DISCOVERY OF FUSARIUM WILT RACE 4 RESISTANCE IN COTTON

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Abstract

Fusarium wilt [*Fusarium oxysporum f.sp. vasinfectum* (FOV) Atk. Sny & Hans)] 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. The cotton host-specific forms of this fungus are comprised of different genotypes called races. Over the past nine years, FOV race 4 has increasingly impacted cotton fields in California's San Joaquin Valley (SJV) and represents an expanding threat to cotton production. Four parents and their derived populations were used in this study: one intraspecific and two interspecific F_1 and F_2 populations. Inheritance and quantitative trait locus (QTL) mapping analyses identified a single resistance gene (*Fov4*) observed in F_2 populations based on inheritance of phenotypes. *Fov4* 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*). Detected QTL and identified SSR markers will be used to validate FOV resistance on additional mapping populations and FOV race resistance-comparisons. In addition, SSR markers will facilitate marker-assisted selection for the introgression of the *Fov4* gene into improved upland germplasm for public release.

Introduction

Fusarium wilt [*Fusarium oxysporum* f.sp. *vasinfectum* (FOV) Atk. Sny & Hans)] 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. FOV represents an expanding threat to cotton production (Kochman et al., 2002; Kim et al., 2005; Ulloa et al., 2006, 2011; Wang et al., 2009). Integrating disease resistance into high-yielding, high-fiber quality cultivars is one of the most important objectives in cotton (*Gossypium* spp.) breeding programs worldwide (Ulloa et al., 2011).

Until the early 2000's, only FOV races 1 and 2 were known to occur in the United States (Smith et al. 1981; DeVay 1986). Races 1 and 2 of FOV are typically found in sandy or sandy-loam soils with significant root-knot nematode [*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), and since then, 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 in the U.S. before 2003. FOV race 4 has caused extensive disease symptoms in cotton plants grown in clay loam and loam soils in which root-knot nematode 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, 2011; Ulloa et al. 2006).

Worldwide, strains and races of FOV 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). 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 Olivera), Verticillium wilt caused by *Verticillium dahliae* Kleb, or black root rot caused by *Thielaviopsis basicola* [(Berk. and Broome) Ferraris] (Ulloa et al. 2011). In recent inheritance and quantitative trait (QTL) studies (Wang et al., 2009; Ulloa et al., 2011; Becerra et al., 2012), resistance to FOV races 1, 7 and the Australian race was reported to be inherited through gene interactions detected in more than one chromosome. In addition, collectively the results from these studies (Wang et al. 2009; Ulloa et al. 201; Becerra et al. 2012) suggested a different gene-specificity of FOV resistance in cotton [*Fov1* (race 1) - chromosome 16, *Fov4* (race 4) – chromosome 14 (Ulloa et al. 2013), FW^R (race 7) - chromosome 17, and FOV Australian race – chromosomes 6, 22, and 25] (Ulloa et al. 2011, 2013).

In this study, we investigated the inheritance of FOV race 4 resistance, detected genomic regions associated with resistance to FOV race 4 using genetic and QTL mapping, and identified molecular markers associated with resistance to FOV. This information will help to unveil the genes involved in the pathogenicity mechanism of FOV resistance and to develop a productive resource for crop improvement and protection through marker assisted selection (MAS).

Material and Methods

Plant material

Plant genotypes used in 2008 at the University of California Kearney Research and Extension Center (UCK, Parlier, CA) greenhouse evaluation and in 2010 at a field site naturally infested with FOV race 4 in Kern County, CA (FKCA) included FOV race 4 susceptible *G. hirsutum* Upland Shorty, moderately tolerant Upland TM-1 cultivar, resistant *G. barbadense* Pima-S6, and susceptible Pima-S7. These cultivars and germplasm were used as parents to develop F_1 and F_2 populations (Table 1). In 2010 at FKCA, we used two F_2 populations - TM-1 x Pima-S6 (63 individuals) and Shorty x Pima-S6 (84 individuals) for QTL analyses (see below).

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 2008 at UCK, Parlier, CA. The isolate originated from a naturally infested field in the San Joaquin Valley 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.

Table 1. 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) with Upland mid-tolerant TM-1, susceptible Pima-S7, and Upland Shorty (S).

