Efficient and economical molecular diagnostic tools for soybean stresses

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Objective 1: *Examine the suitability of selected marker genes for different soybean stresses in SD varieties (Year 1).*

Objective 2: *Examine selected marker genes for quantitative estimation of stresses in SD soybeans (Year 2).*

Objective 3: *Develop a qPCR panel and test is on field conditions (Year 3)*

Progress Report (Year 1)

Summary: One of the major obstacles in achieving the goal of producing 100-bu/ac soybeans is yield losses due to various stresses. Accurate and sensitive diagnosis of stress at the earliest stage is critical for proper management responses to mitigate yield losses. However, many times these stresses go unnoticed at early stages of crop growth due to the lack of visible symptoms. Armed with knowledge from prior research funded by SDSRPC, we propose to develop molecular diagnostic tools that can efficiently and accurately identify specific stresses experienced by soybeans in the field at very early stages. For this purpose we have selected marker genes for five major stresses (drought, salinity, nutrient deficiency, diseases and root pests). In Year 1, we planted 23 soybean cultivars collected from SD and are testing their suitability to diagnose the specific stress under green house conditions. In Year 2, we seek to expand this by and selecting specific expression markers to test their suitability to quantitatively diagnose resistance levels of these cultivars to stress factors and by genotyping these lines for known genes or QTLs. At the end of the project period (three years), we expect to have a diagnostic tool that can be used to identify specific stresses experienced by soybeans in the field so that appropriate management decisions can be made and yield losses minimized/avoided.

Results:

1. General – Sample collection methods and RNA isolation

For successful use of the diagnostic tool, efficient methods need to be established for field collection of samples. This is generally performed using liquid nitrogen to flash freeze the samples. We evaluated two different collection methods (i) using liquid nitrogen and (ii) using

RNA-later, a proprietory commercial sample preservation reagent. Two different methods of sample processing were evaluated, trizol and column-based RNA isolation. The results indicated that the RNAlater reagent can be successfully used to collect samples in the field without the need for flash freezing the samples. This is significant as this will enable easy sample collection by the farmers for sending samples for diagnosis. The trizol method has less contamination from undesirable materials such as DNA or polysaccharides where as the column-based method worked well, but had some contaminants. This RNA preparation was used to validate the primer pairs designed for the marker genes of interest.

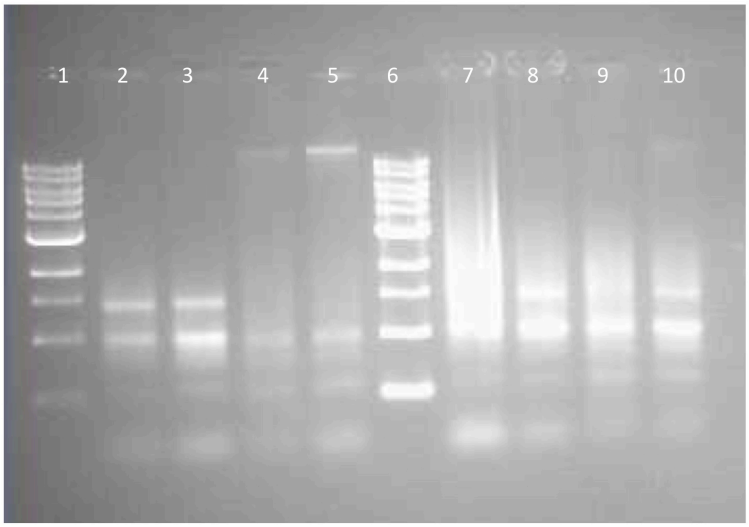
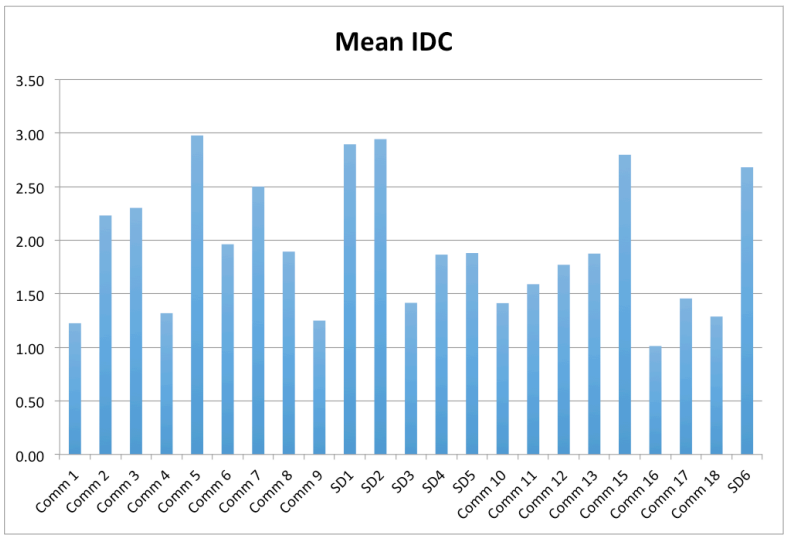


