Theor Appl Genet (2006) 112: 770–777
DOI 10.1007/s00122-005-0183-0

ORIGINAL PAPER

C. Wang · M. Ulloa · P. A. Roberts

Identification and mapping of microsatellite markers linked to a root-knot nematode resistance gene (*rkn1*) in Acala NemX cotton (*Gossypium hirsutum* L.)

Received: 22 July 2005 / Accepted: 30 November 2005 / Published online: 14 December 2005

Abstract Host-plant resistance is the most economic and effective strategy for root-knot nematode (RKN) *Meloidogyne incognita* control in cotton (*Gossypium hirsutum* L.). Molecular markers linked to resistance are important for incorporating resistance genes into elite cultivars. To screen for microsatellite markers (SSR) closely linked to RKN resistance in *G. hirsutum* cv. Acala NemX, F_1, F_2, BC_1F_1, and $F_{2:7}$ recombinant inbred lines (RILs) from intraspecific crosses and an F_2 from an interspecific cross with *G. barbadense* cv. Pima S-7 were used. Screening of 284 SSR markers, which cover all the known identified chromosomes and most linkage groups of cotton, was performed by bulked segregant analysis, revealing informative SSRs. The informative SSRs were then mapped on the above populations. One co-dominant SSR marker CIR316 was identified tightly linked to a major resistance gene (designated as *rkn1*), producing amplified DNA fragments of approximately 221 bp (CIR316a) and 210 bp (CIR316c) in Acala NemX and susceptible Acala SJ-2, respectively. The linkage between CIR316a marker and resistance gene *rkn1* in Acala NemX had an estimated distance of 2.1–3.3 cM depending on the population used. Additional markers, including BNL1231 with loose linkage to *rkn1* (map distance 25.1–27.4 cM), BNL1066, and CIR003 allowed the *rkn1* gene to be mapped to cotton linkage group A03. This is the first report in cotton with a closely linked major gene locus determining nematode resistance, and informative SSRs may be used for marker-assisted selection.

Communicated by P. Langridge

C. Wang · P. A. Roberts (✉)
Department of Nematology, University of California,
Riverside, CA 92521-0415, USA
E-mail: philip.roberts@ucr.edu
Tel.: +1-951-8274442

M. Ulloa
USDA-ARS, WICS, Res. Unit, Cotton Enhancement Program,
Shafter, CA 93263, USA

Introduction

The southern root-knot nematode (RKN) *Meloidogyne incognita* Kofoid and White (Chitwood) is one of the most economically important pests of crops worldwide (Sasser 1977), and causes significant economic losses to cotton (*Gossypium hirsutum* L.) (Goodell and Montez 1994). The RKN damages cotton directly and also forms a disease complex with the *Fusarium* wilt pathogen, resulting in a severe yield loss in some growing regions (Jeffers and Roberts 1993; Abawi and Chen 1998). Nematicides have been successfully used to control nematodes in cotton for many years, but the environmental, health, and safety concerns over their use and relatively high cost in cotton production systems have resulted in increased efforts to develop nematode-resistant cotton cultivars. The development and use of elite-resistant cultivars is considered as one of the most economic and effective strategies for nematode management.

Many advanced cotton breeding lines with high levels of nematode resistance have been released, including *G. hirsutum* Auburn 623 RNR, Auburn 634 RNR and their derived M-lines (Shepherd 1974, 1982; Shepherd et al. 1988, 1989), and N-lines (Hyer and Jorgenson 1984). In 1995, the first commercial *G. hirsutum* cultivar Acala NemX, with high resistance to RKN was released for use in California (Oakley 1995; Ogallo et al. 1997). One single recessive gene for RKN resistance was identified in Clevewilt 6 (Bezawada et al. 2003), and a two-gene model including a dominant and an additive gene was described in breeding line M-315 (McPherson et al. 2004). However, it is not clear whether these resistance sources are the same or different from each other and from the resistance gene(s) in Acala NemX, and whether they localize to the same genomic position in *G. hirsutum*. The identification and mapping of molecular markers linked to nematode resistance genes in cotton are essential steps in understanding these relationships and incorporating the resistance into elite cotton culti-

vars. Once molecular markers linked to resistance genes are developed, wide selection for RKN resistance in cotton breeding can be achieved through marker-assisted selection (MAS), rather than through phenotypic selection which is time-consuming, expensive, and sensitive to screening conditions. Genetic markers will also enable unique resistance genes to be pyramided in one genotype or to be deployed individually. Such strategies can promote the durability of resistance because *M. incognita* is known to become virulent through selection pressure from resistance genes including examples in cotton (Ogallo et al. 1997; Roberts et al. 1998). Combinations of genes pyramided into a cotton genotype could provide broad and higher levels of RKN resistance assuming unique genes are available, as it has proved successful with other pathogen-crop systems (Huang et al. 1997; Sanchez et al. 2000).

Several genetic maps of cotton have been constructed using restriction fragment length polymorphism (RFLP) (Reinisch et al. 1994; Shappley et al. 1998; Ulloa and Meredith 2000; Ulloa et al. 2002), amplified fragment length polymorphism (AFLP) (Abdalla et al. 2001), simple sequence repeats (SSR, microsatellites) (Nguyen et al. 2004), and combined maps (Zhang et al. 2002; Lacape et al. 2003; Rong et al. 2004; Ulloa et al. 2005). Mapped RFLP, AFLP, and SSR markers of known chromosome location can be used for screening flanking markers linked to a resistance gene and for localization of RKN resistance genes in cotton. In addition, the identification and mapping of resistance gene analogs (RGA) in cotton (Tan et al. 2003; He et al. 2004; Hinchliffe et al. 2005) could aid in the identification, characterization, and cloning of nematode resistance genes in cotton.

