DEVELOPMENT OF DIAGNOSTIC MARKERS FOR ROOT-KNOT NEMATODE RESISTANT GENES IN COTTON

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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 (Table 1). 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.

Although more than half of the SSR markers showed genetic variation between the two mapping parents and therefore were useful for genetic linkage analysis, many were later determined to be mapped not to LGA03 or Chromosome 7. In total, we found only 8 SSRs mapped to LGA03 (Fig. 1) and 6 mapped to Chromosome 7 (Fig. 2). These SSRs were tested on whole population consisting of 186 F2 individuals. Statistical analysis to determine linkage was performed on a combined data set from SSRs and RFLPs (see Table 2 and 3). We have determined that the resistance gene on LGA03 is located near the end-point (telomere) of the linkage group, and the most likely position is between the SSR marker CIR316 and the RFLP marker pAR111. This resistance gene has turned out to be the major gene segregating in the mapping population. In addition, we have also identified another SSR marker linked to the resistance gene on Chromosome 7. However, this gene appears to have inherited from the susceptible Pima S6 parent. Further study is needed to determine if the gene from Chromosome 7 is authentic.

In summary, we have achieved our goal in identifying DNA markers linked to the genes that confer resistance to root-knot nematodes. Our next challenge would be to devise a strategy for which these DNA markers can be utilized in a breeding program to help accelerate the development of RKN-resistant cotton cultivars.

Acknowledgements

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References

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

Table 1. Phenotype values for nematode resistance of F1, F2 and their parents.

Trait	Parents		F1	F2				
	Pima S-6	M120		Max	Min	Mean	SD	Skew
Galling	5.7	1.3	1	10	0	2.992	1.192	0.152
Root weight	21.5	10.7	32.7	26.0	0.4	7.249	4.282	1.447
Eggs	91650	550	3120	446400	0	17584	38800	6.667
Eggs/groot	4061.4	56.5	113.1	38483	0	2185.69	3767	4.907

Table 2. QTLs associated with nematode resistance on LGA03 with composite interval mapping.

Trait	Interval	LOD	Additive	Dominance	VAR
Galling	CIR316-pAR111	15.1	2.761	-2.997	62.81
Galling(log)	CIR316-pAR111	6.64	0.1476	-0.1667	35.43
eggs	CIR316-pAR111	5.52	15494	-15750	11.74
Eggs(log)	CIR316-pAR111	6.56	0.2414	-0.4684	14.26
Egg-root	CIR316-pAR111	6.42	1247	-1579	10.3
Egg-root(log)	CIR316-pAR111	5.22	0.2217	-0.4402	13.03

Table 3. QTLs associated with cotton nematode resistance on Chr07 with composite interval mapping.

Trait	Interval	LOD	Additive	Dominance	VAR
Galling	NAU474b-G1158b	3.45	-0.8118	-1.311	7.9
Galling(log)	NAU474b-G1158b	3.43	-0.0542	-0.1283	7.77
rootweight	NAU845-NAU1048	4.82	3.408	-2.905	28.1
rootweight(log)	NAU845-NAU1048	2.95	0.1089	-0.1265	10.91
eggs	NAU474b-G1158b	3.22	-13776	-10042	5.87
Eggs(log)	NAU474b-G1158b	3.27	-13.54	-0.4188	8.02
Eggs/groot	NAU474b-G1158b	2.71	-1298	-822	5.17
Eggs/groot(log)	NAU474b-G1158b	2.17	-0.101	-0.3578	5.9

Note: negative additive effect indicated the effect of increasing galling originated from M120.

