1-26-2019 OSC quarterly report for the quarter of October 1 to December 31, 2018

Objectives:

1. Characterize the *Rag5* gene conferring resistance to soybean aphids.
2. Interrogate the *Rps8* locus conferring resistance to the root rot pathogen *P. sojae.*
3. Assess how the plant hormone auxin contributes to quantitative resistance to seedling pathogens.
4. Develop a novel tool for selecting breeding progeny that harbor resistance genes.

Progresses:

- *Rag5:* We described in the last report that at least five lines of Rag5-transgenic plants, plus five control transgenic plants expressing an unrelated transgene (green fluorescent protein or GFP), were being reared in greenhouse, waiting for seed setting. We have now harvested the Rag5-transgenic seed from two of the Rag5 lines and two control GFP lines. The transgenic seeds have been sowed during the October to December quarter of 2018. The seedlings are now been tested to verify the presence of the Rag5 transgene. Once these testing are complete, the positively transgenic plants will be subjected to feeding with soybean aphids to determine whether they indeed become resistant to the soybean aphids. As noted earlier, the putative Rag5 gene was identified through virus-induced gene silencing (VIGS) of seven different candidate genes. Soybeans that are naturally susceptible to aphids, should become resistant if transformed with the correct Rag5 gene.

- *Rps8:* We have decided recently to use the PI line #399073 as the target plant for VIGS-enabled interrogation of Rps8 gene candidates. This is because (i) the Rps8 gene was originally isolated in PI 399073; and (ii) compared to the breeding lines that acquired the Rps8 gene from PI399073, the PI 399073 itself is substantially more responsible to ALSV VIGS. We are currently carrying out two parallel sets of experiments under this objective. (a) we are further calibrating the condition for ALSV VIGS in the PI399073 in order to maximizing the efficiency of VIGS. (b) we are busy cloning the candidate gene fragments into the ALSV VIGS vector.

- *QTLs on Chromosome 19:* We are using two different approaches to attack the candidate genes mapped to the QTLs. (i) To assess the candidate genes that potentially confer resistance to *Fusarium graminearum* in the seed coat, we are using ALSV VIGS to target the candidate genes CG1, CG2, CG3, CG4, and CG5 in the PI line #567301B resistant to F. graminearum. Our preliminary testing showed that we probably will succeed in silencing these genes in the seed coat, so that their expression would be diminished in the seed coat. The seed harvested from these plants will then be tested for loss of resistance to F. graminearum. (ii) In order to investigate a new gene found in the Conrad/Sloan population that was implicated in resistance to the root rot pathogen P. sojae, we used a hairy root procedure to successfully silence this gene, named as CG6. The resulting hairy roots are being assessed for changes in resistance to P. sojae.

- Using the virus BPMV to interrogate avr proteins encoded by P. sojae. We described in last quarter's report that we sought to screen for the functionality of *Rps* genes function in various soybean cultivars or PI (plant introduction) by expressing their corresponding Avr proteins using a virus (Bean pod mottle virus, BPMV). This idea has now been tested with two Avr proteins, Avr1a and 1c, and revealed a high correlation between development of rusty leaf spots and the presence of the corresponding *Rps* genes in ten different soybean cultivars. We further found

that some cultivars previously thought to have *Rps1a* or *1c* weakly responded to Avr1a or 1c expression, indicating that the *Rps* gene expression could be influenced by other genes present in the cultivars. These results are being repeated in order to finalize a publication.

Activities planned for the next quarter:

- Continue the pursuit of the *Rag5*, *Rps8* genes and disease resistance *QTLs.*
- Repeat and complete the study of using BPMV to express Avr proteins as a way to screen for *Rps* gene functionality.