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Inheritance and QTL mapping of Fusarium wilt race 4 resistance in cotton

Mauricio Ulloa · Robert B. Hutmacher · Philip A. Roberts · Steven D. Wright · Robert L. Nichols · R. Michael Davis

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Abstract Diseases such as Fusarium wilt [*Fusarium oxysporum* f.sp. *vasinfectum* (FOV) Atk. Sny & Hans] represent expanding threats to cotton production. Integrating disease resistance into high-yielding, high-fiber quality cotton (*Gossypium* spp.) cultivars is one of the most important objectives in cotton breeding programs worldwide. In this study, we conducted a comprehensive analysis of gene action in cotton governing FOV race 4 resistance by combining conventional inheritance and quantitative trait loci (QTL) mapping with molecular markers. A set of diverse cotton populations was generated from crosses encompassing multiple genetic backgrounds. FOV race 4 resistance was investigated using seven parents and their derived populations: three intraspecific (*G. hirsutum* × *G. hirsutum* L. and *G. barbadense* × *G. barbadense* L.) F_1 and F_2; five interspecific (*G. hirsutum* × *G. barbadense*) F_1 and F_2; and one RIL. Parents and populations were evaluated for disease severity index (DSI) of leaves, and vascular stem and root staining (VRS) in four greenhouse and two field experiments. Initially, a single resistance gene (*Fov4*) model was observed in F_2 populations based on inheritance of phenotypes. This single *Fov4* gene had a major dominant gene action and conferred resistance to FOV race 4 in Pima-S6. The *Fov4* gene appears to be located near a genome region on chromosome 14 marked with a QTL *Fov4-C14*$_1$, which made the biggest contribution to the FOV race 4 resistance of the generated F_2 progeny. Additional genetic and QTL analyses also identified a set of 11 SSR markers that indicated the involvement of more than one gene and gene interactions across six linkage groups/chromosomes (3, 6, 8, 14, 17, and 25) in the inheritance of FOV race 4 resistance. QTLs detected with minor effects in these populations explained 5–19 % of the DSI or VRS variation. Identified SSR markers for the resistance QTLs with major and minor effects will facilitate for the first time marker-assisted selection for the introgression of FOV race 4 resistance into elite cultivars during the breeding process.

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M. Ulloa (✉)
Cropping Systems Research Laboratory, Plant Stress and Germplasm Development Research, USDA-ARS, SPA, 3810 4th Street, Lubbock, TX 79415, USA
e-mail: mauricio.ulloa@ars.usda.gov

R. B. Hutmacher
Department of Plant Sciences, University of California-Davis, Five Points, CA 93624, USA

P. A. Roberts
Department of Nematology, University of California, Riverside, CA 92521, USA

S. D. Wright
University of California, Cooperative Extension, Tulare, CA 93274, USA

R. L. Nichols
Cotton Incorporated, Cary, NC 27513, USA

R. Michael Davis
Department of Plant Pathology, University of California-Davis, Davis, CA 95616, USA

Introduction

Integrating disease resistance into high-yielding, high-fiber quality cultivars is one of the most important objectives in cotton (*Gossypium* spp.) breeding programs worldwide. Diseases such as Fusarium wilt [*Fusarium oxysporum* f.sp. *vasinfectum* (FOV) Atk. Sny & Hans] represent expanding threats to cotton production (Kochman et al. 2002; Kim et al. 2005; Ulloa et al. 2006, 2011; Wang et al. 2009). FOV is a soil-inhabiting fungus that can survive for long periods in the absence of a host, making it impractical to eradicate from infested fields. This species of fungus is comprised of different genotypes called races. These races were originally classified based on pathogenicity tests on different cotton species including *Gossypium hirsutum* L, *G. barbadense* L., and *G. arboreum* L. (Armstrong and Armstrong 1960, 1978; Ibrahim 1966), and by their pathogenicity on alfalfa (*Medicago sativa* L.), soybean (*Glycine max* L.), and tobacco (*Nicotiana tabacum* L.) (Armstrong and Armstrong 1958, 1978). At least eight of these races (1–8) have been described throughout the world (Fernandez et al. 1994; Skovgaard et al. 2001).

Until the early 2000s, only races 1 and 2 were known to occur in the United States (Smith et al. 1981; DeVay 1986). FOV races 1 and 2 are typically found in sandy or sandy-loam soils with significant root-knot nematode [(RKN) *Meloidogyne incognita* (Kofoid and White) Chitwood] populations (Bell 1984; Veech 1984). In 2003, three additional races (3, 4, and 8) were identified in California (Kim et al. 2005). The described FOV races can be distinguished based on differences in DNA sequences of the translational elongation factor (EF-1α), the phosphate permase (PHO) and the beta-tubulin (BT) genes, and intergenic spacer (IGS) regions, using restriction enzymes. Worldwide, strains and FOV races can be classified into five major lineages (1, 3, 4, 8, and races from Australia) (Fernandez et. al. 1994; Skovgaard et al. 2001; Kim et al. 2005). However, recent analyses with partial sequences from EF-1α, PHO, and BT genes of 61 FOV isolates across the US revealed four additional FOV genotypes. These novel races were pathogenic to at least one cotton cultivar used in the study (Holmes et al. 2009). Even though FOV races can be classified with genemarkers, certain FOV strains are still difficult to classify and place in specific lineages (Geiser et al. 2004). For example, races 4 and 7 were indistinguishable genetically using EF-1α and mtSSU rDNA sequence data (Skovgaard et al. 2001). In a recent study, preliminary partial gene (EF-1α, PHO, BT, and IGS) sequence data were also unable to distinguish races 4 and 7 (M. Davis, unpublished data). However, pathogenicity tests have shown FOV race 7 from China to be different from FOV race 4 of India. Race 4 was more pathogenic to Pima cottons than race 7, while race 7 was more pathogenic to Upland cottons than race 4 (Armstrong and Armstrong 1960). The conflict between pathogenicity tests and genetic studies remains unresolved for distinguishing these two races (4 and 7) (Davis et al. 2006).

Over the past 8 years, FOV race 4 has increasingly impacted cotton fields in California's San Joaquin Valley (SJV) (Hutmacher et al. 2011). This race, first identified in India on Asiatic cottons, had not been identified previously in the US before 2003. FOV race 4 has caused extensive disease symptoms in cotton plants grown in clay loam and loam soils in which RKN populations and root damage from nematodes were absent or extremely low. In field evaluations in the SJV of California, disease expression of race 4 has been most severe in susceptible Pima cotton cultivars, but many Acala and Upland cotton cultivars have also been highly infected by FOV race 4 (Kim et al. 2005; Hutmacher et al. 2005; Ulloa et al. 2006).

In cotton, little is known about the genetic basis for resistance to FOV races, or how these races are affected by environmental factors and interactions with other pathogens (e.g., root-knot and reniform nematodes (*Rotylenchulus reniformis* Linford and Olivira), Verticillium wilt caused by *Verticillium dahlia* Kleb, or black root rot caused by *Thielaviopsis basicola* [(Berk. and Broome) Ferraris] (Ulloa et al. 2011). Resistance to FOV has been evaluated in cotton cultivars and germplasm with different genetic backgrounds and under different evaluation conditions. Phenotypic analyses indicated that resistance to FOV was determined by one or two major genes with complete to incomplete dominance and possibly additional minor genes (Smith and Dick 1960; Mohamed 1963; Ulloa et al. 2006; Wang and Roberts 2006; Wang et al. 2009; Ulloa et al. 2011). A major FOV race 1 resistance gene (*Fov*) in *G. barbadense* cv. Pima-S7 was identified under greenhouse conditions based on phenotypic studies and application of amplified fragment length polymorphism (AFLP) markers of segregating populations from the crosses 'Pima-S7' × 'Acala NemX' and Pima-S7 × 'Acala SJ-2' (Wang and Roberts 2006). Moreover, this recessive *Fov1* gene was co-located with *Fov1-C16* quantitative trait locus (QTL) on chromosome 16, based on phenotypes and application of microsatellites or simple sequence repeat (SSR) markers to segregating populations from the crosses Pima-S7 × Acala NemX and Upland 'TM-1' × Pima '3-79' (Ulloa et al. 2011). In addition to detecting the race 1 *Fov1* resistance gene co-located with *Fov1-C16* QTL locus on chromosome 16, Ulloa et al. (2011) reported gene interactions across five cotton chromosomes harboring QTLs [*Fov1-C06, Fov1-C08, Fov1-C11*$_1$, *Fov1-C11*$_2$, *Fov1-C16,* and *Fov1-C19* loci]. These QTLs explained 8–31 % of the variation in disease severity index of leaves or vascular stem and root staining.

