

## University of Minnesota Sub Report

Aaron Lorenz

Overall, activities related to this project can be divided into those associated with genetic mapping work, breeding work, and screening work. The goal of these interrelated activities is to advance disease resistance in the cultivar development pipelines in parallel with supporting basic research on the underlying genetics of resistance.

### **Genetic mapping**

During this past reporting period we advanced the following populations to the F3 generation. As previously reported, the parents were chosen on the basis of disease susceptibility and resistance to create QTL mapping populations to guide the RenSeq results. F3 seeds were harvested last fall and sent to winter nursery for advancement. These populations will be resources to guide future efforts in gene mapping using RenSeq. However, phenotyping of these populations will depend upon future funds and priorities of this project as well as others. Phenotyping will require significant resources that are not currently part of this project.

#### White Mold Crosses

‘Minsoy’ x ‘Noir1’

‘Minsoy’ x ‘Corsoy79’

‘Minsoy’ x ‘S19-90’

‘Minsoy’ x ‘Vinton 81’

#### Brown Stem Rot Crosses

‘Century 84’ x ‘Bell’

‘Century 84’ x ‘L84-5873’

‘Century 84’ x ‘PI 84946-2’

‘Century 84’ x ‘Iwafune Takiya’

‘Century 84’ x ‘PI 437970’

Additionally, last year we initiated a gene expression experiment using Minsoy and Noir1 to help guide us towards candidate genes in a previously mapped QTL region on chromosome 7. Four biological replicates of each soybean genotype in a white mold inoculation treatment versus control were completed. Good infection was achieved and we extracted RNA from three biological replicates. We are in the process of working with Dr. Ma to send these for sequencing

of the RNA so we can measure gene expression differences between the inoculated and control treatments.

### **Breeding**

We have created and advanced four new breeding populations with the *Rps6* gene, known to confer good resistance to most of the isolates in Minnesota. Despite the effectiveness of this gene in resistance to Phytophthora, it is seldomly used in commercial breeding and mostly unavailable in current commercial cultivars.

Four new breeding populations were advanced in our breeding program. One population was culled based on poor agronomics. The remaining populations were advanced to plant rows where selection for vigor, maturity, and standability will occur in 2026.

We are planning on making many crosses with *Rps11* and *Rps14* this summer once the agreements have been signed by UMN, Purdue and Corteva. We are actively working on this now, with many emails and agreement amendments being passed back and forth. Once approval is received, we will cross these important sources of Phyto resistance into two elite MG 0 and I breeding lines adapted to Minnesota.

### **Screening**

During this past reporting period we initiated screening of our latest advanced breeding lines for resistance to two races of brown stem rot. We are awaiting results. Results from these screenings will assist us in breeding selection and creating mapping populations for new sources of brown stem rot resistance.

## Michigan State University Sub Report

Dechun Wang

### I. Fine mapping of *Rpsan1*:

#### Phenotyping:

Around 1200 F4:5 individual plants were harvested during the previous crop season. These plant families were challenged with the *P. sansomeana* isolate *MPS17-22*. A modified hypocotyl inoculation method was used to inoculate plants. Data were recorded for seven to eight days post-inoculation. The response of each family was evaluated using the ratio of seedlings survived (RSS) expressed as a percentage value, where  $RSS = (2 \times \text{number of survived seedlings} + 1 \times \text{number of survived seedlings with partial damage} + 0 \times \text{number of killed seedlings}) / (2 \times \text{total number of seedlings}) \times 100\%$ . The RSS ranged from 0 to 100%, and a family was considered resistant if RSS was equal to or greater than 70%, segregating if RSS was between 30% and 70%, and susceptible if RSS was less than 30% (Dorrance et al., 2004; Cerritos-Garcia et al., 2023).

#### DNA Extraction:

Plant tissues were collected from all seedlings within each family and pooled. DNA extraction was carried out using a modified 2X CTAB (cetyltrimethylammonium bromide) method. DNA extraction of 900 samples has been completed.

