythium. Regions were mapped in bi-parental populations developed specifically for this project as well as from the soybean checkoff investment to Brian Diers and Jim Specht from the Soybean Nested Association Mapping Populations.

In addition, several new tools for candidate gene analysis have been improved, mainly through virus-induced gene silencing. A bean pod mottle virus gene silencing vector and an apple latent spherical virus gene silencing vector have both been successfully tested.

The results from this project will enable developers and growers to choose from a diverse soybean germplasm base with high levels of resistance to a very diverse and constantly changing group of soil-borne pathogens and diseases. It will provide a database for companies to choose the best form of a gene to combat diseases in specific regions of the U.S., and will also provide a base to ensure that resistance levels do not decline as new traits are incorporated into germplasm. Growers will have varieties that will perform better in high disease environments, especially when heavy rains occur shortly after planting.

In addition, soybean researchers will have a diverse set of tools to study the functions of genes in their systems, and develop even better and more specific varieties to meet the challenges of the future.

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| FY19 SUPPLY RESEARCH | Proposal # | Target Area | Principal Investigator | Contractor | Funding |
|---|---|---|---|---|---|
| | 1920-172-0116-A | Sustainability | Purcell, Larry | University of Arkansas | $425,953 |

# Utilizing Genes from the Soybean Germplasm Collection to Mitigate Drought Stress

By Larry C. Purcell, University of Arkansas

A primary factor affecting the stability of soybean yield from year to year is water. Every year, drought impacts soybean yield in some part of the U.S. Each year from 1986-2016, drought has impacted between 3-59% of the U.S. soybean production area. For 10 of those years, drought affected 20% or more of the soybean production areas. The vulnerability of soybean to drought introduces volatility into the market, and predicted climatic conditions will likely only exacerbate these issues. This project is focused on identifying specific soybean genotypes that provide drought tolerance, and transferring the beneficial genes to high-yielding, elite lines to help provide resilience in the soybean crop of the future. In 2019, the project was funded at the $425,953 level.

The USDA Soybean Germplasm Collection has over 22,000 different soybean genotypes, with everything from ancestral genotypes grown by farmers in East Asia thousands of years ago to more recent, high-yielding genotypes from the U.S. Included in the collection are soybeans from areas with harsh, dry climates that have favorable traits for drought environments.

This research has capitalized on prior USB-sponsored research that provided molecular marker data on all 22,000 accessions. This data allowed for rapid identification of specific DNA regions that contribute to drought tolerance.

Three hundred seventy-three diverse maturity group 4 (MG 4) accessions were evaluated in irrigated and rainfed experiments at sites in Arkansas, Missouri, Mississippi and Arizona. MG 4 accessions are a common link between germplasm in the southern and northern U.S. Favorable DNA regions (alleles) associated with four drought-related traits — high water use efficiency, slow canopy wilting, cool canopy temperature and high nitrogen fixation — were identified.

**Figure 1.** Differences in soybean wilting characteristics: a fast-wilting genotype on the left, versus a slow-wilting genotype on the right.

The favorable alleles for these traits are being transferred from the unadapted accessions into high-yielding, adapted, elite lines using conventional and marker-assisted breeding methods. In 2019, accessions with a large number of favorable alleles for each trait were crossed with an elite line, and the hybrid seed was used to begin inter-trait crosses, including crossing water use efficiency with high nitrogen fixation and crossing slow canopy wilting with cool canopy temperature. In 2020, crosses with the hybrid seed will be made to combine all four traits into the same genetic background. Subsequent research will utilize molecular markers to determine the progeny possessing the most favorable alleles, and those lines will be evaluated for drought tolerance and yield under rainfed conditions.

Often the impacts of drought go undetected, because yield is impacted before there are any visible signs of wilting or stress. This research will provide elite, high-yielding germplasm with a combination of the four traits that confer drought tolerance that will be available for crossing with other cultivars. The drought-resilient soybean cultivars developed will provide an economical means of lessening the impact of drought and ultimately benefit both producers and consumers wherever soybean is grown.

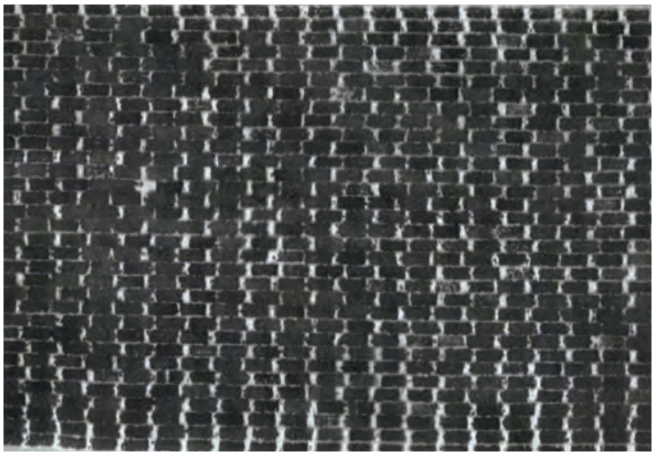

**Figure 2.** Aerial canopy temperature measurements were made from an altitude of 400 feet using a drone and an infrared camera with a sensitivity of 0.10 degrees Fahrenheit . Each rectangle represents a different soybean genotype. Dark colors are cool, and white is hot.

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| FY19 SUPPLY RESEARCH | Proposal # | Target Area | Principal Investigator | Contractor | Funding |
|---|---|---|---|---|---|
| | 1920-172-0116-B | Sustainability | Shekoofa, Avat | University of Tennessee | $56,975 |

# Improving Drought Adaptation by Insertion of the Limited Transpiration Trait into Soybean

By Avat Shekoofa, The University of Tennessee, Knoxville

Drought conditions in the United States are estimated to result in average annual losses in the range of $10 to $14 billion, and these losses will only increase as the climate changes. The identification of genetic mechanisms for tolerating periods of drought, and the incorporation of those genes into current germplasm for breeding stock, is critical to sustaining crop production and yield. Drought tolerance is a complex problem, as there are multiple mechanisms by which a tolerant soybean can overcome water deficiency. This $56,975 project study is examining one mechanism, called limited transpiration or slow wilting. This is a mechanism by which plants are able to limit the water that exits through the leaf.

To identify expression of limited transpiration (TRlim) (i.e., slow wilting) among soybean lines under high evaporative demand (> 2.5 kPa), over 1,000 de-rooted shoots of 150 upper Midsouth soybean lines were tested with silver nitrate. Among 122 of those lines, half of the population showed higher sensitivity (fast wilting) to silver nitrate than their parents. However, several lines showed no or limited changes in water loss (slow wilting) with the silver treatment. Therefore, after the silver treatment, 26 lines were selected for measuring the whole plant transpiration rate under dry air (i.e., high evaporative demand) in a walk-in growth chamber. All 26 lines were divided into three categories: 1) slow, 2) moderate and 3) fast wilting, based on the silver nitrate test results. The whole plant transpiration results indicated that 71% of the slow, 40% of the moderate and 33% of the fast wilting soybean lines expressed limited water loss, which indicates possible water-saving potential under dry air in a controlled environment.

Moreover, DNA is currently being extracted from all 122 lines. Once the sequencing is done and genotypic data is complete, genetic mapping will be used to identify the genomic regions that are controlling limited transpiration (i.e., slow wilting). The soybean lines that showed limited water loss during the whole plant transpiration test will be studied under field conditions in 2020 for seed quality and evaluation as potential drought-tolerant parents in a breeding effort.

The vulnerability of soybean to drought introduces volatility into the market and into growers' revenue streams. Without intervention and planning, predicted climatic conditions for U.S. soybean production regions will likely exacerbate this problem in the near distant future. This project will provide tools to mitigate this issue.

**Figure 1.** Measuring whole plant transpiration rate under high evaporative demand (dry air: > 2.5 kPa) in 2019.

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| Proposal # | Target Area | Principal Investigator | Contractor | Funding |
|---|---|---|---|---|
| 1920-172-0116-C | Sustainability | Stupar, Robert | University of Minnesota | $157,612 |

# Dissecting the Genetic and Molecular Basis of Abiotic Stress Tolerance in Fiskeby III Soybean for Advancing Breeding Outcomes

By Robert Stupar, Ph.D., University of Minnesota

It is mid-June in western Minnesota, near the town of Benson. Jim Johnson is driving down back country roads, passing his fields of soybeans and corn after a few weeks of wet weather. The corn he is not so concerned about. The adequate rainfall has been perfect, and those fields look great. The soybeans have him worried, because Jim knows that in his higher pH soil, and with the steady moisture, they are at risk of iron deficiency chlorosis (IDC). He gets to one of his lower-lying soybean fields that does not drain as well and, sure enough, his beans are starting to turn bright yellow at the top trifoliates, with leaf veins remaining distinguishably green. At this point, Jim knows that there is not much he can do and is wondering just how bad his yield will be this year.

While Jim is a fictitious character, he illustrates the very real problems of soybean growers in regions with high pH soils and high levels of calcium carbonates. IDC, often called "chlorosis," occurs when soybeans cannot get enough iron from the soil. Iron is the fourth most abundant element so it is not missing, but instead is unavailable to plants due to the high soil pH. Iron is a crucial element of many biological processes, including chlorophyll production and photosynthesis. Yield-loss estimates due to IDC are over $100,000,000 annually. The best defenses against IDC are selecting a variety with good tolerance and field application of iron chelates at planting, but this is not always sufficient.

This project, funded at the $157,612 level, will identify a particular IDC tolerance gene(s), and understand at the gene level how to develop varieties that are more tolerant to IDC. The list of potential genes responsible for tolerance has been narrowed from hundreds of possibilities to just a handful. To help identify which of the remaining genes causes IDC, expression of each of these genes in soybean plants is being reduced. Once identified, the specific gene(s) used in the plant and differences between varieties will be studied in order to create more efficient advances in breeding for more tolerant varieties.

Enough is already known to start breeding the source of tolerance into a few elite University of Minnesota breeding lines. The source of the tolerance gene is a cultivar called Fiskeby III, a maturity group 00 variety developed in Sweden in the 1940s for edamame, which does not have the best agronomic qualities but does have high tolerance to many environmental stresses, including drought.

