Identification of Resistance to *Fusarium oxysporum* f. sp. *niveum* Race 2 in *Citrullus lanatus* var. *citroides* Plant Introductions

W. Patrick Wechter¹, Chandrasekar Kousik, Melanie McMillan, and Amnon Levi

U.S. Vegetable Laboratory, United States Department of Agriculture–Agricultural Research Service, 2700 Savannah Highway, Charleston, SC, 29414

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Abstract. *Fusarium* wilt (FW) is a major disease of watermelon in North America and around the world. Control of this disease is difficult because the soilborne causal agent *Fusarium oxysporum* f. sp. *niveum* (*Fon*) produces chlamydospores that remain infectious in the soil for many years. Although various levels of resistance to *Fon* Races 0 and 1 exist in watermelon cultivars, no resistance to Race 2 or 3 has been reported. In this study, we used seed and seedling inoculation procedures to screen 110 U.S. PIs of wild watermelon (*Citrullus lanatus* var. *citroides*) for resistance to Race 2 FW. Of these 110 accessions, 15 showed significantly higher resistance to *Fon* Race 2 than that found in the watermelon cultivars Sugar Baby or Charleston Grey as well as in the *C. lanatus* var. *citroides* PI 296341 that was reported to contain resistance to FW. PI 271769, another *C. lanatus* var. *citroides* that was previously reported as containing resistance to FW, is among the 15 resistant accessions described here. These 15 accessions are potential sources for resistance to Race 2 FW in watermelon breeding.

Fusarium wilt [caused by *Fusarium oxysporum* Schlechtend.:Fr. f. sp. *niveum* (E.F. Sm.) W.C. Snyder & H.N. Hans (*Fon*)] is one of the most serious soilborne diseases of watermelon (Martyn, 1985, 1987; Netzer, 1976). Initial symptoms can vary with chlorosis of leaves being common followed by asymmetrical wilting for the plant. When infection takes place in seedlings, noticeable stunting of the plant occurs followed by chlorosis and death. Necrosis of the stem, in the form of brown streaks in the vascular system produced by both the fungal exudates as well as the dying plant tissues, are characteristic of FW. Although the formae speciales (f. sp.) designation implies host specificity at the species level, there are numerous reports of cross-species and cross-genus level pathogenic interactions in f. sp. *niveum* as well as other *Fusarium* isolates collected from different cucurbits in laboratory studies (Bouhot, 1981; Owens, 1955, 1956; Zhou and Everts, 2003, 2007). In addition, considerable variability in virulence and vegetative compatibility occurs among isolates within this formae speciales (Larkin et al., 1990; Zhou

and Everts, 2007). Currently, there are four described pathogenic Races, 0, 1, 2, and 3 (Martyn and Netzer, 1991; Netzer, 1976; Netzer and Martyn, 1989; Zhou et al., 2010). Resistance to *Fon* Races 0 and 1, but not to Races 2 or 3, was incorporated into various commercial watermelon cultivars. The *Fon* Race 2 is considerably more virulent than Race 0 or 1 and infects all watermelon cultivars. Apart from being a soilborne pathogen, *Fon* is also a seedborne pathogen (Boughalleb and El Mahjoub, 2007; Michail et al., 2002). It can rapidly spread in watermelon production areas and has been reported to reduce yield by 40% in watermelon fields in the southeastern regions of the United States (Keinath et al., 2010). *Fon* Race 2 has been reported in Texas, Oklahoma, Florida, Maryland, Delaware, Georgia, Indiana, and South Carolina (Bruton et al., 1988, 2008; Bruton and Damicone, 1999; Egel et al., 2005; Keinath and DuBose, 2009; Martyn, 1985; Martyn and Bruton, 1989; Zhou and Everts, 2001, 2003).

