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ORIGINAL PAPER

Identification of QTL regions and SSR markers associated with resistance to reniform nematode in *Gossypium barbadense* L. accession GB713

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Abstract The identification of molecular markers that are closely linked to gene(s) in *Gossypium barbadense* L. accession GB713 that confer a high level of resistance to reniform nematode (RN), *Rotylenchulus reniformis* Linford & Oliveira, would be very useful in cotton breeding programs. Our objectives were to determine the inheritance of RN resistance in the accession GB713, to identify SSR markers linked with RN resistance QTLs, and to map these linked markers to specific chromosomes. We grew and scored plants for RN reproduction in the P_1, P_2, F_1, F_2, BC_1P_1, and BC_1P_2 generations from the cross of GB713 × Acala Nem-X. The generation means analysis using the six generations indicated that one or more genes were involved in the RN resistance of GB713. The interspecific F_2 population of 300 plants was genotyped with SSR molecular markers that covered most of the chromosomes of Upland cotton (*G. hirsutum* L.). Results showed two QTLs on chromosome 21 and one QTL on chromosome 18. One QTL on chromosome 21 was at map position 168.6 (LOD 28.0) flanked by SSR markers, BNL 1551_162 and GH 132_199 at positions 154.2 and 177.3, respectively. A second QTL on chromosome 21 was at map position 182.7 (LOD 24.6) flanked by SSR markers BNL 4011_155 and BNL 3279_106 at positions 180.6 and 184.5, respectively. Our chromosome 21 map had 61 SSR markers covering 219 cM. One QTL with smaller genetic effects was localized to chromosome 18 at map position 39.6 (LOD 4.0) and flanked by SSR markers BNL 1721_178 and BNL 569_131 at positions 27.6 and 42.9, respectively. The two QTLs on chromosome 21 had significant additive and dominance effects, which were about equal for each QTL. The QTL on chromosome 18 showed larger additive than dominance effects. Following the precedent set by the naming of the *G. longicalyx* Hutchinson & Lee and *G. aridum* [(Rose & Standley) Skovsted] sources of resistance, we suggest the usage of Ren^{barb1} and Ren^{barb2} to designate these QTLs on chromosome 21 and Ren^{barb3} on chromosome 18.

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Abbreviations

cM	Centimorgans
MAS	Marker-assisted selection
SSRs	Simple sequence repeats
RN	Reniform nematode
QTL	Quantitative trait loci

Introduction

The reniform nematode (RN), *Rotylenchulus reniformis* Linford & Oliveira, is of tropical origin. RN was

introduced into the US and first described in Georgia and Louisiana in the 1940s (Smith 1940; Smith and Taylor 1941). Recently, its range has expanded and RN is now a serious pest of Upland cotton (*Gossypium hirsutum* L.) and soybean (*Glycine max* L.) in the southern United States (Robinson 2007). Cotton lint losses due to RN in Alabama, Arkansas, Louisiana, and Mississippi were estimated at 5.6% in 2008 (Blasingame 2009). In some highly infested fields, growers estimate losses in excess of 300 lb lint ha^{-1}. All Upland cotton cultivars that have been evaluated are susceptible to RN (Robinson et al. 1999; Koenning et al. 2004; Usery et al. 2005; Weaver et al. 2007). Cultural practices, such as rotation with non-host crops, are sometimes impractical because commodity prices make some crops unlikely to return profits in certain years and application of nematicides often is only partially effective for suppressing the damage caused by RN field populations (Koenning et al. 2004). Therefore, the identification of RN resistance genes and their incorporation into Upland cotton breeding lines is highly desirable.

Host resistance to RN has been identified in other *Gossypium* species. Immunity to RN was observed in *G. longicalyx* Hutchinson & Lee, while varying levels of resistance were identified in *G. stocksii* Masters, *G. somalense* (Gürke) Hutchinson, *G. barbadense* L. TX110, and *G. arboreum* L. PI 41895 accessions (Yik and Birchfield 1981, 1984). In addition, these studies reported resistance in *G. herbaceum* L., *G. arboreum* PI 417891 and CB3839, *G. raimodii* Ulbrich, and *G. hirsutum* race Marie Galante accessions TX893 and TX874. Later, 48 *G. hirsutum* and *G. barbadense* accessions from Mexico were screened for resistance to *Meloidogyne incognita* (Kofoid & White) Chitwood race 3 and RN by Robinson and Percival (1997); however, only two *G. barbadense* accessions, TX1347 and TX1348, were found to be RN-resistant.

