QTL Analysis of Transgressive Nematode Resistance in Tetraploid Cotton Reveals Complex Interactions in Chromosome 11 Regions

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Transgressive segregation in cotton (Gossypium spp.) provides an important approach to enhance resistance to the major pest root-knot nematode (RKN) Meloidogyne incognita. Our previous studies reported transgressive RKN resistance in an intraspecific Gossypium hirsutum resistant NemX × susceptible SJ-2 recombinant inbred line (RIL) population and early generations of interspecific cross Gossypium barbadense (susceptible Pima S-7) × G. hirsutum (NemX). However, the underlying functional mechanisms for this phenomenon are not known. In this study, the region of RKN resistance gene rkn1 on chromosome (Chr) 11 and its homoeologous Chr 21 was fine mapped with G. raimondii D5 genome reference sequence. Transgressive resistance was found in the later generation of a new RIL population F2:7 (Pima S-7 × NemX) and one interspecific F2 (susceptible Pima S-7 × susceptible SJ-2). QTL analysis revealed similar contributions to root-galling and egg-production resistance phenotypes associated with SSR marker CIR316 linked to resistance gene rkn1 in NemX on Chr 11 in all seven populations analyzed. In testcross NemX × F1 (Pima S-7 × SJ-2) marker allele CIR069-271 from Pima S-7 linked to CIR316 contributed 63% of resistance to galling phenotype in the presence of rkn1. Similarly, in RIL population F2:8 (NemX × SJ-2), SJ-2 markers closely linked to CIR316 contributed up to 82% of resistance to root-galling. These results were confirmed in BC1F1 SJ-2 × F1 (NemX × SJ-2), F2 (NemX × SJ-2), and F2 (Pima S-7 × SJ-2) populations in which up to 44, 36, and 15% contribution in resistance to galling was found, respectively. Transgressive segregation for resistance was universal in all intra- and inter-specific populations, although stronger transgressive resistance occurred in later than in early generations in the intraspecific cross compared with the interspecific cross. Transgressive effects on progeny from susceptible parents are possibly provided in the rkn1 resistance region of chromosome 11 by tandemly arrayed allele (TAA) or gene (TAG) interactions contributing to transgressive resistance. Complex TAA and TAG recombination and interactions in the rkn1 resistance region provide three genes and a model to study disease and transgressive resistance in polyploid plants, and novel genotypes for plant breeding.

Keywords: root-knot nematodes (RKN), Meloidogyne incognita, Gossypium spp., upland, Pima, allele interactions
INTRODUCTION

Host-plant resistance is a highly effective strategy to manage root-knot nematode (RKN, *Meloidogyne* spp.) damage in crops. The RKN *Meloidogyne incognita* is one of the most important pests of cotton (Goodell and Montez, 1994), and effective resistance is available for improving cotton cultivar performance (Starr et al., 2010). In addition, transgressive segregation is one of the approaches to enhance resistance, in which segregating hybrids exhibit extreme or novel phenotypes compared to the phenotypes of parental lines (Rieseberg et al., 1999, 2003). Improved resistance traits generated by transgressive segregation in progenies derived from interspecific and intraspecific crosses have been reported (Cherif and Harrabi, 1993; Zhang et al., 2001; Imitiaz et al., 2003a,b; Navabi et al., 2004; Bell and Travis, 2005; Zhao et al., 2005; Staal et al., 2006; Aghnoum and Niks, 2011). Transgressive segregation provides one of the major selection sources for enhanced resistance to RKN in cotton (Gossypium hirsutum L.) (Shepherd, 1974; Wang et al., 2006a; Wang C. et al., 2008, 2012; Ulloa et al., 2016). Other cotton-pathogen systems also displayed transgressive resistance, such as Fusarium wilt (Wang and Roberts, 2006a; Ulloa et al., 2011, 2013, 2016), Verticillium wilt (Bolek et al., 2005; Wang H. M. et al., 2008), and bacterial blight (Bayles et al., 2005).

