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ORIGINAL PAPER

Resistance to *Thielaviopsis basicola* in the cultivated A genome cotton

Chen Niu · Harriet E. Lister · Bay Nguyen · Terry A. Wheeler · Robert J. Wright

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Abstract Black root rot (BRR), incited by the soilborne pathogen *Thielaviopsis basicola* has the potential to cause significant economic loss in cotton (*Gossypium* spp.) production. Cultivated tetraploids of cotton (*G. hirsutum* and *G. barbadense*) are susceptible although resistant types have been identified in a possible tetraploid progenitor, *G. herbaceum*. Genetic mapping was used to detect the chromosomal locations of quantitative trait loci (QTL) that confer resistance to the BRR pathogen. A population of F_2 individuals (*G. herbaceum* × *G. arboreum*) and $F_{2:3}$ progeny families were examined. Phenotypic variation between resistant and susceptible reactions could be explained partly by three QTL. The *BRR5.1*, *BRR9.1*, and *BRR13.1* QTL each explained 19.1, 10.3 and 8.5% of the total phenotypic variation, respectively. The combination of all three in a single genetic model explained 32.7% of the phenotypic variation. Comparative analysis was conducted on significant QTL regions to deduce the cotton–*Arabidopsis* synteny relationship and examine the correspondence between BRR QTL and *Arabidopsis* pathogen defense genes. Totally 20 *Arabidopsis* synteny segments corresponded within one of three BRR QTL regions. Each synteny segment contains many potential *Arabidopsis* candidate genes. A total of 624 *Arabidopsis* genes, including 22 pathogen defense and 36 stress response genes, could be placed within the syntenic regions corresponding to the BRR QTL. Fine mapping is needed to delineate each underlying BRR R-gene and possible *Arabidopsis* orthologs. Research and breeding activities to examine each QTL and underlying genes in Upland cotton (*G. hirsutum*) are ongoing.

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Chen Niu, Harriet E. Lister, and Bay Nguyen contributed equally to this work.

C. Niu · H. E. Lister · B. Nguyen · R. J. Wright (✉)
Department of Plant and Soil Science, Texas Tech University, P.O. Box 42122, Lubbock, TX 79409, USA
e-mail: robert.wright@ttu.edu

T. A. Wheeler · R. J. Wright
Texas AgriLife Research, 1102 East FM 1294, Lubbock, TX 79403, USA

Introduction

Tetraploid cotton (*G. hirsutum* and *G. barbadense*) is the number one natural fiber resource for the textile industry, and is an important commodity to the world economy. Diseases can cause substantial yield loss and reduced fiber quality in all cotton producing countries of the world. In the USA alone, estimated annual yield loss due to diseases from 1995 to 2005 was 2,668,262 bales (Blasingame 2006). Considering that the average value of the USA cotton crop was about $7.7 billion ($0.32 per kg) per year, disease accounted for an $897 million annual loss in revenue. Among various cotton diseases, black root rot (BRR) caused by the soilborne fungus, *Thielaviopsis basicola* (Berk. & Broome Ferris, syn. Chalara elegans Nag Raj & Kendrick), is a threat to cotton production (Rothrock 1997; Wheeler et al. 2000). Primary symptoms include necrosis of the tap and lateral roots that result in reduced plant vigor (stunting) and delayed maturity (Minton and Garber 1983). Seedlings with extensive root damage may perish (Walker et al. 2000). The damage is particularly severe when there is an extended period of cool weather in the spring (Rothrock 1992), or if the root-knot nematode, *Meloidogyne incognita* is also present (Walker et al. 1998). *Thielaviopsis basicola* and *M. incognita* interaction results

in substantial damage to the root system (Walker et al. 1999, 2000). Chemical control with triadimenol or myclobutanil has been insufficient to manage BRR (Kaufman et al. 1998).

BRR resistant genotypes have not been available for the breeding of superior germplasm (Wheeler et al. 1999). This is attributed to a lack of resistance in cultivated *G. hirsutum* (Upland) and *G. barbadense*. Traits for which highly resistant germplasm is available, such as bacterial blight caused by *Xanthomonas campestris* pv. *malvaceraum*, are improved readily by breeding of resistant cultivars to reduce or eliminate disease losses (Bird 1986). Wheeler et al. (1999) examined approximately 1,000 tetraploid and diploid accessions, of which an A-genome diploid cotton species, *G. arboreum* (var. PI 1415), had reduced root necrosis when challenged with 337–550 chlamydospores/cm^3 soil from *T. basicola* compared with a susceptible check. However, PI 1415 had significantly higher root necrosis ratings when challenged with 500–1,000 chlamydospores/cm^3 soil compared with *G. herbaceum* var. "A20" (PI 408778) (Wheeler and Gannaway 2007). No other cotton germplasm has been identified with better resistance than A20 (Wheeler and Gannaway 2007). The lack of BRR resistance in cultivated tetraploid cotton dictates a need to identify resistance (R) genes that could be deployed in elite Upland cotton cultivars.