Figure 1. *An agarose gel image showing the quality and quantity of RNA isolated from green house samples using different methods. Lanes 1 and 6 – markers; Lanes 2-3 (liquid N harvested, processed with Trizol); Lanes 4-5 (liquid N harvested, processed with column); Lanes 7-8 (RNAlater harvested, processed with Trizol); Lanes 9-10 (RNAlater harvested, processed with column).*

2. Abiotic stress (iron deficiency)

A collection of 22 cultivars of soybean were evaluated for the resistance to iron deficiency chlorosis (IDC) in a field experiment with four replications. Genotypic variation in IDC was detected in the cultivar collection (ranging 1.1 to 3.2 in the 1 to 5 scales, 1 is the most resistant; (Figure 2A). While a mean IDC score of 2.0 indicated that most cultivars were fairly resistant, the field experiment clearly indicated that the commercial varieties variation can be exploited for developing diagnostic markers of stress tolerance/susceptibility. All cultivar samples are being evaluated for marker gene expression.



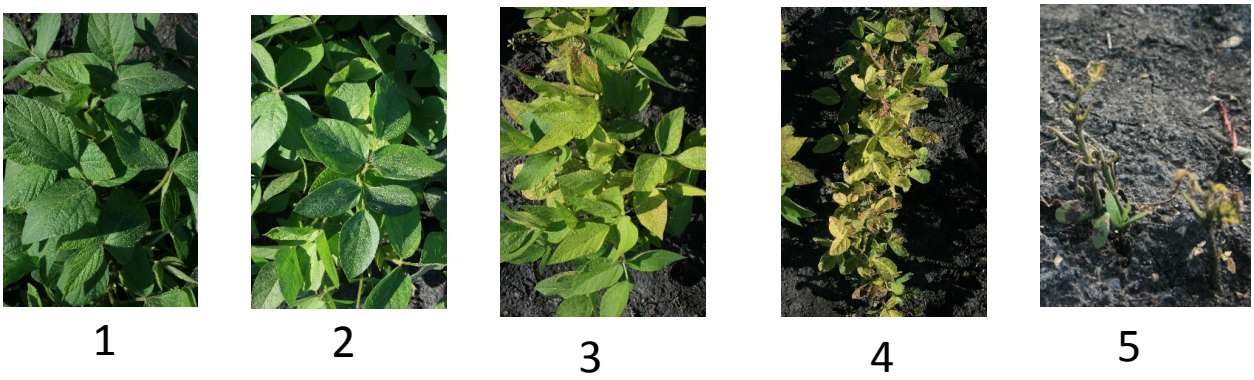


Figure 2A. *IDC scores for the different cultivars evaluated in Year 1. Most cultivars scored towards the "moderately resistant" spectrum. Representative plants with different deficiency scores are shown for comparison.*