Three major germplasm sources of RKN resistance have been utilized in Upland cotton *G. hirsutum*, NemX (Ogallo et al., 1999; Wang et al., 2006a; Roberts and Ulloa, 2010), Clevewilt 6 and derived lines Stoneville LA887 and Paymaster H1560 (Robinson et al., 2001), and Auburn 623 and its derivatives (Shepherd, 1974; McPherson et al., 2004). Highly resistant Auburn 623 RNR was derived from two moderately resistant parental lines, Clevevilt 6-1 and Mexico Wild Jack Jones. The RKN resistance sources Auburn 623 RNR, Auburn 634 RNR, and their derived N-lines (Hyer and Jorgenson, 1984) were reported to be transgressive segregants (Shepherd, 1974; Hyer et al., 1979). Wang C. et al. (2008) reported that a segregating factor (RKN2) from susceptible parent *Gossypium barbadense* Pima S-7 could not function alone but interacted with a major recessive gene rkn1 in *G. hirsutum* NemX (Wang et al., 2006b) to produce a higher resistance phenotype than resistant parental line NemX in progeny of the interspecific cross between Pima S-7 and NemX. Interestingly, the two genes RKN2 and rkn1 were mapped to the same region on Chr 11 (Wang C. et al., 2008). Transgressive resistance was also observed in some intraspecific F_{2:7} (NemX × SJ-2) RI (recombinant inbred) homozygous resistant lines, indicating susceptible parent SJ-2 contributed to higher resistance in progeny than the rkn1 resistance contributed by NemX alone (Wang et al., 2006a). Genetic mapping and quantitative trait loci (QTL) analysis suggested a major telomeric segment on Chr 11 harbors RKN resistance genes from these different resistance sources (Bezawada et al., 2003; Shen et al., 2006, 2010; Wang et al., 2006b; Wang C. et al., 2008; Gutiérrez et al., 2010; Roberts and Ulloa, 2010; Ulloa et al., 2010). A microsatellite marker (SSR) CIR 316 tightly linked to resistance gene rkn1 and other RKN resistance genes on Chr 11 was identified in different segregating populations (Shen et al., 2006, 2010; Wang and Roberts, 2006b; Wang et al., 2006b; Ynturi et al., 2006; Gutiérrez et al., 2010; Roberts and Ulloa, 2010; Ulloa et al., 2010). A resistance gene contributing to suppression of nematode egg production originally derived from Wild Mexico Jack Jones also was identified on Chr 14 (Gutiérrez et al., 2010; He et al., 2014; Kumar et al., 2016), which in combination with a resistance gene on Chr 11 derived from Clevevilt produced transgressive resistance in Auburn 623.

In a parallel study using an interspecific RIL population from a cross between two susceptible parents (*G. hirsutum* TM-1 × *G. barbadense* Pima 3-79), Wang C. et al. (2012) identified four major QTLs (on Chr 3, 4, 11, and 17) and two major QTLs (Chr 14 and 23) which contributed 8–12% transgressive resistance to nematode root-galling (galling index, GI) and nematode reproduction (eggs per gram root, EGR), respectively. In addition, 19 and 15 minor QTLs were identified in the TM-1 × Pima 3-79 population with each QTL accounting for 4–7% of phenotypic variance in GI and EGR, respectively (Wang C. et al., 2012). Although each of these QTLs contributed minor effects on phenotype, combinations of two to four major and/or minor QTLs were shown to dramatically reduce root-galling and nematode egg production by >50%, suggesting epistatic effects among these QTLs (Wang C. et al., 2012).

While these studies establish that transgressive segregation is common in cotton for disease resistance, the underlying functional mechanisms for this phenomenon are not known. For example, the location of the transgressive factor in SJ-2 in RIL population F_{2:8} (NemX × SJ-2) is not known, nor whether it would play a positive role on resistance in other recombinant populations. Other knowledge gaps include the nature of inheritance of the transgressive factor RKN2 in *G. barbadense* Pima S-7 and how it behaves in later generations of the RIL F_{2:7} (Pima S-7 × NemX) population; and how genes interact in the region of rkn1 in different genetic backgrounds. Previous studies demonstrated that a QTL mapping approach could be informative for studying inheritance and gene action to determine further the mechanism of transgressive segregation (Ulloa et al., 2010; Wang C. et al., 2012). Therefore, in this study, we report the development of a new RIL population (Pima S-7 × NemX) and its use in fine-mapping the rkn1 region on Chr 11 and characterizing transgressive resistance with new molecular markers developed from the *G. raimondii* D5 genome reference sequence (Paterson et al., 2012). Through QTL analysis the transgressive segregation for RKN resistance was characterized further in six other genetic populations derived from three parental lines, *G. barbadense* Pima S-7, *G. hirsutum* NemX and SJ-2, and a simple genetic model for transgressive segregation in the Chr 11 region is proposed.

**MATERIALS AND METHODS**

**Plant Materials and Crosses**

Three highly inbred homogeneous parental lines, susceptible *G. hirsutum* cv. SJ-2 (USDA-ARS), resistant *G. hirsutum* cv.
Nematode Resistance Screening

Nematode resistance phenotyping followed the method of Wang et al. (2006a,b). Nematode resistance in cotton populations was screened under controlled greenhouse conditions at 28–35°C during the day and 24°C at night. Five replicate plants of each line in the RIL population and of parental lines were arranged in a complete randomized design. One seed per plastic pot (10-cm-diam. × 17-cm-deep) was sown in blow sand and grown for 3 weeks. Then each plant was inoculated with ~50,000 eggs of *M. incognita* race 3 (isolate Project 77) and fertilized with slow-release fertilizer (Scotts-Sierra Horticultural Products Co) (Wang et al., 2006a). Egg extraction was conducted in NaOCl (Hussey and Barker, 1973). Infection response on cotton plants was evaluated at 60 days post-inoculation using the root-galling scale modified from Bridge and Page (1980), with 0 (no symptom) – 10 (severe symptom). The number of nematode eggs per gram fresh root was also utilized for nematode infection response on cotton. Resistance threshold in each test was determined by the mean values of galling index and eggs per gram root of parents plus standard deviation (SD) (Wang et al., 2006a).

