Phenotypic Expression of *rkn1*-Mediated *Meloidogyne incognita* Resistance in *Gossypium hirsutum* Populations

C. Wang, W. C. Matthews, P. A. Roberts

Abstract: The root-knot nematode *Meloidogyne incognita* is a damaging pest of cotton (*Gossypium hirsutum*) worldwide. A major gene (*rkn1*) conferring resistance to *M. incognita* was previously identified on linkage group A03 in *G. hirsutum* cv. Acala NemX. To determine the patterns of segregation and phenotypic expression of *rkn1*, F_1, F_2, $F_{2:3}$, BC_1F_1 and $F_{2:7}$ recombinant inbred lines (RIL) from intraspecific crosses between Acala NemX and a closely related susceptible cultivar Acala SJ-2 were inoculated in greenhouse tests with *M. incognita* race 3. The resistance phenotype was determined by the extent of nematode-induced root galling and nematode egg production on roots. Suppression of root galling and egg production was highly correlated among individuals in all tests. Root galling and egg production on heterozygous plants did not differ from the susceptible parent phenotype 125 d or more after inoculation, but were slightly suppressed with shorter screening (60 d), indicating that *rkn1* behaved as a recessive gene or an incompletely recessive gene, depending on the screening condition. In the RIL, *rkn1* segregated in an expected 1 resistant: 1 susceptible ratio for a major resistance gene. However, within the resistant class, 21 out of 34 RIL were more resistant than the resistant parent Acala NemX, indicating transgressive segregation. These results suggest that *rkn1*-based resistance in *G. hirsutum* can be enhanced in progenies of crosses with susceptible genotypes. Allelism tests and molecular genetic analysis are needed to determine the relationship of *rkn1* to other *M. incognita* resistance sources in cotton.

Key words: cotton, *Gossypium hirsutum*, *Meloidogyne incognita*, resistance, *rkn1*, root-knot nematode, phenotypic expression, transgressive segregation.

The southern root-knot nematode *Meloidogyne incognita* is an important pest of cotton *Gossypium hirsutum* (Goodell and Montez, 1994) and many other crops worldwide (Sasser, 1977). Nematode infection causes root galling, shoot stunting, and loss of yield. In addition, the presence of root-knot nematodes can increase the incidence, rate of development, and severity of Fusarium wilt (FW) in cotton as a disease complex (Abawi and Chen, 1998). Fusarium wilt symptoms typically are associated with the presence of *M. incognita* in fields with coarsely textured sandy soils (Jeffers and Roberts, 1993). In the San Joaquin Valley of California, where cotton is grown intensively under irrigation, *M. incognita* and FW complex infections occur in up to 20% of the cotton planting area (Goodell et al., 1992; Anonymous, 1996). Restrictions on nematicide use and their relatively high cost in cotton production have expedited the development of root-knot resistant cotton cultivars.

The first highly resistant cotton germplasm available for breeding resistance to root-knot nematode was Auburn 623 RNR (*G. hirsutum*), a transgressive segregant for resistance from a cross of "Clevewilt 6-3-5" and "Mexico Wild" (Shepherd, 1974). Subsequently, Auburn 634 RNR and other derived lines with high levels of resistance, such as the M-line series developed from Auburn 623 RNR and Auburn 56, were released (Shepherd, 1982a; Shepherd et al., 1988, 1996). These lines were not suitable as commercial cultivars but provided advanced breeder line resistant stocks. Early attempts at

genetic analysis of root-knot nematode resistance in these materials indicated the presence of multiple genes with dominant or additive effects and the occurrence of transgressive segregation for resistance in some crosses (Shepherd, 1974, 1986). However, no clear understanding of the genetic control of resistance was revealed. McPherson et al. (2004) reported a two-gene model for resistance in M-315 derived from Auburn 623 RNR. Analysis of an F_2 population indicated that one recessive gene conferred moderate resistance in Clevewilt 6–1 (Bezawada et al., 2003).

In 1995, the upland cotton cultivar Acala NemX (*G. hirsutum*) was released, having been developed as a single line selection in a self-pollinated population with high resistance to *M. incognita* (Oakley, 1995; Ogallo et al., 1997). Acala NemX was developed from the cross Acala B1662 × N-3; N-3 was derived from the nematode resistant line N6072 (Hyer and Jorgenson, 1984). The origin of the *M. incognita* resistance in Acala NemX is not clear from the existing pedigree reports (Hyer and Jorgenson, 1984; Oakley, 1995; Robinson et al., 2001). The nematode resistance in Acala NemX is highly effective in protecting plants from the effects of root infection. The lint yield of Acala NemX was less than that of susceptible Acala Maxxa in noninfested fields or those with low levels of nematode infestation. However, Acala NemX yields decreased only slightly, whereas Acala Maxxa yields were severely decreased with medium or high levels of nematode infestation (Ogallo et al., 1997). The utilization of Acala NemX also can greatly increase the rotational value of cotton for managing root-knot nematodes (Ogallo et al., 1999). In addition, nematode resistance in cotton can protect the plant from FW disease under field conditions (Shepherd, 1986; DeVay et al., 1997; Ogallo et al., 1999). Variation in virulence among *M. incognita* isolates to the Acala NemX resistance has been reported (Ogallo et al., 1997). Recently, we identified a major gene, *rkn1*, in

