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ORIGINAL PAPER

Fine mapping *QMi-C11* a major QTL controlling root-knot nematodes resistance in Upland cotton

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Abstract The identification and utilization of a high-level of host plant resistance is the most effective and economical approach to control root-knot nematode (*Meloidogyne incognita*). In an earlier study, we identified a major quantitative trait locus (QTL) for resistance to root-knot nematode in the M-120 RNR Upland cotton line (*Gossypium hirsutum* L.) of the Auburn 623 RNR source. The QTL is located in a 12.9-cM interval flanked by the two SSR markers CIR069 and CIR316 on the distal segment of chromosome 11. To construct a fine map around the target region, a bulked segregation analysis was performed using two DNA pools consisting of five individuals, with each being homozygous for the two parental alleles. From a survey of 1,152 AFLP primer combinations, 9 AFLP markers closely linked to the target region were identified. By screening an additional 1,221 F_2 individuals developed from the initial mapping population, the *Mi-C11* locus was delimited to a 3.6-cM interval flanked by the SSR marker CIR069 and the AFLP marker E14M27-375. These results further elucidate the genetic fine structure of the *Mi-C11* locus and provide the basis for map-based isolation of the nematode resistance gene in M-120 RNR.

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Introduction

Meloidogyne incognita (southern root-knot nematode; RKN) is the most important endoparasitic pest of Upland cotton (*Gossypium hirsutum* L.) in the US, where it is found in all areas where cotton is grown. Infected plants produce extensive abnormal growth, which forms galls or knots throughout the root system at the nematode feeding site. In the US alone, RKN is estimated to cause an annual loss of 235 million pounds of lint (Cotton Disease Loss Estimate Committee 2008). In addition, RKN greatly increases the incidence and severity of Fusarium wilt (Smith 1941), caused by *Fusarium oxysporum* f. sp. *vasinfectum*, which causes an estimated annual loss of 39 million pounds of lint (Cotton Disease Loss Estimate Committee 2008). Because of the costs of nematicides and their high mammalian toxicity, host plant resistance is the most economical, practical, and environmentally sounds method to provide crop protection against RKN.

Although no highly resistant cultivars have been developed for use in the US cotton belt, three major sources of resistant germplasm have been developed and are available to the cotton breeder. One source is Auburn 623 RNR, which originated from transgressive segregation in a cross between two moderately resistant parents, Clevewilt 6 and Wild Mexican Jack Jones (Shepherd 1974a, b). This source also includes the derivative lines, 'M-120 RNR', 'M-315 RNR', 'M-155 RNR', and 'M-240 RNR', that resulted from crosses with cultivars that were

then considered to have good agronomic performance. The M series demonstrated good resistance to RKN and have good combining ability for both RKN resistance and other agronomic traits (Robinson et al. 2001). Another source is LA434-RKR and its derivative cultivars, Stoneville LA887 and Paymaster H1560. This source also originated from the resistant germplasm line Clevewilt 6 but provides only a moderate level of protection from RKN (Robinson et al. 2001). Finally the Acala NemX source, which is derived from crossing a *Gossypium barbadense* L. genotype and the Acala line 1-2302 (Robinson et al. 2001), also shows resistance to RKN. NemX is only well adapted for cotton production in the San Joaquin Valley of California thus precluding production in much of the US cotton-belt regions.

Recent genetic mapping studies showed that the resistance to RKN in both Acala NemX and the Auburn 623 RNR source is conferred by a major locus located on the same chromosome. Wang et al. (2006a) reported the mapping of a major resistance gene *rkn1* in Acala NemX at a distance of about 2.1–3.3 cM from the SSR marker CIR316 on linkage group A03, which was subsequently renamed to chromosome 11 (Wang et al. 2006b). In the Auburn 623 RNR source, Shen et al. (2006) identified a major QTL *Mi-C11* for resistance to RKN in the M-120 RNR line located on the distal segment of chromosome 11 about 2–4 cM from the same SSR marker CIR316. This QTL was later independently confirmed by Ynturi et al. (2006) and Niu et al. (2007) via QTL analysis using the M-240 RNR and Auburn 634 RNR resistant lines, respectively, which were both derived from Auburn 623 RNR and believed to carry the same resistance genes (Starr et al. 2007). Although the RKN resistance loci from both sources were linked to the bridge marker CIR316, there is presently insufficient marker density around the target region to resolve the two loci to determine if they are allelic. In addition to the major resistance locus on chromosome 11, a number of RKN resistance genes with smaller effects have also been reported, including a QTL for root galling reduction on chromosome 7 (Shen et al. 2006), a QTL for reducing nematode egg production on chromosome 14 (Ynturi et al. 2006), and a transgression factor on chromosome 11 (Wang et al. 2008).

