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| Project Number:  | 1820-152-0106B |
| Project Title:  | Utilizing unique genetic diversity to combine elevated protein concentration with high yield in new varieties and experimental lines |
| Organization:  | University of Nebraska |
| Principal Investigator Name: | George Graef |
| Project Status - What key activities were undertaken and what were the key accomplishments during the life of this project? Please use this field to clearly and concisely report on project progress. The information included should reflect quantifiable results (expand upon the KPIs) that can be used to evaluate and measure project success. Technical reports, no longer than 4 pages, may be included in this section.  |
| **Program Objectives:** * Develop and release improved soybean lines that are higher in seed protein concentration and have improved nutritional bundle and superior yield compared with current high-yield cultivars.
* Characterize high protein sources for the presence of the major protein gene on chromosomes 15 and 20.
* Map new genes for high protein concentration from *Glycine tomentella*
* Characterize protein composition and amino acid profiles of *G. tomentella*-derived lines and selected experimental soybean lines.
* Improve seed quality in early planting soybean production system (ESPS).
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| Did this project meet the intended Key Performance Indicators (KPIs)? List each KPI and describe progress made (or not made) toward addressing it, including metrics where appropriate.  |
| **Key Performance Indicators:*** Most or all major US commercial soybean programs participate in the cooperative wide-area evaluation of our diverse, high-yield soybean lines with improved seed protein concentration and nutritional bundle
* Yes, we have participation from Syngenta, Dow, Schillinger Genetics, and KAPI on the industry side. Pioneer and Monsanto are interested in rejoining our program next year.
* Companies use selected lines from our diversity tests in their breeding programs
	+ UMN is working through licensing agreement on four diverse lines being transferred to Monsanto. Once again, the buyout of Monsanto by Bayer has significantly delayed this. We have reviewed another list of lines with them, which they are interested in transferring for crossing in their program once a boilerplate license agreement can be established.
	+ University of Nebraska is finalizing a MTA for transfer of 13 diverse, high-yield lines to Monsanto during 2018.
* Other public researchers use diverse, high-yield, high-protein lines in their breeding programs
	+ Many high-yield lines with diverse pedigree and improved seed composition have been entered into the USDA Uniform Soybean Yield Tests Northern States, and are used by other state and USDA soybean breeding programs in their breeding efforts.
* Genetic markers for protein QTL on Chr 15 and Chr 20 are available for use by breeding programs
	+ The location and identity of the Chr 20 candidate gene was provided to the soybean research community in a presentation given by Diers at the Soy2018 meeting and this information has allowed breeders and researchers identify markers more closely linked to the gene. For the Chr 15 QTL, markers based on our previous mapping of that QTL are available to the research community.
* Candidate genes for the protein QTL on Chr 20 and Chr 15 are identified
	+ Brian Diers, University of Illinois - Progress has been made in cloning the high protein genes on chromosomes 15 and 20. For the chromosome 15 gene, we are fine mapping it by identifying recombinants close to the gene and testing the progeny of these recombinants for protein and oil and with markers. Using data from several populations that were field tested in 2017, we have tentatively placed the gene into a 267 kb interval. To further narrow the interval, we have planted in the field this spring approximately 40 plant rows derived from each of 9 selected plants that have recombination in the 267 kb interval. These plant rows will be harvested this fall and tested for protein and oil concentration.
	+ To confirm the gene candidate we have identified for the chromosome 20 high protein gene, Tom Clemente at the University of Nebraska made transgenic plants that knock out the low protein allele. At this point, we don’t know whether we need to knock out the low or high protein allele of the candidate gene to show its function. Populations of plants that are segregating for the knock out of the low protein allele are growing in the greenhouse. The plants are filling pods and should be ready to harvest in about a month.
* Soybean lines developed in this program show stable performance for improved yield and seed composition over locations and years
	+ Several lines from the USDA, ARS breeding program at Illinois and the University of Nebraska program have shown superior yield and seed compositional quality over years and locations. Those were highlighted in the March report in the attached 2017 Diversity Program Yield Test report.
	+ MSC09-774089 has showed stable yield over the past three years. It will be release as a germplasm release in 2019 if yield is again good in 2018 regional trials. Breeder’s seed of this line has been made.
* At least one new commercial soybean variety from an industry partner results from crosses made with lines from this program!
