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ORIGINAL PAPER

Re-evaluation of the inheritance for root-knot nematode resistance in the Upland cotton germplasm line M-120 RNR revealed two epistatic QTLs conferring resistance

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Abstract
Key message **We report a second major QTL for root-knot nematode resistance in the highly resistant Upland cotton line M-120RNR and show epistasis between two resistant QTLs with different mechanisms conferring resistance.**
Abstract In an earlier study, we identified a major QTL on Chromosome 11 associated with resistance to root-knot nematode in the M-120 RNR Upland cotton line (*Gossypium hirsutum* L.) of the Auburn 623 RNR source. Herein, we re-evaluated the genetics of the resistance to root-knot nematode in the M-120 RNR × Pima S-6 population by linkage mapping using recently published SSR markers. The QTL analysis detected two regions significantly associated with the resistance phenotype. In addition to the QTL previously identified on Chromosome 11 (*qMi-C11*), a major QTL was identified on Chromosome 14 (*qMi-C14*). The resistance locus on *qMi-C11* originated from the Clevewilt parent, while the *qMi-C14* locus originated from the other resistant parent, Mexico Wild Jack Jones. The *qMi-C14* locus had logarithms of odds score of 17 and accounted for 45 % of the total phenotype variation in egg production. It was also associated with galling index, but the percent variation explained was only 6 %, suggesting that the *qMi-C11* locus had a much stronger effect on root gall suppression than egg production, while the *qMi-C14* locus had a stronger effect on egg production than galling. The results also suggest that the transgressive segregation observed in the development of Auburn 623 RNR was due to the pyramiding of at least two main effect QTLs as well as an additive-by-additive epistatic effects between the two resistant loci. The SSRs markers tightly linked to the *qMi-C11* and *qMi-C14* loci will greatly facilitate the improvement of RKN resistance in cotton via marker-assisted breeding.

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Introduction

The southern root-knot nematode (*Meloidogyne incognita*; RKN) is the most important endoparasitic pest of Upland cotton (*Gossypium hirsutum* L.). Found in all cotton production regions in the US (Koenning et al. 2004; Starr et al. 2005), RKN accounts for an estimated yield loss of

547,728 bales (500 lb/bale), or 2.2 % of the total US cotton production (Blasingame and Patel 2005). At the present time, crop rotations and nematicides are the two primary means of managing nematodes in cotton in the US. Crop rotation, while a recommended cultural practice to lessen soil populations of root-knot nematodes, is not an option for many cotton growers because of the lack of suitable non-host crops with which to rotate their cotton acreages. For example, Peanut (*Arachis hypogaea*) is a non-host crop for the cotton RKN and is a recommended rotational crop for cotton (Koenning et al. 2004). However, about 1.2 million acres of peanut and 12.6 million acres of cotton annually are planted in the US (USDA-NASS), leaving more than 10 million acres of cotton without this suitable non-host crop with which to practice crop rotation as a nematode management tool. Further, RKN also infects corn (*Zea mays*), grain sorghum (*Sorghum bicolor*), soybean (*Glycine max*), and many vegetable crops, leaving many cotton growers with limited options to practice crop rotation as a nematode management tool (Starr et al. 2007). Nematicides are highly effective, but their use adds a significant cost to cotton production, and because they are applied in a single dose at the beginning of the growing season, they do not provide season-long protection from RKN or other nematode species. Further, their future availability is uncertain due to safety and environmental concerns.

Host plant resistance is the most economical, practical, and environmentally sound method to provide crop protection against RKN. Three major sources of resistant germplasm are recognized in Upland cotton (Robinson et al. 2001). The first and most important source of resistance is from Auburn 623 RNR, which originated from transgressive segregation in a cross between two moderately resistant parents, Clevewilt 6 and 'Mexico Wild' Jack Jones, TX-2516 (Shepherd 1974a, b). The resistance was subsequently transferred to several agronomically adapted cultivars through backcrossing, resulting in the release of the M-line series including lines such as 'M-120 RNR' (M120), 'M-135 RNR', and 'M-155 RNR', which greatly improved the agronomic qualities while retaining the almost-immune level of resistance of Auburn 623 RNR. Clevewilt 6 was also used to develop the root-knot nematode resistant line LA434-RKR, which represents the second source of resistance. Finally, there is resistance found in a commercial Acala cultivar, Acala NemX (Ogallo et al. 1997). The original source of resistance in Acala NemX is uncertain, but the donor parent N6072 is thought to have been derived from crossing a *G. barbadense* L. genotype and the Acala line 1-2302 (Robinson et al. 2001).

