2006 Beltwide Cotton Conferences, San Antonio, Texas - January 3 - 6, 2006

PROGRESS IN MAPPING LOCI FOR RESISTANCE TO ROOT-KNOT NEMATODES IN AN INTERSPECIFIC *GOSSYPIUM* POPULATION

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Introduction

Southern root-knot nematodes (*Meloidogyne incognita*; RKN) reduce profits from cotton producers through yield loss, due directly to RKN or indirectly due to other diseases associated with it such as seedling diseases and fusarium wilt (*Fusarium oxysporum*), and increased production cost by nematicide applications. Although nematicides are effective in controlling RKN, they do not provide season-long protection. Also, the future availability of nematicides is uncertain due to environmental concerns. Further, in fields below threshold levels of RKN, small yield losses not justifying cost of nematicide application can occur. With costs of cotton production increasing and prices of fiber at a historical low, any loss of yield can be considered economically significant.

The development and use of cultivars with resistance to RKN offers the best management tool for RKN. However, progress in developing RKN resistant cultivars has been slow because the current screening process to identify resistant genotypes is tedious, time consuming, and destructive. Molecular markers offer an alternative screening process for identifying resistant genotypes in breeding programs. The development of diagnostic markers for genes conditioning RKN resistance will accelerate the transfer of these genes among genotypes or germplasm for new cultivar development. The objective of this study is to develop diagnostic DNA markers for genes conditioning RKN resistance in cotton.

Materials and Methods

The RKN-resistant line M-120 RNR was crossed with Pima S-6, a susceptible *Gossypium barbadense* cotton line, to develop an F_2 population. The resistance of M-120 RNR comes from Auburn 623 RNR via Auburn 634 RNR, which was backcrossed to Coker 201 (Shepherd, 1982). Six plants of each parent, six F_1 plants, and 241 F_2 plants were inoculated with nematodes in a greenhouse. Variables measured were galling (rated on a 0-10 scale, where 0 = no galls and 10 = 91-100% galled), number of eggs extracted per root system, and eggs per gram of root. DNA extractions were obtained from F_2 plants. Approximately 200 restriction fragment length polymorphism (RFLP) markers were selected 20-25 centimorgans apart to cover the entire cotton genome. These markers were used to screen the 16 most resistant and 16 most susceptible F_2 plants. Regression analysis was utilized to test associations between the scores for RFLP markers and the phenotypic variables measured. The markers showing a significant ($P < 0.05$) association in this preliminary screening were used to screen the whole population in order to confirm the association.

Results and Discussion

As expected, M-120 had significantly lower galling, number of eggs, and eggs per gram of root than Pima S-6. The F_1 was highly resistant and was not significantly different from M-120 for any of the three variables, suggesting that one or more dominant genes are involved in the resistance to RKN. Coefficients of correlation among variables were calculated using the F_2 data. The correlation coefficients were significant, suggesting that the three variables measure similar genetic factors. Galling is the easiest and fastest way of measuring resistance to RKN. The phenotypic distribution of the F_2 plants for galling was skewed towards the resistant parent, suggesting that only few genes with dominant effects control RKN resistance.

We screened over 180 RFLP markers, covering all 13 chromosomes of the cotton genome. Statistical analyses performed using the extreme individuals detected seven putative chromosomal regions significantly associated with the resistant phenotype, suggesting that a resistant gene may be present in these regions, although random sampling or scoring errors can not be ruled out at this point. The markers that showed significant association in the preliminary screening were tested on the whole population to confirm the association. Two chromosome regions, chromosome LGA03 and chromosome 7, were significantly associated with the resistant phenotype.

The chromosome regions around the significant markers in LGA03 and Chromosome 7 were investigated in more detail by testing additional PCR-based DNA markers that are mapped to these regions. By searching various scientific publications, we identified 77 Simple Sequence Repeat markers (also commonly called SSRs) that target specifically to the two regions. They include 40 primers from CIRAD, France and 37 from the Brookhaven National Laboratory (BNL). PCR primers were synthesized from all these SSR sequences and tested on 186 F2 individuals where DNA was available. Statistical analysis on the SSR markers showed that at least one additional marker showing linkage with the resistant phenotype at a LOD value of 3 or higher, confirming that a resistance gene is present in these chromosomal regions. More SSR and RFLP derived CAPS markers are being tested on the population to saturate the desirable region and to determine marker(s) tightly linked to the trait so that these could be used in the RKN resistance breeding program.

Acknowledgements

We thank the Georgia Cotton Commission and Cotton Incorporated for financial support for this project.

References

Shepherd, R.L. 1982. Registration of three germplasm lines of cotton. Crop Sci. 22:692.