Response of Melofon Breeding Lines to Powdery Mildew, Downy Mildew, Fusarium Wilt, and Sudden Wilt

R. Cohen, Shoshanna Schreiber, and **H. Nerson,** Department of Vegetable Crops, ARO, Newe Ya'ar Research Center, P.O. Box 90000, Haifa 31900, Israel

ABSTRACT

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The responses of eight melofon (pickling melon, *Cucumis melo*) breeding lines to powdery mildew, downy mildew, Fusarium wilt, and sudden wilt were evaluated. No resistance to races 1 and 2 of *Sphaerotheca fuliginea* (powdery mildew) was found; however, all melofon lines tested except D17-5 were moderately susceptible compared with the highly susceptible melon cv. Ananas Yoqne'am and the cucumber cv. Bet Alfa. Two breeding lines, P22a and P6a-3, were relatively tolerant to *Pseudoperonospora cubensis* (downy mildew), exhibiting the lowest disease index in both experiments conducted. All melofon breeding lines were resistant to races 0 and 1 of *Fusarium oxysporum* f. sp. *melonis*. High susceptibility to *F. o.* f. sp. *melonis* race 2 was evident, while disease severity following inoculation with race 1,2 was moderate in most lines tested. There was a wide variation among melofon breeding lines in responses to sudden wilt: line D17 was very susceptible while P6a-1 did not collapse in field trials. Overall, breeding lines derived from cross P6 were the most promising with respect to disease resistance.

Melofon (*Cucumis melo* L.) is a new crop of the Cucurbitaceae (11,12), developed for the pickle industry, with a concentrated yield suitable for once-over mechanical harvest. Melofons are recombinants derived from crossing cultivars of oriental pickling melons (*C. melo* L. var. *conomon* (Thunb.) Mak.) and snake melons (*C. melo* L. var. *flexuosus* (L.) Naud.) with birdsnest muskmelons (*C. melo* L. var. *reticulatus* Ser.) or with germ plasm having hermaphroditic sex expression (12).

Powdery mildew (*Sphaerotheca fuliginea* (Schlechtend.:Fr.) Pollacci), downy mildew (*Pseudoperonospora cubensis* (Berk. & M. A. Curtis) and Fusarium wilt (*Fusarium oxysporum* Schlechtend.:Fr. f. sp. *melonis* (Leach and Currence) W. C. Snyder & H. N. Hans.), are the most common and severe diseases of melons in Israel (1,3,4,5,18). Sudden wilt of melons, also known as plant collapse or vine decline, is a destructive disease and is a continuous challenge for phytopathological research (2,6). This disease causes a rapid wilt of mature plants late in the season and may lead to a total loss of the crop. The causal agent of this disease has not been identified in all cases. Different soilborne pathogens including *Fusarium solani* (Mart.) Sacc. f. sp. *cucurbitae* W. C. Snyder & H. N. Hans., *F. equiseti* (Corda) Sacc., *Macrophomina phaseolina* (Tassi) Goidanich and *Monosporascus eutypoides* (Petrak) v. Arx. have been isolated from sudden wilted melon plants in Israel (6,8, 15). In Texas, *Monosporascus cannonballus* Pollack & Uecker was determined to be the causal agent of a root rot/vine decline (9,10) and an *Acremonium* sp. was recently reported to be associated with muskmelon collapse in Spain (7). Soil fumigation with methyl bromide or a combination of a reduced rate of methyl bromide with soil solarization has effectively prevented sudden wilt (8,19). Seed treatment or soil drench with the growth retardant paclobutrazol has also proved effective in certain cases (2). Sudden wilt is a disease that is expressed late in the season when plants are under physiological stress. Trickle irrigation applied daily reduced disease incidence compared with irrigation applied every 3 days, possibly as a result of better adaptability of the root system to cope with disease and environmental stresses (15).

Introduction of disease resistance was not originally an objective of the melofon breeding program; however, field observations indicated that breeding lines were relatively resistant to downy mildew, showing lower disease incidence compared with cucumbers and muskmelons. In addition, the *Fom-2* gene conferring resistance to *F. o.* f. sp. *melonis* races 0 and 1 has recently been identified (13) in cv. Freeman's Cucumber, an oriental pickling melon accession, belonging to subspecies *conomon*, and in some parental lines of melofon.

