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QTLs conferring FOV 7 resistance detected by linkage and association mapping in Upland cotton

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Abstract Fusarium wilt is a worldwide disease that affects cotton production. Molecular markers tightly linked to resistance genes can be used for marker-assisted and/or genomic selection. We performed both family-based linkage mapping and population-based association mapping (AM) to detect quantitative trait loci (QTLs) conferring resistance against *Fusarium oxysporum* f. sp. *vasinfectum* race 7 (FOV 7) in Upland cotton. To identify QTLs underlying FOV 7 resistance by linkage mapping, three Upland cotton cultivars/lines, Xuzhou 142, Yumian 21 and Shang 9901, were used to obtain the composite cross population, designated as Xuzhou 142/Yumian 21//Xuzhou 142/Shang 9901. A linkage map containing 185 simple sequence repeat loci and 40 linkage groups was constructed with an average distance of 7.5 cM between adjacent markers. Seven QTLs were detected by linkage mapping, explaining 2.9–6.6 % of the total phenotypic variance. We also performed marker–trait AM with the MLM model (Q + K) in a panel composed of 356 Upland cotton cultivars. In total, 27 loci were significantly associated with FOV 7 resistance at the $\alpha = 0.01$ level ($-\log_{10}P \geq 2$), which were distributed on 16 chromosomes and explained 1.48–12.99 % of phenotypic variation. Three of the 7 QTLs identified by linkage mapping could be detected in AM. We identified the favorable allele for each of the 27 associated loci and investigated the number of favorable alleles in each accession. The results should increase our understanding of the genetic basis of FOV resistance and facilitate future resistance breeding in Upland cotton.

Keywords *Fusarium oxysporum* f. sp. *Vasinfectum* race 7 (FOV 7) · Host resistance · Upland cotton · Linkage mapping · Association mapping

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Introduction

Fusarium wilt (FW), caused by *Fusarium oxysporum* f. sp. *vasinfectum* (FOV), is a widespread disease that

causes huge losses in cotton (*Gossypium* ssp.) production worldwide (Ma 2007; Davis et al. 2006; Ulloa et al. 2006; Constable et al. 2007). FOV is a soil-inhabiting fungus that generally invades seedlings through the root tips, subsequently spreading along the vascular system, which leads to FW symptoms including leaf wilt, necrosis, chlorosis, stunted growth, vascular discoloration, leaf abscission and plant death (Rodríguez-Gálvez and Mendgen 1995). Once established in soil, FOV can survive in the field for several years as Chlamydia spores, even in the absence of a host, and is nearly impossible to eliminate. Although both chemical controls and cultural practices have been employed in protecting plants from damage, the most effective and efficient control should be provided through host resistance (Ma 2007; Ulloa et al. 2006; Constable et al. 2007). In the past several years, eight races (race 1–8) of FOV had been indentified worldwide that use both cotton and non-cotton differential hosts (Davis et al. 2006). Three FOV races (race 3, 7 and 8) had been found in China; race 7 possesses the highest virulence and is the most widely distributed race (Chen et al. 1985; Sun et al. 1999). Recently, DNA-based techniques were employed in conjunction with pathogenicity tests to validate these races and to test new isolates (Skovgaard et al. 2001; Kim et al. 2005; Holmes et al. 2009), and highly virulent isolates of FOV were identified in Australia (Wang et al. 2004) and the United States (Kim et al. 2005; Holmes et al. 2009). Host resistance to FOV races has been widely evaluated in cotton germplasm under both field nursery and greenhouse conditions, and many highly resistant cotton cultivars and breeding lines have been developed through conventional breeding (Ma et al. 2002; Ulloa et al. 2006, 2009). However, little is known about the mechanism and genetic basis of FOV resistance. Some early classical genetic studies have suggested that the inheritance of FOV resistance in cotton is determined by a single gene (Smith and Dick 1960; Netzer et al. 1985; Feng et al. 1996), while other studies have suggested that FOV resistance is controlled by multiple genes (Kappelman 1971; Jiao 1985; Wang et al. 1989).

Large-scale resistance evaluations in breeding programs are time-consuming and labor-intensive, and it is not easy to obtain the ideal genotype simply through phenotypic selection. The cultivar development process has been slow in the face of the emergence of new, highly virulent FOV isolates (Ulloa et al. 2006; Constable et al. 2007). Molecular markers tightly linked to the target genes and QTLs can be used for marker-assisted selection (MAS) and/or genomic selection (GS), thus improving breeding efficiency (Xu and Crouch 2008; Jannink et al. 2010). In the past two decades, the availability of abundant molecular markers has made tagging QTLs harboring functional genes through family-based linkage mapping a routine process (Mackay et al. 2009), and a large number of QTLs for agronomically important traits have been identified in cotton (Zhang et al. 2008; Chen et al. 2011), including QTLs for FOV resistance (Wang and Roberts 2006; Chen et al. 2008; Wang et al. 2009, 2010; Ulloa et al. 2011, 2013; Lopez-Lavalle et al. 2012). However, approximately 80 % of the QTLs identified by linkage mapping could not be confirmed in subsequent studies, and few have actually been applied in breeding programs (Lacape et al. 2010; Rong et al. 2007). This may be due to the fact that most of the QTLs were population-specific, and the limited recombination present in most populations used for linkage mapping makes it difficult to map QTLs at a high resolution, which has severely limited their application in breeding programs.