Data Analysis

Data analysis followed the methods of Wang C. et al. (2008). One-way ANOVA was used to analyze data. The treatment means were compared with Fisher’s Protected LSD test. The transformed log10 (x + 1) data for nematode egg production were used for analysis. Chi-square (goodness of fit) test was used to predict Mendelian inheritance ratios.

Marker Analysis

Since chromosomes 11 and 14 are associated with root-galling index and/or nematode egg production (Shen et al., 2006; Wang et al., 2006b; Gutiérrez et al., 2010; He et al., 2014), markers on Chr11 and its homoeologous chromosome 21, and markers on Chr 14 were screened for nematode resistance linkage in the RIL population Pima S-7 × NemX. In addition, polymorphic markers linked to nematode resistance or Fusarium wilt resistance in the RIL population Pima S-7 × TM1 (Ulloa et al., 2011, 2013; Wang C. et al., 2012; Wang et al., 2015; www.cottonmarker.org) were also used for screening RIL population Pima S-7 × NemX. A total of 186 SSR markers containing 366 polymorphic alleles across the whole genome were obtained for QTL analysis of nematode resistance.

DNA was extracted from fresh or frozen (−80°C) young cotton leaves using the DNeasy® Plant Mini kit (Qiagen, Valencia, CA, USA; Wang et al., 2006b). Primers were synthesized by ITD (IDT, Coralville, IA, USA). The forward primers were synthesized with an M13 forward sequence on the 5’-end (Wang et al., 2006b). The IRD labeled M13 primer (700 or 800 channel: CACGACGTGGTAAAAACGC) was made by LI-COR (LI-COR, Lincoln, NE, USA). The methods of PCR amplification of cotton molecular markers and of electrophoresis and detection were described by Ulloa et al. (2016). The SCAR marker GHACC1 and AFLP markers M9E2, M5E1, and M4E5 linked to *rkn1* were from Wang and Roberts (2006b).

Fine-Mapping Region *rkn1* on Chr 11 and Chr 21 with D<sub>5</sub> Genome

In order to fine-map the resistance region of *rkn1*, 68 primers (Supplemental file 1: Table S1) were designed from about 2.2 M bp of sequence which extended from 58.2 to 60.4 M bp on chromosome 7 (corresponds to Chr 21 in tetraploid cotton) of the diploid D<sub>5</sub> genome (*G. raimondii* v.1.1; Paterson et al., 2012; https://phytozome.jgi.doe.gov) containing markers MUCS088, CIR316 and CIR069 which are associated with RKN resistance (Wang et al., 2006b; Wang C. et al., 2008). The primers were named as UCR+ number, such as UCR1 or UCR2. The 2.2 M bp sequence contained markers CIR316 and CIR069 which were linked to *rkn1* or other resistance genes from different resistance sources on Chr 11 (Shen et al., 2006; Wang et al., 2006b). The parental lines Pima S-7, NemX and SJ-2 were screened for polymorphism with the 71 primers. The identified polymorphic markers were used to screen segregating populations F<sub>2</sub> (NemX × SJ-2), F<sub>2</sub>:8 (NemX × SJ-2), RIL Pima S-7 × NemX, and test-cross NemX × F<sub>1</sub> (Pima S-7 × SJ-2).

Genetic Linkage and QTL Analysis

The linkage groups for chromosomes were developed by using the JoinMap® version 4.0 program (Vank Ooijen, 2006). The Kosambi map function was used to examine Logarithm of odds (LOD) scores of 3–15 for each population. To determine linkage between any two markers, a maximum distance of 40 centiMorgans (cM) and a LOD threshold score >4.0 were used. QTL analyses were conducted on galling index (GI) and egg production per gram root (LogEGR) using MapQTL 5.0 (Van Ooijen, 2004). Non-parametric mapping (Kruskal-Wallis analysis (K*)) test equivalent to the one-way analysis of variance was used for single-marker analysis and interval mapping for analysis of pairs of linked markers. Significant QTLs were set with a more stringent *P* < 0.005 for the K* test (Wang C. et al., 2012).

