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ORIGINAL PAPER

SSR markers closely associated with genes for resistance to root-knot nematode on chromosomes 11 and 14 of Upland cotton

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Abstract Molecular markers closely linked to genes that confer a high level of resistance to root-knot nematode (RKN) [*Meloidogyne incognita* (Kofoid & White) Chitwood] in cotton (*Gossypium hirsutum* L.) germplasm derived from Auburn 623 RNR would greatly facilitate cotton breeding programs. Our objectives were to identify simple sequence repeat (SSR) markers linked to RKN resistance quantitative trait loci (QTL) and map these markers to specific chromosomes. We developed three recombinant inbred line (RIL) populations by single seed descent from the crosses of RKN-resistant parents M-240 RNR (M240), developed from the Auburn 623 RNR source, moderately resistant Clevewilt 6 (CLW6), one of the parents of Auburn 623 RNR, and susceptible parent Stoneville 213 (ST213). These crosses were CLW6 × ST213, M240 × CLW6, and M240 × ST213. RILs from these populations were grown under greenhouse conditions, inoculated with RKN eggs, scored for root gall index, eggs plant^{-1}, and eggs g^{-1} root. Plants were also genotyped with SSR markers. Results indicated that a minimum of two major genes were involved in the RKN resistance of M240. One gene was localized to chromosome 11 and linked to the marker CIR 316-201. This CIR 316-201 allele was also present in CLW6 but not in Mexico Wild (MW) (PI593649), both of which are parents of Auburn 623 RNR. A second RKN resistance gene was localized to the short arm of chromosome 14 and was linked to the SSR markers BNL3545-118 and BNL3661-185. These two marker alleles were not present in CLW6 but were present in MW. Our data also suggest that the chromosome 11 resistance QTL primarily affects root galling while the QTL on chromosome 14 mediates reduced RKN egg production. The SSRs identified in this study should be useful to select plants with high levels of RKN resistance in segregating populations derived from Auburn 623 RNR.

Abbreviations

cM	Centimorgans
MAS	Marker-assisted selection
SSR	Simple sequence repeats
RKN	Root-knot nematode
RIL	Recombinant inbred line
RGI	Root galling index
QTL	Quantitative trait loci
CLW6	Clevewilt 6
ST213	Stoneville 213
M240	M-240 RNR
MW	Mexico wild
J_2	Second-stage juvenile nematode

Introduction

The root-knot nematode (RKN), [*Meloidogyne incognita* (Kofoid & White) Chitwood], is a major pest across U.S.

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cotton (*Gossypium* spp.) production areas (Koenning et al. 2004). The destructive process starts when second-stage juvenile nematodes (J_2) hatch from eggs in the soil and penetrate the cotton roots. Ultimately, the J_2 establishes a feeding site near the vascular cylinder comprised of multinucleated 'giant cells'. It is from this feeding site that the J_2 derives the nutrients required to complete its development (Williamson and Hussey 1996). RKN resistance in cotton has been discovered in exotic germplasm (Miles 1939; Smith 1954; Wiles 1957; Jones et al. 1958; Turcotte et al. 1963; Shepherd 1974a, b, 1982, 1983a; Shepherd et al. 1988; Cook et al. 1997; Robinson and Percival 1997; Robinson et al. 1999, 2004) and is currently available to producers in the cultivars Stoneville LA887 (Jones et al. 1991), Acala Nem-X (Oakley 1995a, b); Paymaster 1560 (Boethel 1997), Stoneville 5599 BR (McCall 2003), and DP174 RF (Santos et al. 2009); however, these cultivars only have moderate levels of resistance to RKN. The source of resistance for the first three cultivars can be traced back to Clevewilt 6 (CLW6) (Jones et al. 1991; Myers et al. 2007). The resistance of CLW6 to RKN was first observed by L. E. Miles in Mississippi in 1938 during the evaluation of cotton varieties in an RKN- and Fusarium wilt-infested field (Miles 1939). The origin of the Acala Nem-X source of resistance to RKN is the germplasm N6072 (Hyer et al. 1978, 1979; Oakley 1995b; Bowman et al. 2006). N6072 came from a cross of FBCX-2, a Missouri breeding line, and AXTE 22, a California breeding line. "The FBCX-2 resistant parent was from the F_2 generation of a diallel cross of five lines derived from germplasm that had in its parentage Sea Island Seabrook 12-B2, Hopi Acala, triple Hybrid (*Gossypium arboreum-thurberi-hirsutum)*, Early Fluff 310 and Auburn 56" (Hyer et al. 1978). It has not been determined if Acala Nem-X and CLW6 carry the same RKN resistance alleles; however, the resistance-linked marker CIR 316-201 allele is present in both lines (Shen et al. 2006; Wang et al. 2006; Niu et al. 2007), which suggests the resistance gene present in Acala Nem-X is on the same chromosome as in CLW6 or may be an allele thereof. In support of this idea, Zhou (1999) reported that the same single recessive resistance gene controlled the resistance to RKN in Acala Nem-X and LA887.

RKN resistance can be classified as moderately resistant, e.g., CLW6 and Acala Nem-X, or highly resistant, e.g., Auburn 623 RNR. Auburn 623 RNR originated as a transgressive segregant in a cross of CLW6 with Mexico wild (MW) (PI 593649) and was the first highly resistant germplasm line developed (Shepherd 1974b). MW was collected by James L. Stulb in the interior of Mexico in 1950 (Jones 1998). This accession was first tested in the spring of 1951 under field nursery conditions by F. Self but, due to photoperiodic sensitivity and late emergence, MW was not determined to be RKN-resistant until a later study (Jones et al. 1958; Jones 1998). Auburn 623 RNR resistance was later transferred to germplasm with better agronomic characteristics by backcrossing (Shepherd 1982; Shepherd et al. 1996).

Experiments to determine the mode of inheritance of the different levels of RKN resistance have been conducted by several independent laboratories. In an early study done by Jones et al. (1958) with F_2 populations and F_3 lines of the cross Deltapine 15 × CLW6, they reported that RKN resistance was inherited as a quantitative trait that was influenced by the environment and controlled by at least two genes. Later, it was shown that moderate resistance to RKN is transmitted as a recessive gene (Robinson 1998; Zhou et al. 1999; Bezawada et al. 2003; Wang et al. 2006). Zhou (1999) showed that resistance in Acala Nem-X and LA887 was due to the same recessive gene in each line. In contrast, the inheritance of the resistance present in Auburn 623 RNR has been reported to be partially dominant and of a polygenic nature (Shepherd 1974a). McPherson et al. (1995) reported two major genes, one dominant and one additive, controlled RKN resistance in the Auburn 623 RNR-derived line M-315 RNR and in the day-neutral converted primitive resistant lines M19-RNR, M25-RNR, M75-RNR, M78-RNR, M188-RNR, and M487-RNR. These two genes were subsequently named Mi_1 (dominant) and Mi_2 (additive) (McPherson et al. 2004). Zhou (1999) and Ynturi et al. (2006), using germplasm derived from Auburn 623 RNR, also reported a two-gene model. Zhou (1999) also reported that two genes were involved in the inheritance of RKN resistance in the cross M-240 RNR (M240) × Deltapine 90; one gene was partially dominant and the other was recessive. Also, Ynturi (2005) and Ynturi et al. (2006) reported that at least one gene on chromosome 11 and one on chromosome 14 were involved in the RKN resistance exhibited by M240 which is derived from Auburn 623 RNR. Zhang et al. (2007), using both Auburn 623 RNR-derived material and Acala Nem-X, also concluded that the minimum number of genes was two; however, they indicated that the Auburn 623 RNR and Acala Nem-X resistance sources were controlled by additive genetic effects.