Co-dominant microsatellite markers (SSR) can be used to distinguish heterozygous- from homozygous-resistant plants and false negatives in breeding programs, and they require relatively simple procedures in screening which make them most suitable for MAS in plant breeding. In our study, we used a broad range of SSR markers distributed throughout the cotton genome, many of which had been mapped previously by Nguyen et al. (2004), Rong et al. (2004), Frelichowski et al. (2005), and Park et al. (2005). The objective of this study was (1) to screen for SSR markers closely linked to an RKN resistance gene (designated here as *rkn1*) in *G. hirsutum* cv. Acala NemX using both intraspecific *G. hirsutum* and interspecific *G. barbadense* × *G. hirsutum* crosses and (2) to localize RKN resistance gene *rkn1* in Acala NemX to the chromosome level in the cotton genome.

Materials and methods

Plant materials and nematode resistance screening

Plant genotypes used in this study were susceptible *G. hirsutum* cv. Acala SJ-2, resistant *G. hirsutum* cv. Acala NemX, and susceptible *G. barbadense* cv. Pima S-7. The populations used for molecular analysis were developed from the intraspecific cross between NemX and SJ-2, and included 69 $F_{2:7}$ recombinant inbred lines (RILs), 96 individual F_2 plants, 97 plants of BC_1F_1 (NemX × F_1), and 48 plants of BC_1F_1 (SJ-2 × F_1). In addition, one interspecific cross was made between NemX and Pima S-7 that generated 94 F_2 individuals. Cotton populations were evaluated for nematode resistance under controlled conditions in a greenhouse. Three-week-old seedlings were inoculated with approximately 50,000 eggs of *M. incognita* race 3 (isolate Project 77). Air temperatures in the greenhouse were maintained between 28 and 35°C during the day and 24°C at night. Cotton was evaluated for resistance reaction 60 days after inoculation for F_2, BC_1F_1 (NemX × F_1), and BC_1F_1 (SJ-2 × F_1), and 125 days for $F_{2:7}$ in order to collect seeds. A 0–10 root-gall rating scale (Bridge and Page 1980), ranging from no galling (0) to severe galling reaction (10) was used to evaluate resistance reaction to nematodes. Cotton resistance was also evaluated by the numbers of nematode eggs per gram fresh root. Eggs were extracted from the roots in NaOCl (Hussey and Barker 1973). Plants were classified as resistant or susceptible based on the susceptible and resistant parent phenotypes in each test. Comparisons with the mean and standard deviation (SD) for galling index and eggs per gram fresh root for each parent were used to determine the threshold for resistance in each test.

DNA extraction

Fresh or frozen (−80°C) young cotton leaves under liquid nitrogen were ground to a fine powder using a mortar and pestle, and genomic DNA was extracted using the DNeasy® Plant Mini kit (Qiagen, Valencia, CA, USA).

Amplification of SSR markers

In order to select the polymorphic markers used in this study, approximately 1,800 SSR markers from Cotton Microsatellite Database (CMD) (http://www.mainlab.clemson.edu/cmd/) and Park et al. (2005), were screened within *G. hirsutum*, and between *G. hirsutum* and *G. barbadense*. Then we chose 284 microsatellite makers that gave genome coverage on an average of 20 cM in the regions of markers availability (Nguyen et al. 2004; Park et al. 2005) and from markers previously assigned to cotton chromosomes by anueploid analysis (Park et al. 2005) and now posted at http://www.mainlab.clemson.edu/cgi-bin/cmd/ViewMarkers.cgi. The above markers were used throughout this study on different analyses and populations. Primers were synthesized by IDT (IDT, Coralville, IA, USA). The reaction was performed in 10 µl volume containing 20 ng DNA, 1× PCR buffer, 3 mM $MgCl_2$, 0.2 mM dNTPs

(Roche Diagnostics GmbH, Mannheim, Germany), 0.1 μM forward primer, 0.1 μM reverse primer, and 0.5 U *Taq* polymerase (Invitrogen, Carlsbad, CA, USA) subjected to electrophoresis in agarose gels. For polyacrylamide gel analysis, a tailed SSR protocol was used and forward primers were synthesized with an M13 forward sequence on the 5′-end. The 0.025 μM IRD-labeled M13 primer (5′-CACGACGTTGTAAAACGAC-3′, LI-COR) was included in the PCR reaction. PCR was performed with a "touchdown" program as follows: initial denaturing at 94°C for 2 min; and then the first cycle of 94°C for 15 s, 60°C for 30 s, and 72°C for 1 min; followed by ten cycles of subsequently lowering the annealing temperature (60°C) by 0.5°C per cycle, while maintaining the 94°C for 15 s and 72°C for 1 min regime in each cycle; followed by 34 cycles of 94°C for 15 s, 55°C or 51°C for 30 s (varied for different primers), 72°C for 1 min; and the final extension at 72°C for 6 min. Amplification was performed at MasterCycler® Gradient (Eppendorf, Hamburg, Germany). To separate the amplified product, 3% agarose gel (Invitrogen) stained with 0.5 μg/ml of ethidium bromide was used and was subjected to electrophoresis in 1× TBE buffer at 80 V for 4–5 h. The bands were visualized by illumination with an ultraviolet light. To obtain better resolution for some primers, denatured DNA fragments were resolved in 25 cm gels (0.25 mm spacer thickness) containing 8% Long Ranger™ polyacrylamide gel (Cambrex, Rockland, ME, USA). Electrophoresis and detection were performed on a two-dye, model 4000 LI-COR IR^2 automated sequencer.