Herein, we report the high-resolution genetic mapping of the *Mi-C11* locus on chromosome 11 and the addition of genetic markers in the flanking QTL region. By screening a large population of F_2 progenies using additional genetic markers, we substantially reduced the likelihood interval and therefore improved the precision of the location of the *Mi-C11* locus. This sets the stage for future research to not only determine if *rkn1* and *Mi-C11* are allelic, but more importantly, for developing a marker assisted selection program to improve RKN resistance and initiating map-based cloning of the gene(s) underlying RKN resistance.

Materials and methods

Mapping population and resistance screening

The mapping population used was derived from the same interspecific cross reported in Shen et al. (2006) from crossing a highly resistant Upland cotton line M-120 RNR (Shepherd et al. 1996) and the susceptible *G. barbadense* cv. Pima S-6. Twelve F_1 plants were self pollinated to yield an F_2 segregating population consisting of 1,252 plants. The root galling index that indicates resistance reaction to RKN was collected using the procedure as described in Shen et al. (2006). The population was divided into three groups of 382, 347, and 523 plants because of limited greenhouse space. Each group of F_2 plants was planted along with 9–13 plants from each of the two parents and the F_1 and inoculated with approximately 8,000 eggs of *M. incognita* race 3 (approximately 450 eggs per 150 cm^3 soil) at 3 weeks after seedling emergence. Nematode eggs were collected from tomato roots (*Lycopersicon esculentum* L.) by agitating roots in 0.6% sodium hypochlorite solution for 2 min (Hussey and Barker 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 57 (first batch), 59 (second batch) and 60 (third batch) days after inoculation. The galling index commonly used to determine resistance reaction to nematodes were collected and 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 (Shen et al. 2006; Bridge and Page 1980).

Molecular genotyping

SSR analysis

Six SSR markers (CIR069, CIR316, CIR196, NAU2016, NAU2152 and BNL1066) closely linked to the *Mi-C11* locus on chromosome 11 (Shen et al. 2006) were used to genotype the entire F_2 population. PCR amplifications were performed as described in Zhang et al. (2002), using a Peltier Thermal Cycler (MJ Research, Waltham, MA, USA), and PCR products were electrophoresed on a 10% non-denatured polyacrylamide gel using a Bio-Rad Protean

II xi electrophoresis apparatus (Bio-Rad, Hercules, CA, USA).

AFLP analysis

The AFLP protocol as descried by Vos et al. (1995) was followed using the enzyme combination *Eco*RI/*Mse*I(+3/+3) and *Pst*I/*Mse*I (+2/+3). Genomic DNA (100 ng) was digested with two restriction enzyme combinations in a volume of 15 µl. Specific double-stranded adaptors were subsequently ligated to the restriction fragment ends. Pre-amplification reaction was carried out in a volume of 10 µl using 50 ng each of primers and 5 ng of adaptor-ligated DNA. The pre-amplification product was diluted (1:40), and an aliquot (1 µl) was used for selective amplification in a volume of 10 µl. For *Eco*RI/*Mse*I(+3/+3) enzyme combination, selective amplification products were electrophoresed on a 6.5% denatured polyacrylamide gel using a Bio-Rad Protean II xi electrophoresis apparatus (Bio-Rad, Hercules, CA, USA) and visualized by silver nitrate staining. For the *Pst*I/*Mse*I(+2/+3) enzyme combination, *Pst*I fluorescent selective primer, IRDye 700-labled, were purchased from Li-COR Biosciences. Selective amplification products were electrophoresed on a 6.5% denatured polyacrylamide gel on Li-COR model 4300 DNA analyzer.