RESULTS

Phenotyping F<sub>2</sub>:7 (Pima S-7 × NemX)

Fifty-six of 108 RI lines had lower (*P* < 0.05) GI than parent NemX (2.7 ± 0.27, SD) accounting for 51.9 % transgressive
QTL Mapping in F$_{2.7}$ (Pima S-7 × NemX) and F$_2$ (Pima S-7 × NemX) Populations

A total of 395 (366 plus 29 listed above) polymorphic markers were used to genotype the F$_{2.7}$ (Pima S-7 × NemX) for QTL mapping. Both Kruskal-Wallis analysis and interval mapping revealed that the significant (P < 0.0001) QTL(s) spanned 38 cM (0–38 cM) on Chr 11, associated with both GI and LogEGR phenotypes (Figure 2A, Supplemental file 2: Table S2A). The contribution to resistance to both galling and EGR was > 40% in a 8.1 cM region spanning from 3.3 cM (CIR112-251/260) to 11.5 cM (MUSB1076-296/306) containing marker CIR316 linked to rkn1 (Figure 2A). The region between the two flanked SSR markers CIR069-271/269 and UCR102-237/236 contributed 52% phenotypic variance of resistance to galling and 57% to egg production (Figure 2A), indicating the rkn1 region contributed to both GI and LogEGR. Interval mapping demonstrated strong additive effects for resistance to both galling (1.62 for UCR102-237/236) and egg production (1.08 for CIR069-271/269; Supplemental file 2: Table S2A), suggesting an epistatic effect on phenotype. Resistance contribution of the locus CIR316-215/221 to both GI and LogEGR is shown in Table 1. Two other significant (P < 0.005) QTLs from Pima S-7 alleles, on Chr 3 (BNL3792-540/0) to GI, Chr 3 (MUS1396-0/300 to LogEGR), and Chr 1 (NAU4045-185/195) to both GI and LogEGR, accounted for ~7–10% phenotypic variance (Supplemental file 2: Table S2A). The homoeologous region on Chr 21 had no resistance contribution to GI or LogEGR even though the order of the marker alleles was similar to those on Chr11, and also no contribution to resistance was found on Chr 14.

F$_2$ (Pima S-7 × NemX)

In order to confirm the rkn1 region on Chr11, markers CIR316, CIR069, and NAU2016 were used to screen 165 F$_2$ (Pima S-7 × NemX) plants which were phenotyped in our previous study (Wang C. et al., 2008). QTL mapping confirmed that the three markers on chr11 were associated with resistance. CIR069 contributed 18.5% phenotypic variance to GI and 23%
FIGURE 2 | Fine mapping of rkn1 region on chromosome (Chr) 11 and its homoeologous Chr 21 in RIL population F_{2.7} (Pima S-7 × NemX) (A) and rkn1 region on Chr 11 in F_{2.8} (NemX × SJ-2) (B) using D5 genome sequence [41]. New markers are underlined in blue. Numbers after the marker name indicate the allele sizes after PCR amplification from the two parent lines, Pima S-7/NemX in the Pima S-7 × NemX RIL population and NemX/SJ-2 in the F_{2.8} (NemX × SJ-2) population. For example, for marker UCR108-194/193 in F_{2.7} (Pima S-7 × NemX), 194 bp amplified from Pima S-7 and 193 bp from NemX. All allelic sizes include the M13 primer tail.

TABLE 1 | Resistance contribution of CIR316 locus in seven interspecific and intraspecific populations.

| Populations       | Locus        | Root galling (GI) | Nematode reproduction (LogEGR) |
|-------------------|--------------|-------------------|--------------------------------|
|                   |              | Kruskal-Wallis Analysis | Interval Mapping | Kruskal-Wallis Analysis | Interval Mapping |
|                   |              | K* | Signif. | LOD | % expl | additive | K* | Signif. | LOD | % expl | Additive |
| **INTERSPECIFIC CROSS** |              |    |         |     |        |          |    |         |     |        |           |
| F_{2} (Px N)      | CIR316-215/221 | 26.7 | 0.0001 | 6.5 | 17 | 0.91513 | 33.0 | 0.0001 | 7.4 | 19.3 | 0.47932 |
| F_{2.7} (PxN)     | CIR316-215/221 | 44.3 | 0.0001 | 15.7 | 48.2 | 1.56367 | 48.8 | 0.0001 | 14.9 | 46.3 | 1.00325 |
| F_{2} (PxS)       | CIR316-215/210 | 15.7 | 0.0001 | 3.5 | 15 | 1.06808 | 22.8 | 0.0001 | 5.8 | 23.7 | 0.32939 |
| NemX × F_{1} (PxS)| CIR316-215/210 | 25.9 | 0.0001 | 9.4 | 57.3 | 1.41808 | 25.6 | 0.0001 | 6.9 | 46.5 | 0.66992 |
| **INTRASPECIFIC CROSS** |              |    |         |     |        |          |    |         |     |        |           |
| F_{2} (Nxs)       | CIR316-221/210 | 33.7 | 0.0001 | 9.2 | 35.8 | 0.95977 | 26.5 | 0.0001 | 5.8 | 24.1 | 0.53658 |
| F_{2.8} (Nxs)     | CIR316-221/210 | 41.3 | 0.0001 | 19.1 | 72 | 2.22059 | 40.9 | 0.0001 | 16.3 | 66.4 | 0.95409 |
| B_{C1} F_{1} NemX × F_{1} (Nxs) | CIR316-221/210 | 39.2 | 0.0001 | 12.1 | 43.8 | 1.87151 |          |          |          |          |           |
| B_{C1} F_{1} SJ-2 × F_{1} (Nks) | CIR316-220/221 | 23.0 | 0.0001 | 6.0 | 44.2 | 1.44089 |          |          |          |          |           |

P, Pima S-7, allele 215 bp; N, NemX, allele 221 bp; S, SJ-2, allele 210 bp.

