1319 Host Plant Resistance to Root-Knot Nematode in Cotton

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ABBREVIATIONS: AFLP (Amplified Fragment Length Polymorphism); BAC (Bacterial Artificial Chromosome); CAPS (Cleaved Amplified Polymorphic Sequence); CPCSD (California Planting Cotton Seed Distributors); EST (Expressed Sequence Tag); FOV (*Fusarium oxysporum* f. sp. *vasinfectum*); FW (Fusarium Wilt); QTL (Quantitative Trait Locus); RAPD (Random Amplified Polymorphic DNA); RIL (Recombinant Inbred Line); RKN (Root-Knot Nematode); SNP (Single Nucleotide Polymorphism); SSR (Simple Sequence Repeat)

Host-plant resistance is economic and highly effective for root-knot nematode (RKN) *Meloidogyne incognita* control in cotton *Gossypium hirsutum*. Nematode resistance can protect cotton plants from direct injury and crop loss from nematode infection, and can protect against the root-knot nematode-Fusarium wilt disease complex caused by combined nematode and fungus infection. In addition, resistance in cotton suppresses soil nematode population densities, thereby protecting other susceptible crops grown in rotation. Recently, nematode R gene mapping in cotton has revealed relationships between resistance sources and linked molecular markers for use in genetic improvement of cotton. Markers are important for the efficiency of incorporating resistance genes into elite cultivars. Microsatellite markers (SSR) linked to RKN resistance in *G. hirsutum* cv. Acala NemX were identified using segregating progenies and recombinant inbred lines from intraspecific crosses and an interspecific cross with *G. barbadense* cv. Pima S-7. Informative SSR were mapped on the above populations and one co-dominant SSR marker CIR316 was identified tightly linked (2.1 to 3.3 cM) to a major resistance gene (designated *rkn1*). Additional markers allowed the *rkn1* gene to be mapped to cotton chromosome 11. Other markers linked to *rkn1* were developed from AFLP screening and converted to CAPS and SNP markers for high throughput screening. Subsequently, a similar location of the major resistance determinant present in the Auburn source of RKN resistance was reported, and additional non-linked minor QTL for resistance identified. Higher levels of resistance in cotton by transgressive segregation were obtained with factors contributed by susceptible parents in intraspecific and interspecific crosses. These gene relationships and use of markers for cotton improvement are discussed.

Key words: cotton, Fusarium wilt, *Fusarium oxysporum* f. sp. *vasinfectum*, genetic mapping, *Gossypium hirsutum*, *Gossypium barbadense*, *Meloidogyne incognita*, phenotypic expression, resistance, *rkn1*, root-knot nematode, rotation, transgressive segregation.

Introduction

The southern root-knot nematode (RKN) *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). In the USA Fusarium wilt symptoms induced by the common mild forms of *Fusarium oxysporum* f. sp. *vasinfectum* (FOV), such as races 1 and 2, typically are associated with the presence of RKN in fields with coarse-textured sandy soils (Jeffers and Roberts, 1993). In the San Joaquin Valley of California, where cotton is grown intensively under irrigation, *M. incognita* and FOV occurs as a complex infection in up to 20% of the cotton planting area (Anonymous, 1996; Goodell et al., 1992). Restrictions on nematicide use and their relatively high cost in cotton production have expedited the development of root-knot resistant cotton cultivars.

Resistance in nematode management

Over the last decade we have been studying the RKN resistance trait in the upland cotton cv. Acala NemX, a variety released by CPCSD (Oakley, 1995) that was bred with nematode resistance for use in the San Joaquin Valley of California. This RKN resistant cotton and the California cotton production system for which it was designed has proved to be a highly informative model system for analysis of RKN and FW pest and disease management and resistance genetics. In multiple field experiments the nematode resistance in Acala NemX was found to be highly effective in protecting plants from the effects of root infection (Ogallo et al., 1997; 1999). The lint yield of Acala NemX was less than that of the standard susceptible cv. Acala Maxxa on non-infested fields or those with low levels of RKN infestation by an average of 6%. However, in fields with medium or high levels of RKN infestation, Acala NemX yields decreased only slightly by 5-23%, whereas Acala Maxxa yields were severely decreased by 59-65% (Ogallo et al., 1997). Subsequently, CPCSD released a higher yielding and agronomically superior version of Acala NemX in 2004, named cv. Acala NemX HY (Anonymous, 2005), carrying the same RKN resistance as Acala NemX and providing a better yielding nematode resistant cotton.