#### Genotyping:

A set of 30 single-nucleotide polymorphism (SNP) markers was selected from the SoySNP50K iSelect BeadChip available at SoyBase (<https://www.soybase.org/tools/snp50k/>). These markers span the previously identified ~1 Mb region on chromosome 03 (Fig. 1).

Assay_name	AlleleX	AlleleY	Sequence	Aliquot_ID	Plate_well	CG%_X	CG%_Y	CG%_Common
Psan27_ss715586806	A	G	AGCTAGCTTCAGAACITTTTACTTGTCTGCCCATGCCATACATG	460262133	1874_022 to ship:D04	18.2	21.2	37.9
Psan1_ss715586346	A	C	ACATAGGAGATGCATGGGTCAGGTTACTCACAACTTGGCCACATC	460262134	1874_022 to ship:A01	37	42.3	39.3
Psan22_ss715586766	A	C	TGTGTTAAATGGAGTTTTCTCAAACACATGCTAACTTTGAAAAAC	460262135	1874_022 to ship:B01	25	28.1	37.9
Psan30_ss715586837	A	C	GATCTAGGAGCGTGGGAACAGTAAATGGTACAAAGGTGAACACG	460262138	1874_022 to ship:E04	44.4	50	50
Psan2_ss715586427	T	C	TTTCTAATAAGTTTAAACACATGAACATAACTTCCACGTAGCTTG	460262139	1874_022 to ship:C01	37	44	37.9
Psan3_ss715586461	G	A	TTTGATCAAAACAAACCTGTATTGGGAGAGGCTCAGATTTTATTC	460262140	1874_022 to ship:D01	52.2	47.8	37.9
Psan4_ss715586505	A	G	TCCCACGTGGCACCCGATACAAAACGTCGGGAGGACGCCGTTAA	460262141	1874_022 to ship:E01	34.5	35.7	48
Psan5_ss715586519	A	G	CTAATCAAGCCATTTATGCAGATCTACAGAAATTTAGAACTAA	460262142	1874_022 to ship:F01	24.2	30	33.3
Psan6_ss715586561	C	T	AATAACAGTTCAAACCAAGCTACAAATGTTTAACTTTCAGG	460262143	1874_022 to ship:G01	47.8	44	37.9
Psan7_ss715586649	T	C	GCAAGGAGAAATGCCTCAACAATAAGAGCAGCACACAATAGG	460262144	1874_022 to ship:H01	42.3	44	37.9
Psan8_ss715586685	A	G	CTCTTCAAATTTGCTTTAGCATTCCGTCGACAGTTCGAGTTAG	460262145	1874_022 to ship:A02	29	31	50
Psan10_ss715586689	C	T	GATGGTGTGATGCCGCGCTCGGAGCAACCAAGGAAAGCGTT	460262146	1874_022 to ship:B02	61.9	57.1	48
Psan12_ss715586697	A	G	ATAAAACAAAACATGACATTAAGTAATCATATTTTCAACATTAAT	460262147	1874_022 to ship:C02	24.2	28.1	23.3