A race 7 resistance gene (FW^R) in Upland cotton was identified in field evaluations (Wang et al. 2009). This FW^R

gene was co-located with a QTL on chromosome 17. In the same study (Wang et al. 2009), QTLs were also reported from chromosomes 7, 15, and 23 for resistance to FOV. Resistance to the Australian *Fov* races was found to be complex, and resistance genes may be co-located with three QTLs on chromosomes 6, 22, and 25 (Becerra et al. 2012). Collectively, these QTL mapping studies (Wang et al. 2009; Ulloa et al. 2011; Becerra et al. 2012) revealed different gene specificity of FOV resistance in cotton [*Fov1*—chromosome 16; FW^R—chromosome 17; Australian *Fov*—chromosomes 6, 22, and 25].

The postulated pathogenicity mechanisms and the inheritance of FOV resistance significantly differ among FOV races for cotton genotypes (Ulloa et al. 2011). When resistant cotton cultivars or germplasm from different genetic backgrounds are challenged by different races of different geographic origin, they may not express similar modes of inheritance of resistance. A QTL mapping approach can be informative for studying quantitative inheritance, for detecting genomic regions associated with disease resistance, and for identifying molecular markers tightly linked to the genes of interest (Ulloa et al. 2007, 2011). Molecular markers can also address fundamental questions regarding the genetics of FOV resistance and can assist in breeding elite cultivars with resistance using marker-assisted selection (MAS). MAS reduces the time and labor spent on phenotypic evaluations to identify resistant genotypes, thereby accelerating the breeding process (Ulloa et al. 2011; Wang et al. 2012).

For this study, the following research objectives were developed based on the availability of genome-wide SSR markers and mapping populations segregating for FOV race 4 resistance: (1) to increase our understanding of the inheritance of FOV resistance in cotton, (2) to detect genomic regions associated with resistance to FOV race 4 using genetic and QTL mapping, and (3) to identify molecular markers associated with resistance to FOV. Examining the inheritance of genomic regions and associations of the molecular markers with resistance to FOV will help to unveil the genes involved in the pathogenicity mechanism of FOV resistance.

Materials and methods

Fungal inoculum

An isolate of *Fusarium oxysporum* f. sp. *vasinfectum* (FOV CA-14) identified as FOV race 4 (Kim et al. 2005) was used in greenhouse evaluations in 2007, 2008, and 2010 at the University of California Kearney Research and Extension Center (UCK, Parlier, CA, USA) and in 2007 at the University of California Davis (UCD). The isolate originated from a naturally infested field in the SJV of California where plants exhibited symptoms typical of Fusarium wilt, which included stunted growth, vascular discoloration, leaf wilt, chlorosis, necrosis, and ultimately leaf abscission or plant death. Inoculum was prepared from 3-week-old potato dextrose agar (PDA) culture plates flooded with water and scraped with a bacterial loop to dislodge conidia. The conidial suspension was then filtered through four layers of cheesecloth to remove hyphae, quantified with the aid of a hemacytometer, and diluted with water to obtain desired conidial concentrations.

Plant material

Plant genotypes used at UCK in 2007, 2008, and 2010 included FOV race 4 susceptible *G. hirsutum* Upland Shorty and two moderately tolerant cultivars, Upland TM-1 and Acala NemX. The trial at UCK also included resistant *G. barbadense* Pima-S6, and susceptible Pima-S7, Pima 3–79, and DP744 (Monsanto: U.S. PVP# 20010004) (Table 1). These cultivars and germplasm releases were used as parents to develop F_1 and F_2 populations. Three intraspecific (*G. hirsutum* × *G. hirsutum* and *G. barbadense* × *G. barbadense*) F_1 and F_2 populations, along with five interspecific (*G. hirsutum* × *G. barbadense*) F_1 and F_2 populations were developed and assayed for race 4 infection (Table 1). In addition, one recombinant inbred line (RIL) population developed from a cross between Upland TM-1 and Pima 3-79 (Kohel et al. 2001) was used to assess reaction to FOV race 4 in 2007 at UCD, in 2008 at UCK, and in 2007 at a field site naturally infested with FOV race 4 in Kern County CA (FKCA). This RIL population was used previously for mapping microsatellite or SSR markers (Park et al. 2005; Frelichowski et al. 2006). In the fall of 2005, a single plant from each RIL was transplanted into a pot in the greenhouse and the resulting seed was harvested from each plant in 2006. In 2007, the seeds from each RIL plant were planted in the field at Shafter, CA for seed increase. One hundred fifty-two RILs produced seed. Seed of the TM-1 × Pima 3-79 RIL population from these different generations was used in FOV race 4 evaluations. These F_2 and RIL populations were also used for genetic and QTL mapping (see below, Table 2). The number of RILs evaluated depended on seed availability or germination. We used 127 RILs (Pop1a) in the UCD experiment (2007), 136 RILs (Pop 1b) in the UCK experiment (2008), and 112 RILs (Pop 1c) in the FKCA experiment (Table 2). In 2010 at FKCA, we used two F_2 populations—TM-1 × Pima-S6 (Pop 2) and Shorty × Pima-S6 (Pop 3)—with 63 and 84 individuals, respectively (Table 2).

Fusarium wilt (FOV) race 4 assays

A root dip method was used in all FOV race 4 greenhouse evaluations. Parents, F_1, F_2, and RIL populations were

Table 1 Mean of vascular stem and root staining (VRS) for parents, F_1 (standard error, SE), F_2, and recombinant inbred line (RIL) cotton (*Gossypium* spp.) populations tested for Fusarium wilt (FOV race 4)

Genotype or progeny	VRS	SE		
Parents				
G. hirsutum				
Upland Shorty[a]	2.0	0.6		
Acala NemX[a]	1.5	0.5		
Upland TM-1[b]	0.3	0.5		
Upland Shorty[c]	2.6	0.6		
Upland TM-1[c]	1.9	0.2		
G. barbadense				
DP744[a]	3.6	0.3		
Pima-S7[a]	3.0	0.0		
Pima-S6[a]	0.2	0.2		
Pima 3–79[b]	3.0	0.7		
Pima-S7[c]	4.0	0.2		
Pima-S6[c]	0.7	0.1		
F_1 intraspecific populations				
Shorty × NemX[a]	2.7	0.2		
NemX × Shorty[a]	1.5	0.5		
Pima-S7 × Pima-S6[c]	1.4	0.1		
F_1 interspecific populations				
DP744 × Shorty[a]	2.6	0.4		
Pima-S7 × Shorty[a]	2.5	0.4		
Shorty × Pima-S7[a]	2.1	0.6		
Shorty × Pima-S6[c]	0.9	0.2		
TM-1 × Pima-S6[c]	0.9	0.1		
		R:S[e]	VRS Range	
F_2 intraspecific populations				
DP744 × Pima-S6[a]	1.2	52:22	0.0–4.0	
Pima-S7 × Pima-S6[a]	0.7	51:10	0.0–4.0	
Pima-S7 × Pima-S6[c]	2.1	109:61	0.0–5.0	
F_2 interspecific populations				
Shorty × Pima-S6[a]	1.1	20:15	0.0–3.0	
NemX × Pima-S6[a]	0.4	71:03	0.0–3.0	
TM-1 × Pima-S6[c]	1.5	105:36	0.0–5.0	
Shorty × Pima-S6[c]	1.2	122:37	0.0–5.0	
Shorty × Pima-S6[d]	1.3	49:30	0.0–3.0	
RIL population				
TM-1 × Pima 3–79[b]	1.3	100:36	0.0–3.8	