The purpose of the present study was to evaluate the responses of melofon, a new crop for the processing industry, to the main cucurbit diseases in Israel. This information is important to complete the background information of this new crop. Moreover, identification of resistance sources may be beneficial for both melofon and sweet melon breeding programs in Israel and elsewhere.

MATERIALS AND METHODS

Plant material. Eight breeding lines of melofon, representing a wide range of genetic sources, were selected from the breeding project. Some breeding lines were derived from crosses within *Cucumis melo* var. *conomon* (BSK and P&G) and *C. melo* var. *flexuosus* (FAQ) and others from crosses between *C. melo* var. *conomon* and *C. melo* var. *reticulatus* (Persia 202) (Table 1). Several muskmelon cultivars were used as controls because of their well-documented responses to specific pathogens. Cultivar Ananas Yoqne'am is highly susceptible to powdery (4,5) and downy (18) mildews. In Israel, cv. Dulce is resistant to powdery mildew races 1 and 2 (5), and moderately resistant to downy mildew (R. Cohen, *unpublished*). Cultivar Noy Yizre'el is resistant to powdery mildew race 1, but susceptible to race 2 (1). Cultivars Noy Yizre'el and Galia are moderately resistant to downy mildew (R. Cohen, *unpublished*). Cultivar En Dor is susceptible to all races of *F. o.* f. sp. *melonis*. Cultivar Makdimon is resistant to races 0 and 1, and cv. Hemed is resistant to races 0 and 2 (5). The cucumber cultivar Bet Alfa is susceptible to powdery mildew race 1 (R. Cohen, *unpublished*).

Table 1. Description of melofon breeding lines: origin and breeding status

Breeding line	Female parent	Male parent
D17-2	P&G[v]	P202[w]
D17-5	P&G	P202
P6a-1	BSK[x]	P&G
P6a-2	BSK	P&G
P6a-3	BSK	P&G
P15a	BSK	D17-135
P20a	P&G	P4[y]
P22a	BSK	P6[z]

[v] Poole and Grimball, a hermaphroditic breeding line from the U.S. (origin China).
[w] Persia 202, a birdsnest breeding line from Iran.
[x] Black Skin, an oriental pickling melon cultivar from Taiwan.
[y] F1 (P&G × FAQ), FAQ = Faquse, a Middle Eastern snake melon.
[z] F1 (BSK × P&G).

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Powdery mildew. Two races of *Sphaerotheca fuliginea* were used. Race 1 was isolated from melon (*C. melo*) cv. Ananas Yoqne'am and race 2 was isolated from melon cv. Galia resistant to race 1. Plants on which inoculum was maintained were grown at 23°C with a 12-h photoperiod (300 μE · m^{-2} · s^{-1}) in a growth chamber and 70% relative humidity. In addition, plants of Noy Yizre'el, which are resistant to race 1 of the pathogen but susceptible to race 2, were placed among the inoculated plants for race identification and for detection of possible contamination of the isolates. The test plants (10 for each genotype) were grown in complete random design in 250-ml plastic pots containing vermiculite and peat (1:1, vol/vol). Plants were maintained in a growth chamber under conditions described above. At the third true leaf stage, plants were inoculated with the respective race by blowing air across an infected plant, which served as the inoculum source, toward the test plants. Although this inoculation procedure is not quantitative, uniform infections were observed previously, using this method (1,4). Disease severity was determined 10 days after the inoculation by visually estimating the percentage of leaf area covered by the pathogen, using a previously described scale (1).