Genotype/ Population	No. Plants	Expected Ratios R:S[a]	Observed Ratio R:S	X^2	*P*-value
Parents					
[b]Upland TM-1	30	All R	(18) R:S (12)		
[c]Upland Shorty	30	All S	(13) R:S (17)		
[b]Pima-S6	10	All R	All R		
[c]Pima-S7	30	All S	(1) R:S (29)		
[c]Pima-S6	30	All R	(28) R:S (2)		
F_1					
[c]Pima-S7 x Pima-S6	30	All R	(27) R:S (3)		
[c]TM-1 x Pima-S6	30	All R	(29) R:S (1)		
[c]Shorty x Pima-S6	30	All R	All R		
F_2					
[c]Pima-S7 x Pima-S6	170	128:42 (3:1) [§]	109:61	11.41	***
[c]TM-1 x Pima-S6	141	105:36 (3:1) [§]	105:36	0.00	NS
[c]Shorty x Pima-S6	159	122:39 (3:1) [§]	122:37	0.10	NS

[a] Expected R:S, number of plants for vascular root stain VRS < 2 as resistant (R) : VRS 2 ≥ as susceptible (S).
[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 in Kern County, CA.
[†]NS = no significant at $X^2_{0.05}$, *significant at > $X^2_{0.05}$, **significant at > $X^2_{0.025}$, ***significant > $X^2_{0.005}$.
[§]One gene model.

Fusarium wilt (FOV) race 4 assays

A root dip method was used in all FOV race 4 greenhouse evaluations. Parents, F_1, and F_2 populations were seeded into a composite medium of vermiculite and peat moss prior to inoculation. When the plants were 3 wk 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 two minutes in a spore suspension of 1×10^5 conidia per ml of water. Seedlings were then transplanted into 6 x 15 cm (500 ml) box pots with one plant in each pot (Ulloa et al. 2006; 2009).

Symptoms of FOV race 4 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). DSI was based on the following scale: 0 = no symptoms; 1 = epinasty of leaves; 2 = 1 to 30% of leaves chlorotic and wilted; 3 = 31 to 80% of leaves chlorotic and wilted; 4 = 81 to 100% of leaves chlorotic and wilted; and 5 = plant death. Also, the upper part of the primary root was cut longitudinally and evaluated for vascular root staining (VRS) at the end of the experiment. The following scale was used for VRS: 0 = no vascular root staining evident, 1 = light vascular root staining 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 days after inoculation (dai), and in 2010, at 31 dai for DSI and VRS to determine disease incidence and severity. In 2010, the F_2 populations 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 race 4 FOV.

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: www. cottonmarker.org) in the various experiments.

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 2µL of DNA template (concentration 10 ng/µL), 0.1 µM each of forward and reverse primers, 1X 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) with a cycling profile of 1 cycle of 10 min at 94°C; 10 cycles of 15 sec at 94°C, 30 sec at 60°C (step -0.5°C/cycle for cycles 2-10), and 1min at 72°C; 35 cycles of 15 sec at 94°C, 30 sec 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 to 5 hrs on a 3% super fine resolution (SFR™) agarose gel (Amresco, Solon, OH) containing 1X TBE at 90 volts, and were visualized by Alphaimager software (v. 5.5, Alpha Innotech Corporation, San Leandro, CA) after staining with ethidium bromide.

Data analyses

Assayed plants from UCK and FKCA 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.

Differences among observed DSI (or VRS) within study entries (parents, and F_1s), 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).

Phenotypic segregation ratios of resistant (R) and susceptible (S) plants were evaluated for F_2 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).

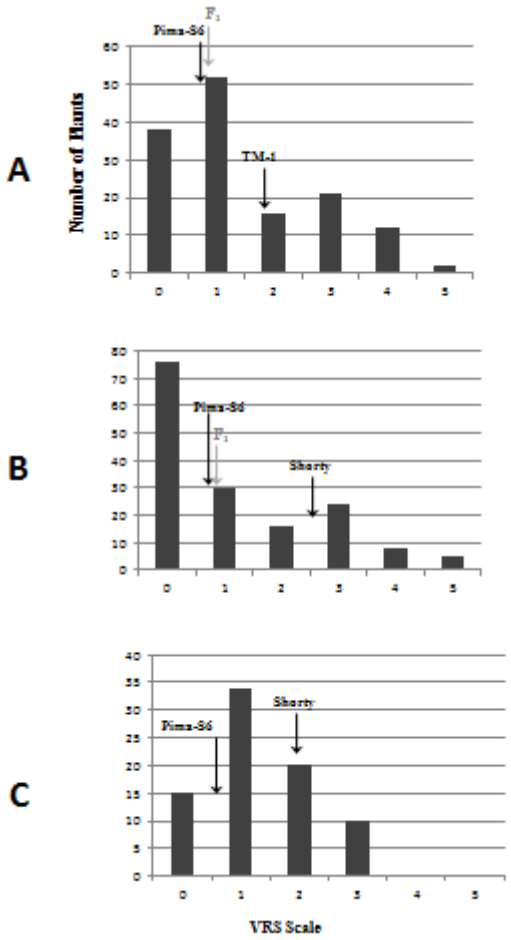

Figure 1. Distribution of vascular stem and root staining (VRS) symptoms caused by Fusarium wilt (FOV race 4) in interspecific F_2 populations screened in two field experiments [A, B (Kern Co. field site, 2010)] and one greenhouse experiment [C (UC Kearney, 2010)]. VRS: 0 to 5 scale; 0 = no symptoms, and 5 = plant dead. Y axis = number of individuals (F_2 or RIL lines) and X axis = VRS scale. Mean values for parents and F_1 included in the same test indicated by arrows.