VRS scale: 0 = no vascular root staining evident, 1 = light vascular root staining evident as spotty areas, 2 = more continuous than 1, but light colored staining covering an area between one quarter and one half of the stem cross-section, 3 = moderate brown/black staining evident in a band encircling most of the stem cross section, 4 = brown/black staining evident across most vascular tissue in stem cross section, and 5 = plant severely damaged or plant death with staining evident throughout a cross-section of root tissue

[a] VRS greenhouse evaluation conducted in 2007 at the University of California Kearney Research and Extension Center, Parlier, CA

[b] VRS greenhouse evaluation conducted in 2008 at the University of California Kearney Research and Extension Center, Parlier, CA

[c] VRS field evaluation conducted in 2010 on a site infested with FOV race 4 at the Kern County field

[d] VRS greenhouse evaluation conducted in 2010 at the University of California Kearney Research and Extension Center, Parlier, CA

[e] Expected R:S, number of plants for vascular root stain VRS <2 as resistant (R):VRS ≥2 as susceptible (S)

Table 2 Summary of phenotypic evaluations for Fusarium wilt (FOV race 4) based on mean vascular stem and root staining (VRS) on F_2 and recombinant inbred line (RIL) populations, and VRS standard deviation (SD)

	Population	No	Mean	SD
Greenhouse Test				
RIL TM-1 × Pima 3–79[a]	Pop 1a	127	1.4	0.90
RIL TM-1 × Pima 3–79[b]	Pop 1b	136	1.3	0.83
Field test				
RIL TM-1 × Pima 3–79[c]	Pop 1c	112	2.5	0.72
F_2 TM-1 × Pima-S6[d]	Pop 2	63	1.6	1.6
F_2 Shorty × Pima-S6[d]	Pop 3	84	1.3	1.4

Phenotypic data of these populations were used for quantitative trait loci (QTL) mapping. VRS scale: 0 = no vascular root staining evident to 5 = plant severely damaged or plant death

[a] VRS greenhouse evaluation conducted in 2007 at the University of California Davis, CA

[b] VRS greenhouse evaluation conducted in 2008 at the University of California Kearney Research and Extension Center, Parlier, CA

[c] VRS field evaluation conducted in 2007 on a infested field with FOV race 4 at the Kern County site

[d] VRS field evaluation conducted in 2010 on a infested field with FOV race 4 at the Kern County site

seeded into a composite medium of vermiculite and peat moss prior to inoculation. When the plants were 3 weeks old, they were removed from the potting medium and the roots were gently washed to remove most of the soil medium. The roots were then dipped for 2 min in a spore suspension of approximately 1×10^5 conidia per ml of water. Seedlings were then transplanted into 6 × 15 cm (500 ml) box pots with one plant in each pot (Ulloa et al. 2006, 2009).

In the greenhouse evaluation at UCD in 2007, plants were evaluated 28 days after inoculation (dai). The UCD experiment used a randomized complete block design with four replications. Each replication of an entry was represented by one plant per pot of each RIL. Symptoms of Fusarium wilt infection include foliar injury (chlorosis, wilting or death) and discoloration of stem and root vascular tissue. Individual plants were rated to determine the response to FOV race 4 infection using the disease severity index (DSI) of leaves and vascular root staining (VRS) (Ulloa et al. 2006, 2009, 2011). DSI was based on the following scale: 0 = no symptoms; 1 = epinasty of leaves; 2 = 1–30 % of leaves chlorotic and wilted; 3 = 31–80 % of leaves chlorotic and wilted; 4 = 81–100 % of leaves chlorotic and wilted; and 5 = plant death. Plant stems and the upper part of the primary roots were cut longitudinally and evaluated for VRS at the end of the experiment. The following scale was used for VRS: 0 = no VRS evident, 1 = light VRS evident as spotty areas or thin line, 2 = more continuous than 1, but light colored staining

covering an area between one quarter and one half of the stem cross-section, 3 = moderate brown/black staining evident in a band encircling most of the stem-root cross-section, 4 = brown/black staining evident across most vascular tissue in stem cross-section, and 5 = plant severely damaged or plant death with staining evident throughout the root tissue.

In the greenhouse evaluation at UCK in 2008, plants were assayed at 24 dai, and in 2010, at 31 dai for DSI and VRS to determine disease incidence and severity. The UCK experiment used a completely randomized design with four replications. Each replication of an entry (RIL) was represented by a single plant in a pot.

In 2007, 112 RILs were planted in a clay-loam soil field site infested with FOV race 4 at FKCA. Plants from previous studies in this field consistently developed severe FOV symptoms, and the site was confirmed to be infested with FOV race 4. Entries were grown in 1-row plots 5 m long with a 1-m row spacing in a randomized complete block design with two replications. Five plants randomly chosen per plot were used as subsamples to obtain estimates of DSI and VRS at 92 days after planting. In addition, plant survival (PS) in each plot was recorded on day 92 after planting. PS was calculated by dividing the total number of surviving plants on each sample date by the initial plant count made 12–14 days after planting and multiplying by 100.

In 2010, F_2 plants from two populations (pop 2 and pop 3; Table 2) were also planted in the clay-loam soil field site infested with FOV race 4 at FKCA.

Marker analysis

We used 1100 SSR markers with wide genome coverage. SSRs averaged 6 cM between two linked markers on cotton chromosomes (Frelichowski et al. 2006; Park et al. 2005; Ulloa et al. 2008; Wang et al. 2006; CMD: http://www.cottonmarker.org). Polymerase chain reaction (PCR) amplification of BNL, CIR, Gh, MUSB, MUCS, MUSS, and NAU markers was performed on a total volume of 15 μL containing 2 μL of DNA template (concentration 10 ng/μL), 0.1 μM each of forward and reverse primers, 1× PCR buffer, 3 mM of $MgCl_2$, 0.2 mM of dNTPs, and 0.5 U of Amplitag Gold Taq polymerase (Applied Biosystems, Foster City, CA, USA) with a cycling profile of 1 cycle of 10 min at 94 °C; 10 cycles of 15 s at 94 °C, 30 s at 60 °C (step −0.5 °C/cycle for cycles 2–10), and 1 min at 72 °C; 35 cycles of 15 s at 94 °C, 30 s at 55 °C, and 1 min at 72 °C; and a final extension cycle of 6 min at 72 °C. PCR products were separated for 4–5 h on a 3 % super fine resolution (SFR^{TM}) agarose gel (Amresco, Solon, OH, USA) containing 1× TBE at 90 V and were visualized by Alphaimager software (v. 5.5, Alpha Innotech Corporation, San Leandro, CA, USA) after staining with ethidium bromide.

We assessed SSR markers located on different cotton chromosomes using bulked segregant analysis (BSA) and QTL analysis (see below). The BSA method included resistant (R, Pima-S6, and moderately tolerant Upland TM-1 and Acala NemX) and susceptible (S, Upland Shorty, Pima-S7, Pima 3-79, and DP744) parents, and R and S bulked F_2 progeny and RILs. Assayed plants from UCD and UCK with a DSI <2 or VRS <2 were classified as R, and assayed plants with a DSI ≥2 or VRS ≥2 were classified as S, using the scale 0 (no symptoms) to 5 (plant, leaves severely damaged or roots severely damaged, respectively) and based on previous observations of the indices corresponding to known resistant and susceptible parents. Bulked samples included at least eight R and eight S entries selected from the F_2 and RIL populations from each experiment.

Data analyses

Differences among observed DSI (or VRS) within study entries (parents, F_1s, and RIL), and among study entries (parents and F_1s) were evaluated for each experiment using PROC GLM (SAS, ver. 9.2, SAS Institute, Cary, NC, USA). After significant difference ($P < 0.05$) was observed from the analysis of variance, mean separations in the various examinations of main effects were conducted using the Waller–Duncan k ratio procedure (Ott 1988). A small summary from the analyses of variance and mean separation from the various experiments are presented in the host-plant resistance section of the results.

Phenotypic segregation ratios of resistant (R) and susceptible (S) plants were evaluated for entries from F_2 and RIL populations in each study. The goodness-of-fit of the observed R:S ratio to the expected Mendelian ratio was assessed by Chi-square analysis (Weir 1996).