Psan13_ss715586703	A	G	GGTAATGGCGTGATGTTGACAGACATAAGCTGAGGCAACATGCAA	460262148	1874_022 to ship:D02	52.2	54.5	36.7
Psan14_ss715586716	T	G	GCCATTGGGACTGAATCGGTTCTGCACCACAGGTTAAGCGTTGA	460262149	1874_022 to ship:E02	45.8	54.5	48
Psan15_ss715586721	C	T	GATGAGAACACACCCTAGCGTACTCGATGACGATGACGACGGA	460262150	1874_022 to ship:F02	46.2	42.3	48
Psan16_ss715586728	A	G	CACITCAAGCGTGGCTGCTCCCTTCTTTTATATTTCTGTTTTAT	460262151	1874_022 to ship:G02	24.2	25	48
Psan17_ss715586733	C	T	ACTGACACGGACCGGTGGGACACCCGACACGGCAAGGGACT	460262152	1874_022 to ship:H02	45.8	44	59.1
Psan18_ss715586750	A	G	TAAGAACACCTTCTCCGCTACAGTTCCTTAAATAATTTTAACTT	460262153	1874_022 to ship:A03	52.2	54.5	48
Psan19_ss715586753	A	G	GTTGATTTGTCACCCCTCTCCCTCCGACGCGGTGCTCCTCCCA	460262154	1874_022 to ship:B03	50	54.5	70
Psan21_ss715586763	T	C	TTACITTTTAAATTTGGAGTTTCTTTTAAATAATTTTAACTT	460262155	1874_022 to ship:C03	14.7	17.1	40.7
Psan23_ss715586779	G	A	GAGTTCGATTTCAAGTTCAGAAATGCATCGTGGTATGTTGGTGC	460262156	1874_022 to ship:D03	57.1	52.2	48
Psan25_ss715586793	C	A	CTCCCAITGAGAACTAGACATAGGATCCACATTAATTTTATG	460262157	1874_022 to ship:E03	44	42.3	37.9
Psan26_ss715586800	A	G	CCCCAAAGAACTCTGATTTTCCCTCTTCCCTTTTAAATGCA	460262158	1874_022 to ship:F03	42.3	44	39.3
Psan28_ss715586817	A	G	TGCATGTTCAATTTCTCCAATTTGCTAATGATAGCAATTTTCAIT	460262159	1874_022 to ship:G03	21.2	24.2	37.9
Psan29_ss715586831	C	T	ACTTCTTTGTAACCCACGCCAATGTAAGCATTAGAGCCATAACT	460262160	1874_022 to ship:H03	44	37	37.9
Psan24_ss715586784	C	T	CAAAGTTTAAATTTGGGTTGCAATGCAATGATGATCGTTCATCG	460262161	1874_022 to ship:A04	42.3	35.7	37.9
Psan11_ss715586691	T	C	GTTTTGCAATTTGGGATCGATGTTTGGAGCATTTAGATATGT	460262187	1874_022 to ship:F04	44	45.8	37.9
Psan20_ss715586760	C	A	AAATAAACACTGCACCAATAACTGTGATTTCTCGGAGTAGTG	460262188	1874_022 to ship:B04	45.8	42.3	46.2
Psan9_ss715586687	G	A	TATCTAACITTTTATACATAATAAGAAATGCTCTGCCCTCAACTC	460262195	1874_022 to ship:C04	40.7	37	37.9