Bulked segregant analysis

Bulked segregant analysis (BSA) was performed according to Michelmore et al. (1991) to identify candidate markers linked to the target region. *Mi-C11* had been mapped on chromosome 11 between SSR markers CIR069 and CIR316. Therefore, two DNA pools were constructed using DNA from plants selected from the original F_2 population (Shen et al. 2006) with each pool consisting of five homozygous Pima S-6 genotypes or five homozygous M-120 RNR genotypes for the SSR loci CIR069 and CIR316. DNA from these two pools and from the two parents was screened with 1,152 AFLP primer combinations.

Conversion of an AFLP marker to a SCAR marker

To convert closely linked AFLP markers into useful SCAR markers, the polymorphic fragments were excised from the polyacrylamide gel, subjected to three freeze–thaw cycles, and centrifuged at 11,000 rpm for 20 min. A 2 µl aliquot of supernatant was used as template for re-amplification using the same primer pairs that generated the polymorphic product. The re-amplified PCR products were checked on a 2% (w/v) agarose gel to ensure that they were of the proper-size. The purified PCR products were cloned into the pGEMT EASY vector (Promega, USA) according to manufacturer's instructions and transformed into JM119 competent cells. For each AFLP fragment, at least fifty clones were screened for insert size by PCR amplification using the corresponding selective primer pairs, and then ten clones that yielded the amplicon of the expected size were sequenced (MWG Biotech, USA). PCR primers for the SCAR markers were designed using the Primer3 software. The average length of primers was 20 nucleotides with an annealing temperature of 55°C. PCR amplification was performed using the protocol as described by Chee et al. (2004). PCR products were electrophoresed on a 10% non-denatured polyacrylamide gel using a Bio-Rad Protean II xi electrophoresis apparatus (Bio-Rad, Hercules, CA, USA).

Mapping and statistical analysis

Genetic mapping and statistical analysis were performed on four data sets: from each of the three batch experiments separately and the entire population by combining the three batches. Linkage maps were constructed using MAPMAKER/Exp Version 3.0b Software (Lander et al. 1987). A LOD score of 4.0 and a 50 cM maximum distance were used. Marker order was confirmed with the "Ripple" command. Recombination frequencies were converted into map distances (cM) using the Kosambi mapping function (Kosambi 1944). QTLs were identified by composite interval mapping (Zeng 1994) using Windows QTL Cartographer 2.5 (Basten et al. 2001).

Results

The galling index frequency distribution for the F_2 population, the F_1, and the parents are shown in Fig. 1. In each batch experiment, the susceptible parent sustained a consistently higher galling index than did the progenies, and the galling of the parental lines exhibited low variance thereby indicating the success of inoculation. The mean galling index differed between the resistant parent M-120 RNR and the susceptible parent Pima S-6 in all three experiments. The average galling index of the 27 F_1 plants, which was 3.67, was comparable to the index value of the resistant parent M-120 RNR, which was 3.41, thus indicating that the nematode resistance in M-120 RNR was under dominant genetic control.

The frequency distribution of the galling index displayed a bimodal distribution in all three separate experiments as well as in the combined F_2 dataset (Fig. 1) indicating that this phenotype was governed by one to a few major genes, and not by multiple genes with small effects, which would show a continuous variation. In the combined dataset, the mean of the resistant parent plus one standard deviation ($3.4 + 2.4 = 5.8$) and the mean of the susceptible parent minus one standard deviation