Genetic linkage analyses

The informative bands were scored as present (+) or absent (−) for a dominant marker (expected ratios 3:1 or 1:3 for a F_2 or 1:1 for a RIL population). If alleles from both parents were present, the marker was scored as co-dominant (expected genotypic ratios 1:2:1 for a F_2 or 1:1 for a RIL population). The JoinMap^R version 4.0 (Van Ooijen 2006) computer program was used to test for Chi-square goodness-of fit for expected versus observed genotypic ratios and to construct the linkage groups/chromosomes for the F_2 and RIL populations. Likelihood ratio (LOD) scores of 3–8 were examined for each population using the Kosambi map function and a maximum distance of 40 cM. LOD

threshold scores >3 were used as a cut-off to determine linkage between any two markers.

Quantitative trait loci (QTL) analyses

Single-marker analysis was conducted using a nonparametric mapping test [Kruskal–Wallis analysis (K*)] equivalent to a one-way analysis of variance (Van Ooijen 2004). The nonparametric analysis was used because in this test no assumptions are being made for the probability distribution(s) of the quantitative trait, and even if the data are distributed normally, the nonparametric test is often as powerful as parametric methods. In addition, the nonparametric test uses all markers genotyped on the population regardless of their linkages (tests each locus separately without the use of the linkage map). QTL analyses were conducted on DSI and VRS phenotypic data using MapQTL 5.0 with interval mapping and the multiple-QTL model mapping procedures. Threshold values for LOD were determined empirically after 1000 permutation tests for all traits (Churchill and Doerge 1994). The threshold for a QTL was determined at LOD ≥3. QTL analyses were performed on DSI and VRS phenotypic data recorded from three data sets of the RIL population (Pop 1a–c) and two F_2 populations (Pop 2 and Pop 3). Table 2 presents the number of plants or RILs that were genotyped and informative for these analyses. QTL analysis was also performed on percent plant survival data recorded at 92 days after planting from the KFCA experiment in 2007.

Results

Host-plant resistance

Differences in severity index of leaves (DSI) and VRS index ratings [resistant (R) as <2 : susceptible (S) as ≥2] were observed between R (Pima-S6) and S (Upland Shorty, Pima 3-79, DP 744 and Pima-S7) parents for FOV race 4 infection. In F_1, F_2 and RIL populations, a wide range of VRS ratings was observed across progeny for FOV race 4 resistance (Table 1). Under greenhouse conditions, the intraspecific Acala NemX (VRS 1.5) × Upland Shorty (VRS 2.0) F_1 population had a VRS index similar (VRS 1.5) to the moderately tolerant parent Acala NemX (Table 1). In the intraspecific Pima × Pima F_1 populations (VRS ≤2.1), VRS was lower than the VRS of the susceptible parents, DP744 (VRS 3.6) or Pima-S7 (VRS 3.0).

Statistically significant differences in VRS index were also observed under greenhouse conditions for parents and F_1 comparisons from the various experiments (data not shown). In the naturally infested field, the VRS index of the intraspecific Pima-S7 × Pima-S6 F_1 population (VRS 1.4) was significantly lower ($P < 0.05$) than the susceptible parent Pima-S7 (VRS 4.0) and higher than the resistant parent Pima-S6 (VRS 0.7). The average plant survival (PS) calculated across all the parents of the cotton populations was 65 % for Upland Shorty and about 30 % for Pima-S7. Pima cottons were more susceptible to FOV. However, a Pima germplasm, Pima-S6, highly resistant to FOV race 4 was identified in field and greenhouse evaluations. When we examined the main effect of mean separation, the minimum significant difference (MSD) for VRS was equal to 0.50 using Waller–Duncan *k* ratio (Ott 1988) in the 2010 experiment at FKCA. F_{1s} from Upland Shorty × Pima-S6 and Upland TM-1 × Pima-S6 had similar VRS index (0.9) to the resistant parent, Pima-S6. Contrary to Pima cottons, where plants resistant to FOV race 4 seem to be highly resistant, no resistant Acala or non-Acala Upland cultivar was identified; only tolerant or moderately tolerant Acala or Upland cottons were observed in these tests (Tables 1, 2 and 3 present VRS index ratings of Upland cottons).

Phenotypic genetic analyses

Modifications in gene action were observed based on VRS index ratings. In four different cross-combinations of R and S parent groups of F_1 (S × R, R × S, S × S, and R × R), the action of resistance genes ranged from dominant to recessive when R and S parents were crossed. Intraspecific F_1 *G. hirsutum* populations showed dominant or recessive gene expression, while interspecific F_1 (*G. hirsutum* × *G. barbadense*) populations only showed dominant gene expression (Tables 1, 2 and 3). The observed gene actions depended on the specific parent cross-combination and genetic background, and whether the combinations were an intra- or interspecific cross (Table 1).

Based on inheritance of phenotypes, one- and more than one-gene models for resistance to FOV race 4 were investigated further in parents, F_1, F_2, and RIL populations (Tables 2 and 3). The segregation of three F_2 populations (DP744 × Pima-S6, TM-1 × Pima-S6, and Shorty × Pima-S6) fit the 3 (R):1 (S) expected distribution for a single gene determining resistance ($\chi^2 = 0.76$, $\chi^2 = 0.00$, and $\chi^2 = 0.10$, respectively, Table 3). This single gene (*Fov4*) was observed with a major dominant gene action and was provided by Pima-S6 in crosses with susceptible Pima DP744 or Upland Shorty, or moderately tolerant Upland TM-1 parents (Table 3). However, distorted Mendelian ratios were observed for a single gene model based on VRS ratings from field and greenhouse experiments on the RIL (moderately tolerant Upland TM-1 × susceptible Pima 3-79) population (Table 3; Fig. 1d–f) and on the F_2 Pima-S7 × Pima-S6 population assessed under FOV race 4 infested field conditions. These

Table 3 Observed and expected values and Chi-square values for one and more than one gene models for Fusarium wilt (FOV race 4) resistance in segregating populations derived from crosses of resistant Pima-S6 (R) and Upland mid-tolerant TM-1with susceptible Pima-S7, DP744, Pima 3-79, and Upland Shorty (S)

Genotype/population	No. plants	Expected ratios R:S[a]	Observed ratio R:S	χ^2	*P* value
Parents					
Upland TM-1[b]	4	All R	All R		
Upland TM-1[c]	30	All R	(18) R:S (12)		
Upland Shorty[d]	30	All S	(13) R:S (17)		
DP744[c]	10	All S	(1) R:S (9)		
Pima-S6[c]	10	All R	All R		
Pima 3–79[b]	4	All S	(1) R:S (3)		
Pima-S7[d]	30	All S	(1) R:S (29)		
Pima-S6[d]	30	All R	(28) R:S (2)		
F_1					
Pima-S7 × Pima-S6[d]	30	All R	(27) R:S (3)		
TM-1 × Pima-S6[d]	30	All R	(29) R:S (1)		
Shorty × Pima-S6[d]	30	All R	All R		
F_2					
DP744 × Pima-S6[b]	74	56:19 (3:1)[e]	52:22	0.76	NS
Pima-S7 × Pima-S6[d]	170	128:42 (3:1)[e]	109:61	11.41	***
TM-1 × Pima-S6[d]	141	105:36 (3:1)[e]	105:36	0.00	NS
Shorty × Pima-S6[d]	159	122:39 (3:1)[e]	122:37	0.10	NS
RIL					
TM-1 × Pima 3–79[c]	136	68:68 (1:1)[e]	100:36	30.12	***
		102:34 (3:1)[f]	100:36	0.18	NS

NS not significant at $\chi^2_{0.05}$, *significant at $>\chi^2_{0.05}$, **significant at $>\chi^2_{0.025}$, ***significant $>\chi^2_{0.005}$

[a] Expected R:S, number of plants for vascular root stain VRS <2 as resistant (R):VRS 2≥ as susceptible (S). Even though TM-1 had plants with VRS >2 and Shorty with <2, we still treated them as conditional R (moderately tolerant cultivar) and S entries, respectively. The overall VRS index of TM-1 and Shorty from all experiments kept the conditional separation between R and S of these two entries