In addition to high relative yield potential under RKN and RKN-FOV infection, the utilization of resistant Acala NemX also can greatly increase the rotational value of cotton for managing RKN in field and vegetable cropping systems (Ogallo et al., 1997; 1999). The underlying principal of this rotation effect is the suppression of nematode reproduction on resistant genotypes resulting in relatively low final soil population densities at the end of the crop season. These in turn have a lower damage potential to susceptible crops than the typically high populations remaining after a susceptible crop (Roberts, 2002). We tested the protection value of resistant cotton to RKN susceptible crops planted in the year following cotton, initially assessing the effects of one year of a resistant cotton genotype on growth of susceptible lima bean (Ogallo et al., 1997). Compared to the average lima bean yield following resistant cotton genotypes including Acala NemX, 'N6072', 'N8577', and sister lines, the lima bean yield following susceptible Acala Maxxa and 'Acala SJ-2' was reduced by 45%.

In more detailed rotation experiments the effects were measured of one or two years of resistant cotton on subsequent susceptible rotation crop yields of lima bean or Acala Maxxa cotton (Ogallo et al., 1999). Lima bean yields were reduced by a mean of 50% after one or two successive crops of susceptible Acala Maxxa compared to lima bean yields after one or

two successive crops of resistant Acala NemX, and yields were not different between treatments of one or two previous crops of Acala NemX (Ogallo et al., 1999). Similar results were obtained in the treatments where susceptible cotton Maxxa was planted after resistant or susceptible cotton, demonstrating the value of RKN resistant cotton in both intensive cotton and mixed crop production systems. The differential nematode multiplication rates in the cotton plantings confirmed the suppressive effects of the RKN resistance in Acala NemX as the basis for the protection of the following susceptible crops.

The RKN resistance in cotton can protect plants from FW disease under field conditions (Shepherd, 1983; 1986; DeVay et al., 1997; Jeffers and Roberts, 1993; Hyer et al., 1979; Ogallo et al., 1997). The earlier studies in California (Hyer et al., 1979) and Mississippi (Shepherd, 1986) had shown that the moderate FW tolerance bred into cotton lines was not effective in protecting cotton yield when the RKN-FOV disease complex was present, but that RKN resistance in cotton, including in the more FW susceptible genotypes, was highly effective in suppressing FW symptoms and wilt-induced yield loss. Analysis of this phenomenon was expanded to better understand the field level interaction of RKN and FOV joint infection on cotton phenology (DeVay et al., 1997), and the effects of FOV tolerant and RKN resistant cotton genotypes on the interaction including the influence of cotton planting date as a regulator of nematode infection potential (Jeffers and Roberts, 1993; Ogallo et al., 1997). These studies confirmed the strong positive relationship between RKN infection and the severity of FW disease, and that tactics to reduce nematode populations in soil, whether nematicides, RKN resistance, or planting date manipulation can be used to control FW caused by the common mild forms of FOV. While resistance to FW has been identified in Pima cottons (Wang and Roberts, 2006b) varieties with strong FW resistance still need to be developed, coupled with analysis of the resistance genetics and marker development. A more virulent form of FOV, Race 4, has been found recently in California and efforts are in progress to breed for Race 4 resistance, for which some Pima cottons are useful sources (Ulloa et al., 2006). The Race 4 type of FOV is not dependent on the RKN co-infection in the field for severe FW to develop, occurring in some fields with finer textured loam and clay-loam soils in which RKN is absent. Thus for virulent FOV, RKN resistance may not be as effective as it is in managing the mild forms of the wilt disease. However, we have found that RKN co-infection with Race 4 FOV can exacerbate FW symptoms, suggesting that RKN resistance could be useful in fields infested with both RKN and FOV Race 4 (Roberts et al., 2006).

