Final Report

Iowa Soybean Association

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Project Title: Evaluation of the *Phytophthora* resistance *Rps12* gene for its utility and identification of tightly linked molecular markers for its selection.

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Progress report for the period from October 1, 2017 to June 30, 2019

Executive Summary

Here we summarize the overall accomplishment of this one-year project.

The goal of this one-year project was to investigate (i) the usefulness of the *Phytophthora* resistance *Rps12* gene that we mapped earlier under the support of another Iowa Soybean Association grant (Sahoo et al. 2017: https://doi.org/10.1371/journal.pone.0169950), and (ii) developed breeder-friendly markers so that the gene can be rapidly introgressed into soybean cultivars.

To accomplish our goal, we identified two desirable recombinant inbred lines (RILs) that most unlikely carry *Rps* genes, other than *Rps12* and unknown *Rps* genes next to *Rps12*. Of the two lines presumably carrying only *Rps12*, one showed susceptibility to six of the *Phytophthora sojae* 33 isolates collected from Iowa soybean fields. Molecular analyses of the two lines revealed that the line showing susceptibility to the six isolates inherited a portion of the chromosome, next to *Rps12,* from the susceptible parent AR12 suggesting that this section of the chromosome from the resistant parent PI399036 contains a new *Rps* gene that is required for resistance against the six of the 33 *P. sojae* isolates collected from the soybean fields of Iowa. In our earlier study, resistance encoded by this new gene *RpsX* was confounded with that of *Rps12* and provided broad-spectrum resistance against many isolates of the pathogen because of the complementary Phytophthora resistance functions of *Rps12* and a neighboring *RpsX* gene. Each of the two genes confer resistance against a subset of *P. sojae* isolates; and together, they confer resistance against many of the *P. sojae* isolates. *Rps12* and *RpsX* are very closely located on Chromosome 18. As a result, they are inherited as a single gene, which is ideal for transferring to new *P. sojae* susceptible cultivars.

Our previous study on the *Phytophthora* resistance *Rps1*-k gene conferring resistance against a large number of *P. sojae* isolates also revealed two functional *Rps* genes (Gao and Bhattacharyya, 2008: https://bmcplantbiol.biomedcentral.com/track/pdf/10.1186/1471-2229-8-29; Gao et al. 2005: https://apsjournals.apsnet.org/doi/pdf/10.1094/MPMI-18-1035).

Our study discovered two *Rps* genes that are tightly linked. It is essential that we select both genes using molecular markers to incorporate the broad-spectrum resistance mechanisms against the *P. sojae* isolates conferred by the two genes. We therefore identified desirable molecular markers to incorporate the two genes into new soybean cultivars.

Detailed Final Report

Goals and Objectives: Based on the responses of recombinant inbred lines (RILs) carrying *Rps 12* to three *P. sojae* isolates that can defeat resistance encoded by most of the characterized *Rps* genes (Sahoo et al. 2017), we ***hypothesized*** that *Rps12* is a very important *Rps* gene provideing soybean with broad-spectrum resistance to most, if not all *P. sojae* isolates. The ***goals*** of this project is to establish the utility of this novel gene through infecting lines carrying this gene against a large number of *P. sojae* isolates and identify molecular markers for pyramiding this gene into commercial cultivars that carry other *Rps* genes.

We proposed to conduct the following two objectives to reach our goal.

Objective 1. Determine the responses of three recombinant inbred lines carrying *Rps12* to a large collection of *P. sojae* isolates.
Objective 2. Identify molecular markers linked tightly to *Rps12*.

Progresses made in the last six months are presented under each objective.

Objective 1. Determine the responses of three recombinant inbred lines carrying *Rps12* to a large collection of *P. sojae* isolates.

It was proposed that the Phytophthora resistant PI399036 line contains several *Rps* genes (Gordon et al. 2007: Phytopathology **97**: 113-118). To identify three RILs containing only *Rps12*, we investigated 42 *Phytophthora* resistant RILs generated from the cross between PI399036 x AR2 for molecular markers linked to the known *Rps* regions.

