**Project Title**: Breeding for high yield, yield protection, and composition traits in soybean for Iowa farmers

Principal Investigator: A.K. SINGH

Budget Amount and Project Year: $305,008 (2015-16)

Report period: Oct 1, 2015 – March 31, 2016.

Brief Statement of Objectives:

The ***objectives*** of this project are:

1. Increase soybean seed yield using genomic and phenomic tools.
2. Incorporate yield protection traits using improved germplasm material.
3. Integrate improved SCN and aphid resistance and study the epistatic effect of the stacked gene combinations on trait expression, and on other important agronomic traits.
4. Study the effectiveness of additional aphid resistance genes.
5. Establish a marker-assisted selection breeding system and repository.

Efforts will be made to broaden the genetic pool by utilizing soybean lines from diversity panels. High -throughput phenomic and genomic tools will be used to achieve the objectives.

Research Progress

Objectives 1 and 2:

Highlights:

* Crossing was successfully conducted in Iowa (IA) and Puerto Rico (PR) to develop new populations to meet the project objectives.
* Breeding populations from 2014 were advanced in IA and Chile (pod pick and/or individual plant pulls) for subsequent yield and other trait selection in 2016 and onwards.
* Genome wide association and epistatic studies conducted on sudden death syndrome, white mold and IDC. New sources of resistance for several diseases, pests and abiotic stress have been identified and will be used as parental stocks.
* Independent studies on plant population and row spacing were conducted in 2015, and research findings were shared at the 2016 ISA on-farm network conference.
* Data analysis and interpretation is on-going from the aerial imagery based phenotyping.
* Advanced data analytics (machine and deep learning) was utilized in the breeding program for application in trait selection.

In the breeding program, we initiated the development of new populations to meet our objectives, and the F1 generation was sent to PR for additional crossing and increase (two cycles). Sixty-six populations were created in the 2015 Summer and Winter Crossing Blocks (SCB, WCB) at Ames, IA and Juana Diaz, PR. Four F2 populations from 2014 summer crossing were increased in 2015 at PR. In addition to high yield parents, yield protection traits included resistance/tolerance to SCN, SDS, white mold, IDC, charcoal rot, brown stem rot, and soybean aphid. Parents included yield diversity soybean genotypes from Dr. R. Nelson’s program. Fifty-nine three-parent crosses were created in PR in cycle 1, and F1 seed were increased in PR cycle 2. Plant stands were reduced due to carlavirus infection leading to lower population size for 22 populations. Estimated harvest date is the third week of May, and the F2 seed received from PR will be planted in Ames in a space-planted nursery. In addition to PR, Chile was utilized as a winter nursery, and six F1 populations created in the 2015 summer crossing block were sent to Rancagua, Chile for a full-season increase. Other material in the Chile block included checks and F2 and F3 increases from the previous year’s crossing. Pod picks and/or individual plant pulls were made in Chile, and the seed returned April 8 will then be planted in bulk populations (for plant pulls in Ames) or progeny rows (bulk row harvest) for selection and continued yield testing.

In our attempt to improve the soybean yield, we have taken two main research approaches: 1) understand the genetic drivers of soybean yield, and 2) increase the genetic diversity of soybean germplasm to breed with novel genes/alleles. Experiments included replicated trials at three locations in Iowa to study elite cultivars vs. plant introductions, varying maturities, growth habit (indeterminate and determinate), planting density, and row spacing (15” and 30”). Meaningful measurements were taken at critical vegetative and reproductive growth stages using multiple sensors (ground) and aerial imagery. Statistical analysis has revealed that, while minimal yield differences are observable at 140K and 210K (seeded) population densities, the physiological processes driving yield are different among genotypes. Significant genotype x row spacing interaction was observed suggesting correct placement of soybean varieties may lead to higher yield in different situations. Presentation on these findings was made at the 2016 ISA On-Farm Network Conference.

To increase the diversity of soybean germplasm, we are currently testing plant introductions for their merit to use as a parent in our breeding program. We planted specialized field (and indoor) nurseries to assess the genetic worth of plant introductions for new sources of stress tolerance/resistance and have now identified accessions that will be used in our plant breeding program that provide unique disease and pest resistance genes. Identification of unique accessions that will complement and increase yield is on-going. Additionally, we are also working with diverse accessions from Dr. R. Nelson’s program. In 2015, 18 heterogeneous soybean populations were planted in Ames. These populations were derived from exotic germplasm which includes both soybean and wild soybean. Single plants were selected by maturity from each population. We selected for group II to early III maturity groups. Each single plant was threshed and will be planted into a 2-row, 15 foot plot in 2016 near Ames. These plots will be blocked by maturity. Selections will be made at the end of the season for advancement through the breeding program.