A population of 200 recombinant inbred lines derived a cross of wild and cultivated soybean was evaluated for IDC in a field with high pH (8.3) soil with four replicates. The population varied in the IDC score from 1 to 5 evaluated on July 1. 15, and 30, and August 15 (Figure 2B). Heritability for the resistance to IDC in the population varied from 0.52 to 0.55. A total of 7 quantitative trait loci (QTL) for the IDC trait were detected in the population (Table 1). Each of the QTL contributed 5% to 11% of the phenotypic variance. The 2014 data confirmed our previous observations that the QTL alleles that enhance the resistance to IDC distribute in both cultivated and wild soybean germplasm and each contributed a relatively small effect on the phenotypic variation.

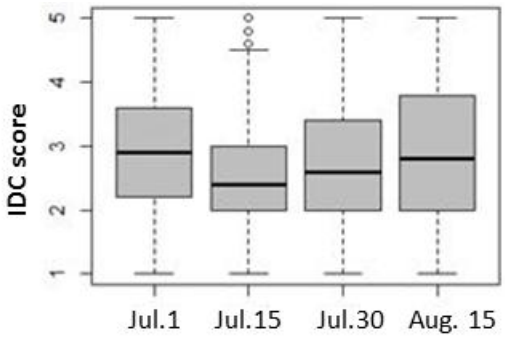


Figure 2B *Distribution of iron deficiency chlorosis (IDC) in the recombinant inbred line population evaluated at four time points in 2014. IDC scores 1 and 5 represent the most resistant and susceptible, respectively.*

Table 1. List of QTL associated with iron deficiency chlorosis detected in a population of recombinant inbred lines from a cross of *Glycine max* and *G. soja*. The population was evaluated on SDSU Larson Farm at four time points in summer 2014.

QTL	Chr.	Nearest marker	Likelihood ratio	% variance explained	Additive effect	Valuation time	Donor of resistance allele
Fe effic 15-1	A1	satt684	14.3	6.8	0.20	Jul. 15	*G.soja*
			16.8	9.5	0.27	Aug. 15	*G.soja*
Fe effic 15-2	A2	satt333	15.3	6.8	-0.20	Jul. 15	*G.max*
Fe effic 15-7	D2	satt186	12.7	5.5	-0.20	Jul. 30	*G.max*
Fe effic 15-8	E	satt411	16.9	10.0	-0.25	Jul. 1	*G.max*
			18.7	10.9	-0.27	Jul. 30	*G.max*
Fe effic 15-9	E	satt045	17.4	7.1	-0.22	Jul. 1	*G.max*
			13.9	5.7	-0.19	Jul. 15	*G.max*
			16.9	6.4	-0.21	Jul. 30	*G.max*
Fe effic 15-10	F	satt114	27.2	10.6	-0.39	Aug. 15	*G.max*
Fe effic 15-13	H	satt142	23.8	9.7	-0.28	Jul. 30	*G.max*
			22.5	9.1	-0.28	Aug. 15	*G.max*

3. Abiotic stress (drought and salt stress)

All 22 cultivars have been grown in the greenhouse for drought and salinity stress imposition. The expectation was to complete all gene expression assays and analysis by the end of May 2015. However, insect infestation at two different experiments in the green house made it impossible for us to complete the experiments on time. However, we did collect plant height data on the insect infected plants after spraying with insecticides. We observed variation in response to both drought and salt stress indicating that the cultivar collection will be suitable for screening molecular markers for stress responses. These samples unfortunately cannot be

used for molecular diagnostic experiments since gene expression changes are expected due to insect damage. The experiment is being repeated now as part of Year 2 experiments.