In addition, the discovery pipeline used for understanding IDC is being applied to uncover the genes useful for drought tolerance, and to breed those genes into elite varieties. The results of this work will allow soybean growers to plant varieties that can better handle stresses such as IDC and drought.

**Figure 1.** Experimental soybean lines that are nearly genetically identical, differing for only a few genes. The line in the background shows superior IDC tolerance to the line in the front, which has very yellow leaves with green veins. This is a classic symptom of iron deficiency.

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| FY19 SUPPLY RESEARCH | Proposal # | Target Area | Principal Investigator | Contractor | Funding |
|---|---|---|---|---|---|
| | 1920-172-0118 | Sustainability | Honeycutt, Wayne | Soil Health Institute | $315,328 |

# Enhancing Productivity, Drought Resilience and Environmental Quality Through Soil Health

By C. Wayne Honeycutt, Soil Health Institute

Drought is one of the biggest problems facing the soybean industry. The impact of climate change on agricultural water availability is projected to suppress soybean yields by approximately 9% by 2040, 12% by 2060, and 14% by 2080. This $315,328 project addresses the problem of reduced agricultural water availability by studying soil carbon. It is well-known that increasing the amount of organic carbon (organic matter) in soil improves its ability to hold plant-available water. It is also known that increasing soil organic matter generally speeds the rate at which water infiltrates into the soil, resulting in less nutrient runoff to nearby waterways. This research will help soybean farmers determine which management practices (such as tillage, cover crops and rotations) will help them improve the water-holding capacity and water infiltration of their particular soils.

This project is developing a field-validated Soil Health Module for the COMET-Farm Decision Support Tool that will enable soybean farmers to make management decisions that will enhance their soil health by improving the plant-available water-holding capacity and water infiltration of their soils. Soybean growers who use this Decision Support Tool will realize additional benefits of soil health management practices, including a reduction in runoff and erosion and thus a reduction in the loss of valuable soil nutrients and contamination of waterways. The Decision Support Tool will be field tested before release to ensure that it is both useful and user-friendly. The goal is to create a valuable tool that will be put to use, not put on a shelf.

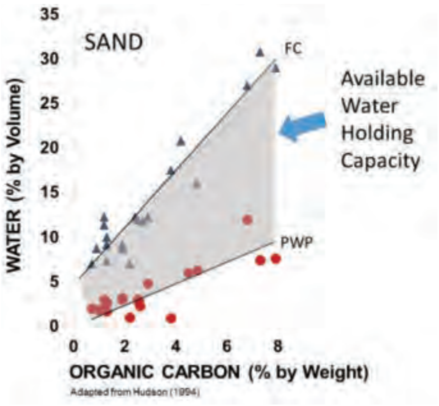

**Figure 1.** Relationship between soil organic carbon and available water-holding capacity in soils of sand-particle size. Measurements taken at field capacity (FC) are represented by triangles, and measurements taken at permanent wilting point (PWP) are represented by circles (adapted from Hudson, 1994).

Preliminary research has shown that the current understanding of the complex relationships between soil properties and plant-available water is limited, and existing data is insufficient for the creation of a detailed tool. There is a need to develop new equations to feed a Decision Support Tool, and work has begun to develop them. These newly developed equations show that decreasing soil bulk density (improving soil tilth) increases plant-available water. Additionally, over 120 long-term agricultural experiment sites have been sampled for a soil health measurement project, which will be used to improve the equations that relate soil organic matter to soybean drought resilience. In addition, connections with growers and other producers have been established, which will prove invaluable when it is time to promote the adoption and use of the Decision Support Tool.

The Decision Support Tool will directly benefit soybean growers. It will be an easy-to-use, accessible tool that will help growers make management decisions without significant added time burden. It will allow farmers to clearly understand the benefits of soil health management practices, and will provide insight on specific ways growers can build drought resilience. This will enable soybean growers to build the long-term health of their farms, not only ensuring they are viable for soybean production well into the future but also producing more stable yields and profits.

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| Proposal # | Target Area | Principal Investigator | Contractor | Funding |
|---|---|---|---|---|
| 1920-172-0119-H | Sustainability | Bradley, Carl | University of Kentucky | $5000 |
| 1920-172-0119-I | | Allen, Tom | Mississippi State University | $5000 |

# Estimates of Soybean Yield Losses Due to Diseases in the United States

By Carl Bradley, University of Kentucky; and Tom Allen, Mississippi State University

Numerous diseases reduce soybean yields, and since 1996, this $10,000 project has provided an annual estimate of soybean yield losses caused by diseases. Soybean pathologists from public universities and governmental agencies estimate the percent yield losses caused by a key set of specific soybean diseases each year. In 2018, estimates from 13 northern states and 16 southern states were received.

In 2018, the total estimated soybean yield loss due to diseases was over 536 million bushels, the highest in the last five years. The top three diseases overall were soybean cyst nematode, Phomopsis seed decay and frogeye leaf spot. The top three diseases were the same for the northern states but differed from the southern states, where Phomopsis seed decay, soybean cyst nematode and root-knot nematode were the top-three diseases.

The estimates are published annually as a scientific paper and extension brief. New this year is an easy-to-use online tool. This tool is available at https://loss.cropprotectionnetwork.org/crops/soybeans and currently contains 21 years of data.

These annual estimates of soybean yield losses allow the prioritization of development of new soybean varieties and tools, as well as regionally specific educational materials, to more effectively manage the most destructive diseases.

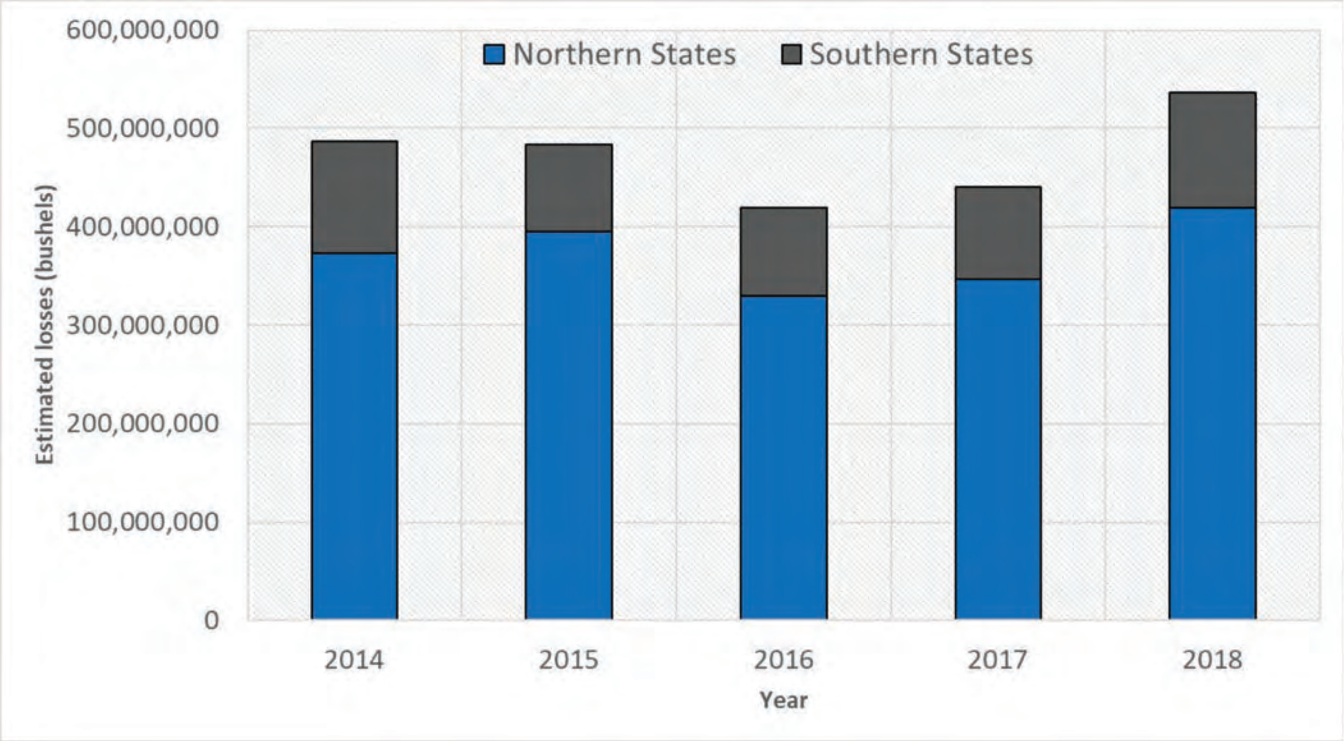

**Figure 1.** Estimated losses (bushels) of soybean yield as a result of plant diseases in the United States from 2014 to 2018.

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| Proposal # | Target Area | Principal Investigator | Contractor | Funding |
|---|---|---|---|---|
| 1920-172-0119-J | Sustainability | Musser, Fred | Mississippi State University | $5000 |

# Research Coordination — Soybean Insect Survey

By Fred Musser, Mississippi State University

Pest insects damage soybean crops and decrease yields annually, but the amount of loss varies. Documenting where and how frequently insects cause loss can aid in prioritizing research objectives and optimizing risk management decisions. University entomologists are knowledgeable and unbiased experts in insect losses on a statewide basis. An annual compilation of their professional opinions provides an imperfect, but practical, objective assessment of soybean insect impacts.

1. In this $5,000 project, soybean loss estimates in 2018 were compiled from 17 states, representing about 40% of U.S. soybean acreage. The data was published in Midsouth Entomologist and is publically available at http://midsouthentomologist.org.msstate.edu/pdfs/Vol12_1/ME19-001.pdf. Primary pests varied regionally, but overall stink bugs and corn earworm accounted for more than 50% of all insect costs in foliar sprays and lost yield.

Over time, annual compilations will permit assessing shifts in management and/or insect pressure, possibly as a result of research or external factors (climate change, economics, etc.).