[b] VRS greenhouse evaluation conducted in 2008 at the University of California Kearney Research and Extension Center, Parlier, CA

[c] VRS greenhouse evaluation conducted in 2008 at the University of California Kearney Research and Extension Center, Parlier, CA

[d] VRS field evaluation conducted in 2010 on a site infested with FOV race 4 at the Kern County field

[e] One gene model

[f] More than one gene model

ratios suggested that more than one gene was responsible for resistance to FOV race 4 in the progeny of these crosses. Upland TM-1 plants ranged from highly resistant (VRS 0.30, Fig. 1e) to moderately susceptible (VRS 2.3, Fig. 1d) to FOV race 4. In the RIL population (TM-1 × Pima 3–79), the Upland TM-1 alleles provided FOV resistance (lower VRS index) (Tables 3). However, a second FOV resistance gene was not detected in the F_2 TM-1 × Pima-S6 population using the VRS index (Table 3). Some progeny from the F_2 and RIL populations were more resistant than the moderately tolerant Upland TM-1and resistant Pima-S6 parents, indicating the presence of transgressive segregants and suggesting that multiple loci governed host-plant resistance to FOV race 4 (Fig. 1). The highly resistant families and RILs may carry two or more minor genes with one or more coming from each parent (Table 4).

Molecular markers and genetic linkage analyses

Using BSA, 550 SSRs on R [VRS <2 (Pima-S6 and Upland TM-1)] and S [VRS ≥2 (Pima 3-79 and Upland Shorty)] parents, and R and S bulked F_2 progeny and RILs were screened (Fig. 2). Twenty-eight SSR markers located on 12 cotton chromosomes were informative for FOV race 4 resistance: five chromosomes from the At-subgenome (3, 5, 6, 8, and 11) and seven from the Dt-subgenome (14, 15, 16, 17, 19, 20, and 25). These SSRs showed different alleles or amplicons associated with assayed R or S genotypes. Eleven of the 28 informative SSRs detected by BSA were further confirmed to be involved in FOV race 4 resistance using the QTL analysis approach (see QTL mapping below).

One thousand and fifty SSR markers that provided wide genome coverage (Park et al. 2005; Frelichowski et al. 2006;

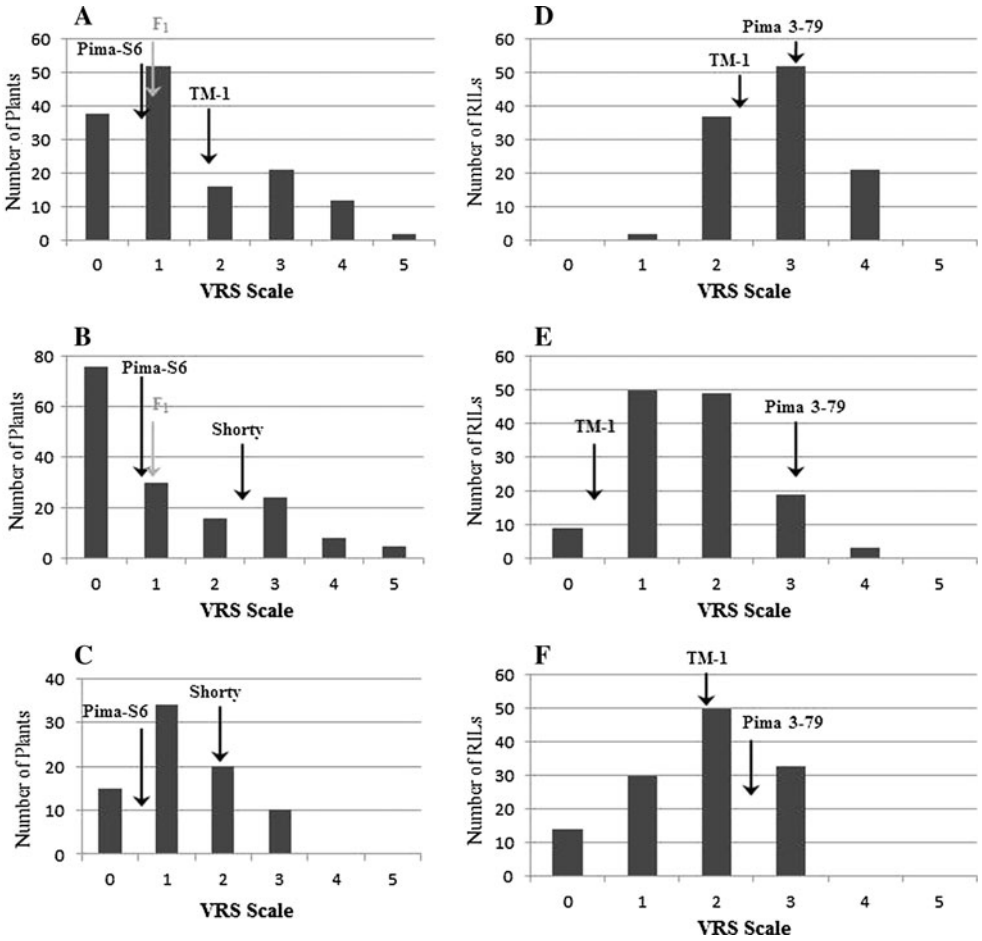

Fig. 1 Distribution of vascular stem and root staining (VRS) symptoms caused by Fusarium wilt (FOV race 4) in interspecific F_2 (**a**–**c**) and RIL populations (**d**–**f**) screened in three greenhouse [**c** (UC Kearney, 2010), **e** (UC Kearney 2008), and **f** (UC Davis, 2007)] and three field experiments [**a** and **b** (Kern Field site, 2010) and **d** (Kern Field site, 2007)]. VRS: 0–5 scale; 0, no symptoms; and 5, plant dead. *Y* axis, number of individuals (F_2 or RIL lines); and *X* axis, VRS scale

Ulloa et al. 2008; CMD, http://www.cottonmarker.org) were tested on the RIL (Upland TM-1 × Pima 3-79) population for association with FOV race 4 resistance. Linkage analyses on the RILs used DNA isolated from FOV race 4-phenotyped plants (VRS) from the experiment in 2008 at UCK, together with a previously developed genetic linkage map (Frelichowski et al. 2006; Ulloa et al. 2008). Twenty-three linkage groups from the 26 cotton chromosomes were developed. Four linkage groups on four chromosomes (8, 14, 19, and 25) were selected from the 12 chromosomes that were involved in FOV race 4 resistance (see below for QTL analyses) (Fig. 3). The linkage groups were developed with LOD >6 to obtain strong linkage between two anchored markers on chromosomes 8, 19, and 25. However, LOD thresholds of 3 and 4 could only be used for the chromosome 14 region.

Quantitative trait loci mapping for Fusarium race 4 resistance

The nonparametric mapping test analogous to one-way analysis of variance (Van Ooijen 2004) revealed 60 SSR markers from twelve chromosomes (3, 5, 6, 8, 11, 14, 15, 16, 17, 19, 20, and 25) associated with FOV race 4 resistance. Based on DSI or VRS ratings, the 60 SSRs were associated with race 4 resistance in at least two different mapping populations (Table 2), using F_2 or RIL genotyped data from FOV race 4-phenotyped plants ($P < 0.05$). Fifty-five of the 60 markers were also detected using a previously developed genetic linkage map (Ulloa et al. 2008) and phenotypes based on DSI and VRS of TM-1 × Pima 3-79 RIL Pop1a–c (Table 2, complete marker data not shown). We selected 11 of the 60 SSRs associated with FOV race 4 resistance; these 11 SSRs amplified alleles from six chromosomal regions (3, 6, 8, 14, 17, and 25) (Table 4). Table 4 presents selected SSR markers associated with FOV race 4 resistance on three mapping populations [RIL TM-1 × Pima 3-79 (Pop 1a–c), F_2 TM-1 × Pima-S6 (Pop 2), and Shorty × Pima-S6 (Pop 3)]. Based on DSI and VRS ratings, two SSRs (BNL0834 and MUSS354) were associated with FOV race 4 resistance in the three mapping populations, Pop1a–b, Pop 2, and Pop 3. Alleles for FOV race 4 resistance were contributed by the tolerant Upland TM-1 parent in Pop 1a-b and by the resistant Pima-S6 parent in Pop 2 and Pop 3 (Table 4). Most of the progeny with homozygous alleles from the resistant parents (Pima-S6 and Upland TM-1) were more