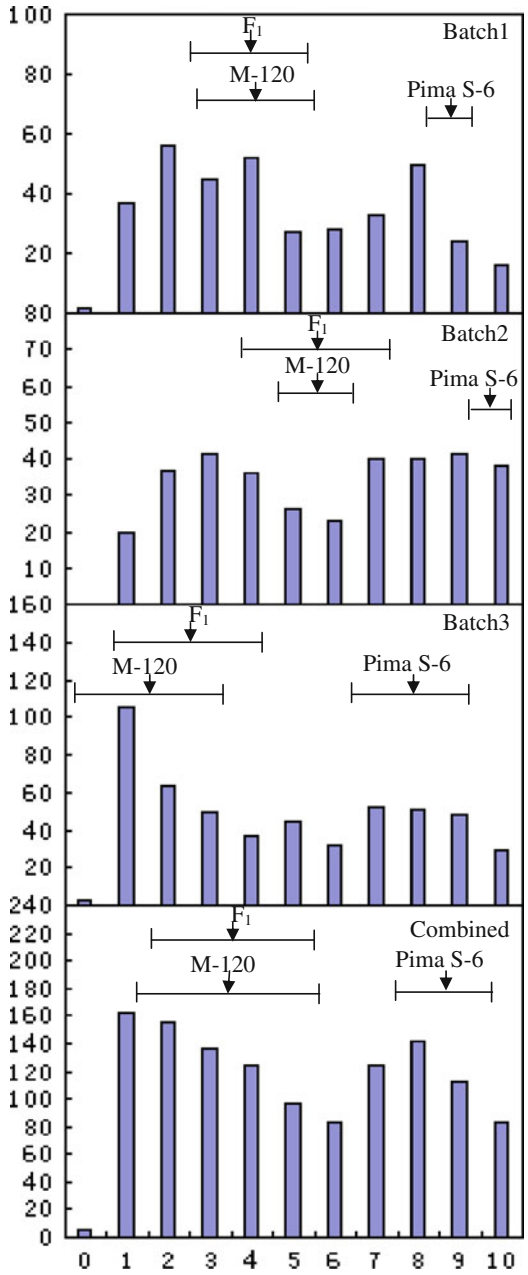


Fig. 1 The frequency distribution of the galling index for the three separate experiments (Batches 1–3) and the combined dataset (*Combined*). The average values and one standard deviation bar of the resistant *M-120* RNR parent, susceptible *Pima-S6* parent, and F_1 hybrid are indicated

$(8.7 - 1.3 = 7.4)$ did not overlap; these numbers were used as the basis to divide the F_2 plants into a resistant or a susceptible class. Using this classification criterion, we classified 765 plants as resistant (gall index ≤ 6) and 456 plants as susceptible (gall index ≥ 7). Chi-square goodness-of-fit test indicated that the segregation of resistant to susceptible progenies significantly deviate from a 3:1 ratio ($\chi^2 = 98.6$, $P < 0.01$) for a single dominant gene.

To assess the allelic effect of the resistance locus, we used the SSR marker closest to the resistance locus (CIR069, discussed below) to group the F_2 population into three genotypic classes; 226 F_2 were homozygous for the resistant parent M-120 RNR allele, 663 F_2 were heterozygous, and 292 F_2 were homozygous for the susceptible parent Pima S-6 allele. The difference in mean galling index between the homozygous M-120 RNR allele class (4.17) and the heterozygous (4.20) was non-significant; however, the two classes were significantly different from that of the homozygous Pima S-6 allele class (7.73). In addition, the mean galling index between the homozygous Pima S-6 allele class (7.73) and the susceptible parent Pima S-6 (8.72) is not significantly different. These results support our previous conclusion that this QTL has a complete dominant effect. The variance of homozygous Pima S-6 class is 4.14 and much lower than that of the homozygous M-120 RNR allele class (6.54), the heterozygous class (6.14), and entire F_2 population (8.56). The lack of phenotypic variation within the homozygous Pima class (no *Mi-C11* effect in this class) suggests that no other major genetic factors are segregating in this population.

