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ROOT-KNOT NEMATODE RESISTANCE IN AUBURN 634RNR: SEGREGATION AND MOLECULAR MAPPING

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Abstract

Clarification of the genetic basis for root-knot nematode (RKN) resistance in the Auburn source and development of closely linked markers to the resistance could finally put the RKN resistance gene(s) to work in cotton breeding. This study was conducted to verify the genetic mechanism of the Auburn source and to verify and identify molecular markers for the RKN resistance. Two F2 populations using Auburn 634 and its derived resistant line M-240 confirmed that the resistance is either controlled by one dominant gene or one dominant gene and one recessive gene. By comparing several pairs of near isogenic lines (NILs) with or without the Auburn source resistance, none of the previously reported RAPD and SSR markers exhibited consistent polymorphism, indicating that they were not closely linked to the RKN resistance. However, several SSR and two RGA markers were identified to be consistently polymorphic between the NILs. These markers will be employed in our mapping populations to evaluate their linkage with the RKN resistant gene(s).

Introduction

Significant progress in nematode resistance (R) in major crops has been made in the recent ten years: (1) major R genes for nematode resistance have been identified; (2) many of the R genes have been utilized in commercial cultivars; (3) Some 30 R genes including 3 R genes for nematode resistance have been cloned; (4) Thousands of R gene analogs (RGA) have been isolated; (5) Many molecular markers tightly linked to R genes have been developed; and (6) tremendous information on signal transduction pathways for R gene functions has been accumulated.

In cotton, many moderately root-knot nematode (RKN) resistant germplasms have been identified and a highly RKN resistant germplasm line Auburn 623 RNR was released by combining the moderately resistances from both of its parents (Clevewilt and Wild Mexico Jack Jones) (Shepherd, 1974a, b). A significant research effort has since been made to investigate the resistance genetics, mechanisms and to develop RKN resistant cultivars (Jenkins et al., 1995; McPherson et al., 1995; Colyer, et al., 2000). The high level RKN resistance from Auburn 623 RNR was transferred into various genetic backgrounds to develop a series of new RKN resistant lines, such as Auburn 634 RNR (Auburn 56 background), M240, and M315 (both in DP 61 background) (Sherpherd et al., 1989) . The resistance from Clevewilt was also used to develop moderately resistant LA 434 RKR, ST LA 887 and PM 1560. A moderately RKN resistant Acala cultivar Nem-X was released with its resistant source unknown (Robinson et al., 1999; Ogallo et al., 1999). However, the highly RKN resistant level from the Auburn source has never been introduced into any commercial cotton cultivars. Furthermore, the lack of reliable selection techniques in RKN resistance breeding also has hindered the research progress. The controversy regarding the genetic basis of its resistance still remains until this day. Shepherd (1974a, b) suggested a polygenic and partially dominant nature for the Auburn source resistance. McPherson (1993, Ph.D. dissertation) in Mississippi indicated that one dominant gene and one additive resistant gene could explain the resistance segregation. Two dominant resistant genes were indicated from Zhou (1999)'s data in Texas. However, one dominant gene was implicated based on Zhang (unpublished). Zhou (1999) further suggested that the RKN resistance in ST LA887 was controlled by one recessive gene, which could be the same in Nem-X. Benzawada et al. (2003) verified the one recessive resistant gene model in Clevewilt. However, Roberts (2003) reported that there were two recessive genes involved in the RKN resistance in Nem-X. Many *G. hirsutum* race stocks and their day-neutral converted lines confer RKN resistance. Based on McPherson (1993), their resistance could be controlled by one additive gene (e.g., in M 19), or one dominant gene (in M 78) or two genes (one dominant and one additive, in M 75). The contradictory data could be complicated by the variation of RKN evaluation method or/and the genetic complexity of RKN resistance.

Materials and Methods

Two segregating populations, SG 747 x Auburn 634 RNR F2 and SG 747 x M240 F2, together with their parents, were planted in the greenhouse in 4" pots on July 9, 2003. In a pot, one F2 plant and 1 plant each from the two parents were

evenly spaced. RKN inoculation (4,000 eggs/pot) was made in the following day. On September 26, 2003, the plants were transplanted to 10" pots followed by re-inoculation on October 17, 2003 and November 25, 2003. Plants were finally evaluated for RKN resistance on January 3, 2004 based on galling, plant size and root mass (Zhang et al., 2004). When comparing with the parents in the same pot, three ratings, resistant (R), moderately resistant (MR), and susceptible (S) were adopted.

Five pairs of near isogenic lines (NIL) on Auburn 56, ST 213, DPL61, Coker 201 and Coker 310 were planted in the greenhouse and leaf tissues were harvested for DNA extraction (Zhang and Stewart, 2000). DNA markers such as SSR, RAPD, and RGA were used to identify polymorphism between the pairs of NILs.