Table 4 Single marker-QTLs associated with disease severity index (DSI) of leaves and vascular stem and root staining (VRS) of Fusarium wilt (FOV race 4) in an interspecific recombinant inbred line (RIL) and two F_2 mapping populations from two greenhouse and two field tests (data detected by nonparametric QTL mapping)

Locus	Chromosome	TM-1	Heterozygote	Pima 3–79	K[a]	*df*	*P* value[‡]
BNL226[b]	3	1.64		0.94	10.55	1	****
MUSB0780_146/141[c]	8	1.41		2.06	7.66	1	***
BNL0834[b]	14	1.12		1.86	15.26	1	****
BNL0834_150/143[c]	14	1.08		1.54	8.52		****
MUSS354_403/397[c]	14	1.21		1.84	11.22	1	****
JESPR224_111/104[d]	25	2.19		2.74	15.98	1	****
Locus	Chromosome	TM-1	Heterozygote	Pima-S6	K[a]	*df*	
MUSB0639_339 h[e]	3	1.78		0.69	4.30	1	**
BNL0834_143/150[e]	14	2.53	1.38	0.93	9.90	2	***
MUSS354_397[e]	14	1.91		0.59	9.90	1	****
JESPR101_124/135[e]	17	1.62	2.08	0.66	7.60	2	**
Gh537_165/174[e]	25	1.80	1.93	0.79	6.56	2	**
Locus	Chromosome	Shorty	Heterozygote	Pima-S6	K[a]	*df*	
NAU2714_176/173[f]	6	2.10	0.93	1.38	8.23	2	**
MUSB0780_132/137[f]	8	1.42	1.40	0.62	4.67	2	** (***)
BNL0834_143/150[f]	14	1.95	1.27	0.61	8.61	2	**
MUSS354_397[f]	14	1.65		0.80	9.53	1	****
BNL2496_129/112[f]	17	1.65	1.21	0.63	6.20	2	* (**)

VRS scale: 0 = no vascular root staining evident to 5 = plant severely damaged or plant death

[a] Kruskal–Wallis analysis (K*) test regarded as the nonparametric equivalent of the one-way analysis of variance (Van Ooijen, 2004)

[b] QTL SSR markers associated with responses to FOV race 4 using recorded DSI on the TM-1 (a—allele) × Pima 3–79 (b—allele) RIL mapping population from a greenhouse evaluation conducted in 2007 at the University of California Davis, CA

[c] QTL SSR marker associated with responses to FOV race 4 using recorded VRS on the RIL Upland TM-1 × Pima 3-79, mapping population from a greenhouse evaluation conducted in 2008 at the University of California Kearney Research, Parlier, CA

[d] QTL SSR markers associated with responses to FOV race 4 using recorded VRS on the TM-1 × Pima-S6 F_2 mapping population from a field evaluation conducted in 2007 on a site infested with FOV race 4 at the Kern County field of CA

[e] QTL SSR markers associated with responses to FOV race 4 using recorded DSI or VRS on the TM-1 × Pima-S6 F_2 mapping population from a field evaluation conducted in 2010 on a site infested with FOV race 4 at the Kern County field of CA

[f] QTL SSR markers associated with responses to FOV race 4 using recorded VRS or DSI on the Shorty × Pima-S6 F_2 mapping population from a field evaluation conducted in 2010 on a site infested with FOV race 4 at the Kern County field of CA

[‡] *P* values are designated as $*P < 0.1$, $**P < 0.05$, and $***P < 0.01$

resistant (lower VRS index) than those with homozygous alleles from the susceptible parents (Pima 3–79 and Upland Shorty) based on the SSR markers on the six chromosomes (Table 4).

A major QTL was also detected between SSRs BNL0834 and MUSS354 on chromosome 14. This major QTL was also identified and validated by interval mapping and multiple-QTL model analyses on the RIL Pop 1a–1b, F_2 Pop 2, and F_2 Pop 3 mapping populations, using VRS or DSI ratings from the various experiments. In Pop1b, this QTL explained up to 18 % of VRS variation and had an additive effect of 0.36 (LOD 3.0, VRS, UCK 2008 experiment). In Pop 2, the QTL explained up to 80 % of VRS variation and had an additive effect of 1.58 (LOD 5.0, VRS, FKCA 2010 experiment). In Pop 3, it explained up to 76 % of DSI variation and had an additive effect of 1.10 (LOD 11.0, DSI, FKCA 2010 experiment) (Fig. 3). This genomic region which marked the QTL *Fov4-C14₁* made the biggest contribution to the FOV race 4 resistance phenotype in the F_2 plants. F_2 plants carrying alleles from Upland TM-1 were observed with a VRS index ranging from 1.0 to 3.5, indicating that TM-1 carried alleles with minor gene effect when it was crossed with susceptible genotypes. In contrast, F_2 plants carrying alleles from Pima-S6 were observed with a VRS index ranging from 0.3 to 0.8, indicating that Pima-S6 carried alleles with major gene effect contributing to the FOV race 4 resistance in progeny when it was crossed with susceptible genotypes.

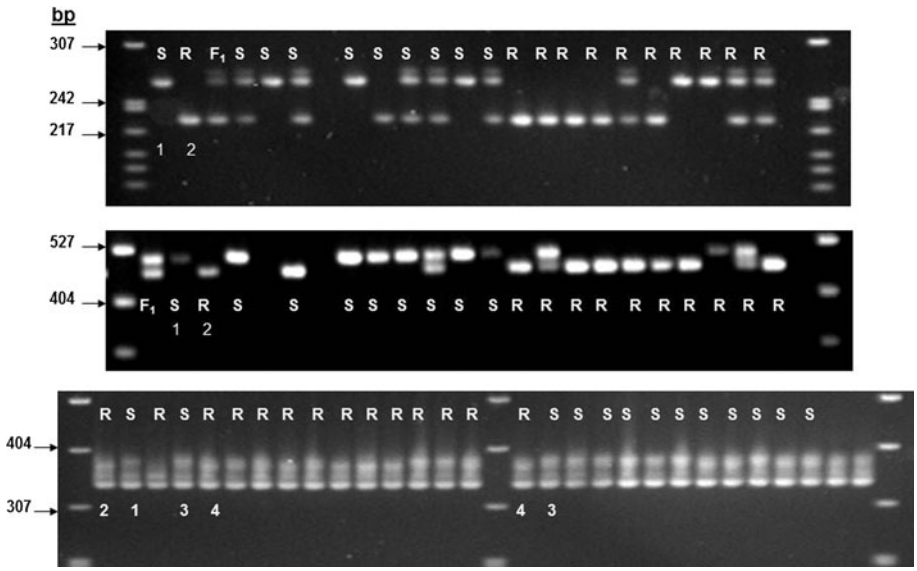

Fig. 2 Three microsatellites or SSR markers evaluated on susceptible (S) Upland Shorty [No. 1, (*Gossypium hirsutum* L.)]; resistant (R) Pima-S6 [No. 2, (*G. barbadense* L.)], S (No. 3) and R (No. 4) bulks of 10 F_2 (Shorty × Pima-S6) cotton plants, and S and R F_2 (Shorty × Pima-S6) single plants

Additional QTLs with minor effect were detected in at least two populations. In Pop 1c, a QTL was detected near SSR JESPR224 (LOD 4.0, VRS, FKCA 2007 experiment) on chromosome 25 explaining up to 15 % of VRS variation and having an additive effect of 0.28. In Pop 2, a QTL was detected near MUSB1155 (LOD 4.0, VRS, FKCA 2010 experiment) chromosome 19 explaining up to 72 % of VRS variation. This QTL had a dominant effect of 1.80 with contribution of resistance from alleles of both parents in this specific population (Table S1).