Effective control of this disease has been achieved by the use of preplant soil fumigants. Recently, the most economical and effective fumigant, methyl bromide, has been withdrawn from the market, leaving no effective alternatives for control of this fungus with conventional chemicals. *Fon* produces chlamydospores that remain dormant in the soil for many years (Martyn, 1996), and there are no alternative fumigants that are as effective as methyl bromide in controlling these dormant spores. For this reason, standard chemical rotation strategies are not effective

in controlling this soilborne disease. Finding resistant germplasm sources and incorporating the resistance into watermelon cultivars should be the most effective strategy for controlling FW in watermelon.

Two U.S. PIs of *Citrullus lanatus* var. *citroides* (PI 296341 and PI 271769) have been reported as resistant to Race 2 of *Fon* (Dane et al., 1998; Martyn and Netzer, 1991). An improved line of PI 296341, 296341-FR, has since been released by the Texas Agricultural Experiment Station (Martyn and Netzer, 1991). PI 271769 was identified by Dane et al. (1998) as resistant to *Fon* Race 2. A third watermelon PI, which was reported to be highly resistant to *Fon* Race 2 by Boyhan et al. (2003) with a rating of 0.9 from a 9-point rating scale, may actually be *Cucumis melo* var. *melo* according to the Genetic Resources Information Network web site (<http://www.ars-grin.gov>) and as such would not normally be considered a host to this formae speciales of *Fusarium oxysporum*. Each of these screens uses slightly different methods and different isolates of *Fon* Race 2. The agreement among these three surveys is that the majority of watermelon accessions are highly susceptible to this race of the pathogen; unfortunately, these studies do not find unanimity as to the lines identified as resistant. In fact, the accession PI 296341, found to be the most resistant by Netzer and Martyn (1989), was found to be quite susceptible in the other two studies. What does appear evident from these surveys is that there are varying levels of resistance in the watermelon germplasm accessions and that there is distinct heterogeneity for this resistance within the individual accessions. Based on the considerable differences in findings of these three surveys, a new survey was justified that used aggressive inoculation techniques and focused on accessions that exhibited a uniformly high level of resistance or had a high frequency of resistant individual in previously published surveys. The *C. lanatus* var. *citroides* PI collection at the USDA, ARS Plant Genetic Resources and Conservation Unit (PGRCU; Griffin, GA) represents wide genetic diversity and is a valuable source for pest resistance genes for use in watermelon breeding (Jarret et al., 1997; Levi et al., 2001, 2011; Thies and Levi, 2007). The objective of this study was to evaluate the USDA, ARS *C. lanatus* var. *citroides* PI collection for resistance to *Fon* Race 2 (using a seed inoculation procedure in addition to a root inoculation procedure) and identify the most resistant accessions that could be useful for enhancing *Fon* Race 2 resistance in watermelon cultivars.

Materials and Methods

Plant materials. *Citrullus lanatus* var. *citroides* PI accessions were obtained from the PGRCU in Griffin, GA. One hundred ten, of the total 133, accessions in the collection were included in these tests (Table 1). In addition to these accessions, the watermelon cultivars Sugar Baby and Charleston Grey

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¹To whom reprint requests should be addressed; e-mail Pat.Wechter@ars.usda.gov.

Table 1. Mean percent survival of *Citrullus lanatus* var. *citroides* accessions in response to *Fusarium oxysporum* f. sp. *niveum* Race 2 in two greenhouse tests.^z