Acala NemX conferring resistance to *M. incognita* and localized *rkn1* to linkage group A03 in the cotton genome using SSR markers (Wang et al., 2006). An understanding of the genetic basis of root-knot nematode resistance in cotton will facilitate breeding of cultivars with improved resistance and indicate possibilities for combining resistance traits to obtain higher or more durable levels of resistance.

The objective of the present work was to determine the phenotypic expression of *rkn1*-mediated resistance to *M. incognita* using nematode-induced root galling and egg production phenotypes of progenies generated from an intraspecific *G. hirsutum* cross between the *rkn1* donor Acala NemX and a related susceptible cultivar Acala SJ-2.

MATERIALS AND METHODS

Plant materials and crosses: Plant genotypes used in this study were susceptible *G. hirsutum* cv. Acala SJ-2 and resistant *G. hirsutum* cv. Acala NemX. Two sets of progenies were produced from separate crosses. In the first set (set I), crosses were made between Acala SJ-2 and Acala NemX to generate F_1, F_2, $F_{2:3}$, $F_{2:7}$ (69 RIL) and BC_1F_1 (32 plants of NemX $\times$ F_1 and 37 plants of $F_1 \times$ NemX) populations. The second set (set II) included F_1, 99 F_2 plants, 100 plants of BC_1F_1 (NemX $\times$ F_1) and 50 plants of BC_1F_1 (SJ-2 $\times$ F_1). In addition, a resistant sister line of NemX, N901, and another susceptible cultivar, Acala Maxxa, were included in the test for the parental and F_1 screening.

Nematode resistance screening: A culture of *M. incognita* race 3 (isolate Project 77), originating from a San Joaquin Valley, CA, cotton field was maintained and multiplied on the tomato cultivar Tropic. The species and race identity of the culture were confirmed by isozyme phenotyping and a host differential test as described

previously (Roberts et al., 1996). Cotton populations were evaluated for nematode resistance under controlled conditions in a greenhouse. Individual cotton seeds were planted into 10-cm-diam. $\times$ 17-cm-deep plastic pots filled with steam-sterilized sand. Plants were fertilized with 17–6-10 controlled release fertilizer (Scotts-Sierra Horticultural Products Co, Marysville, OH). Three-wk-old seedlings were inoculated with approximately 50,000 eggs of *M. incognita*. Inoculum was prepared by extracting eggs from tomato roots with NaOCl (Hussey and Barker, 1973). Pots were drip-irrigated to maintain plant growth. Air temperatures in the greenhouse were maintained between 28 and 35°C during the day and at 24°C at night.

Due to the large numbers of plants evaluated and also to the testing of different generations or populations, the phenotyping experiments were done in different tests. In order to collect F_2, $F_{2:3}$ and $F_{2:8}$ seeds in the set I populations (Tables 1 and 2), F_1, F_2 and $F_{2:7}$ RIL plants were phenotyped for resistance reaction 150, 150 and 125 d after inoculation, respectively. The set I BC populations were phenotyped 146 d after inoculation and the set II F_1, F_2 and BC populations 60 d after inoculation. A 0-to-10 root-gall index (GI) was used to evaluate resistance reaction to nematodes. The GI was modified from the Bridge and Page (1980) root-knot nematode rating chart as follows: 0 = no galls; 1 = few small galls; 2 = small galls with less than 10% of roots infected; 3 = 10% to 30% of roots infected, main roots clean; 4 = 31% to 40% of roots infected; 5 = 51% to 60% of roots infected, galling on parts of main roots; 6 = 61% to 70% of roots infected, galling on main roots; 7 = 71% to 80% of roots infected, majority of main roots galled; 8 = 81% to 100% of roots infected, all main root galled; 9 = all roots severely galled and plant usually dying; 10 = all roots severely galled with diminished

TABLE 1. Classification for resistance to *Meloidogyne incognita* of parental lines and segregating populations derived from crosses between resistant Acala NemX and susceptible Acala SJ-2.

| Parent or generation | Total plants or families | R:S ratio | | χ^2 | *P* value |
		Observed	Expected[a]		
SJ-2	7	0:7	All S		
NemX	9	9:0	All R		
F_1 (SJ-2 $\times$ NemX)	7	0:7	All S		
BC_1 – A ($F_1 \times$ NemX)	32	15:17	16:16	0.125	0.724
BC_1 – B (NemX $\times$ F_1)	37	17:20	18.5:18.5	0.243	0.622
BC_1 – A + B	69	32:37	34.5:34.5	0.362	0.547
$F_{2:3}$ (Seg)[b]	427	104:323	106.75:320.25	0.094	0.759
RIL ($F_{2:7}$)	69 (families)	34:35	34.5:34.5	0.014	0.906
		R:Seg:S ratio			
		Observed	Expected[c]		
$F_{2:3}$	43 (families)	7:29:7	10.75:21.5:10.75	5.233	0.073