Research groups in California and Georgia, working with the Acala Nem-X and Auburn 623 RNR sources, i.e., M-120 RNR, reported that the SSR marker CIR316 was associated with RKN resistance and is located on chromosome 11 (Shen et al. 2006; Wang et al. 2006). In addition, major genes responsible for RKN resistance in Auburn 634 RNR have been located on chromosomes 11 and 14. The chromosome 11 SSR marker BNL1231 and the chromosome 14 short (14sh) markers BNL3661, BNL3644, and BNL3545 have also shown association with RKN resistance (Ynturi et al. 2006). Niu et al. (2007)

identified two random amplified polymorphic DNA markers and one sequence-tagged-site marker associated with RKN resistance located on chromosome 11.

The identification of molecular markers associated with nematode resistance in cotton is routinely plagued by difficulties in accurately assigning susceptible or resistant phenotypes to F_2 segregants. This has confounded attempts to determine RKN resistance inheritance in Upland cotton. In addition, only four types of inter- and intra-specific populations, i.e., F_1, F_2, backcross, and recombinant inbred lines (RILs), have been used in cotton to study the genetics of RKN resistance; however, no intra-specific RILs using the Auburn 623 RNR source have yet been used to evaluate the association of DNA markers with RKN resistance. Unlike F_2 or backcross mapping populations, a RIL population consists of a number of RILs which are highly homozygous and can be used to conduct replicated studies in different environments by different researchers at different times (Bailey 1981; Burr et al. 1988; Burr and Burr 1991); consequently, the use of RIL populations permits a more precise assessment of the phenotype of interest. This experimental advantage offered by the use of RILs can be used to offset the difficulty in accurately scoring individual plants for RKN resistance in segregating populations in the field.

The objectives of the work presented here were: (1) to identify SSR markers linked with RKN resistance genes in lines derived from the Auburn 623 RNR source using three intra-specific RIL populations and (2) to map these markers to specific chromosomes. To meet these objectives, we generated RIL populations where recombinant genotypes could be evaluated for RKN resistance in replicated experiments, or several plants could be used to evaluate an individual RIL. In developing these RIL populations, we made the following assumptions: (1) M240 contains both the CLW6 and the MW source of resistance because it is derived from Auburn 623 RNR, (2) CLW6 only contains the CLW6 source of resistance, and (3) ST213 does not contain either sources of resistance. If our assumptions are correct, the following gene segregations will be observed: M240 × CLW6 should segregate only for the gene from MW; CLW6 × ST213 should segregate only for the CLW6 gene; and M240 × ST213 should segregate for both genes. Thus, we should have RILs with genes from each parent individually and RILs with genes from both parental sources.

Materials and methods

Plant materials

CLW6, moderately resistant, ST213, susceptible, and M240, highly resistant, were chosen as parents for the present study. The three parental genotypes were grown at the Plant Science Research Center (33.4°N, 88.8°W), Mississippi State, MS, USA in the summer of 1998 and the crosses M240 × CLW6, CLW6 × ST213, and M240 × ST213 were made. F_1 plants from each of the crosses were grown and self-pollinated at a winter nursery in Tecomán, Colima, Mexico during the 1998–1999 season to obtain F_2 seed. Following the methods of Brim (1966), F_2 seeds from all three crosses were planted under greenhouse conditions and advanced by single seed descent until they reached the F_6 generation. A total of 97, 78, and 114 F_6 families were obtained for the crosses M240 × CLW6, CLW6 × ST213, and M240 × ST213, respectively.

Greenhouse experiments

A modified RKN screening methodology developed by Shepherd (1979) was used to evaluate the three RIL populations in the greenhouse which were assayed in three separate experiments. Five plants from each F_6 family of the three RIL populations as well as five plants of the parental lines M240, CLW6, and ST213 were planted and grown in 8.9 × 7.6 cm (diameter × depth) plastic pots containing screened, heat sterilized, Wickham sandy loam soil (fine-loamy, mixed, semiactive, thermic Typic Hapludults). RKN race 3 eggs were obtained by soaking infected tomato and cotton plant roots in a 1.05% NaOCl solution on a mechanical shaker for 3 min (Hussey and Barker 1973). Seven days before planting, each pot was inoculated with approximately 10,000 RKN eggs and the bed covered with sequential layers of clear plastic, brown paper, and aluminum foil to induce hatching and dispersal of the J_2 into the soil. For the cross M240 × CLW6, 69 of the 97 RILs were tested for RKN resistance because they were homozygous for the SSR marker CIR316-201 which had been reported to be associated with RKN resistance and had been detected in all RKN-resistant material tested (Shen et al. 2006; Wang et al. 2006; Zhang et al. 2007). An RKN-susceptible cultivar, Fibermax 832 (FM832) (Okra leaf), also was planted in each one of the previously described pots and its root galling index (RGI) was used to determine if the inoculation was successful for each pot. Forty-two days after inoculation, the plants were labeled, cut approximately 5 cm from the soil line, and the soil removed from the roots by gentle washing with water. The washed roots of each RIL plant were placed in a plastic cup filled with water for RGI evaluation. An RGI was determined for each RIL and susceptible check plant by three separate people and the mean RGI of the three individual scorers used for analyses. The RGI consisted of a 1–5 scale: 1 = plants with no galls or very few galls, 2 = plants with a few small sized galls, 3 = plants with a moderate number of medium sized galls, 4 = plants with

many large sized galls, and 5 = all or most of the roots covered with large galls (Shepherd 1983b). A plant was considered resistant when its RGI was less than 2.5, moderately resistant when the RGI was more than 2.5 but less than 3.5, and susceptible when its RGI was greater than 3.5. The number of eggs plant^{-1} was obtained by bulking the roots from the five RIL plants and soaking them in 1.05% NaOCl as described above. Eggs were collected on a 25 μM mesh sieve, thoroughly rinsed with water, and transferred to 50 mL vials. Dilution counting was used to estimate the number of eggs and the resulting counts were used to calculate the number of eggs plant^{-1}. The fresh weight (g) of roots was determined for each of the five plants of each RIL and the average weight used to calculate number of eggs g^{-1} root. Eggs plant^{-1} and eggs g^{-1} root data were transformed to $\log_{10}(x + 1)$ to obtain a normal frequency distribution and remove the dependence of the variance on the mean (Noe 1985).