Genetic mapping of resistance

Screening of SSR markers linked to the root-knot nematode resistance was based on the bulked segregant analysis (BSA) method (Michelmore et al. 1991). Four bulks were made, including two resistant and two susceptible bulks from the $F_{2:7}$ (NemX × SJ-2) RIL population. Each bulk contained seven individuals highly phenotypically resistant or highly phenotypically susceptible. Four bulks together with the two parents were screened for markers showing polymorphisms between the six samples. One hundred and ninety-one SSR markers were used for BSA analysis. The markers with putative linkage to the resistance gene (*rkn1*) in NemX were confirmed by screening the individuals within the bulks. The markers with putative linkage to the resistance gene within the bulks were then tested with the whole $F_{2:7}$ (NemX × SJ-2) RIL population. Subsequently, 93 more markers around the confirmed *rkn1*-linked markers (66 in A03 and D02, 27 in A02 and D03) were screened with parents and $F_{2:7}$ RILs for fine mapping (Frelichowski et al. 2005; Park et al. 2005). The F_2 (NemX × SJ-2), BC_1F_1 (NemX × F_1), BC_1F_1 (SJ-2 × F_1), and F_2 (Pima S-7 × NemX) were then used to confirm the linkage of the markers and for localization of RKN resistance gene *rkn1* in cotton.

The data for resistance and marker segregation ratios were tested for goodness-of-fit to predicted Mendelian inheritance ratios by the Chi-square test ($P = 0.05$). The linkage analysis between the SSR marker and RKN resistance loci was performed with the software JoinMap® 3.0 (Van Ooijen and Voorrips 2001). The linkage maps were used with a LOD score threshold of 4 or more. The recombination fractions were converted into centiMorgans (cM) based on Kosambi's mapping function (Kosambi 1944). The correlations between the resistance (*rkn1*) phenotype and the *rkn1*-linked markers were also analyzed using CORR procedure of the SAS statistical software (SAS Institute, Cary, NC, USA).

Results

Phenotypic and genetic analyses of RKN resistance gene in Acala NemX

In the $F_{2:7}$ population test, the mean galling index of the parents was 2.5 ± 0.48 for NemX and 5.8 ± 0.41 for SJ-2. The distribution of mean galling index values grouped the 69 lines into two distinct classes, with 34 lines in the range 0.25–2.88 classified as resistant (R) and 35 lines in the range 4.88–6.33 classified as susceptible (S). The segregation fit the 1 R:1 S expected distribution for a single gene determining resistance in the RIL population ($\chi^2 = 0.014$, $P = 0.91$). In the F_2, and BC_1F_1 phenotypic screening, the mean galling index (GI) and mean eggs per gram root (EGR) were 1.6 ± 0.38 and 101 ± 90 for NemX, respectively, and 5.0 ± 0.55 and 1,151 ± 600 for Acala SJ-2. Plants with GI ≤ 2 were considered to be resistant, 2 < GI ≤ 4 as heterozygous (H), and >4 as susceptible. Combined with the parental EGR data comparisons, the F_2 segregation fit a ratio of 1:2:1 (23 R:48 H:25 S) ($\chi^2 = 0.083$, $P = 0.96$), a ratio of 1:1 (22 S:26 H) ($\chi^2 = 0.333$, $P = 0.56$) for BC_1F_1 SJ-2 × F_1, and a ratio of 1:1 (46 H:51 R) ($\chi^2 = 0.258$, $P = 0.61$) for BC_1F_1 NemX × F_1. These results confirmed a single gene conferring resistance in NemX.

Marker analysis and mapping with the $F_{2:7}$ (NemX × SJ-2) population

Eight out of 191 SSR markers were identified as polymorphic between NemX and SJ-2. Four (BNL1231, BNL3255, BNL1672, and BNL2449) of these eight polymorphic markers showed putative linkage to the *rkn1* resistance gene based on BSA. After screening within the bulks, two markers, BNL1231 and BNL3255, were identified with potential linkage to the resistance. These two markers were screened with another 32 $F_{2:7}$ (NemX × SJ-2) RILs, and BNL1231 was found to be linked to the resistance gene while BNL3255 was loosely linked to BNL1231.

