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| Project Number:  | 1920-152-0113 |
| Project Title:  | Utilizing unique genetic diversity to combine elevatedprotein concentration with high yield in new varieties and experimental lines |
| Organization:  | University of Nebraska |
| Principal Investigator Name: | George Graef |
| **National Soybean Checkoff Research Database** [**https://www.soybeanresearchdata.com/**](https://www.soybeanresearchdata.com/) **(visible to public)****Please choose one option (if no option is selected, this report will be posted to the website):**[x]  I agree to allow the information contained in this report to be published in its entirety.[ ]  I have included, at the end of this report, a brief non-technical report that can be posted to the website.[ ]  I DO NOT agree to allow the information contained in this report to be published. |
| 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.  |
| The USB Diversity Yield & Protein project continued our success during this FY19 project. We met or exceeded all of the KPIs and Deliverables, with the exception of the uniform MTA across institutions and companies. That is difficult to achieve with so many variables and different institutions, but we have a plan that I think will help us achieve that uniformity and simplicity for our current FY20-21 project. Several diverse breeding lines were transferred to and used by industry programs during FY19, and an important publication from one industry program documents their use of diverse lines and their value in commercial soybean breeding programs for enhancing yield. Thanks to USB for supporting these important long-term efforts. This is key to insuring the success of US soybean improvement to enhance yield and quality and improve profitability and sustainability for US soybean farmers for the next generation.  |
| 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 for 2019

The tests involve university, USDA, and industry cooperators evaluating MG 0, I, II, III, IV and V soybean lines with improved yield and seed protein. The 2019 Diversity Coop Tests have more than 340 entries in 12 tests being grown in 20 locations in 7 states. Industry cooperators during 2019 include Syngenta, KG AgriProducts, and Shillinger Genetics. With the continuing consolidations in the industry, the other companies who participated in the past look forward to participating again after the transitions are complete. Those included Monsanto and Bayer (now Bayer), Pioneer and Dow (now Corteva). * Companies use at least one line from our diversity protein tests in their breeding programs

See the publication: Hegstad et al., 2019. Introgression of novel genetic diversity to improve soybean yield. Theoretical and Applied Genetics 132:2541-2552. <https://doi.org/10.1007/s00122-019-03369-2> This publication documents the characterization and use of diverse germplasm from the Diversity Program by Pioneer/Corteva to improve yield and diversity in a commercial US soybean breeding program. After a long time of negotiating, the University of Minnesota finally signed an MTA with Bayer Crop Science this last year (2019) to allow them to use four of our diversity lines in their breeding program. All lines have some diverse ancestry.**Cross Line**M03-172039 x PI561389A M10-242042M02-495076 x OAC07-06C M10-194097MN1410 x PI561389B M10-249002MN1410 x PI629013 MSC10-562014These lines were used for breeding at Bayer this last year. The line **U14-103015** from University of Nebraska and Dr. Randy Nelson’s USDA diversity breeding program was the #1 line in the 2018 and the 2017-18 2-year average for the USDA Uniform Regional Tests over 25 locations and 2 years. It has diverse *Glycine max* ancestors as well as *G. soja* in the pedigree. This line was shared with other university and industry programs for their use in breeding. Five diversity lines from the Kansas State breeding program were shared with industry programs through MTAs for their use in breeding. * Other public researchers use diverse, high-yield, high-protein lines in their breeding programs to enhance yield and quality

In addition to the 340 entries in the 2019 Diversity Protein Cooperative tests mentioned above, our university and USDA breeding programs have advanced experimental lines with diverse germplasm in the pedigrees being evaluated in the USDA Uniform Regional Tests in Maturity Groups 0, I, II, III, IV and V. These lines are available to other public researchers as well for use in their breeding programs. * Genetic markers for protein QTL on Chr 15 and Chr 20 are available for use by breeding programs by April 2019

The candidate gene for the Chr 20 QTL was identified and information on this gene was shared with the breeding and genetics community at the Soybean Breeder’s Workshop. Based on this gene information, we have developed DNA primers that can be used to test germplasm to determine what allele soybean germplasm has for this gene which is available to other research groups. Work on find mapping the Chr 15 protein QTL has been slow and it remains in an interval with 74 candidate genes which is still too many for producing and testing markers. We hope to reduce the size of this interval soon.* The marker information for Chr 15 and Chr 20 is being used by breeding programs to enhance their breeding efforts for yield and quality