Fusarium wilt. Four races (0, 1, 2, and 1,2) of *F. o.* f. sp. *melonis* isolated in Israel and previously shown to be pathogenic (3) were used. The fungi were periodically isolated from diseased plants and reinoculated to maintain high pathogenicity. The pathogens were maintained on yeast extract medium at 27°C in the dark. Conidial suspensions for seedling inoculation were prepared by macerating 1-week-old cultures with 100 ml of water (3). Seeds of the tested genotypes were sown in sandy loam soil. One to two days after emergence, seedlings were removed from the soil and washed thoroughly to remove adhering soil. Approximately 5 mm of the root ends were cut prior to inoculation, and 40 seedlings for each treatment were inoculated by dipping their roots for 2 min in a 10^6 per ml conidial suspension of *F. o.* f. sp. *melonis* of the appropriate race and transplanted into 250-ml pots containing sandy soil (0.4% organic matter, 3.6% clay, and 96% sand). Each treatment (genotype/pathogen-race combination) contained eight pots with five seedlings per pot arranged in a complete randomized design. Inoculated seedlings were grown in a growth chamber under conditions similar to those used for powdery mildew tests. The number of wilted plants was recorded daily for 14 days, and the disease percentage was calculated. Muskmelon cultivars En Dor, Hemed, and Makdimon were included as race-purity differentials (3).

Downy mildew. A colony of *Pseudoperonospora cubensis* was maintained on *C. melo* (Ananas Yoqne'am) in a growth chamber at 20°C by repeated inoculations. The experimental plants were grown in a growth chamber at 25°C and 300 μE · m^{-2} · s^{-1} with a 12-h photoperiod, in 250-ml plastic pots containing vermiculite and peat (1:1, vol/vol). Plants were inoculated about 3 weeks after sowing (two true leaves). Inoculation was done by spraying eight plants of each genotype, arranged in completely randomized design, with a suspension of the pathogen containing approximately 10^4 sporangia per ml. Inoculated plants were kept in a saturated atmosphere (enveloping well-irrigated plants with polyethylene sheet) at 20°C for 12 h; the temperature was then returned to 25°C. Disease severity was evaluated 5 and 10 (experiment 1) and 5 (experiment 2) days after inoculation by rating the first two leaves: 0 = no visible symptoms; 1 = small lesions (1 mm); 2 = intermediate lesions (3 to 5 mm); 3 = intermediate lesions with partial necrosis of the leaf; and 4 = total collapse of the leaves. Disease rating for each plant was the average rating of the two leaves.

Table 2. Response of melofon breeding lines, muskmelon cultivars, and cucumber to inoculation with *Sphaerotheca fuliginea*[x]

Material	Race 1	Race 2
Melofon		
D17-2	13 c[y]	23 bc
D17-5	69 a	46 a
P6a-1	29 bc	34 ab
P6a-2	15 c	30 abc
P6a-3	23 bc	10 cd
P15a	41 b	30 abc
P20a	16 c	22 bc
P22a	20 c	14 cd
Muskmelon		
Ananas Yoqne'am	72 a	. . .[z]
Dulce	0 d	0 d
Noy Yizre'el	0 d	31 abc
Cucumber		
Bet Alfa	76 a	. . .[z]

[x] Data are mean values of the percentage of mildew coverage of leaves 1 to 3, 11 days after inoculation.
[y] Numbers in columns followed by the same letter are not statistically separated ($P \leq 0.05$).
[z] Not tested.

Table 3. Response of melofon breeding lines and muskmelon cultivars to inoculation with four races of *Fusarium oxysporum* f. sp. *melonis*[y]

Material	Race 0	Race 1	Race 2	Race 1,2
Melofon				
D17-2	0	0	75 b	. . .[z]
D17-5	0	0	100 a	60 bc
P6a-1	0	0	90 ab	46 c
P6a-2	0	0	100 a	53 bc
P6a-3	0	0	97 a	53 bc
P15a	0	0	100 a	56 bc
P20a	0	0	56 c	79 ab
P22a	0	0	92 a	50 c
Muskmelon				
Makdimon	0	0	77 b	47 c
Hemed	0	100	0 d	35 c
En Dor	100	100	100 a	100 a

[y] Percentage of wilted seedlings, 14 days after inoculation. Numbers in columns followed by the same letter are not statistically separated.
[z] Not tested.