Accession	Survival ^b (%)		Accession	Survival (%)	Accession	Survival (%)	
PI482246	56.3	a ^x	PI500335	7.1	PI482315	0.0	f
PI482252	56.3	a	PI505604	7.1	PI482321	0.0	f
PI255136	53.6	a	PI532624	7.1	PI482322	0.0	f
PI296335	50.0	a	PI532664	7.1	PI482324	0.0	f
PI482279	50.0	a	PI532668	7.1	PI482326	0.0	f
PI482282	50.0	a	PI532738	7.1	PI482333	0.0	f
PI482331	46.7	ab	PI596659	7.1	PI482334	0.0	f
PI271779	43.8	ab	PI596668	7.1	PI482335	0.0	f
PI296339	43.8	ab	PI244019	6.3	PI482336	0.0	f
PI299379	43.8	ab	PI270563	6.3	PI482338	0.0	f
PI482273	43.8	ab	PI295850	6.3	PI482342	0.0	f
PI482276	43.8	ab	PI296342	6.3	PI482355	0.0	f
PI482277	43.8	ab	PI296343	6.3	PI482361	0.0	f
PI271769	40.2	abc	PI299378	6.3	PI482379	0.0	f
PI270564	33.0	a–d	PI379243	6.3	PI485581	0.0	f
G15896	18.8	b–e	PI482283	6.3	PI500331	0.0	f
G15897	18.8	b–e	PI482298	6.3	PI500332	0.0	f
PI189225	18.8	b–e	PI482307	6.3	PI500334	0.0	f
PI270562	18.8	b–e	PI482311	6.3	PI500354	0.0	f
PI271771	18.8	b–e	PI482316	6.3	PI525083	0.0	f
PI482257	18.8	b–e	PI485583	6.3	PI526659	0.0	f
PI482309	15.5	cde	PI500308	6.3	PI532666	0.0	f
PI271767	13.4	cde	PI512854	6.3	PI542113	0.0	f
PI542117	13.4	cde	PI525081	6.3	PI542114	0.0	f
PI596662	13.4	cde	PI542123	6.3	PI542118	0.0	f
PI597691	13.4	cde	PI596653	6.3	PI542119	0.0	f
PI244017	12.5	de	PI597677	6.3	PI596656	0.0	f
PI296334	12.5	de	PI597696	6.3	PI596665	0.0	f
PI296337	12.5	de	CHASGRY	6.3	PI596666	0.0	f
PI296341	12.5	de	PI179881	0.0	PI596667	0.0	f
PI482261	12.5	de	PI244018	0.0	PI596669	0.0	f
PI482265	12.5	de	PI255137	0.0	PI596670	0.0	f
PI482300	12.5	de	PI271770	0.0	PI596671	0.0	f
PI482303	12.5	de	PI271775	0.0	PI597675	0.0	f
PI597676	12.5	de	PI288316	0.0	PI606135	0.0	f
PI596658	8.3	ef	PI295842	0.0	SBABY	0.0	f
PI271773	7.1	ef	PI482308	0.0			
PI500303	7.1	ef	PI482312	0.0			

^aTotal number of inoculated seedlings per accession ranged from five to 16.

^bPercent survival for each PI is the mean of two evaluations.

^xMeans followed by the same letter are not significantly different ($P = 0.05$)

CHASGRY = Watermelon cultivar Charleston Grey, moderately susceptible control; SBABY = Watermelon cultivar Sugar Baby, highly susceptible control.

were included as highly susceptible and moderately susceptible controls, respectively. PI 271769 was used as the resistant control in all tests (Dane et al., 1998).

Fungal isolate and growth conditions. *Fusarium oxysporum* f. sp. *niveum* (Fon) Race 2 B05-30 (kindly provided by Anthony Keinath, Clemson University) was originally isolated from a watermelon plot at Clemson University's Coastal Research and Educational Center in Charleston, SC. The isolate was confirmed to be Race 2 by use of differentials as described by Keinath and DuBose (2009). The Race 2 *Fon* isolate was maintained on potato dextrose agar (PDA) plates. Isolates were grown under a 12-h diurnal lighting regime using high-intensity fluorescent lighting at 26 ± 2 °C. Inoculum for tests was generated by placing four 1-cm mycelium/agar plugs from a 10-d-old PDA plate into 500-mL Erlenmeyer flasks containing 200 mL potato dextrose broth. The liquid cultures were grown on a bench shaker at 200 rpm under a 12-h diurnal lighting regime using high-intensity fluorescent lighting at 26 ± 2 °C for 14 d. At 14 d, the

mycelia/conidia culture suspension was filtered through three layers of cheesecloth followed by a final filtration through a single layer of Miracloth (EMD Chemicals, San Diego, CA). Conidia were quantified using a hemacytometer. The conidial suspension was adjusted to a final concentration of 1×10^6 conidia/mL with sterile distilled H₂O.