Earlier pathology studies on the mechanism of RKN resistance in the Auburn 623 RNR source suggest that the expression of resistance is associated with two genes that are involved in suppressing the development of the giant cell at 6-day post-penetration and reducing fecundity of *M. incognita* females at 24-day post-penetration (Creech et al. 1995; Jenkins et al. 1995). Further evidence of a two-gene system was obtained from crosses with the M-series lines (McPherson et al. 1995; Zhou et al. 1999). In an earlier study, using the bulk segregant approach, Shen et al. (2006) identified a major QTL on Chromosome 11 in the resistant line M120 of the Auburn 623 RNR source using an interspecific population derived from crossing the resistant line with the susceptible *G. barbadense* cultivar "Pima S-6". This QTL, which influences root galling and was inherited from the Clevewilt 6 parent (Shen et al. 2006), was independently validated (Ynturi et al. 2006; Niu et al. 2007; Gutierrez et al. 2010) and has recently been fine mapped to a region of 3.6 cM interval (Shen et al. 2010). Working with a different M-series resistant line of the Auburn 634 RNR source, Gutierrez et al. (2010) reported a second QTL located on Chromosome 14. Interestingly, unlike the QTL on Chromosome 11, this QTL appears to largely influence RKN egg production and was inherited from the Mexico Wild Jack Jones parent (Gutierrez et al. 2010).

Since the RKN resistance in the M120 and M-240 RNR lines is derived from the same Auburn 623 RNR source (Robinson et al. 2001), these two highly resistant lines as well as other in the M-series are expected to also carry the same resistance genes. Therefore, the M120 segregating population developed by Shen et al. (2006) provides an opportunity to independently validate the QTL on Chromosome 14 reported by Gutierrez et al. (2010). The objective of this study was to re-evaluate the inheritance of the RKN resistance in the highly resistant line M120. Specifically, we seek to (1) validate the importance of Chromosome 14 in RKN resistance in the Auburn 623 RNR, (2) develop DNA markers that are tightly linked to the genes for RKN resistance, and (3) to elucidate the relationship between different resistant QTLs.

Materials and methods

Mapping population

The F_2 mapping population utilized in this study has previously been described in Shen et al. (2006). Two F_1 plants from an interspecific cross between the highly resistant Upland cotton line M120 (Shepherd et al. 1996) and the susceptible *G. barbadense* cv. Pima S-6 were self-pollinated giving rise to 138 and 107 F_2 progenies, respectively. The two groups of F_2 plants along with 6 plants of each parent and 5 F_1 plants were planted in greenhouse in a completely randomized design. At 3 weeks after seedling emergence, the plants were inoculated with approximately 8,000 eggs of *M. incognita* race 3 (approximately 450 eggs 150 cm^{-3}

soil) (Shen et al. 2006). Nematode eggs were collected from tomato roots (*Solanum lycopersicum* L.) by agitating roots in 0.5 % sodium hypochlorite solution for 2 min (Hussey and Baker 1973) at 1 h before inoculation. Inoculum was distributed into two holes about 2.5 cm deep and covered with soil. Pots were watered immediately following inoculation. The soil temperatures varied between 17 and 28 °C.

Plants were evaluated for their ability to host *M. incognita* reproduction at 56–58 days after inoculation. The phenotypic data commonly used to determine resistance reaction to nematodes were collected; they include root galling index, total number of eggs extracted, and the mean number of eggs per gram of fresh root. Roots were washed free of soil, evaluated for galling, weighed, cut into 5-cm pieces, and agitated in a 1 % sodium hypochlorite solution in a 1-l flask for 4 min (Hussey and Baker 1973). Eggs were collected and rinsed with tap water on nested 150- over 25-mm-pore sieves. Root galling was evaluated using the 0–10 scale where 0 = no galling, 1 = 1–10 % of the root system galled, 2 = 11–20 % of the roots system galled, etc., with 10 = 91–100 % of the root system galled (Bridge and Page 1980). Roots of each plant were harvested and weighed immediately prior to extraction of eggs. Eggs were extracted from the whole root system and counted. The mean number of eggs per gram of fresh root (eggs/g of root) was evaluated to standardize egg counts.