Screening AFLP markers linked to the *Mi-C11* gene

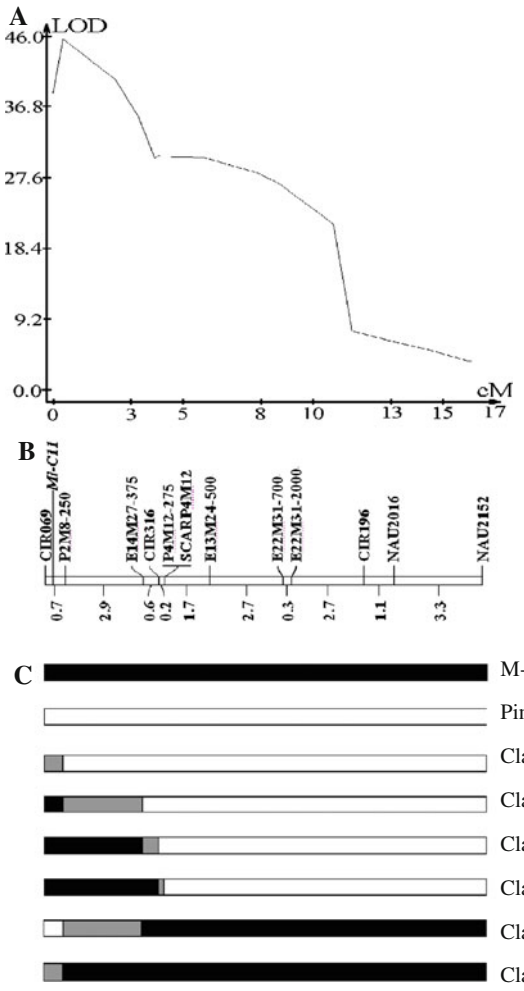
To enrich markers within the *Mi-C11* locus region, a 12.9-cM interval flanked by the SSR markers CIR069 and CIR316 (Shen et al. 2006), a bulked segregant analysis was conducted by using two DNA pools (see "Materials and methods"). Twenty-six candidate markers were identified after screening polymorphisms between the two pools and the two parents with the 1,024 E + 3/M + 3 and 128 P + 2/M + 3 primer combinations. To further examine potential linkage, candidate AFLP markers were examined using the original F_2 population of 198 individuals. Ten AFLP markers linked to the *Mi-C11* allele were obtained and mapped to the interval between the previously obtained SSR marker CIR069 and BNL1066 on chromosome 11. The newly identified AFLP markers are described in Table 1.

Construction of a local genetic map

Six SSR primers (CIR069, CIR316, CIR196, NAU2016, NAU2152, and BNL1066) mapped near the *Mi-C11* locus were used to genotype the 1,221 F_2 plants, and 463 were found to be recombinants in the region. This recombinant subpopulation was further genotyped using the 10 linked AFLP markers identified in the original F_2 population to construct a fine genetic map that spanned 25.8 cM. Two AFLP markers P2M8 and E14M27 were mapped between the interval of CIR069 and CIR316, a 12.9-cM region in which *Mi-C11* was putatively located (Shen et al. 2006).

Table 1 Description of AFLP and SCAR markers linked to the *Mi-C11* gene

Marker	Selective nucleotides	Size (bp)	Codominant/dominant
P2M8	P-GAT/M-CCC	250	Dominant
P4M12	P-GAG/M-CTC	275	Dominant
SCARP4M12	F: CACAACATTCTCAACATGGAGA	165/190	Codominant
	R: GGGAACAACTTCCACATTGA		
E14M27	E-GTA/M-GTC	375	Dominant
E13M24	E-GTT/M-GCG	500	Dominant
E22M31	E-ACG/M-GGC	690	Dominant
E22M31	E-ACG/M-GGC	2,000	Dominant
E20M4	E-ACT/M-CAG	90	Dominant
E21M32	E-ACC/M-GGG	580	Dominant
E6M11	E-GGA/M-CTG	90	Dominant
M1RGA2R	M-CAA/RGA2R	120	Dominant



No. of Recombinant	Resist.	Suscep.
2	1	1
19	15	4
4	3	1
3	2	1
18	0	18
1	0	1

Fig. 2 Interval mapping of root-knot nematode QTL (**a**); Localized linkage map on chromosome 11 (**b**) and graphical genotypes of recombinant lines and their resistance (**c**). *Solid bars* and *open bars* represent the *M-120 RNR* and *Pima S-6* type, respectively. *Gray bars* represent the interval in which recombination has occurred

The marker P2M8 was mapped at 0.7 cM from CIR069, while E14M27 was mapped at 0.6 cM from CIR316 (Fig. 2b). Of the other eight AFLP markers, four were mapped in the interval between CIR316 and CIR196 and three were mapped between NAU2152 and BNL1066.

High-resolution genetic and substitution mapping of *Mi-C11*

Interval mapping showed a single QTL peak near CIR069 with LOD scores ranges from 25.13 to 32.89 for the three

experiments, and of 45.52 for the combined dataset of 1,221 individuals (Table 2). Across the three experiments, this locus explained 32.1% of the total phenotypic variation (Fig. 2a), and the additive and dominant effect was −1.56 and −2.07, respectively.