Variation in virulence among *M. incognita* isolates to the Acala NemX resistance has been reported (Ogallo et al., 1997). The selection of virulent or 'resistance-breaking' forms of RKN and other plant parasitic nematodes by repeated exposure to resistant plants is a major concern in the deployment of resistance, but this has not been observed in commercial cotton fields due to the absence of available RKN resistant cotton cultivars. However, the virulence status of *M. incognita* isolates parasitic on cotton measured by the nematode reproduction potential in controlled greenhouse test inoculations revealed the presence of virulence (Ogallo et al., 1997). Five isolates from commercial cotton fields in the San Joaquin Valley were avirulent to the N6072-derived Acala NemX resistance gene *rkn1*, and to another resistant breeding line N8577, with reproduction factors (multiplication rates) of 1 to 4. However, an isolate from a field at the Shafter Research Station used by Hyer and Jorgenson (1984) in long-term RKN resistance screening, including in the development of the resistant N6072 and N8577 lines, had a significantly higher reproduction factor of 8, representing at least moderate virulence. This result indicated that virulence selection had occurred on resistant genotypes in the cotton breeding program, and demonstrated the potential for virulence selection in the future when resistant cultivars become available for intensive production on infested fields. However, we are not aware of reports of natural RKN virulence to cotton resistance from commercial production fields.

Resistance genetics and breeding

The background to RKN resistance breeding in cotton indicates a rich source of resistance present among *Gossypium* germplasm, especially in the allotetraploid species *G. hirsutum* and *G. barbadense*, and in the A_2 genome donor diploid species *G. arboreum* (Robinson et al, 2001; P. Roberts, M. Ulloa, and C. Wang, unpublished). The first highly resistant cotton germplasm available for RKN resistance breeding was 'Auburn 623 RNR' (*G. hirsutum*), a transgressive segregant for resistance from a cross of 'Clevewilt 6-3-5' and 'Wild Mexico Jack Jones' (Shepherd, 1974). Subsequently, 'Auburn 634 RNR' with a high level of RKN resistance was developed from the cross Auburn 623 RNR x 'Auburn 56', and in turn was used to develop the M-line series ('M-120', 'M-315', etc.) of resistant genotypes (Shepherd, 1982; Shepherd et al., 1988; 1996). These lines were not suitable as commercial cultivars but provided advanced breeder line resistant stocks. Another series of RKN resistant breeding lines including 'LA RN 4-4' and 'LA RN 1032' and the released cv. Stoneville LA 887 was developed from crosses emanating from 'Clevewilt 6' as the likely resistance donor (summarized in Robinson et al., 2001).

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 RKN resistance (Oakley, 1995; Ogallo et al., 1997). Acala NemX was developed from the cross 'Acala B1662' x 'N-3'; N-3 was derived from the nematode resistant line N6072 (Hyer and Jorgenson, 1984). The improved cv. Acala NemX HY has the same RKN resistance source, N6072, and derived from the cross resistant 'N656' x susceptible 'Acala Prema' (pedigree of N656 = 'B1662' x N6072). However, the origin of the RKN resistance in the N6072 pedigree source is not clear from the existing pedigree reports (Hyer and Jorgenson, 1984; Oakley, 1995; Robinson et al., 2001). Line N6072 was developed by Hyer and Jorgenson (1984) who indicated that it was a resistant transgressive segregant selection from the cross of 'AXTE' x 'FBCX-2'. FBCX-2 was chosen because it had 'Auburn 56' and 'Sea Island Seabrook 12-B2' in its pedigree, both reported to have some RKN resistance. The AXTE line pedigree is complex, and included a triple hybrid between *G. arboreum*, *G. thuberi*, and *G. hirsutum* in its background, any of which may have contributed resistance genes (Robinson et al., 2001). In the Acala NemX release the N6072 resistance donor is indicated to have been selected from the cross '12302' x 'Tanguis' (Oakley, 1995). We are currently screening these pedigree lines where available in an attempt to identify the source of gene *rkn1* in Acala NemX.