More recently, 1,866 primitive accessions of *G. hirsutum* and 907 of *G. barbadense* were screened for resistance to *M. incognita* race 3 and RN (Robinson et al. 2004). In that study, *G. hirsutum* accessions TX2469, TX1586, TX748, T25, TX1828, and TX1860 and *G. barbadense* accessions GB49, GB13, GB264, GB171, and GB713 were identified as resistant to RN. By screening 2,102 Upland cotton accessions for resistance to RN, Weaver et al. (2007) identified TX245, TX378, TX500, TX1419, TX1472, TX1565, and TX1765 as accessions that supported lower populations of RN than susceptible controls. The inheritance of resistance to RN has been studied with intraspecific as well as interspecific crosses. Muhammad and Jones (1990), using intraspecific crosses of the *G. hirsutum* lines La RN-910, Auburn 612-RNR, and Deltapine 41 and generation means analysis found that RN resistance was inherited quantitatively and controlled by two or more pairs of genes. Avila et al. (2005, 2006) reported that a single additive-effect gene was responsible for the inheritance of RN resistance in an intraspecific F_2 population of *G. arboreum* using the resistance source A2-19. In addition, LaFoe (2005) observed a ratio of 9 resistant to 7 susceptible individuals in two F_2 populations of resistant *G. arboreum* accessions (A2-190 and A2-019) crossed with the susceptible accession A2-082 and concluded that at least two genes with partial dominance were likely involved in the inheritance of resistance to RN. The inheritance of resistance to RN has also been studied during the process of introgression of resistance to RN from selected *Gossypium* species. A single dominant gene has been identified as responsible for the inheritance of RN resistance obtained from the introgression of *G. longicalyx* into *G. hirsutum* (Robinson et al. 2007). In addition, dominant genes at two different loci have been identified as responsible for the inheritance of resistance to RN obtained from the introgression of *G. arboreum* and *G. aridum* (Rose & Standley) Skovsted (Sacks and Robinson 2007, 2009).

Simple sequence repeat (SSR) markers have been associated with genes conferring RN resistance. The introgression of RN resistance from *G. longicalyx* into *G. hirsutum* was facilitated by the discovery of tightly-linked associations between three SSR markers, BNL3279_114, BNL1066_156, BNL836_215, and one phenotypic marker, green-colored fuzz (Fzg^{lon}) with the RN resistance locus (Ren^{lon}) located on chromosome 11. Two germplasm lines, LONREN-1 and LONREN-2, have been developed and released with the proposal that these molecular markers could assist in selection (Dighe 2007; Robinson et al. 2007; Dighe et al. 2009). More recently, Romano et al. (2009), using progeny derived from a cross between a tri-species hybrid [*G. arboreum* × (*G. hirsutum* × *G. aridum*)^2] and MD51*ne* (*G. hirsutum*), found that two SSR markers, BNL3279_132 and BNL2662_090, were associated with RN resistance. This resistance locus was mapped to chromosome 21 and designated as Ren^{ari} since the SSR fragments associated with resistance were found in *G. aridum* and in the tri-species bridging line G371.

The objectives of the research presented here were to determine the inheritance of resistance to RN in the *G. barbadense* accession GB713, to identify SSR markers linked with QTLs for RN resistance, and to map these markers to specific chromosome(s) to facilitate marker-assisted selection (MAS).

Materials and methods

Plant materials

Accession GB713 PI 608139, a short-day primitive accession (RN-resistant) (Robinson et al. 2004) and the

G. hirsutum cultivar Acala Nem-X PI 590568 PVPO (RN-susceptible) were chosen as parents for the present study. Crosses between GB713 (P_1) and Acala Nem-X (P_2) were made by hand emasculation and pollination in greenhouses in the absence of natural pollinators. The F_1 plants were backcrossed to each of the two parents to produce the backcross generations (BC_1P_1 and BC_1P_2). The F_2 generation was obtained by selfing the F_1 plants.

Nematode resistance assay

Reniform nematode reproduction assays on seedlings were adapted from methods described in Robinson et al. (2004, 2007). Seeds from parents, F_1, BC_1P_1, BC_1P_2, and F_2 were scarified by nicking the seed coat, germinated in moistened, rolled, Whatman No. 3 chromatography paper at 30°C for 24 h and 22°C for an additional 24 h, and transplanted individually into 500 ml cups. Plants were grown in a controlled-environment chamber maintained at 30/26°C day/night with a 14-h photoperiod, 482 μmol m^{-2} s^{-1} photosynthetic photon flux, and humidity ≥50%. Plants were watered daily with water purified by reverse osmosis and were fertilized and sprayed to control insects as needed. The total number of plants evaluated in the growth chamber assays included 30 plants from each of the parents and F_1 generation, 300 plants from the F_2 generation, and 150 plants from each of the backcross generations (BC_1P_1 and BC_1P_2). Twelve additional plants of *G. hirsutum* cultivar Deltapine 16 PI 529251 and GB713 were used as susceptible and resistant controls, respectively. One-third of the plants of each generation was evaluated per experiment for three independent experiments. Data across the three experiments were pooled for analyses.