Based on the linkage map of Nguyen et al. (2004), BNL 3255 localizes in linkage group A02 and BNL1231a in D02. Considering the known association between linkage groups, A02 is homeologous with D03, and D02 with A03. Sixty-one more SSR markers which were distributed along linkage groups A02, D03, A03, and D02 were used to screen the bulks and parents. Of these 61 markers, 7 (BNL2655, BNL3171, BNL3257, BNL3992, CIR61, CIR112, and CIR316) were polymorphic between NemX and SJ-2. The seven polymorphic markers were screened with the 60 $F_{2:7}$ (NemX × SJ-2) lines. One marker, CIR316, was identified tightly linked to resistance gene *rkn1*. The above analysis was based on banding resolution in agarose gels. Both the BNL1231 and CIR316 markers differed in length by only a few base pairs between the two parents, resulting in low resolution in agarose gels, as shown in Fig. 1a for CIR316. To distinguish heterozygous F_1 plants (Fig. 1a) clearly, CIR316 and BNL1231 forward primers were resynthesized with a M13 forward sequence on the 5′-end, and PCR products were subjected to electrophoresis in polyacrylamide gels. The images obtained with the CIR316 [221 bp (CIR316a) and 210 bp (CIR316c) from resistant and susceptible parents, respectively] and BNL1231 [213 bp (BNL1231a) and 207 bp (BNL1231b) from resistant and susceptible parents, respectively] co-dominant markers in polyacrylamide gels are shown in Fig. 1b, c, respectively. The patterns of the F_1 in the heterozygous state were clearly visible (Fig. 1b, c). In addition, multibands were observed in polyacrylamide gels compared to only one band in agarose gel, although the profile in agarose gels could still distinguish the two parents. All the allelic sizes indicated have M13 primer tails included. CIR316 was very close to *rkn1* and was 17 cM distance from BNL1231 (data not shown).

For fine mapping of the resistance gene, an additional set of 32 markers around the BNL1231 and CIR316 markers in linkage groups D02 and A03 were used with M13 labeled primers and were screened with the parents. Three markers (BNL1408, BNL2895, and BNL3649) were identified as polymorphic between NemX and SJ-2, and then were screened with the 60 $F_{2:7}$ (NemX × SJ-2) RILs. However, the three markers were not linked to the nematode resistance. Thus, two markers (CIR316 and BNL1231) linked to resistance gene *rkn1* were identified. The primer sequences of SSR marker CIR316 were 5′-CTTACAGGCACTACCACC and 5′-CCCTTTCTGG CGACTT. The primer sequences of SSR marker

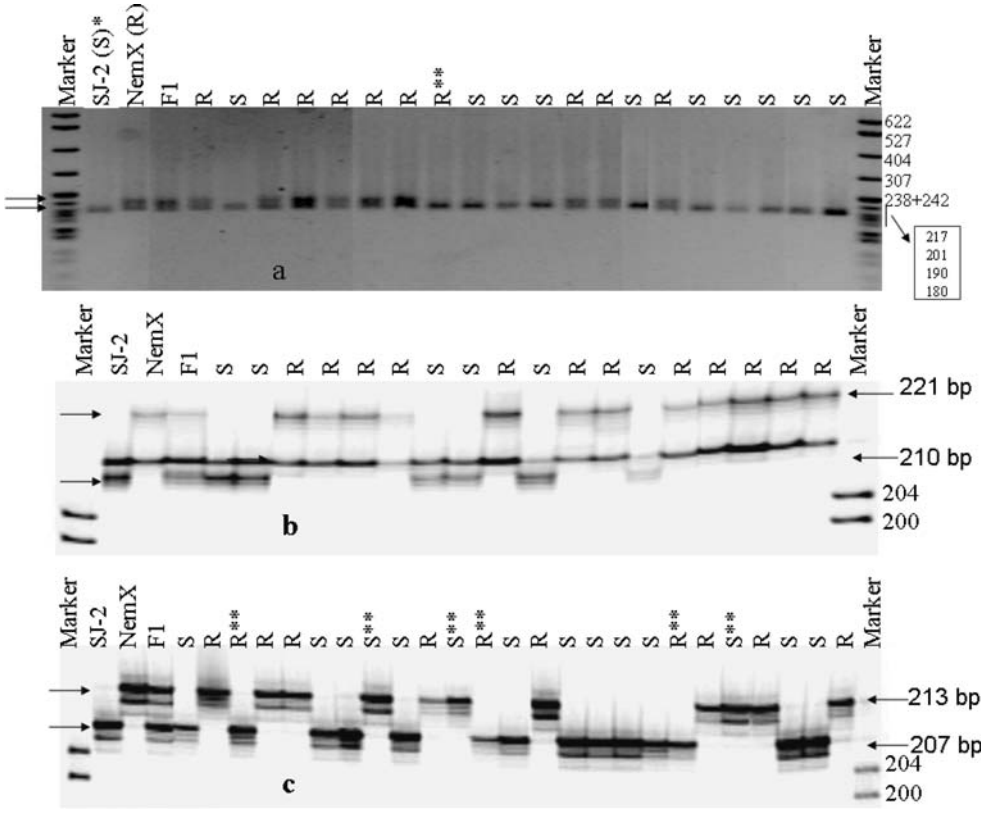

Fig. 1 Images of amplification products with co-dominant marker CIR316 (**a** and **b**) and BNL1231 (**c**) in $F_{2:7}$ (NemX × SJ-2) RIL segregating population electrophoresed on agarose gel (**a**) and polyacrylamide gel (**b**, **c**) with model 4000 LI-COR IR^2 automated sequencer. **SJ-2* susceptible parent, *NemX* resistant parent, F_1 heterozygous, *S* susceptible based on phenotype, *R* resistant based on phenotype. ***R* recombinant line with resistant phenotype (*R*) and susceptible parent marker profile or susceptible phenotype (*S*) and resistant parent marker profile. The *arrows* point to the marker positions, one from NemX and the other from SJ-2. Note all allelic sizes include the M13 primer tail (**b** and **c**)

BNL1231 were 5′-TAATAAAAGGGAAAGGAAAG AGTT and 5′-TATGGCTCTAGAATATTCCCTCG.