%expl, percentage of phenotypic variance explained.

K*, Kruskal-Wallis analysis test regarded as the nonparametric equivalent of the one-way analysis of variance.

Signif., significance level.

QTL Mapping in Testcross NemX × F_{1} (Pima S-7 × SJ-2)

In order to confirm the location of RKN2 in the same region of rkn1 [17], 32 SSR primers on Chr 11 were screened with the testcross population NemX × F_{1} (Pima S-7 × SJ-2) and produced 53 polymorphic markers between Pima S-7 and SJ-2. Of these, 32 were mapped on Chr 11 and 16 on Chr 21 (Figure 3). Interestingly, 18 markers including CIR316 and CIR069 on Chr 11-1 were clustered together in a 3.9-cM interval but over a 16.1 cM interval on the homoeologous region of Chr 21 (Figure 3). Marker MUCS088 closely linked to RKN2 was mapped to the same position as CIR316. Fourteen other markers without clustering were mapped to LogEGR with additive effects (GI: 0.91454; LogEGR: 0.47609) based on interval mapping.
to a region further from rkn1 spanning 42.9 cM (Figure 3, Chr 11-2).

QTL analysis confirmed that the mapped rkn1 region (3.9 cM) was significantly ($P < 0.0001$) involved in both GI and LogEGR phenotypes in the testcross population (Supplemental file 2: Table S2B). Interval mapping analysis revealed that the CIR069 region on Chr 11 contributed up to 62.5% phenotypic variance in GI and 50.3% in LogEGR. Again, there was no contribution to resistance phenotype from Chr 21 and Chr 14.

Among the 18 markers clustered around rkn1, only CIR316 and BNL1231 had polymorphism among three parental lines (Pima S-7, NemX, and SJ-2). The application pattern of CIR316 clearly showed that all lines carried the heterozygous allele (221 bp) linked to rkn1 from NemX (Figure 4). The Pima S-7 allele band (215 bp) was present in the resistant lines and the SJ-2 allele band (210 bp) was present in the susceptible lines except for seven out of 51 lines for which recombination had occurred between CIR316 and the resistance gene based on galling index (Figure 4). An additional Pima S-7 allele band (206 bp) was mapped on Chr 21 (Figure 4).

Phenotyping and QTL Mapping an F$_2$ (Pima S-7 × SJ-2) Population Derived from Susceptible Parents

Since the interactions in the rkn1 region are complex, we examined whether transgressive segregation occurred in the F$_2$ (Pima S-7 × SJ-2) population derived from two susceptible parents and determined whether transgressive factor RKN2 functions in the F$_2$ (Pima S-7 × SJ-2) without the rkn1 allele. The correlation ($R^2$) between galling index and egg production per gram root was 0.5235 (Figure 5). The phenotypic test revealed that 52 out of 106 (49%) F$_2$ (Pima S-7 × SJ-2) plants showed less GI than Pima S-7 (5.85 ± 0.22, SD), SJ-2 (7 ± 0.61, SD), and their F$_1$ (Pima S-7 × SJ-2; 6.9 ± 0.42, SD). Twenty out of 106 F$_2$ plants had low GI < 3 (range 1–3) accounting for 19% resistance. The results indicated that both susceptible parents contributed to transgressive resistance. QTL mapping indicated that the CIR316-215 bp allele from Pima S-7 on Chr 11 contributed 15% phenotypic variance to GI and 24% to LogEGR, respectively (Table 1, Supplemental file 2: Table S2C).
confirmed a previous report (Wang et al., 2006b). The CIR316-221/210 marker contributed 36% phenotypic variance in galling and 24% in LogEGR (Table 1), and the AFLP-derived SCAR marker GHACC1 [37] which was 1.6 cM from CIR316 showed 36% phenotypic variance in galling and 33% in LogEGR (Supplemental file 2: Table S2E), emphasizing that the same rkn1 region contributed to both GI and EGR. No resistance contribution was found from Chr 14 and Chr 21.

