Nebraska Soybean Board
Year-End Research Findings Report

Please use this form to summarize the practical benefits of your research project and what has been accomplished. Your answers need to convey why the project is important and how the results impact soybean production.

Project Title: 90957 - Development of next generation sequencing applications for improving soybean

Contractor & Principal Investigator: Dr. David Hyten

Please check/fill in appropriate box: ☐ ***Continuation research project***
☒ ***x Year 1 of 2 research project (for example: Year 1 of 2)***

1. ***What was the focus of the research project or educational activity?***
   This proposal was part of Dr. David Hyten's start-up funding to focus on developing novel methods that utilize new sequencing technology to better detect markers in the soybean genome.

2. ***What are the major findings of the research or impacts of the educational activity?***

   Objective 1: The sequencer in the UNL soybean genomics lab was set up and was able to produce usable data. The GRA position for the project was filled by the PhD student, Samantha McConaughy and the lab manager position was filled by John Wang. We developed a method to design probes targeted to specific markers in soybean for GBS. The 4K marker probe set was designed and tested. We were able to successfully obtain sequence data from the sequencer with the MIP probe set. This sequencing data identified that we were not getting the probe amplification primers to be cleaved off. After optimization of this critical step we are able to get successful amplification and are awaiting sequencing results to see if all the steps in the protocol were performed successfully.

   Objective 2: We were able to develop a method to sort pollen and extract the DNA into a 96 well plate. This method was able to amplify an SSR marker in 28% of the wells in a 96 well plate. From this amplification we determined our sorting, DNA extraction, and DNA amplification was working on some of the samples but was not a high enough percentage to move to the next stages of the project. We collected bulk pollen samples and developed a method to visualize pollen under fluorescent microscopes to identify size of pollen grains for optimizing flow cytometry. We also developed a method to visualize pollen while testing different DNA extraction methods. This will allow us to determine which chemicals will best lyse the pollen grains to release the DNA for analysis. Pollen grains sorted onto glass slides indicated that some pollen grains were broken upon impact while other pollen grains were not. Also, contamination was observed on the slides. Potentially another method of pollen collection is needed to remove contamination before sorting pollen. We were able to identify that the number of PCR cycles for amplification of pollen DNA needed to be increased.

3. ***Briefly summarize, in lay terms, the impact your findings have had, or will have, on improving the productivity of soybeans in Nebraska and the U.S.***

   Our current results indicate that this new method of genotyping molecular markers in soybean is still a method with great potential. It will be an improvement over current methods for marker genotyping at UNL and will maximize the soybean breeding program's breeding efficiency leading to accelerating the rate of yield gain.

*****This form must be completed and submitted with the fourth quarter report.***

This new protocol for marker genotyping will also be used by the larger soybean breeding community for improving soybean yield nationally as indicated by the additional funding we have been able to leverage with this current project.

Our findings have indicated that with some further protocol efficiency gains it will be possible to detect how the soybean's genome recombines between two parents in a high-throughput manner. This will enable UNL soybean researchers to further study how recombination is effected by the environment and how that affects the breeding program in creating new varieties.

4. Describe how your findings have been (or soon will be) distributed to (a) farmers and (b) public researchers. List specific publications, websites, press releases. etc.

Dr. Hyten has given 3 seminars to public researchers that highlighted the work being performed and the current results in this project. Dr. Hyten hosted a lab tour to the NSB that described the genotyping and the benefits of the genotyping methods being developed from the current findings. A feature article written for the Agronomy & Horticulture Department 2016 Annual Newsletter was written in collaboration with Dr. Graef and Dr. Clemente which describes the benefits of developing new molecular marker technology. This article will be published in the near future.

5. Did the NE soybean checkoff funding support for your project leverage any additional state or Federal funding support? (Please list sources and dollars approved.)

Yes. USB project titled "Utilizing Unique Genetic Diversity to Combine Elevated Protein Concentration with High Yield in New Varieties and Experimental Lines" awarded $79,970

NCSRP titled, "Increasing the rate of genetic gain for yield in Soybean Breeding programs" awarded $212,250