Figure 1. SNP markers selected from SoySNP50K iSelect BeadChip (soybase.org)

These markers were obtained from LGC Technologies and are compatible with KASP (Kompetitive Allele-Specific PCR)-based endpoint genotyping. They were successfully tested on parents (Colfax and Senaki) and are currently being used to genotype the entire population (Fig. 2). Phenotypic and genotypic data will be analyzed to determine closely associated markers and to narrow down the region harboring the resistant QTL.

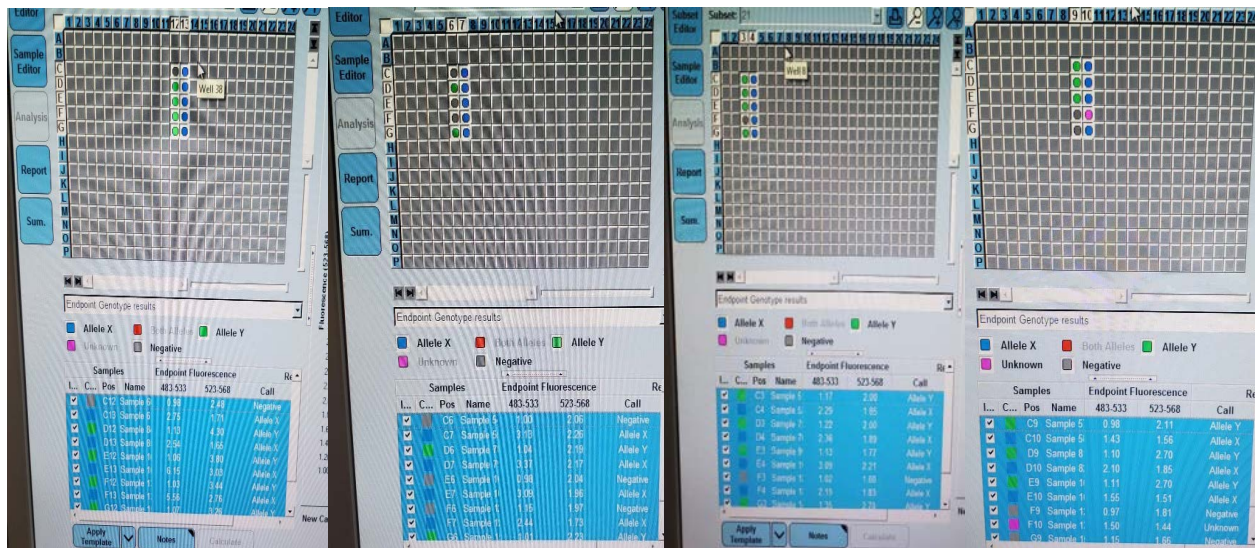


Figure 2. Endpoint genotyping results of resistant (Colfax) and susceptible (Senaki) parents using KASP assays.

## II. Phenotypic screening of resistant and susceptible parents against isolates *Cercospora sojina* causing Frogeye Leaf Spot (FLS).

A set of five populations was developed by crossing susceptible genotypes with a germplasm line previously identified by our group as a resistant genotype. We established our FLS screening protocol in the greenhouse by using the five susceptible and one resistant parent, along with Williams 82 as a susceptible check (Table 1). Five seeds of each genotype were planted in a 6-inch round pot. Each genotype was planted in two pots (2 reps) for each isolate and the control (no inoculation) (Fig. 3). Plants were inoculated at the V2-V3 growth stage, then transferred to humidity chambers for 48 hours, where they were re-inoculated 24 hours after the first inoculation. Plants were removed from the humidity chambers and evaluated after 2-3 weeks post-inoculation. Susceptible genotypes developed disease symptoms characterized by small, irregular, or circular lesions on the upper leaf surface with reddish-brown borders (Fig. 4), whereas the resistant genotype showed no symptoms. The populations developed from these parents will be evaluated in the greenhouse during the summer. We are also exploring the possibility of identifying another reliable susceptible check in the Soybase germplasm panel that could be grown under MG II-III.

Table 1. Susceptible and resistant parents.

Susceptible parents	Resistant parent
E21345-7	PI 532465
E15165T	
E20821-13-2GT	
E18331-34-17	
E20195-3	
Williams 82 (susceptible check)	



Figure 3. Genotypes planted in pots.