**DISCUSSION**

Analysis of RKN resistance phenotypes in multiple segregating cotton populations in this study established the significant and common occurrence of transgressive nematode resistance among progenies of both interspecific and intraspecific populations. Transgressive segregation occurred in both interspecific crosses between *G. barbadense* Pima S-7 and *G. hirsutum* NemX, and between Pima S-7 and *G. hirsutum* SJ-2, and also in the *G. hirsutum* intraspecific cross between NemX and SJ-2. The results also establish that in the case of the crosses with resistant NemX which carries the recessive R gene *rkn1* (Wang et al., 2006b), the susceptible parent in each case, whether intraspecific (SJ-2) or interspecific (Pima S-7) contributes at least one transgressive factor that enhances resistance. More unexpectedly, the interspecific cross between two susceptible parents [Pima S-7 (RKN2) (Wang C. et al., 2008) and SJ-2 (designated as RKN3)] also results in combinations of transgressive factors which produce some progenies with resistance phenotypes. Beside the R gene *rkn1*, these resistance-enhancer factors may be the results of epistasis or complex recombinations and interactions of tandemly arrayed alleles (TAA) or genes (TAG). Copy number of multiple genes in a 31-kb segment at the *Rhg1* locus for resistance to cyst nematode in soybean has been shown to determine the level of expressed resistance, with copy number ranging from 1 (susceptible), to 3 (partially resistant) to 10 (highly resistant) in different genotypes (Cook et al., 2012). Whether or not a comparable arrangement of multiple genes with varying copy
number determining the level of resistance expression occurs in the cotton rkn1 region will require identification of the gene sequences determining resistance phenotype.

The refined mapping by QTL analysis of the genome region harboring rkn1 on Chr 11 and its homoeologous region on Chr 21 provided further insight into the transgressive resistance control by this region. Of the original 40 cM mapped region of rkn1 which contributed resistance to both root-galling and egg production phenotypes in the F$_2$$_7$ (Pima S-7 × NemX) population, 8 cM of the region containing marker CIR316 accounted for more than 40% of the phenotypic variance. This result indicated that genes cluster together in the region but cannot be separated only by phenotype. Strong positive additive effects on resistance phenotypes were found in both the F$_2$ and F$_2$$_7$ (Pima S-7 × NemX) RIL populations, suggesting that epistasis plays a role in the RKN transgressive resistance in this interspecific cross. Although the interval bracketed by markers HAU1809 and Gh288 in the rkn1 region was 25–27 cM in length on Chr 11 andChr 21, in the testcross population we found at least three-fold shorter interval on Chr 11 than on Chr 21, indicating complex recombination between the rkn1 region and its homoeologous Chr 21. This shorter genetic distance on Chr 11 in the testcross population, which contributed 50–60% phenotypic variance, suggested that the complex recombination in the rkn1 region produced transgressive resistance and that the level of resistance depends on the specific parent combination (Ulloa et al., 2010). The non-separated markers might result from homologous/repetitive sequence or repetitive transposable elements in the rkn1 region (Wang et al., 2015). In addition, more QTLs around the rkn1 region in RIL population NemX × SJ-2 (Figure 2B) might exist but were not detected because of fewer polymorphic markers available between NemX and SJ-2, suggesting more complexity in this region.

The homoeologous region of chromosome 21 consistently failed to show any detectable resistance contribution even though the order of marker alleles amplified from the same primer pair appeared similar in various segregating populations developed from crosses between the three parental lines. Although the sequence is highly conserved between Chr 11 and Chr 21 (Paterson et al., 2012; Wang K. et. al, 2012; Li et. al., 2014, 2015; Zhang et al., 2015), only Chr 11 contributed to RKN resistance, emphasizing that the unique structure and gene combination on Chr 11 is the primary basis for RKN resistance control and that minor differences between Chr 11 and Chr 21 result in phenotypic change, as reported earlier with only one nucleotide difference (SCAR marker GHACC1) between NemX and SJ-2 causing phenotypic change for RKN resistance (Wang and Roberts, 2006b). An additional result of importance from this study was that no contribution of resistance to root-galling or egg production was found on Chr 14, supporting that the resistance in NemX is based on rkn1, and thus different from the resistance in other cotton lines such as Wild Mexico Jack Jones, Auburn 623 and its derived M-lines which all carry a resistance gene on Chr 14 effecting egg production but not galling response (Gutiérrrez et al., 2010; He et al., 2014; Kumar et al., 2016).

Analysis of the CIR316-215/210 marker alleles in the F$_2$ (Pima S-7 × SJ-2) population, which accounted for 15–24% of phenotypic variance, confirmed that the 215 bp allele in Pima S-7 enhanced resistance to both root-galling and egg production. We identified F$_2$ progenies with GI phenotypes outside the range of the susceptible parents Pima S-7 and SJ-2, including ones with high resistance (GI < 3) and others with about 50% higher GI than the susceptible parents. Thus, both transgressive resistant and transgressive susceptible progenies were present in the F$_2$, providing evidence that both parents contributed to the transgressive resistance. In this F$_2$ population, which lacks the rkn1 locus resistance, the transgressive factor in Pima S-7 (RKN2) might be dominant over the transgressive factor(s) in SJ-2 (RKN3) and allele interaction might occur in the region of CIR316, as found in the testcross. The behavior of RKN2 from Pima S-7 showed dominant action in our previous study (Wang C. et. al, 2008), where plants heterozygous or homozygous for RKN2 were effective in enhancing resistance phenotype in the presence of rkn1. Comparing different generations of Pima S-7 × NemX progenies, about 25% transgressive resistant lines were found in the F$_2$ population and in F$_2$×3 families (Wang C. et. al, 2008) and 52% in the F$_2$×7 RIL population in this study. It is likely that this is also the situation in progenies with RKN2 coupled with RKN3 in Pima S-7 × SJ-2 crosses, but to result in transgressive resistant phenotypes, the SJ-2 factor (RKN3) must be in the homozygous condition. This would explain transgressive progenies occurring in the F$_2$ but not in the F$_1$ which is susceptible to RKN (Wang et al., 2006b).