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| Expected Outputs/Deliverables - List each deliverable identified in the project, indicate whether or not it was supplied and if not supplied, please provide an explanation as to why. |
| **Expected Outputs/Deliverables:*** Distribute final report of all field and seed composition data for experimental lines tested jointly with commercial companies to all participants and other interested soybean breeders by January 31
	+ Results from the 2017 Diversity Protein Tests were distributed to all university, industry, and USDA cooperators by January 31, 2018. We continue to make great progress in identifying high-yielding soybean lines with increased genetic diversity that meet yield and composition targets. With our USDA, university, and industry partners, we evaluated the six different Diversity Protein Tests in 7 MG2 environments, 12 MG3 environments, and 10 MG4 environments (See Figure 1 below). In 2017, which was generally a lower protein year for much of the central USA, the average of all lines from our diversity protein tests was in the range of protein and oil that produced a 48% meal and 11 pounds of oil per bushel. The best lines in the tests exceeded the yield of the checks and produced higher protein meal (See Figure 2 below). Note that the high-yield check lines from the USDA Uniform Regional Tests in MG2, MG3, and MG4 did not even fall in the shaded area of the graph, so will not produce a 48% meal.

**Figure 1. Test locations for 2017 Diversity Protein Tests.** Figure 2. Seed Protein & Oil concentration averages for checks and Diversity Test lines. The 2018 Diversity Protein Tests have 204 entries total and will be grown in 19 locations in NE, KS, MO, IA, IL, AR, and MD, as well as additional tests in MN for the MG1 and earlier maturities organized by Aaron Lorenz. Each test will have 9 to 12 locations of evaluation, more than for the 2017 tests. * Continue and strengthen industry cooperative evaluations of new, diverse, high-yield lines with improved seed composition and nutritional bundle, facilitating exchange and adoption of germplasm by industry programs.
* We completed transfer of over 135 lines in the 2018 USB Diversity Protein Tests to project cooperators and our industry partners. Due to ongoing mergers, Pioneer and Monsanto are not able to participate this year. But we have participation from Syngenta, Dow, Schillinger Genetics, and KAPI on the industry side. These tests are MG2, MG3, and MG4, with a preliminary and an advanced test in each MG at 9-12 locations each. I spoke with people from the other companies, and they are interested in rejoining our program next year.
	+ UMN has maintained communication with Corteva and Bayer Crop Sciences regarding germplasm sharing. They are particularly interested in germplasm from our program with diverse genetic backgrounds. We discussed the possibility of shared testing with them during the summer of 2018, but the major mergers that have happened during the last year and are ongoing prevented us from doing this. We hope to resume the cooperative testing next summer.
* Develop a common, testing-only MTA acceptable to all commercial, university, and USDA participants to facilitate exchange of seeds for the cooperative tests by January 31, 2018
	+ I have talked with people from Monsanto and other companies regarding key items and protocols that will facilitate this. One important consideration is that some companies require testing for adventitious presence (AP) in all seed lots coming from outside their system. That adds expense and time. In addition, at least one company requires that MTAs for the current season be completed before January 15 of that year. That will be challenging. One approach we talked about is developing a preliminary test for entries that are in their first year of testing, and an advanced test for entries that are retained for a second or third year of testing. That will allow those industry partners who require more time for AP testing and MTA processing to participate in the advanced tests the second year. Another option we discussed is outsourcing of that company testing. That was just a suggestion in a discussion; we don’t know if it is possible or will be allowed by the company. But these may present options for 100% participation by our industry partners in the USB Diversity Protein Tests.
	+ Minnesota is doing this independently since we are the only University testing with companies in the earlier MG zone.
* Make germplasm releases of experimental lines with improved yield and enhanced protein concentration and nutritional bundle to be used by both public and private sector breeders to develop new varieties
	+ For plots in Mississippi, all plots received damage from Dicamba drift during the spring/summer. The damage could have come multiple times from multiple points. Differences for damage among breeding lines were noted. Harvest of late III/early IV yield trials has been completed. Harvest of mid-to-late IVs is temporarily on hold due to rain bands associated with Tropical Depression Gordan and associated storms from the gulf. We expect seed damage from this harvest delay, which will hopefully allow for the separation of genotypes for seed damage. The paper work for a germplasm release has been submitted to the National level and is awaiting approval.
	+ New crosses were made to develop new populations with diverse germplasm and improved seed protein/oil/carbohydrate composition.
	+ MN produced Breeders’ Seed of MSC09-774089 which has PI5677516C as a parent. They will make a germplasm release of this line.
* Develop a list of high-protein germplasm accessions indicating the presence or absence of the high-protein gene on chromosome 20 that can help select new sources of high protein that are genetically different from what is currently being used
	+ High protein accessions from the germplasm collection were identified that do not have the chromosome 20 high protein allele. Nine of these accessions were planted in the crossing block (IL) and were crossed with germplasm with normal protein levels to develop germplasm that can be used to map alternative alleles that can increase protein levels and potentially not have a negative impact on yield.