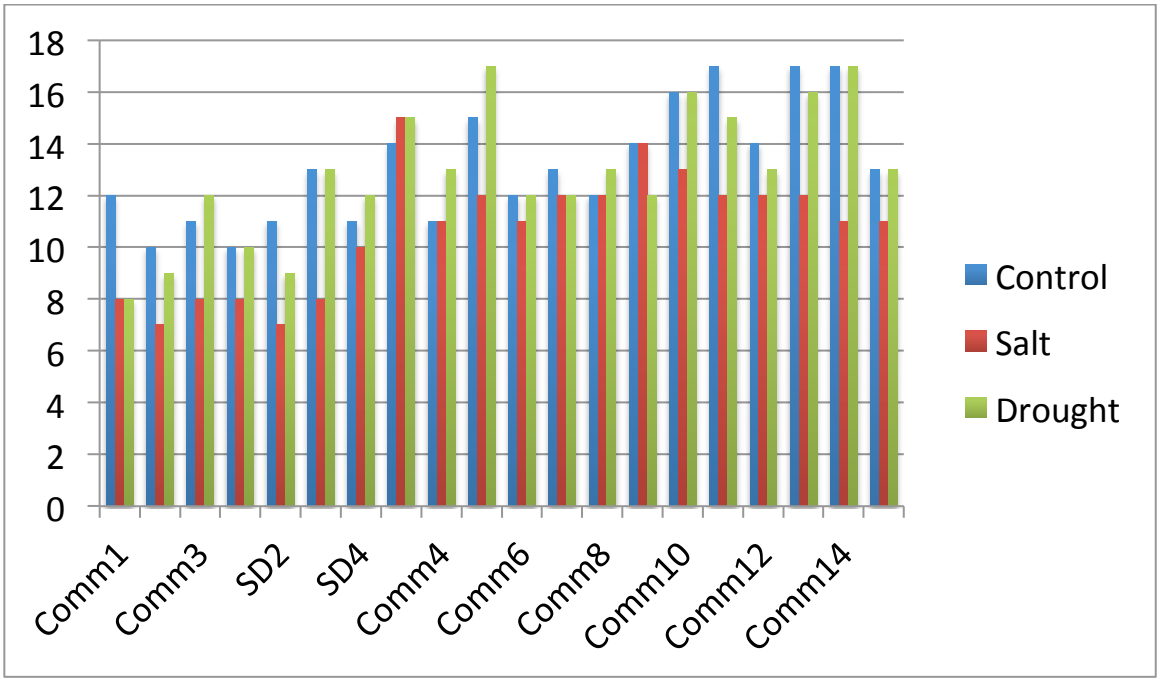


Figure 3. *Plant height (inches) in response to salt and drought stress. Reduction in plant height is indicative of stress response. It is notable that a number of lines did not show a reduction in plant height in response to drought stress while nearly all lines had reduced plant height in response to salinity stress.*

4. Biotic stress (**Response to SCN and *Phytophthora sojae)***

The same collection was screened for response to soybean cyst nematode (SCN) and two SD ioslates of *Phytophthora sojae*, the root rot pathogen in collaboration with Dr. Emmanuel Byamukama. The cultivar collection had significant variation in their resistance against SCN (Figure 4). Indeed, none of the cultivars tested showed benchmark resistance against SCN (which is 10% female reproductive index relative to a susceptible check). Nevertheless, the variation would be suitable for use in diagnostic marker development. Leaf samples have been harvested from these plants and are being evaluated for marker gene expression.

Similarly, we observed significant variation in terms of resistance against the root rot pathogen *P. sojae*. While several varieties displayed strong resistance, there were several varieties with very high susceptibility in terms of seedling lethality (Figure 5). Leaf samples have been collected from infected plants and are being evaluated for marker gene expression.

We had some issues with RNA quality from leaf samples with significant damage from pathogen infection. However, the diagnostic tool is more likely to be used with samples with less obvious symptom. Therefore, this should not be a big bottleneck.