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 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 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 populations. Likelihood ratio (LOD) scores of 3 to 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.

Results

Evaluation of Fusarium wilt (FOV) Resistance

In the naturally infested field, the vascular root staining (VRS) index of the intraspecific Pima-S7 x 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). Pima cottons were more susceptible to FOV than Upland/Acala cottons. However, a Pima germplasm, Pima-S6, highly resistant to FOV race 4 was identified in both 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 1998) in the 2010 experiment at FKCA. F_{1s} from Upland Shorty x Pima-S6 and Upland TM-1 x Pima-S6 had VRS index (0.9) similar 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 (Table 1 and Fig. 1).

Genetic analysis of FOV race 4 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 and Pima-S7) parents for FOV race 4 infection. We classified as R and 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 R and S parents. Modifications in gene action were observed based on VRS index ratings. The action of resistance genes ranged from dominant to recessive when R and S parents were crossed. Interspecific F_1 (*G. hirsutum* x *G. barbadense*) populations showed dominant gene expression (Table 1).

Based on inheritance of phenotypes, a one-gene model for resistance to FOV race 4 was investigated in parents, F_1, and F_2 populations (Table 1). The segregation of two F_2 populations (TM-1 x Pima-S6, and Shorty x Pima-S6) fit the 3(R):1(S) expected distribution for a single gene determining resistance ($X^2 = 0.00$, and $X^2 = 0.10$, respectively, Table 1). This single gene (*Fov4*) was observed with a major dominant gene action, and was provided by Pima-S6 in crosses with susceptible Upland Shorty or moderately tolerant Upland TM-1 parents (Table 1). However, distorted Mendelian ratios were observed for a single gene model based on VRS ratings from an experiment on the F_2 Pima-S7 x Pima-S6 population. Some progeny from the F_2 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 may carry two or more minor genes with one or more coming from each parent (Fig. 1).

Table 2. 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 F_2 mapping populations from two field tests (data detected by nonparametric QTL mapping).

Locus	Chromosome	TM-1	Heterozygote	Pima-S6	K†	Df	*P - Value*‡
§BNL0834_143/150	14	2.53	1.38	0.93	9.9	2	***
§MUSS354_397	14	1.91		0.59	9.9	1	****
Locus	**Chromosome**	**Shorty**	**Heterozygote**	**Pima-S6**			
††BNL0834_143/150	14	1.95	1.27	0.61	8.61	2	**
††MUSS354_397	14	1.65		0.8	9.53	1	****

†Kruskal-Wallis analysis (K*) test regarded as the nonparametric equivalent of the one-way analysis of variance (Van Ooijen, 2004).
‡*P*-values are designated as *$P < 0.1$,** $P < 0.05$, and *** $P < 0.01$.
§ QTL SSR markers associated with responses to FOV race 4 using recorded DSI or VRS on the TM-1 x 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, CA field.
††QTL SSR markers associated with responses to FOV race 4 using recorded VRS or DSI on the Shorty x 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, CA field.
VRS scale: 0 = no vascular root staining evident to 5 = plant severely damaged or plant death.

Genetic and QTL mapping of FOV race 4 resistance

One thousand and fifty SSR markers that provided wide genome coverage (Park et al. 2005; Frelichowski et al., 2006; Ulloa et al., 2008; CMD, www. cottonmarker.org) were previously used on a recombinant inbred line [RIL (Upland TM-1 x Pima 3-79)] population to develop twenty-three linkage groups from the 26 cotton chromosomes (Frelichowski et al., 2006; Ulloa et al., 2008, 2011). Of these, SSR markers from four linkage groups on four chromosomes (8, 14, 19, and 25) were selected, which were involved in FOV race 4 resistance (Ulloa et al., 2013). 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 (QTL) mapping for Fusarium race 4 resistance

The nonparametric mapping test analogous to one-way analysis of variance (Van Ooijen 2004) revealed SSR markers from four chromosomes (8, 14, 19, and 25) associated with FOV race 4 resistance (Fig. 2), using F_2 genotype data from FOV race 4-phenotyped plants ($P < 0.05$). Table 2 presents selected SSR markers associated with FOV race 4 resistance on two mapping populations (F_2 TM-1 x Pima-S6 and F_2 Shorty x Pima-S6). Based on DSI and VRS ratings, two SSRs (BNL0834 and MUSS354) were associated with FOV race 4 resistance on these mapping populations. Most of the progeny with homozygous alleles from the resistant parents (Pima-S6) were more resistant (lower VRS index) than those with homozygous alleles from the Upland TM-1 and susceptible Upland Shorty parents (Table 2).