Plant growth and fungal inoculations. A modified version of the inoculation method of Dane et al. (1998) was used as follows: eight seeds were sown into a 1:1:1 (v:v:v) mixture of Metromix 360 (The Scott's Co., Maryville, OH), autoclaved sand, and vermiculite (Therm-O-Rock, New Eagle, PA) in 50-cell plug trays (T.O. Plastics, Clearwater, MN). Each cell was then inoculated by pipette with 20 mL of this inoculum suspension. Only one seed was planted per cell. Eight seeds of each accession were planted per test separated in two blocks of four seed each. Each plug tray was placed in a 56 × 28 × 5-cm containment tray and then placed on the greenhouse bench where temperatures ranged from 27 to 32 °C. Seedlings were watered from the bottom as needed to maintain a moist

soil mix. Seedlings were re-inoculated with an additional 15 mL of fresh inoculum prepared as described 14 d after seeding. Plants were rated as dead or alive after 21 d. The screen was repeated.

Surviving plants of the best performers (15 accessions) from both the first and second rounds of tests were re-inoculated a third time as described previously and then transplanted into 10-cm plastic pots in Metromix 360. After 3 weeks of additional growth in the greenhouse, the plants were transferred to 2-gallon (7.6 L) plastic pots filled with Metromix 360 and self-pollinated to generate an S₁ population.

Fon screen of S₁ populations. Viable S₁ seeds were obtained from 12 of the 15 resistant accessions (Table 2). Disease screening of the 12 S₁ populations were performed as follows: 10 seeds were sown into a 1:1:1 (v:v:v) mixture of Metromix 360, autoclaved sand, and vermiculite in 50-cell plug trays. Each cell was then inoculated by pipette with 20 mL of this inoculum suspension. Plants were maintained as described previously on greenhouse benches. Each plug tray cell was re-inoculated with an additional 15 mL of fresh inoculum 14 d after the original inoculation. Plants were rated as 1 = healthy, 2 = stunted/chlorotic, or 3 = dead 5, 21, and 28 d after initial inoculation (Table 3). The screen was repeated.

Statistical analysis. Data on percentage of survivors from the initial two tests were arcsine transformed and analyzed using the PROC GLM procedure of SAS (SAS Institute Inc., Cary, NC). Means for each accession were separated using Fisher's protected least significant difference (LSD) ($P = 0.05$) (Table 1). Individual plant ratings collected over time using the 1–3 scale for the S₁ generation of each putatively resistant accession or the checks ('Charleston Grey' and 'Sugar Baby') were analyzed as repeated-measures data using the nonparametric analysis described by Brunner et al. (2002) and Shah and Madden (2004). The macro F1_LD_F1 was used to calculate the analysis of variance (ANOVA)-type statistic to test for the overall effect of a factor on disease severity and for testing pairwise comparisons of the relative treatment effects (RTE) within factors (Akritas and Brunner, 1997). In short, the relative effect for the *i*th treatment, p_i , describes the stochastic tendency of the normalized distribution $F_i(x)$ for the random variable X_i relative to the weighted average of all the $F_i(x)$ s in the experiment. An estimate of the relative treatment effect can be obtained from the observed midranks. If R_{ik} is the rank of X_{ik} among all N observations, the mean rank for the *i*th treatment is estimated by $\bar{R}_{i\bullet} = 1/n_i \sum_{k=1}^{n_i} R_{ik}$, where n_i is the number observations in the *i*th treatment, and the relative treatment effect is estimated as $\hat{p}_i = 1/N(\bar{R}_{i\bullet} - 0.5)$ (Brunner et al., 2002; Shah and Madden, 2004). The macro files can be downloaded for free from the following web site (<http://www.ams.med.uni-goettingen.de/de/sof/ld/makros.html>). Pairwise comparison of the effects (RTE) of FW among the various

Table 2. Fifteen *Citrullus lanatus* var. *citroides* accessions with the highest survival percentage in response to two controlled inoculations with *Fusarium oxysporum* f. sp. *niveum* (Fon) Race 2 in two greenhouse tests and their origin and reported resistances to other pathogens.