Confirmation of marker linkage to resistance in intra-specific NemX × SJ-2 F_2 and BC_1F_1 populations

To confirm the linkage of both CIR316 and BNL1231 co-dominant markers to nematode resistance gene *rkn1*, the F_2 and two BC_1F_1 (NemX × F_1 and SJ-2 × F_1) populations were screened and PCR products were subjected to electrophoresis in polyacrylamide gels. The two markers segregated in expected ratios in the three segregating populations. The amplification patterns of the F_2 (NemX × SJ-2) population with the CIR316a marker closely linked to gene *rkn1* are shown in Fig. 2. In the F_2, three genotypes (homozygous resistant, heterozygous, and homozygous susceptible) could be distinguished (Fig. 2). In the backcross population of NemX × F_1, only heterozygous and resistant genotypes were observed, and only heterozygous and susceptible genotypes were present in the backcross population of SJ-2 × F_1. Three out of 96 individual F_2 plants showed recombination between CIR316 and *rkn1*. Three out of 97 plants in the backcross population of NemX × F_1 and 1 out of 48 plants in the backcross of SJ-2 × F_1 also showed this recombination. Linkage analysis confirmed that BNL1231 and CIR316 were still associated with nematode resistance in each of the three segregating populations. The CIR316a marker was tightly linked to the *rkn1* gene with an estimated distance of 2.6 cM in F_2 (Fig. 3), 2.1 cM in BC_1F_1 (SJ-2 × F_1), and 3.3 cM in BC_1F_1 (NemX × F_1). Moreover, the genetic distance between BNL1231 and CIR316 was similar in the three populations, being 29.2 cM in the F_2, 27.4 cM in the NemX × F_1 population, and 25.4 cM in the SJ-2 × F_1 population. Correlation analyses of gene *rkn1* resistance phenotype classes (R, H, and/or S) and the presence or absence of the SSR markers CIR316a and BNL1231 are presented in Table 1 for all used populations.

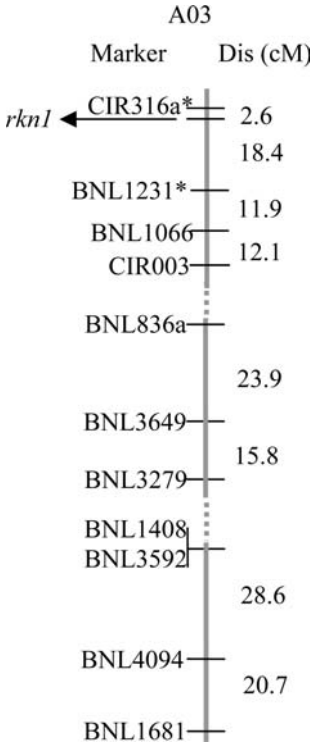

Fig. 3 Location of the resistance gene *rkn1* relative to SSR markers in cotton A03 linkage group combining populations of F_2 (NemX × SJ-2) and F_2 (Pima S-7 × NemX). *Asterisks* indicate that the markers are present in both populations. *Dash lines* indicate that the clusters above and below may connect together. CIR316a, 221 bp amplified from NemX; BNL1231, 213 bp amplified from NemX

Localization of the two markers (BNL1231 and CIR316) with the interspecific F_2 population (Pima S-7 × NemX)

To obtain more markers around BNL1231 and CIR316, the interspecific segregating population from a cross between Pima S-7 (*G. barbadense*) and NemX (*G. hirsutum*) was used. In the linkage groups A03 and D02, 13 markers, already tested in the F_2 (NemX × SJ-2) that

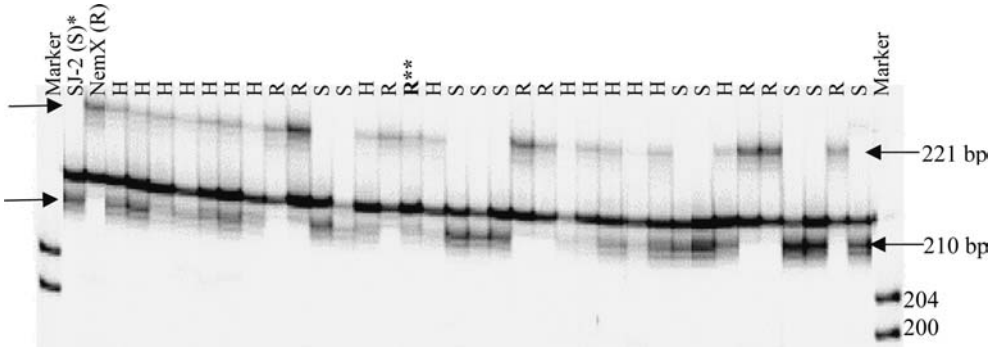

Fig. 2 Images of amplification products with co-dominant marker CIR316 in the segregating population F_2 (NemX × SJ-2) electrophoresed on polyacrylamide gel with model 4000 LI-COR IR^2 automated sequencer. **SJ-2* susceptible parent, *NemX* resistant parent, *H* heterozygous, *S* susceptible, *R* resistant, all based on phenotype. ***R* recombinant line with resistant phenotype (*R*) and susceptible parent CIR316 marker profile. The *arrows* point to the marker positions, one of 221 bp (CIR316a) from NemX and the other of 210 bp (CIR316c) from SJ-2. Note all allelic sizes include the M13 primer tail

