Identifying New Sources of Resistance to Brown Stem Rot in Soybean

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Abstract

Breeding for pathogen resistance is an important objective to improve and protect soybean yields. In 2010, 14.4% of the total soybean yield (~ $5.59 billion) was suppressed by diseases (fungi, microbes, and nematodes). Brown stem rot (BSR) caused by the fungus *Phialophora gregata,* reduces yield up to 38%. To date, three dominant BSR resistant genes have been identified: *Rbs1, Rbs2*, and *Rbs3.* Other resistance loci might be present within the soybean germplasm collection. This research was conducted to determine the inheritance of BSR resistance in soybean accessions PI 594858B, PI 594637, PI 594638B, and PI 594650A crossed to the three known resistance genes. BSR severity was assessed in growth chambers 5 weeks after inoculation based on three measurements: 1) plant vigor, 2) incidence of discoloration and 3) recovery of *P. gregata*. Allelism tests from $F_{2:3}$ plants of PI 594638B, PI 594858B and PI 594650A fit a 15:1 segregation ratio, indicating non-allelism to any of the three genes. The progeny from PI 594637 segregated in a 3:1 ratio indicating that there is only one dominant resistance gene present and that PI 594637 is susceptible to BSR. Molecular characterization of the populations is in progress and will provide additional information about resistance to BSR.

Introduction

Brown Stem Rot (BSR) of soybean, caused by the fungus *Phialophora gregata* W. Gams 1971 f. sp. *Sojae,* is a major disease affecting yields in the Northern US. Yield losses of up to 38% have been reported. Genetic resistance to BSR in soybean is one of the most effective means to control the disease.

Upon infection of soybean plant roots by *P. gregata*, the fungus colonizes the pith and vascular system, moving through the stem to the leaves of susceptible plants. Leaf visual symptoms are caused by one genotype of *P. gregata,* identified as Genotype A or Type I. Genotype B or Type II does not produce leaf symptoms, and is only detectable when soybean stems are split at harvest and the pith and vascular system discoloration becomes visible. Three independent resistance genes, *Rbs1, Rbs2*, and *Rbs3,* have been identified and mapped to chromosome 16, Molecular Linkage Group (MLG) J. Each gene was identified from plant introductions from the National Soybean Germplasm Collection.

Searching for new sources of BSR resistance, Perez et al. (2010) evaluated four plant introductions from south-central China that had previously shown resistance to BSR. The authors indicated that the PIs could contain novel sources of resistance different from the known genes.

The objective of our research was to determine if the resistant PIs had alleles that were different to the known genes by conducting allelism tests and additional molecular analyses.

Materials & Methods

Plant Material

Table 1. To conduct the allelism tests, $F_{2:3}$ lines were developed from each of 12 populations by crossing each potential PI to lines containing the three known BSR resistance genes.

Populations	
L78-4094 *(Rbs1)* x PI 594858B *(Rbs?)*	L78-4094 *(Rbs1)* x PI 594638B *(Rbs?)*
PI 437833 *(Rbs2)* x PI 594858B (*Rbs?*)	PI 437833 *(Rbs2)* x PI 594638B *(Rbs?)*
PI 437970 *(Rbs3)* x PI 594858B *(Rbs?)*	PI 437970 *(Rbs3)* x PI 594638B (*Rbs?*)
L78-4094 *(Rbs1)* x PI 594637 *(Rbs?)*	L78-4094 *(Rbs1)* x PI 594650A *(Rbs?)*
PI 437833 *(Rbs2)* x PI 594637 *(Rbs?)*	PI 437833 *(Rbs2)* x PI 594650A *(Rbs?)*
PI 437970 *(Rbs3)* x PI 594637 *(Rbs?)*	PI 437970 *(Rbs3)* x PI 594650A *(Rbs?)*

Growth Chamber and Inoculation Procedures

- A total of 60 individual F_3 plants per cross were used for the inheritance study.
- For each population, the parents, two resistant controls, 'BSR 101' & 'IA 1006,' and two susceptible controls, 'Corsoy 79' & PI 437654, were also included in the test.
- *P. gregata* f. sp *sojae* isolate $Oh_{2\text{-}3}$ cultures were a single-spore isolate of strain Oh_2, also used by Tabor et al. (2003), Type I.
- Two-week old plants were stab inoculated with *Phialophora gregata* (Perez et al., 2010).

Disease Assessment Criteria - Severity Assays

BSR severity was assessed in growth chambers 5-weeks after inoculation based on three measurements: 1) plant vigor, 2) incidence of discoloration, and 3) recovery of *P. gregata*.