[a] Expected number of plants for a single recessive gene model of resistance segregating 1R:1S in BC_1 [(SJ-2 $\times$ NemX) $\times$ NemX and NemX $\times$ (SJ-2 $\times$ NemX)] and in $F_{2:7}$ (recombinant inbred lines); segregating 1R:3S in segregating $F_{2:3}$ families; and segregating 1R:2Seg:1S among $F_{2:3}$ families.

[b] Classification of individuals as resistant or susceptible was based on root galling and nematode egg production phenotypes in parent, F_1, and BC_1 populations, and on root galling in $F_{2:3}$ families and $F_{2:7}$ RIL populations.

[c] R = Resistant; S = Susceptible; Seg = Segregating.

TABLE 2. Segregation data for *Meloidogyne incognita* resistance in the F_2 population and derived $F_{2:3}$ families of the cross Acala NemX × SJ-2.

		Parents or F_2 (grouped by genotype)				Parent or corresponding $F_{2:3}$ families									
		Mean (range)					# plants/gall rating category					Mean (range)			
	#[a]	Gall rating	Eggs/root system (×1000)	EGR[b] (×1000)	Gen[c]	#[a]	0–3.5	4	4.5	5–5.5	≥6	Gall rating	Eggs/root system (×1000)	EGR (×1000)	Res[d] class
SJ-2	7	5.6 (4.5–6.5)	256 (141–412)	4.129 (1.6–8.1)	AA	7				1	6	6.3 (5.0–7.0)	313 (163–672)	4.70 (1.7–12.9)	S
NemX	9	1.2 (0–2.0)	15 (0.1–51)	0.29 (0.02–1.0)	aa	8	8					2.6 (2.0–3.5)	18 (4.0–44)	0.45 (0.1–0.8)	R
F_2	7	0.6 (0–2.0)	12 (1.8–35)	0.35 (0.03–1.1)	aa	97	92	5				2.2 (1.3–2.7)	16 (15–17)	0.47 (0.4–0.6)	HR
	7	6 (2.0–7.5)	322 (296–365)	7.80 (4.6–10.1)	AA	77				21	56	6 (5.7–6.6)	345 (276–414)	6.35 (6.1–6.6)	HS
	29	3.8 (0–7.0)	103.6 (4.4–263)	2.52 (0.07–5.7)	Aa	427	91	13	72	93	158	4.8 (3.4–6.1)	92.8 (139–203)	3.83 (2.5–5.3)	Seg

[a] #, number of plants. [b] EGR, Eggs per gram root. [c] Gen, Genotype. [d] Res class: Resistance class; S, Susceptible; R: Resistant; HR: Homozygous resistant; HS, Homozygous susceptible; Seg, Segregating.

root system and plant usually dead. Cotton resistance also was evaluated by the numbers of nematode eggs per gram fresh root. Eggs were extracted from the roots in NaOCl (Hussey and Barker, 1973).

Due to the different lengths of the tests and the influence of time of year in the greenhouse, the GI and number of eggs per gram root varied among tests for the same parental genotypes. Therefore, the criterion for classifying individuals as resistant or susceptible was based on the separation of the parent phenotype scores in each test. The mean and SD for GI and eggs per gram root for each parent were used to determine the threshold for resistance in each test. Plants with a score equal to or less than the resistant parent mean plus 1 SD value were classified as resistant. For the GI, the threshold in the set I tests (Table 1) was ≤ 1.9 (F_1), ≤ 3.0 ($F_{2:7}$) and ≤ 3.5 (F_2, $F_{2:3}$ and BC_1), and ≤ 2.0 in the set II tests. For egg production, plants with ≤ 500 eggs/g root were classified as resistant and > 500 as susceptible in set I. On almost all plants, the galling and egg scores provided a matching classification. However, on a few test plants, a galling score slightly above the resistance threshold was matched with a typically resistant egg production score, and these plants were classified as resistant in the segregation analysis. This occurred in the $F_{2:3}$ segregating progenies, where 13 individuals with a GI of 4.0 were classified as resistant based on low egg production scores (< 500) (Tables 1 and 2). In segregating F_2 populations, homozygous resistance was identified when all individual plants in a $F_{2:3}$ family were resistant, homozygous susceptible when all individual $F_{2:3}$ plants were susceptible, and heterozygous when plants in a $F_{2:3}$ family were segregating susceptible and resistant.

Data analysis: Data were subjected to one-way ANOVA analysis. Fisher's Protected LSD test was used to compare the treatment means. Data for the nematode egg production were transformed to $\log_{10} (x + 1)$ for analysis. The data for resistance segregation were tested for goodness-of-fit to predicted Mendelian inheritance ratios by χ^2-test.