To further define the QTL position, a total of 47 recombinants representing a different recombination breakpoint between CIR069 and P4M12 were grouped into six recombinants classes. Their graphical genotypes are shown in Fig. 2c. Recombinant classes 1–6 carried different M-120 RNR segments in the target region in the Pima S-6 background, which had recombination events between the intervals CIR069 to P2M8, P2M8 to E14M27, E14M27 to CIR316, and CIR316 to P4M12, respectively. Recombinant Class-1 had two F_2 individuals that had the CIR069 to P2M8 M-120 RNR fragment of which one showed resistance and one was susceptible; recombinant Class-2 had 19 F_2 individuals that carried the CIR069 to E14M27 M-120 RNR fragments of which 15 recombinants showed resistance and 4 were susceptible; recombinant Class-3 had 4 F_2 individuals that carried the CIR069-CIR316 M-120 RNR fragment of which 3 recombinants showed resistance and one was susceptible; and recombinant Class-4 had 3 individuals that carried the CIR069 to P4M12 M-120 RNR fragment of which 2 recombinants showed resistance and one was susceptible. Finally, recombinants Class-5 and -6 that carried different Pima S-6 segments in the target region in the M-120 RNR background had recombination events between the intervals P2M8 to E14M27, and CIR069 to P2M8, respectively, were all highly susceptible to RKN. These results indicate the chromatin segment in the CIR069 to P2M8 interval from M-120RNR had a positive effect on nematode resistance and suggests that *Mi-C11* is located in a 3.6-cM interval flanked by CIR069 and E14M27-375.

Conversion of AFLP marker into SCAR marker

Three AFLP markers (P2M8-250, E14M27-375 and P4M12-265) closely linked to *Mi-C11* were selected for conversion to SCAR markers. Ten clones per marker that yielded amplicons of the expected size were sequenced. The sequence data revealed the presence of a selective amplification primer sequence, confirming the correct identification of the fragments. Based on this sequence information, two pairs of primers per sequence were designed for amplification of the corresponding loci from parents' genomic DNA. Of the three AFLP loci, only primers from P4M12 fragment (fwd: 5′-CACAACAT TCTCAACATGGAGA; rev: 5′-GGGAACAACTTCCAC ATTGA) detected polymorphism between the two parents with the resistant parent M-120 RNR showing a single 190-bp fragment and the susceptible parent Pima S-6 a single 165-bp fragment (Fig. 3). Analyzing the whole population of 1,221 individuals indicated this co-dominant SCAR marker co-segregated with the original AFLP marker P4M12-275.

Table 2 Biometrical parameters QTL associated with the *Mi-C11* locus

	LOD	Add	Dom	d/a[a]	PV (%)
Batch 1	26.03	−1.596	−2.150	1.347	39.40
Batch 2	32.89	−2.106	−1.595	0.757	37.59
Batch 3	25.13	−1.605	−1.780	1.109	27.44
Combined	45.52	−1.56	−2.070	1.327	32.10

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

Discussion

The highly resistant line Auburn 623 RNR is believed to carry more than one genetic factor for RKN resistance because it was a transgressive segregant derived from crossing two moderately resistant parents (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 M-78RNR, M-120 RNR, M-315 RNR, and M-155 RNR. In working with M-315 RNR and M-78 RNR, McPherson et al. (1995, 2004) reported that the RKN resistance in the Auburn 623 RNR source was simply inherited and proposed a two-gene model for resistance with one dominant and one additive gene named Mi_1 and Mi_2, respectively. Our QTL mapping results corroborate with those observed in previous experiments first reported by Shen et al. (2006) and subsequently by Ynturi et al. (2006) and Niu et al. (2007) in assigning the position of a major RKN resistance locus in the Auburn 623 RNR resistance source to the telomeric region of chromosome 11. Collectively, these results strongly indicate that the dominant factor Mi_1 is located on chromosome 11 previously mapped as a major dominant QTL. The resistance allele in this locus explained 32.1% of the total phenotypic variation, reducing galling index by 2.1 units, and appeared to be insensitive to genetic background (Ynturi et al. 2006; Niu et al. 2007). To more accurately describe this resistance locus, we propose revising the nomenclature thereby renaming this dominance resistant gene in the Auburn 623 RNR source to *QMi-C11* (see Shen et al. 2006 for the criteria used in naming QTLs).