SSR analysis

One-thousand and four-hundred publicly available SSR markers from six different sources including NAU (Guo et al. 2007, 2008; Han et al. 2004, 2006), BNL, JESPR (Reddy et al. 2001), CGR (Xiao et al. 2009) CIR (Nguyen et al. 2004) and STV (Taliercio et al. 2006) were screened for polymorphisms between the M-120 RNR and Pima S-6 parents. The SSR primer sequences were downloaded from the Cotton Marker Database (http://www.cottonmarker.org) (Blenda et al. 2006) and were commercially synthesized by Operon (Eurofins MWG Operon, Huntsville, AL, USA). The PCR amplifications were performed on a Peltier Thermal Cycler (MJ Research, Waltham, MA, USA) as described in Shen et al. (2010) and PCR products were electrophoresed on a 10 % non-denatured polyacrylamide gel using DYZ-30 electrophoresis apparatus (Liuyi, Beijing, China). The DNA fragments were visualized by staining with silver nitrate as described (Zhang et al. 2002). The SSR primer pairs showing polymorphisms between the parents were tested on the mapping populations.

Data analysis

Genetic mapping and QTL analyses were performed on the combined dataset consisting of 245 F_2 individuals. Linkage maps were constructed using the Mapmaker/EXP (Lander et al. 1987) software. Logarithms of odds (LOD) score of 8.0 and maximum recombination fraction of 30 cM were set as grouping thresholds. Recombination units were converted into genetic distances using the Kosambi mapping function (Kosambi 1944). Detection of QTL and estimation of genetic parameters were performed with composite interval mapping (CIM) function of the software WinQTL Cartographer version 2.5 (Wang et al. 2005). QTLs were declared significant if the corresponding LR score was greater than 11.5 (equal to an LOD score of 2.5). The percent of phenotypic variance (PV) explained by a QTL (R^2) was estimated at highest probability peaks. QTL cartographer assumes that the quantitative data under consideration are normally distributed (Wang et al. 2005). However, as with most quantitative disease resistance phenotypes, none of the data for root-knot nematode resistance in this study fits this assumption (Table 1). A common method to normalize the data used in QTL mapping is to conduct $\text{Log}_{10}(x + 1)$ transformation (Noe 1985). Therefore, for the total egg count and eggs g^{-1} of root phenotypes, both the non-transformed and Log transformed data were used for the QTL analysis. Epistatic interaction effects were estimated using the mixed-model based QTL Network 2.0 program (Yang et al. 2008). Permutation test of 1,000 times at a significance level of $p = 0.005$ was conducted for minimizing the experimental type-I error rate.

DNA sequence analysis and STS markers development

For EST-SSR markers that were not polymorphic between the two parents, the sequences flanked by primers were submitted to BLASTN for sequence comparison against Genbank databases to obtain the full-length cDNA sequence from cotton and other species. The sequence data were then exported to a PC workstation for editing and sequence alignment analysis using the geneDOC software (Nicholas and Nicholas 1997). The phylogenetic analysis was conducted with the PHYLIP program of the ClustalX software package (Version 1.81) (Thompson et al. 1997). Sequence Tagged Site markers were designed using the Primer 5.0 software.

Results

Phenotypic evaluation

The resistance reaction to RKN for the parents and the F_2 population was previously reported (Shen et al. 2006). The resistant parent M120 was associated with a lower galling index, total egg count and eggs/g of root as compared with the susceptible parent Pima S-6 (Table 1). The

Table 1 Phenotype values for nematode resistance of F_1, F_2 and their parents

Trait	Parents		F_1	F_2				
	Pima S-6	M120 RNR		Max	Min	Mean	SD	Skew
Galling index	5.7	1.3	1.0	10	0	3.1	2.70	1.19
Eggs	91,650	550	3,120	446,400	0	18,302	39,475	6.66
Eggs g^{-1} root	4,061	56	113	38,483	0	2,251.5	3,830	4.90

F_1 plants showed high levels of resistance, with the galling index (1.0), total egg count (3,120, ranges from 600 to 6,600) and eggs/g of root (113, ranges from 14.3 to 289.5) not significantly different from the resistant parent M120 (1.3, 550, and 57 for galling index, total egg count, and eggs/g of root, respectively). In the F_2 population, all three phenotypes displayed a non-normal distribution (skewness exceeding 1.0), with individuals skewed toward the resistant parent (Table 1). These data indicated that resistance was mainly governed by dominant and/or additive, but not recessive genes. Galling index was correlated ($p < 0.01$) with eggs/g of root at a value of $r = 0.57$, and with total egg count at $r = 0.53$, whereas eggs/g of root was correlated with total egg count at $r = 0.91$. The moderate positive correlation between root galling and egg production suggests that either the two phenotypes measure the same genetic factor but with different precision or the two phenotypes may be governed by unlinked genes with different modes of action in suppressing RKN infection and reproduction.