A RN population collected from Baton Rouge, LA, USA, was maintained on cotton and tomato plants grown in a silt loam soil in the greenhouse. Nematodes used as inoculum for assays were obtained from this soil via Baermann funnels, held at 30°C the night before inoculation (Robinson and Heald 1991), and gently aerated for 1–3 h before being introduced into pots. Nematodes were 95–99% motile. Seedlings were inoculated at 7 and 14 days after germination with a total of 4,000 nematodes per plant (2,000 per inoculation). Pots were randomized before inoculating, and nematodes were introduced into pots in an aqueous suspension by inserting a hypodermic syringe needle into the bottom of the pot halfway between the plant and the pot wall and injecting 0.6 ml as the needle was withdrawn. The needle tip was modified to force water to spray in all directions. Each pot was inoculated at ten equidistant spots corresponding to 6 ml of nematode suspension.

The inoculated pots were placed in the controlled-environment chamber in a randomized complete block array with one resistant (GB713) and one susceptible (Deltapine 16) control per block and held for 7 weeks, at which time three soil cores, extending from the top to the bottom of the pot and weighing ca 40 g total, were removed from each pot, weighed, and processed to extract nematodes. Nematodes were extracted by the Baermann funnel and counted. Nematode counts were taken from negative control pots containing either no plants or non-host plants at various times during the 7-week incubation to determine nematode survival, and to confirm that conditions within pots did not allow significant survival of the primary nematode inoculum that could be extracted at the end of the assay and thereby bias the reproduction estimates. Selected plants were retained for breeding by transplanting to 10 L pots containing RN-free potting medium.

SSR amplification and analysis

Young leaves from each of the parents, F_1, and F_2 generations were collected, lyophilized, ground to a fine powder, and stored at −20°C in capped vials. Genomic DNA was isolated from 20 mg (dry weight) of cotton leaf tissue as previously described (Gutiérrez et al. 2009). A total of 1,419 SSR primer pairs was used to screen the parents. Sequences for all SSR primers are available at http://www.cottonmarker.org/projects/. Bulk segregant analysis (Michelmore et al. 1991) was then carried out with SSR primers that showed polymorphism in the parental screening. Individual bulked DNA samples from P_1, P_2, F_1, 10 of the most resistant F_2, i.e., those F_2 showing the least amount of RN reproduction, and 10 of the most susceptible F_2 were prepared by combining 13.5 μg of DNA from each of the 10 individual plants. PCR reactions contained a total of 50 ng of bulked DNA as template where each of the 10 plants of the bulk contributed 5 ng DNA. Polymorphic SSR primers from the bulk segregant analysis were then run on the total F_2 population (300 plants). PCR parameters and capillary electrophoresis using the automated ABI PRISM 3130 XL Genetic Analyzer™ (Applied Biosystems, Foster City, CA, USA) were as described previously (Gutiérrez et al. 2009). Computer-assisted analysis of the data was performed with GeneMapper™ 4.0 software (Applied Biosystems, Foster City, CA, USA) using the default values for the local Southern method. All data were also visually examined to verify accuracy of calling of alleles by the software.

Chromosome assignments

Chromosomal locations of the polymorphic SSR markers associated with RN resistance in each population were

obtained by hypoaneuploid-based deletion analysis following the methods of Gutiérrez et al. (2009). The F_1 plants used to identify chromosomal locations of the polymorphic SSR markers were the monotelodisomes for 1*Lo*, 1*sh*, 2*Lo*, 2*sh*, 3*Lo*, 3*sh*, 4*Lo*, 4*sh*, 5*Lo*, 6*Lo*, 6*sh*, 7*Lo*, 7*sh*, 8*Lo*, 9*Lo*, 10*Lo*, 10*sh*, 11*Lo*, 11*sh*, 12*Lo*, 12*sh*, 14*Lo*, 15*Lo*, 16*Lo*, 16*sh*, 17*Lo*, 17*sh*, 18*Lo*, 18*sh*, 20*Lo*, 20*sh*, 21*sh*, 22*Lo*, 22*sh*, 25*Lo*, and 26*sh* and monosomes H1, H2, H3, H4, H6, H7, H9, H10, H11, H12, H16, H17, H18, H20, H21, H23, H25, and H26. In addition, to provide coverage of the genome outside that supplied by monosomic and monotelodisomic stocks we used two types of translocation-based segmental hypoaneuploids, including tertiary monosomics and segmental trisomic–monosomics, also known as duplication-deficiency. These interspecific segmental aneuploids NTN 4–5, NTN 4–15, NTN 6–14, NTN 7–11, NTN 10–19, Df11 Fr 11–13, Df11 Fr 11–15, NTN 12–11, NTN 12–19, Df13 FR 11–13, Df13–Dp19, NTN 16–15, NTN 17–11, Df19 FR 8–19, Df19 FR 13–19, and Df21L Dup 19L were also derived from crosses between hypoaneuploid *G. hirsutum* parents and *G. barbadense*, *G. tomentosum* Nutall ex Seemann, and/or *G. mustelinum* Watt. All these cytogenetic stocks were kindly provided by Dr. David M. Stelly of Texas A & M University. Finally, the confirmation of the markers was done using logical patterns of absence/presence of contiguous blocks of markers ordered by previous linkage mapping, primarily according to Nguyen et al. (2004) available at the Cotton Microsatellite database (http://www.cottonmarker.org/cmap/).