Linkage disequilibrium (LD) based association mapping (AM), which has the potential to exploit most recombination events that have occurred in the evolutionary history of a plant and to simultaneously evaluate the effects of many alleles of target traits, has become a powerful approach to dissecting complex traits in many crops (Mackay et al. 2009; Zhu et al. 2008), including cotton (Kantartzi and Stewart 2008; Abdurakhmonov et al. 2008, 2009; Zeng et al. 2009; Zhang et al. 2013). However, several inherent constraints to natural populations have limited the successful use of association mapping in plants, such as genetic relatedness and rare alleles (Gupta et al. 2005; Myles et al. 2009). Genetic relatedness among individuals can result in spurious marker-trait associations (Gupta et al. 2005). Several statistical strategies have been developed to account for issues related to population structure and relatedness (Price et al. 2006; Yu et al. 2006; Yu et al. 2008). Given that the number of individuals with a specific genotype is quite small, the effect of rare alleles on mapping can go far beyond the effect of small population sizes (Myles et al. 2009). However, family-based linkage mapping can make use of alleles that occur at low frequencies in natural populations by designing crosses to create artificial

populations with inflated frequencies of those alleles. Therefore, joint linkage and association mapping was recommended as an alternative approach to overcome some of the inherent limitations of both linkage and association mapping (Gupta et al. 2005; Myles et al. 2009), and this technique has proven to be a powerful approach to detecting QTLs underlying complex traits (Nemri et al. 2010; Lu et al. 2010; Brachi et al. 2010; Korir et al. 2013; Cadic et al. 2013).

In the present study, we performed joint family-based linkage mapping and population-based association mapping to detect QTLs underlying FOV 7 resistance in Upland cotton. In addition, we identified a set of favorable alleles and representative germplasm resources. The results of this study should provide useful information for further understanding the genetic basis of FOV resistance and for facilitating future resistance breeding by GS in Upland cotton.

Materials and methods

Plant materials

A composite cross population (CP) with three parents was developed for linkage map construction and QTL mapping. Three Upland cotton cultivars, Xuzhou 142, Yumian 21 and Shang 9901, were chosen as parents according to many years of FOV 7 resistance evaluation. Xuzhou 142, an obsolete cultivar selected from STV 2B in the 1970s with large boll size and high lint percentage that is severely infested by FOV 7, was selected as the susceptible parent. Yumian 21 was released in 1999 and is currently used as a resistant control in national cotton regional trials due to its extremely high resistance to FOV 7. The cultivar Shang 9901 is an anonymous breeding line with high yield potential and moderately high resistance to FOV 7. Two single crosses (Xuzhou 142/Yumian 21 and Xuzhou 142/Shang 9901, SF_1) were made in Hainan, China in the winter of 2008. The two SF_1 were double-crossed to develop the CP (Xuzhou 142/Yumian 21// Xuzhou 142/Shang 9901, DF_1) at Jiangpu breeding station at Nanjing Agricultural University (NAU), Nanjing, China during the summer of 2009. The DF_1 seeds were planted in Hainan in the winter of 2009 to collect leaves for DNA extraction and to produce $DF_{1:2}$ families for resistance evaluation. A total of 241 DF_1 were self-pollinated, but only 239 individuals produced seeds enough for FW resistance evaluation. Meanwhile, a total of 356 representative Upland cotton cultivars and breeding lines were selected from the cotton germplasm collection in our laboratory and at the Cotton Research Institute, Chinese Academy of Agricultural Sciences (CRI-CAAS) and assembled to construct an association mapping panel. All accessions were self-pollinated for more than six generations; detailed information about the accessions is summarized in Table S1.

Resistance evaluation

Accessions of the association panel were tested in both the greenhouse and field nursery, while the CP progenies were only tested in the greenhouse due to limited seed production. In both assays, the cultivars Yumian 21 and Simian 3 were treated as the resistant and susceptible control, respectively. The 356 entries of the association panel were tested in the greenhouse of NAU by artificially inoculating the plants with FOV 7 in the winter of 2009, and the 239 $DF_{1:2}$ families of the CP (for evaluating resistance of DF_1 individuals) were tested in the same way in the winter of 2010. The FOV 7 isolate was provided by the Institute of Plant Protection, Jiangsu Academy of Agriculture Sciences (PPI-JAAS), China. To produce the inoculum, boiled wheat seeds were mixed with sand in a 3:1 ratio and sterilized at 120 °C for 2 h in an autoclave. The mixture was inoculated with pre-cultured FOV pathogen on a clean bench, incubated at 25 °C for 10 days and air-dried for use. The culture soil was sterilized at 160 °C for 2 h in a drying oven and mixed with 2 % inoculum (by weight) to produce culture medium. The medium was loaded into paper cups ($\sim$250 g per cup) and irrigated prior to sowing. A randomized complete block design with two replications was carried out. Each replication comprised 15 cups; six to eight acid-delinted seeds were sown into each cup and only two seedlings remained after emergence; so about 30 plants per genotype were tested in each replication. The room temperature was 18 to 20 °C at night and 25 to 28 °C in the daytime. Symptoms appeared 18 days after planting and leaf damage was measured every 5 days. The susceptibility of each plant was scored according to China's national standard for FW evaluation (Ma 2007). The disease grades of 0, 1, 2, 3 and 4 for symptoms were scored as follows: 0 indicates healthy, with no disease symptoms; 1 indicates

<25.0 % of the leaf surface exhibited disease symptoms; 2 indicates 25.1–50.0 % of the leaf surface exhibited disease symptoms or plants were slightly dwarfed in stature; 3 indicates 50.1–75.0 % of the leaf surface exhibited disease symptoms or plants obviously dwarfed in stature; and 4 indicates >75.0 % of the leaf surface exhibited disease symptoms or plants completely defoliated or died. The disease index (DI) of each accession was calculated using the following formula:

$$\mathrm{DI} = 100 \times \left(\sum_{i=1}^{n} x_i y_i\right) \Big/ 4\alpha$$

where DI is the FW disease index of each accession, x is the disease grade of 0–4, y is the number of plants with corresponding disease grade, and α is the total number of investigated plants of each accession. *F* test and multiple comparisons (LSD) for DIs of three parents and 239 $DF_{1:2}$ families were performed using the software SPSS 13.0. The additive (*A*) and dominance (*D*) effects of FOV resistance were estimated in the two single crosses according to the methods described by Kong (2006).