RESULTS

Phenotype of parents and F_1: The resistant sister lines, Acala NemX and N901, had lower ($P < 0.05$) GI (mean 1.17 and 1.35, respectively, Fig. 1A) and supported fewer ($P < 0.05$) numbers of nematode eggs per gram of roots (291 and 175, respectively, Fig. 1B) than the two susceptible parent genotypes Acala SJ-2 (GI = 5.58 and 4,129 eggs) and Acala Maxxa (GI = 5.85 and 6,430 eggs). In separate tests with the different segregating populations derived from Acala NemX and Acala SJ-2, the two parents were included in each test. Acala NemX and Acala SJ-2 differed from each other for GI and egg production ($P < 0.05$) in each test; the parent means are presented in Table 2 and Figures 1–4. The four F_1 from resistant x susceptible crosses of the four parents did not differ from two susceptible parents Acala SJ-2 and Maxxa in GI scored at 150 d after inoculation (Fig. 1A). Based on egg production at 150 d, the two F_1 from NemX and N901 crossed with susceptible SJ-2 did not differ from SJ-2, and the two F_1 from NemX and N901 crossed with susceptible Maxxa did not differ from Maxxa (Fig. 1B). The mean values of eggs per gram root (Fig. 1B) and total eggs per root system (data not shown) of susceptible Maxxa and SJ-2 did not differ. However, the F_1 (Maxxa × N901) with 462,000 eggs/root system and 7,784 eggs/g root supported greater nematode reproduction and root galling ($P < 0.05$) than F_1 (SJ-2 × N901) with 147,000 eggs/root system and 2,487 eggs/g root. The resistant x resistant F_1 (NemX × N901) supported less egg production (65 eggs/g root) and root galling (0.75) ($P < 0.05$) than the resistant parents.

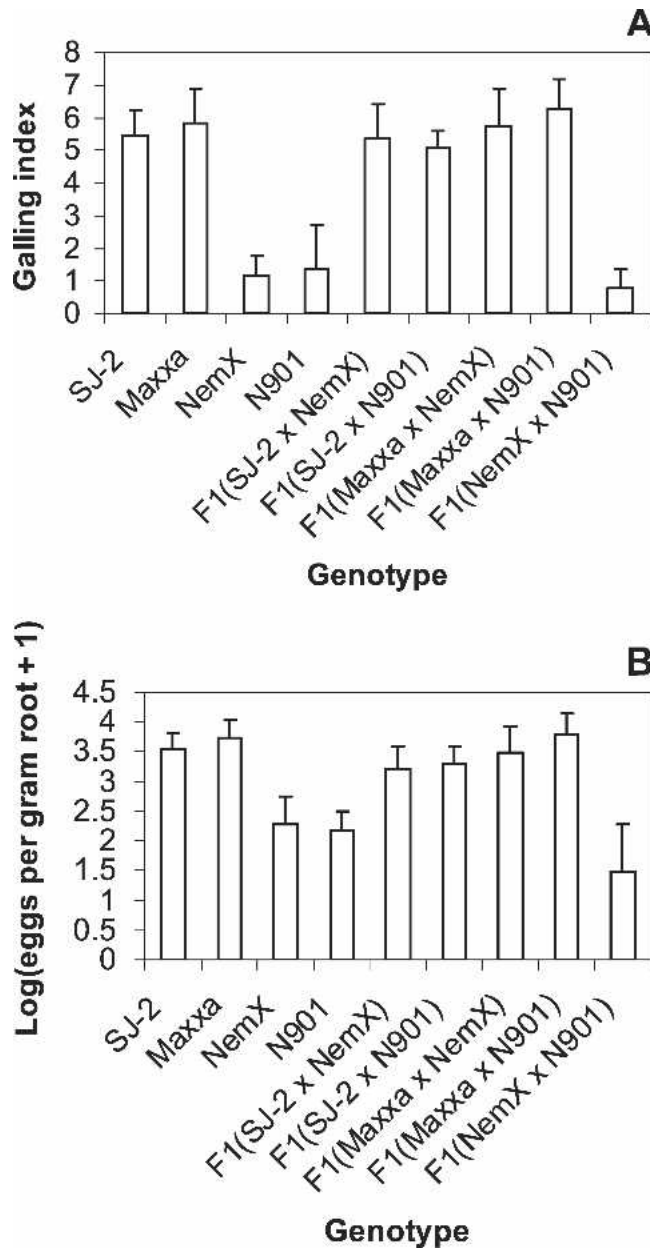In a shorter screening 60 d after inoculation, the F_1

FIG. 1. Root galling (A) and egg production (B) of *Meloidogyne incognita* on susceptible (Acala SJ-2, Acala Maxxa) and resistant (Acala NemX, N901) cotton cultivars and breeding lines and their F_1 from the first set of crosses. Log_{10} (x + 1) transformed data were used for analysis of eggs per gram of root. Galling Index: 0 to 10 scale; 0 = no galling, and 10 = severe galling. Bars represent 1 standard deviation.