Statistical analyses

We calculated reproduction based on RN larva in soil cores and expressed the data as RN g^{-1} soil. Robinson et al. (2007) reported that across six genotypes, including experimental controls, the population variance was linearly related to the mean, suggesting that a log transformation should be properly used on the nematode data. Following their research, we transformed the data in two ways. One was to express reproduction as a ratio of susceptible parent, Acala Nem-X, and the other was to express reproduction as $[\log_{10}(X + 1)]$, (Noe 1985). These data were used for the analysis of variance using the PROC MIXED procedure (SAS Institute, 2003). Experiments were considered random effects and generations were considered fixed effects. In addition, a weighted least square analysis (Rowe and Alexander 1980), using the PROC REG procedure (SAS Institute, 2003), was used to estimate genetic effects using Hayman's generation means analysis (Hayman 1958, 1960). In addition, five estimates of the minimum number of genes (n_1, n_2, n_3, n_4, and M) involved in the inheritance of resistance to RN were calculated following the methodology described by McPherson et al. (2004). Parental means (P_1 and P_2) and the segregating genetic variance estimates (S_s^2) were used in the following formulas developed by (1) Castle (1921), (2–5) Lande (1981), and (6–7) Cockerham (1986).

The Castle–Wright formula is Eq. 1:

$$n_x = (P_1 - P_2)^2 * (8s_{sx}^2)^{-1} \quad (1)$$

Lande (1981) provided four equations to calculate segregating genetic variance (S_s^2) of the Castle–Wright formula shown in Eqs. (2–5).

$$S_{S_1}^2 = S_{F_2}^2 - S_{F_1}^2 \quad (2)$$

$$S_{S_2}^2 = S_{F_2}^2 - \left(0.5S_{SF_1}^2 + 0.25S_{SP_1}^2 + 0.25S_{SP_2}^2\right) \quad (3)$$

$$S_{S_3}^2 = 2S_{F_2}^2 - S_{BC_1P_1}^2 - S_{BC_1P_2}^2 \quad (4)$$

$$S_{S_4}^2 = \left(S_{BC_1P_1}^2 + S_{BC_1P_2}^2\right) - \left(S_{F_1}^2 + 0.5S_{P_1}^2 + 0.5S_{P_2}^2\right) \quad (5)$$

Cockerham (1986) provided another estimate for gene number as shown in Eqs. 6 and 7.

$$M = [(P_1 - P_2)^2 - (S_{P_1}^2 N^{-1} + S_{P_2}^2 N^{-1})] * (8S_{S_5}^2)^{-1} \quad (6)$$

where N = number of plants and

$$S_{S_5}^2 = 0.2\,(4S_{F_2}^2 + S_{BC_1P_1}^2 + S_{BC_1P_2}^2) - 0.4\,(S_{P_1}^2 + S_{P_2}^2 + S_{F_1}^2) \quad (7)$$

The nematode count data were expressed as percentage of reproduction on Acala Nem-X and number of RN g^{-1} soil and each was transformed to $[\log_{10}(x + 1)]$ and used for estimating gene number.

Linkage maps were constructed using JoinMap 4.0 (Van Ooijen 2006) using the 300 plant F_2 data classification scheme and JoinMap 4.0 default settings except as noted below. Linkage map calculations were performed using the raw segregation marker data, and the Kosambi mapping function (Kosambi 1944) was used to convert recombination units into genetic distances. A LOD grouping threshold minimum of 2 and maximum of 10 with a step of 1 was used to calculate the linkage groups. A LOD of 4.0 was the minimum value used to select the linkage groups.

QTL analyses were performed using Windows QTL Cartographer 2.5 (Wang et al. 2007). Single marker analyses, interval mapping, and composite interval mapping were used to identify QTLs associated with RN resistance. Phenotype data (expressed as percentage of reproduction on Acala Nem-X, RN g^{-1} soil, and log RN g^{-1} soil) from 300 F_2 plants were used for QTL/marker association detection. In the composite interval mapping (CIM) method the option model 6 and forward and backward regression methods were used. One thousand permutations were used to estimate the threshold log of the likelihood ratio score with a significance level of $P = 0.05$. In the

CIM analysis, QTL peaks were selected when they had a minimum log of the likelihood ratio of 2.5 and a minimum distance of 5 cM.

Polymorphic SSR loci were named conventionally, i.e., by primer pair designation with the addition of the fragment size that corresponded to the resistant parent (GB713) in the cross, e.g., BNL3279_106.

Results

Phenotypic evaluation of the generations

The data collected was RN reproduction on individual plants of each population. Because these are RN count data, it is appropriate to transform the data to $\log_{10}(X + 1)$. There were significant differences among the means of the generations (Fig. 1). Individual generation population data are graphed in Fig. 2a–f, where the *x*-axis is scaled linearly; however, the *x*-axis data are in logs and the *y*-axis data are the number of plants. The means of the F_1 and F_2 populations were about equal and strongly skewed toward the resistant parent (Fig. 2c, d). In addition, the mean value of the backcross to the resistant parent (BC_1P_1) was skewed toward the resistant parent (P_1) (Fig. 2a, e). In contrast, the mean of the backcross to the susceptible parent (BC_1P_2) was skewed toward the susceptible parent (P_2) (Fig. 2b, f). These results suggest that the inheritance of the resistance to RN in the GB713 × Acala Nem-X cross may be characterized as partial dominance. Results from previous studies with *G. arboreum* indicated similar gene action (LaFoe 2005).