Table 1 Correlation analysis of gene *rkn1* resistance phenotype classes (R, H, and/or S) and the presence or absence of the SSR markers CIR316a and BNL1231

		F_2 (NemX × SJ-2)		BC_1F_1 (SJ-2 × F_1)		BC_1F_1 (NemX × F_1)	
		CIR316	BNL1231	CIR316	BNL1231	CIR316	BNL1231
rkn1	PCC	0.8909	0.3475	0.9164	0.5255	0.9580	0.5674
	P	< 0.0001	0.0006	< 0.0001	< 0.0001	< 0.0001	< 0.0001
CIR316	PCC		0.3423		0.6115		0.6140
	P		0.0007		< 0.0001		< 0.0001

PCC Pearson correlation coefficient, *P* probability

were associated with BNL1231 and CIR316, and polymorphic between Pima S-7 and NemX, were used to screen 94 individual F_2 (Pima S-7 × NemX) plants and tested for linkage relationships to each other and to *rkn1* (Fig. 3). BNL1231 and CIR316a (dominant marker, the 221 bp band amplified from NemX) were linked together, and another two markers, BNL1066 and CIR003 in A03 (Nguyen et al. 2004), were associated with BNL1231, indicating that the *rkn1* gene is located on cotton linkage group A03. Four markers (BNL1408, BNL3592, BNL4094, and BNL1681) were linked together as a separate cluster, and three markers (BNL836a, BNL3649, and BNL3279) as another cluster (Fig. 3). In addition, both CIR316b (214 bp) and CIR316d (204 bp) markers amplified from Pima S-7 were linked together with CIR112, but not associated with *rkn1*.

Discussion

The *rkn1* gene conferring RKN resistance in Acala NemX was localized on cotton linkage group A03 based on different segregating populations from both intraspecific and interspecific crosses with Acala NemX. The three segregating populations derived from the *G. hirsutum* intraspecific cross consistently confirmed BNL1231 and CIR316a markers linked to the *rkn1* gene in NemX (Fig. 3, Table 1). Because of the low polymorphism between the related *G. hirsutum* cvs. Acala NemX and Acala SJ-2, the intraspecific mapping populations were not informative enough for fine mapping of the *rkn1* resistance gene. Except for BNL1231 and CIR316, there were no other polymorphic SSR markers found around the two markers in the *G. hirsutum* cross in our study, prompting the use of the interspecific cross between *G. barbadense* and *G. hirsutum*. We found 6.3% (18/284) polymorphism between intraspecific populations, compared to 55.6% (158/284) for the interspecific population. Low levels of polymorphism within *G. hirsutum* and high levels of polymorphism between interspecific *G. barbadense* and *G. hirsutum* were also reported by Frelichowski et al. (2004) and Rungis et al. (2005) based on SSR analysis.

Published reports differ about the position of the BNL1231 marker in linkage groups A03 and (or) D02, and may reflect the amplification of alleles from homeologous genes by the same primers in tetraploid cotton. Nguyen et al. (2004) reported BNL1231a linked to BNL3279 and CIR316 on linkage group D02 based on the cross of *G. hirsutum* cv. Guazuncho 2 × *G. barbadense* cv. VH8-4602. In the cotton database (Cotton DB, http://www.cottondb.tamu.edu), BNL1231B was linked to BNL3279 in linkage group LG22 and to BNL1231A in LGA based on the cross of *G. hirsutum* cv. TM1 × *G. barbadense* cv. 3–79. Meanwhile, Rong et al. (2004) reported that BNL1231a (co-dominant) mapped to linkage group A03 and BNL1231b (recessive) to linkage group D02 based on the cross of *G. hirsutum* race "palmeri" with *G. barbadense* acc. "K101". In our population of *G. barbadense* cv. Pima S-7 × *G. hirsutum* cv. NemX, BNL1231 was a co-dominant marker and closely linked to BNL1066 and CIR003 (Fig. 3). BNL1066 is located on A03 (Nguyen et al. 2004) and in LG22 (http://www.cottondb.tamu.edu). Moreover, BNL1066 was associated with CIR003 localized on A03 in the map of Nguyen et al. (2004). In addition, CIR316a, BNL1231, BNL1066, and CIR003 mapped to linkage group A03 based on the interspecific segregating population *G. hirsutum* cv. TM1 × *G. barbadense* cv. 3–79 (Frelichowski, M. Ulloa et al., in preparation), further confirming the location of *rkn1* in linkage group A03.

Comparing all markers from the previous studies (Nguyen et al. 2004; http://www.cottondb.tamu.edu), the same orders of BNL1066, BNL836, BNL3649, BNL1408, BNL3592, and BNL4094 were found indicating that A03 is equivalent to LGA. The order of the markers, BNL1408, BNL3592, BNL4094, and BNL1681, was the same in our Pima S-7 × NemX F_2 population (Fig. 3) and in the A03 linkage group of Nguyen et al. (2004). For linkage analysis, the lack of closely linked markers along the linkage group resulted in separate clusters being identified. This explains why BNL1408, BNL3592, BNL4094, and BNL1681 (Fig. 3) appeared as a separate cluster from BNL1066 and CIR003 (Fig. 3), and also why BNL836a and BNL3649 were in a separate cluster (Fig. 3). If more markers were associated with both markers at the ends of these clusters, all the markers in the separate clusters should be linked together in one linkage group, A03 (Fig. 3). Moreover, BNL1408, BNL3592, and BNL836 were also

mapped to linkage group A03 in the segregating population of *G. hirsutum* cv. TM1 × *G. barbadense* cv. 3–79 (Frelichowski, M. Ulloa et al., in preparation). Therefore, we concluded that BNL1231, CIR316, and the *rkn1* gene are located on linkage group A03.