(NemX × SJ-2) had lower galling (GI = 5.1) ($P < 0.05$) and numbers of eggs per gram root (7,252 eggs) ($P < 0.05$) than susceptible parent Acala SJ-2 (GI = 5.6; 12,431 eggs) and higher GI and greater number of nematode eggs than resistant parent Acala NemX (GI = 1.8; 518 eggs/g root).

Galling index and egg production in backcross populations: The combined backcross populations of NemX × F_1 and F_1 × NemX had 69 individual plants which showed a close fit to a 1:1 segregation between resistance and

susceptibility (Table 1). Root-galling index was highly correlated ($r = 0.744$) with egg production in the backcross population (Fig. 5), confirming the results from the parent and F_1 phenotype reactions (Fig. 1). Based on egg production, 32 plants had < 500 eggs/g root and 37 plants had > 500 eggs/g root, whereas the parents had 374 eggs/g root in Acala NemX and 2,841 eggs/g root in Acala SJ-2. Galling index (Fig. 5) gave the same distribution, with 32 plants having a GI ≤ 3.5 and 37 plants having a GI > 3.5, with parent phenotypes having a mean GI =1.9 (range 1 - 3) for Acala NemX and GI = 5.7 (range 5–6.5) for Acala SJ-2.

A second set of progenies developed from separate crosses of NemX × SJ-2 included 100 individual backcross plants from resistant NemX × F_1 and 50 backcross plants from susceptible SJ-2 × F_1 (Fig. 2). In the backcross population to resistant Acala NemX, 54 plants had a GI ≤ 2 and 46 plants had a GI > 2 (Fig. 2A). In the backcross population to susceptible parent Acala SJ-2, 46 out of 50 plants showed moderately to highly susceptible galling phenotypes (GI = 2.5–6.0) (Fig. 2B). Four individuals had resistant responses based on galling and egg production. They were confirmed to be
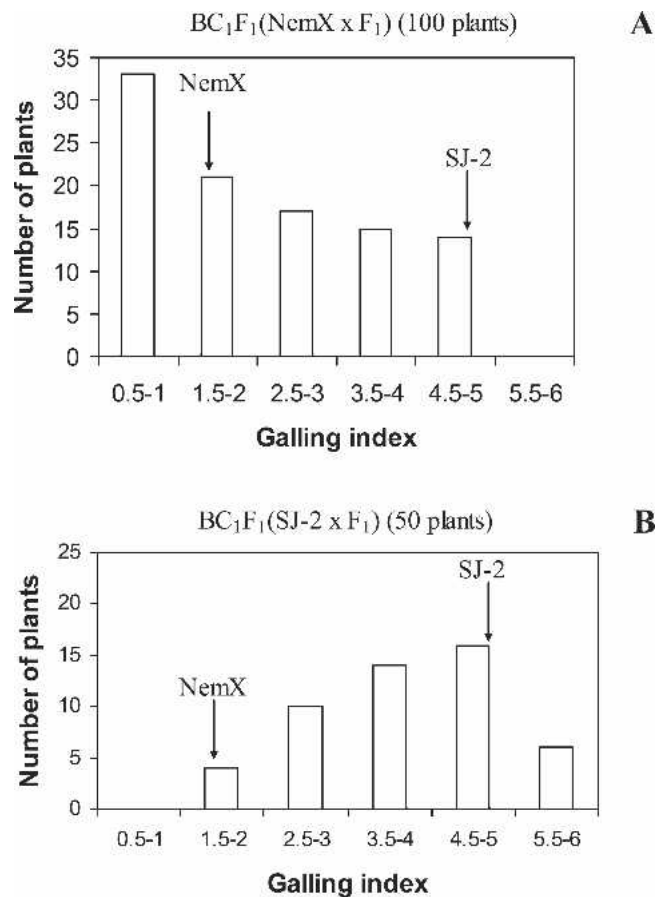

FIG. 2. The distribution of different classes of resistance reaction to *Meloidogyne incognita* of backcross plants from the second set of crosses based on root-galling index. Galling Index: 0 to 10 scale; 0 = no galling, and 10 = severe galling. A: NemX × F_1 (NemX × SJ-2); B: SJ-2 × F_1 (NemX × SJ-2).

true heterozygotes for the resistance region based on subsequent marker analysis (data not shown) and probably were infection escapes with small root systems. The mean GI of the parents were 1.6 for Acala NemX and 5.0 for Acala SJ-2. Even though the phenotype screens for the second set of progenies had less infection overall compared with the first set, classification of resistance phenotype based on galling index as 54 resistant: 46 susceptible for BC_1F_1 NemX × F_1 ($P = 0.424$) and 4 resistant: 46 susceptible for BC_1F_1 SJ-2 x F_1 conformed to an expected segregation for the *rkn1* gene determining resistance in Acala NemX.