Genomic DNA extraction

Leaf tissues were lyophilized, ground to a fine powder, and stored at −20°C in capped vials. DNA was isolated from 20 mg (dry weight) of cotton leaf tissue using the Mixer Mill MM 300 (Qiagen, Santa Clarita, CA, USA) for tissue disruption and the DNeasy Plant Mini Kit (Qiagen, Santa Clarita, CA, USA) following the manufacturer's protocol with minor modifications. Sodium metabisulfite was added to the lysis buffer at a concentration of 10 mM (Horne et al. 2004) from a freshly prepared 1 M stock solution. In addition, the lysis incubation time at 65°C was increased to 45 min. Because the three RIL populations were at the F_6 generation and had reached a high degree of homozygosity, leaves from one arbitrarily selected plant for each RIL were used for DNA extraction.

SSR amplification and analysis

A total of 1,419 SSR primer sets were used to screen M240, CLW6, and ST213 for polymorphisms. Only markers that were polymorphic between the parents of each RIL population were run and scored against the individual RIL. The sequences for all SSR primers (BNL, CM, JESPR, CIR, MGHES, TMB, NAU, DPL, GH, MUCS, MUSB, MUSS, and STV) used in this study are available at http://www.cottonmarker.org/projects/.

PCR amplification was performed as described in Gutiérrez et al. (2009). Capillary electrophoresis was performed using the automated ABI PRISM 3130XL Genetic Analyzer™ (Applied Biosystems, Foster City, CA, USA). 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.

Chromosome assignments

Chromosomal locations of the polymorphic SSR markers associated with RKN resistance in each of the RIL populations were confirmed or obtained by hypoaneuploid-based deletion analysis following the methods of Gutiérrez et al. (2009). In addition, the confirmation of the markers was done by 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

Single-factor analyses of variance were performed using SAS Proc GLM (SAS Institute 2003) to estimate additive effects associated with each marker (Edwards et al. 1987). Markers that had significant individual effects for RKN resistance were then incorporated sequentially into multiple marker models using SAS Proc GLM. For example, the marker with highest significance was entered into the multiple marker model first, followed by the locus with second-highest significance, and so forth. At each step, the significance of each locus was re-tested based on Type III sums of squares. Any loci not significant at $P < 0.05$ in the multiple locus models were eliminated from the model. This process continued until no additional markers could be added that remained significant in the multiple marker model, resulting in a final model. In addition, each significant marker locus was tested for epistatic interactions with all other significant marker loci using SAS Proc GLM (Holland 1998). Standard errors of the means in the multiple marker model were calculated using SAS Proc GLIMMIX (SAS Institute 2003).

Linkage maps were constructed using JoinMap 4.0 (Van Ooijen 2006) using the RIX data classification scheme and JoinMap 4.0 default settings except as noted below. Linkage map calculations were performed using the raw segregation 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 MapQTL5 (Van Ooijen 2004). QTL mapping was done using simple interval mapping and the 99% threshold level for calling QTL was determined by permutation tests (3,000 permutations in each case). Finally, single polymorphic SSR loci were named

Table 1 Mean phenotypic values of three recombinant inbred line (RIL) populations and corresponding susceptible check FM832 evaluated for root-knot nematode resistance

	M240 × CLW6 RILs ($n = 69$)					Susceptible check FM832				
	Mean	SE	Minimum	Maximum	Skew	Mean	SE	Minimum	Maximum	Skew
Root gall index	2.32	0.54	1.00	4.00	0.22	4.76	0.29	3.80	5.00	−1.06
log eggs plant^{-1}	3.66 (4,603)[a]	0.56	2.15 (140)	4.62 (41,300)	−0.89	4.87 (74,764)	0.18	4.61 (41,040)	5.12 (134,840)	0.05
log eggs g^{-1} root	3.32 (2,100)	0.54	1.83 (68)	4.40 (25,000)	−0.54	4.63 (44.388)	0.23	4.23 (16,903)	4.87 (74,463)	−0.87
	CLW6 × ST213 RILs ($n = 78$)					Susceptible check FM832				
	Mean	SE	Minimum	Maximum	Skew	Mean	SE	Minimum	Maximum	Skew
Root gall index	3.42	0.70	2.00	4.80	0.010	4.54	0.40	3.80	5.00	−0.24
log eggs plant^{-1}	4.06 (11,470)	0.50	2.10 (125)	5.11 (131,701)	−0.79	4.67 (47,227)	0.46	4.22 (16,702)	5.63 (428,350)	1.89
log eggs g^{-1} root	3.86 (7,221)	0.47	2.21 (161)	4.79 (61,972)	−0.57	4.56 (36,269)	0.43	4.12 (13,227)	5.46 (288,452)	1.81
	M240 × ST213 RILs ($n = 114$)					Susceptible check FM832				
	Mean	SE	Minimum	Maximum	Skew	Mean	SE	Minimum	Maximum	Skew
Root gall index	3.10	0.65	1.73	4.6	0.04	4.73	0.31	3.80	5.00	−1.15
log eggs plant^{-1}	3.67 (4,723)	0.50	2.37 (233)	4.86 (73,196)	−0.18	4.36 (22,765)	0.20	4.05 (11,301)	4.55 (35,825)	−0.71
log eggs g^{-1} root	3.48 (3,053)	0.49	2.11 (130)	4.82 (66,742)	−0.17	4.17 (14,830)	0.39	3.51 (3,241)	4.49 (30,950)	−1.59

[a] Values in parentheses are means back-transformed to their original value of eggs plant^{-1} and eggs g^{-1} root

conventionally, i.e., by primer pair designation with the addition of the fragment size that corresponded to the most resistant parent in the cross, e.g., CIR316-201.

Results

Phenotypic evaluation of RIL populations

Distributions of RGI, eggs plant^{-1}, and eggs g^{-1} root for all three RIL populations and the corresponding susceptible check FM832 are presented in Table 1. RGI, eggs plant^{-1}, and eggs g^{-1} root for FM832 indicated that nematode infection levels were sufficient for evaluating RKN resistance within all three RIL populations in their respective experiments. For the M240 × CLW6 RIL population, RGI, eggs plant^{-1}, and eggs g^{-1} root ranged from 1.00 to 4.00, 140 to 41,300, and 68 to 25,000, respectively (Table 1). The mean RGI of the population was 2.32 and the number of individuals with resistant to moderately resistant values, i.e., an RGI less than 3.5, was greater than the number of susceptible individuals (Fig. 1a). RILs derived from CLW6 × ST213 showed RGI, eggs plant^{-1}, and eggs g^{-1} root values that ranged from 2.00 to 4.80, 125 to 131,701, and 161 to 61,972, respectively (Table 1). The RGI mean of this RIL population was 3.42 with the majority of individuals belonging to the moderately resistant and susceptible categories (Fig. 1b). RILs derived from the third cross, M240 × ST213, showed RGI, eggs plant^{-1}, and eggs g^{-1} root values that ranged from 1.73 to 4.60, 233 to 73,196, and 130 to 66,742, respectively (Table 1). The mean RGI for this population was 3.10 and it contained individuals that belonged to all phenotypic categories, ranging from resistant to susceptible (Fig. 1c).