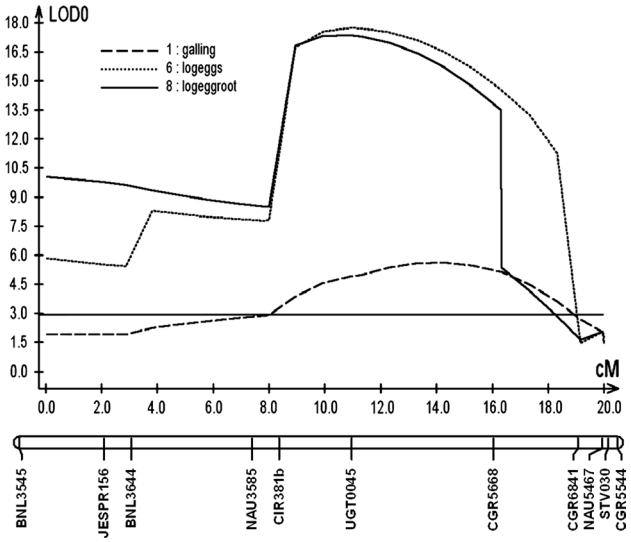

Fig. 1 QTLs associated with nematode resistance detected on Chromosome 14

QTL mapping

A total of 276 (19.7 %) SSR primers were polymorphic between M120 and Pima S-6, producing 328 marker loci that mapped to 26 linkage groups with a total map distance of 2,886 cM or about 88 % of the cotton genome, which estimated to be about 3,295 cM (Rong et al. 2004). Because the phenotypes displayed a non-normal distribution, only log transformed data for total eggs and eggs/g of root phenotypes were used for further analysis. QTL analysis detected two regions significantly associated with the resistant phenotypes; a region on the long arm of chromosome 11 near CIR316-CIR069 and a region on the short arm of chromosome 14 near CIR381-CGR5668 (Fig. 1). The QTL on chromosome 11 has previously been described in Shen et al. (2006). This QTL, which was given the name *qMi-C11*, had an LOD score of 19.21 and accounted for 63.7 % of the PV for galling index. The total eggs and eggs/g of root phenotypes also showed significant association, but the PVs explained by this locus were only 9 and 15 %, respectively. This QTL showed a completely dominant gene action and the allele from the resistant parent M120 increased nematode resistance for all phenotypes. The *qMi-C11* was later fine mapped to a 3.6 cM interval flanked by the SSR marker CIR069 and the AFLP marker E14M27-375 (Shen et al. 2010).

The QTL on chromosome 14 has not been reported in the resistant line M120. This resistant QTL, which has a large genetic effect on total eggs and eggs/g of root (discussed below), was given the name *qMi-C14* after the conventional nomenclature for QTL, where acronym of the scientific name of the pathogen causing the disease is in lowercase followed by the chromosome number (Shen et al. 2006). To develop markers more closely linked to the resistance locus, a functional EST-SSR marker NAU5499 was identified to have previously mapped near the QTL (Guo et al. 2008), but was monomorphic between M120 and Pima S-6. The sequence of NAU5499 clone (NCBI ID DR458210) was blasted through the NCBI EST database to obtain 3 overlapping cotton EST sequences (DW490390, DT047508, DT050033). Sequence analysis of the 1,105 bp contig indicated the presence of the complete ORF, 5-UTR, 3-UTR and the Poly (A) tail suggesting that a full-length cDNA was obtained. Comparison of this cDNA to sequences in NCBI databases showed that it encodes the gene for endoplasmic reticulum retention defective 2 protein. Three STS markers were designed in the different regions of the ERD2 and one primer (UGT0045;

Table 2 Biometrical parameters of the *qMi-C14* locus on roots galling and RKN eggs production