Tetraploid cotton ($2n = 4x = 52$) arose some 1–2 million years ago through the hybridization of an Old World taxon of the A-genome cytogenetic group, related to the species *G. arboreum* and *G. herbaceum* ($2n = 2x = 26$), with a taxon of the D-genome group related to the New World species *G. raimondii* or *G. gossypioides* ($2n = 2x = 26$) (Beasley 1940; Wendel et al. 1992, 1995). During the domestication and improvement of Upland cotton, intensive selection for agronomic and quality traits led to a narrowing of the gene pool. The utilization of tertiary germplasm sources to mitigate the consequences of this narrowing of the gene pool was demonstrated in the 1940 s when genes conferring resistance to *X. campestris* pv. *malvacearum*, were successfully transferred from *G. arboreum* into tetraploid cotton (Knight 1948, 1955). These R-genes historically have been an important source of resistance to bacterial blight. A detailed examination of a trait's heredity using molecular genetics may lead to a molecular breeding strategy to exploit underrepresented germplasm resources and ancient gene complexes and reveal knowledge about cotton evolution (Jiang et al. 1998; Wright et al. 1998; Brubaker et al. 1999; Abdalla et al. 2001; Rong et al. 2004, 2005; Desai et al. 2006).

Cotton has several interesting evolutionary features that include the divergence of R-genes through the radiation of species, genetic bottleneck events such as polyploidy, and the domestication of a small subset of *Gossypium* species. The implications of these events for host–pathogen interactions are of interest. Analysis of the subgenomic (A_t vs. D_t) distribution of genes conferring resistance to bacterial blight in tetraploid AD-genome cottons provided information on the impact of allopolyploid formation on host–pathogen interactions (Wright et al. 1998). Among seven resistance genes derived from tetraploid cottons, six (86%) mapped to D-subgenome chromosomes. This suggests that the D-subgenome of tetraploid cotton has a higher propensity to give rise to new R-gene alleles than the A-subgenome, and may indicate that polyploid formation offers novel avenues for phenotypic response to selection. However, the divergence of R-gene alleles does not always parallel that of speciation. As a result, alleles (allelic frequency) can be lost or fixed in descendant populations (species). The examination of R-genes alleles believed lost during speciation events may help clarify the host–pathogen relationship in descendant species. To examine the evolution/divergence of R-genes in *Gossypium*, research that examines the genetic control of cotton disease resistance may benefit from *Arabidopsis* genetic resources and knowledge. *Gossypium* and *Arabidopsis* are thought to have shared common ancestry about 83–86 MYA (Benton 1993), and cotton may be the best crop outside of the *Brassicales* in which to employ translational genomics from *Arabidopsis* (Rong et al. 2007).

The present research was designed to examine the inheritance of resistance to *T. basicola* in the progenitor A-genome diploid cotton using molecular markers to identify QTL that explain the phenotypic variation of resistant and susceptible disease reactions.

Materials and methods

Population development and phenotyping of the mapping population

A population segregating for resistance to *T. basicola* was created by crossing the highly resistant *G. herbaceum* var. A20 (hereafter *GH*) parent (Wheeler and Gannaway 2007) with the partially resistant *G. arboreum* var. PI 1415 (hereafter *GA*) parent (Wheeler et al. 1999). Progeny (F_2) were produced from self-fertilized F_1 plants to maintain a strict control of purity. Resistant and susceptible disease reactions were determined for 129 F_2 individuals and the $F_{2:3}$ derived families. BRR phenotypes were assessed based on the following method. The fungus was grown on carrot agar for at least 6 weeks, the chlamydospores were washed from the plates into water, blended for 11 s, and poured through a 230-μm pore-sized sieve over a 37-μm pore-sized sieve. The contents from the smaller pored sieve were washed with tap water, stirred for several hours, quantified

with a hemacytometer and adjusted to 40,000–50,000 chlamydospores ml^{-1}. A measure of 2 ml of the spore solution were added to 100 cm^3 soil (77% sand, 9% silt, and 14% clay), and hand mixed for 1 min. The inoculated soil (800–1,000 spores/cm^3 soil) was added to a 115-cm^3 container (model RLC7, Stuewe & Sons, Inc., Corvallis, OR) and planted with a single seed. The 129 F_2 individuals were placed in a growth chamber at 19 ± 3°C in a completely randomized design which contained the resistant (*GH*) and susceptible (*GA*) parents. At ~25 days after planting, the soil was carefully washed from the roots. Disease ratings (0–100%) were assessed visually based on the percentage of necrotic root tissue. A 50% disease rating denotes that necrosis was observed on half the total root area. After visual assessment, each F_2 plant was then carefully transplanted and grown to maturity. The seed from each plant ($F_{2:3}$) was harvested and the progeny tested again to obtain an improved disease rating. A randomized complete block design with 25 replications was used to assess resistant and susceptible disease reaction of the progeny. Each replication contained a single individual from each $F_{2:3}$ family and the *GA* and *GH* parents.

The average (mean) disease score of each $F_{2:3}$ family was calculated and used as a second phenotypic data set. A third data set (F_2-adjusted) was created to offset possible disease escapes based on single F_2 plant phenotypes. This adjusted data set was based on the following modification of the F_2 data set. The difference (Δ) between F_2 and $F_{2:3}$ disease reactions (measures) were calculated and when $\Delta \geq 30\%$, F_2 phenotypes were adjusted to reflect the higher (more susceptible) disease score. In no case did this deviation reflect an $F_{2:3}$ phenotype less susceptible ($\Delta \geq 30\%$) than observed in the F_2. All three data sets were used in the QTL analysis.

