Origin of Fusarium Wilt Resistance in Texas AES Muskmelon Cultivars

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ABSTRACT

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The mode of inheritance of resistance to Fusarium wilt, caused by *Fusarium oxysporum* f. sp. *melonis* race 2, in muskmelon (*Cucumis melo*) cvs. Mango Melon and Smith's Perfect and in Texas AES (TAES) cvs. Rio Gold, Wescan, and Dulce was determined by analyzing wilt resistance segregation of F_1, F_2, and BC_1 populations of crosses with susceptible cv. Top Mark. The ratios obtained indicate that resistance to race 2 in these cultivars is conferred by a dominant gene. In allelism tests, resistance in Mango Melon, Smith's Perfect, and the TAES cultivars was determined to be controlled by the gene *Fom 3*, or an allele of this gene, which also confers resistance in Perlita. Mango Melon, Smith's Perfect, and the TAES cultivars were resistant to race 2 at inoculum concentrations of 0.5×10^6 spores per milliliter and susceptible at 1.0×10^6. Circumstantial evidence indicates that Fusarium wilt resistance in Rio Gold, Wescan, Perlita, and Dulce stems from the Fusarium wilt-resistant Mango Melon and Smith's Perfect. Resistance to downy mildew, caused by *Pseudoperonospora cubenis*, was introduced into the TAES cultivars from Smith's Perfect. It is proposed that the breeders were not aware of Fusarium wilt resistance in their breeding lines or cultivars and that the gene *Fom 3*, or an allele of this gene, is associated with the downy mildew resistant trait and was incorporated into the TAES cultivars during the selection for downy mildew resistance.

Fusarium wilt, caused by *Fusarium oxysporum* Schlechtend.:Fr. f. sp. *melonis* W.C. Snyder & H.N. Hans., presents a serious problem in growing muskmelons (*Cucumis melo* L.) in many parts of the world. Control of such a soilborne disease is practically limited to breeding cultivars inherently resistant. A survey of muskmelon germ plasm resistant to *F. o.* f. sp. *melonis* race 2 revealed that several commercial seed lots of the cultivar Perlita, developed by the Texas Agricultural Experiment Station (TAES), were segregating for resistance (11). Inheritance studies revealed that resistance is conferred by the dominant gene *Fom 3* (10). Mango Melon, Hale's Best, Smith's Perfect, Rio Gold, and Wescan appear in the pedigree of Perlita (2).

This paper reports the reaction of Mango Melon, Smith's Perfect, Rio Gold, Wescan, and Dulce to *F. o.* f. sp. *melonis* race 2; the mode of inheritance of resistance in these cultivars; the genetic relationship between these sources of resistance; the probable source of resistance in the TAES cultivars Rio Gold, Wescan, Perlita, and Dulce; and the apparent association in the TAES cultivars of resistance to downy mildew, caused by *Pseudoperonospora cubenis* (Berk. & M.A. Curtis) Rostovzev, and to Fusarium wilt.

MATERIALS AND METHODS

Standard hybridization techniques for muskmelon (9) were used to make crosses between the homozygous cultivars resistant to *F. o.* f. sp. *melonis* race 2 (Mango Melon, Smith's Perfect, Rio Gold, Wescan, and Dulce) and a cultivar (Top Mark) susceptible to *F. o.* f. sp. *melonis* races 0, 1, 2, and 1-2 in order to determine the mode of inheritance of resistance. A second series of crosses was made between the five resistant cultivars and Perlita FR, a University of California selection resistant to *F. o.* f. sp. *melonis* race 2, to identify alleles for resistance.

All isolates of *F. o.* f. sp. *melonis* used in these studies were obtained from Thomas Gordon, University of California, Berkeley. Races are classified according to the nomenclature of Risser et al (8). Inoculum of race 2 consisted of a mixture of macroconidia and microconidia prepared from potato-dextrose agar slant cultures grown for 10 days at 20–24 C under continuous illumination. The spores were washed from the agar surface and the suspension was filtered through two layers of cheesecloth. The filtrate was diluted with distilled water to obtain the desired inoculum concentration. A hemacytometer was used to quantify inoculum. Resistance of Mango Melon, Smith's Perfect, Rio Gold, Wescan, Perlita FR, and Dulce to race 2 was tested at inoculum concentrations of 0.05×10^6, 0.1×10^6, 0.5×10^6, and 1.0×10^6 spores per milliliter. The inoculum concentration used in the inheritance studies was 0.10×10^6 spores per milliliter.