In partitioning the generation mean squares in the generation means analysis, only additive effects were significant ($P = 0.01$), and R^2 values ranged from 0.83 to 0.93. Estimates of the minimum number of genes involved in the resistance to RN suggested that one or more genes are involved (Table 1).

Polymorphism evaluation

We used a total of 1,419 SSR primer pairs distributed throughout the cotton genome to screen for polymorphisms between the parental genotypes. Bulk segregant analysis identified 137 primers that showed some association with RN resistance. The 137 polymorphic primers yielded 164 loci, which mapped to 17 linkage groups that included chromosomes 2, 3*sh*, 4, 7, 8, 9*Lo*, 10, 11*sh*, 11*Lo*, 17, 18, 18*Lo*, 20, 20*Lo*, 21, 22, and 24. These 137 primers were subsequently run against all 300 F_2 plants. The majority of these primers were not highly associated with resistance when genotyped over the 300 plants.

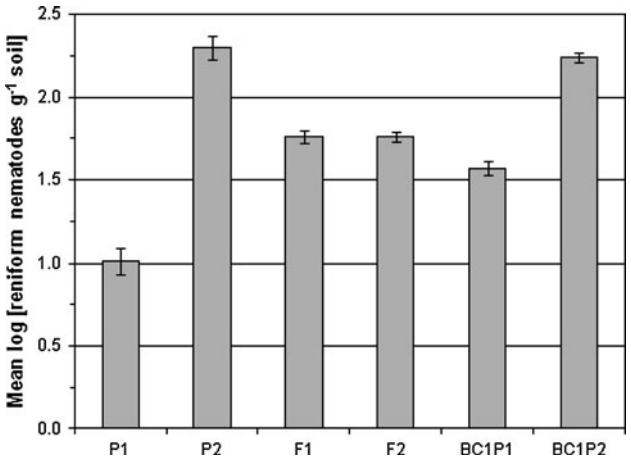

Fig. 1 Mean ± SE of parents GB713 (P_1) and Acala Nem-X (P_2), F_1, and segregating generations (F_2, BC_1P_1, and BC_1P_2) in log RN g^{-1} soil

SSR markers associated with RN resistance

Single marker analyses of variances

The association of each marker with RN reproduction was tested separately by a one-way analysis of variance. BNL3649_176, BNL1551_162, GH132_199, BNL4011_155, BNL3279_106, GH288_133, STV067_105, NAU2152_224, GH561_75, DPL475_171, CIR316ND_184, BNL3402_202, CIR069_246, TMB0294_200, and CIR112-229, all located in the long arm of chromosome 21, between 153.4 and 219.0 cM (Fig. 3) were significantly associated with RN resistance (LOD > 2.5). Marker BNL 3279_106 allele at location 184.5 on chromosome 21 showed the highest R^2 values and explained 37, 29, and 20% of the variation expressed as percentage of Acala Nem-X, RN g^{-1} soil, and log RN g^{-1} soil, respectively (data not shown).

WinQTL Cartographer composite interval mapping

The WinQTL Cartographer composite interval mapping results for each of the expressions of resistance indicated that two major QTLs located on chromosome 21 and one minor QTL located on chromosome 18 were associated with RN resistance (Table 2, Figs. 4, 5). The chromosome 21 map had 61 markers (Fig. 3) covering 219 cM and a major QTL was identified at map position 168.2 (LOD > 13.8, R^2 0.09–0.15) and flanked by SSR markers BNL 1551_162 and GH 132_199 (Fig. 3). A second major QTL, also on chromosome 21, was identified at map position 182.7 (LOD > 16.7, R^2 0.12–0.17) and flanked by SSR markers BNL 4011_155 and BNL 3279_106 (Fig. 3). In addition, the chromosome 18 map had 9 markers covering 81.2 cM (Fig. 3), and a third minor QTL was identified at map position 42.0 (LOD > 3.1, R^2 0.03–0.06) and