SSR marker genotypes

Genomic DNA of the parental and F_2 individuals was isolated from young leaf tissue using a CTAB based method (Paterson et al. 1993). DNA quantity was measured using the NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE USA) and adjusted to a working concentration of 20 ng/µl. DNA integrity of each sample was checked on a 0.8% agarose gel against Lambda DNA/HindIII Markers. Parental genotypes of 437 SSR loci were determined, of which 157 were polymorphic and selected to genotype the entire mapping population. These SSRs include BNL, CIR, CMS, JESPR, MGHES, NAU and TM, which have been described in detail (Reddy et al. 2001; Nguyen et al. 2004; Han et al. 2004; Qureshi et al. 2004). Polymerase chain reactions (PCR) were performed in a 25 µl reaction volume that contained 50 ng template DNA, 1 × PCR buffer (10 mM Tris–HCl, 50 mM KCl, pH 8.3), 1% PVP-10, 1 mg/ml BSA, 0.1 mM dNTPs, 0.4 µM of each forward and reverse primer, 1.5–3.0 mM $MgCl_2$ (primer dependent), and 0.05 U/µl Taq polymerase. Each reaction was initially denatured at 94°C for 5 min, followed by 40 cycles of 94°C for 45 s, 51–61°C (optimized for individual primer pair) for 45 s, and 72°C for 2 min, and a final extension at 72°C for 7 min using an eppendorf Mastercycler epgradient (Eppendorf AG. Hamburg, Germany). SSR fragments were resolved on 4% metaphor agarose gels (Cambrex, Bio Science Rockland, Inc. Rockland, ME USA) or 5–7% non-denaturing polyacrylamide gels (Omnipur Acrylamide:Bis Solution 19:1 DMD Chemicals Inc. Gibbstown, NJ. USA). All gels were stained with ethidium bromide and recorded by using AlphaImager® HP (Alpha Innotech, San Leandro, CA USA). Polymorphic fragments were scored manually as dominant or co-dominant loci.

Linkage and QTL analysis

A linkage map was constructed from the 157 polymorphic loci using MAPMAKER/EXP 3.0 (Lander et al. 1987). A LOD score of 3.0 and recombination fraction of 0.4 (Kosambi mapping function) were used as the threshold criteria for linkage (Kosambi 1944). The colinearity of each linkage group was compared to a diploid map (Desai et al. 2006) and the tetraploid maps of Han et al. (2004), Nguyen et al. (2004) and Rong et al. (2004) to assist in the assignment of each linkage group. Quantitative trait loci (QTL) were identified by composite interval mapping using Windows QTL Cartographer 2.5 (Wang et al. 2007). The position of each QTL was inferred if the LR score exceeded a threshhold calculated from 1,000 permutation runs. Allelic effects, and the percentage of phenotypic variance (PV) explained by each QTL, were calculated at the likelihood peak.

Comparative analysis of BRR QTL

Comparative analysis was conducted on significant QTL regions to deduce the cotton–*Arabidopsis* synteny relationship and examine the correspondence between BRR QTL and *Arabidopsis* pathogen defense genes. Each QTL region was aligned with the corresponding cotton consensus map (Rong et al. 2005; http://chibba.agtec.uga.edu/cgi-bin/cmap/viewer) based on conserved marker loci to locate BRR QTL on the consensus map. Markers that flanked the 99% confidence interval of each QTL were used in this step. Consensus fragments were subjected to both Fast Identification of Segmental Homologs (FISH) (Calabrese et al. 2003) and CrimeStatII (Levine 2002) software analysis to identify putatively duplicated genomic regions with the hypothetical ancestral cotton, as well as putatively corresponding regions between cotton and *Arabidopsis* (Rong et al. 2005). FISH

preprocesses the matched locus data between two genomes to enforce symmetry and remove noise from the data set, and then identifies sets of neighbors. The CrimeStatII (Levine 2002) software was initially developed for the analysis of crime-occurrence data, the package performs measurements of central tendency, spatial autocorrelation, and hot-spot analysis that can be applied to any spatial data set. This package has proven to be a powerful tool for assessing spatial patterns in genomic data sets (Rong et al. 2005).

Results

Phenotypic variation for *Thielaviopsis* reactions

Individual plant (F_2) reactions to *T. basicola* infestation were distributed continuously from resistant to susceptible (with a mean of 25.0% and standard deviation of 21.9%) but skewed toward disease resistance (Fig. 1a). The partially resistant *G. arboreum* parent (susceptible at concentrations above 500 spores/cm^3 soil) had an average disease rating of 57% compared to less than 5% in the highly-resistant *G. herbaceum* parent. The few disease lesions observed on the *GH* parent were a series of small infection sites rather than a concentrated area of necrosis.

In the evaluation of $F_{2:3}$ families, root necrosis values fit a normal distribution, with mean of 46.5% and standard deviation of 33.7% (Fig. 1b). Heritability (H^2), estimated using the variance of the parental and $F_{2:3}$ disease scores, was 36.5%. The mean value of each $F_{2:3}$ family was used in the QTL analysis.

The F_2-adjusted data set was created to resolve potential susceptible escapes in the F_2 data (Fig. 1c). The result was a distribution less skewed toward resistance with a mean of 40.2% and standard deviation of 24.8%.