2. The information provided by this compilation will allow both growers and researchers to make decisions that impact insect management with a more accurate assessment of the current and historical situation than previously possible.

| INSECT | % ACRES INFESTED | % ACRES > THRESHOLD | % ACRES SPRAYED | $ LOSS + COST/ACRE | % ALL LOSSES + COSTS |
|---|---|---|---|---|---|
| Stink Bugs (all) | 75 | 19 | 18 | 6.99 | 34.3 |
| Corn Earworm | 19 | 9 | 9 | 4.36 | 21.4 |
| Soybean Looper | 20 | 7 | 7 | 1.87 | 9.2 |
| Bean Leaf Beetle | 57 | 4 | 4 | 1.18 | 5.8 |
| Japanese Beetle | 46 | 1 | 1 | 0.83 | 4.1 |

**Figure 1.** Most costly soybean insects in surveyed states during 2018.

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| Proposal # | Target Area | Principal Investigator | Contractor | Funding |
|---|---|---|---|---|
| 1920-172-0122-A | Sustainability | Li, Zenglu | University of Georgia | $310,170 |

# Discovery and Deployment of Novel Genes for Durable Resistance to Multiple Nematode Populations in Soybean

By Zenglu Li, University of Georgia

Soybean cyst nematodes (SCN) and root-knot nematodes (RKN) are the most yield-limiting soybean pests in the U.S. SCN is by far the No. 1 yield-limiting pest, with an estimated yield loss of over 128 million bushels in 2018. RKN is a major problem, particularly in the sandy or sandy loam soils in the southern U.S., with yield loss estimates of over 13.5 million bushels in 2018. Although cultural practices in soybean production fields can help reduce some yield loss from nematode damage, these soilborne pathogens have a wide range of hosts and can survive for many years in the soil. Currently, the development of nematode-resistant soybean varieties is the most economical and effective means of preventing nematode damages in soybean production.

Over 95% of SCN-resistant cultivars used in U.S. soybean production derive their resistance from only two sources: PI 88788 or Peking. Unfortunately, continued use of the same two resistance genes is causing selection pressure on SCN field populations, which are starting to develop resistance to these two genes, and a shift in the SCN HG types. This has been observed in infested fields. Identifying and implementing novel sources of resistance before the nematodes develop immunity to the two existing sources is critical, and increasing the available genetic diversity for SCN and RKN resistance is critical for long-term sustainability of the soybean crop.

The prevalence and impact of these pests vary by location due to environmental factors that influence the distribution of these pests and the stress they impose on the growing plants. Soybean researchers need to develop genetic resistance to key pests in each region. This project aims to identify and utilize novel nematode resistance genetics from cultivated, exotic or wild soybean sources to develop multiple nematode-resistant germplasm lines and breeder- friendly DNA marker assays for marker-assisted selection to support commercial breeding efforts. The project was funded for $310,170 in 2019, and involves six scientists from five soybean-producing states, covering maturity groups 0 through VIII.

**Figure 1.** Drs. Zenglu Li and Melissa Mitchum from the University of Georgia in the greenhouse.

During the period from 2017 to 2019, a total of 24 nematode-resistant germplasm/cultivars derived from diverse genetic backgrounds across maturity groups (MG 0-VIII) were released. These lines include some that are resistant to both SCN and RKN, and others resistant to multiple HG types of SCN as well as fungal diseases. Some of these lines contain SCN resistance from sources other than PI88788 and Peking. A total of 125 MTAs were signed, and 86 germplasm were transferred to commercial and public breeders. The nematode-resistance breeding team published 17 articles in peer-reviewed journals and presented 30 posters, oral presentations and field days.

Two major QTLs associated with resistance to Southern RKN were mapped to chromosomes 10 and 18, and DNA markers associated with the candidate genes were developed and widely employed for marker-assisted selection. Robust and breeder-friendly marker assays were developed for Rhg1 and Rhg4 SCN-resistance genes, which have been routinely used in marker- assisted selection.

Over this same time period, several new sources of unique nematode-resistant lines have been identified using a novel genomic strategy. This is the first step toward finding new genes that will provide broader and more durable resistance in soybean. More than 10 genetic populations incorporating these novel sources of resistance have been developed. These populations will be used for mapping QTLs/genes for nematode resistance, and for developing improved soybean germplasm with resistance to multiple nematode species. Currently, some of these populations are being used in QTL mapping, and a strong pipeline of soybean germplasm lines with resistance to SCN or RKN and competitive yield across all maturity groups (MG 0-VIII) is in place.

**Figure 2.** Greenhouse assays for screening nematode resistance.

**Figure 3.** Root-knot nematode infection on soybean roots.

Soybean nematode resistance is one of the most important traits for new commercial varieties. Results from this project include new resistance genes, DNA markers and enhanced soybean germplasm and improved soybean cultivars, which will directly benefit both commercial and public soybean breeding programs, as well as soybean producers, by providing new genes or new resistant sources to develop high-yielding and resistant varieties. Soybean growers will be able to reduce yield loss from nematodes by using these improved varieties, and the fact that they are adapted to local growing conditions will enhance yields even more, and add value to the entire soybean value chain. DNA markers and QTL information generated from this project will benefit all soybean researchers seeking a better understanding of the genetics and mechanisms underlying resistance. Finally, it will produce desirable source materials for commercial and public breeders to use in their crossing programs for development of high- yielding and nematode-resistant germplasm and cultivar.

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| FY19 SUPPLY RESEARCH | Proposal # | Target Area | Principal Investigator | Contractor | Funding |
|---|---|---|---|---|---|
| | 1920-172-0122-B | Sustainability | Bent, Andrew | University of Wisconsin-Madison | $213,706 |

# Rhg1, cqSCN Loci and Epigenetic Determinants of Resistance to Soybean Cyst Nematode

By Andrew Bent, University of Wisconsin-Madison

Soybean cyst nematode (SCN) is the No. 1 yield-robbing pest facing the U.S. soybean industry. This project aims to improve the resistance of soybean to SCN. A major focus is to increase understanding of Rhg1, the most important SCN-resistance gene segment, in order to improve its effectiveness in commercially grown varieties of soybean. In addition, two genes from wild soybean that confer improved SCN resistance are being characterized and used to improve soybean resistance to SCN.

Soybean breeders and growers are increasingly challenged by the fact that more SCN populations in growers' fields are able to overcome resistance from PI 88788, the most common source of resistance to SCN. This is a topic of strategic importance to sustained profitable soybean production in the U.S. With FY19 USB funding of $213,706, much work has been done by the project team to advance discoveries and develop new germplasm resources.

Several years ago, it was discovered that one reason some soybean plants are resistant to SCN is they have multiple copies of the Rhg1 locus. Both the number and the version of the Rhg1 locus affect resistance. Several varieties of soybean with different numbers of copies of Rhg1 have been developed, and it has been shown that more copies means more resistance in the soybean. This is a promising avenue for developing new SCN-resistant cultivars.

To enable this research, a rapid and inexpensive test to measure how many copies of the Rhg1 locus are present was developed. However, it was discovered that the copy number alone was insufficient. Information on how much the gene was expressed was also required to explain resistance, implying that resistance is also controlled by expression level. Methods of measuring expression have been developed, and work is now underway to manipulate levels of expression to increase resistance.

The group also made a major discovery regarding mechanisms of Rhg1-mediated SCN resistance by discovering that a gene encoding an unusual variant of an "NSF" protein is required for the viability of soybean lines that carry resistance-conferring Rhg1 types.

In addition, finding new genes (not related to Rhg1) has been a goal of this project. An SCN-resistant Plant Introduction (PI) from the soybean germplasm collection was identified a number of years ago that is a wild relative of soybean (species Glycine soja). After a lot of crossing and mapping, the present project transitioned more to gene sequencing and cloning. Resistance was narrowed down to a small number of genes. The team is continuing to understand what these genes are and how they work and to incorporate them into lines for commercialization.

The group also generated soybean germplasm carrying novel combinations of Rhg1 genes that cannot be generated by conventional soybean breeding, but which may recover the effectiveness of Rhg1-mediated resistance against many SCN populations.

This is a key program for USB in terms of SCN research. It focuses both on understanding the mechanism of SCN resistance, and also on practical development of germplasm with enhanced SCN resistance. In the long term, as growers experience more and more SCN overcoming PI88788, they will need more tools and resources to develop SCN-resistant seed and other solutions. This project will deliver the needed tools and resources.