Of the 30 *Rps*-linked SSR markers evaluated, 10 SSR markers were found to be polymorphic between the two parents and were applied in evaluating 42 RILs homozygous for *Rps12*. From the polymerase chain termination reaction (PCR) assays of 420 combinations of 42 RILs and 10 SSR markers, we identified two RILs (RIL12 and RIL14) that carry molecular marker alleles, specific to the *Phytophthora* susceptible parent AR2. We have obtained 33 *P. sojae* isolates from the Robertson lab that were collected earlier from the Iowa soybean fields. The isolates were characterized for their pathotypes by inoculating a set of 14 soybean lines that are considered to be differential lines for 14 individual *Rps* genes. The isolates were used also to infect the two selected RILs (RIL12 and RIL14) and the two parents, PI399036 and AR2, used to develop the RILs. Although RIL12 and RIL14 were shown to contain *Rps12* earlier (Sahoo et al. 2017: PLoS ONE **12**: e0169950), RIL12 is susceptible to six of the 33 *P. sojae* isolates collected from the Iowa soybean fields. Based on the genetic make-ups of RIL12 and RIL14 for molecular markers of the *Rps12* region and distinct responses of the two RILs to six isolates, it appeared that there could be a functional *Rps* gene next to *Rps12*, which we named temporarily *RpsX*. The preliminary mapping results were reported on October 1, 2018.

We conducted additional mapping experiments to conform the presence of *RpsX* in PI399036. Six RILs, RILs 6, 9, 12, 42, 49 and 81, containing genetic rearrangements between *Rps12* and *RpsX* due to exchange of chromosomal segments from the two parents were studied to confirm the new *RpsX* gene (Table 1). Due to absence of *RpsX,* the RILs 6, 9, 12, 42, and 49

were susceptible to the *P. sojae* isolate V13. The five lines however contain *Rps12* (Table 1); and therefore, resistant to the mixture of the isolates, R17 and Val 12-11. On the contrary, RIL81 contains *RpsX* but not the *Rps12* gene. Therefore, this RIL is resistant to V13 and susceptible to the mixture of R17 and Val 12-11 isolates (Figure 1).

Table 1. Chromosomal rearrangement in the *Rps12* region due to crossing over between the chromosomes of the parent PI399036 (shown with white color) and AR2 (shown with yellow).

Lines /Markers	SSR1820	SSR1830	SSR1840	*Rps12*	NBSLRR130	NBSLRR533	Sat_064	SSR1859	*RpsX* V13	SSR1860	SSRG60684K	SSR1861	SBP57.12	SBP57.14
RIL6	A	B	B	*R12*	A	A	A	A	*rx*	A	A	A	A	A
RIL9	B	B	B	*R12*	A	A	A	A	*rx*	A	A	A	B	B
RIL10	A	A	A	*R12*	B	B	B	B	*Rx*	B	B	B	B	B
RIL12	B	B	B	*R12*	B	B	B	A	*rx*	A	A	A	A	B
RIL14	B	B	B	*R12*	B	B	B	B	*Rx*	B	B	B	A	A
RIL42	A	B	B	*R12*	B	B	B	A	*rx*	A	A	A	A	A
RIL49	B	B	B	*R12*	B	B	B	A	*rx*	A	B	B	B	A
RIL81	A	A	A	*r12*	B	B	B	B	*Rx*	B	B	B	B	B
PI399036	B	B	B	*R12*	B	B	B	B	*Rx*	B	B	B	B	B
AR2	A	A	A	*r12*	A	A	A	A	*rx*	A	A	A	A	A