GH × *GA* genetic map

A total of 157 polymorphic SSR markers were used to construct a map of the *GH* × *GA* population. With nine SSR markers unlinked to any groups, the map consisted of 12 linkage groups (LGs) including 148 SSR markers with a combined total genetic distance of 1,216 cM. The genomes of *G. arboreum* and *G. herbaceum* differ by a single reciprocal translocation (Menzel and Brown 1954). This chromosomal difference has been shown to cause pseudolinkage between markers near the interchange breakpoint of the chromosomes involved (Brubaker et al. 1999; Desai et al. 2006). Consequently, the map contains the 12 LGs that represent the collinear chromosomes (LG.A1, 3, 4, 6, 8, 9, 10, 12, 13, 14, 16) and a single linkage group (LG.A5) that has been implicated in the A genome translocation. This observation is consistant with two previous studies (Brubaker et al. 1999; Desai et al. 2006). The 148 SSR loci have an average spacing of 8.27 cM and ample colinearity (3–13 loci/linkage group) with one of several tetraploid maps to determine the orthologous LGs of A and A_t (tetraploid A-subgenome) (Nguyen et al. 2004; Rong et al. 2004; Rong et al. 2005; Desai et al. 2006). The linear order of 72 common SSR loci generally agreed with the published cotton maps. A single change in order was noticed on LG.A1, 5, 6, and 14 and two order changes were found on LG.A3, 10, and 13 (Fig. 2). Chromosome recombination length varies from 16.9 to 193.2 cM. The total recombinational length (1,216 cM) of this map is similar to the A-genome map (1,147 cM) reported by Desai et al. (2006).

QTL conferring BBR resistance

The chromosomal locations of three QTL (*BRR5.1*, *BRR9.1*, and *BRR13.1*) associated with the resistance phenotype, based on F_2, $F_{2:3}$, and F_2-adjusted measures of disease reaction, are presented in Table 1 and Fig. 3. A region on LG.A5 (*BRR5.1*) explained 5.8–19.1% (LOD 2.74–5.75) of the phenotypic variation in all three data sets (Table 1, Fig. 3). In each case, a likelihood peak was observed in the interval between markers *BNL1693* and *NAU1072*. The *GH* allele improved resistance in each data set. Linkage Group 9 contained a second QTL (*BRR9.1*) that explained 8.2–10.3% (LOD 2.86–3.94) of the phenotypic variation in the F_2-adjusted and $F_{2:3}$ data sets (Table 1, Fig. 3). The likeli-

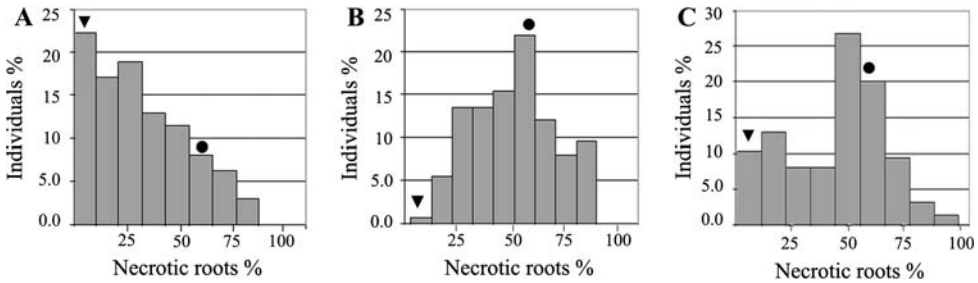

Fig. 1 Frequency distribution of percentage root necrosis in the *G. herbaceum* × *G. arboreum* population. **a** F_2 disease reactions, **b** Average $F_{2:3}$ disease reactions, and **c** F_2-adjusted disease score. The *triangle and circle* represent mean disease scores for the *G. herbaceum* and *G. arboreum* parents, respectively

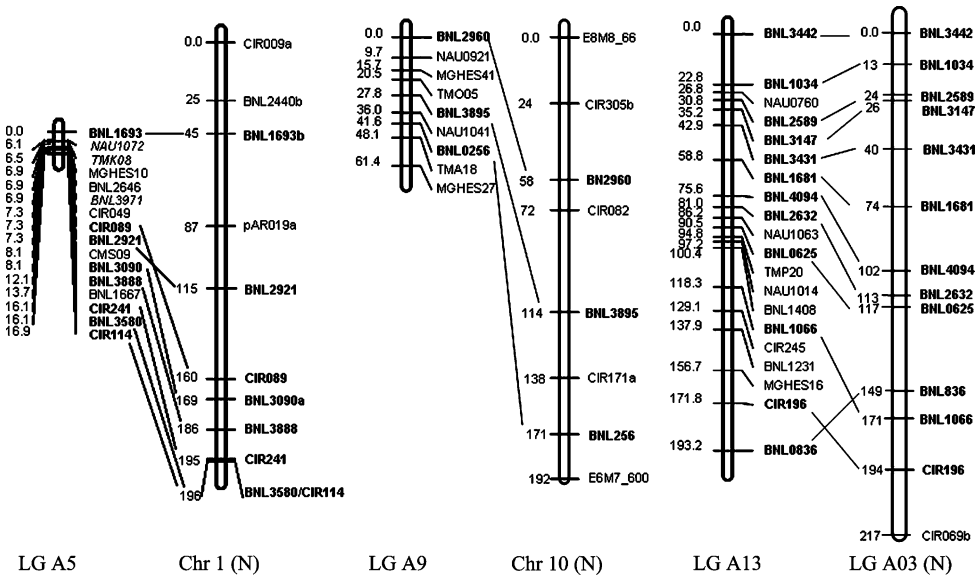

Fig. 2 Colinearity of the *G. herbaceum* × *G. arboreum* linkage map with the Nguyen et al. 2004 tetraploid map (N)

