Mapping of the Arabidopsis gene *Pss18* involved in nonhost resistance against soybean pathogens, *Phytophthora sojae* and *Fusarium virguliforme*

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**Introduction:**
Soybean is an important global crop providing oil and protein. Worldwide soybean production is threatened by various diseases, such as SDS (sudden death syndrome). SDS is caused by a fungal pathogen, *Fusarium sojae*. It is a major threat to soybean production. Nationally, the estimated soybean yield suppression from SDS in 2010 was 2.1% of the total United States' yield valued at $820 million. In Iowa, the estimated financial loss was nearly $332 million.

Nonhost resistance (NHR) is the most common and robust form of plant disease resistance. The first layer of NHR suppresses the invasion by non-adapted pathogens at the pre-haustorial (before forming feeding thread) level by preventing the penetration of the fungal pathogen. It has been shown that the Arabidopsis mutant *pes1-1* is penetrated by the oomycete *Phytophthora sojae*. Presumably, the first layer of defense in Arabidopsis against the nonhost pathogen *P. sojae* is lost in *pes1-1*. Therefore, *pes1-1* was used for creating a mutant population using Ethyl methanesulfonate (EMS).

EMS-induced mutant plants were screened and identified for possible infection by *P. sojae*. Thirty putative *pss* mutants were identified. The mutants were named *pss* through *pss*30. A map-based molecular approach was applied to identify nonhost resistance genes in Arabidopsis. Here, molecular markers such as sequence-based polymorphic (SBP) and simple sequence length polymorphism (SSLP) were used to map the *Pss18* to a region on the Arabidopsis chromosome.

**Objective:**
The objective of this study was to map the Arabidopsis nonhost resistance gene *Pss18* that confers broad-spectrum resistance to both the soybean pathogen *F. virguliforme* and the host pathogen *P. sojae*.

**Molecular map based cloning, a method of rapid isolation of nonhost resistance (NHR) gene**

**Methods and Results**

1. **Plant Material**
   - Seeds of Arabidopsis *pss18 F2* families were sown (Fig. 1A) on LC1 soil-less mixture (Sun Gro Horticulture, Bellevue, WA) with a 16 h light and 8 h dark regime at 21°C with approximately 60% relative humidity. Seedlings were transplanted (Fig. 1B) ten days after sowing into new LC1 mixture and covered with humidity domes. Thereafter, seedlings were watered every fourth day. After transplantation, seedlings were allowed to grow for 11 days to inoculate the *P. sojae* spores (Fig. 1C).

2. **Fungal Pathogen**
   - Preparing the Pathogen
   1. *P. sojae* cultured on nutrient-rich V8 media plates (Fig. 2A). After 7 days, the plates were soaked overnight in water and were washed the following day with autoclaved water every thirty minutes for 9 h (Fig. 2B). The plates were left covered overnight at 22°C to allow the release of zoospores.
   2. Fungal Inoculation and Scoring
   3. A leaf inoculation method for the phenotypic analysis of the *pss18* mutant was used. Three leaves were detached from 21-day-old plants and placed on moistened filter paper in petri dishes. Each leaf was inoculated with 20 μl of *P. sojae* zoospores in suspension at a concentration of 4 x 10⁷ spores/ml (Fig. 2C). Symptoms were evaluated after 48 h of post inoculation (pi) for either a resistant or susceptible phenotype (Fig. 4).
   4. Leaves were scored based on necrotic symptoms observed in response to fungal penetration and proliferation (Fig. 4). Symbols “R” and “S” were used to denote resistant and susceptible phenotypes, respectively.

3. **Extraction of Genomic DNA from *pss18 F2* families**
   - Arabidopsis genomic DNA was extracted by CTAB method. Young leaf tissue was collected for DNA extraction as described in Naranjo et al. (2010) to prepare DNA for PCR reactions.

4. **Genetic Mapping of the *Pss18* gene**
   - In order to map the *Pss18* gene, bulk segregant analysis (BSA) was performed using SSLPs and SBPs markers. Bulk1 (B1) is carrying nine *F2* susceptible families and bulk2 (B2) is carrying 16 *F2* susceptible families. *Columbia-0* (Col-0) and *Nederland-0* (Nd-0) were included as a control. Samples were grouped as “RRR” and “SSS” confirming respective resistance or susceptibility to *P. sojae*. (Fig. 4).

5. **Polymerase chain reaction (PCR):**
   - Each PCR reaction mixtures contained master mix (MgCl2, dNTPs and Taq polymerase), forward and reverse primer. PCR was performed at 94°C for 3 min (Initial denaturation), and then 32 cycles of 94°C for 30 s (Denaturation), 50°C for 30 s (Annealing) and 72°C for 30 s (Extension), and finally the reaction was incubated at 72°C for 10 min (Final extension). The amplified PCR products were resolved on a 4% agarose gel by running at 100 V for 9 h. The ethidium bromide stained PCR products were visualized under UV light.

**Figure 6. Mapping of the *Pss18* region.**

A) Molecular mapping of *Pss18* using SSLP (simple sequence length polymorphism) and SBP (sequenced based polymorphism) markers across the Arabidopsis chromosome. Bi is bulk DNA from 9 susceptible *F2* families; B2 is bulk DNA from 16 susceptible *F2* families, C is Col-0, N is Nd-0. C + N is the DNA from both the parents.

B) Location of different markers (SSLPs and SBPs) on the 18 chromosomes of Arabidopsis. Arrow indicates the location of marker as shown in Figure 5A.

**Conclusion**
- *F2* families developed from a single cross between *pss18* (Col-0) and Nd-0 (ecotype of Arabidopsis thaliana, completely impervious to *P. sojae*).
- A total of 110 *F2* plant leaves were inoculated and scored for a susceptible phenotype. At least 25 *F2* families showed susceptible to *P. sojae*.
- For identification of *Pss18* gene, a map-based cloning approach was applied. *P. sojae* susceptible *F2* families were applied to bulk segregation analysis (BSA) for mapping of *Pss18*.
- We performed BSA using SSLPs and SBPs markers in all chromosomes of Arabidopsis.

**Future Work**
Once the *Pss18* locus is identified, the genomic DNA from the individual *F2* susceptible families will pool and send to next generation sequencing (NGS). By comparing sequences of the *Pss18* locus of the bulk susceptible *F2* families homologous for the *pss18* allele with that of the ecotype Col-0, we will identify SNPs of the *pss18* region. Analysis of T-DNA insertion mutant lines of the *pss18* region will identify the *Pss18* gene. Further, *Pss18* gene will be cloned and transformed into soybean to develop the transgenic plants against SDS disease.

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**References**