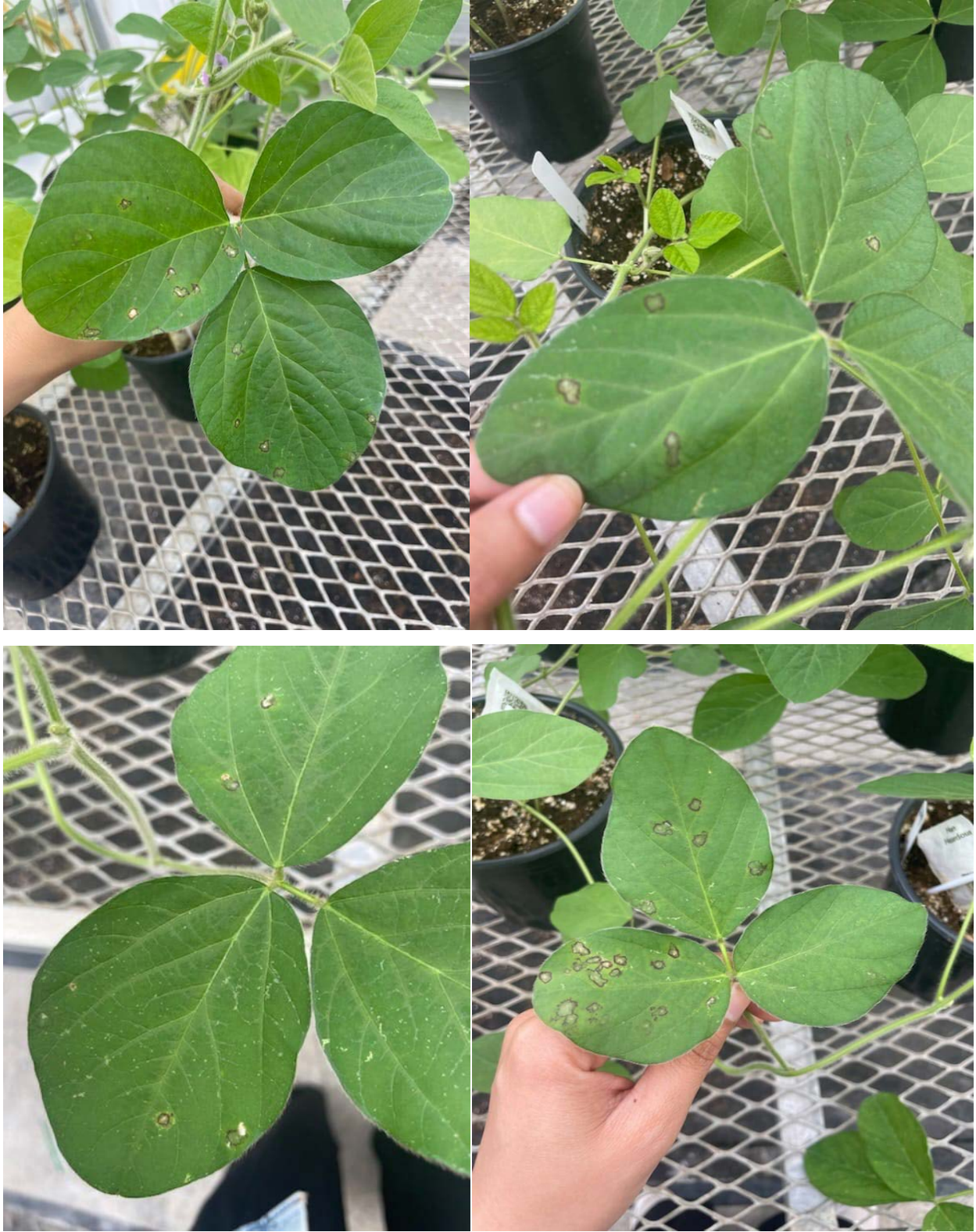


Figure 4. Symptoms of frog eye leaf spot infection on different genotypes.

## Iowa State University Sub report

Madan Bhattacharyya

The Progress made during six months are presented under each of the three objectives.

### 1. Map NLR genes to four Rps regions of the Chromosomes 3, 7 and 18.

Bhattacharyya lab will use the NLR sequences identified in Ma lab map to the four Rps regions: (i) one from Chromosome 3; (ii) two Rps regions from Chromosome 7 and (iii) one Rps region carrying three Rps genes from Chromosome 18.

Deliverables: NLR sequences mapped to four genomic regions.