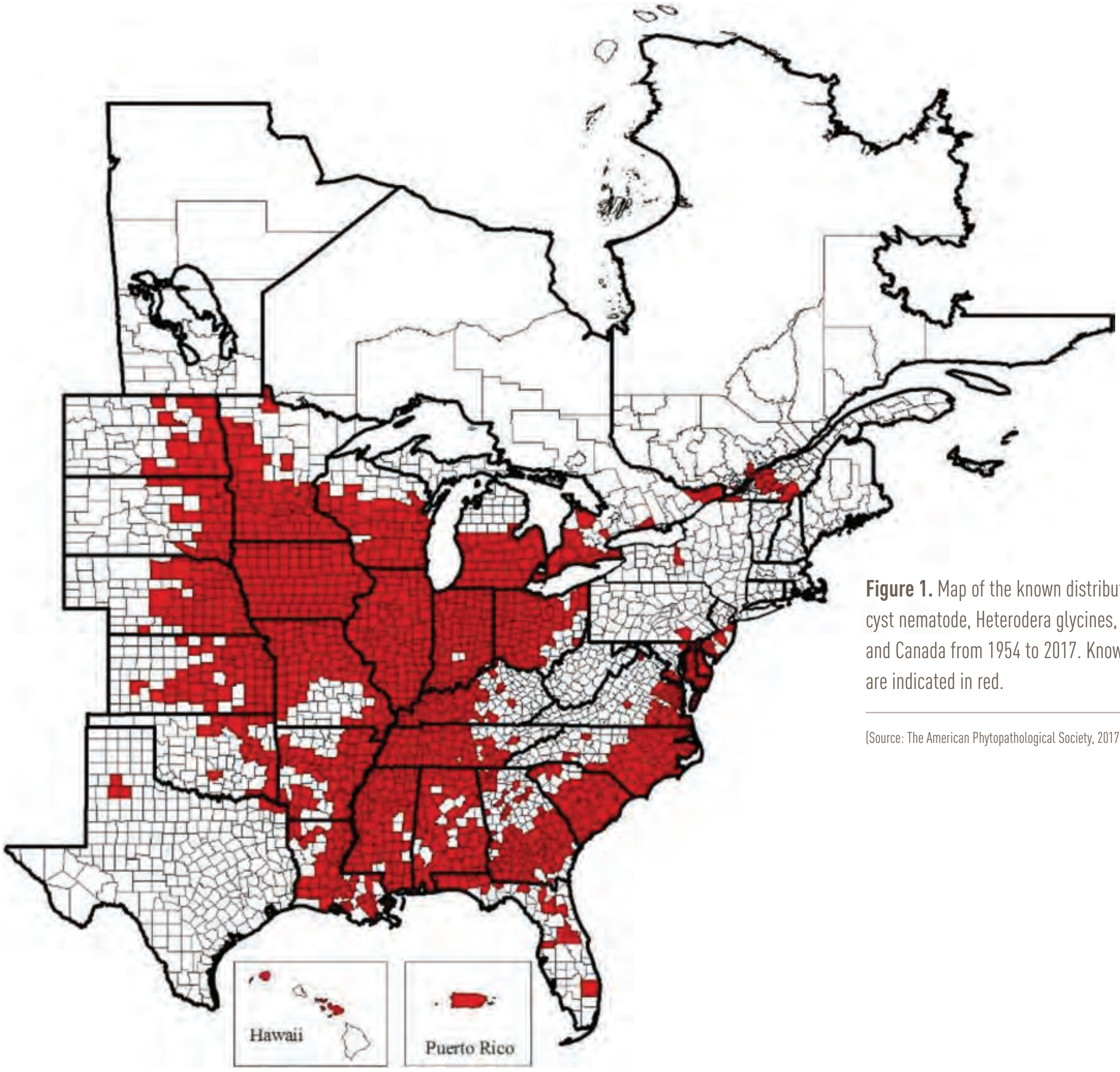

**Figure 1.** Map of the known distribution of the soybean cyst nematode, Heterodera glycines, in the United States and Canada from 1954 to 2017. Known infested counties are indicated in red.

(Source: The American Phytopathological Society, 2017)

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**FY19 SUPPLY RESEARCH**

| Proposal # | Target Area | Principal Investigator | Contractor | Funding |
|---|---|---|---|---|
| 1920-172-0123 | Sustainability | Markell, Sam | North Dakota State University | $586,880 |

# Expanding the SCN Coalition: Resistance Management and Awareness Campaign

By A Public-Private Partnership of More than 30 Organizations, Led by North Dakota State University

Soybean cyst nematode (SCN) is the most important yield-limiting biological threat to soybeans in the United States. Currently, genetic resistance is the primary tool used by soybean growers to manage SCN. However, the nematode is adapting to the genetic resistance found in approximately 95% of the SCN-resistant soybean varieties in the United States (PI88788), leaving many "SCN-resistant" soybean varieties vulnerable to yield loss from SCN. Furthermore, many growers are unaware of the ever-changing threat posed by SCN, so they are not proactively preparing and could suffer additional yield loss in the future.

The objective of this project was to develop an SCN Resistance Management and Awareness campaign that would increase awareness of the evolving yield threat from SCN throughout the soybean-producer community. This would allow growers to prepare and act proactively, to minimize the currently increasing levels of yield loss among soybean crops.

A public-private partnership called the second SCN Coalition was launched in February 2018 at the Commodity Classic, the largest farmer-led and farmer-focused trade show. The SCN Coalition now includes over two dozen universities, half a dozen private companies, many checkoff organizations (including USB) and several media partners. With financial and in-kind support (advertising/marketing) from both the soybean checkoff and industry partners, the SCN Coalition has worked to unify messaging and speak with one voice on how best to manage SCN as the nematode adapts to genetic resistance (Figure 1). USB contributed $586,880 to this project in 2019.

**Figure 1.** "Take the test — beat the pest" logo of the SCN Coalition.

Since the launch, the SCN Coalition has developed a robust online resource center (www.thescncoalition.com), training tools, videos and animated graphics that describe the problems and possible solutions to the yield threat that is presented by SCN. Similarly, the SCN Coalition has worked to deliver this information to growers through trade shows, press conferences, news releases and other traditional and social media. Between the launch of the SCN Coalition and December 1, 2019, the SCN Coalition has generated over 3 million earned media impressions in the farm press.

The SCN Coalition was designed, and continues to work, to help prevent yield loss on all growers' farms that are threatened by SCN. As a coalition of over 30 public and private partners (Figure 2), the strength of this unified messaging will provide soybean growers with the best information possible to manage SCN. Growers can see materials developed

**Figure 2.** Public and private partners of the SCN Coalition.

**The SCN Coalition recommends** that farmers work with their advisors and develop a plan to actively manage SCN:

- Test your fields to know your numbers.
- Rotate resistant varieties.*
- Rotate to non-host crops.
- Consider using a nematode-protectant seed treatment.

*SCN populations can adapt to individual resistant varieties as well as to sources of resistance such as PI 88788 and Peking. So, rotating to a different resistant variety – even if it's still PI 88788 – may help slow the buildup of SCN populations.

**Figure 3.** Pillars of SCN management supported by the SCN Coalition.

by the SCN Coalition being used by companies and universities across the country, which also provides peer support for prevention efforts.

Healthy and productive soybean crops are critical to many tens of thousands of growers in the United States, as well as the processors and end users who depend on those products. The SCN Coalition helps growers limit yield loss caused by SCN and SCN-related diseases, such as Sudden Death Syndrome, by delivering clear, consistent management information to help growers select the best management tools for their specific farm and growing conditions.

**Figure 4.** SCN Coalition panelist at a 2019 Commodity Classic Learning Center session hosted by Tyne Morgan of the U.S. Farm Report and sponsored by corporate partners of the SCN Coalition.

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| Proposal # | Target Area | Principal Investigator | Contractor | Funding |
|---|---|---|---|---|
| 1920-172-0124-A | Sustainability | Fakhoury, Ahmad | Southern Illinois University | $373,041 |

# Developing a Comprehensive Management Program for Foliar Diseases of Soybean (Phase II)

By Ahmad M. Fakhoury, Southern Illinois University Carbondale

Each year, U.S. soybean growers lose as much yield to disease as the entire soybean production of China, the world's fourth largest producer. Foliar diseases cost U.S. soybean producers $700 million/year, and some, such as Cercospora leaf blight (CLB) and frogeye leaf spot (FLS), are increasing in geographical range and frequency. To make matters worse, some of these pathogens are evolving fungicide and genetic resistance faster than new fungicides, and resistant cultivars can be developed. This $373,041 program will develop cost-effective, sustainable management options for major foliar diseases of soybean by gaining a better understanding of the biology of foliar diseases.

Many of the pathogens causing foliar diseases of soybean are historically understudied. For example, CLB was historically thought to be caused exclusively by Cercospora kikuchii. From a collection of > 700 pathogen isolates from 10 soybean-producing states, it was determined that C. flagellaris is actually the predominant pathogen associated with CLB in the U.S., followed by C. sigesbeckiae. C. kikuchii has not been found in the U.S.

An association mapping approach was used to identify regions of the C. sojina genome that correlated with FLS, leading to the discovery of two regions of the C. sojina genome that are strongly associated with different races of the pathogen.

Most commercial soybean varieties are susceptible to FLS, and three genes that condition resistance are known, but less than 5% of commercial varieties contain them. Five naturally occurring strains of C. sojina that infected soybean cultivar Davis (the original source of Rcs3) at consistent, albeit low, levels were identified. Advanced breeding lines were evaluated for resistance to C. sojina, and five with high crop potential will be introduced to uniform soybean tests in 2020, and advanced as breeding material.

Toward other goals, strobilurin fungicide-resistant strains of C. sojina were confirmed in 250 counties/parishes in 15 states from 2010 to 2018. These findings helped soybean farmers make better-informed decisions about foliar fungicide products to best control FLS in their area, increasing awareness and showing the benefit of integrated disease management.

First reported in 2008, soybean vein necrosis virus (SVNV) has since been reported in all soybean-producing areas of the U.S. SVNV is transmitted by soybean thrips, which are difficult to control. Little is known about the yield losses or effective management. Both field and growth chamber studies have been used to evaluate the effects of the virus and thrips on plant productivity and to evaluate management approaches using host plant resistance. Data indicates that while thrips carrying SVNV can obliterate soybean plants, soybean genotypes differ and exhibit various levels of resistance.

This work will provide both immediate-term benefits and long-term solutions to minimize the risks of soybean foliar pathogens, including CLB, FLS and Septoria brown spot pathogen. A combination of new disease-resistant cultivars and management practices will benefit growers, and ultimately the entire soybean community.

**Figure 1.** Soybean plants inoculated with soybean thrips infected and non-infected with SVNV. (A) PI 417136 infested with infected thrips at growth stage V1 and thrips killed after two weeks; (B) PI 171451 infested with SVNV-infected thrips at V1 and thrips killed after two weeks; and (C) PI 417136 infested with SVNV-infected thrips at V1 and thrips killed after one day.

**Figure 2.** Frogeye leaf spot (FLS). (Photo by D. Mueller)

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| Proposal # | Target Area | Principal Investigator | Contractor | Funding |
|---|---|---|---|---|
| 1920-172-0124-B | Sustainability | Bond, Jason | Southern Illinois University | $268,113 |

# Seedling Diseases of Soybean: Characterization and Education

By Jason P Bond, Southern Illinois University

Soybean crops are susceptible to soilborne seedling and root diseases, which reduce yields by more than 35 million bushels annually in the U.S. Seedling diseases rank among the top four pathogen threats to soybean; insidious nature makes them difficult to diagnose and control. It is difficult to predict when seedling and root diseases will take a heavy toll until it happens. This project received $268,113 in 2019 to address these critical challenges.