Sequences from within the Chr 20 candidate gene were used to genotype soybean experimental lines from one other breeding program to provide information on whether lines have the high protein allele at this locus.* Candidate genes for the protein QTL on Chr 20 and Chr 15 are identified by September 2019

The candidate gene for the Chr 20 QTL was identified and confirmed using transgenic plants which showed an impact on seed protein concentration from down regulating the gene. A manuscript is being prepared which show these results. Work on find mapping the Chr 15 protein QTL has been slow and a specific candidate gene has not been identified.* Soybean lines developed in this program show stable performance for improved yield and seed composition over locations and years (Evaluate in subsequent years).

M12-439036 (Sheyenne X PI 639637) was entered into the 00 regional trials in 2018. It was equivalent in yield to the check, ND Henson, but had two points more protein than the check. This line also showed good yield and protein performance in 2017 Minnesota advanced yield trials across three Minnesota locations, being equivalent to the check, MN0083 in yield and maturity. For the Diversity Protein Cooperative Tests in MG II, II, and IV, we evaluated seed composition on the top 50% yielding lines. Of those lines, at least 90% met the quality targets to be able to produce at least 10,5 pounds of oil per bushel and a soybean meal with at least 47.5% protein. The lines also meet or exceed the yield of the checks. The specific information is available in the March quarterly report in the attached files. * At least one new commercial soybean variety from an industry partner results from crosses made with lines from this program (This is our long-term end goal!)

 See information under KPI #2 above.  |
| 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 each year

The final summary report was shared with cooperators in January, and a copy was shared with USB via Lisa Weaver at Smith Bucklin. Electronic copies are available to USB members upon request. * 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

I have commented on our efforts here in past reports, citing some important considerations in industry programs. Those include stringent requirements on acceptance of outside material into their program, concerns about adventitious presence (AP) and the need for AP testing, and ability to evaluate conventional material in their industry programs. While this has been relatively straight forward and easy in the past, current industry structure and culture makes it more difficult. I continue to talk with contacts at all major companies to develop a plan that allows their continued participation in our cooperative testing to facilitate their adoption and use of diverse germplasm. It seems that one of the solutions with highest probability of implementation and success would be to arrange for contract testing of this material near locations for each of the industry cooperators to that the companies do not need to receive the material directly. We continue to work on this to maintain high level of industry involvement and use. * Develop a common, testing-only MTA acceptable to all commercial, university, and USDA participants to facilitate exchange of seeds for evaluation in the cooperative tests. Complete by January 31, 2019

This was not completed. * Make available experimental lines with improved yield and enhanced protein concentration and nutritional bundle to be used by both commercial and public programs.

All of these lines were shared. Determination of what will be transferred is in progress. Those already licensed are listed above:Line FPH Parentage Reason Yld trial notes Approx. RMM13-194018 P-T-Y M06-381085 X HEFENG 50 DIV 3rd year 1.8M13-194009 P-G-Y M06-381085 X HEFENG 50 DIV 3rd year 1.6M13-194022 P-G-Y M06-381085 X HEFENG 50 DIV 3rd year 1.4M13-194051 P-G-Y M06-381085 X HEFENG 50 DIV 3rd year 1.5M13-204003 W-T-Y M03-158071 X PI612739 DIV 3rd year 0.4M13-198033 W-G-Y PI639633A X PI639554 DIV 3rd year 0.6M13-190024 P-T-Y HEFENG 50 X M08-154093 DIV 3rd year 0.2M13-194010 P-T-Y M06-381085 X HEFENG 50 DIV 3rd year 1.7M12-437045 W-T-Y M02-495076 X M06-381077 DIVERS 4th year 1.6M12-439036 P-G-Y SHEYENNE X PI639637 DIVERS 4th year 0.9M12-454061 P-G-M ND07-2205 X PI639637 DIVERS 4th year 0U14-103015 was used by university and industry breeding programs. See information in KPI #2 above. The University of Illinois breeding program shared breeding lines that it developed with five public breeding programs and three private breeding programs. A total of 26 lines were shared with these breeding programsEight breeding lines with diverse pedigree were disclosed to ISU Research Foundation after two years of multi-location testing in 2017 and 2018. These lines are undergoing IA state-wide testing in 2019 with commercial checks in the test. Most promising lines will be entered in 2020 Uniform tests and prepared for commercial release.* Develop a list of high-protein germplasm accessions indicating the allele status the high-protein gene on chromosome 20 and chromosome 15 that can help select new sources of high protein that are genetically different from what is currently being used

