Inheritance of Resistance to Downy Mildew in *Cucumis melo* PI 124112 and Commonality of Resistance Genes with PI 124111F

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ABSTRACT

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The monoecious, downy mildew-resistant *Cucumis melo* PI 124112 was made homozygous for resistance by selfing and screening for seven generations and crossed with either the downy mildew-susceptible cultivar Ananas-Yokneam (AY) or the downy mildew-resistant breeding line PI 124111F (possessing the resistance genes *Pc1* and *Pc2*). F_1, F_2, BCs, and TC progeny plants were inoculated with *Pseudoperonospora cubensis* pathotype 3 and assessed for reaction type 8 days postinoculation. Progenies of the cross PI 124112 × AY segregated in a manner supporting the hypothesis that resistance in PI 124112 was conferred by two partially dominant genes. Progenies of the cross PI 124112 × PI 124111F segregated in a manner supporting the hypothesis that PI 124112 shares one common gene (*Pc1* or *Pc2*) with PI 124111F and another, newly assigned gene, *Pc4*. The lack of identity of the genes from these germ plasm lines means that the level of downy mildew resistance may be improved in newly developed muskmelon cultivars.

Additional keywords: Cucurbitaceae, genetics

Downy mildew, caused by the fungus *Pseudoperonospora cubensis* (Berk. and M. A. Curtis) Rostovzev is a major threat to cucurbit production in humid areas of the world (3,12). This fungus is classified into five pathotypes inciting downy mildew in six genera of Cucurbitaceae (20). Chemical control of the disease with protectant and/or systemic fungicides is widely practiced. However, protectants are now in a process of reregistration, and the most efficient systemic fungicide, metalaxyl, lost efficacy because of the prevalence of resistant strains of the fungus (15). Resistant cultivars, therefore, may serve as a useful means of combatting the disease.

In muskmelons (*Cucumis melo* L.), several sources of resistance to downy mildew are known including: PI 124111 (4), PI 124111F (6), MR-1 (17), PI 124112 (3,10,16), PI 414723 (9), PI 164323 (3), and PI 165449 (3). The first three plant introductions were extensively studied by Cohen et al (4–6,8,11), Thomas et al (17–19), and Epinat and Pitrat (9). Although Cohen and associates (4,11) and Thomas et al (19) reported on two incompletely dominant genes (*Pc1* and *Pc2*) conferring resistance in PI 124111F and MR-1, Epinat and Pitrat reported on four factors responsible for resistance in MR-1 and PI 124112 (9). Pitrat (13) designated the partial resistance gene in PI 414723 as *Pc3*.

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In spite of the fact that PI 124112 was discovered and used for resistance breeding in the early 1940s (10), no genetic studies are available on the inheritance of resistance to downy mildew in this source except that of Epinat and Pitrat (9), who used leaf disks and pathotype 3 of the fungus (14) for their study. These authors claimed that four genes appeared to control resistance in PI 124111 when data were analyzed according to the biometrical method of Mather and Jinks (see 9), but two genes appeared to control this resistance when they used the grouping method of Thomas et al (19).

In this article, we report on the inheritance of resistance to pathotype 3 of *P. cubensis* in *C. melo* PI 124112 when crossed with the susceptible cultivar Ananas-Yokneam (AY) and on the commonality of resistance genes between PI 124112 and PI 124111F.

MATERIALS AND METHODS

Germ plasm. The monoecious resistant parent PI 124112 was originally obtained in 1979 from G. Sowell, Jr., U.S. Department of Agriculture, Experiment, GA. PI 124112 is characterized by long cotyledon leaves, serrated true leaves, and long fruits. PI 124112 was made homozygous-resistant to pathotype 3 of *P. cubensis* and races 1 and 2 of *Sphaerotheca fuliginea* (Schlechtend.: Fr.) Pollacci (inciting powdery mildew) by seven generations of selfing and selection (Y. Cohen and H. Eyal, *unpublished data*). Unlike PI 124111F (6), it is susceptible to races, 0, 1, 2, and 1-2y of *Fusarium oxysporum* Schlechtend.: Fr. f. sp. *melonis* W. C. Snyder & H. N. Hans. (R. Kassas-Etinger and Y. Cohen, *unpublished data*). The downy mildew-susceptible parent, AY, was purchased from Hazera Seed Company, Haifa, Israel. PI 124111F was from our germ plasm stock (6).

Crosses. The following crosses were made in the greenhouse: PI 124112 × AY F_1, AY × PI 124112 E_1, PI 124112 × AY F_2, AY × PI 124112 E_2, (PI 124112 × AY) × PI 124112 BC_R, (AY × PI 124112) × PI 124112 BC_R, (PI 124112 × AY) × AY BC_S, (AY × PI 124112) × AY BC_S, PI 124112 × PI 124111F F_1, PI 124112 × PI 124111F F_2, and (PI 124112 × PI 124111F) × AY TC (testcross).