This project focuses on identifying the impact of environmental conditions, stress syndromes, cover crops and specific production practices on early season root pathogens. In addition, the interactions between root and seed pathogens and seed quality, emergence and seedling vigor are being evaluated.

Fusarium oxysporum and Fusarium graminearum are two of the most prevalent pathogens that infect soybean roots, but little is known about the conditions that favor infection and enhance disease development. This research is investigating how soil moisture, texture and pH affect the severity of seedling disease. Root rot caused by F. oxysporum was found to be enhanced in soybeans planted in warm conditions (25-27 degrees Fahrenheit) and in moderately acidic soils (pH 6). Root rot caused by F. graminearum was also favored by moderately acidic soil and was most severe in moisture-limiting soil conditions, particularly in sandy vs. loam soils.

Two additional Fusarium species, F. solani and F. tricinctum, are also common root rot pathogens. Both cause severe root rot and kill seedlings under high disease pressure. This research has shown that the amount of pathogen in the soil, the soil type, moisture and temperature are key factors in the development of root rot. In a sandy loam, disease is favored when the soil moisture content is greater than 40%. F. solani is the more aggressive pathogen and infects soybean roots over a broader temperature range and at cool soil temperatures where soybean germinates slowly. Lighter soils tend to favor infection and higher disease severity, while heavy clay soils are generally associated with lower disease severity.

Soil type and contents can also favor the activity of one pathogen over the other. For example, the presence of soybean cyst nematode at high populations can increase seedling root rot caused by these pathogens. Salinity was thought to be a major factor predisposing seedling to root rot, but the research showed that the effect of salinity on root growth was more important than the effect of the pathogen. These new findings clarified will help soybean growers make more informed management decisions about how to reduce risk from these pathogens.

Another line of investigation looked at the role of Fusarium in the development of iron deficiency chlorosis (IDC). Three years of field studies comparing root colonization by Fusarium species in plants with and without IDC were conducted. There was no difference in the frequency of Fusarium species on roots of plants with and without IDC, suggesting that infection by Fusarium is not a key driver in the development of this syndrome.

The effects of seed treatment and soybean seed quality, as well as planting date, were determined in fields infested with soybean pathogens. Seed quality had the greatest impact on stands and yields at all locations and at both planting dates. Most seed treatments evaluated resulted in greater stands and yields than the untreated control with low-quality seed. There were no differences between treatments in stands or yields when high-quality seed was used. Seed treatments applied to seeds with low quality were equally effective May and June plantings. In some conditions, seed treatments actually lead to a reduction in stands and yields, especially when low-quality seed is used.

The promotion of cover crops implements a different scenario for soybean growers. Cover crops can increase soil organic matter, water infiltration and soil structure; however, these conditions could be favorable for certain soilborne diseases. In cover crop trials, soybean seed treatments increased stands across different cereal rye termination treatments. Planting was more difficult into green cereal rye than into cereal rye that had been terminated by herbicide.

Taproot decline is an emerging soybean disease caused by the Xylaria fungus. Members of this group are commonly observed in the forest producing a fruiting body known as "dead man fingers," surviving on dead plant material and forest litter. In soybean fields, it has been associated with plant debris, rich in organic matter. Cover crops that increase organic matter could potentially increase the prevalence of pathogens like taproot decline. Also, cover crops can serve as alternative hosts for soilborne pathogens. Inoculation methods were adapted to study the potential of Xylaria to survive and colonize different cover crops. The cover crops studied included winter pea, radish, wheat, mustard and cereal rye. The responses varied significantly among the cover crops, either reducing, not affecting or increasing the cover crop biomass. The potential survival of Xylaria on cover crops is important in considering crop rotations for management of taproot decline.

The ultimate goals of this research project are to increase knowledge about seedling disease progression, to help farmers manage diseases more effectively and increase economic returns, and to improve yields and quality of the U.S. soybean. To this end, several webinars on seedling disease management were created, and research summaries are being developed in conjunction with the Crop Protection Network (https://cropprotectionnetwork.org/). In addition, interactive opportunities are in process, where soybean farmers can learn about soybean seedling diseases at trade shows and other events. By disseminating the results of this research widely, and in a variety of different formats, this project is helping growers manage these diseases more effectively and develop comprehensive management plans. More effective management will increase economic returns and improve yield and quality of the U.S. soybean crop.

**Figure 1.** Dr. Mandi Warner culturing seedling pathogens and potential biocontrol agents collected in this study.

**Figure 2.** F. oxysporum inoculated soil (greenhouse experiment). Fusarium oxysporum damage shown at four weeks after planting includes poor or slow emergence, brown vascular tissue in root and stems, and wilt or blight.

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| Proposal # | Target Area | Principal Investigator | Contractor | Funding |
|---|---|---|---|---|
| 1920-172-0125-A | Sustainability | Price, Trey | LSU Ag Center | $213,851 (USB)<br>$160,000 (MSSB) |

# Enhanced Pest Control Systems for Midsouth Soybean Production

By Paul Price III, Louisiana State University Agricultural Center

Soybean growers lose yield to both diseases and pests every year. Disease losses due to anthracnose, Cercospora leaf blight (CLB), frogeye leaf spot, pod and stem blight, Rhizoctonia aerial blight, Septoria brown spot and soybean rust ranged from $142 to $302 million per year over the past five growing seasons in the southern states. The problem is increasing, as many diseases are now showing fungicide (especially strobilurin) resistance. The most sustainable way to manage soybean diseases is by choosing resistant varieties; however, for many of these diseases, including CLB, few or no resistant soybean varieties have been identified.

In addition to diseases, insect pests such as stink bugs reduce soybean yield and quality. Estimates from the 2018 USB-funded insect loss survey (USB project #1920-172-0119-J) of southern soybean-producing states reported that stink bugs were the most important insect pest, resulting in an estimated economic loss of $244 million.

The brown stink bug represents about 50% stink bug damage in soybean overall in southern states. While the redbanded stink bug accounts for only about 3% overall, it causes 70% and 40% of the damage in Texas and Louisiana, respectively. It continues to expand as a threat because it is already tolerant of many insecticides.

A main goal of this FY19 project was to identify new soybean germplasm with resistance to diseases and stink bugs. The overall budget for the project is $373,851, of which USB has funded $213,851 and the Mid-South Soybean Board funded $160,000. Approximately 500 plant introductions (PIs) from the USDA soybean germplasm collection were planted in 18 locations over three years, in order to identify novel sources of resistance.

Another goal was to identify commercial and public varieties with resistance to CLB and stink bugs. Entries were planted in 15 locations in seven southern states over three seasons then evaluated for disease resistance, yield and quality. Several locations were planted and monitored for stink bug infestation as well.

In collaboration with USB project #1920-172-0124A and entomologists throughout the U.S., over approximately 1,000 Cercospora spp. isolates were collected and characterized, revealing that at least three different Cercospora species responsible for CLB. Isolates were also tested for sensitivity to different fungicides and will remain in storage as an important genetic archive and resource.

CLB resistance screening is time-consuming and inefficient. The group is working on two methods, one involving cercosporin accumulation and the other developing correlation with iron levels.

To date, eight PIs with very high levels of resistance to CLB have been identified and are being incorporated into elite lines and cultivars adapted to the southern United States. In addition, several breeding lines and PIs with resistance to stink bugs have been identified, and are currently being incorporated into breeding programs. Preliminary results from three seasons indicate publicly available sources of moderate resistance to stink bugs.

The development of new commercial varieties with improved genetic resistance to diseases and stink bugs will result in higher yields, with less fungicides and insecticides, directly benefiting soybean producers and seed companies across the country.

**Figure 1.** Adult southern green stink bug.

**Figure 2.** Cercospora leaf blight (Photo by D. Mueller)

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| Proposal # | Target Area | Principal Investigator | Contractor | Funding |
|---|---|---|---|---|
| 1920-172-0125-B | Sustainability | Leandro, Leonor | Iowa State University | $347,543 |

# Biology and Management of Soybean Stem Diseases

By Leonor Leandro, Iowa State University

Soybean stem diseases, such as sudden death syndrome (SDS), white mold, charcoal rot and stem canker, can severely limit crop yields across the U.S. In order to protect soybean yield, development of genetic resistance to these diseases, improved management practices and a deeper understanding of how the different pathogen strains affect soybean would all be valuable. This $347,543 project will deliver soybean varieties with improved resistance to stem diseases, and will advance knowledge of the biology of the pathogens that cause those diseases, allowing for improvements in management of soybean stem diseases.

The main goals of this project are to:

- Increase availability of genetic resistance to stem diseases in soybean varieties
- Understand the identity and diversity of soybean pathogens that cause stem disease and determine fungicide resistance present in those pathogens
- Advance ability to predict stem disease progression
- Improve stakeholder awareness of stem disease pathogens and best management practices for diseases

Initial plant-breeding efforts focused on characterizing advanced breeding lines with resistance to SDS, charcoal rot, white mold and stem canker. Field trials were established in Iowa, Michigan and South Dakota. Successful development of disease symptoms allowed assessment of breeding lines for resistance, and a range of resistance levels were observed from replicated tests. These efforts will allow release of soybean varieties with disease ratings, so that growers can make decisions based on their needs. In addition, private seed companies will be able to utilize this information and, after licensing public germplasm, will be able to develop more disease-resistant cultivars.

Next, genetic studies were conducted to determine the pathogenic variability, host preference and fungicide sensitivity among isolates of Macrophomina phaseolina, a seed and soilborne pathogen that causes many of these stem diseases. Genomic regions related to fungicide insensitivity were identified and are being analyzed from 96 isolates. Protocols were developed and optimized to conduct infection studies with Macrophomina phaseolina, charcoal rot pathogen and Fusarium virguliforme, the SDS pathogen.

In order to expand the white mold disease forecasting model nationally, the existing soybean Sclerotinia stem rot (SSR) advisory tool, Sporecaster, was revised to account for varietal resistance in soybean by including new thresholds determined by field trials in Wisconsin and Michigan.