Based on our analysis, CIR316a is closely linked to the *rkn1* resistance gene in NemX and was mapped to linkage group A03. As shown in Fig. 3, CIR316a was 21 cM from BNL1231 and 32.9 cM from BNL1066. According to the order of BNL1231a and BNL1066 in LGA (http://www.cottondb.tamu.edu), CIR316a should be located above BNL1231. In the map of Rong et al. (2004), BNL1231a was mapped to a position of 13.1 cM on linkage group A03. Therefore, the CIR316 co-dominant marker from the cross of NemX × SJ-2 also mapped to linkage group A03. In summary, the major gene in Acala NemX conferring resistance to RKN, designated *rkn1*, was found to be closely linked to CIR316a which mapped to linkage group A03. This is the first report in cotton with a closely linked major gene locus determining nematode resistance, and informative SSRs may be used for marker-assisted selection.

Acknowledgement This study was funded in part by a Cooperative Research Agreement from Cotton Incorporated and a grant from the University of California Discovery Grant (BioSTAR) Program. The authors thank Steven Oakley, California Planting Cotton Seed Distributors for providing cotton seed; Mikeal Roose for helpful advice; and William Matthews, Kathie Carter, and Teresa Mullens for technical help.

References

Abawi GS, Chen J (1998) Concomitant pathogen and pest interactions. In: Barker KR, Pederson GA, Windham GL (eds) Plant and nematode interactions. Am Soc Agron, Crop Sci Am, and Soil Sci Soc, Madison, pp 135–158

Abdalla AM, Reddy OUK, El-Zik KM, Pepper AE (2001) Genetic diversity and relationships of diploid and tetraploid cottons revealed using AFLP. Theor Appl Genet 102:222–229

Bezawada C, Saha S, Jenkins JN, Creech RG, McCarty JC (2003) SSR marker(s) associated with root-knot nematode resistance gene(s) in cotton. J Cotton Sci 7:179–184

Bridge J, Page SLJ (1980) Estimation of root-knot nematode infestation levels on roots using a rating chart. Trop Pest Manage 26:296–298

Frelichowski JE, Ulloa M, Tomkins JP, Palmer M, Main D, Stelly D, Cantrell RG (2004) New BAC-end derived microsatellite markers in cotton (*Gossypium hirsutum* L.) Acala 'Maxxa'. In: ASA-CSSA-SSSA Annual Meeting Abstracts

Frelichowski JE, Ulloa M, Palmer MB, Main D, Tomkins JP, Stelly DM, Cantrell RG, Kohel RJ, Yu J (2005) Genetic, physical and QTL mapping assessments of BAC-end derived microsatellite markers developed from cotton (*Gossypium hirsutum* L.) Acala 'Maxxa'. In: Plant and animal genome XIII conference, San Diego

Goodell PB, Montez GH (1994) Acala cotton tolerance to southern root-knot nematode, *Meloidogyne incognita*. In: Proceedings of Beltwide cotton production research conference, National Cotton Council of Am, Memphis, pp 265–267

He L, Du C, Covaleda L, Xu Z, Robinson AF, Yu JZ, Kohel RJ, Zhang H-B (2004) Cloning, characterization, and evolution of the NBS-LRR-encoding resistance gene analogue family in polyploid cotton (*Gossypium hirsutum* L.). Mol Plant Microbe Interact 17:1234–1241

Hinchliffe DJ, Lu Y, Potenza C, Segupta-Gopalan C, Cantrell RG, Zhang J (2005) Resistance gene analogue markers are mapped to homeologous chromosomes in cultivated tetraploid cotton. Theor Appl Genet 110:1074–1085

Huang N, Angeles ER, Domingo J, Magpantay G, Singh S, Zhang G, Kumaravadivel N, Bennett J, Khush GS (1997) Pyramiding of bacterial blight resistance genes in rice: marker-assisted selection using RFLP and PCR. Theor Appl Genet 95:313–320

Hussey RS, Barker KR (1973) A comparision of methods of collecting inocula of *Meloidogyne* spp. including a new technique. Plant Dis Rep 57:1025–1028

Hyer AH, Jorgenson EC (1984) Root-knot nematode resistance in cotton breeding: techniques and results. In: Proceedings of Beltwide cotton production research conference, Natl Cotton Council of America, Memphis, pp 377–379

Jeffers DP, Roberts PA (1993) Effect of planting date and host genotype on the root-knot nematode-*Fusarium* wilt disease complex of cotton. Phytopathology 83:645–654

Kosambi DD (1944) The estimation of map distances from recombination values. Ann Eugen 12:172–175

Lacape JM, Nguyen TB, Thibivilliers S, Bojinov B, Courtois B, Cantrell RG, Burr B, Hau B (2003) A combined RFLP-SSR-AFLP map of tetraploid cotton based on a *Gossypium hirsutum* × *Gossypium barbadense* backcross population. Genome 46:612–626