In the field trial, cotton seeds of the 356 entries were sown directly into an FW nursery at Shihezi Cotton Research Institute, Xinjiang Autonomous Region, China, in April, 2010. The nursery had been artificially inoculated with FOV 7 for many years and was heavily and evenly infested by the pathogen. A randomized complete block design with two replications was performed; each plot included a single 4 m row with a 15 cm plant-to-plant distance and a 60 cm row-to-row distance. 20–25 plants for each accession were assayed at the seedling stage, in early June, ~35 days after planting. The disease grades of individual plants were scored and the DI of each accession was calculated with methods described above. The mean DI value of two replications for each accession was used for QTL detection.

SSR genotyping

Young leaves from three parents, two SF_1 and 241 DF_1 individuals of the CP population and 356 accessions of the association panel were collected and stored at −20 °C. Total genomic DNA was extracted from the samples as described by Guo et al. (2007). The three parents, Xuzhou 142, Yumian 21 and Shang 9901, were first screened with 6,300 pairs of SSR primers to search for polymorphism. Polymorphic markers were then used to genotype two SF_1 and 241 DF_1 individuals. Individual genotypes were scored according to the method described in the JoinMap 4.0 manual (Van Ooijen and Voorrips 2006), and Chi square tests of goodness-of-fit were performed on segregation data for all loci to determine their agreement with the expected ratios. To fingerprint the entries of the association panel, 381 pairs of SSR primers evenly distributed in the tetraploid cotton genome (one marker per 10 cM) were selected according to a dense genetic linkage map constructed in our laboratory (Guo et al. 2007). The procedure for PCR-amplification and product analysis followed our published methods (Zhang et al. 2002; Zhao et al. 2012a).

Linkage map construction and QTL mapping

A CP with three homozygous parents (P1/P2//P1/P3) is analogous to a 4WC population (P1/P2//P3/P4) and can be regarded as a 4WC population with a common parent, P1. The methods of linkage analysis and QTL mapping in a full-sib family of outbreeding species can be applied to a four-way cross (4WC) population without any modification (Qin et al. 2008), and therefore, the methods can be extended to the CP with three parents (Zhang et al. 2012). The software JoinMap 4.0 (Van Ooijen and Voorrips 2006) was employed to construct the linkage map. Log-of-odds (LOD) scores ≥4.0 were used to determine linkage groups. The Kosambi map function was used to convert recombination frequencies to map distances. Previously chromosome-anchored SSR markers (Guo et al. 2007; Zhao et al. 2012b) were used to assign the linkage groups to chromosomes. QTL analyses were conducted on DI data using MapQTL 5.0 (Van Ooijen 2004) with the Multiple-QTL model (MQM). The significance thresholds for LOD scores were calculated by permutation tests in MapQTL 5.0, with a genome-wide significance level of $\alpha = 0.05$, $n = 1{,}000$ as a significant QTL and a linkage group-wide significance level of $\alpha = 0.05$, $n = 1{,}000$ as a suggestive QTL (Van Ooijen 1999). The determination of a QTL depended on the highest peak LOD and the directions of additive effects. The graphic representation of the linkage groups and QTLs was created by MapChart 2.2 (Voorrips 2002). QTL nomenclature was adopted using the method developed in rice (McCouch et al. 1997).

Marker-trait association mapping

Genetic diversity in 356 accessions was evaluated using the software PowerMarker 3.25 (Liu and Muse 2005). The Bayesian model-based program STRUCTURE 2.3 was used to infer the population structure with 66 unlinked and/or weakly linked SSR markers (Pritchard et al. 2009). Based on the correct k, each accession was assigned into a subpopulation for which the membership value (Q value) was >0.5 (Pritchard et al. 2000), and the population structure matrix (Q) was generated for further marker-trait association mapping. The software SPAGeDi was used to calculate the pair-wise relatedness coefficients (K, kinship matrix) to estimate the genetic relatedness among individuals with the negative value of kinship set as zero (Hardy and Vekemans 2002). The mixed linear model (MLM) considering both Q and K implemented in the TASSEL software package was used to perform marker-trait association, and the P value and R^2 of each association were determined (Yu et al. 2006; Bradbury et al. 2007).

Favorable allele identification

Based on the results of linkage and association mapping, QTL alleles of loci significantly associated with FW resistance were further analyzed. The phenotypic allele effect was estimated through comparisons between the average phenotypic value over accessions with the specific allele and that of all accessions:

$$a_i = \sum x_{ij}/n_i \; - \sum N_k/n_k$$

where a_i is the phenotypic effect of the ith allele; x_{ij} is the phenotypic value over the jth material with the ith allele; n_i is the number of materials with the ith allele; N_k is the phenotypic value over all accessions and n_k is the number of all accessions. If $a_i > 0$, the allele is determined to be positive, and $a_i < 0$ corresponds to a negative allele. The favorable allele was then identified according to breeding objectives of the target trait (Zhang et al. 2013).