Finally, field trials were conducted in Wisconsin, Iowa and Mississippi to study the role of management strategies, including the use of genetic resistance on disease development. One goal was to identify soybean varieties with a high level of resistance to white mold that are stable across the North Central region. Several commercial varieties were identified that appear to have good physiological resistance in the greenhouse and acceptable field resistance in multiple environments. These varieties were tested in Wisconsin, Michigan and Iowa in the 2019 field season. Disease data was collected, and yield data collection is ongoing.

The information obtained will be shared with stakeholders in multiple formats, through outreach materials designed to improve stakeholder awareness of stem disease pathogens and best management practices for diseases.

As more is learned about the causes, progression and management of soybean stem diseases, more can be done to manage these devastating problems, allowing growers to increase yield and improve soybean profitability.

**Figure 1.** Soybean genotypes with different levels of resistance to white mold. (Photo credit: Damon Smith)

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| Proposal # | Target Area | Principal Investigator | Contractor | Funding |
|---|---|---|---|---|
| 1920-172-0125-C | Sustainability | Wilkerson, Tessie | Mississippi State University | $249,896 |

# Breeding Soybeans for Resistance to Mature Soybean Seed Damage

By Teresa Wilkerson, Mississipi State University

Reductions in soybean grain quality can occur as a result of delayed harvest and/or late-season diseases, with more than 7% damage in some years. Phomopsis sp. infection in grain causes losses in all regions, and increases with overhead irrigation or rainfed environments. In 2017, as reported by the Southern Soybean Disease Workers, an estimated 52,500 metric tons (over 1.9 million bushels of soybean) of grain production were lost across 16 southern states. Symptoms include seed that is shriveled, elongated, moldy, chalky, discolored, weathered and with reduced germination and vigor. Harvested grain will incur reductions in monetary value (dockage) at the grain elevator if the combined seed damage ("damaged kernel total" [DKT]) is greater than 2%. Infection of developing soybean grain may be more severe in early-maturing cultivars, when the harvest is delayed and environmental conditions continue to be warm and humid during advanced growth stages. Current management strategies include crop rotation with non-hosts, tillage, fungicide applications during pod-fill, and resistant cultivars. However, information regarding cultivar resistance in commercial offerings is limited. This $249,896 project aims to improve soybean resistance to reduced seed quality by identifying novel sources of genetic resistance for biotic and abiotic stresses, and developing new and improved breeding lines.

Breeding lines were tested in environments prone to severe seed damage, and lines with significantly lower seed damage were identified. A combination of laboratory and field research is being implemented. The availability of genetic resistance to quality reduction in harvested grain is being increased by providing commercial and public breeders with access to the best (lowest DKT scores) lines for use in making crosses. In addition, by 2020, line(s) with significantly lower DKT and improved seed yield will be released for general use.

Field trials were conducted under shelters equipped with overhead irrigation, to ensure environmental conditions are conducive to reducing harvested grain quality. Plots include germplasm selected by breeders from yield trials exhibiting the least amount of seed damage.

Fungicides were applied during late reproductive stages, under shelters subjected to extreme conditions, to determine efficacy in prevention of losses associated with a reduction in quality.

Ultimately, one or more varieties, tested and grown in three different states, possessing differing qualities of seed, will be identified. Seed will be treated with different active ingredients to determine which products are effective and monitored for resistance to these active ingredients. In addition, treated and untreated seed will be observed at different planting populations to assess the benefit of treatment, increase planting population or both to compensate for poor seed quality.

Finally, outreach materials were developed to improve stakeholder awareness, preventing losses associated with a reduction in harvested grain quality.

This research addresses the need for better soybean tolerance to mature seed damage, which will have a direct impact on growers by helping them select the best genetics for resistance to mature seed damage and understand what management practices are effective for limiting reduced quality of harvested grain.

**Figure 1.**

Photo credit: Teresa Wilkerson

**Figure 2.** Field plots under two shelters (equipped with overhead irrigation equipment) located in Stoneville, Mississippi.

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| Proposal # | Target Area | Principal Investigator | Contractor | Funding |
|---|---|---|---|---|
| 1920-172-0126-A | Sustainability | Young, Brian | Purdue University | $659,000 |
| 1920-172-0126-B | | Johnson, Bill | Purdue University | $314,153 |

# Research to Integrate Best Management Practices for Herbicide-Resistant Weeds in Soybean Production Systems and Take Action – Multi-State Herbicide-Resistant Crops and Weeds Educational Program

By Dr. William (Bill) Johnson and Dr. Bryan Young, Purdue University

Weeds are present in every field of soybeans grown every year, and the progressive evolution and spread of herbicide-resistant weeds continues to challenge soybean production and grower profitability. The economic impact of herbicide-resistant weeds on soybean production is difficult to quantify, but a conservative estimate is an average increase in herbicide costs by $20 per acre (approximately $1.8 billion total for U.S. soybean production), with local variability from $0 per acre to $60 or more per acre.

Current soybean production systems are compromised by the evolution of glyphosate (Roundup)-resistant weeds. Over 30 species of weed have now evolved resistance to glyphosate worldwide, 15 of these are found in the U.S. Nine glyphosate-resistant biotypes are major threats to soybean production because they are resistant to Roundup as well as other herbicides, and at more than one herbicide site of action. No new herbicide sites of action have been introduced since the early 1990s, and new herbicides are unlikely to appear because of the relative safety of glyphosate and because of recent consolidations in the agriculture industry. Furthermore, the effectiveness of these herbicides in the past has diminished the value of managing weed escapes, so many weed management practices were reduced.

Despite evidence of higher grower awareness of herbicide-resistant weeds, the number of areas infested is increasing at an alarming rate. For example, Palmer amaranth first started showing signs of herbicide resistance in the late 1980s. The weed showed confirmed resistance to glyphosate in 2006 in Macon County, Georgia. Since then, glyphosate-resistant Palmer amaranth, which can produce more than 600,000 seeds per female plant, has spread across 13 states. There continues to be a need to understand and communicate to growers the impact of herbicide-resistant weeds, best practices for and diversification of weed management, and proper management of new soybean herbicide-resistance traits to slow the inevitable evolution of resistant weeds.

This dual project was funded for $659,000 in 2019 to refine non-chemical weed management strategies that could be integrated into regional best management practices (BMPs) for weed control, with an additional $314,153 to disseminate this information to growers through the Take Action workshops and educational materials.

The herbicide-resistant waterhemp and Palmer amaranth plants that escape management have been a research focus for several years. Up to 15% of the weed seeds that drop to the soil surface remain viable after 12 months in a no-till system, and up to 20% remained viable when seeds were buried to simulate tillage. Whether on the surface or buried in the soil, about 5% of the weed seed was still viable after three years. A 95% loss of seed mortality is good, but a single herbicide-resistant plant can produce hundreds of thousands of seeds, leaving tens of thousands of potential herbicide-resistant seeds after three years. Management to prevent weed seed production should be practiced, either manually, through hand-weeding or by other methods. About 99% of the seed on waterhemp and Palmer amaranth plants does not shatter from the plant through soybean maturity, which allows the entire end of the growing season to be used to manage weed escapes and devitalize seeds.

One new strategy that was investigated in 2019 was the use of a mechanical mill attached to the back of the combine, to process the chaff and destroy the weed seed. Mechanical mills from Integrated Harrington Seed Destructor and Seed Destroyer were installed on commercial combines and evaluated, but the results have been inconsistent. This may have been due to the moisture of weed and soybean plants when harvested prior to a killing frost, making the chaff difficult to feed through the mills and reducing the efficiency of the mill. Further adaptations are being evaluated, but the technology is not currently ready for broad grower adoption.

The results of this and other research have been shared through outreach and extension meeting presentations and a webinar, and incorporated into materials produced by the Take Action weed initiative.

Take Action, the multi-state education project, began six years ago to inform growers about BMPs for new soybean herbicide-resistance traits. Most of the early work focused on workshops and development of training materials for control of herbicide-resistant weeds in Roundup Ready and non-GMO soybeans. Several hundred meetings, workshops and field days were conducted during the first six years of the project, and over 500,000 copies of printed materials were distributed. These materials were designed to provide structured guidance on how to control herbicide-resistant weeds to the individuals who participated in those events.

During the course of this multi-year effort:

- Over 100 meetings and field days have been held each year, across the nine participating states
- Over 30,000 farmers have been instructed annually
- Over 40,000 copies of the educational publications have been distributed each year
- An average of 10 new training videos have been produced each year

Over 30% of the growers surveyed through these outreach efforts have adopted revised management strategies for herbicide-resistant weeds as a direct result of these educational efforts.

Because of the rapid expansion of acres infested with glyphosate-resistant weeds, utilization of LibertyLink and Xtend soybeans has grown, and cover crop adoption has expanded. Thus, the emphasis of this project has shifted toward education and demonstration, using these technologies to control weeds. However, as new information is obtained about application technologies to keep herbicides from moving off-target, the use of diverse cover crops as a weed control tool and other topics, such as the need for education, will continue. In addition, regulatory changes, such as the recent approval of Enlist soybean and additional herbicide-resistant soybean varieties for commercial growth, will bring additional educational requirements.

In order to utilize effective strategies for control of herbicide-resistant weeds, growers need to understand BMPs, and how they change over time as weeds evolve. This two-part project is addressing both sides of the issue, thus prolonging the utility of herbicides and benefitting soybean growers nationwide.

**Bill Johnson** @BillJohnson53 · Sep 23
Purdue weed science graduate student Matt Oosterholt showing his dicamba + surfactant soybean injury research to our weed science and AAPCO group.

**Figure 1.** The researchers have active social media followings to draw attention to issues of herbicide-resistance development and best management practices for defeating weeds. Here, demonstration plots funded by USB are showcased to farmers at a state-level meeting.

**Figure 2.**

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| Proposal # | Target Area | Principal Investigator | Contractor | Funding |
|---|---|---|---|---|
| 1920-172-0128 | Sustainability | Young, Bryan | Purdue University | $446,004 |

# Investigations of Off-Target Movement of Dicamba

By Bryan Young, Purdue University

The commercial launch of Xtend (dicamba-resistant) soybean in 2017 provided growers with an alternative site of action for the control of herbicide-resistant weeds. However, applications of dicamba in Xtend soybean resulted in significant off-target movement of dicamba to sensitive plants, primarily non-Xtend soybeans. The amount of soybean acres affected, and the level of soybean damage, was beyond acceptable levels in some areas. In many cases, traditional models or assumptions of spray particle drift could not explain how adjacent soybean fields were exposed to injurious levels. Secondary movement of dicamba in the air or volatility was often suspected. This $446,004 project aims to characterize the factors and practices that contribute to off-target movement of dicamba.

