Differential Expression of Resistance to Powdery Mildew Incited by Race 1 or 2 of *Sphaerotheca fuliginea* in *Cucumis melo* Genotypes at Various Stages of Plant Development

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Thirty-nine genotypes of *Cucumis melo* (plant introduction entries, open-pollinated cultivars and F_1 hybrids) were evaluated for resistance to powdery mildew under either natural field conditions or artificial inoculation in growth chambers at the cotyledonary stage and the 2-true-leaf stage. Results confirmed that susceptibility in cotyledons was not necessarily associated with susceptibility in either true leaves in growth chambers or adult plants in the field. However, resistance at the 2-true-leaf stage in growth chambers was highly correlated with resistance of field-grown plants. Results also showed that 20 muskmelon genotypes resistant to race 1 at the cotyledonary stage were also resistant at the 2-leaf-stage and as adult plants in the field. The same was true for ten genotypes with race 2 inoculations. Because muskmelon genotypes expressing resistance in cotyledons were also resistant in true leaves in growth chambers or the field, the use of plants at the cotyledonary stage is recommended for screening for powdery mildew resistance caused by race 1 or race 2 of *S. fuliginea*. When cotyledons are susceptible, screening should be done at the 2-true-leaf stage.

KEY WORDS: Melon; *Cucumis melo*; powdery mildew; *Sphaerotheca fuliginea*; genetics; screening for resistance; breeding.

INTRODUCTION

Powdery mildew caused by *Sphaerotheca fuliginea* (Schlecht. ex Fr.) Poll. is a devastating disease of cucurbits with a wide geographical distribution (13,18). In Israel the fungus attacks *Cucumis melo*, *Cucumis sativus*, *Citrullus lanatus*, *Cucurbita pepo*, *Cucurbita maxima* and *Lagenaria vulgaris* (5), causing considerable damage in the open field and greenhouse; fungicide treatments are frequently required to save the crops. *S. fuliginea* was found to produce sexual fruiting bodies only rarely on muskmelons in nature (4,8,10) and infection *via* ascospores was never established (Y. Cohen and H. Eyal, unpublished). The other species of powdery mildew in cucurbits, *Erysiphe cichoracearum* DC. ex. Merat (13), has never been recorded in cucurbits in Israel.

Three races of *S. fuliginea* and many genes for resistance to these races have been recorded (1,6,9,13,14,16,17,20,21). In Israel only races 1 and 2 were reported (5). McCreight *et al.* (14) reported on seven not previously described genes in muskmelon against *S. fuliginea*: one (in line 92417) against race 1 and six (in lines PI414723 and

Received Jan. 5, 1995; received in final form March 24, 1995.

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WMR29) against race 2. Cohen and Cohen (3) and Kenigsbuch and Cohen (11) reported a new gene against race 2 (*Pm-6* in PI124111F).

Environmental and economic considerations emphasize the need for development of commercially acceptable resistant cultivars. Screening for resistance is a laborious task which breeders wish to minimize without affecting the reliability of the results. A laboratory bioassay with cotyledons (22) or leaf discs (2) is usually preferable to greenhouse evaluation of resistance, and the latter is preferable to field tests. However, little is known on the relationships between a cultivar's response to mildew attacks in small-scale bioassays (cotyledons, leaf discs) and that in the greenhouse and/or the field.

In this study we compared the reaction of 39 genotypes of muskmelon to *S. fuliginea*, race 1 or race 2, at the cotyledonary stage of development, at the 2-true-leaf stage, and in small plots in the field. In conclusion, a procedure is recommended for screening of resistant genotypes.

MATERIALS AND METHODS

Thirty-eight genotypes of muskmelon (*Cucumis melo* L.) were tested with race 1 and 39 genotypes with race 2.