Seeds of plants to be assayed for disease reaction were treated with 5% calcium hypochlorite solution for 5 min, rinsed in water, and planted into cell-type plastic growing trays (one seed per 55-ml cell) filled with a sterilized potting mix of peat and vermiculite (1:1, v/v). Each tray containing seeds was placed on top of a flat that was also full of potting mix. Each cell of the tray had an enlarged drainage hole that allowed seedling roots to grow into the lower flat of soil. When the seedlings were in the first- to second-leaf stage of growth, the upper tray was lifted from the lower flat. Ruptured roots protruding from the drainage holes in the cell bottoms were rinsed with a fine spray of water before the entire tray was placed in a shallow basin (cafeteria tray) containing 1 L of inoculum. The tray was allowed to sit in the spore suspension until all of the inoculum was absorbed (approximately 45 min), then was placed back on the lower flat. Control plants were dipped in water only. To hasten inoculum absorption into the soil, seedling trays were not watered the day before inoculation. Inoculated seedlings were kept in a greenhouse at 20–27 C.

Plants were examined periodically, and the number of stunted, necrotic, or dead seedlings was recorded. Final assessments of the wilt reaction were made 42 days after inoculation. Plants free of wilt symptoms were considered resistant. Differential cultivars Charentais T, Doublon, and CM 17-187, as well as the parents of the cross being studied, were included in each test to ensure that there were no changes in pathogen virulence or race.

RESULTS

Cultivar reactions to race 2 inoculum concentrations. Mango Melon, Smith's Perfect, Rio Gold, Wescan, Dulce, and Perlita FR were resistant at inoculum concentrations of 0.05×10^6, 0.1×10^6, and 0.5×10^6. A few plants of Mango Melon, of Smith's Perfect, and of each TAES cultivar were recorded as susceptible after inoculation at the highest concentration of 1.0×10^6 (Table 1). Doublon was resistant at all inoculum concentrations tested. Charentais T, CM 17-187, and Top Mark were equally susceptible at each inoculum concentration.

Inheritance of resistance to race 2. Crosses of resistant Mango Melon, Smith's Perfect, Rio Gold, Wescan, and Dulce with susceptible Top Mark produced F_1 progenies resistant to race 2. The segregation observed in the F_2 generation (Table 2) suggested simple inheritance (3:1) of the disease reaction, with resistance determined by a single

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dominant gene.

To verify the mode of resistance, the F_1 progenies were backcrossed to susceptible Top Mark. The BC_1 progenies of this cross gave a good fit to a 1:1 ratio of resistant to susceptible plants. The F_1 progenies backcrossed to resistant Mango Melon, Smith's Perfect, Rio Gold, Wescan, and Dulce resulted in all resistant progenies (Table 2). The segregation of the BC_1 progenies indicated that resistance is conferred by a single dominant gene.

Allelism test. Crosses were made between Perlita FR, with the dominant gene *Fom 3* conferring resistance to race 2, and Mango Melon, Smith's Perfect, Rio Gold, Wescan, and Dulce, shown to have a dominant gene conferring resistance to race 2. F_1 plants produced F_2 progenies that did not segregate (Table 3). The progenies from the crosses (resistant Perlita FR × resistant TAES cultivar) × susceptible Top Mark did not segregate for wilt resistance (Table 3). The allelism tests indicate that the same dominant gene *Fom 3*, or alleles of this gene, confer resistance to race 2 in Mango Melon, Smith's Perfect, Rio Gold, Wescan, and Dulce.

DISCUSSION

Fusarium wilt of muskmelon was confirmed in Texas for the first time from the Lower Rio Grande Valley in 1986 (5). Random plants in commercial plantings of Perlita displayed Fusarium wilt symptoms. Based on the reaction of differential cultivars, the isolate was designated *F. o.* f. sp. *melonis* race 0. It is not known if the pathogen was recently introduced or if it is indigenous to the area. Jacobson and Gordon (4) reported that both the Maryland and the Texas isolates of race 0 are included with European isolates of race 0 in the vegetative compatibility group 0134, and it is possible that these infestations resulted from recent introduction of the pathogen from Europe. Of interest is the observation by Zink et al (11) that three commercial seed lots of Perlita segregated for resistance to *F. o.* f. sp. *melonis* race 2. Furthermore, the University of California selection for resistance to *F. o.* f. sp. *melonis* race 2 within Perlita (designated as Perlita FR) is resistant to the Texas isolate race 0 at inoculum concentrations of 0.5×10^6 (*unpublished data*). Race 0 incites disease only on those muskmelon genotypes that lack genes resistant to Fusarium wilt (e.g., Charentais T, Top Mark).