Polymorphism evaluation

Initially, we screened 1,349 SSR primer pairs distributed throughout the cotton genome for polymorphisms between the parental genotypes of each cross. Chromosomes 11 and 14 had previously been implicated in RKN resistance (Ynturi 2005; Shen et al. 2006; Wang et al. 2006; Ynturi et al. 2006; Niu et al. 2007); therefore, an additional 70 SSR markers that either mapped to chromosomes 11 or 14 or had been localized to these chromosomes by hypoaneuploid-based deletion analysis were also screened. SSR allele sizes ranged from 84 to 348 bp. Markers that were polymorphic between any two parents were run against the RILs derived from the respective cross. Between M240 and CLW6 there were 162 primer pairs (11.7%) that revealed 167 polymorphic loci. Primer pairs BNL3408, BNL3442, BNL3545, BNL4071, and CIR055 amplified loci on two different chromosomes, indicating the presence of duplicated loci. Between CLW6 and ST213, 154 primers (10.8%) amplified 158 polymorphic loci. Primer pairs BNL3914, BNL4067, JESPR274, and NAU0998 amplified duplicated loci. Between M240 and ST213, 149 primers (10.5%) yielded 155 polymorphic loci. In this instance, primers BNL632, BNL3280, BNL3408, BNL3545, CIR055, and CIR105 amplified duplicated loci.

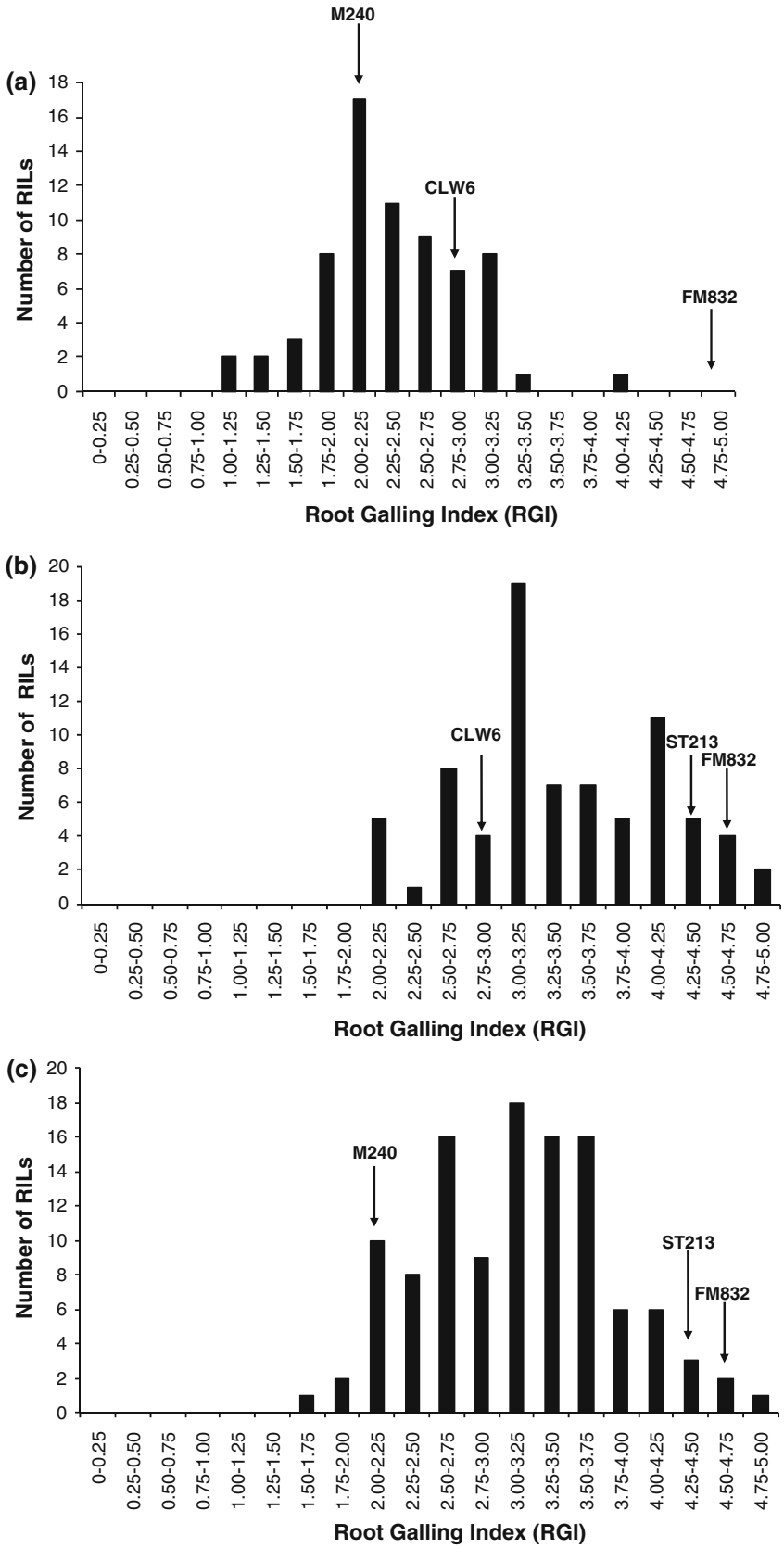

Fig. 1 Distribution of mean root galling index (RGI) among recombinant inbred line (RIL) families from the crosses **a** M240 × CLW6, **b** CLW6 × ST213, and **c** M240 × ST213. RGI values of the corresponding parents and susceptible check FM832 are indicated by *arrows*

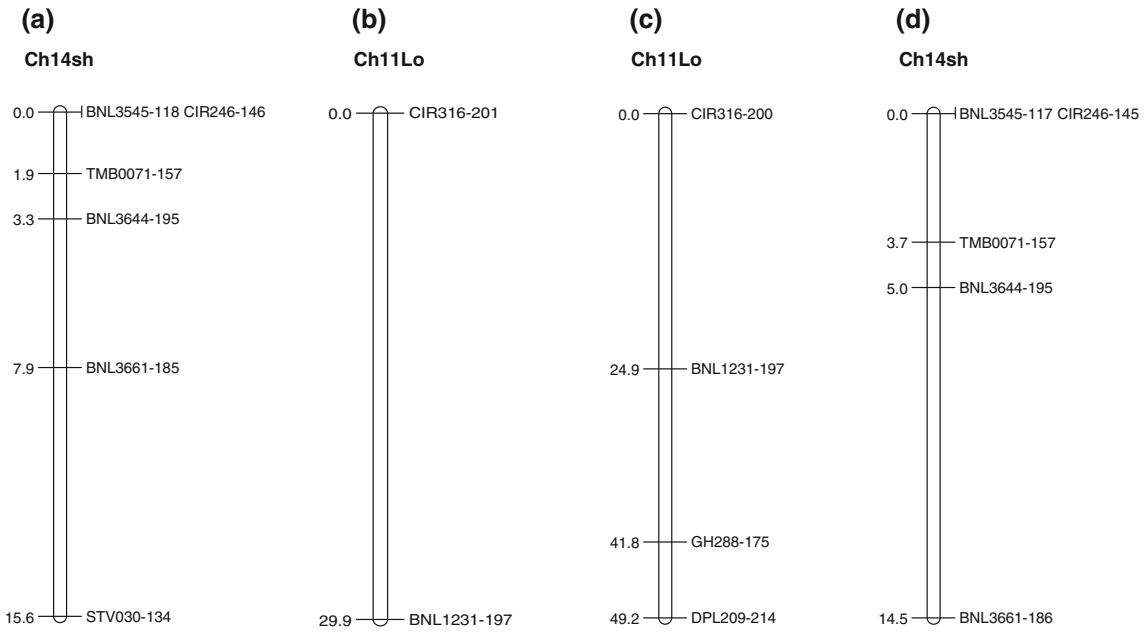

Fig. 2 **a** Chromosome 14 short (Ch14sh) linkage group as determined using the recombinant inbred line (RIL) population from the cross M240 × CLW6, **b** Chromosome 11 long (Ch11Lo) linkage group as determined using the RIL population from the cross CLW6 × ST213, **c** Ch11Lo and **d** Ch14sh linkage groups showing SSR loci associated with root-knot nematode resistance as determined using RILs from the cross M240 × ST213

Collectively, these results supported previous reports regarding the low levels of polymorphism that exist within *G. hirsutum* (Gutiérrez et al. 2002; Rungis et al. 2005).