Cotyledon tests

Ten plants were grown in 10-cm-diam plastic pots filled with a 3:1:1 (v/v/v) mixture of pasteurized sandy loam, peat and vermiculite in the greenhouse (19–34°C). One week after germination when cotyledonary leaves were 3-4 cm long, pots were transferred to growth chambers for inoculation (23°C, 12:12, L:D photoperiod, 120 $\mu E.m^{-2}.s^{-1}$, 50–70% r.h.).

Two-leaf plant tests

Two plants were grown in 15-cm-diam pots as above, two pots per entry. At ~3 weeks after sowing, when the second true leaf was fully expanded, plants were transferred to growth chambers for inoculation, as above. Inoculated plants were kept in growth chambers for disease development.

Field tests

Ten-m^2 plots with 20 plants in each were grown in two fields located on the Bar-Ilan University campus. Plants were sown in early May, inoculated in mid June and evaluated for mildew development in early August.

Fungus

Two races of *S. fuliginea* were used: race 1 and race 2. Race 1 was grown and maintained on *C. melo* cv. 'Ananas Yokneam' (AY, universal susceptible) and race 2 on cv. PMR-45 (resistant to race 1, susceptible to race 2) in separate chambers at 20°C (12:12, L:D). Two field isolates of race 1 (1A and 1B) and two isolates of race 2 (2A, 2B) were used. Isolate 1A was collected from a cucumber field in the coastal plain in 1987, and isolate 1B from a summer squash field in the central coastal plain in autumn 1988. Isolate 2A was collected from spontaneously infected muskmelon plants in our greenhouse in summer 1986, and isolate 2B from a cucumber field in the central coastal plain in summer 1987.

Inoculation

AY plants infected with race 1 or PMR-45 plants infected with race 2 were used 8 days after inoculation. Old conidia were blown away from donor plants one day before being used. For inoculation, donor plants were shaken above the recipient plants so that approximately 50–150 conidia/cm^2 settled on glass slides placed on the bench between the inoculated plants.

Assessment of disease development

Disease development in cotyledonary plants and 2-leaf plants was examined with the naked eye 8 days after inoculation, and that in the field was examined 50 days after inoculation. In potted plants in growth chambers, disease was assessed according to the following scale: 0, no disease symptoms apparent; 1, one or two fungal colonies per leaf; 2, 3–10 discrete colonies per leaf; 3, most or all of the leaf surface covered with coalescing fungal colonies. When fungal colonies were associated with host necrosis, the designation 'HR' (hypersensitive response) was given. In the field, the following scale was used: 0, no disease symptoms apparent; 1, crown leaves covered with sparsely sporulating mildew on both surfaces; 2, crown leaves desiccated due to the mildew, middle leaves covered with numerous discrete colonies on both surfaces, and younger leaves mostly disease-free; 3, crown and middle leaves heavily desiccated due to the mildew, and youngest leaves showing discrete development of colonies.

Statistical analysis

Tests with cotyledonary plants and 2-leaf plants were conducted with each of the two isolates of each race (a total of eight experiments) with ten and four plants per entry genotype, respectively, in each experiment. Because the reaction of the cultivars to isolates 1A and 1B was similar and that to isolates 2A and 2B was similar in either cotyledonary or 2-leaf plants, data from the two isolates of each race were pooled and mean disease severity data are presented for each race. Field tests were conducted once with race 1 (isolate 1B) and once with race 2 (isolate 2B). The correlation between the amount of disease developed on cotyledonary plants compared with 2-leaf plants, and between either one compared with the amount of disease developed in the field was estimated using Pearson's correlation coefficients.

For comparison purposes, we classified the muskmelon genotypes tested into six groups according to their resistance or susceptibility to the mildew [with disease severity <1.0 = resistant (R) and disease severity ≥ 1.0 = susceptible (S)] in cotyledonary plants, 2-true-leaf plants and field-grown plants, respectively: RRR, RRS, RSS, SSS, SSR and SRR. These three-letter designations were termed 'resistance formulae'.