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, and had indicated earlier that resistance in some other M lines such as 'M-75' and 'M-78' might be different from the M-315 resistance. Analysis of an F_2 population indicated that one recessive gene conferred moderate resistance in 'Clevewilt 6-1' (Bezawada et al., 2003). Zhou et al. (1999) reported a single recessive or additive gene control of RKN resistance in Acala NemX based on small F_2 family analysis.

Based on these analyses, the origins of and the genetic relationships between the various RKN resistance traits remain to be determined with the application of molecular markers for the resistance determinants. In efforts to locate RKN resistance genes in the cotton genetic linkage map, as described further on, several very useful RKN resistance gene markers have been identified that provide some insight into the gene relationships and their origins. In

mapping the major resistance determinant *rkn1* in Acala NemX, a single, incompletely recessive gene, we identified both SSR (Wang et al., 2006c) and AFLP and CAPS (Wang and Roberts, 2006a) markers tightly linked to *rkn1* that are informative for comparing resistant genotypes. In marker screens of resistant germplasm, the same molecular patterns were amplified with both the SSR marker CIR316 and the CAPS marker GHACC1 among resistant cotton genotypes Acala NemX, Clevewilt 6, Auburn 623 RNR, Auburn 634 RNR, M-120, M-315, LA RN 4-4 and LA RN 1032, with the exception of Auburn 623 RNR with the CIR316c marker (Wang and Roberts, 2006a). These results suggested that Acala NemX may have the same resistance source as Clevewilt 6, and that these other lines derived from Clevewilt 6 may carry the same gene as Clevewilt 6. In order to confirm these resistance relationships, crosses of Acala NemX and Acala SJ-2 with Clevewilt 6 and other resistant lines have been made and segregating populations are being screened for resistance phenotype and marker profiles. The SSR marker CIR316 is especially useful because its co-dominance enables the differentiation of heterozygous from homozygous individuals in progeny screening and selection (Wang et al., 2006c).

Recessive gene(s) can be masked in the presence of a dominant gene. McPherson et al. (2004) reported that M-315 carried one dominant gene and one additive gene for resistance. The GHACC1 and CIR316 markers should be useful to test this population to confirm whether M-315 carries the recessive *rkn1* gene. Our finding of CIR316 as an important major resistance locus marker was confirmed by Shen et al. (2006) in a QTL mapping study of the RKN resistance in line M-120, and they also detected the CIR 316 resistance marker profile in Clevewilt 6. Our marker screens also showed that M-75, M-78, and 'M-188' had the same kind of marker profiles as susceptible Acala SJ-2 and they were different from M-315 with both markers, suggesting M-75, M-78, and M-188 lines may not carry the *rkn1* gene and that they have different resistance genes from M-315 (Wang and Roberts, 2006a). These results supported the hypothesis of McPherson et al. (1995) based on phenotypic tests that M-188 and M-78 with moderate resistance might have different resistance genes to those found in M-315, but not that M-75 may have the same genes as M-315. In addition, we found that Wild Mexico Jack Jones had the same marker profiles as Acala SJ-2 with CIR316 and different profiles with GHACC1, indicating recombination between the two markers in this genotype.

Subsequently, we analyzed the behavior of the *rkn1* markers developed from Acala NemX with newly developed RAPD and STS markers for the resistance in Auburn 634 RNR (Nui et al., 2007). This resistance, thought to be controlled by dominant and additive genes *Mi1* and *Mi2* (Mcpherson et al, 2004), had close marker associations that allowed the placement of a major resistance determinant, presumably *Mi2*, in the same chromosome region as *rkn1*, but not at the same genetic location (Nui et al., 2007). In addition to the marker analysis, we resistance phenotype-screened an F_2 population from the cross Acala NemX x Auburn 634 RNR, and found no susceptible recombinants among the progeny of 200 individuals (Nui et al., 2007). The phenotype data supported the close genomic arrangement between these genes, while the marker results suggested that *rkn1* and *Mi2* are different but linked genes. We hypothesize they represent divergent tandem repeated genes that may be homologues, similar in arrangement to that between the *Mi-9* and *Mi-1* RKN resistance genes and their homologues we are studying in tomato (Jablonska et al., 2007). In another study of the Auburn 623 RNR-derived and Acala NemX RKN resistance traits, Zhang et al. (2007) used a nine-parent diallel analysis of F_1 and parent genotypes. They concluded that the resistance in these sources was partially dominant, and that the general combining ability was more important than the specific combining ability for RKN resistance.