F_2 and $F_{2:3}$: In the second set of progenies, 99 F_2 plants were tested for resistance based on galling index (Fig. 3) and egg production (data not shown). Twenty plants with a GI ≤ 2 were classified as resistant, and 79 plants with GI > 2 were classified as susceptible (parent mean GI were 1.6 for Acala NemX and 5.0 for Acala SJ-2). This segregation distribution fit a 1 resistant: 3 susceptible ratio ($P = 0.270$) expected for *rkn1* behaving as a recessive gene for resistance.

In the first set of progenies, 43 families of $F_{2:3}$ were developed from individual F_2 plants that were screened for resistance. Each $F_{2:3}$ family, represented by 10 to 16 plants/family, was then screened for resistance. The resistance categories of the F_2 individuals and the $F_{2:3}$ families, based on root galling and egg production phenotype screens together with their predicted genotypes, are given in Table 2. The homozygous resistant lines had low GI, low total eggs per root system and low eggs per gram root phenotypes for both individual F_2 plants (mean values were 0.6, 12,000, and 350, respectively) and their derived $F_{2:3}$ families (mean values were 2.2, 16,000, and 470, respectively). The homozygous susceptible lines had correspondingly high GI and egg production per root system and gram root phenotype scores for F_2 (mean values were 6, 322,000, and 7,800, respectively) and $F_{2:3}$ (mean values were 6, 345,000, and 6,350, respectively). In the segregating $F_{2:3}$ families derived from heterozygous F_2 plants, the progenies showed a range of phenotypes for root galling and egg

production that included both resistant and susceptible responses (Table 2). Based on the F_2 and $F_{2:3}$ phenotypes, the segregation of the 43 lines was 7 resistant: 29 segregating: 7 susceptible, conforming to a 1:2:1 distribution for homozygous resistant: heterozygous (segregating in $F_{2:3}$): homozygous susceptible genotypes expected for a single gene determining resistance (Table 1). The 427 individuals pooled from the 29 segregating $F_{2:3}$ families segregated in a 1 resistant (104 individuals): 3 susceptible (323 individuals) ratio, further confirming the recessive condition of gene *rkn1* in Acala NemX (Table 1).

Recombinant inbred lines (RIL): For the phenotypic test of 69 $F_{2:7}$ RIL, 4 plants/line were screened for nematode resistance. The distribution of mean GI values grouped the 69 lines into two distinct classes, with 34 lines with GI of 0.25 to 2.88 classified as resistant and 35 lines with GI of 4.88 to 6.33 classified as susceptible (Fig. 4). This segregation fit the 1 resistant: 1 susceptible expected distribution for gene *rkn1* determining resistance in the RIL population (Table 1), in which lines are either homozygous resistant or homozygous susceptible for *rkn1*. Within the susceptible group, all lines were similar to Acala SJ-2 (GI = 5.8 ± 0.41). However, within resistant lines, 21 (GI of 0 - 2.0) were more resistant ($P < 0.05$) than resistant parent Acala NemX (GI = 2.5 ± 0.48). In addition, eggs were extracted from a few lines from the resistant and susceptible RIL groups. Similar to the root-galling reactions, the two groups were differentiated ($P < 0.05$), e.g., three resistant lines had a mean of 14,111 eggs/root system and 268 eggs/g root, compared to a typical susceptible line with 345,000 eggs/root system and 6,001 eggs/g root.

DISCUSSION

The phenotypic analysis of *rkn1*-mediated resistance in multiple progenies developed from an intraspecific
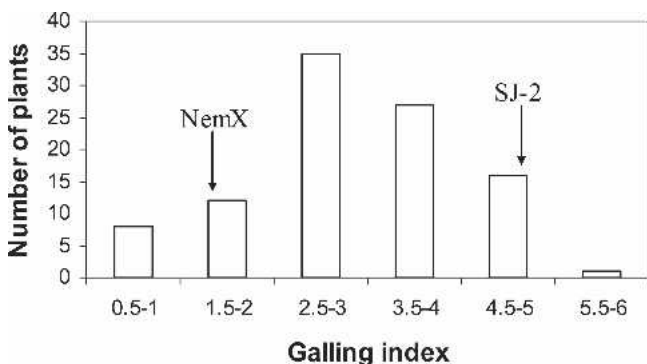