RESULTS

Muskmelon genotypes – were divided into three groups according to their response to race 1 inoculation: 20 genotypes expressed the RRR formula, eight genotypes – the SRR, and nine genotypes – the SSS (Table 1). Among the genotypes exhibiting the RRR formula, only PI124112, Male-sterile #1, PMR-5, PMR-6, Perlita, Dulce, Rio Gold and Tam Uvalde were symptomless at 8 days after inoculation. The other RRR entries showed varying degrees of fungal development and/or early necrosis (HR) (Table 2).

TABLE 1. Distribution of resistance formulae among muskmelon genotypes in response to inoculation with *Sphaerotheca fuliginea*

Resistance formula[z]	Number of genotypes	
	Race 1	Race 2
RRR	20	10
RRS	0	1
RSS	0	0
SSS	9	20
SSR	0	2
SRR	8	3

[z] R = resistant (disease severity <1.0); S = susceptible (disease severity ≥1.0).
First letter, response of cotyledonary plants at 23°C; second letter, response of true leaves in 2-leaf plant at 23°C; third letter, response of field-grown plants (May – August).

A similar analysis conducted with race 2 inoculations showed that muskmelon genotypes could be divided into five response groups: 20 genotypes expressed the SSS formula, 10 – the RRR, 3 – the SRR, 2 – the SSR, and 1 (the F_1 hybrid Sucdor) – the RRS formula (Table 1). We found one entry (PI124112) that remained symptomless after inoculation with race 2 (Table 2). All RRR-reacting entries exhibited some mycelial development (graded 0.25–0.5) and/or some early necrosis (HR) in growth chambers. Unlike the reaction to race 1, to which most entries were resistant at all stages of development, reaction to race 2 in most entries was susceptible.

Table 3 presents the correlation coefficients between disease severities on the various plants for each race. Disease severity in cotyledons inoculated with race 1 was moderately correlated (r=0.6442) with that on true leaves or with disease development in the field (r=0.6339). On the other hand, disease severity in true leaves was strongly correlated (r=0.9239) with that seen in the field.

Only eight entries were resistant to both races at all developmental stages (the RRR resistance formula); five of them (PI124111F, MR1, PI124112, PMR-5, PMR-6) are widely used for breeding purposes. In PI124111F, the genes *Pm-3* and *Pm-6*, which confer resistance against race 1 and race 2, respectively, are independently inherited (11). In PI124111, *Pm-5* – which confers resistance against race 1, and *Pm-4* – which confers resistance against race 2, are also independently inherited (12). In PMR-6, *Pm-1* confers resistance against race 1 and *Pm-1* + *Pm-2* (with modifiers) are responsible for conferring resistance against race 2 (1,6,12).

TABLE 2. Severity of powdery mildew caused by race 1 and race 2 of *Sphaerotheca fuliginea* on genotypes of *Cucumis melo*