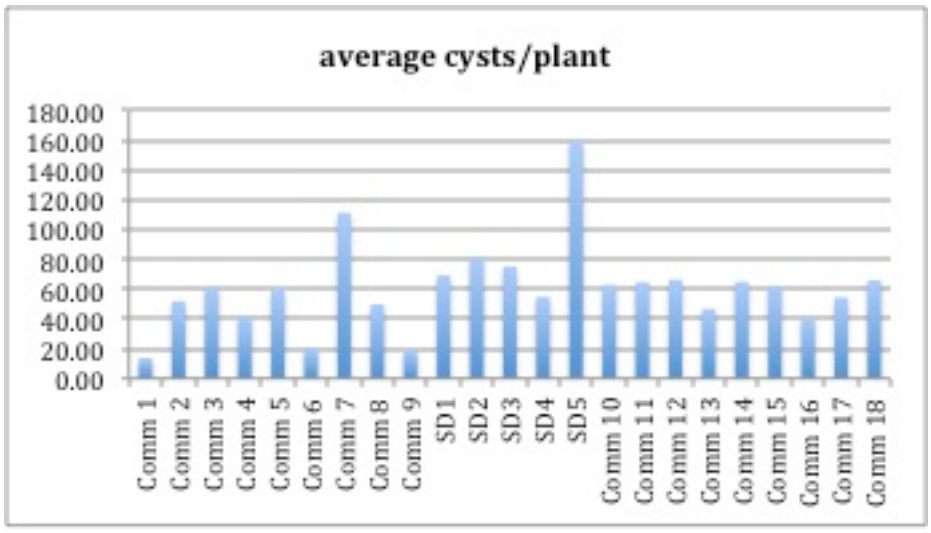


Figure 4. *Average number of SCN cysts per plant (2-3 replicates each; cysts counted at 3 and 4 weeks post inoculation). None of the tested lines were "resistant", but a few of them were moderately resistant while most were susceptible.*

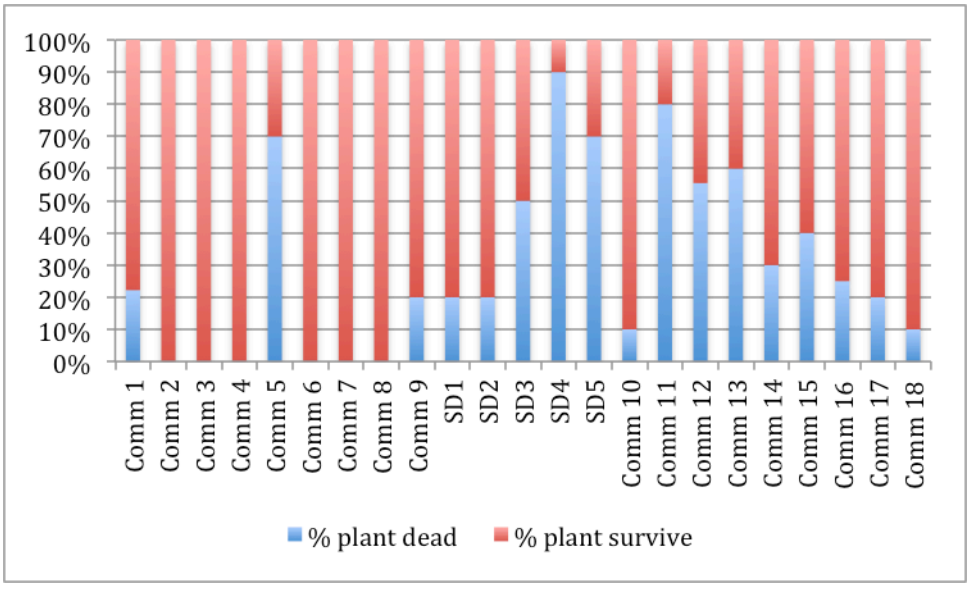


Figure 5. *Seedling survival as a measure of resistance against P. sojae root rot in the cultivars tested. A few lines were "resistant" while the majority had a range of susceptibility scores.*

Conclusions:

1. Sample collection method without the need for freezing was tested and found to be suitable
2. For each of the five stresses tested, there appears to be significant variation in observable phenotypes among the 23 cultivars tested. This is suitable to test the marker genes.
3. Primer pairs have been developed for marker genes of interest for all stresses and tested to work appropriately