1) Plant vigor

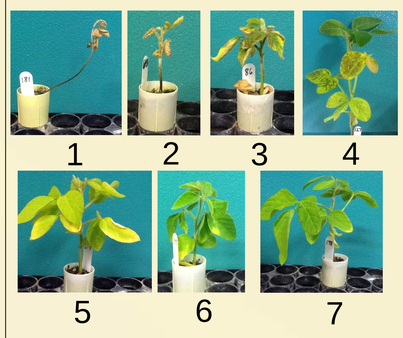

Figure 1. Visual assessment of plant vigor scale of BSR severity in soybean plants, 5 weeks after inoculation: 1 = dead plant; 2 = green stem with no leaves; 3 = leaves that were predominantly chlorotic and necrotic; 4 = plant had some stunting with mosaic chlorosis and necrosis on leaves; 5 = normal leaf area with some leaves showing yellowing; 6 = plant appeared small but healthy; 7 =plant appeared healthy and normally tall.

2) Incidence of Discoloration

- Plant stems were cut lengthwise from top to bottom.
- The amount of tissue discoloration and damage inside the vascular tissue was measured in cm from the inoculation point up.
- A plant was considered discolored if there was any visible dark brown discoloration on the vascular tissue or the pith.
- The discoloration length was divided by the total plant height x 100 to obtain the percentage of discoloration in the stem.

Figure 2: Incidence of discoloration was measured as the length of browning in the stem divided by the total plant height.

3) Recovery of *P. gregata*

- Five 1 cm stem segments were cut from the plant and plated in sequential order on green bean extract agar. Plates were placed in the dark at 5°C to promote fungal growth.
- Stems were evaluated for the presence of *P. gregata* infection measured as any colony growth around the stem segment on the plate after two and four weeks of growth.
- The average of the two measurements was used to assess BSR resistance and susceptibility.

Figure 3: Recovery of *P. gregata* measured as colony growth around the stem segment.

References

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Data Analysis

- Means for vigor, incidence of discoloration, and recovery of *P. gregata* were calculated for each genotype by PROC MEANS of SAS v. 9.1 (SAS Institute, 2003).
- The CONTRAST statement of PROC GLM of SAS v. 9.1 was used to compare each genotype to the resistant and susceptible standards.
- Lines were declared resistant or susceptible if they were not significantly different from the resistant or susceptible standards. Lines that were significantly different from the resistant and susceptible standards were classified as intermediate. For the analyses, resistant and intermediate categories were pooled together.
- Using the resistant/intermediate counts and susceptible counts, a chi-square test was calculated for each population to determine goodness-of-fit between observed and expected segregation ratios.
- Theoretical inheritance ratios were tested for the one gene hypothesis (3:1 resistant to susceptible), and for the two gene hypothesis (15:1 resistant to susceptible).

Results

- PI 594638B, PI 594858B, and PI 594650A segregated in a 15:1 ratio with each of the known resistance genes, indicating the PIs may contain novel source of resistance (Table 2).
- PI 594637 segregated in 3:1 ratio with each of the known resistance genes, indicating there is only one dominant gene present (Table 2), and PI 594637 is susceptible to BSR.
- Molecular characterization of the segregating populations obtained with these accessions is in progress, and will provide additional information about resistance to BSR.

		Number of Plants				
		Observed Ratio				
Cross/Allelism test	Disease assessment criteria	R	I	S	Expected Ratio	P value
***Rbs1* gene x ?**						
L78-4094 x PI 594650A	Vigor	62	12	8	15:1	0.423093
L78-4094 x PI 594650A	Incidence of Discoloration	8	64	10	15:1	0.084317
L78-4094 x PI 594650A	Recovery of *P. gregata*	17	57	8	15:1	0.423093
***Rbs2* gene x ?**						
PI 437833 x PI 594650A	Vigor	12	58	7	15:1	0.588427
PI 437833 x PI 594650A	Incidence of Discoloration	11	60	6	15:1	0.855322
PI 437833 x PI 594650A	Recovery of *P. gregata*	25	48	4	15:1	0.929452
***Rbs3* gene x ?**						
PI 437970 x PI 594650A	Vigor	57	17	3	15:1	0.694843
PI 437970 x PI 594650A	Incidence of Discoloration	3	56	18	3:1	0.947325
PI 437970 x PI 594650A	Recovery of *P. gregata*	20	49	8	15:1	0.324336

Table 2: Segregation ratios for $F_{2:3}$ plants of each of three populations developed by crossing PI 594650A with each of the sources of the *Rbs* resistance genes. Individuals were screened for BSR reaction on the basis of three disease assessment criteria, by the lower 95% confidence limit of vigor, percent stem severity, and recovery of *P. gregata.* The three populations consisted of PI 594650A crossed to L78-4094 (*Rbs1*), PI 437833 (*Rbs2*), and PI 437970 (*Rbs3*). Only one of the PI lines studied was included due to limitations of space.

Future Work

- Molecular mapping of population L78-4094 (*Rbs1*) x PI 594650A.
- Bulk-segregant analysis is being conducted with an Illumina BARCSoySNP6K BeadChip using susceptible progeny only.
- Allelism test will be conducted between each of the three PIs with a potential new source of resistance.

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