A major QTL was also identified and validated by interval mapping and multiple-QTL model analyses on these F_2 Populations, using VRS or DSI ratings from the various experiments. This major QTL was detected between SSRs BNL0834 and MUSS354 on chromosome 14. In the F_2 TM-1 x Pima-S6, the QTL explained up to 80 % of VRS variation and had an additive effect of 1.58. In the F_2 Shorty x Pima-S6, it explained up to 76 % of DSI variation and had an additive effect of 1.10. 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. This genomic region which marked the QTL *$Fov4_1$-C14* made the biggest contribution to the FOV race 4 resistance phenotype in the F_2 plants.

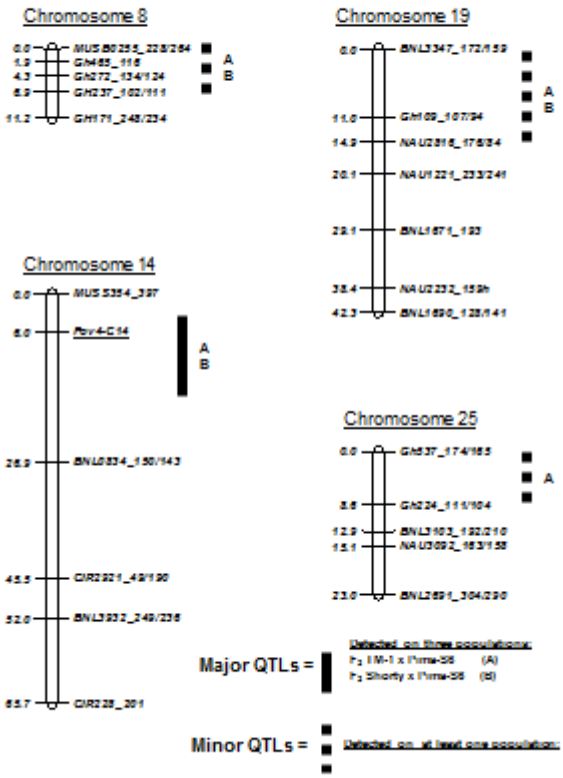

Figure 2. Linkage maps of four chromosome regions (8, 14, 19, and 25) showing relationships between molecular markers and QTLs for Fusarium wilt (FOV race 4) resistance in three mapping populations.

Common QTLs with minor effect (LOD < 3.0) were also detected in these two mapping populations (Fig. 2) based on DSI and VRS data sets corresponding to the various experiments. Figure 2 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. QTLs were detected on the same locus or in the same vicinity of the two populations (Fig. 2).

Discussion

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 vascular root staining (VRS) index. Additional comprehensive genetic and QTL analyses yielded SSR markers which indicated the involvement of additional minor genes in the inheritance of FOV race 4 resistance across four chromosomes (8, 14, 19, and 25). QTLs with minor effect were detected explaining 5% - 19 % of the DSI or VRS variation. The SSR markers for the resistance QTLs reported herein may have important application for breeding FOV race 4 resistance into elite cultivars by marker-assisted selection (MAS).

A wide range of DSI and VRS infection indices was observed on progeny across tested F_2 populations. Resistance segregation in the Pima-S7 x Pima-S6, Upland TM-1 x Pima-S6, and Upland Shorty x Pima-S6 F_1 and F_2 populations (Table 1 and 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 parents

increased FOV resistance in some progeny (data not shown). 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, Pima 3-79 and Pima-S7, which had moderate to susceptible resistance response to root-knot nematode (RKN) (Shepherd 1974; McPherson et al. 2004; Wang et al. 2008; Ulloa et al. 2010; Wang et al. 2012) and Fusarium wilt race 1 (Ulloa et al. 2011). Progeny from these parents should be useful for breeding cultivars with strong levels of resistance.

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$_1$-C16* - chromosome 16, Ulloa at el. 2011), race 4 (*Fov4$_1$-C14* - chromosome 14, reported herein; Ulloa et al., 2013), race 7 (*FW^R* - chromosome 17, Wang et al. 2009), and the Australian race [several 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 and *FW^R* - chromosome 17 – Australian *Fov* – chromosomes (6, 22, and 25)].

The genome region that marked the QTL *Fov4$_1$-C14* 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$_1$-C19* locus (explaining 62 % of the variation in VRS with a dominance effect of 1.80) which was contributed by heterosis effect (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.

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