	Entry	Origin	Mean disease severity (0–3 visual scale)					
			Cotyledonary plants[z]		2-leaf plants[y]		Field-grown plants[x]	
			Race 1	Race 2	Race 1	Race 2	Race 1	Race 2
	PI's and Breeding lines							
1.	PI124111F	Own	0.5 CH[w]	0.5	0	0	0	0
2.	MR1	USDA	0 5	0	0	0	0	0
3	PI124112	USDA	0	0 25	0	0.1	0	0
4	Male-sterile #1	USDA	0	0 5 HR[v]	0	0 2 HR	0	0
5.	PMR-5	USDA	0	0 HR	0	0.6 HR	0	0
6	PMR-6	USDA	0	0 HR	0	0	0	0
	Open-pollinated cultivars							
7.	Ananas Yokneam	Hazera	3	3	3	2.9	3	3
8.	Charantais-T	INRA	2	3	3	2.9	3	3
9	Vedrantais	Vilmorin	2 5	2 5	2 5	2 7	3	3
10	Delicious 51	Petoseed	2 5	2 5	3	2.9	2	3
11	Top Mark	Asgrow	2 5	2 5	2	2.5	3	2
12.	Golden Perfection	USDA	2.5	2 5	2 5	nt	1	nt
13	En Dor	Hazera	2.5	2 5	0	2.8	0	2
14.	Gulfstream	Hollar	0 5	2 5	0	1 3	0	1
15	Gulfcoast	Hollar	0.5	3	0 3	1.7	0	1 5
16.	Hale's Best 36	Hollar	2	3	3	3	2	2
17.	Mainstream	Petoseed	1 5	2	0	seg[u]	0	seg
18.	Doublon	INRA	1	3	2.5	3	1.5	3
19.	PMR-45	Petoseed	1	2 5	0	2 5	0	3
20	Georgia 47	USDA	0.25 HR	0.75 HR	0	0 5 HR	0	0
21	Edisto 47	Petoseed	0 5	2	0	2.2	0	0
22.	Cinco	USDA	0.75	1.5 HR	0	0 3	0	0
23	Perlita	Petoseed	0	2 HR	0	0.4 HR	0	0
24	Dulce	USDA	0	0.5 HR	0	0 1 HR	0	0
25	Rio Gold	USDA	0	nt	0	1 3 seg	0	1 5
26	Tam Uvalde	Hollar	0	0 25	0	0 3	0	0
27	Green Ice	USDA	1	1	nt	0 9	nt	0.5
28	Noy Yisrael	Hazera	nt	2	nt	3	nt	3
	F_1 hybrid cultivars							
29	Aragon	Asgrow	0 25	2.5	0.1	2	0	3
30.	Hiline	Asgrow	0 25 HR	1.5 HR	0	1.6	0	1
31	Galia	Hazera	2	3	0	3	0	3
32	Prior	Vilmorin	1 HR	1 5 HR	0.7 HR	1 6	0 5	3
33.	Sucdor	Vilmorin	0 5 HR	0.75 HR	0 5 HR	0.9	0	2
34	Charity Ball	Sakata	0.1 HR	2.5	0	1.4	0	0.5
35	Emerald Pearl	Sakata	2 5	2	0 2	3	0	3
36	Hy Mark	Petoseed	0.5 HR	1.5 HR	0	1.7	0	1
37	Mayan Sweet	Sun Seeds	2 5	0 5 HR	0 5	0 1	0	0
38	Spanish Rocket	Fito Femillas	1.5	2.5	3	3	2	2
39.	Sky Rocket	Known-You	nt	2	nt	3	nt	3
40	Sharon	Hazera	2	nt	0	nt	0	nt

[z]Results from two experiments with 20 plants in each.
[y]Results from four experiments with 10 plants in each
[x]Results from a single field experiment in a 10 m^2 plot, with 20 plants per entry.
[w]CH = early chlorosis
[v]HR = early necrosis
[u]seg = segregating for resistance.
nt = not tested.

TABLE 3. Pearson's correlation coefficients (and significance level, in parentheses) for disease severity of powdery mildew incited by *Sphaerotheca fuliginea* in cotyledonary plants, 2-true-leaf plants and field-grown plants of muskmelon genotypes

Race	Category	Correlation coefficient	
		2-true-leaf plants	Field-grown plants
1	Cotyledonary plants	0.6442	0.6339
(n=37)		(0.0001)	(0.0001)
	2-true-leaf plants		0.9239
			(0.0001)
2	Cotyledonary plants	0.8461	0.7119
(n=36)		(0.0001)	(0.0001)
	2-true-leaf plants		0.8613
			(0.0001)

DISCUSSION

Attempts to correlate powdery mildew development at the various stages of muskmelon plant development were made as early as 1942, by Pryor and Whitaker (16). They observed that resistance against race 2 of *E. cichoracearum* (probably *S. fuliginea*) in young plants in the greenhouse was not always associated with resistance in the field. Nevertheless, the greenhouse method of eliminating susceptible lines was more efficient and reliable than field trials. Whitaker and Pryor (22) later examined the development of *E. cichoracearum* in the greenhouse and the field in F_3 families segregating for resistance. They found that in the field susceptible progenies were attacked with equal severity on leaves and stems, whereas in resistant progenies fungal colonies were present only on leaves. In the greenhouse, there was a high degree of correlation between disease severity on leaves, cotyledons and stems of the same plant.