FIG. 3. The distribution of different classes of resistance reaction to *Meloidogyne incognita* of 99 F_2 (NemX × SJ-2) plants from the second set of crosses based on galling index. Galling Index: 0 to 10 scale; 0 = no galling, and 10 = severe galling.
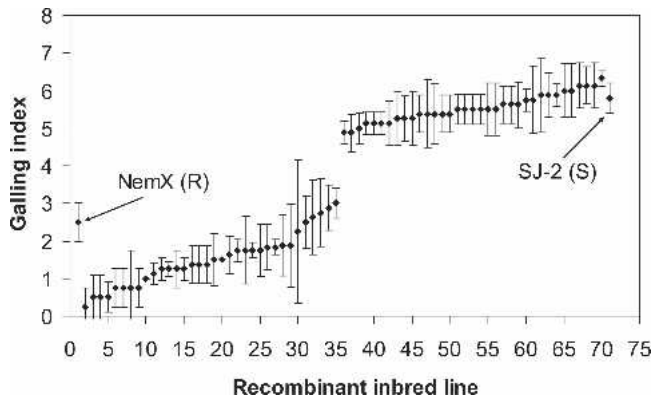

FIG. 4. The distribution of different classes of resistance reaction to *Meloidogyne incognita* of $F_{2:7}$ RIL (NemX × SJ-2) from the first set of crosses based on galling index. Mean values of four plants per line plus standard deviation bar. Score of the resistant (NemX) and susceptible (SJ-2) parents are indicated. Galling Index: 0 to 10 scale; 0 = no galling, and 10 = severe galling.
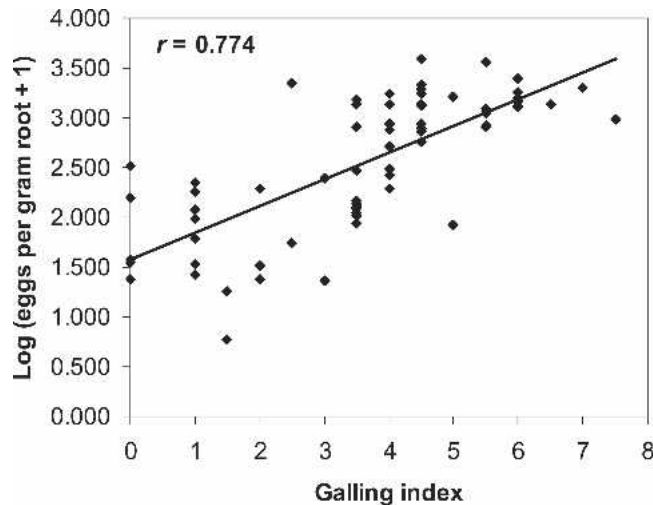
FIG. 5. The relationship between galling index and egg production in the combined segregating backcross populations of $F_1 \times$ NemX and NemX $\times$ F_1 from the first set of crosses. Log_{10} (x + 1) transformed data were used for analysis of eggs per gram of root. Galling Index: 0 to 10 scale; 0 = no galling, and 10 = severe galling.

G. hirsutum cross demonstrated that *rkn1* showed a typical bimodal pattern of segregation for a major gene. The *rkn1* gene explained the main resistance phenotype effects in Acala NemX of suppressing both nematode-induced root galling and nematode reproduction. Further, the level of resistance expression in heterozygous plants or populations including the F_1 indicated that *rkn1* operated as a recessive gene or incompletely recessive gene depending on the screening conditions. Root-galling reactions in heterozygous plants were not different from homozygous susceptible plants in tests that were terminated 125 d or longer after inoculation. However, the heterozygous plants screened for this gene for the short duration of 60 d after inoculation showed slight suppression of nematode egg production and root galling compared to the susceptible parent. This confirmed our finding with SSR markers for *rkn1*, in which the co-dominant marker CIR316 in the heterozygous condition correlated with plants in the susceptible class that had root-gall indices between 2 and 4, with homozygous susceptible plants having gall ratings mostly greater than 4 in short duration screenings (Wang et al., 2006). Because of the sensitivity of the resistance phenotype to test conditions, markers will be especially helpful in distinguishing heterozygous plants from homozygous susceptible plants. Levels of galling and egg production varied among tests, being lower in the shorter duration screening of the second set of progenies. However, we found that including resistant and susceptible parents in each test and basing the resistance threshold on the mean plus 1 SD of the resistant parent score provided a definitive classification of resistance for each test.

In a preliminary study based on F_1 (NemX $\times$ Deltapine 90) and 43 F_2 individuals, Zhou et al. (1999) sug-

gested that Acala NemX contained a recessive or neutral gene for *M. incognita* resistance. McPherson et al. (2004) reported a two-gene model for *M. incognita* resistance in M-315 RNR (*G. hirsutum*) that was developed by backcrossing Auburn 634 RNR to Deltapine 61 (Shepherd et al., 1996), with one dominant gene (Mi_1) and an additive gene (Mi_2). However, they hypothesized that Acala NemX may have the same additive gene Mi_2 as in M-315 and M78-RNR and that homozygous Mi_2 may confer a low level of resistance (McPherson et al., 2004). In our study, *rkn1* in Acala NemX acted as a major resistance gene in suppressing root galling and nematode reproduction. Therefore, *rkn1* in Acala NemX may be different from Mi_1 and Mi_2 in M-315.