Table 1 Biometrical parameters of individual QTL conferring resistance to black root rot (BRR) of cotton

Data set	QTL	Locus	Interval[a]	Position[b]	LOD	% Var	Mode of QTL action[c]		
							a	*d*	*d*/*a*
F_2	*BRR5.1*	LG.A5	*BNL1693–NAU1072*	6.01	2.74	5.8	−8.86	−0.80	0.09
$F_{2:3}$	*BRR5.1*	LG.A5	*BNL1693–NAU1072*	5.01	5.75	19.1	−15.14	2.18	−0.14
F_2-adjusted	*BRR5.1*	LG.A5	*BNL1693–NAU1072*	6.11	3.93	7.2	−11.00	−2.44	0.22
$F_{2:3}$	*BRR9.1*	LG.A9	*BNL2690–NAU0921*	2.01	2.86	10.3	−9.83	1.56	−0.16
F_2-adjusted	*BRR9.1*	LG.A9	*BNL2690–NAU0921*	3.01	3.94	8.2	−10.63	−3.36	0.32
$F_{2:3}$	*BRR13.1*	LG.A13	*BNL3442–BNL1034*	2.01	3.02	8.5	−9.08	11.15	−1.23

[a] Markers flanking the QTL likelihood peak

[b] Position of the QTL likelihood peak (centi-Morgan from top)

[c] Biometrical parameters were calculated using dominance and recessiveness to refer to the behavior of the *G. herbaceum* alleles

hood peak for each mapped within the interval delineated by markers *BNL2690* and *NAU0921*. The *GH* allele increased resistance to *T. basicola.* A third QTL (*BRR13.1*), explained 8.5% of phenotypic variation among the $F_{2:3}$ families (Table 1, Fig. 3). The interval between markers *BNL3442* and *BNL1034*, on LG.A13, contained the likelihood peak (LOD 3.02). The *GH* allele contributed to the increased BRR resistance (Table 1). The three QTL collectively explained 32.7% of the phenotypic variation in reaction to *T. basicola* among $F_{2:3}$ families.

Comparative analysis of BRR QTL

The three BRR QTL were projected onto the cotton consensus map, which was inferred to resemble the DNA marker arrangement of the hypothetical ancestor of the two subgenomes of tetraploid cotton (Rong et al. 2005). Totally 20 *Arabidopsis* synteny segments corresponded to one of the three BRR QTL regions (Table 2). Cotton–*Arabidopsis* synteny within the *BRR5.1* QTL was defined by three FISH (DS08.76, D03.75, and D10.17) and three CrimeStatII (D03.98, D05.99, and D05.100) segments (Fig. 4). Eight *Arabidopsis* segements (DS01.260, DS02.262, D05.261, D11.263, D14.264, D14.39, D14.171, and D23.170) with inferred synteny with *BRR9.1* were identified. The *BRR13.1* QTL corresponding regions in *Arabidopsis* were delineated by six segments (DS05.166, DS02.34, D15.164, DS02.69, D03.143, and D12.68). The gene ontology of 624 *Arabidopsis* genes located in syntenic regions revealed 22 pathogen defense and 36 stress response genes (Table 2).

Discussion

Congruence among F_2, $F_{2:3}$, and F_2-adjusted mapping data

There was general agreement among the three data sets with regard to the detection of QTL inferred from statistical analysis. The *BRR5.1* QTL was detected in all three data sets (F_2, $F_{2:3}$, and F_2-adjusted) with the likelihood peak

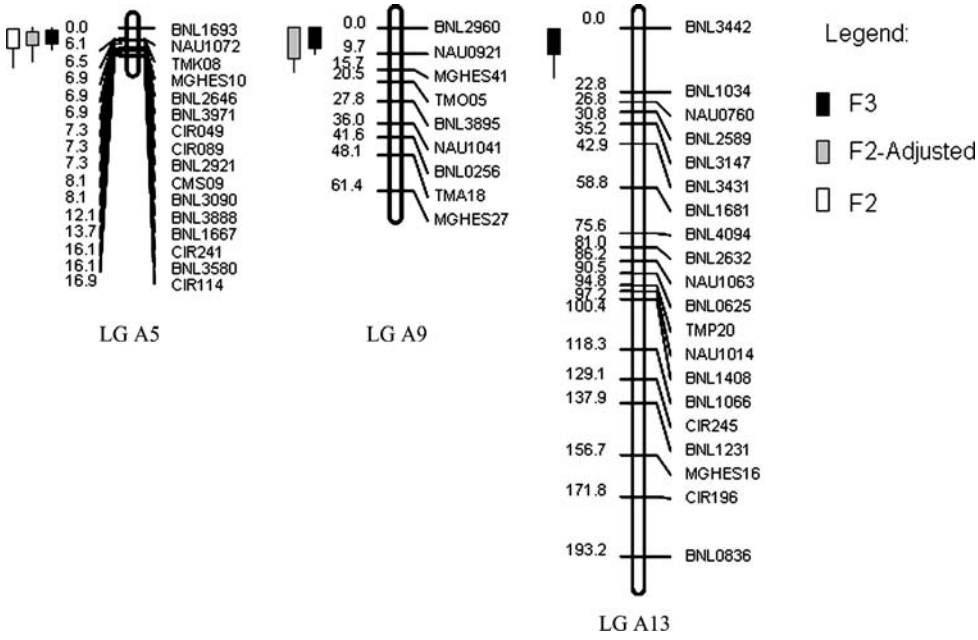

Fig. 3 Chromosomal location of QTL conferring resistance to *Thielaviopsis basicola*. *Bars* along the linkage groups indicate 90% (1-LOD) likelihood intervals for the QTL, and *whiskers* indicate 99% (2-LOD) likelihood intervals. Marker names and genetic distances (in centi-Morgan) are shown at the *right* and *left* of each linkage group