Table 4. Response of melofon breeding lines and muskmelon cultivars to inoculation with *Pseudoperonospora cubensis*[y]

	Experiment 1		Experiment 2
Material	5 days	10 days	5 days
Melofon			
D17-2	2.5	4.0	1.5
D17-5	2.2	2.5	3.2
P6a-1	1.2	1.5	0.9
P6a-2	1.0	1.0	1.4
P6a-3	0.7	1.4	0.5
P15a	2.1	1.6	1.8
P20a	2.2	2.2	3.0
P22a	0.7	0.4	0.6
Muskmelon			
Galia	1.0	1.3	1.0
Dulce	1.0	1.3	1.2
Ananas Yoqne'am	2.7	4.0	2.9
Noy Yizre'el	0.4	1.1	0.7
Significance of genotype effect[z]	<0.001	<0.001	<0.001

[y] Evaluation of responses of the first two leaves to the disease was obtained according to 1 to 4 scale: 0 = no visible symptoms; 1 = small lesions (1mm); 2 = intermediate lesions (3 to 5mm); 3 = intermediate lesions to partial necrosis of the leaves; 4 = total collapse of the leaves.
[z] Kruskal-Wallis one-way analysis of variance.

Sudden wilt. Four melofon breeding lines, one experimental hybrid and two commercial muskmelon cultivars, Gala and Arava, used as controls, were planted (four replicates with 10 plants each) in three field trials: the first in June, the second in July, and the third in August of 1993, in different plots in the Arava valley with a history of sudden wilt. In contrast to other commercial fields in this area, the experimental plots were not fumigated with methyl bromide prior to planting. At the stage of full fruit size and fruit maturation, the severity of plant collapse in each plot was visually evaluated: 0 = no collapse; 1 = initial wilting; 2 = collapse of ~50% of the plants; 3 = collapse of ~75% of the plants; and 4 = total collapse of all plants.

Statistical analysis. All inoculation tests for each pathogen were conducted at least twice. For powdery mildew and Fusarium wilt the results of different trials resembled each other, so the results of one representive trial are given. Significant ($P \leq 0.05$) differences in each category tested are presented by Duncan's multiple range test (Tables 2 and 3). For downy mildew (Table 4), in which results are expressed by indices, the rating was analyzed by Kruskal-Wallis one-way analysis using PROC Freq (17). For sudden wilt (Table 5), Friedman two-way analysis was conducted using PROC Rank followed by PROC GLM of SAS (16).

RESULTS

Powdery mildew. No resistance to either race of *Sphaerotheca fuliginea* was detected among the lines evaluated; however, disease severity was generally moderate (Table 2). Only line D17-5 was as susceptible to race 1 as the muskmelon and cucumber controls.

Fusarium wilt. All melofon breeding lines were resistant to Fusarium races 0 and 1 but susceptible to races 2 and 1,2 (Table 3). Line P20a was less susceptible to race 2, but was the most susceptible to race 1,2 of all melofon lines tested.

Downy mildew. Significant genetic variation for the response to downy mildew was found in the two experiments conducted. Melofon lines showed various responses to inoculation with downy mildew (Table 4). The most tolerant melofon lines were P22a and P6a-3. In the case of P22a there was a slight decline (0.7 → 0.4) in disease index in the second recording date due to vigorous vegetative growth that was not accompanied by an increase in disease symptoms.

Sudden wilt. Different responses to sudden wilt were observed in the various melofon breeding lines under field conditions (Table 5); these were significant. Line D17-5 was highly susceptible, whereas line P6a-1 in three experiments and line P20a in two experiments were relatively tolerant (index <1). The experimental hybrid P6a-1 × D17-5 was as tolerant as the maternal parent in two field trials.

DISCUSSION

Melofons are pickling melons (*C. melo*) developed as a new item for the processing industry, suitable for mechanical once-over harvest. Among the four cucurbit diseases tested in the present study, Fusarium wilt is specific to melons, whereas the foliar diseases (powdery and downy mildews) are common in both melons and cucumbers. In Israel, sudden wilt is common in melons but also occurs in watermelons.