McPherson et al. (1995) postulated that highly resistant Auburn 623 RNR may carry two genes, with one coming from each parent, Clevewilt 6-3-5 and Mexico Wild Jack Jones. Bezawada et al. (2003) reported that one recessive gene in Clevewilt 6-1 may control root-knot nematode resistance in crosses with Stoneville 213. Assuming Clevewilt 6-3-5 has the same gene as Clevewilt 6-1, Auburn 623 RNR should have one recessive gene controlling *M. incognita* resistance. Auburn 634 RNR, a highly resistant breeding line, was developed by backcrossing Auburn 623 RNR to Auburn 56, a moderately resistant cultivar (Shepherd, 1982b) with less resistance than Clevewilt 6 (Shepherd, 1983). Therefore, Auburn 634 RNR may carry resistance genes from both Auburn 623 RNR and Auburn 56. If M-315 contains only one dominant gene and one additive gene for resistance, the recessive gene in the pedigree may have been lost during breeding selection. Whether the recessive gene in Clevewilt 6-1 is the same as *rkn1* in Acala NemX is not known, but could be determined by allelism tests with the cross NemX $\times$ Clevewilt 6. In a study of crosses of root-knot-resistant $\times$ susceptible *G. barbadense* L. breeding stocks (Turcotte et al., 1963), two recessive genes were reported to determine *M. incognita* resistance in this tetraploid cotton species. Resistance genes found in a related species background should be tested for their relationship to the *rkn1* gene in Acala NemX.

In tracing the origin of the resistance in Acala NemX, different accounts of the pedigrees were found (Robinson et al., 2001). The advanced line donor of the Acala NemX resistance was breeding line N6072, which Hyer and Jorgenson (1984) reported that they had developed from the cross of a Missouri line, FBCX-2, with a Shafter AXTE line. FBCX-2 was moderately resistant and developed from the cross of Auburn 56, carrying some resistance, and Sea Island Seabrook 12-B2. Oakley (1998) indicated that N6072 was developed from the cross Acala 1-2302 $\times$ Tanguis, with Acala 1-2302 derived from susceptible Acalas SJ-1 and SJ-2. N6072 had greater resistance than Auburn 56 (Hyer et al., 1979), whereas the level of resistance in Acala NemX was simi-

lar to that in N6072 (Ogallo et al., 1997). The greater resistance in N6072 or Acala NemX may be due to transgressive inheritance, which is quite common in cotton, such as that reported for Auburn 623RNR, a transgressive segregant for root-knot nematode resistance from the F_6 generation of a cross of Clevewilt 6-3-5 and Mexico Wild, and Auburn 61 from an F_6 of the cross of Hybrid 257 and Mexico Wild (Shepherd, 1974). Three lines (N9281, N9308, and N9311) also had greater resistance than their resistant parent N6074, one of the sister lines of N6072 (Hyer and Jorgenson, 1984).

In our study, evidence for transgressive segregation involving the *rkn1* gene in Acala NemX was found. The F_1 between Acala NemX and its resistant sister line N901 had greater resistance than either parent. Further, in the $F_{2:7}$ RIL population, the significant variation in the level of resistance among the 34 resistant lines, with 21 being more resistant than Acala NemX, indicated transgressive segregation in this *G. hirsutum* cross. Presumably, a resistance-enhancing factor was contributed from susceptible Acala SJ-2, in which the enhanced resistance was achieved when both the *rkn1* gene from Acala NemX and the Acala SJ-2 factor were present in the homozygous condition. The susceptible RIL did not differ from susceptible parent Acala SJ-2, indicating that the Acala SJ-2 factor had no measurable effect on susceptibility in the absence of the *rkn1* gene from Acala NemX. However, differences in F_1 susceptibility, with those produced from Maxxa as susceptible parent being more susceptible than when Acala SJ-2 was the susceptible parent, may indicate a minor influence of the transgressive factor in the susceptible background. Such minor effects would require more stringent phenotype testing to be more clearly characterized.

In summary, the *M. incognita* resistance in Acala NemX determined by gene *rkn1* was conferred in an incomplete recessive manner in an intraspecific *G. hirsutum* cross. This gene is effective in suppressing both nematode-induced root galling and nematode reproduction on cotton roots as measured by numbers of eggs produced during two or more months from inoculation. The reduced galling and egg production resistance phenotypes are highly correlated among individuals of different segregating populations. The *rkn1*-based resistance was found to be enhanced in some $F_{2:7}$ recombinant inbred lines by a modifying gene or genes contributed by the susceptible parent genotype Acala SJ-2. These results suggest that the Acala NemX resistance level can be improved in *G. hirsutum* crosses depending on the transgressive interaction with additional genes in susceptible *G. hirsutum* genotypes. Understanding relationships between resistance sources and development of molecular markers will expedite the transfer of the resistance genes into commercial cotton and determine their value in gene combinations pyramided into cultivars to produce more durable and higher levels of root-knot nematode resistance.

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