Chr. 14 CIR381-CGR5668	LOD	Add	Dom	*d*/*a*[a]	PV(%)
Total eggs	17.3	−0.47	0.44	−0.94	45.7
Eggs/g of root	17.6	−0.47	0.46	−0.98	44.9
Galling index	5.7	−0.19	1.72	−9.05	8.3

[a] Dominant gene action when the absolute *d*/*a* ratio is greater than 1

5′-CGGTATCACAAAATAGTTCG-3′ and 5′-GCACCAAGGAGAAACACATA-3′) was polymorphic between M120 and Pima S-6. This marker was subsequently mapped to a region between the SSR marker CIR381 and CGR5668 on chromosome 14 (Fig. 1).

Genetic effects of *qMi-C14*

The inclusion of UGT0045 in the CIM placed the *qMi-C14* locus in the interval flanked by the markers CIR381b and UGT0045, and about 0.2 cM from UGT0045 (Fig. 1). Based on the transformed data, this QTL had an LOD score of 17.3 and 17.6 and accounted for 45.7 and 44.9 % of the phenotypic variation (PV) in total eggs and eggs/g of root phenotypes, respectively (Table 2). These results indicated that *qMi-C14* has a large genetic effect on suppressing total egg production. The *d*/*a* ratio was 0.94–0.98 indicating that the gene action for this QTL approaches dominance effects.

The outcome of the linkage analyses with galling index phenotype was also highly significant, but the LOD score was only 5.7 and the PV explained by this locus was 8.3 %, which was much lower than from the total egg count phenotype. The low PV indicated that this locus only had a minor effect on suppressing root galling. The absolute *d*/*a* ratio was greater than one, indicating that this QTL showed over dominant gene action. The allele from the resistant parent M120 increased nematode resistance for all phenotypes.

Epistasis analysis

Three distinct genotypic classes of individuals were identified based on the genetic makeup of the QTLs conferring resistance to RKN. The first class included eight individuals carrying both *qMi-C11 and qMi-C14* (homozygous for M120 alleles at the marker intervals CIR069-CIR316 and NAU3584-CGR5668), the second class included eight individuals carrying only *qMi-C11* (homozygous for M120 alleles at the marker interval CIR069-CIR316 but homozygous for Pima alleles at marker interval NAU3584-CGR5668) and the third genotypic class included three individuals carrying only *qMi-C14* QTL (homozygous for M120 alleles at the marker interval NAU3584-CGR5668 but homozygous for Pima alleles at the marker interval CIR069-CIR316). The phenotypic changes in terms of percent deviation from the population mean for the galling index, total egg count and eggs/g of root are shown in Fig. 2. When only the *qMi-C11* locus was present, 22 % reduction in galling was observed with no change in total egg production or eggs/g of root. However, when only the *qMi-C14* locus was present, galling was reduced by 12 %, total eggs production was reduced by 11 % and eggs/g of root was reduced by 15 %. Finally, when both QTLs were present, up to 54 % reduction in galling, 30 % reduction in total eggs production and 35 % reduction in eggs/g of root were observed. These results suggest the existence of epistatic interaction between the two RKN resistance loci. Significant additive × additive interaction was detected between the two resistance loci for total egg count and eggs/g of root phenotypes but not for galling (Table 3). Therefore, while main additive effects serve as the major genetic basis for the two resistance QTLs, epistasis is an important genetic component for reducing eggs production resulting in higher degree of resistance exhibited by M120. These interaction effects explained 4.9 and 6.0 % of the phenotypic variation for total eggs production and eggs/g of root, respectively.

Origin of *qMi-C14* in the M120 line

The marker UGT0045 was tested on a panel of RKN resistance lines involved in the development of the resistant parent M120; Auburn 623 RNR, Auburn 634 RNR, Clevewilt 6, Mexico Wild Jack Jones, Coker 201, and Auburn 56. The result showed that the 300 bp DNA fragment amplified using primer UGT0045, which showed tight linkage to the *qMi-C14* resistant locus, was present in M120, Auburn 623 RNR, Auburn 634 RNR, and Mexico Wild Jack Jones, but was absent in Clevewilt 6, Auburn 56, and Coker 201 (Fig. 3). This result indicated that the *qMi-C14* resistant locus in M120 line likely was originated from the moderately resistant line Mexico Wild Jack Jones.