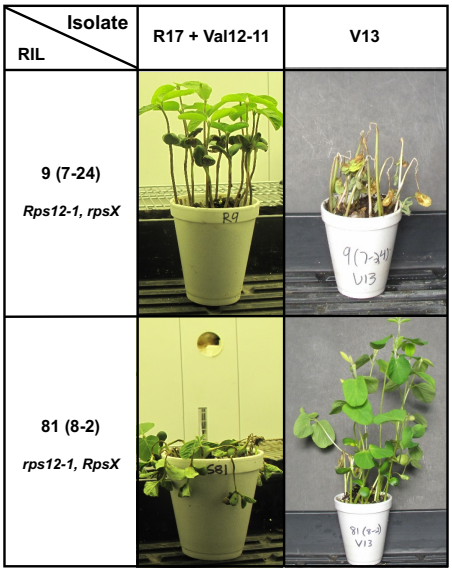

Figure 1. Two RILs differing alleles for the *Rps12* and *RpsX* loci showed distinct responses to *P. sojae* V13 isolate and the mixture of *P. sojae* R17 and Val12-11 isolates. Note that *P. sojae* isolate V13 cannot overcome *RpsX* gene, but can *Rps12*; whereas, the mixture of R17 and Val12-11 isolates can overcome *RpsX*, but not *Rps12*.

KPIs/Performance Metrics: In the one-year grant period, under the Objective 1 we expected to accomplish the following:

1. By the end of the one-year grant period, we will complete the evaluation of three selected RILs carrying only *Rps12* for their responses to at least 100 *P. sojae* isolates.

Accomplishment: We were able to identify only two RILs carrying only *Rps12* based on molecular analyses of 42 RILs, available to us. The two lines were sufficient enough to test our hypothesis that the *Rps12* gene governs broad-spectrum *Phytophthora* resistance. While testing our hypothesis, we discovered that the broad-spectrum resistance of *Rps12* is because of the complementary Phytophthora resistance functions of *Rps12* and a neighboring *RpsX* gene. Each

of the two genes confer resistance against a subset of *P. sojae* isolates; and together, they confer resistance against many of the *P. sojae* isolates.

The outcome was unexpected. We, therefore, instead of investigating the RILs 12 and 14 against additional 67 *P. sojae* isolates as proposed, we utilized our resources to map the new *Rps* gene and identify molecular markers linked to the two *Rps* genes, *Rps12* and *RpsX*, to facilitate *Phytophthora* resistance soybean breeding programs for this complex locus.

Objective 2. Identify molecular markers linked tightly to *Rps12*.

Using the unassembled genome sequences of the two parents of the RILs, PI399036 and AR2, and the reference Williams 82 genome sequence, our collaborator Dr. Anindya Das, identified single nucleotide polymorphic (SNP) loci of the *Rps12* region. We generated sequence-based polymorphic (SBP) markers as follows (Sahu et al. 2012: BMC Genomics **13**:20). First, we predicted the SNPs between the two parents of the RILs, PI399036 and AR2, by comparing their short-read sequences with sequences of the reference Williams 82 genome sequence. PCR amplified DNA fragments containing this SNPs were then digested with the respective restriction endo nucleases and run on an agarose gel. Examples of SBP markers are shown in Figure 2.