The reactions of Mango Melon, Smith's Perfect, Rio Gold, Wescan, Dulce, and Perlita FR in the inoculum concentration test were similar: resistant at 0.05×10^6, 0.1×10^6, and 0.5×10^6 and partially susceptible at 1.0×10^6 spores per milliliter. These data suggest that the resistant gene *Fom 3* reported in Perlita FR (11) also confers resistance in Mango Melon, Smith's Perfect, Rio Gold, Wescan, and Dulce and is in agreement with the allelism tests. In contrast, wilt did not develop at the highest concentration of 1.0×10^6 in Doublon, which is reported to have the dominant gene *Fom 1* (7).

The TAES cultivars have complex pedigrees (Fig. 1) originating from a cross made in 1941 between Mango Melon and the Fusarium wilt-susceptible Hale's Best (1,2). This F_1 hybrid was crossed with Smith's Perfect to introduce downy mildew resistance into the pedigrees of TAES cultivars. Several generations of inbreeding and selection led to the release of Rio Gold in 1943. Rio Gold was reported to have excellent resistance to downy mildew (2). Wescan was released in 1963 as highly resistant to downy mildew and resistant to race 1 of powdery mildew caused by *Sphaerotheca fuliginea* (Schlechtend.:Fr.) Pollacci (1). Perlita and Dulce were

Table 1. Reaction of Mango Melon, Smith's Perfect, Rio Gold, Wescan, Perlita FR, Dulce, Top Mark, and the differential cultivars Charentais T, CM 17-187, and Doublon over a range of inoculum concentrations of race 2 of *Fusarium oxysporum* f. sp. *melonis*

	Fusarium wilt reaction[a] at inoculum concentration							
	0.05×10^6		0.1×10^6		0.5×10^6		1.0×10^6	
Cultivars	R	S	R	S	R	S	R	S
Mango Melon	40	0	40	0	40	0	34	6
Smith's Perfect	40	0	40	0	40	0	37	3
Rio Gold	40	0	40	0	40	0	35	5
Wescan	40	0	40	0	40	0	38	2
Perlita FR	40	0	40	0	39	0	37	3
Dulce	40	0	40	0	40	0	36	4
Top Mark	0	20	0	20	0	20	0	20
Charentais T	0	18	0	20	0	19	0	18
CM 17-187	0	20	0	20	0	20	0	20
Doublon	40	0	40	0	38	0	40	0

[a] R = number resistant, S = number susceptible.

Table 2. Segregation in progenies from crosses between resistant Mango Melon, Smith's Perfect, Rio Gold, Wescan, and Dulce and susceptible Top Mark after inoculation with race 2 of *Fusarium oxysporum* f. sp. *melonis*

Parents and crosses	Expected ratio[a]	Observed reaction[b] R	Observed reaction[b] S	χ^2	*P*
Mango Melon (MM)	All R	76	0	...	...
Top Mark (TM)	All S	0	20	...	...
F_1MM × TM	All R	47	0	...	...
TM × MM	All R	50	0	...	...
F_2MM × TM	3:1	67	26	0.70	0.50–0.30
BC_1(MM × TM) × TM	1:1	46	47	0.01	0.95–0.70
BC_1(MM × TM) × MM	All R	98	0	...	...
Smith's Perfect (SP)	All R	86	0	...	...
Top Mark (TM)	All S	0	20	...	...
F_1SP × TM	All R	46	0	...	...
TM × SP	All R	48	0	...	...
F_2SP × TM	3:1	68	25	0.17	0.70–0.50
BC_1(SP × TM) × TM	1:1	40	52	1.56	0.30–0.20
BC_1(SP × TM) × SP	All R	92	0	...	...
Rio Gold (RG)	All R	64	0	...	...
Top Mark (TM)	All S	0	24	...	...
F_1RG × TM	All R	49	0	...	...
TM × RG	All R	46	0	...	...
F_2RG × TM	3:1	106	42	0.90	0.50–0.30
BC_1(RG × TM) × TM	1:1	65	78	1.18	0.30–0.20
BC_1(RG × TM) × RG	All R	87	0	...	...
Wescan (W)	All R	49	0	...	...
Top Mark (TM)	All S	0	24	...	...
F_1W × TM	All R	48	0	...	...
TM × W	All R	50	0	...	...
F_2(W × TM)	3:1	67	28	1.01	0.50–0.30
BC_1(W × TM) × TM	1:1	51	41	1.08	0.30–0.20
BC_1(TM × W) × W	All R	95	0	...	...
Dulce (D)	All R	48	0	...	...
Top Mark (TM)	All S	0	26	...	...
F_1D × TM	All R	44	0	...	...
TM × D	All R	50	0	...	...
F_2D × TM	3:1	70	28	0.67	0.50–0.30
BC_1(D × TM) × TM	1:1	46	41	0.10	0.50–0.30
BC_1(D × TM) × TM	All R	91	0	...	...