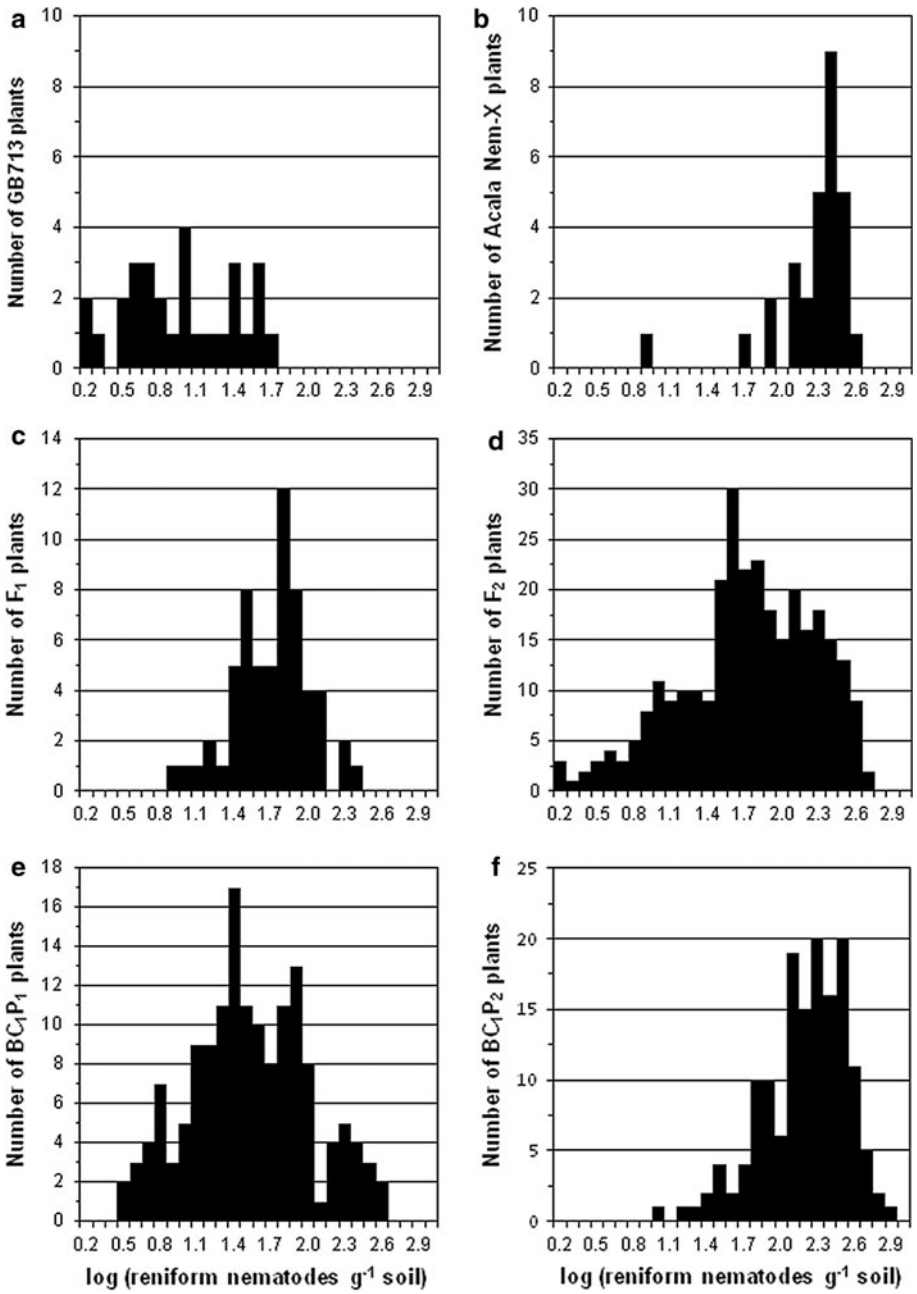

Fig. 2 Frequency distribution of the log RN g^{-1} soil of the parents **a** GB713 (P_1), **b** Acala Nem-X (P_2), **c** the F_1, and the segregating generations, **d** F_2, **e** BC_1P_1 and **f** BC_1P_2

Table 1 Estimated minimum number of genes involved in the resistance to reniform nematode in *Gossypium barbadense* accession GB713

Estimates	Log RN g^{-1}soil
n_1	1.07
n_2	1.15
n_3	0.96
n_4	1.43
M	1.23

n_1, n_2, n_3 and n_4 were calculated using the formulas developed by Lande (1981), and M was estimated using the method developed by Cockerham (1986) Equations in "Materials and methods"

flanked by markers BNL 1721_178 and BNL 569_131 (Fig. 3). Large significant additive and dominance effects for each of the QTLs were found except for the log-transformed data. The lack of significant dominance genetic effects, when the data were analyzed as log RN g^{-1} soil, is a scale effect due to the log transformation (Table 2). Significant reductions in reproduction were attributed to each of the three QTLs. The negative effects indicate a reduction in reproduction for the QTL alleles from GB713. As expected, the LOD scores using count data expressed in three ways all agreed on QTL locations. The region flanking the two QTLs on chromosome 21 covered 30.3 cM; however, the QTL at 168.2 is flanked by

Fig. 3 Genetic linkage map of chromosomes *21* and *18* resulting from 300 F_2 plants of the cross GB713 × Acala Nem-X

markers 23 cM apart and the QTL at 182.7 is flanked by markers 3.9 cM apart (Fig. 3). In each case, the QTL is about equidistant from its two flanking markers. The QTL on chromosome 18 is flanked by markers 15.3 cM apart; however, the estimated QTL position is within 0.9 cM of marker BNL 569_131 (Fig. 3). Thus, one should be able to select for the three QTLs using the SSR markers identified in this research. Finally, we suggest the designation of the GB713 genes as *Ren*^{barb1}, *Ren*^{barb2}, and *Ren*^{barb3} for the genes located at 168.2 and 182.7 on chromosome 21 and at 41.4 on chromosome 18, respectively.