Accession	Country of collection	Reported phytopathogen resistance
PI 255136	South Africa	None
PI 270564	South Africa	None
PI 271769	South Africa	Fon Races 1 and 2 (Dane et al., 1998)
PI 271779	South Africa	Anthrachnose (Sowell et al., 1980)
PI 296335	South Africa	None
PI 296339	South Africa	None
PI 299379	South Africa	Anthrachnose (Sowell et al., 1980)
PI 482246	Zimbabwe	None
PI 482252	Zimbabwe	Watermelon Mosaic Virus (GRIN, NGRP)
PI 482273	Zimbabwe	Powdery mildew Race 2 (Tetteh et al., 2010); Fon Race 2 (Boyhan et al., 2003)
PI 482276	Zimbabwe	Gummy stem blight (Gusmini et al., 2005)
PI 482277	Zimbabwe	Powdery mildew race 2 (Tetteh et al., 2010)
PI 482279	Zimbabwe	Bacterial fruit blotch (Hopkins and Thompson, 2002)
PI 482282	Zimbabwe	None
PI 482331	Zimbabwe	None

Table 3. Responses of the first self-pollinated (S1) generation of 12 *Citrullus lanatus* var. *citroides* accessions and two watermelon cultivars to controlled inoculation with *Fusarium oxysporum* f. sp. *niveum* Race 2 in two greenhouse tests as expressed by relative treatment effect (RTE), disease rating 28 d post-inoculation, and percent survival of plants.

Accession	First screen				Second screen				Mean survival (%)	
	RTE ^z		Final rating ^y	Survival ^x (%)	RTE		Final rating	Survival (%)		
PI 482246	0.319	g ^w	1.2	f	100	0.341	de	1.4	de	100
PI 482252	0.319	g	1.2	f	100	0.327	e	1.3	e	100
PI 271769	0.458	ef	1.7	ef	89	0.515	bc	1.9	cde	89
PI 296335	0.471	ef	1.9	de	75	0.406	de	1.4	de	100
PI 296339	0.438	f	2.1	cde	57	0.428	de	1.9	cde	88
PI 482279	0.488	ef	2.1	cde	63	0.452	cd	2.0	bcd	75
PI 482276	0.435	fg	1.7	ef	89	0.438	cd	2.2	bc	44
PI 299379	0.534	de	2.1	cde	75	0.554	bc	2.2	bc	56
PI 271779	0.547	de	2.1	cde	63	0.508	bcd	2.0	bcd	67
PI 270564	0.609	b	2.3	bcd	67	0.607	b	2.4	abc	56
PI 482331	0.599	bc	2.4	bcd	50	0.580	b	2.4	abc	50
PI 482282	0.592	bcd	2.6	abc	30	0.562	bc	2.4	abc	40
‘Charleston Grey’	0.605	b	2.8	ab	20	0.600	b	2.6	ab	44
‘Sugar Baby’	0.695	a	3	a	0	0.703	a	3.0	a	0

^zRTE = relative treatment effect of each PI is based on the mean of three ratings taken on each PI at 5, 21, and 28 d after inoculation.

^yFinal rating is based on the mean of the 1–3 scale, where 1 = healthy, 2 = wilted/chlorotic, and 3 = dead, rated 28 d after inoculation.

^xPlants that did not die at 28 d after inoculation and rated less than 3 were considered as survivors.

^wMeans followed by the same letter in a column are not significantly different ($P = 0.05$).

'Charleston Grey' = moderately susceptible control.

'Sugar Baby' = highly susceptible control.

accessions over time was done using the output generated by the mentioned SAS macros at $P = 0.05$ (Table 3) (Akritas and Brunner, 1997; Brunner et al., 2002). Detailed descriptions of the statistical methods are provided by Shah and Madden (2004). The final ratings taken 28 d after inoculation were analyzed using the PROC GLM procedure of SAS. Means for each accession were separated using Fisher's protected LSD ($P = 0.05$).