Results

FW susceptibility of assayed accessions

The FW disease indices of three parents, two SF_1 crosses and 239 $DF_{1:2}$ families of the CP were shown in Table 1 and Table 2. Highly significant differences were observed between the S (Xuzhou 142) and R (Yumian 21 and Shang 9901) parents and 239 $DF_{1:2}$ families by F test. The additive (A) and dominance (D) effects of FOV resistance were estimated through classical quantitative genetics methods (Kong 2006) and were shown in Table 1. The dominant and additive effect ratios in Xuzhou 142/Yumian 21 and Xuzhou 142/Shang 9901 were 0.57 and 0.60 (Table 1), respectively, which shows that partially dominant action of the FOV resistance gene occurred in both single crosses. The DIs of 239 $DF_{1:2}$ families followed a normal distribution, and transgressive segregations were found (Table 2), which suggests that FW resistance in Upland cotton may be controlled by multiple genes. The FW indices of 356 accessions of the association panel were scored in both the greenhouse and field nursery; summary statistics were shown in Table 2. The DIs of 356 entries in the greenhouse and field nursery evaluations averaged 33.15 and 20.97, with ranges of 1.56–66.67 and 0–97.70, respectively. The DIs obtained in the greenhouse followed a normal distribution, while those obtained in the field nursery did not. This result may be due to the fact that some of the entries escaped from pathogen infection under field nursery conditions. Nonetheless, a significant positive correlation ($r = 0.424$, $P \leq 0.01$) was found between the two measurements.

Table 1 Mean of DI of 3 parents, 2 DF_1 of the CP evaluated in greenhouse (2010)

Material	Mean	A	D	$[D]/[A]$
Xuzhou 142	43.01A	–	–	–
9901	14.74B	–	–	–
Yumian 21	2.65C	–	–	–
Xuzhou142/Yumian21)	11.37	20.18	−11.46	0.57
Xuzhou142/Shang9901	15.93	21.51	−12.95	0.60

A, D, and $[D]/[A]$ indicate additive effect, dominance effect and dominance and additive effect ratio, respectively; Highly significant differences ($\alpha = 0.01$) were observed between the S (Xuzhou 142) and R (Yumian 21 and Shang 9901) parents

QTLs detected through linkage mapping

A total of 6,300 SSR primer pairs were used to screen the polymorphisms among Xuzhou 142, Yumian 21 and Shang 9901. Of these primer pairs, 216 (3.5 %)

Table 2 Distribution of FW DI of 239 $DF_{1:2}$ families and 356 accessions

Population	Environment	Mean	Min.	Max.	SD	Skewness	Kurtosis
CP	Gh (2010)	24.28**	0	66.67	11.61	0.68	0.16
AP	Gh (2009)	33.15	1.56	75.00	11.59	0.37	0.23
AP	FN (2010)	20.97	0	97.70	20.97	1.12	0.15

CP and AP indicate composite population and association mapping population, respectively; Gh and FN indicate greenhouse and field nursery condition, respectively

**Highly significant differences ($\alpha = 0.01$) were observed among the S (Xuzhou 142) and *R* (Yumian 21 and Shang 9901) parents and 239 $DF_{1:2}$ families

showed polymorphism and amplified 219 loci when they were used to genotype the 241 CP individuals. A Chi square test for goodness of fit was used to assess Mendelian segregation ratios, including 1:1, 1:2:1, and 1:1:1:1 in CP, and showed that 28 loci significantly deviated from the expected segregation ratios ($P \leq 0.05$), accounting for 12.79 % of the total. A linkage map with 185 loci and 40 groups was obtained when 191 loci were used to construct the linkage groups (Table S3), leaving six loci unlinked. The map spanned 1,378.8 cM, with an average distance of 7.5 cM between adjacent markers, accounting for approximately 37.6 % of the entire tetraploid cotton genome (Guo et al. 2007; Zhao et al. 2012b). Thirty-nine of 40 linkage groups were assigned to 20 chromosomes, and one could not be assigned. Nineteen linkage groups were assigned to the A-subgenome, containing 80 loci and spanning 623.7 cM, with an average distance of 7.8 cM. Twenty-one linkage groups were assigned to the D-subgenome, containing 103 loci and spanning 748.3 cM, with an average distance of 7.3 cM (Table S3).

QTL analyses were performed with MQM implemented in MapQTL 5.0 (Van Ooijen 2004). A total of seven suggestive QTLs with 2.9–6.6 % of the total phenotypic variance explained were detected; these QTLs were localized to six chromosomes (Fig. 1). A summary of the QTLs, including the chromosome position, LOD score, percentage of phenotypic variance explained by the QTL (PVE), additive effects of a_1 (additive effect of the first single cross) and a_2 (that of the second single cross) and overall dominance effect d was shown in Table 3. The a_1 values of four QTLs were positive (*qFW-A3-1*, *qFW-A12-1*, *qFW-D3-1* and *qFW-D5-1*, with values of 2.29, 1.48, 2.19 and 0.98, respectively), which indicates that these alleles, from the resistant parent Yumian 21, led to an increase in FOV 7 resistance (decreasing DI). Four QTLs with positive a_2 values (*qFW-A12-1*, *qFW-D3-1*, *qFW-D5-1* and *qFW-D8-1* with 1.48, 2.19, 2.58 and 2.22, respectively) were detected, which suggests that the other resistant parent, 9901, contributed favorable alleles on these loci. Furthermore, the effects of alleles from the two resistant parents on *qFW-A12-1* and *qFW-D3-1* were equal; they were maybe the same alleles or different alleles with same effects. Interestingly, the susceptible parent Xuzhou 142 actually contributed favorable alleles on locus *qFW-D13-1* and *qFW-D13-2* (with negative a_1 and a_2 values). In addition, a single-marker analysis was also conducted among DIs and 34 non-located markers using the Kruskal–Wallis model (Van Ooijen 2004). Four of the 34 markers were significantly associated with FW resistance (K** to K***). According to our saturated linkage map, NAU3695, NAU3519 and Gh401 were assigned to chromosome A11, A12 and D13, respectively (Guo et al. 2007; Zhao et al. 2012a, b), while NAU2666 could not be assigned to any chromosome.