SSR markers associated with RKN resistance

Using single-factor analyses of variance, associations between SSR loci and RGI, log eggs plant^{-1}, and log eggs g^{-1} root were identified. For the M240 × CLW6 RIL population, the SSR loci BNL3545-118, TMB0071-157, BNL3644-195, BNL3661-185, and STV030-134, all located on the short arm of chromosome 14 (Fig. 2a), were associated with RKN resistance (Table 2). BNL3661-185 was associated with 20, 53, and 55% of the variation in RGI, log eggs plant^{-1}, and log eggs g^{-1} root, respectively (Table 2). In addition, MapQTL5.0 interval mapping results (Table 3) indicated that a major QTL close to BNL3661-185 explained 20, 58, and 60% of the variation in reduction of RGI, log eggs plant^{-1}, and log eggs g^{-1} root, respectively. The QTL peak was located at 10.90 cM for the RGI trait and 8.90 cM for both log eggs plant^{-1} and log eggs g^{-1} root with LOD values of 2.87, 11.48, and 12.47, respectively.

In the RIL population from the cross CLW6 × ST213, SSR loci CIR316-201 and BNL1231-197, located on the long arm of chromosome 11 (Fig. 2b), were associated with RKN resistance (Table 2). Results from the one way analysis of variance indicated that CIR316-201 explained 30, 32, and 36% of the variation in RGI, log eggs plant^{-1}, and log eggs g^{-1} root traits, respectively; whereas, BNL1231-197 explained 9, 5, and 9% of the variation in RGI, log eggs plant^{-1}, and log eggs g^{-1} root, respectively (Table 2). MapQTL5.0 interval mapping results (Table 3) showed that a major QTL located between CIR316-201 and BNL1231-197 accounted for 59, 43, and 57% of the phenotypic variation of RGI, log eggs plant^{-1}, and log eggs g^{-1} root, respectively.

In the M240 × ST213 RIL population, the SSR loci CIR316-201, BNL3545-118, TMB0071-157, BNL3644-195, and BNL3661-185 were associated with RKN resistance (Table 2; Fig. 2c, d). SSR locus CIR 316-201, located on chromosome 11, accounted for 28, 5, and 6% of the variation for RGI, log eggs plant^{-1}, and log eggs g^{-1} root, respectively (Table 2). Chromosome 14sh SSR loci BNL3545-118, TMB0071-157, BNL3661-185, and BNL3644-195 explained 10, 8, 8, and 8% of the variation for RGI; 20, 22, 24, and 25% for log eggs plant^{-1}, and 20, 23, 25, and 23% for log eggs g^{-1} root, respectively (Table 2). MapQTL5.0 interval mapping results (Fig. 2c) revealed that allele CIR316-201 exhibited a QTL peak located at 6.0 cM that accounted for 31% of the phenotypic variation for the RGI (LOD = 6.6). Neither log eggs plant^{-1} nor log eggs g^{-1} root showed more than 8% association with the CIR316-201 allele. In contrast, SSR loci located on chromosome 14sh showed associations with all three measures of RKN resistance (Fig. 2d). A QTL peak that explained 12% (LOD = 2.44) of the phenotypic variation for RGI was detected between BNL3661-185 and BNL3664-195 at 10.05 cM. Chromosome 14sh SSR loci

Table 2 Genetic effects and coefficients of determination associated with individual chromosome 11 long (11Lo) and 14 short (14sh) SSR markers and root-knot nematode resistance traits in three recombinant inbred line populations

Cross	Trait evaluated	Chromosome	SSR Loci	R^2	Mean A[a]	Mean B[b]	Additive effect
M240 × CLW6	Root gall index	14sh	BNL3545-118	0.06*	2.17	2.44	0.14*
M240 × CLW6	Root gall index	14sh	TMB0071-157	0.08*	2.15	2.45	0.15*
M240 × CLW6	Root gall index	14sh	BNL3644-195	0.13**	2.09	2.49	0.20**
M240 × CLW6	Root gall index	14sh	BNL3661-185	0.20***	2.07	2.53	0.23***
M240 × CLW6	Root gall index	14sh	STV030-134	0.11**	2.11	2.47	0.18**
M240 × CLW6	log eggs plant^{-1}	14sh	BNL3545-118	0.36***	3.29 (1,934)[c]	3.97 (9,299)	0.34***
M240 × CLW6	log eggs plant^{-1}	14sh	TMB0071-157	0.39***	3.27 (1,855)	3.97 (9,345)	0.35***
M240 × CLW6	log eggs plant^{-1}	14sh	BNL3644-195	0.38***	3.29 (1,927)	3.97 (9,388)	0.34***
M240 × CLW6	log eggs plant^{-1}	14sh	BNL3661-185	0.53***	3.20 (1,590)	4.02 (10,462)	0.41***
M240 × CLW6	log eggs plant^{-1}	14sh	STV030-134	0.32***	3.32 (2,083)	3.93 (8,424)	0.30***
M240 × CLW6	log eggs g^{-1} root	14sh	BNL3545-118	0.40***	2.95 (882)	3.63 (4,279)	0.34***
M240 × CLW6	log eggs g^{-1} root	14sh	TMB0071-157	0.41***	2.94 (862)	3.63 (4,247)	0.35***
M240 × CLW6	log eggs g^{-1} root	14sh	BNL3644-195	0.40***	2.95 (899)	3.63 (4,258)	0.34***
M240 × CLW6	log eggs g^{-1} root	14sh	BNL3661-185	0.55***	2.87 (743)	3.68 (4,730)	0.40***
M240 × CLW6	log eggs g^{-1} root	14sh	STV030-134	0.34***	2.98 (958)	3.58 (3,822)	0.30***
CLW6 × ST213	Root gall index	11Lo	CIR316-201	0.30***	3.11	3.89	0.39***
CLW6 × ST213	Root gall index	11Lo	BNL1231-197	0.09**	3.20	3.62	0.21*
CLW6 × ST213	log eggs plant^{-1}	11Lo	CIR316-201	0.32***	3.83 (6,846)[c]	4.41 (25,778)	0.29***
CLW6 × ST213	log eggs plant^{-1}	11Lo	BNL1231-197	0.05*	3.94 (8,692)	4.16 (14,579)	0.11*
CLW6 × ST213	log eggs g^{-1} root	11Lo	CIR316201	0.36***	3.63 (4,287)	4.21 (16,416)	0.29***
CLW6 × ST213	log eggs g^{-1} root	11Lo	BNL1231-197	0.09**	3.71 (5,136)	4.00 (9,946)	0.14**
M240 × ST213	Root gall index	11Lo	CIR316-201	0.28***	2.77	3.47	0.35***
M240 × ST213	Root gall index	11Lo	BNL1231-197	0.02	3.05	3.20	0.08
M240 × ST213	log eggs plant^{-1}	11Lo	CIR316-201	0.05*	3.57 (3,678)	3.79 (6,192)	0.11*
M240 × ST213	log eggs plant^{-1}	11Lo	BNL1231-197	0.00	3.64 (4,377)	3.69 (4,856)	0.02
M240 × ST213	log eggs g^{-1} root	11Lo	CIR316-201	0.06**	3.36 (2,313)	3.61 (4,090)	0.12**
M240 × ST213	log eggs g^{-1} root	11Lo	BNL1231-197	0.00	3.46 (2,915)	3.48 (3,052)	0.01
M240 × ST213	Root gall index	14sh	BNL3545-118	0.10***	2.85	3.28	0.21***
M240 × ST213	Root gall index	14sh	TMB0071-157	0.08**	2.87	3.25	0.19**
M240 × ST213	Root gall index	14sh	BNL3644-195	0.08**	2.88	3.25	0.18**
M240 × ST213	Root gall index	14sh	BNL3661-185	0.08**	2.90	3.27	0.19**
M240 × ST213	log eggs plant^{-1}	14sh	BNL3545-118	0.20***	3.40 (2,486)	3.85 (7,151)	0.23***
M240 × ST213	log eggs plant^{-1}	14sh	TMB0071-157	0.22***	3.37 (2,350)	3.85 (7,072)	0.24***
M240 × ST213	log eggs plant^{-1}	14sh	BNL3644-195	0.24***	3.36 (2,309)	3.86 (7,271)	0.25***
M240 × ST213	log eggs plant^{-1}	14sh	BNL3661-185	0.25***	3.40 (2,544)	3.90 (7,921)	0.25***