delineated by the same markers. The *BRR9.1* QTL detected in two ($F_{2:3}$ and F_2-adjusted) of three data sets was also delineated by a common set of marker loci. These results suggest that the relative position of a QTL is not altered among the datasets but the power of detecting QTL is improved when phenotypes are replicated. This observation is evident by the higher LOD score, smaller likelihood interval, and greater phenotypic variation explained in the $F_{2:3}$ dataset. This observation is consistent with QTL mapping of replicated phenotypic data (Zhang and Xu 2004).

The identified QTL on LG.A5, 9 and 13 indicated the presence of at least three genes conditioning reactions to *T. basicola.* For each of the three QTL, as expected, the *GH* alleles contributed the increased resistance. It is possible that additional QTL remain undetected; however, the three QTL model explained almost all (32.7%) of the genetic variability estimated by broad-sense heritability (36.5%). This suggests that the remaining non-genetic variation (63.5%) probably does not reflect undetected QTL.

Disease escapes

In the assessment of individual F_2 plants it was clear that individual plant disease ratings were not necessarily accurate and that some susceptible individuals were rated similarly to resistant types (i.e., escapes). However, a common QTL (*BRR5.1*) with the largest phenotypic effect was identified in both F_2 and $F_{2:3}$ data sets, indicating the utility of F_2 data in this study. Assessing the disease reactions of each $F_{2:3}$ family substantially improved the reliability of the QTL analysis, identifying two QTL that could not be resolved in F_2 analysis alone. This is evident by the congruence among *BRR5.1* mapping results and the two additional QTL detected in the $F_{2:3}$ dataset (Figs. 1, 3).

Colinearity of resistance QTL

Defense response genes tend to cluster in plant genomes (Pflieger et al. 2001; Monosi et al. 2004). Although a small number of QTL or diagnostic markers associated with disease resistance have been mapped in cotton, *BRR9.1* appears to map within a region that is homoeologus to a bacterial blight resistance QTL (Qb_{6c}) on tetraploid Chromosome 20 (formerly LGD04) (Wright et al. 1998) (Fig. 5a). Although LG.A9 and Chromosomes 20 are homoeologous, it is not clear whether or not the *BRR9.1* and Qb_{6c} QTL map to corresponding locations. Both map within 10 cM of the homoeologous marker pAR09D03. Additional DNA markers will be needed to better delineate the comparative organization of these two chromosomes in order to determine whether the underlining QTL are truly homoeologous. A region on Chromosome 11 (LGA03) has been shown to confer resistance to Verticillium wilt (*Verticillium dahliae* Kleb) (Bolek et al. 2005; Wang et al. 2006b). More recently, a molecular marker and QTL associated with root knot nematode resistance (*rkn1* and *Mi-C11*) also were detected on LGA03 (Wang et al. 2006a; Shen et al. 2006). The colinearity of these genes/QTL was compared with the orthologous A-genome region of LG.A13. The *BRR13.1* QTL mapped at the opposite ends of the linkage group (Fig. 5b). It is possible that these BRR QTL are located coincidentally with other known resistance loci in the

Table 2 Pathogen defence and stress response genes on the *Arabidopsis* synteny segments

QTL	*Arabidopsis* synteny segments[a]	*Arabidopsis* locus[b]	Gene function
BRR5.1	DS08.76 (91)	At3g47600	Response to stress
		At3g47830	Response to stress
		At3g47950	Response to stress
		At3g48030	Response to stress
		At3g48090	Defense
		At3g48425	Response to stress
	D03.75 (2)		
	D10.17 (47)	At2g21050	Defense
		At2g21110	Defense
		At4g38580	Response to stress
		At4g38620	Response to stress
	D05.100 (83)	At1g20440	Response to stress
		At1g20450	Response to stress
		At1g20510	Response to stress
		At1g20620	Response to stress
		At1g76670	Defense
		At1g76680	Response to stress
		At1g76690	Response to stress
		At1g76930	Response to stress
		At1g77000	Response to stress
		At1g77100	Response to stress
		At1g77120	Response to stress
	D05.99 (26)	At1g22900	Defense
		At1g13010	Response to stress
		At1g23120	Defense
		At1g23130	Defense
	D03.98 (37)	At1g09740	Response to stress
		At1g09760	Response to stress
		At1g09770	Defense
		At1g09780	Response to stress
		At1g58170	Defense
		At1g58200	Response to stress
BRR9.1	DS01.260 (14)	At1g04400	Response to stress
	DS02.262 (6)		
	D05.261 (2)		
	D11.263 (3)	At2g38750	Response to stress
	D14.264 (3)		
	D14.39 (80)	At3g11170	Response to stress
		At3g11410	Response to stress
		At3g11480	Defense
		At3g11650	Defense
		At3g11660	Defense
		At5g06150	Response to stress
		At5g06320	Defense
	D14.171 (4)		
	D23.170 (109)	At5g63980	Response to stress
		At5g64100	Response to stress
		At5g64110	Response to stress