* Provide information on genetic markers to identify the specific genes on chromosomes 15 and 20 that increase protein concentration
	+ Information on the chr 20 candidate gene was released at the Soy2018 meeting which has provided information to that can be used to identify markers closer to the gene.
* Obtain preliminary data indicating if the genes controlling high protein concentration in *G. tomentella*-derived lines are different from the major genes known in soybean
	+ Several G. tomentella-derived lines are included in our cooperative Diversity Protein Tests and are being evaluated for yield and seed compositional quality at multiple locations by industry and university cooperators as part of the coop test.
* Identify specific protein components that are responsible for the increased protein concentration in experimental lines derived from *G. tomentella*
* Provide data on protein quality for both high protein soybean lines and high protein *G. tomentella*-derived lines that will help select lines with higher concentration of sulfur-containing amino acids
	+ See below for data on high-protein lines from exotic germplasm
* Identify genes in elite and exotic germplasm pools that influence seed protein concentration
	+ High-resolution two-dimensional (2-D) analysis of the UP2 high protein lines (UPPC7(S3)-0115, UPPC7(S3)-0230, UPPC7(S3)-0064, and UPPC7(S3)-0033) revealed the accumulation of a unique high-molecular weight protein in these lines. We have partially purified this unique protein and identified this protein by mass spectrometry. MALDI-TOF-MS analysis of this protein followed by peptide mass searches revealed significant peptide matches with soybean 11S glycinin protein. Antibodies raised against soybean glycinin protein also reacted against this unique protein indicating that this protein is most likely an unprocessed glycinin precursor. This possibility is currently being verified by cloning the glycinin gene from these UP2 high protein lines. We used two long-term recurrent selection populations to investigate how selection for increasing seed protein concentration might affect seed protein composition and quality. The UP2 population was developed from seven 100% exotic, unadapted, high-protein soybean accessions. The UP3 population was developed from 100% elite, high-yielding lines with average or above-average seed protein concentration. High-resolution two-dimensional (2-D) analysis of seed proteins of 7 exotic parent lines of UP2 and the 10 highest protein lines after five cycles of recurrent selection has been completed. This analysis demonstrated several of the UP2 high protein lines (UPPC7(S3)-0115, UPPC7(S3)-0230, UPPC7(S3)-0064, UPPC7(S3)-0033) failed to accumulate Gy4 protein. Interestingly, all these high protein lines accumulated a unique high-molecular weight protein. The identity of this protein is currently being investigated by mass spectrometry. The relationship, if any, between the high-protein trait and the presence of this unique high-molecular weight protein needs further investigation.
	+ Amino acid profile of 7 exotic parent lines of UP2 and the 10 highest protein lines after five cycles of recurrent selection and 7 original elite parental lines of UP3 and the 10 highest protein lines after five cycles of recurrent selection was determined by high-performance liquid chromatography. Based on this analysis the following conclusions can be derived.
		- Methionine content of UP3 high protein lines (1.2%) is slightly lower than their parents (1.4%).
		- Cysteine content of UP3 high protein lines are very similar to that of parents.
		- Alanine, Isoleucine, Threonine and Valine content of UP3 high protein lines are slightly lower than their parents.
		- UP3 high protein lines have higher content of Arginine and Serine than their parents.
		- Methionine and cysteine content of UP2 high protein lines is very similar to that of parents.
		- Threonine and valine content of UP2 high protein lines are slightly lower than their parents.
* Identify unique loci for yield and seed composition traits in exotic sources that are not present in the commercial gene pool
	+ We are working on genotyping the lines in the KPI above from the elite UP3 population and the exotic UP2 population to help answer this question.
	+ Data presented by Corteva at the Soy 2018 conference showed distinct contributions from lines with exotic pedigree from this program and their elite commercial germplasm
* Develop at least one new soybean line with superior yield and seed composition and quality for the ESPS in the southern US
	+ Yield trials in the early production system of the Mid-south at Stoneville, MS were planted 18 April 2018, with the breeding nursery planted 20 April 2018. Field conditions were cool and wet in early April, but progressed to hot and dry in May. We initiated furrow irrigation the 8th and 9th of June. The plots look very good and rows will soon be lapping (covering the inter-row spaces). So far, there has been no Dicamba drift damage from our neighbors.
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| Describe any unforeseen events or circumstances that may have affected project timeline, costs, or deliverables (if applicable.) |
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| What, if any, follow-up steps are required to capture benefits for all US soybean farmers?Describe in a few sentences how the results of this project will be or should be used. |
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| **List any relevant performance metrics not captured in KPI’s.** |
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