**Iowa Soybean Association**

**Mapping and introgression of *Rps*12 for resistance to Phytophthora root rot into elite soybean lines**

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**Accomplishments**

* Identified a new locus for resistance to *P. sojae, Rps*12
* Mapped the locus to a genomic region of Chromosome 18 that harbors several *Phytophthora* resistance genes
* Identified markers that soybean breeders can use when incorporating the gene into new varieties.
* Backcrossing *Rps*12 into elite soybean germplasm lines is in progress

**Project Goal:** The long-term goal of our research is to develop elite soybean lines with improved resistance to *Phytophthora sojae,* the causal organism of Phytophthora root rot

**Objectives**

1. Generate molecular markers linked to *Rps*12.
2. Backcross *Rps*12 into elite soybean germplasm lines.
3. Identify candidate *Rps*12 genes.

**Objective 1.** Generate molecular markers linked to *Rps*12.

A previous report by Gordon et al. (2007) [62] indicated that PI399036 contained multiple *Rps* genes including at least one novel *Rps* gene. Our recent mapping study (Abeysekara et al. 2016) [63] using a mixture of three *P. sojae* isolates suggested an *Rps* gene located on the lower arm of Chromosome 18 (ISA Proposal Identification of quantitative trait loci for partial resistance

to *Phytophthora sojae* in soybean).

The AX20925 recombinant inbred line (RIL) population was developed by crossing PI399036 (National Germplasm Collection) with the germplasm line AR2, released by Iowa State University (Cianzio et al., ISURF Docket # 03381). A cup assay was used to assess susceptibility or resistance of the RIL population to *P. sojae* and 120 RILs consisting of homozygous lines for either resistance or susceptibility were used to analyze the inheritance of the *Rps12* gene. Eleven plants of each RIL were inoculated with *P. sojae* using the hypocotyl inoculation method. Fifty-nine homozygous resistant and 61 homozygous susceptible F5:7 families were used for the mapping experiments. Eleven plants from each resistant or susceptible homozygous F7 family were scored for responses to the *P. sojae* isolates in each experiment, which was repeated two more times.

In this study, to further characterize the novel *Rps* gene, 25 F2 plants obtained from the cross between PI399036 X AR2 were inoculated with the mixture of the Val 12-11 and R17 isolates. These two isolates together were shown to be virulent on soybean lines carrying all genes that were previously mapped to the Chromosome 18, except the *RpsJS* gene. It is unknown if the *RpsJS* gene can encode resistance against the mixture of *P. sojae* R17 and Val12-11 isolates. Of the 25 F2 plants, 19 were resistant: 6 susceptible to the *P. sojae* R17 and Val12-11 isolate mixture. The segregation ratio fits to the expected 3:1 (R:S) ratio for a single gene with dominance of resistance over susceptibility (**χ**2 withdf 1= 0.013, *P*=0.908). Screening of the 120 RILs of the AX20925 population with an inoculum mixture of *P. sojae* R17 and Val12-11 or P7074 alone resulted in 59:61 (R:S) lines indicating again single gene action for *Phytophthora* resistance (χ2df=1 0.03 , *P*=0.855) in the novel *Rps* locus. *P. sojae* P7074 isolate is virulent on soybean lines with *Rps8,* but PI399036 was not susceptible to infection suggesting this line does not have *Rps*8.

In our previous study, preliminary data suggested that a novel Rps gene could be present in the *Rps4/6* region. We therefore evaluated 33 SSR markers mapped to the *Rps4*/6 region for polymorphisms between the PI399036 and AR2 parents considered in this study. The SSR markers selected for this study encompass the genomic region that includes *RpsJS*, *4* and *6* genes. Of these 33 SSR markers, only 14 were polymorphic between PI399036 and AR2 parents and were considered for bulked segregation analyses (BSA). BSA analyses of the markers suggested that the novel *Rps* gene is mapped to the *Rps4*/6 region (Figure 1).

In addition to the 14 polymorphic SSR markers, we designed primers and amplified two PCR products of 130 and 533 bp in length using *Rps4*-NBS-LRR-specific primers, from both PI399036 and the resistant bulked DNA sample, but not from either AR2 or the susceptible bulked DNA sample. BSA analysis suggested that the amplified *Rps4*-NBS-LRR-type sequences co-segregate with the putative novel *Rps* gene. Moreover, the sequenced 130 bp and 533 bp PCR fragments showed 100% and 99% nucleic acid sequence identity to the *Rps4*-NBS-LRR sequence identified by Sandhu et al. (2004) [44]. The 130 and 533 bp NBS-LRR-type fragments were named as NBSLRR130 and NBSLRR533, respectively.

The two dominant markers, NBSLRR130 and NBSLRR533, and nine co-dominant SSR markers were used to map the putative novel *Rps* gene. The gene mapped in between SSR markers, BARCSOYSSR\_18\_1840 and Sat\_064 (BARCSOYSSR\_18\_1858) (Figure 1). Both NBSLRR130 and NBSLRR533 markers were mapped at 2.2 cM distal to the locus suggesting that the novel *Rps* gene is unlikely allelic to *Rps4* (Figure 1).



Figure 1. Genetic map of chromosome 18 showing location of Rps12

The *RpsJS* gene has been mapped also to the *Rps4*/6 genomic region [45]. We therefore included the molecular markers BARCSOYSSR\_18\_1859 and SSRG60752K that flanked the *RpsJS* gene. Both these markers mapped distal to Sat\_064, which co-segregated with the *Rps4/6* locus. *Rps5* is very loosely linked to *Rps4* and mapped closely to XXX [77], which proximal to the novel *Rps* locus. Our mapping data suggest that the novel *Rps* gene is located in region distal to Rps5 and proximal to *Rps4, 6* and *JS*. We named this novel *Phytophthora* resistance gene as *Rps12*.

**Objective 2:** Backcross *Rps12* into elite soybean germplasm lines.

Backcrossing Rps12 into elite soybean germplasm was delayed but will start in Fall 2016.

**Objective 3:** Identify candidate *Rps12* genes

This work is in progress.

**Publications and presentations**

1. Sahoo, D.K., Abeysekara, N.S., Cianzio, S., Robertson, A.E., and Bhattacharrya, M.K. XXXX. A novel *Phytophthora* resistance gene mapped tightly to the *Rps4/6* region in soybean*.* PLoS ONE.