Table 2 continued

QTL	*Arabidopsis* synteny segments[a]	*Arabidopsis* locus[b]	Gene function
		At5g64120	Defense
		At5g64250	Defense
		At5g64290	Defense
		At5g64630	Response to stress
		At5g64750	Response to stress
		At5g64840	Defense
		At5g64900	Defense
		At5g64930	Defense
		At5g64960	Defense
BRR13.1	DS05.166 (11)	At3g48900	Response to stress
	DS02.34 (3)		
	D15.164 (2)	At3g23830	Response to stress
	DS02.69 (3)		
	D03.143 (29)	At1g12110	Response to stress
		At1g12210	Defense
		At1g12220	Defense
		At1g12270	Response to stress
	D12.68 (75)		

[a] Parentheses indicate the total number of *Arabidopsis* gene on each segment

[b] *Arabidopsis* locus tag ID

same chromosome. BRR and other resistance gene functions will need to be further investigated to determine whether they coincide and reveal more information on cotton R-gene evolution.

Comparative analysis

Comparative analysis linked to synteny-based information revealed several regions of correspondence between BRR QTL and twenty *Arabidopsis* synteny segments. This is an important benchmark to identify candidate genes using *Arabidopsis* genetic resources. The inferred position of 624 *Arabidopsis* genes can be placed within BRR QTL regions, which include 22 pathogen defense and 36 stress response genes. This synteny based comparison may provide clues regarding possible cotton defense responses genes. However, fine-mapping is needed to validate that the underlying BRR R-gene(s) do map within an *Arabidopsis* syntenic region. This will also condense the potential list of canadidate genes to a manageable number for analysis.

Resistance mechanism

The mechanism that confers resistance to BRR in cotton has not been intensively examined. In tobacco (*Nicotiana debneyi*), BRR resistance is controlled by a single dominant gene (Clayton 1969). This R-gene reduces lesion size, number, and secondary inoculum production of *T. basicola* (Hood and Shew 1996). The reduced root necrosis in the *GH* and other resistant progeny may resemble the resistant response in tobacco, though conidia production was not evaluated. Further characterization of the resistance mechanism will require a better understanding of the fungus-host coexistance that leads to disease and resistant reactions. By studying the function of each gene(s) underpinning each QTL(s) will ultimately lead to a better understanding of the resistance mechanism.

Gene deployment (breeding)

The *BRR5.1*, *BRR9.1* and *BRR13.1* QTL provide a novel source of resistance that could be used to improve cultivated cotton. While tetraploid cotton is often reproductively incompatible with diploid cotton (Beasley 1942), the transfer and deployment of R-genes was accomplished successfully (Knight 1948). Knight successfully transferred bacterial blight resistance genes from diploid *G. arboreum* into tetraploid cotton by creating a synthetic tetraploid followed by successive back crossing to the tetraploid parent (Knight 1948, 1955). These R-genes historically have been a very important source of resistance to the most virulent bacterial blight causing races, including Race 18 (Bird 1982; Wright et al. 1998).

Cotton breeding is rapidly shifting from traditional phenotypic selection to genetic technologies that enable the direct selection and examination of genes or alleles. DNA markers that detect *BRR5.1*, *BRR9.1* and *BRR13.1* will aid in the introgression of BRR resistance by improving selection efficiency and shortening the intro-