We have completed identifying the NLR genes from the complex Rps region containing three functional Rps genes, Rps4/6/13, 12, and JS. We generated a 1.22 Mb contig using long read sequences. Sequencing was performed on a near-isogenic Williams isoline, L89-1581, carrying Rps6 using the Oxford Nanopore Technologies (ONT) at Iowa State University. The identified NLR genes are presented in Figure 1.

Figure 1. The Rps region mapped to the south arm of on soybean Chromosome 18.

The physical map was constructed based on the assembled 1.22 Mb region containing the three NLR gene clusters. The rps12 gene cluster is located to the northern region (~0.16–0.29 Mb), while the Rps6 gene cluster is positioned in the middle (~0.41–0.44 Mb). The rpsJS containing two NLR genes mapped to the south (~0.71–0.72 Mb), close to the telomeric region.

We will identify the NLR genes of the Chromosomes 3 and 7 by applying the RenSeq technology in collaboration with the Ma lab this summer.

### 2. Functionally characterized one Rps gene through genome editing.

Bhattacharyya Lab will conduct soybean transformation to knockout candidate NLR genes mapped to the Rps6 gene, which has shown high durability and broad-spectrum *Phytophthora* resistance.

Deliverables: Candidate Rps6 NLR genes mutated and the Rps6 gene identified.

We have applied a PCR approach to clone the three candidate Rps6 NLR genes (Figure 1) and sequenced using Sanger sequencing platform. The genes will be overexpressed and mutated in transgenic soybean lines to identify the functional Rps6 gene.

3. Introgress the Rps11, Rps14 and Rpsan1 genes into a soybean cultivar of the maturity group 3 suitable for the major soybean belt including Illinois, Indiana and Iowa.

Bhattacharyya Lab will apply a marker assisted back-crossing program to introgress the Rps11, Rps14 and Rpsan1 genes into a commercial cultivar.

Deliverables: A near-isogenic soybean cultivar carrying the Rps11, Rps14, and Rpsan1 genes.

We plan to stack Rps11, Rps14 and Rpsan1 genes in the cultivar IAR 3001. IAR 3001 is a maturity group 3 (MG 3) cultivar and is ideal for growing in the latitudes that cover a major soybean growing areas including Iowa, Illinois, and Indiana. IAR 3001 carries SCN resistance genes from PI438489B and PI88788 and contains Rps8. We have collected PI 573008 (Rpsan1). The donors for Rps11 and Rps14 will be received from Purdue University for planting this summer in the field. The material transfer agreement (MTA) was signed by Corteva and Iowa State University (ISU). The second MTA between Purdue University and ISU will be signed shortly. We hope to start the hybridization work in the field this summer.

## University of Missouri Sub Report

Feng Lin/Mariola Usovsky

During the 2025 season, eight crosses incorporating *Rps11* and *Rps14* were successfully completed (Table 1). These crosses involved high-yielding elite lines and PI donor lines carrying *P. sojae* resistance. Seeds were harvested in November, and F<sub>1</sub> seeds were planted in the greenhouse on January 8, 2026. The F<sub>1</sub> generations are currently at the flowering stage, with harvest anticipated in June. The resulting F<sub>2</sub> seeds will be inoculated in July–August, and the surviving plants will be backcrossed to the recurrent elite parents.

**Table 1:** Crosses made in 2025 growing season at the University of Missouri, Portageville.

Cross	Female	Male	Generation
C1	S22-1294	PI 594527 ( <i>Rps11</i> )	F1
C2	S22-1294	PI 340029 ( <i>Rps14</i> )	F1
C3	S16-13165	PI 594527 ( <i>Rps11</i> )	F1
C4	S22-3859	PI 594527 ( <i>Rps11</i> )	F1
C5	S22-3859	PI 340029 ( <i>Rps14</i> )	F1
C6	S20-2227	PI 594527 ( <i>Rps11</i> )	F1
C7	S19-10701	PI 594527 ( <i>Rps11</i> )	F1
C8	S19-10701	PI 340029 ( <i>Rps14</i> )	F1

## **NDSU-Sub Report**

Carrie Dottey/Miranda

Dottey/Miranda lab at NDSU continued advancing mapping population for resistances to white mold and brown stem rot and received F5 seeds in the fall of 2025 for mapping.

