North Central Soybean Research Program

Development of soybean genotypes with enhanced capacity of nitrogen fixation

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Progress report for 2016

Soilborne seedling and root diseases of soybean significantly reduce yields in the North Central region of the United States. Seedling diseases rank among the top four pathogen threats to soybean, because their insidious nature makes them difficult to diagnose and control. It is nearly impossible to predict when they will take a heavy toll, until it happens. The challenges and failures of managing soilborne diseases and pathogens of soybean and other crops are based in part on limitations in knowledge and methods.

This project will address critical limitations in identifying and managing seedling diseases. Producers and industry will see benefits in the form of rapid diagnostics and management recommendations. This benefit will also help industry in their assessments in pesticides and germplasm development.

Project Objectives

1. Development and deployment of a panel of QPCR probes to identify and quantify fungal seedling pathogens of soybean
2. Curate the collection of fungal pathogens collected during the first phase of this project
3. Improve understanding of the biology of *Rhizoctonia solani* as a seedling pathogen of soybean
4. Improve understanding of the biology of *Fusarium* sp. as a seedling pathogen of soybean
5. Improve understanding of the biology of *Pythium* as a seedling pathogen of soybean
6. Evaluate the effect of multiple pathogen interactions on seedling disease
7. Impact of seed treatments on the interaction of seedling pathogens
8. Communicate research results with farmers and stakeholders

Reporting Period Accomplishments

Objective 1: We have developed qPCR assays targeting the following most frequently found fungal species associated with seedling diseases in soybean, as determined in the first phase of the USB-NCSRP project. Currently assays are being optimized and tested for specificity against non-target species.

Objective 2: We have finished cataloguing 3000 fungal isolates that are now stored and maintained at SIU. A searchable site is still under construction and will be publically available soon.

Objective 3: In May and June of 2015, we surveyed for seedling disease in 14 different counties, an area of approximately 24,500 square miles and representing the major soybean production areas in Nebraska. From each field, we collected 10 to 20 soil samples and (when present) symptomatic seedlings from a total of 20 grower fields. A total of 195 soil and plant samples were collected.

Objective 4:Most *F. proliferatum* isolates are more resistant to fludioxonil that those of other pathogenic species of Fusarium. However, *F. proliferatum* isolates that were resistant to fludioxonil were not resistant to azoxystrobin. Most germplasm screened using *F. proliferatum* in the rolled towel assay (30C, pH 6.5) resulted in medium to high severity ratings. However, some materials are highly resistant to this fungus (e.g., AR AES R09-430, Bayer HBK RY4721, Midland 3884NR2, NK S39-T3, and Nutech 7414). Among the 2014 KSVT germ plasm entries, a positive relationship appears to exist between *F. proliferatum* disease severity and that observed for SDS (R2 = +0.52) and SCN (R2 = +0.13) in the field.

Representative isolates of *Pythium lutarium, P. oopapillum, P. sylvaticum,* and *P. torulosum* were retrieved from the OSCAP culture collection housed at the MSU Chilvers lab and sent to ISU Robertson lab for fungicide sensitivity testing. The OSCAP culture collection is comprised of isolates from across 12 US states and Ontario, Canada. The Chilvers lab at MSU has been developing a high-throughput fungicide sensitivity assay. Testing and validation of the assay has been completed for mefenoxam sensitivity and additional rounds of testing are being conducted for ethaboxam sensitivity determination.

Objective 5: The pathogenicity and fungicide sensitivity of three representative isolates of each species from each state in the NC region was evaluated at 55 F and 73 F. All species from all states, except AR, were more sensitive (>50% mycelia inhibition) to metalaxyl and ethaboxam than to azoxystrobin. Isolates of *P. lutarium* from IA, ND, and NE were less sensitive to azoxystrobin at 23°C compared to 13°C. At 13°C isolates of *P. oopapillum* from all states, except AR, were less sensitive to all fungicides compared to 23°C. Isolates of P. sylvaticum from IA, ND, NE, SD, and WI were less sensitive to all fungicides at 23°C compared to 13°C. At 13°C isolates of *P. torulosum* from IA, IN, ND, NE, and WI were less sensitive to all fungicides tested compared to 23°C.A significant three-way interaction was detected between cold stress, inoculation and seed treatment ($P<0.0001$). In general, emergence of

untreated seeds was reduced more than 70% when subjected to periods of cold stress ($P<0.05$). Emergence was improved with seed treatments ($P<0.0001$).

Objective 6a: A preliminary analysis of the data from the two seedling disease projects has been done. Hierarchical clustering was used to identify groups of isolates frequently recovered together from the same field. When data were analyzed including all fungi and oomycetes, regardless of pathogenicity, six distinct clusters were identified. Within each cluster were fungal and oomycete pathogens and non-pathogens. When data were reanalyzed with only pathogenic species, four distinct clusters were present. Cluster 3 contained only Fusarium species, while cluster 2 contained several Pythium species as well as *Phytophthora sojae, Phytophthora sansomnea* and *F. verticiliioides*. The remaining two clusters contained both Fusarium and Pythium species.

Objective 7: Impact of seed treatments on the interaction of seedling pathogens
A greenhouse experiment has been established at SIU to test the effect of seed treatments on seedling pathogens. In a first experiment, *Fusarium oxysporum* and *Rhizoctonia solani* were used to inoculate the soil one day using infected sorghum seeds. Next, treated seeds were planted and covered with a thin layer of soil. Roots will be collected 3 weeks after planting and qPCR assays will be conducted to quantify each pathogen.

Objective 8:
The following resources were developed and distributed over the past year:
Scouting card of common seedling diseases/disorders
Full length publication on soybean seedling diseases
17,500 publications downloaded/distributed
Updated soybean fungicide seed treatment efficacy table
Two seed treatment publications in development
Three videos released and hosted on SRII, another video in development