A total of 190 plant introductions (PIs) were tested for the candidate gene on Chr 20 to identify high protein lines without this gene.* Identify genes in elite and exotic germplasm pools that influence seed protein concentration

To identify new genes for protein in exotic germplasm, crosses were made between high protein PIs without the Chr 20 high protein allele and a low protein cultivar. F2 populations from nine of these crosses were grown in the field this summer.We showed that long-term selection for increased seed protein concentration in both elite and exotic backgrounds led to increases in both 7s and 11s seed storage proteins, compared with the initial parent lines.Several of the high protein lines failed to accumulate Gy4 protein, instead accumulated a unique high-molecular weight protein. Protein composition of all the UP2 (Exotic) and UP3 (Elite) lines along with the respective parents were analyzed by one and two-dimensional gel electrophoresis. * Identify unique loci for yield and seed composition traits in exotic sources that are not present in the commercial gene pool

Protein composition of all the UP2 (Exotic) and UP3 (Elite) lines along with the respective parents were analyzed by one and two-dimensional gel electrophoresis. Some unique proteins were identified in the derived high-protein lines in both genetic backgrounds. High-resolution two-dimensional (2-D) analysis separated these unique protein into more than 5 discrete protein spots. Mass spectrometry have confirmed that these high molecular proteins are closely related to Gy1/Gy2/Gy3. Analysis confirmed significant increase in expression of Gy3 in the several of the high-protein progeny lines.Positive desired effects on sulfur amino acid composition in the high-protein progeny lines were observed; indicating that in these populations, selection for increased protein concentration in the seed also resulted in higher levels of sulfur-containing amino acids. * Develop at least one new soybean line with superior yield and seed composition and quality for the ESPS in the southern US

Three new soybean breeding lines with superior yield and seed composition and quality for the ESPS were developed and tested in the USDA Uniform Regional Tests in 2019. Regional 2019 data for the three lines are not yet available, but 2018 Stoneville trials in the ESPS showed that late IV MGs DS1169-512 (72.6 Bu/A) and DS1260-260 (72.6 Bu/A) yielded similarly to commercial cultivars AG4835 (73.3 Bu/A) and AG46X7 (70.1 Bu/A). In addition, both DS lines had at least 48% meal protein and had at least 80% standard germination after experiencing warm wet harvest conditions. The commercial cultivars also had 48% meal protein, but had germinations of 43% and 40%, respectively. Early IV MGs DS31-243 yielded 55.7 Bu/A, significantly higher than cultivar LD06-7620 (47.8 Bu/A), over eight locations in the 2018 USDA Uniform Tests, and produced 48.2% protein meal and 11.4 pounds of oil per bushel, meeting quality targets. Federal Grain Inspection Service mature seed damage ratings at Stoneville in 2018 were 5.9% for DS31-243, compared to 48.9% for LD06-7620. In the ESPS at Stoneville in 2019, DS31-243 yielded 56.2 Bu/A, compared to AG39X7 (51.1 Bu/A), AG4135 (50.1 Bu/A), and P40A47X (53.4 Bu/A) and DS1260-260 yielded significantly higher (67.8 Bu/A) than AG4835 (59.5 Bu/A). Clearly, we met this KPI. |
| 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. |
| There has been good, long-term and continued interest from all of our industry cooperators, which includes all the major soybean seed companies and some smaller and specialty seed companies. The follow-up to this project is the funded project which builds on our integrated and coordinated approach to develop ways to facilitate best choice and use of diverse germplasm to deliver improved yield and quality to the US farmer. We include genomics, biotechnology, proteomics, quantitative genetics, and breeding in a coordinated research and development program in our integrated, collaborative approach in the project “Increasing genetic diversity, yield, and protein of US commercial soybean germplasm.” |
| **List any relevant performance metrics not captured in KPI’s.** |
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| **Non-technical report (this information will be posted to website in place of above report):** |
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