McPherson MG, Jenkins JN, Watson CE, McCarty JC (2004) Inheritance of root-knot nematode resistance in M-315 RNR and M78-RNR cotton. J Cotton Sci 8:154–161

Michelmore RW, Paran I, Kesseli RV (1991) Identification of markers linked to disease resistance genes by bulked segregant analysis: a rapid method to detect markers in specific genomic regions by using segregating populations, Proc Natl Acad Sci USA 88:9828–9832

Nguyen TB, Giband M, Brottier P, Risterucci AM, Lacape JM (2004) Wide coverage of the tetraploid cotton genome using newly developed microsatellite markers. Theor Appl Genet 109:167–175

Oakley SR (1995) CPCSD Acala C-225: a new nematode-resistant Acala variety for California's San Joaquin Valley. In: Proceedings of Beltwide cotton production research conference, Natl Cotton Council of Am, Memphis, p 39

Ogallo JL, Goodell PB, Eckert J, Roberts PA (1997) Evaluation of NemX, a new cultivar of cotton with high resistance to *Meloidogyne incognita*. J Nematol 29:531–537

Park YH, Alabady MS, Sickler B, Wilkins TA, Yu J, Stelly DM, Kohel RJ, El-Shihy OM, Cantrell RG, Ulloa M (2005) Genetic mapping of new cotton fiber loci using EST-derived microsatellites in an interspecific recombinant inbred line (RIL) cotton population. Mol Gen Genomics (in press)

Reinisch A, Dong J, Brubaker CL, Stelly DM, Wendel JF, Paterson AH (1994) A detailed RFLP map of cotton, *Gossypium hirsutum* × *Gossypium barbadense*: chromosome organization and evolution in a disomic polyploid genome. Genetics 138:829–847

Roberts PA, Matthews WC, Veremis JC (1998) Genetic mechanisms of host-plant resistance to nematodes. In: Barker KR, Pederson GA, Windham GL (eds) Plant and nematode interactions. Am Soc Agron, Crop Sci Am, and Soil Sci Soc, Madison, pp 209–238

Rong J , Abbey C, Bowers JE, Brubaker CL, Chang C, Chee PW, Delmonte TA, Ding X, Garza JJ, Marler BS, Park C, Pierce GJ, Rainey KM, Rastogi VK, Schulze SR, Trolinder NL, Wendel JF, Wilkins TA, Williams-Coplin TD, Wing RA, Wright RJ, Zhao X, Zhu L, Paterson AH (2004) A 3347-locus genetic recombination map of sequence-tagged sites reveals features of genome organization, transmission and evolution of cotton (*Gossypium*). Genetics 166:389–417

Rungis D, Llewellyn D, Dennis ES, Lyon BR (2005) Simple sequence repeat (SSR) markers reveal low levels of polymorphism between cotton (*Gossypium hirsutum* L.) cultivars. Aust J Agric Res 56:301–307

Sanchez AC, Brar DS, Huang N, Li Z, Khush GS (2000) Sequence tagged site marker-assisted selection for three bacterial blight resistance genes in rice. Crop Sci 40:792–797

Sasser JN (1977) Worldwide dissemination and importance of the root-knot nematodes, *Meloidogyne* spp. J Nematol 9:26–29

Shappley ZW, Jenkins JN, Meredith WR, McCarty JC (1998) An RFLP linkage map of upland cotton, *Gossypium hirsutum* L. Theor Appl Genet 97:756–761

Shepherd RL (1974) Transgressive segregation for root-knot nematode resistance in cotton. Crop Sci 14:872–875

Shepherd RL (1982) Registration of three germplasm lines of cotton. Crop Sci 22:692

Shepherd RL, McCarty JC, Jenkins JN, Parrott WL (1988) Registration of twelve nonphotoperiodic lines with root-knot nematode resistant primitive cotton germplasm. Crop Sci 28:868–869

Shepherd RL, McCarty JC, Jenkins JN, Parrott WL (1989) Notice of release of nine root-knot nematode resistant germplasm lines of upland cotton *Gossypium hirsutum* L. USDA Miss Agric and Forestry Exp Stn Memo

Tan H, Callahan FE, Zhang X-D, Karaca M, Saha S, Jenkins JN, Creech RG, Ma D-P (2003) Identification of resistance gene analogs in cotton (*Gossypium hirsutum* L.). Euphytica 134:1–7

Ulloa M, Meredith WR Jr (2000) Genetic linkage map and QTL analysis of agronomic and fiber quality traits in an intraspecific population. J Cotton Sci 4:161–170

Ulloa M, Meredith WR, Shappley ZW, Kahler AL (2002) RFLP genetic linkage maps from four F2:3 populations and a joinmap of *Gossypium hirsutum* L. Theor Appl Genet 104:200–208

Ulloa M, Saha S, Jenkins JN, Meredith WR Jr, McCarty JC, Stelly DM (2005) Chromosomal assignment of RFLP linkage groups harboring important QTLs on an intraspecific cotton (*Gossypium hirsutum* L.) joinmap. J Hered 96:132–144

Van Ooijen JW, Voorrips RE (2001) JoinMap® 3.0, software for the calculation of genetic linkage maps. Plant Research International, Wageningen

Zhang J, Guo W, Zhang T (2002) Molecular linkage map of allotetraploid cotton (*Gossypium hirsutum* L. × *Gossypium barbadense* L.) with a haploid population. Theor Appl Genet 105:1166–1174