In addition, we tested seven RKN resistant germplasm** from the M-series lines derived from Auburn 634 RNR source (M-92 RNR, M-240 RNR, M-249 RNR, M-272 RNR, M-315 RNR, M-331 RNR, and M-725 RNR) as well as the two RKN resistant cultivars Stoneville LA887 and Acala NemX. The results showed that the 300 bp DNA fragment amplified by the primer UGT0045 was present in all the tested M-series lines but was absent in Stoneville LA887, which originated from a cross between Clevewilt 6 and Deltapine 15 (Robinson et al. 2001), and Acala NemX, which shared no known common parentage with Auburn 623 RNR or Stoneville LA887 (Robinson et al. 2001). These results further indicate that the RKN resistance *qMi-C14* locus in M120 was originated from Mexico Wild Jack Jones, and this resistant gene was not present in Stoneville LA887 and Acala NemX.

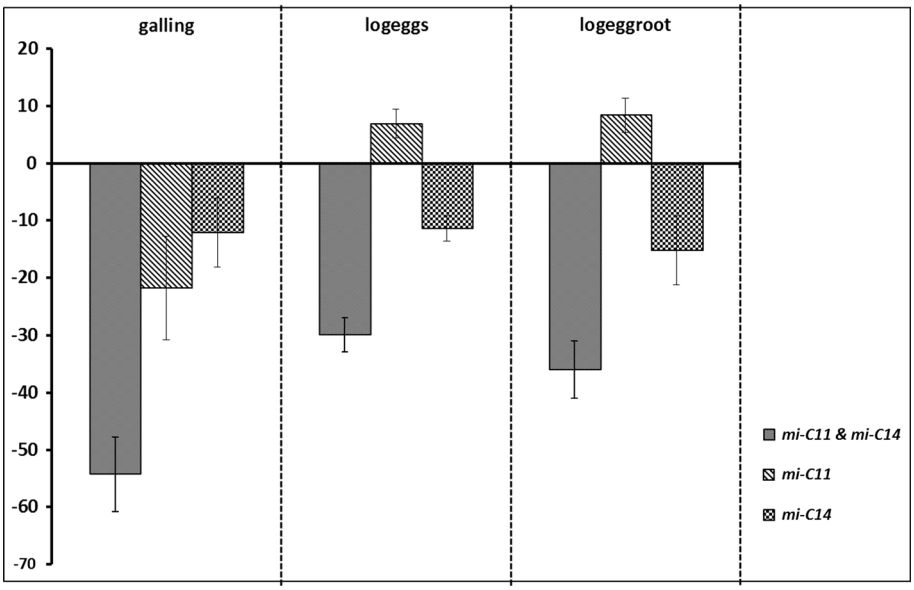

Fig. 2 Genetic effects of q*Mi-C11* and q*Mi-C14* on the suppression of galling and egg production as indicated by percent deviation from population mean

Table 3 Epistatic interaction between two RKN QTLs

Trait	QTLi	Interval-i	QTLj	Interval-j	AA	*p* value	H^2(aa)
Total eggs	1–6	UGT0045-CGR5668	2–1	CIR069-CIR316	−0.3007	0.001509	0.0494
Eggs/g of root	1–5	CIR381-UGT0045	2–1	CIR069-CIR316	−0.3586	0.000016	0.0596

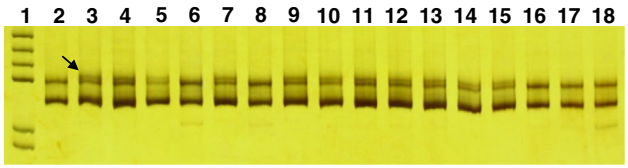

Fig. 3 Root-knot nematode resistance locus on chromosome 14 amplified by STS primer UGT0045. Lane 1 Marker, 2 Pima S-6, 3 M120, 4 Auburn 634 RNR, 5 Auburn 623 RNR, 6 Clevewilt 6, 7 Mexico Wild Jack Jones, 8 Coker 201, 9M-92 RNR, 10M-240 RNR, 11M-249 RNR, 12M-272 RNR, 13M-315 RNR, 14M-331 RNR, 15M-725 RNR, 16 Auburn 56, 17 Acala NemX, 18 LA887