Transgressive segregation and resistance phenotypes

The *rkn1* gene in Acala NemX is effective in suppressing both nematode induced root-galling and nematode reproduction on cotton roots (Wang et al., 2006a). This high positive correlation between root galling and nematode reproduction (Fig. 1) was also found in tests with resistant and susceptible upland cotton by Zhang et al. (2006). Use of the simpler and more cost-effective index of root galling as a measure of RKN resistance is adequate for most RKN resistance breeding purposes. In our progeny screens the heterozygous F_1 did not differ in root galling reactions from the susceptible parent Acala SJ-2, and had slightly suppressed nematode egg production, indicating an incomplete recessive behavior of *rkn1* (Wang et al., 2006a). Moreover, we observed transgressive segregation for nematode resistance in some $F_{2:7}$ RI homozygous resistant lines, indicating susceptible genotype Acala SJ-2 contributed to the level of resistance (Wang et al, 2006a). The galling reaction phenotypes of the individual RIL in this population are shown in Fig. 2, in which the distinct separation of resistant and susceptible classes based on presence and absence of *rkn1*, respectively, is apparent, as is the separation of 'Acala NemX equivalent' and 'higher than Acala NemX' resistant sub-classes.

Another component of our findings comes from analysis of the *rkn1* gene in interspecific crosses between *rkn1* donor Acala NemX and susceptible *G. barbadense* Pima S-7 (Wang, 2006). We first noticed that F_1 plants were much more resistant than the NemX parent, and quite different from the recessive behavior of *rkn1* found in the crosses with Acala SJ-2. In advanced segregating progenies we were able to confirm the presence of transgressive segregants with resistance phenotypes beyond the range of the parent (Fig 2). Test-cross progenies generated from Acala NemX crossed with the F_1 of Pima S-7 x Acala SJ-2 also were highly informative in showing the two distinct resistant and susceptible classes, in which the resistant class contained one allele of both the *rkn1* and the Pima S-7 transgressive factor genes, and the susceptible class only one allele of *rkn1*.

The transgressive segregation effects on cotton traits present a valuable resource for cotton improvement because extreme genotypes beyond the parent range are generated that, when advantageous such as with stronger resistance or higher quality fiber, can be selected in breeding programs. This is certainly true for the enhanced RKN resistance in both intraspecific and interspecific crosses involving gene *rkn1*. However, in genetic analyses of RKN resistance, it appears that transgressive segregation plays an important part in the uncertainty about the number of resistance genes, whether they are dominant or recessive relative to phenotypes of heterozygous plants, the uniqueness of genes in different background sources, and the resistance gene origins in breeding pedigrees. The behavior of gene *rkn1* from Acala NemX exemplifies this challenge. In the heterozygous condition in progenies from our crosses with highly susceptible upland and Pima cotton parents, *rkn1* behaved as recessive or incompletely recessive in Acala SJ-2 crosses or completely dominant in Pima S-7 crosses, and partially dominant in the diallel analyses of Zhang et al. (2007). Transgressive segregation is quite common in a wide range of plants and animals (Bell and Travis, 2005; Rieseberg et al., 2003), but seems to be especially common in cotton, not only for nematode resistance, but also for resistance to Verticillium and Fusarium wilts and bacterial blight (Bayles et al., 2005; Bolek et al., 2005; Wang and Roberts, 2006b), and for agronomic traits including fiber quality (R. G. Cantrell, personal communication). The functional basis of this process, which could involve epistatic, pleiotropic, or additive gene interactions, is not known, but identity of gene locations and their subsequent cloning should provide the opportunity to unravel this valuable phenomenon in cotton.