Table 2 continued

Cross	Trait evaluated	Chromosome	SSR Loci	R^2	Mean A^a	Mean B^b	Additive effect
M240 × ST213	log eggs g^{-1} root	14sh	BNL3545-118	0.20***	3.21 (1,633)	3.66 (4,590)	0.22***
M240 × ST213	log eggs g^{-1} root	14sh	TMB0071-157	0.23***	3.18 (1,524)	3.66 (4,580)	0.24***
M240 × ST213	log eggs g^{-1} root	14sh	BNL3644-195	0.25***	3.17 (1,490)	3.67 (4,723)	0.25***
M240 × ST213	log eggs g^{-1} root	14sh	BNL3661-185	0.23***	3.23 (1,694)	3.70 (4,995)	0.24***

* Significant at 0.05 level

** Significant at 0.01 level

*** Significant at 0.001 level

[a] Indicates the plant carries the allele from the resistant parent

[b] Indicates the plant carries the allele from the susceptible parent

[c] Values in parentheses are means back-transformed to their original value of eggs $plant^{-1}$ and eggs g^{-1} root

were also strongly associated with log eggs $plant^{-1}$ and log eggs g^{-1} root with the major QTL being located between BNL 3661-185 and 3664-195 with a peak at 11.05 cM which explained 33 and 32% (LOD = 7.99, 7.88) of the variation in reduction of log eggs $plant^{-1}$ and log eggs g^{-1} root. Lastly, SSR marker CIR246 was found to be associated with RKN resistance in the M240 × CLW6 and M240 × ST213 RIL populations, but since it was a redundant marker and mapped to the same location as BNL3545-118 in both populations, only BNL 3545-118 was used in the QTL analysis.

Our results also indicated that all the SSR alleles across the three RIL populations that were strongly associated with RKN resistance exhibited significant additive effects (Tables 2, 3). Potential dominant effects could not be estimated in this study because RIL populations lack heterozygous genotypes and thus can only detect additive effects. Several combinations of chromosome 11Lo- or 14sh-specific SSR loci, that were also associated with RKN resistance among RILs derived from M240 × ST213, were analyzed in multiple regression models to determine which combination of loci, representing both chromosomes, accounted for the highest percentage of phenotypic variation. The first regression model considered only two SSR loci at a time and their interaction in order to estimate additive × additive epistasis. Only significant additive genetic effects were detected for the three traits using the two marker model; however, no significant additive × additive epistasis was found by any of the models or with the use of the EPISTACY program (Holland 1998). A subsequent collection of multiple regression models that included all significant SSR loci was examined. The model that showed the most significant R^2 value was that which included the SSR loci CIR316-201, BNL3545-118, and BNL3661-185. This model explained 41, 42, and 40% of the variation of the RGI, log eggs $plant^{-1}$, and log eggs g^{-1} root, respectively (Table 4). Also, significant additive main effects were detected for all of these SSR loci with the exception of RGI for BNL3661-185 and the three way interaction. Results from a generalized linear mixed model (GLMM) analysis indicated significant additive × additive × additive interactions for log eggs $plant^{-1}$ and log eggs g^{-1} root. Three SSR markers associated with RKN resistance are shown in Table 5. The RGI was 2.96 when marker CIR316-201 on chromosome 11 was detected; however, when BNL3545-118 or BNL3661-185 on chromosome 14sh were present the RGI was reduced to 2.60. In addition, a sixfold reduction in the amount of eggs $plant^{-1}$ and eggs g^{-1} root was observed in plants that had the three SSR loci CIR 316-201, BNL3545-118, and BNL3661-185 compared to plants having none of these loci (Table 5).

Table 3 Quantitative trait loci (QTL) associated with root-knot nematode resistance calculated by interval mapping for the traits root gall index, log eggs plant^{-1}, and log eggs g^{-1} root in three recombinant inbred line populations

Cross	Chromosome	Trait evaluated	Flanking markers	Marker position	Map position of QTL	LOD score	Additive effects	R^2
M240 × CLW6	14sh	Root gall index	BNL3661-185-STV030-134	7.895–15.619	10.90	2.87	−0.25	0.20
M240 × CLW6	14sh	log eggs plant^{-1}	BNL3661-185-STV030-134	7.895–15.619	8.90	11.48	−0.43	0.58
M240 × CLW6	14sh	log eggs g^{-1} root	BNL3661-185-STV030-134	7.895–15.619	8.90	12.47	−0.42	0.60
CLW6 × ST213	11Lo	Root gall index	CIR316-201-BNL1231-197	0.000–29.945	10.00	7.15	−0.55	0.59
CLW6 × ST213	11Lo	log eggs plant^{-1}	CIR316-201-BNL1231-197	0.000–29.945	9.00	5.56	−0.33	0.43
CLW6 × ST213	11Lo	log eggs g^{-1} root	CIR316-201-BNL1231-197	0.000–29.945	13.00	6.77	−0.36	0.57
M240 × ST213	11Lo	Root gall index	CIR316-201-BNL1231-197	0.000–24.851	6.00	6.60	−0.37	0.31
M240 × ST213	11Lo	log eggs plant^{-1}	CIR316-201-BNL1231-197	0.000–24.851	6.00	1.07	−0.13	0.06
M240 × ST213	11Lo	log eggs g^{-1} root	CIR316-201-BNL1231-197	0.000–24.851	6.0	1.29	−0.14	0.08
M240 × ST213	14sh	Root gall index	BNL3644-195-BNL3661-185	5.048–14.526	10.05	2.44	−0.23	0.12
M240 × ST213	14sh	log eggs plant^{-1}	BNL3644-195-BNL3661-185	5.048–14.526	11.05	7.99	−0.29	0.32
M240 × ST213	14sh	log eggs g^{-1} root	BNL3644-195-BNL3661-185	5.048–14.526	11.05	7.88	−0.28	0.32