Field research is being conducted across multiple states to advance knowledge of how and why dicamba movement occurs. This information will be shared with applicators and growers involved with dicamba stewardship and Xtend soybean. Specific parameters being investigated include air concentrations of dicamba following application across different landscapes and weather conditions, the influence of rainfall within 24 hours of application and spray solution characteristics.

Initially, label restrictions prevented the application of dicamba formulations approved for use in Xtend soybean if rain was predicted to occur within 24 hours of application. It was demonstrated that half an inch of overhead irrigation (simulated rainfall) at six hours after dicamba application reduced dicamba air concentrations by at least 75% in the 18 hours immediately following irrigation. Furthermore, no dicamba volatility was detected after the first 24 hours where irrigation was applied, compared with as long as three days without irrigation.

Off-target herbicide movement was defined as “primary” drift, which occurs during the herbicide application, and “secondary” drift, which occurs after the first 30 minutes. The 30-minute window allows any spray droplets (particles) to settle in the target area or move with the wind. Both primary and secondary drifts were evident, with the primary movement explained by prevailing wind direction during application. Secondary off-target movement has not been fully explained, but could be due to temperature inversions and/or dicamba volatility.

Controlled environment experiments demonstrated that drift reduction agents (DRAs) and spray water contaminated with organic matter or sediment did not increase the volatility of Clarity (not approved for Xtend crops), Xtendimax or Engenia. Furthermore, a spray solution with pH from four to six did not increase the volatility when applied to Xtend soybeans. The only spray pH that increased volatility was a pH of three, which would seldom be reached. The addition of ammonium sulfate (AMS) increased dicamba volatility by as much as 16 times. Inclusion of AMS in dicamba solutions had a minor influence on spray pH (0.1 pH units); thus, some other factor is responsible for the increased volatility of dicamba with AMS, and it should be avoided in combination with dicamba for commercial applications.

Results were presented at scientific meetings and extension meetings, and discussed with the U.S. EPA and state regulatory agencies. It may have informed the U.S. EPA when the 24-hour restriction related to rainfall was modified to provide greater flexibility in dicamba applications.

A greater understanding of how herbicide off-target movement occurs supports the overall goal of improving herbicide and environmental stewardship.

**Figure 1.** Weather instruments involved with large-scale research for off-target movement of herbicides. (Photo credit: John Obermeyer)

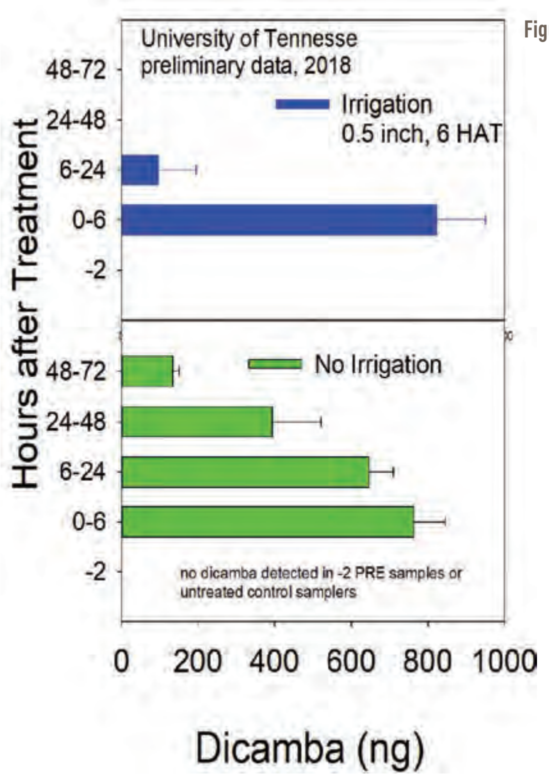

**Figure 2.**

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**FY19 SUPPLY RESEARCH**

| Proposal # | Target Area | Principal Investigator | Contractor | Funding |
|---|---|---|---|---|
| 1920-172-0129 | Sustainability | Nguyen, Henry | University of Missouri | $348,525 |

# Genetic Improvement of Flood Tolerance and Best Management Practices for Sustainable Soybean Production

By Henry T. Nguyen, University of Missouri

Flooding is a major abiotic stress for soybean crops, causing economic losses to U.S. agriculture estimated at $1.5 billion per year (USDA, 2009), a figure which is expected to increase due to climate volatility. Flood damage to crops can be caused by extreme rainfall events, excessive irrigation or by rainfall that occurs after an irrigation event. In the North Central and the Mississippi Delta regions, early-season flooding stress is frequent due to excessive rains in spring and early summer. In fact, the spring floods of 2019 hit the key row-crop growing regions of the U.S. (Figure 1A), impacting more than 66% of the lower 48 states and causing devastation in key agricultural production states. Major disaster declarations were issued by the Federal Emergency Management Agency (FEMA) to parts of Minnesota, Mississippi, Kansas, Alabama, Nebraska, Iowa, Tennessee, Kentucky and Missouri, all states with significant soybean and maize production acres.

As a major crop in the U.S., soybean is particularly sensitive to soil flooding, and yield is especially subjective to flooding stress. As little as two days of soil flooding can lead to a more than 25% reduction in crop yield, and under extended soil-flooding periods, complete yield losses are expected. Moreover, the National Aeronautics and Space Administration's (NASA) weather simulation models predict an alarming 30% increase in heavy rains by the year 2030 due to global changes in climate, which will significantly increase the frequency of, and losses caused by, flooding. Heavy precipitation causes soil-flooding stress in crop plants in the lower parts of any field due to poor drainage (Figure 2A), which reduces the exchange of oxygen between the soil and the atmosphere. As few as four days of soil flooding can kill young soybean plants at early stages (Figure 2B) or cause severe injury of larger plants at later growth stages, which may result in delays in plant growth and shorter plants with fewer nodes (Figure 2C). In addition, excessive rains during the seed-filling stage are known to cause significant reduction in seed quality and composition across the entire U.S. soybean production region, as was observed during the 2018 growing season.

Therefore, it is imperative that flooding-tolerant and high-yielding cultivars/germplasm be identified and developed to enable the development of new, flood-tolerant soybean crops. This $348,525 project aims to build long-term, value-chain resilience to an increasingly present source of shock — the stress caused by flooding. This project has taken a three-pronged approach: identifying flooding-tolerant genetic resources resistant to both early- and mid-season flooding stress; accelerating the development of flooding-tolerant and high-yielding varieties/germplasm; and optimizing management practices to protect yield from excess water.

Progress has been made toward all these goals. A well-developed, flood-tolerant breeding pipeline has been established, along with field-screening sites that can be flooded on demand in all five participating states. Specific accomplishments are listed below.

1) Novel genetic resources were identified, including six exotic and 10 wild-type soybean lines, which have high tolerance for flooding. From these, six flooding tolerance genes and their associated DNA markers were identified for use in molecular breeding.

2) Six populations of soybean germplasm were developed for gene mapping and breeding, utilizing the newly identified genetic resources shown to be flood tolerant.

3) Early- and mid-season flooding tolerances were found to share some common features and lineage, but they differed in the tolerance mechanisms, as a low correlation was observed between the genes responsible for early- and mid-season flooding stresses.

4) New flooding-tolerant germplasm, such as lines of S12-1362, S15-19625, R04-342 and R07-6669, are in the process of being released as conventional high-yielding germplasm with flooding tolerance (Figure 3).
5) Effects of flooding on yield were quantified for the Mississippi Delta region, and a raised-bed production strategy appeared to be promising after field testing several options.

Although significant progress has been made, efforts continue to increase flood resilience in soybean. New genetic resources for flooding tolerance at both early- and mid-seasons continue to be identified in both exotic and wild soybean lines. Multiple genes that lead to flood tolerance can be stacked in a single germplasm, and this stacking is expected to develop stronger flooding tolerance. New breeding strategies aimed at fully utilizing marker-assisted selection are currently being evaluated, and novel flood-tolerant wild soybean is being used in applied breeding, in order to incorporate new genetics for flood tolerance into existing, high-yielding elite lines.

Furthermore, the negative effect of excessive rains at the seed-filling stage on soybean seed quality and composition are being examined. In the short term, novel genetic resources for flooding tolerance will be identified and utilized to develop new and improved flooding-tolerant germplasm to support variety development. Best management practices will be evaluated, validated and recommended for flooding-prone areas.

The long-term benefit of this project will be its contribution to the development of flooding-tolerant varieties for flooding-prone areas, along with the latest production techniques to maximize profit opportunities. Flood-tolerant germplasm grown with experimentally proven flood-resistant management can improve yield up to 50-70% under flooding stress.

The development of new germplasm that is significantly more flood-tolerant, and a better understanding of flood management practices, will benefit U.S. soybean producers across the country, wherever excessive water from heavy precipitation and irrigation is a problem. Especially as flooding increases over the next 20 years, this research will become ever more important.

THE LELAND PROGRESS

BUSINESS

New Federal Rules Allow Landowners To Enroll Frequently Flooded Farmland In Conservation Easements

Jimmy Doolittle Recognized As

**Figure 1.** A nation concern of flooded farmland. (A) NOAA 2019 spring flood forecast map. Purple, red and orange areas indicate major, moderate and minor flooding, respectively. (B) Flooding is recognized as a national problem.

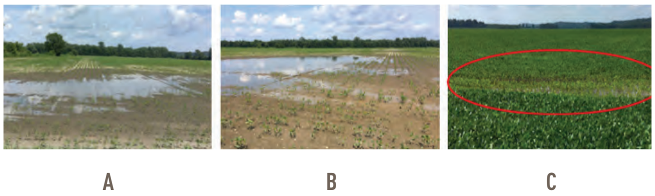

**Figure 2.** Soil flooding in the field. (A) Standing water at 4-days after a heavy precipitation in Missouri. (B) Damage of soil flooding at seeding stage. (C) Slowing growing and injury of plants at lower spots of the field.