Interestingly, we found no evidence for the presence of another major QTL contributed by the resistant M-120 RNR parent. Although the segregation of resistant to susceptible progenies significantly deviate from a 3:1 ratio for a single dominant gene, the frequency distribution of the

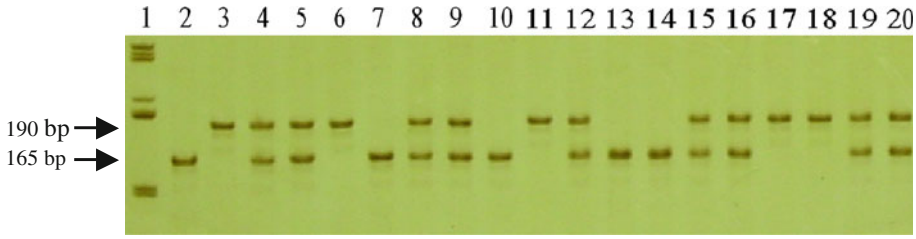


Fig. 3 Polymorphisms analysis of SCAR marker P4M12 in mapping parents and individuals. *1* molecular marker; *2* Pima S-6, *3* M120 RNR, *4* F_1, *5–20* some individuals of F_2 population

galling index was bimodal and after eliminating the genetic effect of the *Mi-C11* locus, all the F_2 plants with the homozygous Pima S-6 allele at the *Mi-C11* locus showed a high galling index and lacked significant variation in this trait. Also, we have performed a thorough QTL analysis on the initial population reported by Shen et al. (2006) using 275 additional SSR markers to provide a complete coverage of the cotton genome and detected no additional significant association with root galling index (He and Chee, in preparation). These results support our argument that the *Mi-C11* locus confers a majority of the genetic variance for the root galling reaction phenotype. However, the data also suggest that other QTLs with small effects, such as the minor QTL from the susceptible Pima S6 parent (Shen et al. 2006) or perhaps transgressive segregation factors such as RKN2 (Wang et al. 2008), may be segregating in the population.

It is important to note that the present QTL analysis is based on phenotyping by means of root galling phenotype, which is easy to evaluate and widely used to infer resistance response to RKN in cotton. We previously observed that root galling and RKN egg production, another commonly used means to phenotype for determination of resistance, is only moderately correlated with a coefficient of determination of $r = 0.53$–0.67 (Shen et al. 2006). Further, Shepherd (1979) reported that one of the parents of Auburn 623 RNR, "Mexico Wild", was more susceptible to root galling but had a much lower RKN egg production. Therefore, it may be conceivable that the control of resistance to root galling and resistance to reproduction may be genetically independent in the Auburn 623 RNR source. Although not frequently documented, such differential infection response pathways to nematode root galling and reproduction have been observed in several legume species (Fassuliotis et al. 1970; Harris et al. 2003; Roberts et al. 2008).

The separation of galling and nematode egg production has been suggested in the resistant cotton line M-315 RNR (Jenkins et al. 1995). Recent QTL analyses have identified a major QTL on chromosome 14 conferring reduction in eggs production but this locus apparently has little effect on root galling (Ynturi et al. 2006; He and Chee, in preparation). Further, while the *Mi-C11* locus was inherited from Clevewilt 6 (Shen et al. 2006; Ynturi et al. 2006), the locus on chromosome 14 was inherited from the Mexico Wild parent (Ynturi et al. 2006). Therefore, an intriguing 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. Experiments are in progress investigating the hypothesis of independent resistance genes controlling plant responses to suppression of root galling and nematode reproduction by developing and testing near-isogenic lines carrying only the resistance gene on chromosome 11 or chromosome 14, but not both. Such lines can be created by backcrossing the M-120 RNR lines to its recurrent parent Coker 201 (Robinson et al. 2001) followed by selfing the F_1 and selecting lines which differ only by the individual RKN resistance genes using the DNA markers linked to the respective QTLs.