QTLs identified by association mapping

Of the 381 SSR markers evenly distributed across the tetraploid cotton genome, 145 were polymorphic in the 356 accessions, and a total of 415 alleles were detected (Table S2). The allele number, gene diversity and PIC values of the 145 loci averaged 2.86, 0.32 and 0.27, with ranges of 2–9, 0.01–0.73 and 0.27–0.68, respectively (Table S2). Model-based evaluation of the population structure of the 356 accessions showed that the LnP(D) value corresponding to each hypothetical k kept increasing along with the k value and did not show any peak or platform, while the Δk value showed a much higher likelihood at $k = 2$ than at $k = 3$–10 (Figure S1), which suggests that the panel should be divided into two major subpopulations. The corresponding Q-matrix was used for subsequent

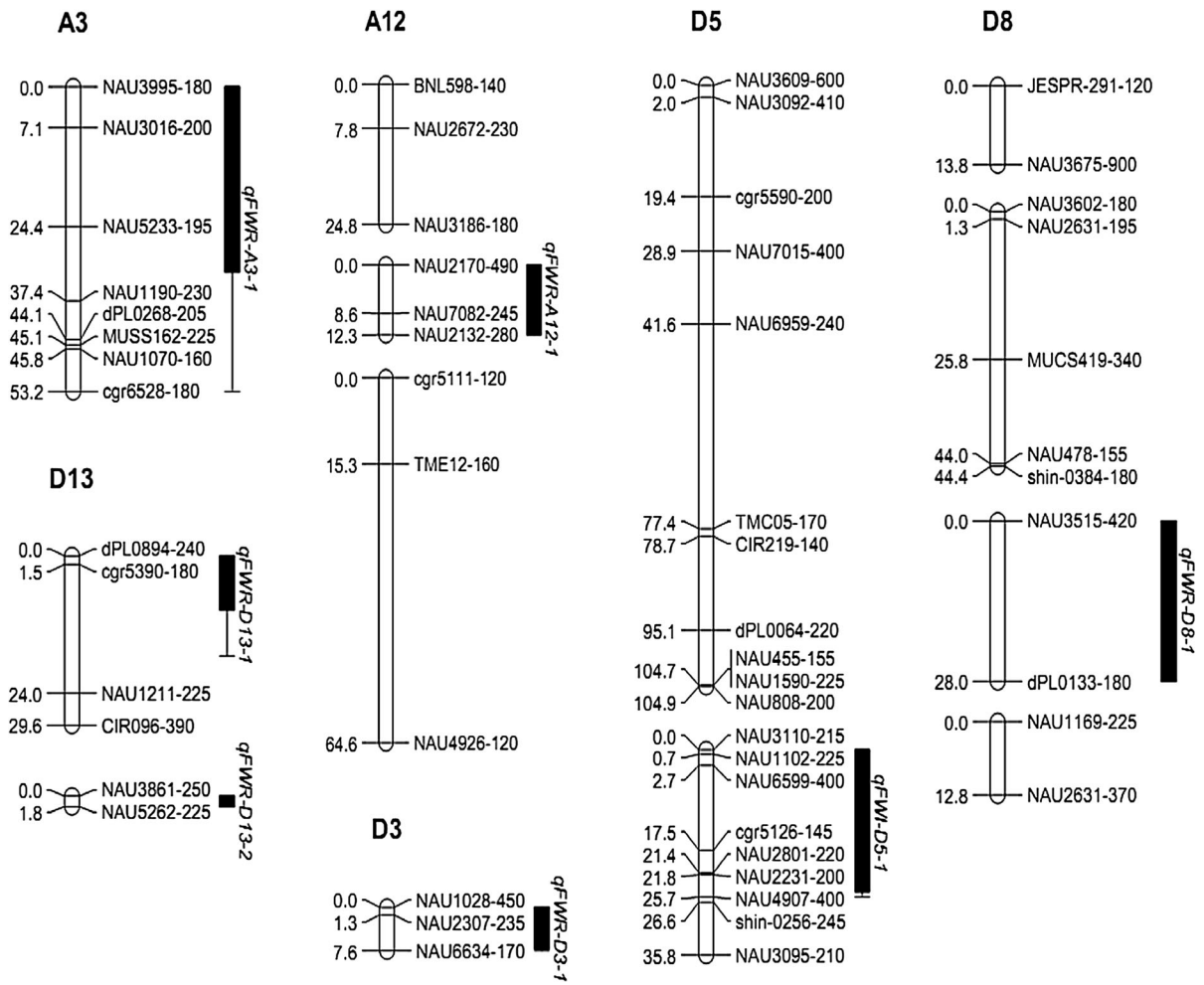

Fig. 1 QTLs for FOV 7 resistance detected by linkage mapping in a composite CP. SSR band sizes on the map are the alleles from Xuzhou142

Table 3 QTLs conferring FOV 7 resistance detected by linkage mapping in CP

QTL	Chr.	Nearest marker	LOD	Threshold	a_1	a_2	d	PVE (%)
qFW-A3-1	A3 (Chr. 3)	NAU3995	2.27	2.6	2.29	−0.46	−0.85	4.3
qFW-A12-1	A12(2) (Chr.12)	NAU7082	1.58	1.7	1.48	1.48	0.72	3.1
qFW-D3-1	D3 (Chr.17)	NAU1028	3.43	1.6	2.19	2.19	0.08	6.6
qFW-D5-1	D5(2) (Chr.19)	cgr5126	2.72	2.3	0.98	2.58	−8.30	5.7
qFW-D8-1	D8(3) (Chr. 24)	dPL0133	1.71	1.1	0	2.22	0	4.1
qFW-D13-1	D13(1) (Chr.18)	dPL0894	3.45	1.9	−1.74	−1.74	−1.99	6.6
qFW-D13-2	D13(2) (Chr.18)	NAU5262	1.22	0.9	0	−2.11	0	2.9

The LOD threshold values of each linkage group were evaluated by 1,000 permutation; a_1, a_2 and d indicate the additive effect of the first, second single cross and total dominance effect, respectively; PVE indicate phenotypic variation explained by individual QTL

association mapping. Of the kinship coefficient values, 86.85 % were less than 0.05, 8.56 % had a range of 0.05–0.10 and the remaining 4.59 % showed various degrees of genetic relatedness (data not shown). Based on the relatedness among accessions, a K matrix was constructed.