[a] If resistance is a dominant gene.
[b] R = number resistant, S = number susceptible.

Table 3. Allelism tests for the dominant gene *Fom 3* in Mango Melon, Smith's Perfect, Rio Gold, Wescan, Perlita FR, and Dulce that confers resistance to race 2 of *Fusarium oxysporum* f. sp. *melonis*

Crosses[a]	Expected ratio[b]	Observed reaction[c] R	S
F_2(MM × P)	15:1	84	0
(MM × P) × TM	3:1	91	0
F_2(SP × P)	15:1	88	0
(SP × P) × TM	3:1	96	0
F_2(RG × P)	15:1	83	0
(RG × P) × TM	3:1	94	0
F_2(W × P)	15:1	88	0
(W × P) × TM	3:1	97	0
F_2(D × P)	15:1	92	0
(D × P) × TM	3:1	87	0

[a] MM = Mango Melon, SP = Smith's Perfect, RG = Rio Gold, W = Wescan, P = Perlita FR, Dulce = D, TM = susceptible cultivar Top Mark.
[b] If different dominant genes confer resistance.
[c] R = number of resistant plants, S = number of susceptible plants.

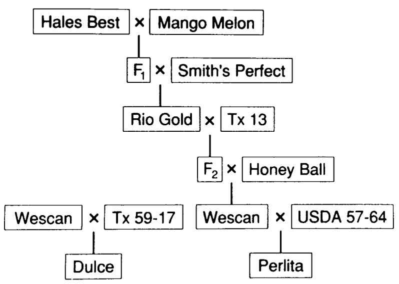

Fig. 1. Pedigree of Texas muskmelon cultivars Rio Gold, Wescan, Dulce, and Perlita.

released in 1965 and 1968, respectively, and were reported to be resistant to downy mildew and to races 1 and 2 of powdery mildew (1). The TAES breeding line 13 and 59-17, the Honeyball variant, and the USDA breeding line 57-64 that appear in the pedigree could not be tested for resistance to *F. o.* f. sp. *melonis* race 2 because seed of these lines was no longer available.

No mention of Fusarium wilt resistance was made in the release notices for the TAES cultivars or in the breeding and evaluation techniques described by Bohn et al (1), suggesting that the breeders were not aware of Fusarium wilt resistance in their breeding lines and cultivars. This is consistent with the fact that Fusarium wilt of muskmelon was only recently identified in Texas (5). The genetic studies indicate that resistance to *F. o.* f. sp. *melonis* races 0 and 2, conferred by the gene *Fom 3*, was introduced into the pedigree of the TAES cultivars by Mango Melon and introduced a second time by Smith's Perfect. Circumstantial evidence suggests that Fusarium wilt resistance conferred by the dominant gene *Fom 3* is associated with the partially dominant downy mildew trait found in Smith's Perfect (3).

Although linkage of the gene *Fom 3* with the downy mildew-resistant trait has not been demonstrated, pathologists and breeders should be aware that cultivars with the Smith's Perfect source of downy mildew resistance may also be resistant to *F. o.* f. sp. *melonis* races 0 and 2. Georgia 47 is such a cultivar. Georgia 47 originated from a cross between Heart of Gold and the USDA breeding line 29554. A fifth generation from this cross was then crossed with Smith's Perfect, and after several generations of selection, this breeding line was released by the Georgia AES as having high resistance to downy mildew, medium resistance to powdery mildew, and some resistance to aphids (6); there was no mention of resistance to Fusarium wilt in the release notice. Investigations of Georgia 47 revealed that it is resistant to *F. o.* f. sp. *melonis* race 2 (11). Furthermore, the resistance was determined to be conferred by a dominant gene, and allelism tests indicate that this is the same gene as, or an allele of, *Fom 3*, which confers resistance in Smith's Perfect (*unpublished data*). These facts give additional support to the hypothesis that there is an association between the gene *Fom 3* and the downy mildew-resistant trait.

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