Discussion

Muhammad and Jones (1990) reported that RN resistance was "inherited in a quantitative manner and controlled by two or more genes" when using intraspecific crosses of *G. hirsutum* lines La RN-910, Auburn 612-RNR, M019-RNR and Deltapine 41 with generation means analysis model. In research conducted in our laboratory, LaFoe (2005), utilizing crosses of resistant and susceptible *G. arboreum*, reported that the F_1 generation was skewed toward the resistant parent and concluded that partial dominance and probably more than one gene was involved in the inheritance of resistance to RN in *G. arboreum.*

Romano et al. (2009) reported that BNL 3279_132 and BNL 4011_133 were associated with resistance at a locus they designated as *Ren*^{ari} in reference to a *G. aridum* bridge line used in the construction of a tri-species hybrid. The authors also reported that BNL 3279_132 and BNL 4011_133 were located on chromosome 21 and that a 67 and 63% probability existed that plants possessing these markers in their population would score in the resistant class (Romano et al. 2009). Our results show that these same two primer pairs but amplifying fragments of a different size than reported by Romano et al. (2009), are associated with one of the QTLs (*Ren*^{barb2}) on chromosome 21 for resistance derived from GB713. Our associations of markers with QTLs from GB713, however, are considerably stronger than the marker associations detected for the *Ren*^{ari} locus (Romano et al. 2009). Dighe et al. (2009) and Robinson et al. (2007) also found BNL 3279_114 but on chromosome 11 and tightly associated with the *Ren*^{lon} resistance from *G. longicalyx.*

A region associated with both root-knot nematode [*M. incognita* (Kofoid & White) Chitwood] and RN resistance has been identified on chromosome 11 (Robinson et al. 2007; Dighe et al. 2009). On the other hand, Romano et al. (2009) found association between alleles located on chromosome 21 and RN resistance. In both cases, the SSR primer pair, BNL3279, was responsible for the amplification of alleles associated with RN resistance. It is interesting that the marker BNL 3279 with different fragment lengths mapping to different chromosomes has been associated with QTL for RN resistance from the *G. longicalyx, G. aridum,* and our *G. barbadense* source. This suggests that there may be a common ancestral source of RN resistance for each of these species. Our findings of three QTLs in GB713, on two different chromosomes suggest that the RN resistance in GB713 may be significantly different from the other two sources, yet have some similarities. Since the chromosomes 11 and 21 are homeologous, and BNL 3279 is associated with introgression of *Ren*^{barb1}, *Ren*^{barb2}, and *Ren*^{ari} on chromosome 21 and introgression of *Ren*^{long} on chromosome 11, a reasonable assumption is

Table 2 Quantitative trait loci calculated with composite interval mapping for RN reproduction expressed as percentage of Acala Nem-X, RN g^{-1} soil, and log RN g^{-1} soil

Chromosome and QTL	Flanking marker marker position		Map position of QTL	LOD score	Additive effects	Dominance effects	R^2
Expressed as percentage of reproduction on Acala Nem-X							
21*Ren*^{barb1}	BNL 1551_162	154.2	168.4	39.7	−21.31	−22.55	0.12
	GH 132_199	177.3					
21*Ren*^{barb2}	BNL 4011_155	180.6	182.7	34.1	−23.82	−17.98	0.16
	BNL 3279_106	184.5					
18*Ren*^{barb3}	BNL 1721_178	27.6	41.4	5.0	−13.80	2.12	0.05
	BNL 569_131	42.9					
Expressed as RN g^{-1} soil							
21*Ren*^{barb1}	BNL 1551_162	154.2	168.6	28.0	−49.98	−60.78	0.09
	GH 132_199	177.3					
21*Ren*^{barb2}	GH 4011_155	180.6	182.7	24.6	−57.42	−42.35	0.12
	BNL 3279_106	184.5					
18*Ren*^{barb3}	BNL 1721_178	27.6	39.6	4.0	−29.45	−2.93	0.03
	BNL 569_131	42.9					
Expressed as log RN g^{-1} soil							
21*Ren*^{barb1}	BNL 1551_162	154.2	173.5	13.8	−0.31	−0.03	0.15
	GH 132_199	177.3					
21*Ren*^{barb2}	GH 4011_155	180.6	184.0	16.6	−0.32	−0.02	0.17
	BNL 3279_106	184.5					
18*Ren*^{barb3}	BNL 1721_178	27.6	42.0	3.1	−0.20	0.01	0.06
	BNL 569_131	42.9					