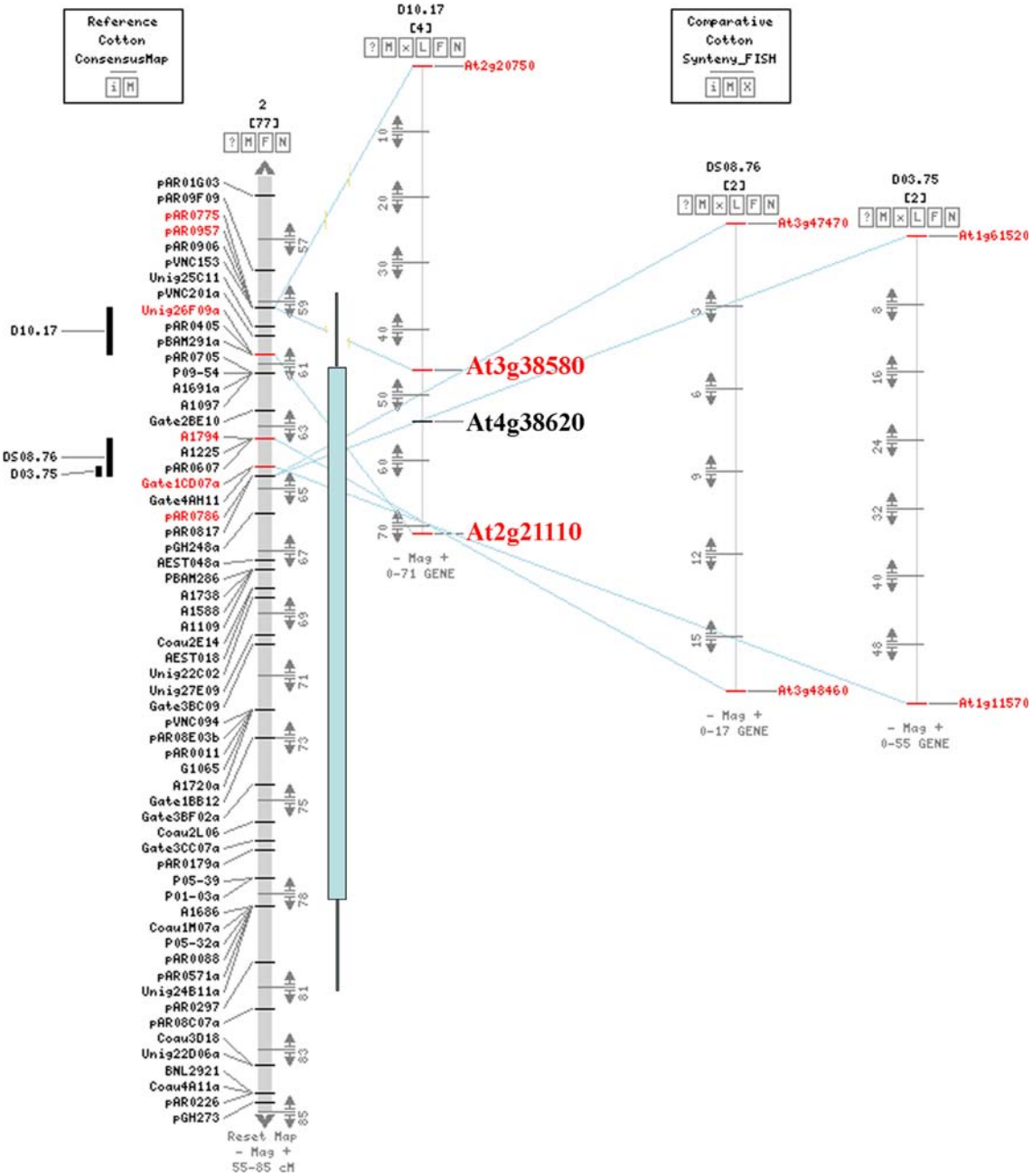

Fig. 4 A CMap display showing the inferred *Arabidopsis* syntenic regions (D03.75, DS08.76, and D10.17; FISH analysis) to *BRR5.1* on cotton consensus chromosome C02 (from 55 to 85 cM). *Solid lines* link putative orthologs between cotton and *Arabidopsis* which were used to infer correspondence in this region. Identifiers in bold are defense or stress response genes used to align syntenic regions. The *bar* on the *right* of the linkage group indicates the 90% (1-LOD) likelihood interval and *whiskers* indicate 99% (2-LOD) likelihood interval

gression time-line. The implications of this shift for breeding and using novel germplasm sources have recently been realized. The development of resistance to reniform nematodes (*Rotylenchulus reniformis*) from F-genome diploid *G. longicalyx* (Robinson et al. 2007) used a strategy that merged new and traditional technologies to accelerate the discovery and transfer of resistance into elite cotton. Concurrently, fine-mapping will better delineate each QTL region and eventually lead to the physical isolation and cloning of each R-gene sequence. The direct transformation of isolated R-gene(s) will provide an alternative strategy for the development of resistant Upland cotton. Once transferred, via transformation or conventional breeding, it is important to assess the

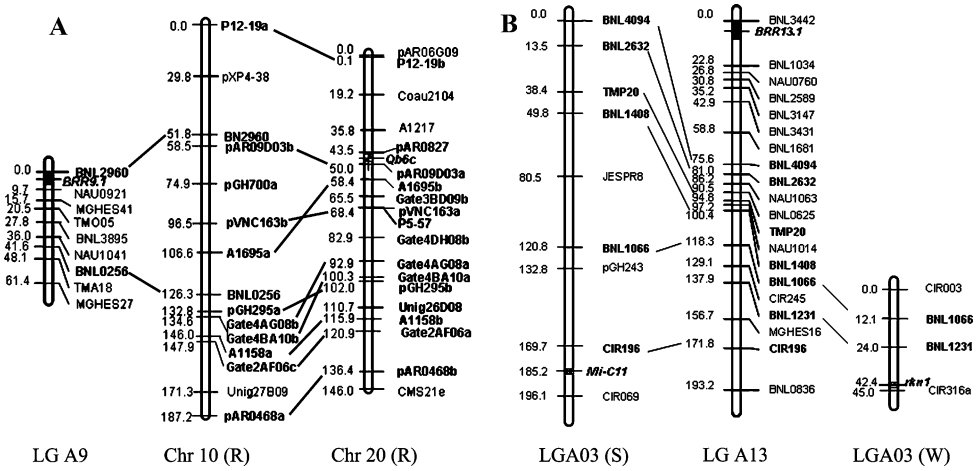

Fig. 5 **a** Colinearity of *BRR9.1* and bacterial blight resistance Qb_{6c} (Wright et al. 1998) based on homoeologous marker loci (Rong et al. 2004) (R). **b** Colinearity of *BRR13.1* and root knot nematode resistance *Mi-C11* (Shen et al. 2006) (S) and *rkn1* (Wang et al. 2006a) (W). *Solid lines* link common marker loci (*bold*) among the different mapping experiments

efficacy of each R-gene and its breeding value in the tetraploid genome.

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