Common QTLs with minor effect (LOD <3.0) were detected in two mapping populations (Table 4) based on DSI and VRS data sets corresponding to the various experiments. Figure 3 presents four chromosomes (8, 14, 19, and 25) where QTLs with high LOD and with minor effect were detected explaining from 5 to 19 % of the DSI or VRS variation. Using the DSI and VRS data sets corresponding to the various experiments from Pop 1a–c, Pop 2, and Pop 3 (Table 2), QTLs were detected on the same locus in two of three populations (Fig. 3) or on the same chromosome in different locations of these populations.

Discussion

Gene action in cotton governing FOV race 4 resistance was studied by a comprehensive approach of combining conventional inheritance, and genetic and QTL mapping with molecular markers. Initially, a single resistance gene (*Fov4*) model was observed in F_2 populations based on inheritance of phenotypes. This single *Fov4* gene was observed with a major dominant gene action and conferred resistance to FOV race 4 in Pima-S6. The *Fov4* gene appears to be located near a genomic region on chromosome 14 marked with QTL *Fov4*$_1$*-C14*, which made the biggest contribution to FOV race 4 resistance in generated F_2 progeny based on disease severity index (DSI) of leaves or VRS index. Additional comprehensive genetic and QTL analyses yielded a selected set of 11 SSR markers. These SSR markers indicated the involvement of more than one gene and gene interactions in the inheritance of FOV race 4 resistance across six chromosomes (3, 6, 8, 14, 17, and 25). Common QTLs with minor effect were also detected in these populations explaining 5–19 % of the DSI or VRS variation. The SSR markers for the resistance QTLs reported herein have important application for breeding FOV race 4 resistance into elite cultivars by MAS during the breeding process. Our study provided the first comprehensive results and insight into FOV race 4 resistance that will help speed up the development of resistant cultivars and combat this pathogen in the SJV of California.

Previous genetic resistance studies have indicated that FOV resistance is under the control of one or two major genes with complete to incomplete dominance (Smith and Dick 1960; Mohamed 1963; Wang and Roberts 2006; Ulloa et al. 2006; 2011). Herein, we report a major gene (*Fov4*) with dominant gene effect for resistance to FOV race 4 in Pima-S6 (Tables 1, 3, 4). However, a wide range of DSI and VRS infection indices was also observed on progeny across tested F_2 and RIL populations. In the RIL population (TM-1 × Pima 3-79), Upland TM-1 alleles provided FOV resistance (lower VRS index), but a second FOV resistance gene was not detected in the F_2 TM-1 × Pima-S6 population using the VRS index. It is possible that the Upland TM-1 gene(s) provides minor effect for FOV race 4 resistance or in the F_2 population the Upland TM-1 gene(s) is masked by the strong dominant *Fov4* gene effect from Pima-S6 when VRS or DSI scales are used. In

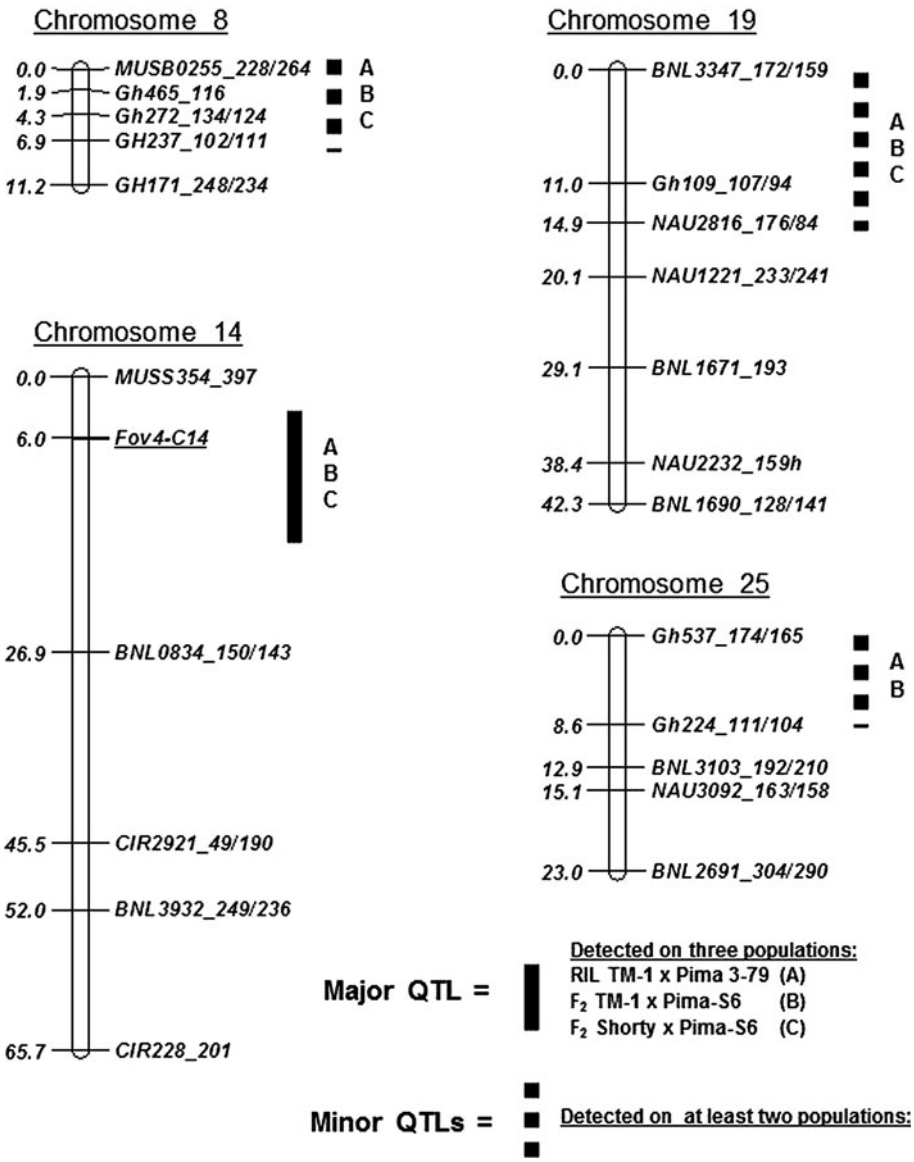

Fig. 3 Linkage maps of four chromosome regions (8, 14, 19, and 25) showing relationships between molecular markers and QTLs for Fusarium wilt (FOV race 4) resistance in three mapping populations

addition, different inoculum levels of the fungus in the soil (and greenhouse vs. field tests, Fig. 1) or differences in environmental conditions during assays may have prevented detection or expression of these minor gene-effects. Based on tested progeny, the current study indicates the expression of additional minor genes under different evaluation conditions when using cultivars or progeny of phenotypes with different genetic backgrounds.

Symptom severity varied in different experiments, especially for heterozygous progeny. Resistance segregation in the Pima-S7 × Pima-S6, Upland TM-1 × Pima-S6, and Upland Shorty × Pima-S6 F_1 and F_2 populations, and Upland TM-1 × Pima 3-39 RIL population by the generation means and distributions (Tables 1, 2, 3; Fig. 1) might also indicate that allelic interaction and epistasis operated in these crosses. We observed that additional alleles from moderately-tolerant Upland TM-1, and susceptible Upland Shorty and Pima 3–79 parents increased FOV resistance in progeny. These results support previous studies in which highly resistant transgressive segregants were observed in progeny derived from crosses between parents Upland Wild Mexican Jack Jones, Upland TM-1, and Pima-S7, which had moderate to susceptible resistance response to RKN (Shepherd 1974; McPherson et al. 2004; Wang et al. 2008; Ulloa et al. 2010a; Wang et al. 2012) and Fusarium wilt race 1 (Ulloa et al. 2011). In the current study, parents may have contributed one or more genes that enhanced resistance levels in progeny. Progeny from these parents should be useful for breeding cultivars with strong levels of resistance.