Data analysis of genome-wide studies including several hundred PI accessions from the USDA Soybean Germplasm collection from MG I, II, and III, to identify genomic regions controlling disease resistance (white mold, sudden death syndrome, charcoal rot, iron deficiency chlorosis, viruses, bacterial blight, etc.) is ongoing through ISA and other supporting/funding agencies. The outcome of this work will be the identification of superior parental stock and genomic regions for eventual integration into our high yield germplasm. Results will be shared with Dr. S. Cianzio and other soybean collaborators so that they can utilize this information for soybean research.

Objectives 3 and 4:

Highlights:

* Experiments on testing the effectiveness of stacked aphid resistance are near completion, and results indicate that the triple stacks will be useful for long-term viability of soybean aphid resistance.
* Experiments on the role of trichome density on aphid resistance suggest that trichome density does not provide protection against soybean aphid but does increase the number of insects that are natural enemies of aphid.
* New sources of aphid resistance and molecular insights for better molecular markers for aphid resistance were identified.

To study the effectiveness of stacked aphid resistance several experiments were done.

Experiment#1: Soybean aphid biotypes 1, 2, 3, and 4 were screened on 13 treatments with different *Rag* gene combinations. One of the aims of this screening was to determine whether the three-gene combination would succeed in suppressing aphid populations among all biotypes. This was tested by applying five mixed age aphids to the soybean plant and taking population growth measurements 11 days after initial infestation. To date, all of the biotypes except for biotype 1 have been screened with three repetitions. The last repetition for biotype 1 is currently in progress and should be completed within the coming weeks.

* B1 Results: Biotype-1 *A. glycines* survived poorly on all treatments containing *Rag* genes. Two of the three repetitions are completed for this biotype, and the third will be completed soon.
* B2 Results: Biotype-1 *A. glycines* did well on *Rag1* but did poorly on all treatments containing the three-gene combinations.
* B3 Results: Biotype-3 *A. glycines* did well on *Rag2* but did poorly on all treatments containing the three-gene combinations.
* B4 Results: Biotype-4 *A. glycines* performed well on *Rag1, Rag2, Rag1/2,* and on the three-gene combinations containing *Rag1+2+4.* However, the populations were reduced on the three-gene combinations containing *Rag1+2+3.*

These are very promising results in our effort to breed for aphid resistance.

**Experiment#2**: We also conducted experiments to test whether trichomes prevent or diminish damage by soybean aphid; this result can make it a breeding objective and provide additional protection to farmers against soybean aphid. Separate lab and field experiments were conducted.

Lab Experiment: We hypothesized that the *A. glycines* populations on soybean plants would vary depending upon trichome density. We used 10 varieties of varying trichome densities all derived from a similar background (Clark). To determine if this impact was comparable to *Rag*-genes, we included an aphid susceptible (IA3027) and aphid-resistant (IA3027RA12) varieties as susceptible and resistant controls. A total of 16 replications of each variety was tested.

Field experiment: We hypothesized that trichome density would affect *A. glycines* in a field setting with natural enemies present. We tested a glabrous variety and a variety with the most trichomes (extra-dense) compared to our susceptible and resistant controls (described above).

Each variety was both caged and left uncaged to determine the impact of natural enemies on *A. glycines*. Every combination of variety and caging was replicated 10 times at the ISU Horticulture Farm in a randomized complete block design. Soybean aphid and SA natural enemy were monitored and counted.

Results:

* Uncaged soybeans had fewer *aphids* than caged after two weeks.
* A glabrous variety had higher biological control (BSI) compared to an extra-dense variety over a two-week period suggesting that trichome density did not seem useful to protect against aphid damage.
* More natural enemies were observed on the extra dense variety and the least on the glabrous variety suggesting that the extra dense varieties may promote beneficial insects for biological control.

Experiment #3: Furthermore, we conducted transcriptome analysis to understand the molecular response of different *Rag* genotypes to feeding by soybean aphids to develop better molecular markers and characterize the molecular effect of stacking *Rag1* and *Rag2* aphid resistance genes.

