Mapping of Arabidopsis Nonhost Resistance Gene Pss5 that Confers Immunity to Soybean Pathogens, *Phytophthora sojae* and *Fusarium virguliforme*

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**Abstract**

The soybean root and stem rot pathogen, *Phytophthora sojae*, can penetrate single cells of the Arabidopsis penetration deficient mutant, pen1-1. A mutant screen was undertaken in pen1-1 to identify mutants that are infected by *P. sojae*. Thirty putative *P. sojae* susceptible (pss) mutants showing visible necrosis following inoculation with *P. sojae* zoospores were identified. Here we determine the genetic map location of the Arabidopsis Pss5 gene that confers resistance to both *P. sojae* and *Fusarium virguliforme*.

**Introduction**

Soybean sudden death syndrome (SDS) is a major threat to soybean production. Nationwide, the estimated soybean yield suppression from SDS in 2010 was 2.1% of total yield, valued at $0.82 billion; and in Iowa, the estimated financial loss was nearly $332 million. It is caused by a fungal pathogen, *Fusarium virguliforme*.

Nonhost resistance is the most revelant resistance mechanism found in nature. The first layer of NHR suppresses the invasion by non adapted pathogens at the pre-haustorial level by preventing the penetration of the fungal pathogen. It has been shown that pen1-1 is penetrated by *P. sojae*. Presumably, the first layer of defense in Arabidopsis against the nonhost pathogen *P. sojae* is lost in pen1-1. Therefore, pen1-1 was used for creating a mutant population.

Arabidopsis displays nonhost resistance against *F. virguliforme* as well as *P. sojae*. We screened and identified EMS-induced mutant plants for possible cell death and infection by *P. sojae* and identified 30 putative pss mutants. We named the mutants, pss1 through pss30. We applied a map-based molecular approach to identify nonhost resistance (NHR) genes in Arabidopsis. Here, we used molecular markers such as sequence-based polymorphic (SSBP) and simple sequence length polymorphism (SSLP) to map Pss5 to a region on the northern arm of chromosome 5.

**Objective**

The objective of this study was to map the Arabidopsis nonhost resistance gene Pss5 gene that confers broad-spectrum resistance to both the soybean SDS pathogen *F. virguliforme* and the root rot pathogen *P. sojae*.

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

**Methods**

**Phenotypic Analysis**

1. **Plant Material**

   Seeds of Arabidopsis *pss* F2 were grown (a) on LC1 soilless mixture (Sun Gro Horticulture, Bellevue, WA) with a 16 h light and 8 h dark regime at 21°C with approximately 60% relative humidity. The intensity of the light was maintained at 120-150 µM/m²/s. Seedlings were transferred (b) ten days after sowing into new LC1 mixtures and covered with humidified domes and thereafter watered every fourth day. A fertilizer mixture of 15:15:15:N-P-K (%) was applied to the seedlings seven days after transplantation. After transplantation, plants were watered to individual trays and allowed to grow (c).

2. **Fungal Preparing the Pathogen**

   - Nutrient rich V8 media plates were used to culture *P. sojae* (d). After 8 days, the plates were soaked overnight in water and were washed (e) the following day with autoclaved water every thirty minutes for 9 h. The plates were left covered overnight at 22°C to allow the release of zoospores.

3. **Fungal Inoculation and Scoring**

   - We used a leaf inoculation method for the phenotypic analysis of the pss5 mutant. Three leaves were detached from 21-day old plants and placed on moistened filter paper in petri dishes. Each leaf was inoculated (f) with 10 µl of *P. sojae* zoospores in suspension at a concentration of 3×10⁶ spores/ml. Symptoms were evaluated 72 h post inoculation (h) for either a resistant or susceptible phenotype (4A & B).

   - Leaves were scored based on necrotic symptoms observed in response to fungal penetration and proliferation. Symptoms "R" and "S" were used to denote resistant and susceptible phenotypes, respectively.

4. **Mapping of the Pss5 gene**

   - In order to map the Pss5 gene, we applied bulked segregant analysis (BSA). One bulk of *P. sojae* susceptible plants each carrying 14 *F. sojae* susceptible families and one bulk of *P. sojae* resistant plants each carrying 6 *F. sojae* resistant plants were generated. Columbia-0 (Col-0) and Niederzen-D (Nd-0) were included as a control. Samples were grouped as, "RRR" and "SSS" confirming respective resistance or susceptibility to *P. sojae*.

   - SSBP and SSLP molecular markers were used in this study to map the Pss5 gene.

**Conclusion**

- Preliminary results clearly indicate that Pss5 is linked to marker SBP5 8.40 on the north arm of chromosome 5.
- This study revealed that Pss5 gene plays an important role in Arabidopsis defense against the soybean pathogens *P. sojae* and *F. virguliforme* and lay the foundation to determine the role of Pss5 in plant-pathogen interaction and its role in adaptive immunity.

**Future Work**

Equal amounts of DNA from these 14 susceptible families of *pss5* were pooled and their bulked genomic DNA sent to prepare a genomic library for next generation sequencing (NGS). By comparing sequences of the pss5 region of the bulked susceptible *F. sojae* families homozygous for the pss5 allele with that of the ecotype Col-0, we will identify SNPs of the Pss5 region. Analysis of T-DNA insertion mutant lines of the Pss5 region will identify the Pss5 gene.

**Acknowledgements**

This project was supported by the National Institute of Food and Agriculture, Grant no. 2013-68004-20074 by the United States Department of Agriculture (NIFA-USDA).

**References**


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