Greenhouse and field evaluations have revealed interactions in disease response between cultivars and breeding

lines, FOV races, and evaluation sites (greenhouse vs. infested field) (Wang et al. 2009; Ulloa et al. 2010b, 2011). These interactions in disease response may be the result of different gene expression under different environmental conditions (Rebouillat et al. 2009). In the SJV of California, some Upland cotton cultivars and germplasm lines appeared "tolerant" to FOV race 4, in terms of good growth rate, lack of visual DSI symptoms, and high percent survival rate. However, many of these Upland cultivars and germplasm have been severely infected by race 4 (Hutmacher et al. 2011). Overall, Pima cottons in the SJV of California are more susceptible to FOV race 4 than Acala or Upland cottons (Ulloa et al. 2006).

The modern allotetraploid Acala, Upland, and Pima cottons are the products of a presumed single polyploidization event between the At-subgenome (chromosomes 1–13) and Dt-subgenome (chromosomes 14–17) diploids that occurred around 1–2 million years ago (Wendel and Cronn 2003). There are 26 disomic pairing gametic chromosomes. During paring exchange of genomic regions occurred in these chromosomes. In this study, a major QTL (SSRs BNL0834 and MUSS354) was detected for FOV race 4 on chromosome 14 based on phenotypes of interspecific (*G. hirsutum* × *G. barbadense*) progeny, while for FOV race 7 a major QTL (JESPR304 and CIR35) was reported on chromosome 17 based on intraspecific (*G. hirsutum* × *G. hirsutum*) progeny (Wang et al. 2009). Both genes (*Fov4* and FW^R, respectively) share the same dominance-inheritance pattern. Pathogenicity tests have shown race 7 from China to be different from race 4 of India, with race 4 being more pathogenic to Pima cottons than Upland cottons (Armstrong and Armstrong 1960). However, races 4 and 7 were indistinguishable genetically using EF-1α and mtSSU rDNA sequence data (Skovgaard et al. 2001). More research is needed to resolve the conflict between pathogenicity tests and genetic studies for distinguishing these two races.

Herein, we have proposed that major genes or gene forms are harbored on chromosome 14 and 17, one acting upon Upland genetic backgrounds (FW^R, Wang et al. 2009) and one acting upon genetic backgrounds of Pima and Upland × Pima interspecific progeny (*Fov4*, reported herein; Table 4). These genes may not be allelic and may reside at different loci or on different chromosomes. To determine the chromosome location of SSRs JESPR304 and CIR35, we used our existing mapping populations and placed JESPR304 on chromosome 2. A similar location for the JESPR304 marker on chromosome 2 was also reported by Yu et al. (2011) and Yu et al. (2012). If races 4 and 7 are genetically similar based on DNA sequence data (Skovgaard et al. 2001), but they have a different pathogenic mode of action on Upland and Pima cottons (Armstrong and Armstrong 1960), further investigation may be needed to resolve the discrepancy in chromosome-position of these R genes (Wang et al. 2009; in this study).

The present study, in combination with earlier reports, confirms that race specificity occurs in *F. oxysporum* f. sp. *vasinfectum*, a condition typically associated with major R gene-based qualitative resistance. These results support that different genes or QTLs on different chromosomes confer FOV resistance to each race. Several major gene-QTLs are involved in FOV resistance: in race 1 ($Fov1\text{-}C16_1$ - chromosome 16, Ulloa at el. 2011), race 4 ($Fov4\text{-}C14_1$-chromosome 14, reported herein), race 7 (FW^R—chromosome 17, Wang et al. 2009), and Australian races [several chromosomes (6, 22, and 25), Becerra et al. 2012]. It is interesting to note that FOV resistance genes may be provided by the D genome [chromosomes 14 and 17 (races 4 and 7), chromosome 16 (race 1), and chromosomes 22 and 25 (Australian races] (Wang et al. 2009; Ulloa et al. 2011; Becerra et al. 2012), while nematode resistance genes may be contributed by the A genome (chromosome 11; Roberts and Ulloa 2010).

Additional QTL analyses revealed that cotton genotypes and progeny share quantitative resistance genes for plant defense against FOV races (1, 4, 7, and Australians) (Wang et al. 2009; Ulloa et al. 2011; Becerra et al. 2012) and other biotrophic pathogens such as RKN (Ulloa et al. 2010a). In a recent FOV race 1 study, Ulloa et al. (2011) reported gene annotations from marker-DNA, new DNA sequences of BAC clones, and QTLs associated with FOV resistance. These annotations revealed that defense genes were detected upon pathogen infection. In the current study, some of the same genes associated with race 1 resistance were associated with FOV race 4 resistance based on the marker-DNA sequence annotations (Ulloa et al. 2011). Chromosomes 6, 8, 11, 16, and 19 appear to share common genes or genome regions for FOV plant defense response (e.g., NAU2716, MUSB0780, MUSB0827, BNL 0580, and Gh109, respectively). In addition, QTLs have been identified in similar genome regions [chromosomes 6 and 25 (races 1, 4, and Australians)], which contribute to FOV resistance from Pima-S6 and 3–79, and Upland MCU-5 (Ulloa et al. 2011; Becerra et al. 2012; data herein and data not shown from this study). It is possible that some of these genes are constantly involved in host-plant disease resistance, but their expression depends on the genetic background of the tested progeny, and sources of variance in testing conditions, including environmental conditions and pathogen genotype effects. This recent detection of quantitative resistance genes with minor effects will help in developing plant resources for crop improvement (Ulloa et al. 2011; Wang et al. 2012). More research is needed to confirm that detected genes underlying QTLs have a function in resistance to nematodes and FOV races 1, 4, 7, and Australian races.

Herein, we propose naming the QTLs reported in this study following the conventional nomenclature used by Ulloa et al. (2010a, 2011) where the acronym of the scientific name of the pathogen causing the disease is presented in lower case followed by the chromosome number. Moreover, the race number is added after the scientific name of the pathogen, and a number in subscript distinguishes multiple QTLs identified on the same chromosome. The following QTLs that explained from 5–80 % of variation in DSI or VRS are designated here: *Fov4-C03*$_1$ near marker MUSB0639 and *Fov4-C03*$_2$ near marker BNL226 on chromosome 3; *Fov4-C06*$_1$ near marker MUSB0754 on chromosome 6; *Fov4-C08*$_1$ near marker MUSB0780 and *Fov4-C08*$_2$ near marker MUSB0255 on chromosome 8; *Fov4-C11*$_1$ near marker MUSB0827 on chromosome 11; *Fov4-C14*$_1$ between markers BNL0834 and MUSS354 on chromosome 14; *Fov4-C16*$_1$ near marker BNL0580 on chromosome 16; *Fov4-C19*$_1$ between markers JESPR236 and MUSB1155, and *Fov4-C19*$_2$ near marker Gh109 on chromosome 19; and *Fov4-C25*$_1$ near marker JESPR224 on chromosome 25 (Table 4; Fig. 3).

The genome region that marked the QTL *Fov4-C14*$_1$ locus made the largest contribution to the FOV race 4 resistance phenotype contributed by Pima-S6 to the F_2s, explaining 80 % of the variation in DSI or VRS with an additive effect up to 1.58. The second largest contribution was made by the genome region that marked the *Fov4-C19*$_1$ locus (explaining 62 % of the variation in VRS with a dominance effect of 1.80) which was contributed due to heterosis (Pima-S6 and TM-1 to the F_2s). We conclude that these QTLs have a significant role in conferring FOV race 4 resistance in different cotton backgrounds and should be primary targets for cotton breeding using MAS.

Genetic and physical framework mapping in cotton are being used to discover putative gene sequences involved in resistance to FOV, nematodes, and possibly other common soil pathogens (M Ulloa, P. Roberts, C. Wang, C. Saski, unpublished data). For example, chromosome 11 houses resistance to FOV race 1 (Ulloa et al. 2011), FOV race 4 (reported herein), RKN (Wang et al. 2006), reniform nematode (Dighe et al. 2009), and Verticillium wilt (Bolek et al. 2005). Genetic and QTL mapping coupled with physical alignment of genomic regions into chromosomal maps will expedite discovery of R or pathogen-induced R genes underlying QTLs and will offer candidate gene sets for functional analyses of plant defense against Fusarium wilt and other biotrophic pathogens. In addition, this research strategy will provide important information to dissect polygenic forms of disease resistance and to connect genome research with crop improvement.

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