Table 4 SSR loci significantly ($-\log_{10}P \geq 2.0$) associated with FOV 7 resistance and their explained phenotypic variation in greenhouse and field nursery evaluations

Marker loci	Chr.	Position (cM)	$-\log_{10}P$		R^2 (%)	
			Gh	FN	Gh	FN
NAU2437	A01(Chr.01)	15.248	ns	2.19		5.21
NAU4073	A01(Chr.01)	85.025	2.03	ns	1.48	
JESPR304	A02(Chr.02)	22.517	ns	7.09		9.65
NAU934	A05(Chr.05)	19.421	ns	4.61		5.78
NAU3273	A05(Chr.05)	37.244	ns	2.85		2.81
NAU797	A05(Chr.05)	143.032	2.04	ns	3.22	
BNL3255	A08(Chr.08)	81.913	ns	3.20		4.41
STV031	A10(Chr.10)	12.751	2.09	2.82	3.34	4.34
NAU440	A10(Chr.10)	42.017	2.80	2.57	2.81	2.51
Gh369	A11(Chr.11)	84.701	ns	2.29		3.61
BNL1066	A11(Chr.11)	134.486	ns	2.12		3.94
BNL1707	A13(Chr.13)	104.694	2.29	ns	1.59	
NAU1070	D02(Chr.14)	13.885	2.47	5.89	3.10	7.56
CIR246	D02(Chr.14)	112.473	2.55	3.14	3.65	4.56
BNL3590	D03(Chr.17)	39.284	ns	2.77		3.66
BNL834	D03(Chr.17)	81.098	ns	5.07		5.46
NAU6966	D04(Chr.22)	36.51	ns	2.47		4.16
NAU3095	D05(Chr.19)	10.634	4.20	4.04	5.12	4.99
NAU2816	D05(Chr.19)	160.89	ns	2.62		4.20
BNL3594	D06(Chr.25)	7.66	7.87	4.12	12.99	7.92
NAU3911	D07(Chr.16)	28.658	ns	3.52		3.93
NAU6752	D07(Chr.16)	74.671	ns	3.35		3.44
NAU478	D08(Chr.24)	67.677	2.08	ns	3.86	
NAU3207	D08(Chr.24)	90.625	ns	5.21		5.70
NAU1350	D08(Chr.24)	108.529	ns	4.38		4.69
NAU5418	D11(Chr.21)	75.156	ns	3.71		6.98
NAU3084	D12(Chr.26)	0	ns	2.36		2.27

Gh and FN indicate the results evaluated in greenhouse and field nursery, respectively; ns indicates non-significant at $\alpha = 0.01$ level

Marker–trait association mapping was performed with the MLM model (Q + K) implemented in TASSEL software. A total of 35 markers were significantly associated with FOV 7 resistance at the $\alpha = 0.05$ level ($-\log_{10}P \geq 1.30$) and were distributed on 18 chromosomes (Table S4), while at the $\alpha = 0.01$ level ($-\log_{10}P \geq 2.0$), a total of 27 loci were significant and were localized to 16 chromosomes (Table 4). Of these loci, 23 were detected under field nursery conditions, 10 were detected in the greenhouse and six were detected under both conditions. The proportion of phenotypic variation explained by the markers ranged from 1.48 to 12.99 %, with an average of 4.21 %.

Favorable QTL alleles and their representative materials

The phenotypic effects of each QTL allele of the 27 associated loci were estimated in both the greenhouse and field nursery evaluations according to the method mentioned above, and therefore, favorable alleles for FOV 7 resistance were identified (Table 5). The phenotypic effects of the 27 loci in the greenhouse

Table 5 Favorable QTL alleles, phenotypic effects (a_i) and their representative materials