Discussion

The resistant germplasm Auburn 623 RNR and the elite breeding lines derived from it remain the most important source of RKN resistance because they exhibit the highest level of resistance known to date in Upland cotton. In this respect, the identification and characterization of the resistance genes in this resistance source are of great interest to cotton nematologists and breeders. In a previous study using the bulk segregant analysis (Michelmore et al. 1991), we mapped a major RKN resistance QTL in the M120 resistant line located in the telomeric region of Chromosome 11 (Shen et al. 2006, 2010). Herein, by utilizing linked SSRs markers covering all 26 linkage groups and more than 88 % of the cotton genome, QTLs analysis revealed that in addition to the previously reported *qMi-C11* locus on Chromosome 11, a major RKN resistance QTL *qMi-C14* was present on chromosome 14 in the M120 resistant line. Prior genetic segregation analysis using the resistant line M-315 (McPherson et al. 2004) and more recent genetic mapping analysis using the M-240 (Gutierrez et al. 2010) suggest that resistance to RKN in this line is conferred by two major genes mapped on Chromosomes 11 and 14. Since the RKN resistance in M120, M-315, and M-240 was all derived from the Auburn 623 RNR source, collectively these results have now established that two major genes located on Chromosome 11 and 14 mostly confer the near-immunity reaction in this source of resistance.

The Auburn 623 RNR was derived from the cross of Clevewilt 6 and Mexico Wild Jack Jones, both of which are only moderately resistant to RKN (Shepherd 1974a, b). Consequently, it has long been debated which parental line contributed the genes for the RKN resistance in Auburn 623 RNR. Our results from tracing the origin of the two resistance genes in the M120 line confirmed previous observation in the M-240 line that the *qMi-C11* locus was inherited from the Clevewilt 6 parent, whereas the *qMi-C14* locus was inherited from the Mexico Wild Jack Jones parent (Shen et al. 2006; Gutierrez et al. 2010). Further, the SSRs allele linked to both QTLs were present in all the

M-series lines tested in this study and in Shen et al. (2006) suggesting that resistance in these lines is mainly conferred by these loci (Fig. 3). Therefore, the emerging picture is that both Clevewilt 6 and Mexico Wild Jack Jones carry at least one major resistance gene and the RKN resistance in the Auburn 623 RNR is due to the effects of pyramiding two major genes inherited from the different parents.

It is interesting to note that our QTL analysis indicated that the *qMi-C11* locus had a much stronger effect on root gall suppression than egg production while the *qMi-C14* locus had a stronger effect on egg production than galling (Fig. 2). Gutierrez et al. (2010) also observed the same results in the genetic population derived from the resistant line M-240, which carry the same QTLs but in Deltapine 61 genetic background. This is not surprising because the correlation between galling and eggs was only 0.53 (Shen et al. 2006), suggesting that the two phenotypes, while commonly used to measure resistance reaction to RKN, could very well be partly genetically independent. The separation of galling and nematode egg production has been observed in different cotton germplasm with resistance to RKN (Jenkins et al. 1995, Mota et al. 2012). Further, Shepherd (1979) reported that Mexico Wild Jack Jones was more susceptible to root galling than Clevewilt 6 but had a much lower RKN egg production. These results strongly suggest that the resistance loci originally inherited from Clevewilt 6 and Mexico Wild Jack Jones may have different mechanisms for resistance. Full characterization of the genes underlying *qMi-C11* and *qMi-C14* will advance our understanding of the different defense mechanisms against parasitism from RKN, and elucidate the host-pathogen interactions at the molecular level.

Histopathological studies suggest that resistance in the Auburn 623 RNR source involves a two-stage mechanism (Creech et al. 1995; Jenkins et al. 1995). In the early defense mechanism, parasitism is suppressed after second-stage infective juveniles (J2s) penetration or during its initial tissue migration at around 6–8 days post-penetration. The second defense mechanism occurs at about 21–24 days post-penetration and further limits the development of nematodes and the formation of giant cells. A recent histological study showed that the early defense reaction was similar to an hypersensitive response where programmed cell death occurs around the area of infection when nematodes penetrate the epidermis and migrate through the root cortex while the second resistance stage involved a mechanism that impedes the formation of functional feeding sites, thereby suppressing the development of later stage juveniles into female adults (Mota et al. 2012). Because the *qMi-C11* locus mostly affects root gall suppression, we hypothesized that *qMi-C11* locus confers an early defense mechanism in the form of a hypersensitive reaction, which blocks J2s that have penetrated the roots from developing giant cells and forming well-developed galls. Further, we hypothesized that the *qMi-C14* locus does not inhibit the establishment of feeding sites but confers a delayed hypersensitive response occurring at a later stage infection resulting in the collapse of the developing giant cells, preventing the nematode from developing into reproductive maturity to begin egg production. Experiments are in progress investigating the hypothesis of independent resistance genes controlling plant responses to suppression of root galling and nematode reproduction by testing near-isogenic lines carrying only the resistance gene on *qMi-C11* locus or *qMi-C14* locus, but not both.