While genotyping and phenotyping a large population size such as described herein is labor intensive and costly, it provides the power to address three important issues that could not be studied in the genetic mapping populations used in the previous RKN mapping studies based fewer than 200 individuals. First, the ability to precisely resolve closely spaced markers by linkage analysis is directly proportional to the size of the mapping population; therefore, genetic mapping utilizing less than 100 individuals can only accurately assign the order of markers which are several cM apart (Ferreira et al. 2006). The current population with 1,221 F_2 individuals would allow us to determine the orientation of markers at the resolution of less than 1 cM apart and such resolution is critical for future attempts in positional cloning of the resistant gene. In addition, a larger population size will provide a more robust estimation of recombinant frequencies because the detection of rare crossovers is subject to chance. Therefore, the observed smaller linkage distance between markers near the *Mi-C11* locus, such as the 4.2 cM between CIR069 and CIR316, likely is a better reflection of the true genetic distance than previously observed.

Finally, the appreciably larger number of recombinants recovered in this work enabled us to further dissect the interval of the *Mi-C11* locus by utilizing the substitution mapping approach (Paterson et al. 1989) that relies on the ability to distinguish overlapping chromosomal segments with different phenotypic effects. By adding new AFLP

markers to the QTL interval previously defined via SSR markers (Shen et al. 2006), the size of the inter-marker interval to which the QTL can be assigned was reduced from 7.8 to 2.9 cM; and after examining the phenotype of the recombinants classes carrying different recombination breakpoints between CIR069 and P4M12, the most plausible location of *Mi-C11* is in a 3.6-cM interval flanked by CIR069 and E14M27-375 (Fig. 2). Although additional experiments are needed to assess the relationship between the frequency of meiotic recombination (cM) and the physical distance (kilobases) between markers in the vicinity of the target locus, this work serves as a major step for which the QTL is assigned to an interval sufficiently small for physical mapping and a starting point toward cloning the gene underlying the QTL.

Currently, the SSR marker CIR316 is being utilized in numerous cotton breeding programs across the US to screen for resistant progenies. Although the redesigning of this SSR marker with a longer primer sequence has improved the PCR amplification (Shen et al. 2006), it did not reduce the amplification of multiple additional DNA fragments that are not associated with RKN resistance, but could contribute to scoring errors rendering the marker less effective at predicting progeny phenotype in breeding populations. The new codominant SCAR marker developed from AFLP fragment P4M12-265 that produces a single DNA fragment that can clearly discern the resistant and susceptible parents (Fig. 3) could be a valuable replacement for CIR316 marker in screening for RKN resistance progenies. Selection for the SCAR marker P4M12-265 in conjunction with CIR069 would ensure the recovery of the resistant *Mi-C11* allele, especially when used in backcrossing to rapidly introgress the resistant phenotype into the genetic background of elite cultivars because the probability of double recombination in a 3-cM region is low.

In addition to the *Mi-C11* locus, this particular telomeric region of the long arm of chromosome 11 appears to also contain numerous resistance genes for nematodes as well as to other pathogens. For example, as previously noted, both the RKN resistant genes *rkn1* in Acala NemX and a transgressive factor RKN2 in Pima S-7 were mapped near the SSR markers CIR316 and MUSC088 on chromosome 11 (Wang et al. 2006a, 2008; Roberts and Ulloa 2010). He et al. (2010) reported the detection of another major QTL for RKN resistance on chromosome 11 from the wild RKN resistant germplasm line M-495 RNR. Mapping about 20 cM from CIR316, this QTL is likely to be a different locus than *Mi-C11* and *rkn1*. In addition to RKN, genes conferring resistance to reniform nematode (*Rotylenchulus reniformis*) introgressed from *G. longicalyx* (Dighe et al. 2009) and possibly from *G. aridum* (Romano et al. 2009) into Upland cotton are known to be mapped on chromosome 11 or its homeolog chromosome 21. Finally, this genomic region also is known to harbor QTLs for resistance to Verticillium wilt (Bolek et al. 2005) and Fusarium wilt (Starr et al. 2007). Collectively, these results indicate that this region on chromosome 11 is likely a hot spot for resistance genes and worthy of further dissection at the molecular and physical level.

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