North Central Soybean Research Program

Characterization and enhancement of soybean genetic resources for soilborne disease resistance

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Soilborne diseases caused by various oomycete and fungal pathogens have been a major limitation to soybean production. *Phytophthora sojae*, *Pythium ultimum*, *Pythium irregulare*, and *Fusarium graminearum* are the major pathogens in the North Central Regions.

Development and planting disease-resistant soybean cultivars remains the most practical, economical, and environmental-friendly solution for eliminating or reducing soybean yield losses from these pathogens. Due to rapid changes of the pathogen, most known resistance genes have become ineffective or partially effective.

The primary goals of this project are to characterize, identify, and/or isolate novel genes/QTLs conferring resistance or partial resistance to *P. sojae*, *P. ultimum*, *P. irregulare*, and *F. graminearum*, and to deploy the new sources of resistance towards effective disease management in the North Central region.

Project Objectives

- Evaluate soybean germplasm for resistance or partial resistance to *P. sojae*, *P. irregulare*, *P. ultimum and F. graminearum*).

- Identify QTLs underlying resistance to *P. sojae, P. irregulare, P. ultimum and F. graminearum by association mapping.*

- Fine map, isolate and functionally verify two uncharacterized *Rps* genes conferring resistance to *P. sojae.*

- Develop highly adapted soybean cultivars, or experimental lines, with major resistance QTLs and *Rps* genes by marker-assisted selection.

Benefit to Soybean Farmers

The goal of this research is to capitalize on molecular methods for identifying novel resistance to seed and seedling rot pathogens. The potential impact of this research

would add to the options available to soybean farmers for managing these diseases. The potential benefit for American soybean farmers would be an increase in yield of as much as 10% based on estimates of yield lost to *P. sojae* and the complex of seedling diseases that include *Fusarium* and *Pythium* species.

The research is necessitated by changing patterns in the occurrence of diseases common in the corn-soybean cropping system. Identification of additional highly effective *Rps* genes is a necessity because of the appearance of new and complex *P. sojae* pathotypes capable of overcoming currently available resistance genes. Resistance to *F. graminearum* is necessitated by the increased prevalence of this fungus as a pathogen of soybeans grown in rotation with corn.

Resistance to *Pythium* species is increasingly important as the prevalence of *Pythium* species that infect both corn and soybean become more common with planting of sequences consisting of only these two crops. Because resistance would be available as a varietal trait, the benefit would be available at the lowest cost. In addition, effective control of seed and seedling rots would protect the value of seed, currently the largest single expense in soybean production. In combination with seed treatment fungicides and management practices, varietal resistance would enhance the ability of these tactics to limit losses caused by seed, seedling, and root rots.

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Objective 1
Seed, seedling, and root rot caused by *F. graminearum* and *P. ultimum var.ultimum* was evaluated in six experiments conducted to
1) confirm repeatability of results obtained in inoculated assays (three experiments) and
 2) to phenotype all 280 ancestral lines for resistance to *F. gramearum, P.ultimum,* and *P. irregulare* seedling rot (three experiments).

In preparation for phenotyping the ancestral lines, the response of a selected subset consisting of 24 lines of the 280 ancestral lines were evaluated for their response to the seed and seedling rot caused by inoculation with *Pythium ultimum* and by inoculation with *P. irregulare.* The evaluations were conducted twice with three reps for both pathogens. The seed rot assay results were compared by correlation analysis to determine the repeatability of the results obtained using the in vitro inoculation method.

Correlation analysis of results within each Pythium species indicated that root length or root mass yielded the most consistent responses to inoculation (P. ultimum : r2 of 0.87 and 0.91 for root length and root mass respectively).

Response to seed root (in vitro assay) was not correlated with the response to seedling rot.

Three replicates of 280 lines are being evaluated for resistance response to inoculation with *P. ultimum* and to inoculation with *P irregulare* in the seedling assay planted in growth media. This trial was just completed and inoculated plants are being evaluated and assigned a root necrosis score and the ratio of uninoculated to inoculated root length and root dry weight are being calculated and data recorded. Three replicates of the seed assay for resistance to P. ultimum were planted.

Three replicates of 280 lines were tested for response to inoculation with *F. graminearum* in a seedling assay planted in growth media. Inoculated plants were assigned a root necrosis score, the ratio of uninoculated to inoculated root length and root dry weight are being determined, results are being calculated and data recorded for analysis.

Objective 2
Data from phenotyping for resistance to 1) *Fusarium graminearum*, 2) *P. ultimum*, and 3) *P. irregulare* in the seedling assay is being prepared for association analysis.

Objective 3
We have measured expressional changes of candidate genes for RpsUN1 and RpsUN2, respectively, at different time points (2, 4, 8, 12, 24 hours after inoculation) by quantitative real-time PCR, and cloned the candidate genes for RpsUN1 and RpsUN2 into a plasmid vector, and confirmed that the integrated gene sequences were correct by sequencing. We have been in the process of transferring the plasmids with integrated sequences of RpsUN1 and RpsUN2 candidate genes into Williams, a soybean variety that is susceptible to *Phytophthora sojae* for resistance evaluation suing whole plant as well as the hairy root systems.

We are using a special *Phytophthora sojae* strain with a molecular (Green Florescence Protein) tag to inoculate soybean hairy roots produced using Williams. This system will be used to evaluate phenotypic variation between transgenic and non-transgenic hairy roots

We are continuing the backcrossing process assisted with marker assisted selection towards the introgression of RpsUN1 and RpsUN2 into four Purdue elite cultivars. The backcrossing progeny seeds are being planted in the greenhouse and the progeny seeds from backcrosses in the greenhouse will be planted in the filed in early June.

Objective 4
Progeny seed of crosses of RpsUN1 and RpsUN2 into prebreeding lines and elite breeding lines was increased in Chilean winter nursery.