Results and Analysis

Segregation Analysis in Two F2 Populations

For data analysis, the F2 plants in the pots where R parental plants did not show resistance (R or MR) or/and S parental plants did not show susceptibility (S) were excluded. The results were shown in Table 1. The Chi-square test revealed that the segregation in the two F2 populations fitted to a 3:1 ratio, indicating one dominant resistant gene involved in the RKN resistance from Auburn 634 and its derived line M-240. Our data seemed to support one gene model; however, the data for the second F2 population also did not violate a 13:3 ratio (one dominant R gene and one recessive R gene). In this case, Rk1-Rk2- (9/16), Rk1-rk2rk2 (3/16), and rk1rk1rk2rk2 (1/16) are resistant or moderately resistant, while rk1rk1Rk2- (3/16) are susceptible, assuming that Rk1 is dominantly resistant and rk1 is recessively resistant. Further study will be needed to separate the genetic effects of the two genes and their interactions.

Marker Development for RKN Resistance

Two chromosomal locations from Jim Starr's lab were identified to be associated with RKN resistance: LG A02 (18.6 cM from RFLP marker B1-3) and C14 (21.6 cM from RFLP marker pAR815). Also Zhang (unpublished) lab also reported some potential polymorphic RAPD markers amplified by UBC 10-mer primers. More recently, Benzawada et al. (2003) reported two polymorphic SSR markers that could explain less than 10% of the RKN resistance in a F2 population from a cross between Clevewilt 6 and ST 213. However, the polymorphism was not detected between the 5 pairs of near isogenic lines (NILs) using these putative RAPD and SSR markers, indicating that these markers were not closely linked to the RKN resistant genes. Based on our SSR and RGA marker analysis, several SSR markers (Figure 1) and two RGA markers (Figure 2) were found to be polymorphic between the pairs of NIL-S and NIL-R lines. The linkage of these markers to the RKN resistance will be further tested in our mapping populations.

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Table 1. Segregation analysis in two F2 populations

Population	No. R	No. S	Exp. ratio	χ^2
SG747 x A634 F2	48	24	3:1	2.67
SG747 x M-240 F2	47	16	3:1	0.00

$\chi^2(0.05)= 3.84$

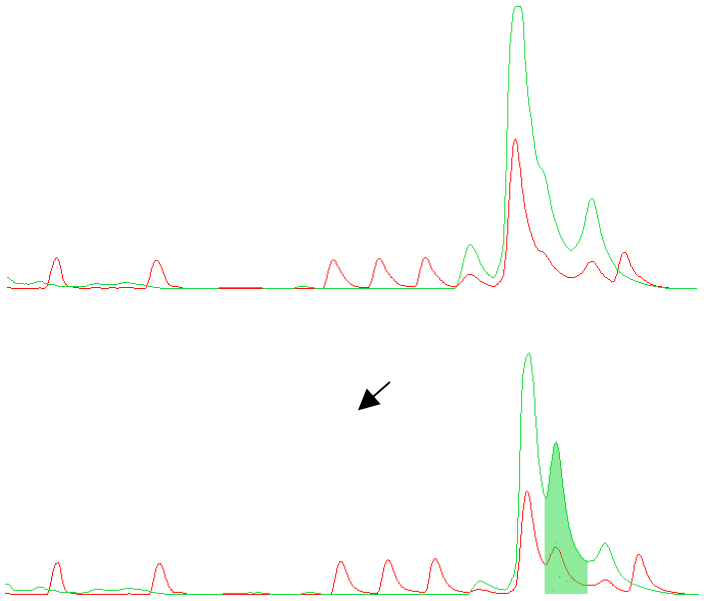

Figure 1. A potential SSR marker for RKN resistance. The arrow indicates an extra band in the NIL-R line

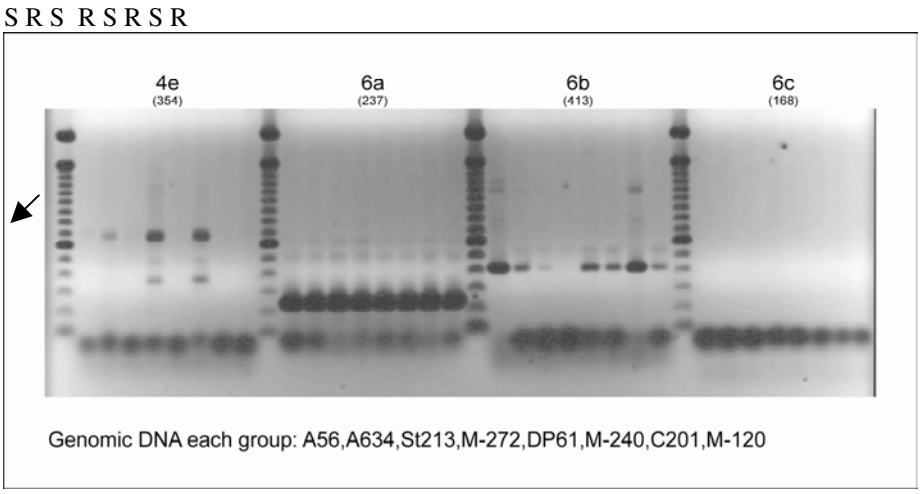

Figure 2. A potential RGA marker for RKN resistance. The arrow indicates a RGA marker only in NIL-R lines.