Molecular Markers Linked to Soybean Sclerotinia Stem Rot Resistance Using Bulk Segregant Analysis

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Abstract

Sclerotinia stem rot is a common disease of soybean in the northern growing regions of the U.S. One method to manage this disease is by host resistance. The objective of this experiment was to determine if bulk segregant analysis could be used to develop markers associated with genes or genomic regions for resistance. Plants of F4:5 derived lines of PI194634 x Merit were visually scored for resistance 2 weeks after inoculation using the cut stem method. Disease assessed by measuring lesion lengths and using a 0 (most resistant) to 9 (most susceptible) scale. Two bulk samples of DNA were extracted based on those lines that were identified as resistant and susceptible, respectively, using the two assessment methods. The bulk samples were screened for differences using fluorescently labeled SSR primers and non labeled primers to identify polymorphic markers associated with genes for resistance to Sclerotinia stem rot. Using bulked segregant analysis, polymorphic markers were identified and a number of markers closely linked to a specific region of the genome were identified based on the disease assessment. Linkage analysis was performed to estimate the genetic distance of the linked markers. Significant markers tightly linked to the gene may prove to be instrumental in positional cloning and hasten the development of soybean lines with resistance to Sclerotinia stem rot.

Overview

Sclerotinia sclerotiorum causes Sclerotinia stem rot or white mold in many crop plants. On soybeans, the disease has increased dramatically in the north central United States since 1990. The disease was ranked as the second-most important disease based upon 1994 data.

The pathogen produces oxalic acid and enzymes that degrade tissues, causing serious economic loss by impacting crop quality and yields. The annual losses for soybean as high as $300million (National Sclerotinia Initiative, 2006).

Host plant resistance has been one option to reduce the impact of Sclerotinia stem rot; however, no sources of complete resistance have been identified. Due to the complexity of the resistance to this pathogen, it is difficult to develop partially resistant cultivars.

Attempts have been made to identify QTL for resistance to Sclerotinia stem rot in soybean. Arahana et al. (2001) identified 28 putative QTL for the resistance in five recombinant inbred line (RIL) populations while Kim and Diers (2000) mapped three QTL on molecular linkage groups (MLG) C2, K and M in F3 derived populations.

Vuong et al. (2003) identified three putative QTL on MLG K, D1b, and J. Although many QTL were identified, no major QTL for the resistance to Sclerotinia stem rot has been detected. Co-dominant microsatellite SSR DNA markers are distributed throughout the soybean genome and some clustering of markers are also observed (Cregan et al., 1999).

Objective

Identify markers associated with Sclerotinia stem rot resistance in soybean using bulk-segregant analysis based on separate resistant and susceptible bulks using two disease assessment methods.

Materials and Methods

Plant materials and Disease Assessment

- Bulks were made from PI194634 (resistant) x Merit (susceptible), F4:5 derived population comprising of 92 individuals.
- Individual genotypes were either qualitatively (visual assessment from 0-9 scale) or quantitatively (stem lesion lengths measured) rated 2 weeks after inoculation using the cut stem method.
- Based on the visual scale, 0-3 (resistant) and 6-9 (susceptible) plants were bulked separately. Similarly, based on lesion length measurements, resistant and susceptible plants were bulked. DNA was extracted using CTAB method from the bulk tissues of 6- to 8-day-old seedlings for each method.

Fig 1. Inoculated soybean plants under mist conditions.

Fig 2. Level of susceptibility 2 weeks after cut stem inoculation.

Development of Mapping Population

Selected resistant and susceptible plants for each method of evaluation were kept separately.

Two ug of DNA of each individual homozygous for one of the other allele for the target gene was bulked together. The number of individuals in each bulk varied from 15 to 20 plants. The bulks of each group and the parents together were screened using randomly chosen SSR primers based on linkage groups for polymorphism. Over 150 SSR primer pairs were used in the initial screening.

The markers that showed polymorphism between the bulk and the parents were used to genotype the individuals in each group separately. Genotype data of each group based on co-segregation in the F3:4 population was used to construct a map.

Fig 3. Fluorescently labeled SSR Primers multiplexed with 3 primers for genotying bulk DNA individual genotypes.

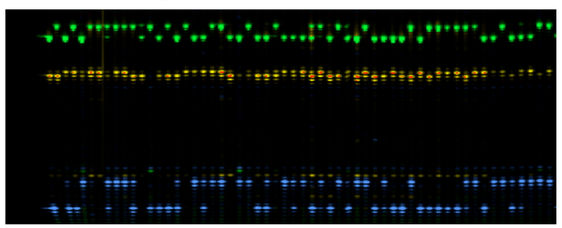


Fig 4. Two promising markers, satt147 (LG-D1a) and satt424 (LG-A2), from a segregating population.

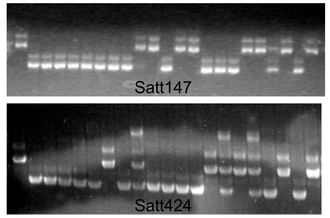


Fig 5. Map from bulk genotypes phenotyped by a qualitative assessment.

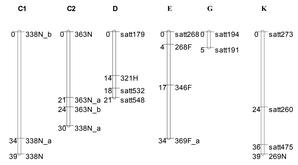
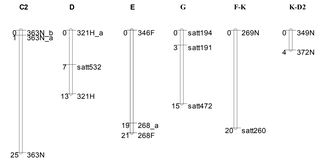


Fig 6. Map from bulk genotypes phenotyped by a quantitative assessment.



Conclusions

- Markers satt273 and satt260 mapped cM apart in LG–K 24 based on the qualitative assessment method. However, satt260 was not mapped based on quantitative assessment method. These two alleles were reported to be associated with decreased lesion size (Arahana et el.2001), and affected resistance directly (Kim and Diers, 2000).
- Marker satt191 mapped in LG-G and was associated with lower quantitative measurements. This marker is reported to be linked with Rps4 and Rps5 genes, and could be used in MAS.
- In LG-C2 and LG-E, there were markers mapped; however, bulk individuals in each group are small in size and more markers will be needed to determine if more markers are associated with resistance genes.
- More work on this population is in progress to study the QTLs associated with stem rot resistance to see whether or not the same alleles can be identified as the two methods of assessment.

References

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