Fungus, inoculation, and evaluation of resistance in progenies. A colony of *P. cubensis* (pathotype 3, collected from a muskmelon field in the Coastal Plain of Israel in 1988) was maintained on AY by repeated inoculations in growth chambers at 20 C. Test plants were grown in the greenhouse (18–26 C) in peat/vermiculite (1:1, by volume) in 56-well polystyrene trays (one plant per 5 × 5 cm well) and inoculated when the first two true leaves were fully developed (about 25 days after sowing). Inoculation was done by spraying the adaxial leaf surfaces with a sporangial suspension containing about 10,000 sporangia per milliliter. Inoculated plants were kept in a dew chamber at 18 C in the dark for about 20 hr and then returned to the greenhouse. Plants were again placed in a dew chamber (as above) on the seventh night to allow for fungal sporulation. The following reaction types (RT), as expressed on the eighth day after inoculation, were used to identify resistance to downy mildew (11): 1 = susceptible reaction typical of AY (10- to 15-mm irregular lesions with profuse sporulation that may extend beyond the apparent margins of the lesions); 2 = moderately resistant reaction (type 1 lesions mixed with type 3 lesions); 3 = moderately resistant reaction (3- to 4-mm, irregular to circular chlorotic lesions with water-soaked margins beneath and sparse sporulation); and 4 = resistant reaction typical of PI 124111F and PI 124112 (1-mm, circular chlorotic lesions with necrotic centers and water-soaked margins beneath and limited or no apparent sporulation).

Leaf 1 and leaf 2 (from the stem base)

were evaluated separately using the RT index just described (11). The assignment of a separate RT to each leaf resulted in a two-digit numerical classification in which the first digit represented the RT for leaf 1 (from the stem base) and the second digit represented the RT for leaf 2. Reaction types were categorized as follows: 11 = susceptible; 12, 13, 22, 23, 24, and 33 = moderately resistant; and 34 and 44 = resistant (11). Plants with RT 14 were not detected. Reaction types 12 and 13 were considered moderately resistant rather than susceptible (19), because they never appeared on the susceptible parent AY.

Data analysis. Two populations per pedigree were tested. Parents and F_1 plants were included in each inoculation test for comparison purposes. Segregation ratios of F_2 and BC populations were tested for goodness-of-fit to theoretical ratios with chi-square tests. E_1 and E_2 represent pedigrees of reciprocal crosses of F_1 and F_2, respectively.

RESULTS

Inheritance of resistance in PI 124112. The susceptible parent plants all had an RT index of 11. About one-third of the resistant parent plants showed the highest resistance level, RT44, and about two-thirds showed a RT index of 34. The F_1 (AY × PI 124112) and the reciprocal E_1 (PI 124112 × AY) plants showed a moderate level of resistance to downy mildew with about 76% showing RT 23, 13% showing RT 22, and 11% showing RT 24 (Table 1).

The moderate level of resistance in F_1 and E_1 plants indicated that resistance in PI 124112 was partially dominant. F_1 plants did not differ from E_1 plants in RTs (Table 1), indicating that no cytoplasmic (maternal) factors were involved in the expression of resistance to downy mildew. The F_2 and E_2 populations segregated 1:14:1 susceptible/moderately resistant/resistant (Table 1), supporting the hypothesis of two partially dominant genes conferring resistance against downy mildew in PI 124112.

Progenies of the backcrosses of F_1 to the susceptible parent AY segregated 1:3 susceptible/moderately resistant, whereas the backcross progeny of F_1 to the resistant parent PI 124112 segregated 1:3 resistant/moderately resistant (Table 1). Thus, the backcross data further supported a digenic partial dominant inheritance of resistance to *P. cubensis* in PI 124112.

Commonality of genes for resistance in PI 124112 and PI 124111F. Both resistant parents, PI 124112 and PI 124111F, produced resistant RTs after inoculation with *P. cubensis* (Table 2). PI 124111F was previously shown to carry *Pc1* and *Pc2*, two partially dominant genes, when crossed with the susceptible parents AY (19) or WI998 (11). F_1 and F_2 progenies were produced between these two resistant parents to examine whether or not *Pc1* and *Pc2* are allelic with the two resistance genes of PI 124112.

