Evaluation of the Genetic Architecture of Soybean Cyst Nematode Resistance at the *Rhg1* Locus

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Abstract

Plant genetic resistance is the most sustainable management strategy to combat soybean cyst nematode (SCN). Traditionally, both PI 90763 and Peking were classified into Peking-type resistance sources, yet they show differences in the degree of resistance to virulent SCN populations including TN22. Thus, a recombinant inbred line (RIL) population of 144 $F_{3:4}$ individuals was developed crossing PI 90763 and Peking to investigate the allele status at the *Rhg1* locus. The seeds from each of the F_3 plants were individually harvested and phenotyped for SCN resistance. After extracting the DNA from the $F_{3:4}$ population, QTL mapping confirmed that both parents carried the same *rhg1-a* allele and revealed two novel QTL for SCN resistance in PI 90763. This research contributes to understanding Peking-type resistance and provides more genetic diversity for breeding superior SCN-resistant soybean cultivars in the future.

Introduction

- Soybean cyst nematode (*Heterodera glycines*, Ichinohe) is the most destructive pathogen of soybean [*Glycine max* (L.) Merr.], causing over $1 billion in economic loss annually (Figure 1). Current SCN management methods include seed treatment, crop rotation, and use of plant genetic resistance. Developing cultivars with SCN resistance is the most cost-effective, environment-friendly, and reliable method for SCN management.

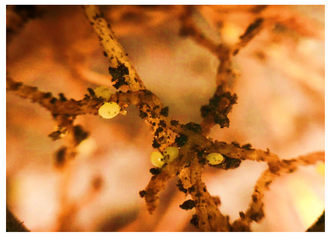


Figure 1. Cysts intact on roots

- Genetic diversity of SCN resistance in soybean is underutilized and limited to cultivars derived from plant introduction PI 88788, which is becoming less effective in SCN resistance over time. PI 88788 carries *rhg1-b* allele at *Rhg1* locus while the Peking-type resistance is mediated by *rhg1-a* allele at the *Rhg1* locus and the *Rhg4* locus. Continuous use of PI 88788-type resistance has been leading to the reduced effectiveness of SCN resistant cultivars over time. Therefore, there is a need for more diverse sources of plant genetic resistance cultivars to control this pathogen in the field. Moreover, searching for alternative sources of resistance is of high importance.

- Currently, Peking and PI 90763 are categorized under Peking-type resistance. However, PI 90763 exhibits higher resistance to some virulent SCN populations including TN22 (HG-type 1.2.5.7). Understanding the genetic difference between PI 90763 and Peking is crucial for developing new SCN-resistant soybean cultivars.

Objective

- Understanding the genetic difference between PI 90763 and Peking is crucial for developing new SCN-resistant soybean cultivars. Therefore, the objective of this study is to investigate *Rhg1* alleles status at the *Rhg1* locus through QTL mapping.

Materials & Methods

POPULATION DEVELOPMENT

- In summer of 2019, a cross was made between parental lines PI 90763 and Peking. The F_1 seeds were grown in a winter nursery located in Kauai, HI for two generations. F_3 seeds were space planted in rows in summer of 2020 in Columbia, MO. After assessing the true hybrid status of RILs with phenotypic markers, each plant was tagged, tissue was sampled, and seeds from individual plants were harvested separately (Figure 2).

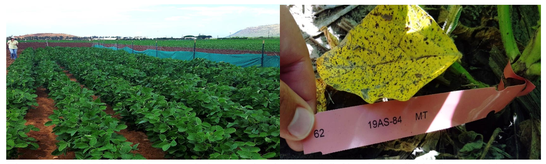


Figure 2. PI 90763 x Peking population growing in Hawaii (left); Plant individually tagged in the field (right).

SCN PHENOTYPING

- Evaluation of SCN resistance was conducted using the standardized cyst evaluation 2008 protocol. Five seedlings from each line were planted in separate PVC pipes and inoculated with 1,200 eggs of SCN population TN22 (Figure 3).

Figure 3. SCN phenotyping experimental design (left); Population in greenhouse after inoculation (right).

- After 30 days, cysts were harvested and manually counted under a microscope. Female Index (FI) was calculated based on the following formula:

  FI = [(mean number of females on a test soybean line) / (mean number of females on the standard susceptible)] × 100

- SCN resistance was scored as resistant (R, FI<10), moderately resistant (MR, FI=10-30), moderately susceptible (MS, FI=31-60) and susceptible (S, FI>60).