Mapping RKN Resistance Genes in Cotton

The first detailed restriction fragment length polymorphism (RFLP) linkage maps for cotton were generated through hybridization between *G. hirsutum* and *G. barbadense* (Reinisch, et al., 1994) and included 705 RFLP loci assembled into 41 linkage groups and 4675 cM. The cotton genome was estimated to contain about 400-kb DNA per cM (Reinisch, et al., 1994). Since that time, a series of updated and denser, more informative maps have been developed. These include those based primarily on compilations and integration of multiple marker types (Lacape et al., 2003; Nguyen et al., 2004), including one from Rong et al. (2004) with more than 3000 markers assigned. More recently, cotton genetic maps have been based on the newly available EST-derived (Han et al., 2006; Park et al., 2005) and BAC-end derived (Frelichowski et al., 2006) SSR markers, allowing additional assignments of linkage groups to cotton chromosomes. A complete assignment of linkage groups to chromosomes was achieved recently by translocation and fluorescence in situ hybridization (FISH) mapping (Wang et al., 2006b). The availability of the cotton linkage maps and the high levels of polymorphism between the *G. hirsutum* and *G. barbadense* parental genotypes (e.g. 'TM-1' x '3-79'; Acala Nemx, Acala SJ-2 or 'Acala 44' x Pima S-7) make the localization of nematode and wilt resistance genes and map-based gene cloning feasible.

In our analyses of RKN resistance in the Acala NemX, we identified the major resistance determinant *rkn1* using a series of progenies (F_1, F_2 and $F_{2:3}$, TC, BC_1F_1 and RIL) from crosses of Acala NemX with susceptible Acala SJ-2 and Pima S-7 (Wang et al., 2006a,c). The *rkn1* gene mapped to cotton LG A03 (Wang et al. 2006c), and A03 was subsequently assigned to chromosome 11 (Wang et al., 2006b). Our mapping work was based primarily on screening existing SSR markers place throughout the genome. From these screens, markers CIR316 and BNL1231 in particular were highly informative for mapping. CIR316 maps within 2-4 cM of *rkn1* depending on the population used (Wang et al., 2006b), and this marker showed 0.89 – 0.96 correlation (Pearson correlation coefficient) with the resistance phenotype classes in F_2 and BC populations. In addition, we identified AFLP markers linked to *rkn1*, one of which we converted to a CAPs marker (GHACC1), and ultimately a SNP marker for high throughput screening (Wang and Roberts, 2006a). The AFLP and its converted CAPS markers co-segregated and mapped 2.6 cM from the *rkn1* locus. These and other markers linked to *rkn1* on chromosome 11 are shown in Fig. 3. In addition, we have evidence that at least one transgressive factor interacting with *rkn1* also maps to this same region (C. Wang, M. Ulloa, and P. Roberts, unpublished), and we have started to map some of the BAC-end sequence derived SSR markers into the chromosome 11 map. Thus we have a good start to saturation mapping in this region.

In other RKN resistance mapping efforts, Shen et al. (2006) reported that one major dominant gene (accounting for 63.7% of phenotypic variation in resistance) in M-120 RNR (ex. Auburn 634 RNR) was also associated with SSR marker CIR316 localized on chromosome 11, and one minor gene (accounting for 7.7% phenotypic variation) mapped to chromosome 7 when crossed with *G. barbadense* Pima S-6. They noted that the chromosome 7 factor was associated with the Pima S-6 susceptible parent and was present in one but not the other of two F_2 populations used in the QTL analysis. In a second study employing SSR markers in one cross within *G. hirsutum* of resistant (from Auburn 634 RNR) x susceptible near-isolines, Ynturi et al. (2006) identified two independent genes contributing to RKN resistance, one additive and dominant on chromosome 14 (with three markers explaining 12, 19, and 21% variation in root galling) and the other additive on chromosome 11. The chromosome 11 gene with only additive effects (11% variation in root galling explained) was linked to marker BNL1231, thus placing it within the same region as *rkn1* from Acala NemX. This work is consistent with the RAPD and STS marker associations

with Auburn 634 RNR derived resistance on chromosome 11 determined by Nui et al., 2007, in which a gene from Auburn 634 RNR, presumably *Mi2*, was placed in this same chromosome 11 region. Thus, a suite of genes for root-knot resistance have been identified and several map to the same region of chromosome 11, however their relationships to one another are unclear.