Further aspects of the transgressive behavior contributed by the susceptible SJ-2 factor RKN3 were revealed in the NemX × SJ-2 RIL population, in which SJ-2 markers closely linked to CIR316 contributed up to 82% of resistance to root-galling. This result indicated that the RKN3 factor from SJ-2 also interacts with rkn1-based resistance in NemX in the rkn1 resistance region. In our previous study of this population, about 62% of the RILs carrying the homozygous rkn1 resistance were significantly more resistant than the level of resistance in the rkn1 donor parent NemX and up to 30% of total RI lines showed transgressive resistance (Wang et al., 2006a,b). It is likely that this highly resistant set of RILs, which we confirmed to be homozygous for the recessive rkn1 from NemX, were also homozygous for the RKN3 transgressive resistance factor contributed by SJ-2. This was also confirmed in the BC$_1$F$_1$ population SJ-2 × F$_1$ (NemX × SJ-2) in which the marker CIR316-210/221 contributed up to 44% of phenotypic variance to GI resistance. In the BC$_1$F$_1$, transgressive progenies showed less galling than both susceptible parent lines [SJ-2 and F$_1$ (NemX × SJ-2)]. The stronger transgressive resistance which occurred in later than in early generations in the intraspecific NemX × SJ-2 cross compared with interspecific crosses between Pima S-7 and NemX or SJ-2 may reflect the increased levels of homozygosity of the interacting factors. Further, the presence of either homozygous or heterozygous rkn1 improves resistance, and the increasing contribution of resistance is influenced by different recombination backgrounds.

Based on GI and LogEGR phenotypic data in the rkn1 region in different populations and QTL analysis, we constructed a simple genetic model to better conceptualize the transgressive resistance among the three parental lines and their progenies (Table 2). The inferred genetic model is based on the linkage of
The three gene model in five segregating populations based on phenotypic data and QTL analysis.

![Table 2](image-url)

The three gene model in five segregating populations based on phenotypic data and QTL analysis. The number is based on the highest value of GI phenotypic variance in the interval mapping on the Chromosome 11 region.

# Chi-square test is based on galling index (GI) data since root galling and nematode reproduction had similar result.

The pairs of loci (rkn1 with RKN2, rkn1 with RKN3, and RKN2 with RKN3) as indicated by mapping the respective alleles of the closely linked CIR316 marker.

Allele interactions and/or linked gene interactions in the rkn1 region clearly play a major role in controlling transgressive resistance. We labeled the NemX resistance gene rkn1 as aA, Pima S-7 transgressive factor RKN3 as BB, and the SJ-2 transgressive factor RKN3 as CC. Analysis of the F2 population (Pima S-7 × NemX) published previously (Wang C. et al., 2008), showed that the segregation for root-galling response followed a two-gene model for resistance (R, genotypes AaB-, aaB-, and aabb) and susceptibility (S, genotypes AAb- and A-bb). However, 66% resistant lines (71R:37S) based on GI were observed in the new F2 population which did not match the expected 1:1 ratio if only rkn1 was involved. Since the transgressive resistance depended on the recombination background as discussed above, the greater proportion of resistant lines in this RIL population indicated that multiple genes or alleles are present in the rkn1 region, which was also supported by significant effect QTL(s) spanning 38 cM (0–38 cM) in the rkn1 region associated with both GI and LogEGR phenotypes. Extending the model, the exact fit to a 3R:13S ratio between resistant genotypes (BBC-) and susceptible genotypes (BBcc, BbCc, bbCc, and bbc) in the F2 (Pima S-7 × SJ-2) population was predicted because both parents (BBcc and bbc) and the F1 (BbCc) had susceptible phenotypes. The RKN3 gene in SJ-2 (AACC), as with RKN2 in Pima S-7, was expected not to express resistance on its own but produce transgressive resistance in the presence of homozygous rkn1 because the F1 (NemX × SJ-2) (AaCc) was susceptible. This result might explain why in our earlier study (Wang et al., 2006a), 21 (aaC-) out of 34 resistant lines had higher resistance than the resistant parent NemX (aacc) in the NemX × SJ-2 RIL population for which a 3:1 ratio (25.5HR: 8.5R) for GI ($\chi^2 = 3.176, P = 0.075$) was found between the highly resistant (HR) genotype (aaC-) and resistant NemX type (aacc).