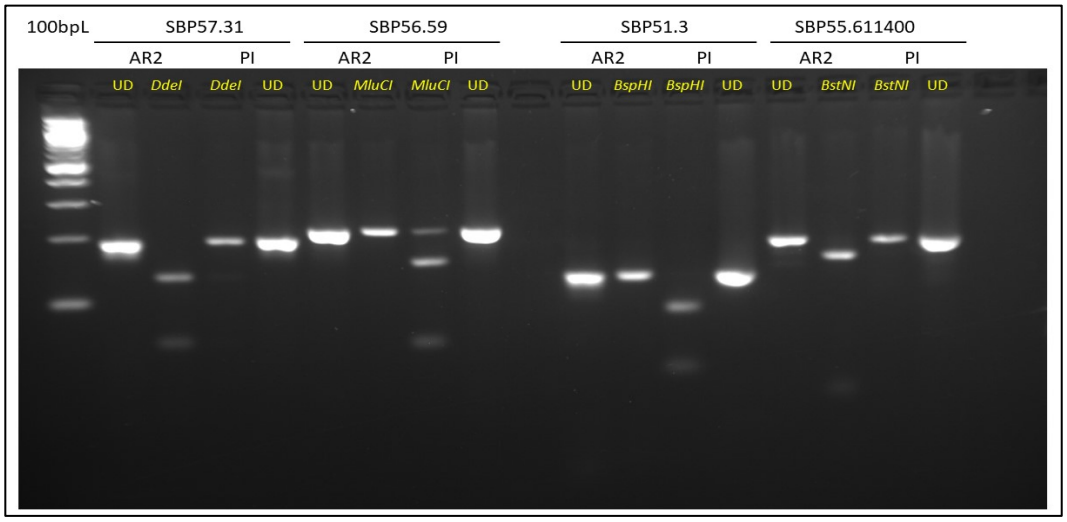

Figure 2. Phenotypes of four SBP markers linked to *Rps12* and *RpsX* genes. AR2, susceptible parent AR2; PI, resistant parent PI399036; UD, undigested DNA fragments; respective restriction enzymes used to digest the PCR products are indicated on the top of each lane with yellow font.

The current status of the genetic and physical maps showing the locations of molecular markers in the *Rps12* region are presented in Figure 3.