Results and Discussion

Of the 110 *C. lanatus* var. *citroides* accessions examined in this study, 15 showed a significantly higher level of resistance than any of the other accessions to Fon Race 2 with a survival rate of 33.0% to 56.3% 28 d after the initial Fon inoculation (Tables 1 and 2). Most (67%) of the 110 accessions had

either zero plant survival or only a single plant survival in one but not both screens (Table 1). In these cases, the surviving plant was stunted and somewhat chlorotic yet not dead. Isolations from crown tissue from some of these stunted plants resulted in recovery of the pathogen (data not shown). Based on these findings, we used a disease rating scale based on 1 = healthy, 2 = wilted/chlorotic, and 3 = dead for the S₁ screens.

Most *C. lanatus* var. *citroides* accessions tested were found to be as susceptible or more so compared with the commercial watermelon (*Citrullus lanatus* var. *lanatus*) cultivars Sugar Baby and Charleston Grey, the highly and moderately susceptible checks, respectively (Table 1). Although most of the accessions tested in this study were uniformly susceptible (i.e., all plants died), no accession was uniformly resistant (i.e., all plants survived). Fifteen of the accessions had significantly

more surviving plants than all other accessions and cultivars tested and may be considered as potential sources of resistance (Table 2). These accessions had 33% or greater survival rate with plants remaining healthy throughout the initial testing (Table 1). Here, PI 271769 and PI 296341, which were reported as resistant to Fon Race 2 (Dane et al., 1998; Martyn and Netzer, 1991), had a survival rate of 40.2% and 12.5%, respectively (Table 1). Although PI 271769 showed intermediate resistance, PI 296341 was significantly more susceptible than the top performers. The most resistant line in the Boyhan et al. (2003) study, PI 534536, is listed as *Cucumis melo* var. *melo* in the Genetic Resources Information System and so would not be an actual host to this pathogen and thus was not included in our survey. On the other hand, PI 482273, listed as one of the 11 resistant watermelon PIs identified by Boyhan et al. (2003), was also found to be in the top 15 resistant lines in our study with 43.8% survival (Table 1). It is both interesting and troublesome that in the three previous published surveys as well as the one presented here, no consensus is found as to one or more resistant lines. This may in part be attributable to the particular isolate used for screening the lines, the type of inoculation method used, the age of the plant, and/or environmental conditions under which the assays were performed. On the other hand, it is evident from all of these studies that new putative sources of host plant resistance to Race 2 Fon exist among the *C. lanatus* var. *citroides* accessions. Healthy, inoculated plants from the 15 most resistant accessions were selfed and the S₁ seed tested in subsequent experiments (Table 3). We obtained viable S₁ seed from all but PI 255136, PI 482273, and PI 482277.

The ANOVA-type statistics indicated highly significant ($P \leq 0.00001$) differences in FW development of the Race 2 inoculated S₁ generations. A highly significant ($P < 0.00001$) interaction between accessions and rating period (time, accession $\times$ T) was observed in both of the S₁ screens, indicating that disease progression occurred at different rates among the accessions and the susceptible checks. Disease progression was further evaluated by calculating the RTE, which takes into account each of the three ratings and compares them with the ratings from the other accessions. Disease progressed very rapidly on the susceptible control 'Sugar Baby' as indicated by the high relative treatment effect (0.695 and 0.703) for both screens and was significantly faster in progression than the other susceptible control, 'Charleston Grey', as well as the other accessions. All the tested S₁ individuals of PI 482246 and PI 482252 survived in both screening procedures, and disease progress was significantly slower on these two accessions compared with the susceptible checks and susceptible accessions identified in this study. PI 482246 and PI 482252 also had the lowest final ratings of all other accessions in both screening procedures. Disease progress was