Table 4 Genetic effects and coefficients of determination associated with multiple SSR markers and root-knot nematode resistance traits in recombinant inbred lines from the cross M240 × ST213

Trait evaluated	SSR loci	R^2	Mean A[a]	Mean B[b]	Additive effect
Root gall index	CIR316-201 + BNL3545-118 + BNL3661-185	0.41***			
Root gall index	CIR316-201	0.21	2.71	3.48	0.39***
Root gall index	BNL3545-118	0.03	2.94	3.25	0.16*
Root gall index	BNL3661-185	0.01	3.01	3.18	0.09
Root gall index	CIR316-201 × BNL3545-118 × BNL3661-185	0.02			0.08
log eggs plant^{-1}	CIR316-201 + BNL3545-118 + BNL3661-185	0.42***			
log eggs plant^{-1}	CIR316-201	0.09	3.51 (3,253)[c]	3.88 (7,632)	0.19***
log eggs plant^{-1}	BNL3545-118	0.06	3.54 (3,505)	3.85 (7,083)	0.15**
log eggs plant^{-1}	BNL3661-185	0.07	3.53 (3,365)	3.87 (7,377)	0.17***
log eggs plant^{-1}	CIR316-201 × BNL3545-118 × BNL3661-185	0.06			0.12*
log eggs g^{-1} root	CIR316-201 + BNL3545-118 + BNL3661-185	0.40***			
log eggs g^{-1} root	CIR316-201	0.10	3.31 (2,041)	3.70 (5,020)	0.20***
log eggs g^{-1} root	BNL3545-118	0.05	3.36 (2,296)	3.65 (4,462)	0.14**
log eggs g^{-1} root	BNL3661-185	0.07	3.34 (2,201)	3.68 (4,654)	0.16***
log eggs g^{-1} root	CIR316-201 × BNL3545-118 × BNL3661-185	0.05			0.96*

* Significant at 0.05 level

** Significant at 0.01 level

*** Significant at 0.001 level

[a] Indicates the plant carries the allele from the resistant parent

[b] Indicates the plant carries the allele from the susceptible parent

[c] Values in parentheses are means back-transformed to their original value of eggs plant^{-1} and eggs g^{-1} root

Origin of the RKN resistance QTL on chromosome 14

In order to identify the source of the resistance QTL on chromosome 14 which mediates decreased RKN egg production, MW and CLW6, in addition to several germplasm lines that show a range of susceptibility to RKN, were genotyped with CIR316-201, BNL3545-118, BNL3644-195, and BNL3661-185 (Table 6). We discovered that the CIR316-201 allele was absent in all RKN-susceptible lines but was present across all moderately resistant and resistant germplasm with the exception of MW and M-155 RNR. In contrast to CIR316-201, the chromosome 14 SSR locus,

Table 5 Root-knot nematode resistance trait means of recombinant inbred lines derived from the cross M240 × ST213 with genotypes that correspond to three SSR markers located on chromosomes 11 or 14 short (14sh)

CIR316-201 Chr. 11	BNL3545-118 Chr. 14sh	BNL3661-185 Chr. 14sh	n	RGI		log eggs plant^{-1}		log eggs g^{-1} root	
				Mean	SE	Mean[a]	SE	Mean[a]	SE
A/A[b]	A/A	A/A	17	2.60	0.13	3.25 (1,779)	0.09	3.07 (1,168)	0.10
A/A	A/A	B/B[c]	5	2.67	0.24	3.51 (3,248)	0.17	3.32 (2,101)	0.18
A/A	B/B	A/A	7	2.60	0.20	3.47 (2,925)	0.15	3.24 (1,720)	0.15
A/A	B/B	B/B	26	2.96	0.10	3.82 (6,623)	0.08	3.61 (4,110)	0.08
B/B	A/A	A/A	15	3.08	0.14	3.31 (2,022)	0.10	3.15 (1,417)	0.10
B/B	A/A	B/B	4	3.40	0.27	4.11 (12,911)	0.20	3.90 (7,988)	0.20
B/B	B/B	A/A	5	3.73	0.24	4.08 (12,186)	0.17	3.92 (8,246)	0.18
B/B	B/B	B/B	24	3.71	0.11	4.03 (10,660)	0.08	3.83 (6,800)	0.08

Chr chromosome

[a] Values in parentheses are means back-transformed to their original value of eggs plant^{-1} and eggs g^{-1} root

[b] A/A: homozygous for the allele coming from the resistant parent (M240)

[c] B/B: homozygous for the allele coming from the susceptible parent (ST213)

Table 6 Relationship between the presence or absence of certain SSR alleles and the root-knot nematode resistance or susceptibility of various cotton germplasm lines

Germplasm	Phenotype	CIR316-201	BNL3545-118	BNL3644-195	BNL3661-185
ST213	S[a]	−	−	−	−
Auburn 56	S[b]	−	−	−	−
G. barbadense 3-79	S[c]	−	−	−	−
Pima S-6	S[d]	−	−	−	+
Pima S-7	S[d]	−	−	−	+
FM832	S[1]	−	+	+	−
Clevewilt 6	MR[1]	+	−	−	−
Mexico Wild	MR[c]	−	+	+	+
Acala Nem-X	MR[c]	+	−	−	−
Auburn 623 RNR	R[c]	+	+	+	+
Auburn 634 RNR	R[c]	+	+	+	+
M-92 RNR	R[b]	+	+	+	+
M-120 RNR	R[c,b]	+	−	−	+
M-155 RNR	R[b]	−	+	+	+
M-240 RNR	R[a,b]	+	+	+	+
M-249 RNR	R[b]	+	+	+	+
M-272 RNR	R[b]	+	+	+	+
M-315 RNR	R[e,b]	+	+	+	+
M-331 RNR	R[c,b]	+	+	+	+
M-725 RNR	R[c,b]	+	+	+	+

S susceptible to RKN, *MR* moderately resistant to RKN, *R* resistant to RKN

−, resistance-associated allele absent; +, resistance-associated allele present

[a] This report, [b] Shepherd et al. (1996), [c] Niu et al. (2007), [d] Robinson et al. (1999), [e] McPherson et al. (2004)

BNL3661-185, was the only resistance-associated allele present in MW and all resistant RKN germplasm but not CLW6. These results indicate that the SSR allele BNL36631-185, associated with reduced RKN egg production, originated from MW but not CLW6.