To address the above research objectives, transcriptome analysis was conducted using RNA sequencing to investigate the molecular response of four near isogenic soybean lines (one aphid-susceptible and three aphid-resistant: *Rag1*, *Rag2* and *Rag1Rag2*) to aphid feeding at 6 hours and 12 hours, respectively. Comparison of mock-treated samples versus aphid-treated samples for each soybean line showed that the transcriptional response of the soybean lines to aphid feeding involved unique sets of genes and shared genes respectively at both time points. At the early time point (6 hours), each of the soybean lines tested had a distinctive transcriptional response to aphid feeding. However at the 12 hour time point, more common biological processes were significantly modified by aphid feeding for all four soybean lines tested. This study also characterized the molecular effect of increased resistance in the *Rag1Rag2* soybean line that results from stacking these two aphid resistance genes. The data from this study will also be utilized for *de novo* synthesis of prospective loci for *Rag1* and *Rag2* resistance genes. It will give more insight on the molecular mechanisms of resistance to soybean aphids and also guide future approaches to genetic improvement of soybean resistance to aphids. Currently, data analysis and manuscript writing for this study is ongoing.

Experiment#4: In a separate study aimed at identifying new sources of resistance to soybean aphids, a diverse panel of soybean lines has been screened for aphid resistance using choice tests. In this panel, 16 soybean lines were found to be resistant (7 lines) or moderately resistant (9 lines) to soybean aphids. Data analysis to identify possible candidate resistance genes in these 16 soybean lines is in progress. Additionally, follow-up experiments to determine the type of resistance in the resistant soybean lines are being conducted. The expected publication date for this research paper is by December 31, 2016.

Overall, findings from both the above studies will broaden the knowledge base on the molecular mechanisms of resistance to soybean aphids. This information will be useful for the management of not only soybean aphids but also several other phloem-feeding insect pests of other cultivated crop species, ultimately reducing the economic losses that result from damaging insect pests. The aphid-resistant soybean lines identified from the aphid-resistance screening studies will be utilized in plant breeding programs to develop more soybean lines with durable aphid resistance, and these will be made available to soybean growers.

Objective 5:

Highlights:

* Molecular marker and genomics lab set-up and functional
* Data management system has been implemented

The molecular breeding lab, capable of handling marker-assisted selection, SNP discovery and screening, and expressions studies has been set up in the reporting period. Equipment for this lab was purchased through other synergistic funders including private company investments, but this lab is now functional and able to handle molecular breeding components. The data management system has also been fully implemented and integrated in the Singh group’s breeding and research activities.

Benefit of Soybean Farmers: (This explains the potential impact to farmers based on research project being done) 250 words

The direct benefit of this research to farmers will be the development of soybean cultivars with superior yield and production potential. Our work will lead to the development of high yielding genotypes to be released as commercial cultivars or used as parents to develop elite breeding lines for commercial application and for further breeding genetic stocks. The ISA, ISU researchers, and extension personnel can also utilize this information we are generating on the interaction of genetics with cultivation practices, in their own work for the benefit of the farmers. The use of the modern and sophisticated solutions of phenomics and genomics may enhance the rate of genetic gain. Information on the molecular basis of aphid (and other traits) resistance will be beneficial in developing breeding strategies for future pest protection.

Performance Metrics: (These are measurement tools we use to measure the success of the project for the association who funded it) 250 words

We used the following performance metrics:

1. Were the objectives completely or mostly met?

Yes. See project highlights and progress.

1. Did the outcome of this research lead to further advancement of soybean profitability?

Yes. See project highlights and progress – population advancement to increase yield, field trials to study genotype x crop management interactions, identification of new parental stocks, better molecular markers, use of imaging technology to phenotype, identification of improved disease and pest resistance, and establishment of genomic capabilities.

1. Did this project lead to successful partnerships and collaborations with the funding agency and within the research team members?

Yes. Meaningful and strategic productive partnerships were established among the PIs and other collaborators. Evidence of joint projects, papers, posters.

Funding acknowledgement (verbal presentations and research articles):

1. Presentations made by A.K. Singh and his group acknowledges ISA support. These presentations were made at SD state university, Baker symposium, Data Driven science workshop, Seed science center, Phenotypic prediction conference at ISU, and in Brazil.
2. Zhang J, A Singh, D Mueller\*, AK Singh\*. 2016. Genome-wide association and epistasis studies unravel the genetic architecture of sudden death syndrome resistance in soybean. The Plant Journal 10/2015; DOI:10.1111/tpj.13069.
3. News articles: AgWeb, CALS-ISU