**Figure 3.** Evaluation of flooding tolerance of the developed germplasm lines. (A) Image of lines immediately after flooding water removal. (B) Image of lines after recovery for 7 days. Left row is the new germplasm line S15-1965 and the right row is the commercial check.

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| Proposal # | Target Area | Principal Investigator | Contractor | Funding |
|---|---|---|---|---|
| 1920-172-0133 | Sustainability | Carter, Tommy | USDA | $465,002 |

# Yield Potential of Commercial Varieties Under Drought: Identifying and Overcoming Weakness Via the Public Breeding Pipeline

By Dr. T. E. Carter. Jr.; USDA-ARS; Raleigh, North Carolina

Summer drought has always been the most important barrier to profitable soybean production. Irrigation is expensive — only about 8% of the U.S. soybean acreage is irrigated. A solution is to create new, drought-tolerant varieties of soybean. To that end, a $465,002 grant was provided to Team Drought to create a national public breeding pipeline of novel, drought-tolerant varieties and breeding stocks.

Surprisingly, private-sector breeders are not pursuing a coordinated attack on drought. Studies from 15 years ago suggest that private-sector varieties generally wilt fast when drought hits. More recent evaluations in 2016-2018 confirm this is still the case. Less than 15% of current private varieties of soybean show some level of drought tolerance.

New genetics and genome sources are needed. Team Drought evaluated thousands of varieties from around the globe, and identified a select few with good levels of drought tolerance. These novel Asian soybeans are being hybridized with local U.S. varieties to produce the first drought-ready varieties. Breeding and development is taking place at special drought-prone field sites in North Carolina, Georgia, South Carolina, Arkansas, Missouri and Kansas.

After screening thousands of exotic soybean types from around the world, the first drought-tolerant, slow-wilting soybean was discovered in the 1980s in Japan (PI 416937). Two more were discovered in the 1990s in Nepal and Korea (PI 471938 and PI 407859-2). In recent years, more drought-tolerant soybeans have been identified in China and Sweden. These exotic drought-tolerant types are the foundation of the USB's Public Breeding Pipeline for Drought-Tolerance.

Slow-wilting adapted breeding lines coming out of this pipeline appear to be drought-tolerant, with yield response as much as 3 to 8 bushels/acre, depending on the severity and timing of the drought. This yield boost associated was observed in North Carolina, South Carolina, Missouri and Kansas.

The first drought-tolerant variety, USDA-N8002, was released in 2015, as well as two germplasms, R10-2436 and R10-2710, more recently. Many more breeding lines are in the pipeline and are candidates for release in the near future.

In September, more than 10 commercial breeders from BASF, Pioneer and Bayer visited the drought-testing site at the Sandhills Research Station, located near the Pinehust golf courses in North Carolina. Commercial breeders got a firsthand look at USB's public breeding pipeline. Drought was severe, and the new drought-tolerant materials showed themselves well. An impromptu "in the field" brainstorming session developed on methods to put the new lines to good private-sector use.

To date, one commercial variety has been released, and many more are in the pipeline. These new stocks are not only available to growers, but they can also be used as commercial breeding stock by private breeders. By encouraging commercial use of these genetic materials, deployment of drought tolerance across the industry will be maximized, and the entire value chain will benefit.

**Figure 1.** New drought-tolerant type on left vs. drought-susceptible variety on right during severe drought at the Sandhills Research Station near Pinehurst, North Carolina, in September 2019. The drought-tolerant type on the left is a USDA product from the USB public breeding pipeline. (Photo by T. Carter)

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| FY19 **SUPPLY RESEARCH** | Proposal # | Target Area | Principal Investigator | Contractor | Funding |
|---|---|---|---|---|---|
| | 1920-172-0201-B | Sustainability | Nelson, Scott | Iowa Soybean Association | $100,000 |

# Use of High-Density Protein Maps and Remote Sensing to Understand Protein Levels in U.S.-Produced Soybeans

By Scott Nelson, Iowa Soybean Association

Soybeans produced in the northern regions of the United States typically have lower protein content than those produced in the southern U.S. regions and Brazil. This difference in protein content is a major concern for U.S. soybean farmers, due to the associated potential for loss of U.S. soybean export share on the international market.

The declining soybean protein content in the northern areas can be attributed to several factors, including a shorter growing season and prevalence of lower protein varieties. Weather, terrain attributes, soil fertility levels and soybean varieties' planting dates have also been shown to affect soybean protein content levels.

The selection of soybean varieties that naturally produce higher levels of protein is an important step in closing the United States protein deficit; however, there are large within-field and between-field variations in protein levels of commercial soybean varieties. This $100,000 project collected quantitative data about within-field variation of soybean protein content, which will lead to new insights regarding the parameters that affect protein content. These insights will be used to identify best management practices for existing varieties of soybean that lead to higher protein levels.

Having accurate protein maps of production soybean fields will open many opportunities to identify factors that drive higher or lower protein levels. In addition, protein maps will allow growers to segregate fields and zones with higher protein, in order to capture potential price premiums.

High-density maps of soybean protein content across landscape positions and soils will provide opportunities to understand and develop management practices to increase the total field or farm protein production.

The aim is to enable producers to estimate soybean protein levels before harvest, using multi-spectral vegetation indexes, and then apply the protein map to quantify the total protein yields from different parts of the fields.

Spatial NIR samples were collected during the 2018 field season in six fields covering approximately 630 acres total. Grain samples were collected using a custom grain bypass on the combine to allow the soybean samples to be scanned with NIR, and a subset of samples to be collected in order to collect calibration data.

To understand the effects of terrain and soil attributes on protein concentration, six of the fields were grid sampled for organic matter, phosphorous, potassium and cation-exchange capacity. The historical corn and soybean yield data for those same fields were also collected. Aerial imagery for the six fields was collected every 10-15 days during the season using a commercial aerial imagery system.

Collecting and analyzing quantitative data on the relationships between protein maps, soil and terrain attributes and crop inputs will provide valuable insights into the parameters that are associated with higher protein content, and this will allow growers to better control and profit from the protein content of their soybean crops.

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| **abiotic stress** | Negative impact of environmental factors on plants, such as drought, flooding or extreme temperatures. |
| **accession** | A genetic type (e.g., land race, breeding line or variety) often collected from abroad and usually preserved in the USDA Soybean Germplasm Collection. It is usually unadapted to the U.S. |
| **allele** | Variations of a gene found in the same location of the chromosome. An example is one allele that determines blue eye color and another green eye color. |
| **association mapping** | A genomic tool used by breeders to identify the approximate chromosomal location of genes that control important traits. |
| **backcrossing** | Breeding with a parent or close relative. A good breeding method to incorporate one or a few new genes into an existing elite variety. |
| **biotic stress** | Negative impact of living organisms on plants, such as fungi, nematodes, insects or weeds. |
| **breeding line** | An unreleased pre-variety developed by crossing contrasting parents and followed by selection until the soybean is true breeding. All varieties are breeding lines prior to release. |
| **CRISPR (CRISPR-Cas9)** | A newly discovered method for gene editing. It is more efficient and precise than older methods. |
| **cultivar** | A synonym for "released soybean variety." |
| **diversity** | Lines with different alleles or combinations of alleles. Diverse soybean lines may have differences in composition, resistance to disease or yield. Poor diversity in a crop makes it difficult to breed improved cultivars and makes it more vulnerable to pests and diseases. |
| **DNA markers** | A gene or specific sequence with a known location on a chromosome that can be used to determine the presence of a specific trait. |
| **essential amino acid** | An amino acid that cannot be synthesized by an animal or human; it is needed for life and must be obtained from the diet. Amino acids are the building blocks of proteins. |
| **fatty acid** | The primary component of soybean oil. Oil consists of many types of fatty acids or lipids, which affect shelf life and taste of soybean cooking oil. Fatty acids have important biological functions, especially for energy storage and cell membranes. They are the primary component of soybean oil. |
| **gene** | A unit of DNA that controls one or more traits, it is the basic unit of inherited genetic information. |
| **gene editing** | A method to precisely modify a gene. |
| **gene knockout** | A gene whose function has been disabled. |
| **genetic resistance** | An allele or set of alleles that, when present in a plant, confers protection against infection by a pest or disease. It can also refer to the alleles in a pest or weed that make them protected from insecticides, fungicides or herbicides. |
| **genome/genomic** | Of or relating to the complete set of genes or genetic material present in an organism. |

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| **genome-wide association** | A form of association mapping that takes into account the entire genome, often using populations of plants that are closely related to one another. |
| **genotype** | The complete genetic makeup of a particular or individual organism. Varieties and breeding lines are examples of genotypes. |
| **germplasm** | A collection of individual plants that contains distinct genetic variation. It can refer to wild relatives or landraces, or it can refer to "pre-breeding" material containing specific traits of interest but not yet ready for commercialization. The USDA Germplasm Collection preserves more than 22,000 distinct soybean types. |
| **phenotype** | The observable or measurable characteristics of a plant. For instance, a purple flower or a high oleic acid content. |
| **plant introduction (PI)** | A true breeding soybean type or accession that is preserved in the USDA soybean germplasm collection. Each genotype in the collection is assigned a unique plant introduction number (PI). |
| **plant regeneration** | A method of developing a whole plant from a single cell, often done after gene editing or plant transformation, to ensure the new genetic change is carried into subsequent generations. |
| **plant transformation** | A method of introducing a new gene into the genome plant; the gene can come from the same, similar or completely different species. |
| **quantitative trait loci (QTL)** | A region or regions on chromosomes that contribute to a phenotype. There may be many regions that collectively define a specific trait. |
| **susceptibility** | A plant that is unable to defend or resist an abiotic or biotic stress. The result is yield loss or even plant death. |
| **tolerance** | Often referred to as the ability of a plant to withstand abiotic stresses. Similar to genetic resistance from biotic stresses. |

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