**Purdue University/USDA-ARS**

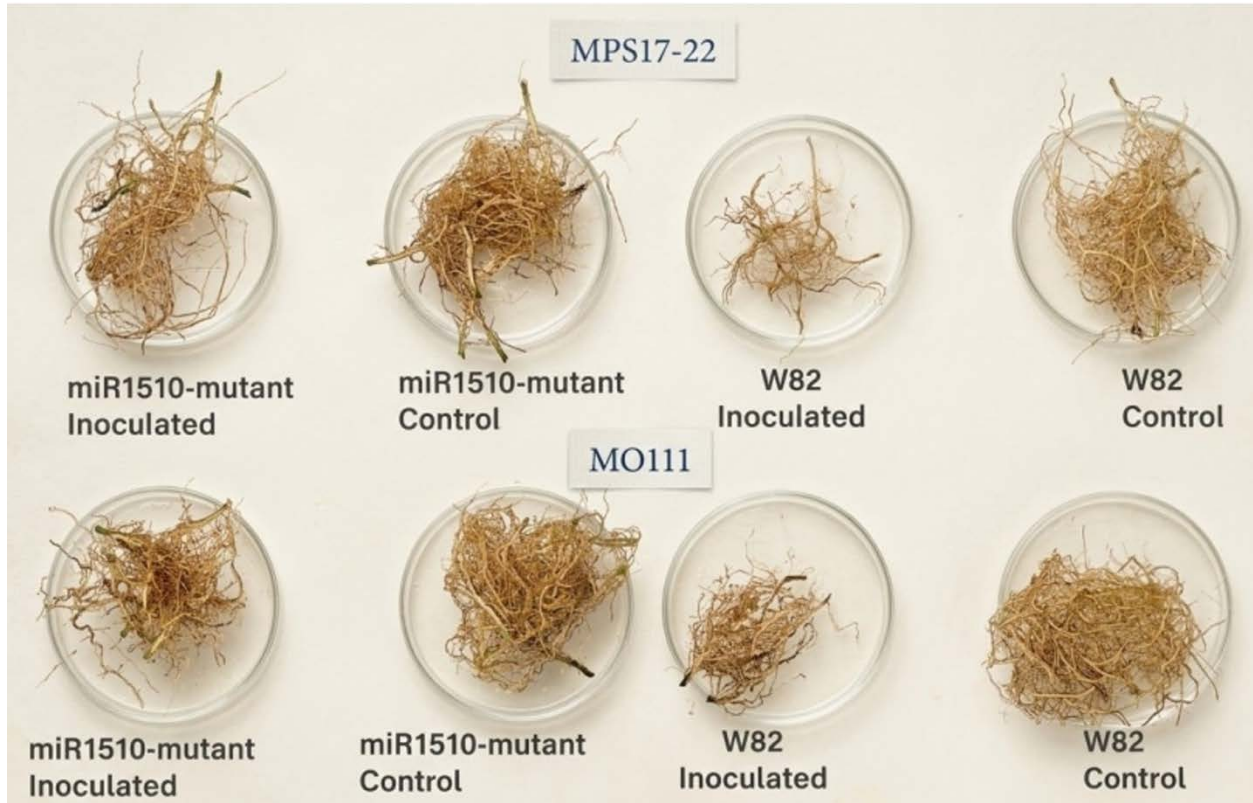
Jianxin Ma/Guohong Cai

(some interesting data from gene-editing plants)

Below shows gene-editing approach to create resistance to stem and root rot caused by *P. sansomeana*, Fusarium, and frogeye spots



Resistance to Furarium



Resistance to *P. sansomeana*



Resistance to Frogeye