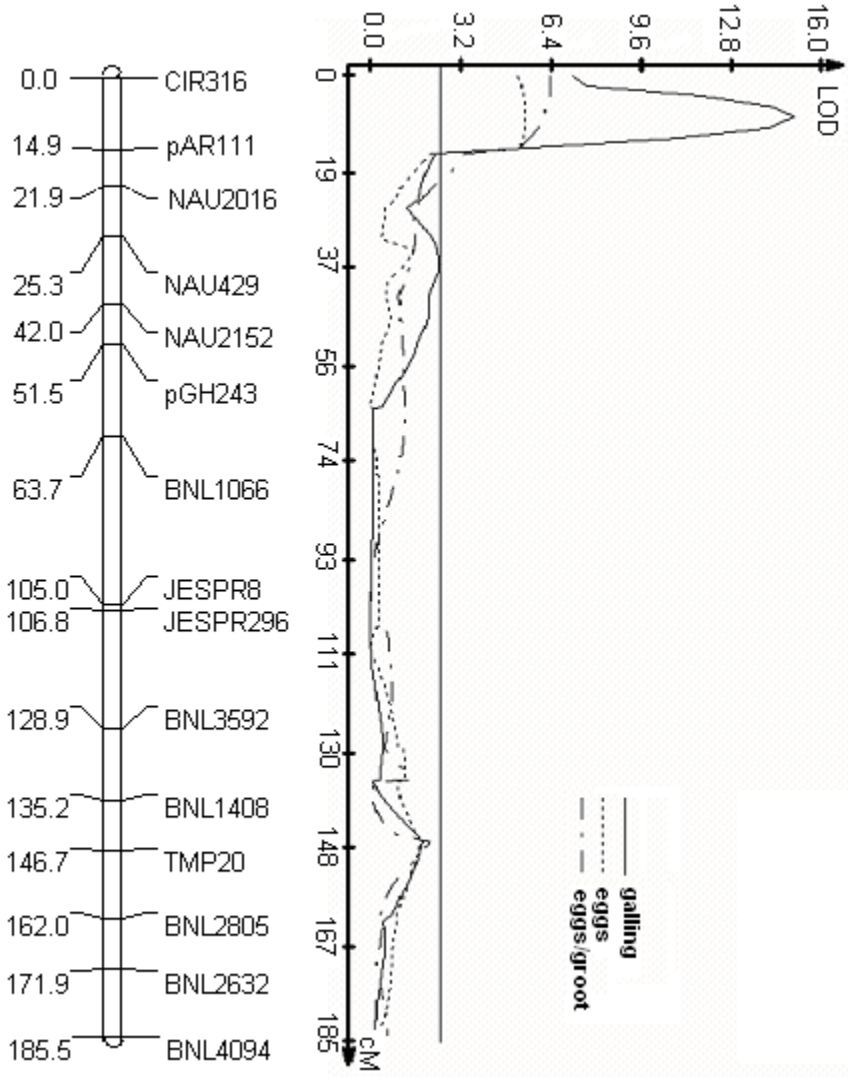

Figure 1. QTL map figure for LGA03

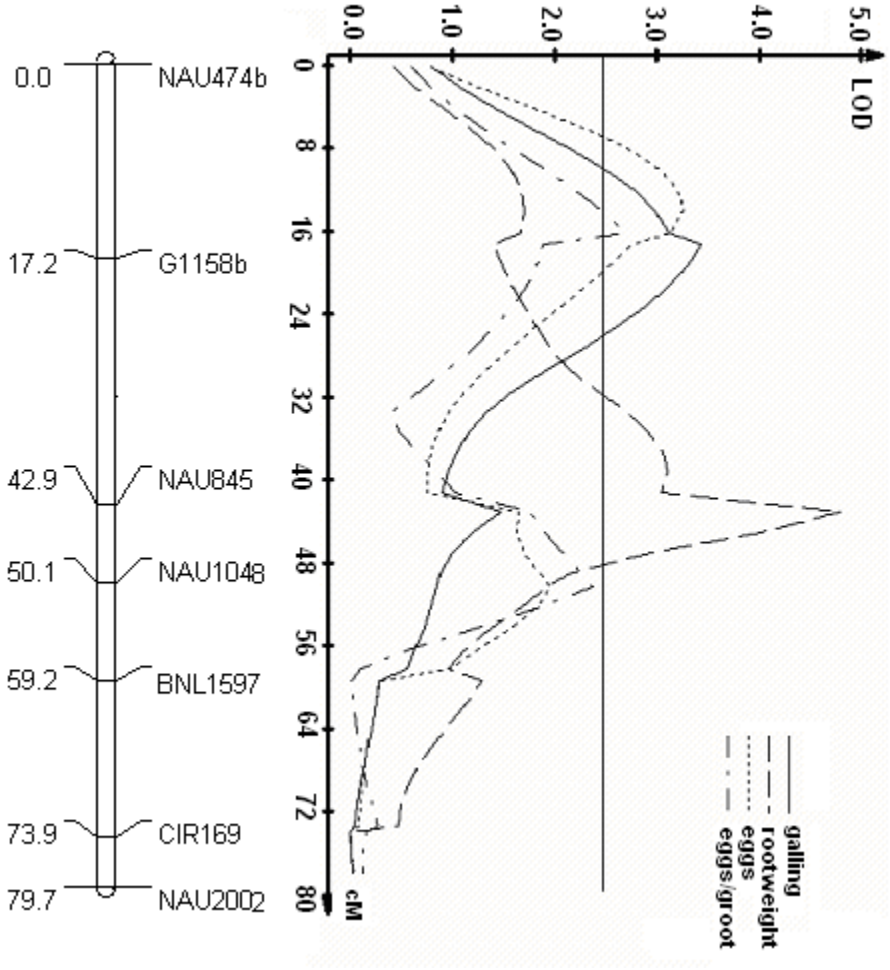

Figure 2. QTL map figure for Chr 07