Chromosome 11 is especially interesting because it also contains other resistance genes. Three QTL (CM12, STS1, and 3174-2) with large effect on resistance to Verticillium wilt were mapped to chromosome 11 with SSR markers by Bolek et al. (2005) in Pima S-7 based on a cross with Acala 44. The recent introgression of reniform nematode (*Rotylenchulus reniformis*) resistance from *G. longicalyx* into upland cotton by Robinson et al. (2007) also is based on a trait that maps to chromosome 11 (R. Nichols, personal communication). Thus chromosome 11 represents a rich resource for resistance genes, including a cluster of RKN resistance gene specificities, and presents an excellent opportunity for resistance gene exploitation.

Further study with molecular markers linked to the resistance genes will help to clarify the relationships between the RKN resistance genes, and indicate possibilities for combining resistance traits to obtain higher or more durable levels of resistance by taking advantage of novel resistance phenotypes generated through transgressive segregation. The deployment of RKN resistance gene markers should expedite development of resistant cultivars via marker-assisted selection. New technologies developed through molecular analyses including physical mapping and gene cloning should enable deployment of resistance in superior cotton cultivars.

Figure legends

Fig 1. The relationship of root galling index and RKN egg production in reciprocal backcross populations of F_1 (Acala SJ-2 x Acala NemX) x Acala NemX segregating for gene *rkn1*. Galling Index: 0 to 10 scale; 0 = no galling, and 10 = severe galling. (From J. Nematol. 38:250-257).

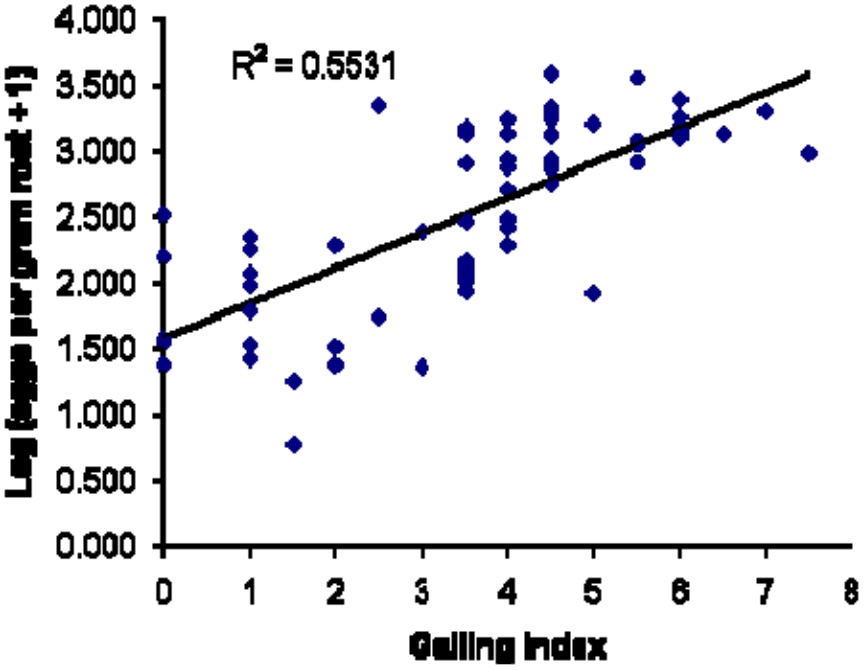


Fig. 2. Distribution of different classes of RKN resistance reaction of $F_{2:7}$ RIL (Acala NemX x Acala SJ-2) based on galling index. Mean values of four plants per line plus SD bar. Mean scores of the resistant (Acala NemX) and susceptible (Acala SJ-2) parents are indicated. Galling Index: 0 to 10 scale; 0 = no galling, and 10 = severe galling. (From J. Nematol. 38:250-257).