Discussion

A cross between CLW6 and MW, both moderately resistant to RKN, produced the highly resistant transgressive segregant Auburn 623 RNR, from which M240 and many other RKN-resistant M-lines have been derived (Shepherd et al. 1996). The relative contribution of CLW6 and MW to the resistance observed in the M-lines has remained obscure. In this report, using RIL populations derived from the crosses M240 × CLW6, CLW6 × ST213, and M240 × ST213, we demonstrate that the high level of RKN resistance shown by M240 is likely manifested as two separate phenotypes: (1) a reduction in root galling index as mediated by a QTL located on chromosome 11 and derived from CLW6, and (2) reduced RKN egg production

as mediated by a QTL on chromosome 14 and derived from MW.

One of our initial hypotheses was that both CLW6 and M240 harbored the same RKN resistance QTL located on chromosome 11; consequently, the RIL population derived from these lines would not be expected to segregate for this QTL. This assumption was validated by our finding that no chromosome 11-specific SSR alleles could be associated with RKN resistance in the M240 × CLW6 RIL population. In fact, only the RKN resistance QTL contributed by the M240 ancestor MW would be expected to segregate among these RILs. The chromosome 14-specific SSR loci BNL3545-118, BNL3644-195, and BNL3661-185 were found to be associated with RKN resistance in this RIL population. Interestingly, these same SSR alleles were present in the MW parent, further supporting our hypothesis that RKN resistance QTLs contributed by the CLW6 and MW parents to the M-lines are independent and reside on different chromosomes. Finally, these results corroborate an earlier report by Ynturi et al. (2006) where the SSR alleles BNL3545-118, BNL3644-195, and BNL3661-185 on chromosome 14 were linked to RKN resistance.

QTL mapping results using RIL populations derived from the CLW6 × ST213 and M240 × ST213 crosses were in complete agreement with the findings described above. For example, in the RIL population from the cross of CLW6 × ST213, only the chromosome 11-specific SSR alleles CIR316-210 and BNL 1231-197 were found to be associated with resistance. No chromosome 14-specific SSR alleles were detected in the CLW6 × ST213 RIL population. These results also agree with those of Wang et al. (2006, 2008) and Shen et al. (2006) who stated that CIR316-201 was associated with RKN resistance. Ynturi et al. (2006) also reported that BNL 1231-197 was associated with RKN resistance.

Taking into account the conclusions garnered from the M240 × CLW6 and CLW6 × ST213 RIL mapping populations, we would expect to detect RKN resistance QTLs on both chromosomes 11 and 14 among RILs derived from M240 × ST213. This is precisely what occurred where we observed that chromosome 11-specific SSR alleles (CIR 316-201 and BNL1231-197) as well as chromosome 14-specific SSR alleles (BNL3545-118, BNL3644-195, and BNL3661-185) were associated with RKN resistance. Furthermore, for the M240 × ST213 RIL population, 103 out of a total of 114 lines were homozygous for the 8 expected classes of allele combinations for the SSR markers CIR 316-201, BNL3545-118, and BNL3661-185. As expected, no linkage was detected between CIR316-201 and BNL3661-185 since they mapped to different chromosomes. If no linkage was present between the BNL3545-118 and BNL3661-185 SSR resistance alleles we would expect an equal number of plants in each genotypic class; however, the observed ratio (24:9:12:50) did not fit such a scenario, thus, linkage between these markers indicate the co-localize to chromosome 14sh. This result also confirmed a previous report by Ynturi et al. (2006) that SSR alleles BNL3661-185 and BNL3664-195 accounted for 21 and 19% of the variation for RGI. Finally, the M240 × ST213 RIL population, which we hypothesized would show segregation on chromosomes 11 and 14, revealed that SSR alleles from markers located on these chromosomes (CIR 316-201, BNL3545-118, TMB0071-157, BNL3644-195, BNL3661-185) were associated with RKN resistance. The average linkage disequilibrium measurement in cotton is about 10–20 cM (Abdurakhmonov et al. 2009) which agrees with the small amount of recombination observed between the chromosome 14sh SSR markers.

When the SSR resistance alleles BNL3545-118 and BNL3661-185 on chromosome 14 were present in combination with the CIR316 susceptible allele we observed an 81% reduction (2,022 vs. 10,660) in the number of eggs plant^{-1} and a 79% reduction in eggs g^{-1} root (1,417 vs. 6,800) compared to plants having the susceptible alleles of all three markers. Conversely, when only the CIR316-201 resistance allele was present, eggs plant^{-1} and eggs g^{-1} root were reduced by only 38 and 40%, respectively. These data strongly suggest that the chromosome 14 resistance QTL, contributed by MW, largely influences RKN egg production as opposed to root galling. This conclusion is supported by Shepherd (1979) who stated that "MW was more susceptible to root galling as indicated by a root-knot index of 2.3 but had a much lower egg production". Additional corroboration stems from recent work performed by Shen et al. (2010) who have observed a significant association between chromosome 14 and reduced RKN egg production in a segregating F_2 population derived from a M-120 RNR × Pima S-6 cross. Significant additive × additive × additive epistasis has also been observed in genes conditioning soybean resistance to reniform nematode (Ha et al. 2007).

The chromosome 11 resistance QTL linked to CIR316-201 appears to be primarily associated with the reduction of RGI. We determined that CIR316-201 was present in CLW6, Acala Nem-X, and almost all RKN resistance sources with the exception of MW and M-155 RNR. It is likely that a recombination event between CIR316-201 and the resistance QTL on chromosome 11 occurred at some point in the development of M-155 RNR because this line shows a resistant phenotype (Shepherd et al. 1989). The chromosome 14 resistance marker alleles BNL3545-118 and BNL3661-185 are present in MW and the majority of the Auburn 623-derived RKN-resistant germplasm with the exception that the BNL3545-118 allele is not present in M-120 RNR; however, the resistance QTL and BNL 3661-185 are present in M-120 RNR. Neither BNL3545-118 nor

BNL3661-185 is present in either CLW6 or Acala Nem-X. These facts, coupled with our genetic analyses, further supports the conclusion that the RKN resistance present in Auburn 623 RNR-derived lines shows a different mode of inheritance than that shown by Acala Nem-X and is controlled by at least one additional QTL.

A recent report by Roberts and Ulloa (2010), based upon the RKN phenotype and SSR fingerprint of some *G. hirsutum* and *G. barbadense* lines and a number of diploid *Gossypium* spp., contends that only the CIR316 resistance allele can be significantly associated with RKN resistance and used reliably to identify resistant cotton genotypes. One possible explanation for this contradictory finding is that only the root galling phenotype was evaluated in the author's experiments (Roberts and Ulloa 2010). As can be clearly seen from the data presented here, in addition to being observed by Shen et al. (2010), the chromosome 14 resistance QTL affects total RKN egg production; consequently, it is not surprising that this locus went undetected in that study (Roberts and Ulloa 2010).

In summary, the data presented here provide compelling evidence in support of there being RKN resistance QTLs on both chromosomes 11 and 14 which were contributed by the CLW6 and MW parents, respectively, in the cross that produced Auburn 623 RNR and subsequent RKN-resistant germplasm. The SSR markers presented herein can be used to select for RKN resistance genes on both chromosome 11 and chromosome 14 in applied breeding programs using the nine M-lines previously released (Shepherd et al. 1996).

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