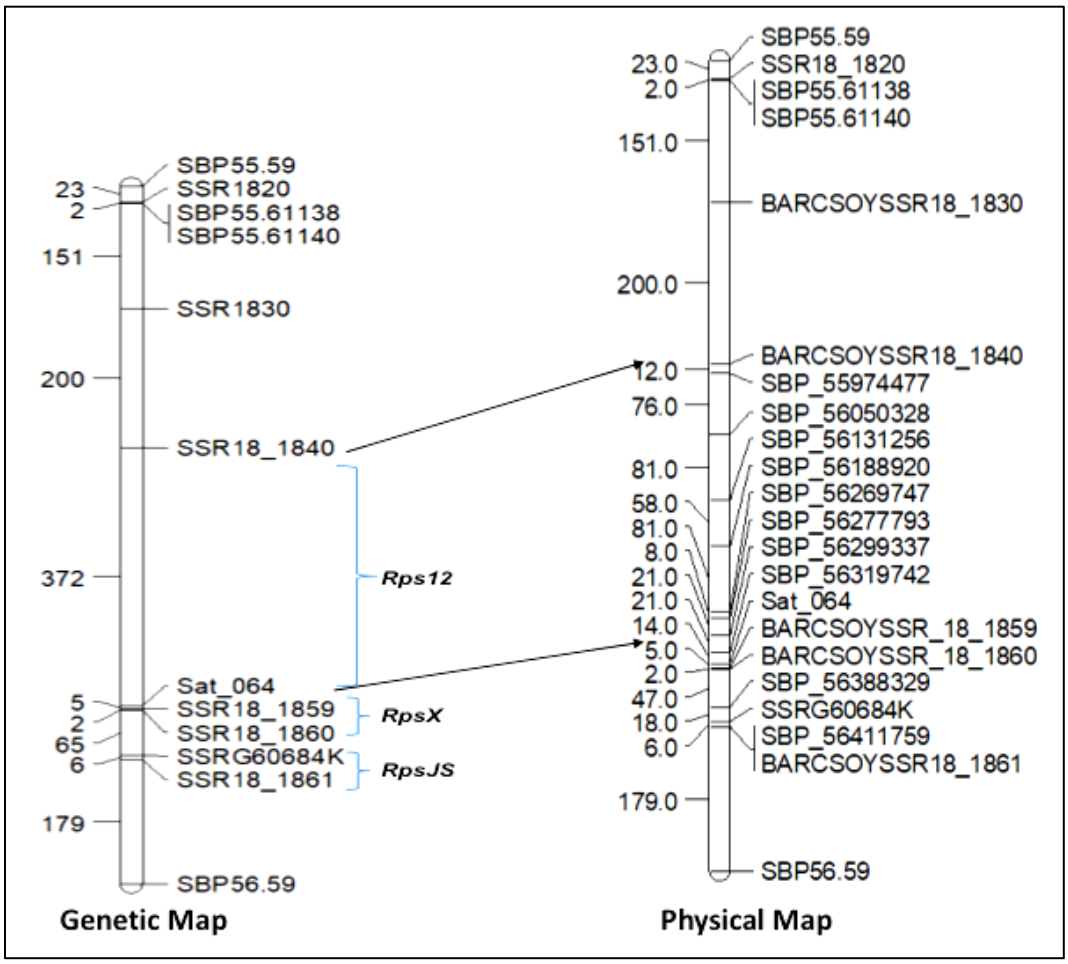

Figure 3. Genetic and physical maps of the *Rps12* region. The genetic map shows the region containing *Rps* genes. *Rps12* and *RpsX* have been identified by us; whereas, *RpsJS* was by a Chinese lab. The genetic distances between adjacent loci in cM map units are presented on the left side of the map. The physical map of the *Rps* region is based on the positions of the SSR and SBP markers on the genome sequence of the cultivar 'Williams 82', which has been sequenced. The physical distances between adjacent loci are presented in kilobase pair DNA molecules (shown on left side of the physical map). Note that the new *RpsX* gene is mapped to a 72 kilobases DNA fragment located in between Sat_064 and SSRG60684K markers. This distance is based on the Williams 82 genome. Exact size of the fragment in the PI399036 chromosome containing the *RpsX* gene is expected to be different. Eight additional SBP markers have recently been identified for the *Rps12* region, are presented in between SSR18_1840 and Sat_064 of the physical map.

Our study discovered two *Rps* genes that are tightly linked. It is essential that we select both genes using molecular markers to incorporate the broad-spectrum resistance mechanisms against the *P. sojae* isolates conferred by the two genes. We therefore identified desirable molecular markers to introgress the two genes into new soybean cultivars. The Figure 4 shows the molecular markers that can be used in marker assisted selection of the *Rps12* and *RpsX* genes in breeding Phytophthora resistant soybean cultivars,