Considering the resistance contribution of the rkn1 region in all seven segregating populations, the three resistance genes might represent multiple genes in tandem linkage, TAG or multiple alleles, TAA of unknown arrangement in one gene, possibly similar to the arrangement of resistance locus Rhg1 in soybean (Cook et al., 2012). Although we did not have a population which allowed analysis of the three genes together, based on the interaction of the three possible gene pairs which
we have analyzed here, the three possible combination pairs of the different loci or genes produce novel resistance (aaB-, A-B-, aacC- and BBCC) or susceptibility (AAbb, AAcc, bbcC) phenotypes in the progenies. The interspecific crosses (aa + BB, BB + CC) produced transgressive resistance (aaB-, A-B-, aacC- and BBCC) in earlier progeny generations than interspecific crosses (aa + CC). In the presence of rkn1 (a-B- or aaCC), stronger transgressive resistance occurred than without the rkn1 gene (AABB and BBCC).

Sequence tandem repeats result from unequal crossing over during genetic recombination, and these tandem repeats (TR), TAAs, or gene cluster-TAGs are abundant across all domains of life. Little is known about their distribution and contribution to proteins. However, it is known that TR enriched leucine-rich repeats (LRRs) are commonly found in R genes (Schaper and Anisimova, 2015). Evidence of complex recombination between the rkn1 region and its homoeologous chromosome 21 is presented in this study. The behavior of RKN2 and RKN3 showed the presence of TAAs or TAGs enhancing resistance phenotype in the presence of rkn1, resulting in transgressive resistant phenotypes. In addition, resistance to other soil-borne diseases mapped to Chr 11 also indicated the unique structure and resistance gene clustering on Chr 11 (Wang et al., 2015), such as found in resistance to reniform nematode (Dighe et al., 2009), Fusarium wilt (Ulloa et al., 2011, 2013), Verticillium wilt (Bolek et al., 2005), and black root rot (Niu et al., 2008). More research is needed to determine if TRs (e.g., SSRs), TAAs, or TAGs play a major role in transgressive resistance and facilitate resistance to emerging pathogen diseases.

Recently published genome-wide SNP or SSR linkage maps for fiber, yield, or other trait analysis (Yu et al., 2011, 2012; Zhao et al., 2012; Hulse-Kemp et al., 2015; Li et al., 2016; Iqbal and Rahman, 2017; Khan et al., 2017) and the available marker or genome sequence information might shed more light on the mechanism of transgressive resistance. For example, segregation distortion was commonly observed in cotton and greater genetic distance between parents could result in higher distortion (Yu et al., 2011; Zhao et al., 2012; Khan et al., 2017). The distortion could result from translocations, chromosome rearrangements, and other genomic structure variations (Khan et al., 2017). Therefore, deeper sequencing of Chr 11 would increase understanding of resistance gene clusters on Chr 11.

From a practical plant breeding standpoint, the SSR marker CIR316 not only has proven to be a powerful marker to study transgressive resistance in cotton, but also this marker plus the newly developed markers in the rkn1 region reported here are important for marker-based breeding selection to develop nematode resistant cotton varieties. Moreover, through selection of highly resistant progenies resulting from transgressive segregation, valuable novel sources of resistance are available which can be tracked for breeding advancement by the reported marker profiles in the rkn1 region. Although not proven, it is likely that the novel transgressive resistant progenies will be more durable than single source resistance when utilized in cotton production systems, because nematode populations frequently exposed to the resistance would have to overcome the combined effects of multiple allele or multiple gene action.

CONCLUSIONS

In this study, QTL mapping of seven intraspecific and interspecific segregating populations generated from Pima S-7, NemX, and SJ-2 crosses revealed the allele profile of marker CIR316 in all tested populations corresponding with phenotypic profile, demonstrating that a transgressive factor (designated as RKN3) from susceptible SJ-2 exists in the region of rkn1. Allele or gene interactions between NemX (rkn1), SJ-2 (RKN3), and Pima S-7 (RKN2) contributed to transgressive resistance. Stronger transgressive resistance occurred in later than in early generations in the interspecific cross NemX × SJ-2 but not in the interspecific cross. All positive contributions to resistance phenotype came from the rkn1 region on Chr 11, while no evidence was found for any resistance contribution from the homoeologous region of Chr 21. The rkn1 region had similar resistance contribution to both root-galling and egg production in each population. The complex TAA and/or TAG recombination and interactions in the rkn1 resistance region in the NemX background provide a model to study transgressive resistance in plants.

AUTHOR CONTRIBUTIONS

CW, PR, and MU conceived and designed the study. CW and TD performed the laboratory work. CW, PR, and MU analyzed the data and wrote the manuscript. All the authors read and approved the final manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpls.2017.01979/full#supplementary-material
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**Conflict of Interest Statement:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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