The near immunity to RKN parasitism in Auburn 623 RNR, and presumably in a number of elite breeding lines derived from it, was due to transgressive segregation because it was developed from a cross between two moderately resistant parents (Shepherd 1974a, b). Therefore, a possible explanation of the transgressive segregation in Auburn 623 RNR is that it may be due to the stacking of genes from two moderately resistant parents with different mechanisms for resistance (Shen et al. 2010; Gutierrez et al. 2010). Interestingly, while main effect QTLs appeared to serve as the major genetic basis in conferring resistance for both galling and eggs production phenotypes, our analysis also suggests that additive × additive interaction was important in suppressing nematode eggs production (Table 3), resulting in a higher level of resistance exhibited by M120. Therefore, our data suggest that the transgressive phenotype observed was due to the pyramiding of two moderately resistant QTLs as well as an epistatic interaction between the two resistant loci (Fig. 2). This result is consistent with the observation that the level of resistance was significantly lower if only one of the resistance loci was present (Gutierrez et al. 2010). While not common, epistatic interaction among nematode resistance QTLs has been observed in several crops including soybean (Wu et al. 2009) and cotton (Wang et al. 2008, 2012).

It is interesting to note that while the inheritance of the resistance to root-knot nematodes has been well established and germplasm with a high level of resistance has been extant since the 1970s (discussed above), cotton breeding programs have only been moderately successfully in deploying the resistance genes in elite cultivars. The challenge in developing RKN resistant cultivars is mostly that the phenotypic screening process is tedious, time consuming, and destructive. In addition, the mode of resistance from Auburn 623 RNR and other germplasm derived from it appears to be imparted by two major genes (Ynturi et al. 2006; McPherson et al. 1995; Gutierrez et al. 2010), and resistance is greatly reduced if either gene is lost during cultivar development. Finally, the phenotypic distribution of the F_2 population (Table 1) as well as the QTL analyses (Table 2) is consistent with previous observations in demonstrating the dominance

of the resistant allele on either one or both the *qMi-C11* and *qMi-C14* locus (Ynturi et al. 2006; McPherson et al. 1995; Jenkins et al. 2012). Therefore, the phenotypic similarity between plants that are homozygous and heterozygous for the two resistant alleles complicates selection of homozygous resistant plants in a breeding population. In the backcross breeding that lead to the development of the highly resistant germplasm line GA120R1B3, after the initial M-120 RNR/PD 94042 cross, Davis et al. (2011) performed 3 backcrosses into PD 94042 followed by eight generations of self-pollination with phenotypic selection of only the most resistant plants in each generation. Despite that the population was advanced via single-seed descent, the progenies appear to continue to segregate for resistance, and a highly resistant true-breeding line was obtained only after progeny testing of lines with the resistant phenotype and reselection into the F_8 generation. Shepherd (1974a, b) reported a similar account in the difficulty of fixing the resistant trait in the development of the Auburn 623 RNR resistant line.

Jenkins et al. (2012) demonstrated the effectiveness of utilizing a single genetic marker linked to each of the two RKN resistance loci in selecting for highly resistant plants. The use of tightly linked, flanking markers could greatly improve the marker-assisted selection for the *qMi-C11* and *qMi-C14* locus by detecting and discarding plants that carry the diagnostic marker but not the resistant gene due to genetic recombination. In our earlier work, the *qMi-C11* locus was fine mapped to a region of 3.6 cM interval flanked by CIR069 and E14M27-375 (Shen et al. 2010), and in this study, the *qMi-C14* locus was mapped to a region of about 2 cM interval flanked by CIR381b-UTG0045. Together, these markers will greatly facilitate the improvement of RKN resistance in cotton via marker-assisted breeding.

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Conflict of interest The authors declare that they have no conflict of interest.

Ethical standards The experiments comply with the current laws of the country in which they were performed.

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