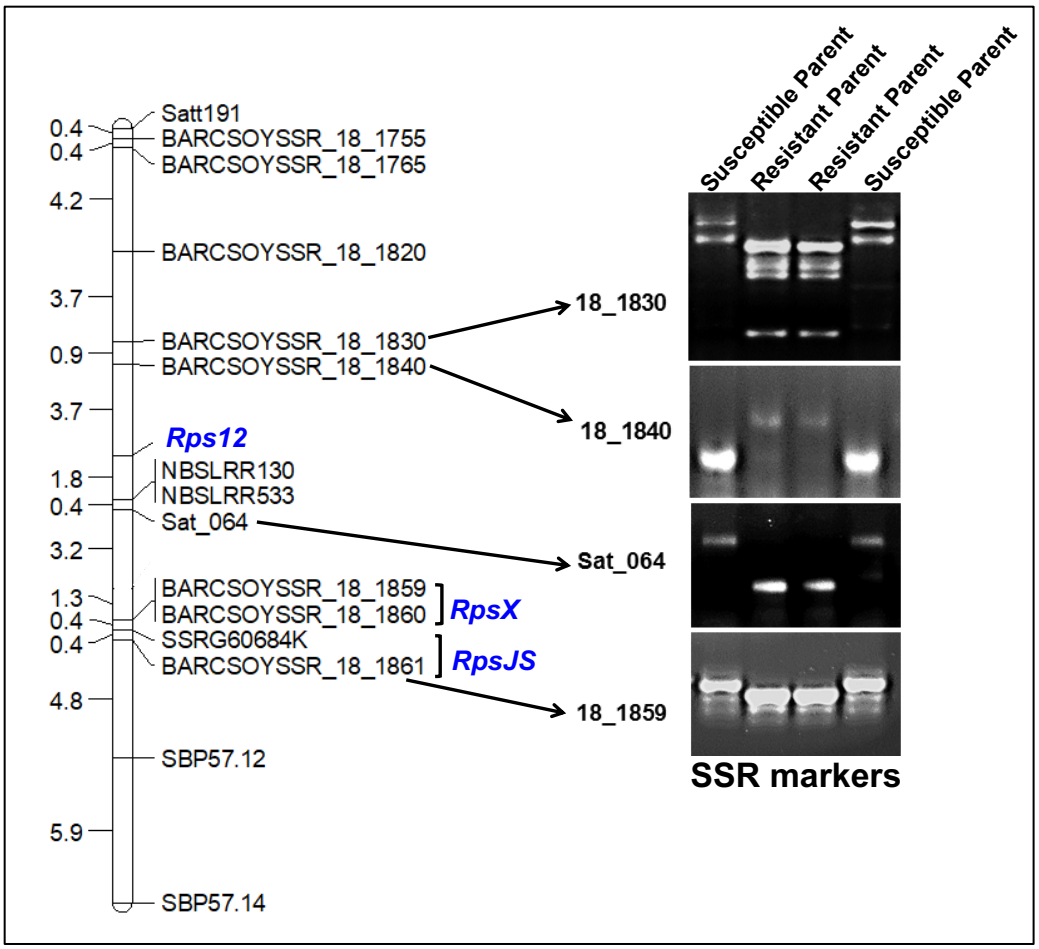

Figure 4. Genetic mapping of *Rps12* and *RpsX* and their associated molecular markers for breeding them into soybean cultivars. The genetic map, on the left, shows the region containing *Rps* genes. *Rps12* and *Rpsx* have been identified by us; whereas, *RpsJS* was by a Chinese lab. Phenotypes of the simple sequence repeat (SSR) breeders' friendly markers selected for breeding the two *Rps* genes into soybean cultivars are shown on the right side.

KPIs/Performance Metrics: In the one-year grant period, under the Objective 2 we expected to accomplish the followings:

1. We would map at least 20 molecular markers of the *Rps12* region by September 30, 2018.
2. We would identify the candidate *Rps12* genes by September 30, 2018.

Accomplishment: In our proposal, we proposed to identify 20 molecular markers to saturate the *Rps12* region. We have mapped 12 molecular markers in the region containing *Rps12* and *RpsX* genes. Eight additional molecular markers physically mapped to the region. Genetic mapping of these markers will be completed shortly. The genetically mapped molecular markers are sufficient to breed the two genes into soybean cultivars.

We have not been able to identify the candidate *Rps12* genes for the following reason. The *Rps12* is most likely comprised of simple sequences making it difficult to assemble the sequences. Our collaborators Drs. Anindya Das and Xiaoqiu Huang failed to generate the continuous sequence for the *Rps12* region. The resistance gene regions are novel and cannot be predicted from the genome sequence of Williams 82, which has been sequenced. Our

collaborators requested to sequence the PI399036 genome for additional coverages to facilitate the *de novo* assembly of the genome sequence.

Here it is worth noting that although genome of Williams 82 was sequenced in 2010 and again recently, the *Rps1*-k region is missing in the assembled genome sequence. We cloned the gene using conventional approaches involving cloning and isolation of bacterial artificial chromosomes and subsequently published the sequence of *Rps1* region (Gao and Bhattacharyya, 2008: https://bmcplantbiol.biomedcentral.com/track/pdf/10.1186/1471-2229-8-29; Gao et al. 2005: https://apsjournals.apsnet.org/doi/pdf/10.1094/MPMI-18-1035). Thus, our failure to identify the candidate *Rps12* genes was not unexpected.

Although identification of the candidate *Rps12* and/or *RpsX* genes was not possible due to lack of sufficient sequences, the outcomes of this project however provided adequate information for conducting marker-assisted selection of the two novel *Rps* genes for developing *Phytophthora* resistant soybean cultivars.