Ferriere and Molot, working with race 2 of *S. fuliginea*, observed (7) that muskmelon cotyledons are so susceptible to the fungus that they can not be used for screening for resistance. They concluded that leaf 3 provided the best information on the resistance or susceptibility of genotypes to the disease.

Takada and co-workers (19,20), studying progenies of the powdery mildew susceptible cv. 'Pearl' and the resistant cvs. 'Georgia 47', 'Rio Gold' and 'Wescan', concluded that inoculation of the first true leaf of 2-true-leaf plants was effective for screening resistant individuals, and that cotyledon inoculation was inferior to using leaf 1.

In the present study we observed a high correlation between susceptibility or resistance to powdery mildew in the first two true leaves and susceptibility or resistance in adult plants growing in the field. We found that 28 muskmelon entries that were resistant to race 1 and 13 entries that were resistant to race 2 at the 2-true-leaf stage in growth chambers at 23°C were also resistant to the respective race in the field. In contrast, 9 and 20 entries that were susceptible to race 1 and race 2, respectively, at the 2-leaf stage were susceptible in the field. There were three exceptions to this rule, all with race 2: one F_1 cultivar (Sucdor) was resistant indoors and susceptible in the field, and two (Edisto-47 and Charity Ball, F_1) were susceptible at the 2-leaf stage and resistant in the field.

For simplicity, we constructed a 'resistance formula' for each entry to describe its reaction to infection with the powdery mildew pathogen at each of the three development stages tested. The RRR formula (plants resistant in cotyledons, 2-leaf stage, and the field) was twice as common with race 1 as with race 2, whereas the SSS formula was twice as common with race 2 as with race 1, thus reflecting the dominant nature of the genes conferring resistance against race 1 (*Pm-1, Pm-3, Pm-5*) (6,9,12,14,15,18) as opposed to the partially dominant nature of the genes conferring resistance against race 2 (*Pm-2, Pm-4, Pm-6*) (1,4,6,11,12,15).

It also became apparent that resistance (but not susceptibility) in the cotyledons of cotyledonary plants was strongly associated with resistance in 2-leaf plants and in mature plants in the field. Thus, all 20 entries resistant to race 1 and ten of the 11 entries resistant to race 2 in cotyledons, were resistant at the later stages of plant development (resistance formula RRR). In contrast, susceptibility in cotyledons was equally associated with susceptibility or resistance to race 1 at later stages of plant development, and was mostly associated with susceptibility to race 2 in 2-leaf plants and in the field.

It is noteworthy that genotypes with resistance to race 2 are resistant to race 1 but not *vice versa*, except the F_1 Mayan Sweet – which is RRR with race 2 and SRR with race 1.

Based on these data, we conclude that screening muskmelon entries for resistance against powdery mildew at the cotyledonary stage of development is a useful procedure which would save time, labor and space. Entries expressing resistance at this stage will most probably express resistance in the field. Susceptibility in cotyledons will force the breeder to search for resistance at the 2-leaf stage.

We are currently expanding our study with segregating F_2 families in order to determine the validity of this recommended procedure in segregating progenies. In addition, we are attempting to adapt the multiple-pathogen screening technique developed for cucumber cotyledons (23), to include a droplet inoculation with a conidial suspension of *S. fuliginea*.

ACKNOWLEDGMENT

The authors acknowledge the useful assistance of Abraham Cohen of this department.

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