Although no complete resistance to the two races of powdery mildew was found in melofon breeding lines, disease severity was relatively low; it ranged between 10 and 46% for both races, compared with 72% for the susceptible melon cv. Ananas Yoqne'am and 76% for cucumber cv. Bet Alfa. High disease incidence (69% leaf area covered with powdery mildew) was evident in only one line, D17-5, following inoculation with powdery mildew race 1 (Table 2). This particular line was also the most susceptible to race 2 (46%). The high susceptibility of this line to powdery mildew is not surprising, as it was derived from a cross in which the super-susceptible line P202 (1,14) was the donor parent. D17-2, derived from the same cross, was significantly less susceptible than D17-5, probably because it was selected early, at F2, with more resistance available from the P&G (Table 1) parent.

Downy mildew is a limiting factor in cucumber and melon production in Israel. In several field experiments in which the horticultural performance of melofons was compared with that of cucumbers serving as controls, we observed that, generally, melofons exhibited symptoms later and disease development was much slower than in cucumbers. The present data revealed that melofon lines P6a-3 and P22a were only slightly infected by downy mildew. Possible commercialization of tolerant melofon lines, identified in growth chamber trials, may contribute to reduction in pesticide usage in the pickling industry. This melofon germ plasm may also be used for introducing tolerance to downy mildew into sweet melon breeding programs, along with other characteristics such as yield concentration and other disease resistances.

All melofon breeding lines tested exhibited resistance to *F. o.* f. sp. *melonis* races 0 and 1. Different levels of susceptibility were evident following inoculation with races 2 and 1,2. Recently, we have shown (13) that the Fusarium-resistant gene *Fom-2* conferring resistance to races 0 and 1 exists in Freeman's cucumber, an oriental pickling melon. Since some of the melofon germ plasm is genetically close to Freeman's cucumber, the same gene may be responsible for the Fusarium resistance in the lines tested in the present work.

To the best of our knowledge, no genetic resistance is available against sudden wilt as it occurs in Israel. The breeding line P6a, highly tolerant to sudden wilt, may offer germ plasm for breeding for commercial melon cultivars tolerant to this disease. Preliminary results (Table 5) showing no collapse in F1 plants of a cross between resistant and susceptible lines, indicate that tolerance may have a dominant nature. It should be emphasized that the same germ plasm may differ in its response to the disease under different conditions and in different growing seasons. Therefore, the inheritance of disease resistance should be further investigated. Previous studies conducted in Israel concerning the sudden wilt phenomenon (2,15,19) used susceptible material only. Genetic material with varied levels of tolerance to sudden wilt may provide a tool for basic research. Testing pathological factors such as pathogenicity and colonization of plants by various microorganisms, as well as plant performance under various environmental stresses, may lead to a better understanding of the factors causing this disease.

As was evident in field observations,

Table 5. Sudden wilt of melofon breeding lines at fruit maturity under field conditions in the Arava Valley, spring and autumn 1993

Material	Sudden wilt index[x]		
	June 1993	September 1993	November 1993
Melofon			
D17-5	3.7	4.0	2.3
P6a-1	1.0	0.5	0.6
P15a	2.5	...[y]	...[y]
P20a	0.5	3.2	0.0
P6a-1 X D17-5(F1)	...[y]	0.25	0.7
Muskmelon			
Gala	2.7	3.0	1.7
Arava	2.5	2.7	1.1
Significance of genotype effect[z]	0.0283	0.0001	0.0186

[x] Evaluation of sudden wilt was obtained according to a 1 to 4 scale: 0 = no collapse; 1 = initial wilting (overview of the plot); 2 = collapse of 50% of plants; 3 = collapse of 75% of plants; 4 = total collapse of all plants.

[y] Not tested.

[z] Friedman two-way analysis of variance.

some melofon lines have exhibited low susceptibility to powdery mildew and downy mildew, and tolerance to sudden wilt. In the present study, conducted mostly in growth chambers, in which the conditions were more conducive to disease development, resistance responses were evident in breeding lines based on oriental pickling melon genetic background. Overall, lines derived from the cross P6 are the most promising material for breeding use in respect to disease resistance. P202, used in some crosses for improving yield concentration, is extremely susceptible to powdery mildew (14), downy mildew, and soil-borne pathogens (R. Cohen, *unpublished*). Indeed, the more susceptible lines found in this study included genes from P202 (Table 1).

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