Favorable alleles	a_g	a_f	No. sample	Representative cultivars
NAU2437-4	−3.27	−8.49	26	Yumian21, Xingmian2, Shiyuan321, Xiangmian16, Zhongmiansuo21
NAU4073-2	−1.46	−0.68	172	Xinluzao24, Xingmian2, Xinluzao28, GK44, Shiyuan321
JESPR304-1	−0.45	−2.69	316	Yumian21, Jimian14, Xinluzao24, Xingmian2, Zhongmiansuo3
NAU934-2	−3.81	−4.68	39	Xinluzao24, Zhongmiansuo3, Xinluzao28, GK44, Xinluzao12
NAU3273-1	−0.13	−0.79	341	Yumian21, Jimian14, Xinluzao24, Xingmian2, Zhongmiansuo3
NAU797-2	−1.51	−1.03	172	Yumian21, Jimian14, Xinluzao24, Xingmian2, Xinluzao28
BNL3255-1	−0.01	−2.33	284	Yumian21, Jimian14, Xinluzao24, Xingmian2, Zhongmiansuo3
STV031-1	−2.09	−4.91	137	Yumian21, Xingmian2, Zhongmiansuo3, Shiyuan321, Zhongmiansuo15
NAU440-1	−0.33	−0.60	347	Yumian21, Jimian14, Xinluzao24, Xingmian2, Zhongmiansuo3
Gh369-2	−1.02	−3.33	10	Xinluzao24, Xinluzao28, Emian13, Jinmian28, Esha28
BNL1066-4	−1.96	−4.14	15	Zhongmiansuo27, Jimian20, Emian13, Liaomian10, Yumian18
BNL1707-2	−1.93	−4.79	112	Yumian21, Xinluzao24, Xingmian2, Xinluzao28, Xinluzao9
NAU1070-1	−1.73	−5.84	199	Jimian14, Xinluzao24, Xingmian2, Zhongmiansuo3, Xinluzao28
CIR246-3	−2.77	−8.65	16	Ejing1, Ekangmian3, Zhongmiansuo49, Zhong51504, Liaomian13
BNL3590-1	−0.06	−1.04	315	Yumian21, Jimian14, Xinluzao24, Xingmian2, Zhongmiansuo3
BNL834-1	−0.24	−1.70	312	Yumian21, Jimian14, Xingmian2, Zhongmiansuo3, Xinluzao9
NAU6966-3	−1.89	−14.88	37	Xinluzao24, Xinluzao28, GK44, Yumian1, Sumian12
NAU3095-1	−2.30	−4.55	192	Yumian21, Xinluzao24, Xingmian2, Zhongmiansuo3, GK44
NAU2816-2	−1.70	−3.43	152	Xinluzao24, Jimian14, Zhongmiansuo3, Zhongmiansuo9, Xinluzao28
BNL3594-1	−2.58	−5.23	201	Jimian14, Xingmian2, Zhongmiansuo9, Xinluzao9, Shiyuan321
NAU3911-1	−0.33	−1.91	282	Yumian21, Jimian14, Xingmian2, Zhongmiansuo9, Shiyuan321
NAU6752-1	−0.14	−0.83	341	Yumian21, Jimian14, Xinluzao24, Xingmian2, GK44
NAU478-1	−0.21	−0.20	239	Yumian21, Jimian14, Xingmian2, Zhongmiansuo3, Shiyuan321
NAU3207-1	−0.17	−2.27	301	Yumian21, Xinluzao24, Xingmian2, Zhongmiansuo3, Zhongmiansuo9
NAU1350-1	−0.03	−0.62	348	Yumian21, Xinluzao24, Jimian14, Xingmian2, Zhongmiansuo3
NAU5418-1	−2.90	−2.44	47	Yumian21, Jimian14, Yumian20, Chuanjian1, Xinluzao23
NAU3084-1	−0.42	−1.13	326	Yumian21, Jimian14, Xinluzao24, Xingmian2, Zhongmiansuo3

a_g and a_f indicate phenotypic effects evaluated in greenhouse and field nursery conditions, respectively; Representative materials are the top-five accessions with low DI

and field nursery averaged −1.31 and −3.45, with ranges of −3.81 to −0.01 and −14.88 to −0.20, respectively. Among the favorable alleles, NAU934-2 had the most negative phenotypic effect in the greenhouse assay and was able to decrease FW DI by 3.8, while NAU6966-3 had the most negative phenotypic effect in the field nursery and was able to decrease FW DI by 14.88. A wide variation of the favorable alleles processed in the 356 accessions was observed, with an average of 14.84 and a range of 7–21 (Table S1). Pearson correlation analysis between the number of favorable alleles and FW DI was carried out, and highly negative significance was found in both the greenhouse ($r = -0.344$, $P < 0.001$) and the field nursery ($r = -0.488$, $P < 0.001$) evaluations. The top-5 resistant accessions for each favorable allele were listed as representative materials in Table 5.

Discussion

QTLs for FOV 7 resistance identified by linkage and association mapping

FOV 7 is the most widely distributed FOV race in China (Chen et al. 1985; Sun et al. 1999). Molecular tagging and favorable allele mining of FOV 7 resistance QTLs/genes will be highly beneficial for cotton improvement. In previous studies, Chen et al. (2008) detected four QTLs conferring FOV 7

resistance using an interspecific cross (*G. hirsutum* 98134 × *G. barbadense* Xinhai 14) and located these QTLs to Chr 3 (A3), 15 (D1), 23 (D9) and 26 (D12), respectively. Wang et al. (2009) detected four QTLs associated with FOV 7 resistance in Upland cotton and located these QTLs to four chromosomes [Chr 7 (A7), 15 (D1), 17 (D3) and 23 (D9)], with a major QTL on Chr 17 (D3) that explained 52.5–60.9 % of the total phenotypic variation. In a subsequent report, Wang et al. (2010) detected five QTLs associated with FW 7 resistance in two intraspecific populations, with one located on Chr 15 (D1) and four on Chr 2 (A2) or 17 (D3), and indicated that the latter four QTLs were linked to JESPR304 or CIR 35; it is possible that these QTLs were the same as the major QTL previously detected (Wang et al. 2009). In addition, the authors also detected two major QTLs on Chr 9 (A9) and Chr 12 (A12) or 26 (D12) in another interspecific population. In the current study, we detected 7 suggested QTLs conferring FOV 7 resistance using linkage mapping, and we localized the QTLs to six chromosomes (A3, A12, D3, D5, D8 and D13; Fig. 1; Table 3), which greatly exceeded that detected in previous studies (Chen et al. 2008; Wang et al. 2009, 2010). The results showed that using a composite CP with more than two parents is more powerful for QTL detection (Qin et al. 2008; Zhang et al. 2012) than the populations used in previous studies, although it is not easy to compare QTL mapping results from different studies due to divergent plant materials and markers used. Three (*qFW-A3-1*, *qFW-A12-1*, *qFW-D3-1*) of the 7 QTLs were similar in map position to that detected in previous studies (Chen et al. 2008; Wang et al. 2009, 2010), while the LOD scores (1.22–3.45) and PVEs (2.9–6.6 %) were somewhat lower in the current study. This result may be due to the fact that resistance evaluation was carried out only at the seedling stage, when the susceptible plants had not time enough to develop severe symptoms. Therefore, resistance should be evaluated at more than one plant developmental stages under greenhouse conditions.