SNP GENOTYPING

- DNA was extracted from 144 F_3 plants and the parent lines using CTAB method and checked for quality control with gel electrophoresis (Figure 4). The samples were submitted to the Soybean Genomics and Improvement Laboratory, USDA-ARS, where genome-wide fingerprinting occurred for 6,000 single nucleotide polymorphic (SNP) markers, using the Illumina Infinium BARCSoySNP6K Beadchip.

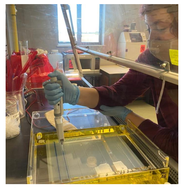


Figure 4. DNA samples undergoing gel electrophoresis after extraction.

QTL MAPPING ANALYSIS

- QTL analysis was conducted to find marker associations between resistant phenotypes and their positions within the soybean genome. The matrix of 6,000 SNP markers was used to create a genetic linkage map with Rstudio software using the RQTL package. QTL mapping was performed using MapQTL 5.0 software and presented using MapChart 2.3 software.

Results

- Indicator lines confirmed the correct responses of the TN22 SCN population as HG type 1.2.5.7. The parental lines PI 90763 and Peking scored a FI of 0 and 19.8, respectively. The mapping population was not normally distributed and skewed toward the resistant phenotype with a mean of 7.3 and a range of 0 to 24.1 (Figure 5).

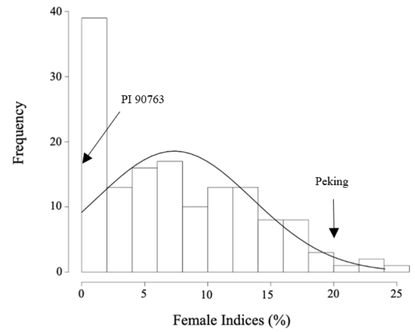


Figure 5. Frequency distribution of female indices for SCN population TN22 (HG type 1.2.5.7) in 144 $F_{3:4}$ lines from population PI 90763 x Peking.

- For population PI 90763 x Peking, 1,135 polymorphic markers were positioned on 20 soybean chromosomes (Figure 6).

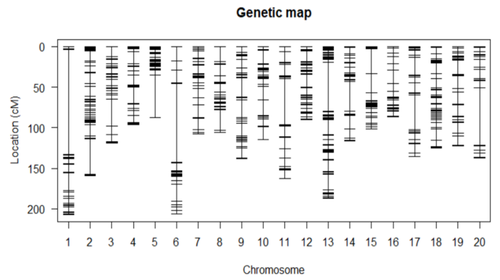


Figure 6. Genetic linkage map created for 144 $F_{3:4}$ lines from population PI 90763 x Peking.

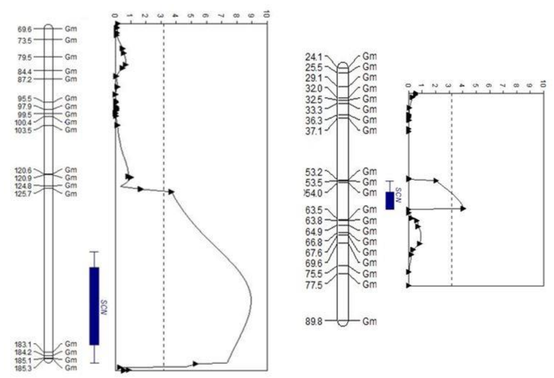


Figure 7. Quantitative trait loci (QTL) controlling soybean cyst nematode (SCN) resistance to TN22 (HG type 1.2.5.7) detected in PI 90763 x Peking population.

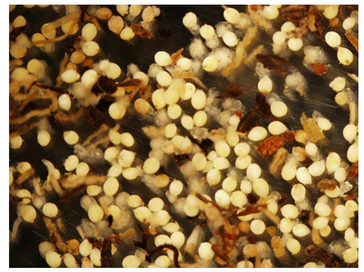
- The permutation test determined a significant likelihood of determination (LOD) threshold of 3.2. No QTL was found at the *Rhg1* locus on chromosome 18. Two QTL were detected by composite interval mapping method (Figure 7). A major QTL explained 22.9% of the phenotypic variation in FI score with a LOD value of 8.9. A minor QTL explained 9.6% of the phenotypic variation with a LOD score of 4.1.

Conclusions

- No marker association was found on chromosome 18, indicating that PI 90763 and Peking carry the same *rhg1-a* allele of the *Rhg1* locus.

Therefore, the difference in resistance to TN22 is caused by two novel QTL from PI 90763.

- These two novel QTL can be used in a marker-assisted selection (MAS) program for developing new soybean cultivars with SCN resistance from PI 90763.



Future Studies

- Fine-mapping of novel QTL from PI 90763 x Peking population to identify potential candidate genes
- Diversify SCN resistance in modern soybean cultivars with two novel QTL by using MAS in the northern soybean breeding program at the University of Missouri

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