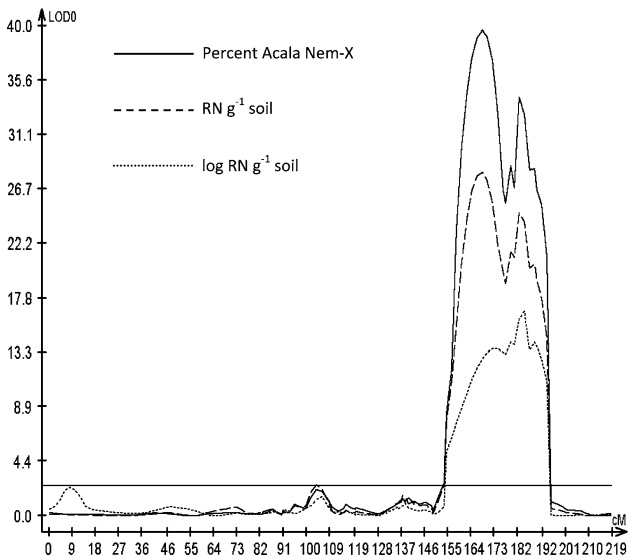

Fig. 4 QTL regions associated with RN resistance for the chromosome 21 linkage group as determined using 300 F_2 plants from the cross GB713 × Acala Nem-X based on reproduction expressed as percentage of Acala Nem-X, RN g^{-1}soil and log RN g^{-1}soil and obtained by composite interval mapping using WinQTL Cartographer 2.5

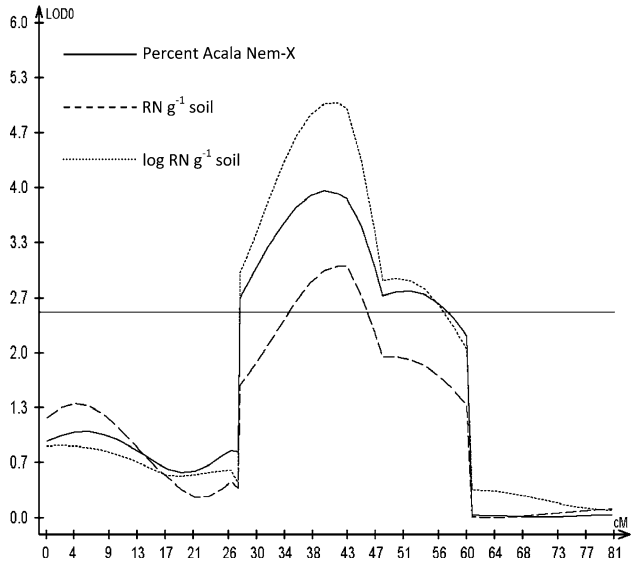

Fig. 5 QTL region associated with RN resistance for the chromosome 18 linkage group as determined using 300 F_2 plants from the cross GB713 × Acala Nem-X based on reproduction expressed as percentage of Acala Nem-X, RN g^{-1}soil, and log RN g^{-1}soil and obtained by composite interval mapping using WinQTL Cartographer 2.5

that these data may be detecting the action of similar or homeologous genes. However, the detection of the involvement of three QTLs in the GB713 and not in the others could indicate new additional genes for resistance in GB713. These genes are clearly associated with RN resistance in this population and the relationship of the

QTLs in GB713 to the other sources of resistance is undefined at this time. Only a comparison of the sequences of each allele among the different sources can fully answer this question.

In our present study, a dense linkage map of chromosome 21 with 61 loci has been constructed and a major region associated with RN resistance in GB713 has been detected. SSR loci BNL1551_162, GH 132_199, BNL4011_155, and BNL3279_106 on chromosome 21 and BNL 1721_178 and BNL 569_131 on chromosome 18 have been found to be significantly associated with three QTLs for RN resistance. This chromosome 21 region of approximately 30 cM contains two QTLs that have a major association with RN resistance in GB713. In addition, a 15 cM region on chromosome 18 also has an association with RN resistance in GB713, but with a much lower LOD and smaller genetic effects. Fine-mapping of these two areas with SSR or SNP markers should be pursued in order to obtain markers that are even more closely associated with the three QTLs in GB713. Detailed fine-mapping may also provide information related to the resistance reported in *G. aridum* since it is reported to involve a related region on chromosome 21.

GB713 provides an excellent additional source of resistance to RN. Since this resistance source is *G. barbadense*, which is a cultivated tetraploid species, it should be more readily compatible with Upland cotton than the diploid sources in *G. arboreum, G. aridum,* and *G. longicalyx*. SSR loci BNL1551_162, GH 132_199, BNL4011_155, and BNL3279_106 on chromosome 21 and BNL 561_131 on chromosome 18 should be helpful to plant breeders for the selection of plants with high levels of RN resistance in segregating populations that have used GB713 as a source of RN resistance. This study offers effective markers that can be used to select for RN resistance genes in GB713 on both chromosomes 21 and 18 in applied breeding programs since these markers have shown polymorphism across several *G. hirsutum* lines and GB713.

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Conflict of interest None.

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