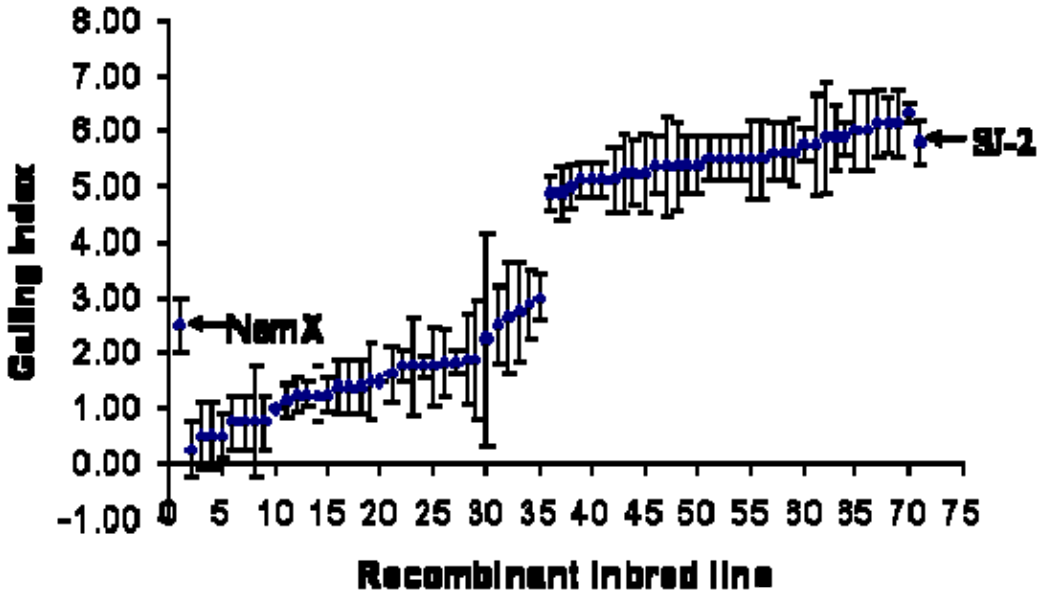
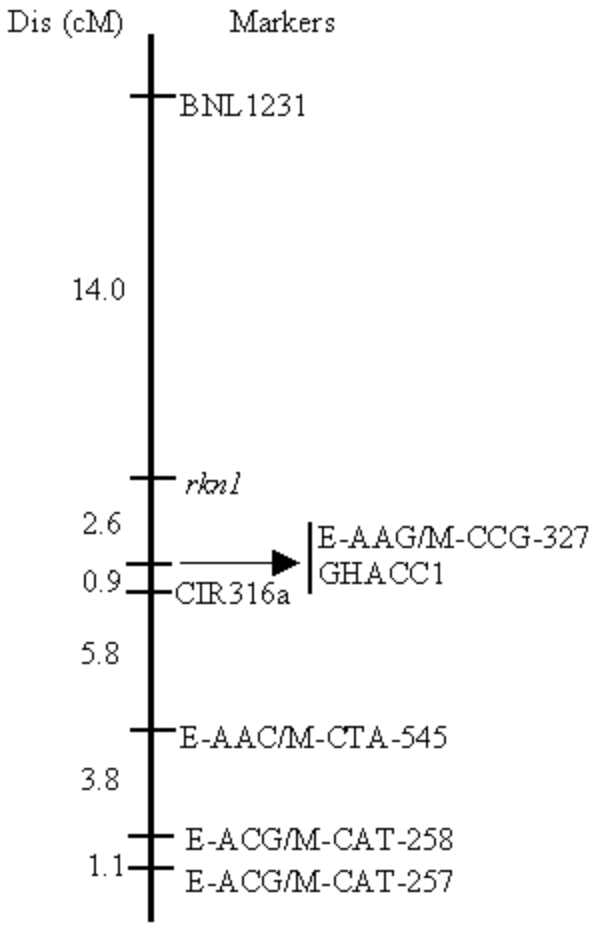


Fig. 3. Location of the resistance gene *rkn1* on chromosome 11 relative to four AFLP, one CAPS (GHACC1) and two SSR markers (CIR316a and BNL1231) in an $F_{2:7}$ (Acala NemX x Acala SJ-2) segregating RIL population. Distances are reported in Kosambi cM. (From Euphytica 152:185-196).



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