Association mapping can be affected by many factors, such as population structure, relatedness among accessions, small sample size and low frequency of specific alleles; these factors may increase the detection of false positive associations (Gupta et al. 2005; Yu et al. 2006). In this study, AM was performed with a moderately large-sized panel (356 accessions) using the optimal model of MLM, which considers both population structure and relatedness, to detect SSR markers associated with FOV 7 resistance. Nonetheless, it is still not easy to determine which significance level is the most appropriate. The use of stringent probability thresholds reduces the danger of false positives but poses the risk of rejecting true positives caused by setting the thresholds too high (Yan et al. 2011). In the present study, 35 loci were significantly associated with FOV 7 resistance at the $\alpha = 0.05$ level (Table S4), and 27 loci were significant at the $\alpha = 0.01$ level (Table 4). If a more stringent threshold by the Bonferroni correction ($P \leq 0.05/145$, $-\log_{10}P \geq 3.46$) is adopted (Lander and Botstein 1989), only 10 associations were significant. Five (*qFW-A3-1*, *qFW-A12-1*, *qFW-D3-1*, *qFW-D5-1* and *qFW-D8-1*) of the 7 QTLs identified by linkage mapping could be detected by association mapping at the $\alpha = 0.05$ level (Table S4), while only three QTLs (*qFW-D3-1*, *qFW-D5-1* and *qFW-D8-1*) could be detected at the $\alpha = 0.01$ level (Table 4). The marker JESPR304, which is closely linked with a major QTL [Chr 17 (D3)] (Wang et al. 2009, 2010), was indeed significantly ($-\log_{10}P = 7.09$) associated with FOV 7 resistance in our association mapping study, while this locus should be located on Chr 2 (A2) (Guo et al. 2007; Zhao et al. 2012a, b; Ulloa et al. 2013). Unfortunately, JESPR304 did not amplify polymorphic bands among the three parents of our CP.

Inheritance and genetic basis of FOV resistance in cotton

As FW has long been and will continue to be an important disease that affects cotton production, most studies of this disease have been performed to unravel the genetics of FW resistance. In early classical genetic studies, the inheritance of FOV resistance in cotton was considered to be controlled by a single gene (Smith and Dick 1960; Netzer et al. 1985; Feng et al. 1996) or multiple genes (Kappelman 1971; Jiao 1985; Wang et al. 1989) with partially dominant effects. In the current study, partial dominance was also observed in the two single crosses, Xuzhou 142/Yumian 21 and Xuzhou 142/Shang 9901, with dominant-additive ratios of 0.57 and 0.60, respectively (Table 1).

Wang and Roberts (2006) identified a major resistance gene (*Fov1*) against FOV 1 in *G. barbadense* cv. Pima-S7 and indicated that one or more minor genes in Acala NemX could delay wilt

symptoms. Recently, Ulloa et al. (2011) performed QTL mapping in an F_2 (Pima-S7 × Acala NemX) and a recombinant inbred line (RIL; *G. hirsutum* TM-1 × *G. barbadense* Pima 3-79) population. The authors detected six QTLs (Fov1-C06, Fov1-C08, Fov1-C11$_1$, Fov1-C11$_2$, Fov1-C16 and Fov1-C19) conferring FOV 1 resistance and confirmed a major QTL (Fov1-C16) in different genetic backgrounds. Lopez-Lavalle et al. (2012) used an intraspecific cross between the Upland cotton MCU-5 and Siokra 1-4 to detected QTLs conferring resistance to Australian FOV races and found that MCU-5 resistance is complex, with 3 QTLs identified in F_3 and 8 ones in F_4. The QTLs were localized to chromosomes A6, D4 and D6. Ulloa et al. (2013) investigated three intraspecific (*G. hirsutum* × *G. hirsutum* L. and *G. barbadense* × *G. barbadense* L.), five interspecific (*G. hirsutum* × *G. barbadense*) and one RIL population in four greenhouse and two field experiments and identified a set of 11 SSR markers across six linkage groups/chromosomes (3, 6, 8, 14, 17 and 25) associated with FOV race 4 resistance. Integrating information mentioned above with the QTL mapping results for FOV 7 in our study,, it suggests that the inheritance of FOV resistance in cotton should be far more complex than has been elucidated to date. Interestingly, the map positions of some QTLs conferring resistance against different FOV races coincided, suggesting that these genes may play the same or similar roles in the process of different host-pathogen and/or environment interactions.

Favorable alleles and their potential use in breeding programs

Host resistance has always been considered to be an effective way to manage plant diseases. Although significant progress in the production of FOV-resistant cotton has been made by conventional phenotype-based selection, this process has been slow and inefficient in the face of emergence of new, highly virulent isolates of FOV in Australia and the United States (Wang et al. 2004; Kim et al. 2005). The development of effective markers for FOV resistance offers the promise of increasing the selective gain while ensuring sustainable targeted breeding progress. In the current study, favorable alleles for each of the 27 associated loci were identified, and the number of favorable alleles in each of 356 accessions were also investigated (Table 5, Table S1). Pearson correlation analysis showed that there was highly negative significance between DIs and the number of favorable alleles. While great differences in the resistance of cotton genotypes against FOV races have been demonstrated in many evaluations worldwide, a genotype with a high level of resistance against all FOV races has not been found. Given that FW is the result of interactions among the host, pathogen and environment, many genes must be involved in this process. The accumulation of resistance alleles, even with relatively minor individual effects, will result in higher levels of resistance. The Upland cotton cultivars from China and Africa have shown more resistance to Australian FOV races (Constable et al. 2007). Therefore, the favorable alleles and their typical germplasm resources indentified in this study should have great potential for developing highly resistant Upland cotton cultivars in future molecular breeding programs.

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