significantly slower in PI 271769 S₁ individuals when compared with either cultivar in the first screen and when compared with 'Sugar Baby' in the second screen. The PI 271769 S₁ population had 89% survival in both screens. PI 271769 has previously been reported as a potential source of resistance by other researchers (Dane et al., 1998). Disease progress and final ratings were not significantly different from the control 'Charleston Grey' for S₁ generation of PI 482282 and PI 482331. Highly significant correlations ($P \leq 0.0001$) between the RTE and final ratings were observed in the first ($r = 0.95$) and the second ($r = 0.94$) S₁ screens. Similarly, the number of survivors was also significantly ($P \leq 0.0001$, $r = 0.85$) correlated with the RTE in both the S₁ screens.

Of the 15 resistant accessions chosen for tests of the S₁ generation, all were collected from southern Africa, the center of origin of *Citrullus* spp. (Table 2). Eight of these accessions were collected in Zimbabwe: PI 482246, PI 482252, PI 482279, PI 482282, PI 482331, PI 482273, PI 482276, and PI 482277, whereas seven accessions were collected in South Africa: PI 255136, PI 296335, PI 296339, PI 299379, PI 270564, PI 271769, and PI 271779. The exact lineage or initial origin of these accessions is not known. How the genetic make-up of the accessions reported here compares with that of PI 296341 or PI 271769 also is unknown. Although all of the identified resistant germplasm is from the African continent, the collection points differ. Both PI 296341 and PI 271769 were acquired from South Africa, whereas the two most resistant accessions reported here, PI 482246 and PI 482252, are from Zimbabwe.

Zhang and Rhodes (1993) using controlled crosses of PI 296341 with the cultivar New Hampshire Midget and root inoculations with *Fon* Race 2 reported a 13 susceptible:three resistant ratio, but their actual data are also an acceptable fit to a 3:1 ratio ($\chi^2 = 1.86$, $P = 0.18$) expected for the simpler, single gene model. Unfortunately, they do not include the test cross to the resistant parent, which would be expected to segregate one susceptible:one resistant in a single-gene model or two susceptible:one resistant in a two-gene model. This omission, combined with the vagueness of the statistics used and a less than perfect fit to either a 3:13 or a 1:3 ratio, makes it difficult to determine inheritance. Genetic control of resistance to *Fon* Race 2 in PI 296341-FR has not been determined with any certainty.

Xu et al. (1999, 2000) studied the heritability of resistance to *Fon* Race 1 in PI 296341 and reported that the resistance is conditioned by a single dominant gene. Henderson et al. (1970) also found that *Fon* Race 1 resistance was conditioned by a single gene in the resistant watermelon cultivar Summit. Netzer and Weintall (1980) examined F₁, F₂, F₃, and backcross progenies of crosses between the *Fon* Race 1-susceptible watermelon cultivar Mallali and two resistant cultivars, Calhoun Gray and Summit, concluding that the resistance is conferred by a single dominant gene,

Fo-1 (Wehner, 2008). These observations indicate that there may be more than one FW resistance gene in PI 296341.

Several other studies of fusarium resistance in cucurbits have identified the involvement of single dominant genes for resistance. In the cucurbit *Cucumis melo* (cantaloupe), resistance to the FW pathogen *Fusarium oxysporum* f. sp. *melonis* Race 1 is conferred by a single dominant gene (*Fom-2*) and resistance to Race 2 is controlled by another dominant gene (*Fom-1*) with both genes also conferring resistance to Race 0 (Joobeur et al., 2004; Schreuder et al., 2000; Wang et al., 2000; Wechter et al., 1995, 1998; Zink and Thomas, 1990). The confusion and difficulties associated with determining the inheritance of resistance to *Fon* Race 2 in PI 296341 may be why, to date, no markers for this resistance have been reported and why no commercial cultivars with resistance to Race 2 have been developed.

Work is currently being carried out to determine the genetics involved in resistance in a number of these accessions. These studies should lead to the identification and development of molecular markers linked to FW Race 2 resistance for use in introgression of the identified resistance into breeding lines and ultimately watermelon cultivars.

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