The F_1 progeny plants (PI 124112 × PI 124111F) all were highly resistant to *P. cubensis* (Table 2). The F_2 offspring plants segregated 13:3 resistant/moderately resistant with no susceptible individuals detected (81% of the plants produced RTs 34 and 44 and 19% produced RTs 23, 24, and 33), suggesting that one gene in both parents is allelic (common) and the other gene is distinct but weakly linked to the first.

The testcross plants (PI 124112 × PI 124111F) × AY all were moderately resistant with a slight skewing toward susceptibility (plants with RTs 11–13 were not observed) compared with F_2, supporting again the hypothesis that one of the two resistance genes in PI 124112

Table 1. Segregation for downy mildew resistance in a cross between the resistant PI 124112 and the susceptible Ananas-Yokneam (AY) muskmelons

		Number of plants										Expected ratio[b]		
		Reaction type (RT)									Total			
Pedigree	Generation	11	12	13	22	23	24	33	34	44	S:MR:R[a]	S:MR:R[a]	χ^2	*P*
AY	P_1	53	...	...	...	...	...	...	...	...	...	...	...	...
PI 124112	P_2	...	...	...	...	...	...	...	42	19	...	...	...	...
$P_1 \times P_2$	F_1	...	...	...	8	68	13	...	...	...	...	...	...	...
$P_2 \times P_1$	E_1	...	...	...	16	75	10	...	...	...	...	...	...	...
$P_1 \times P_2$	F_2	18	41	24	32	68	16	4	9	4	18:185:13	1:14:1	1.603	0.45
$P_2 \times P_1$	E_2	16	44	22	29	71	11	1	8	3	16:178:11	1:14:1	1.059	0.59
Homogeneity		...	...	...	...	...	...	...	...	...	...	...	0.12	0.94
Combined		34	85	46	61	139	27	5	17	7	34:363:24	1:14:1	2.527	0.28
$F_1 \times P_1$	BC_S	23	30	2	9	8	...	...	...	...	23:49:0	1:3:0	1.85	0.17
$E_1 \times P_1$	BC_S	15	12	2	10	7	4	...	...	...	15:35:0	1:3:0	0.66	0.42
Homogeneity	BC_S	...	...	...	...	...	...	...	...	...	...	...	0.052	0.97
Combined	BC_S	38	42	4	19	15	4	...	...	...	38:84:0	1:3:0	2.45	0.12
$F_1 \times P_2$	BC_R	...	...	...	7	24	12	...	8	1	0:43:9	0:3:1	1.64	0.20
$E_1 \times P_2$	BC_R	...	...	...	5	22	14	...	10	3	0:41:13	0:3:1	0.02	0.88
Homogeneity	BC_R	...	...	...	...	...	...	...	...	...	...	...	0.692	0.71
Combined	BC_R	...	...	...	12	46	26	...	18	4	0:84:22	0:3:1	1.01	0.31

[a]S = Susceptible (RT 11); MR = moderately resistant (RTs 12, 13, 22, 23, 24, 33); R = resistant (RTs 34 and 44).
[b]Based on a model of two complementary incompletely dominant genes for resistance.

Table 2. Segregation for downy mildew resistance in a cross between the two resistant muskmelons, PI 124112 and PI 124111F

		Number of plants										Expected ratio[b]		
		Reaction type (RT)									Total			
Pedigree	Generation	11	12	13	22	23	24	33	34	44	S:MR:R[a]	S:MR:R[a]	χ^2	*P*
PI 124112	P_1	...	...	...	...	...	...	...	16	26	...	...	...	...
PI 124111F	P_2	...	...	...	...	...	...	...	26	13	...	...	...	...
$P_1 \times P_2$	F_1	...	...	...	...	...	...	...	24	46	...	...	...	...
$P_2 \times P_1$	F_2	...	...	...	...	18	59	8	187	178	0:85:365	0:3:13	0.006	0.96
$F_1 \times AY$[c]	TC	...	...	...	14	100	81	13	...	...	0:208:0	...	...	...
$F_1 \times P_1$	BC_1	...	...	...	...	...	...	...	67	24	...	...	...	...
$F_1 \times P_2$	BC_2	...	...	...	...	...	...	...	73	10	...	...	...	...

[a]S = Susceptible (RT 11); MR = moderately resistant (RTs 12, 13, 22, 23, 24, 33); R = resistant (RTs 34 and 44).
[b]Based on a model of two complementary incompletely dominant genes for resistance.
[c]Ananas-Yokneam muskmelon.

Table 3. Five hypothetical models and expected segregation ratios for resistance to downy mildew caused by *Pseudoperonospora cubensis* in a cross between two resistant parents, PI 124112 and PI 124111F

	Genes present		F_1		F_2	
					Expected	Observed
Model	PI 124111F	PI 124112	Expected	Observed	S:MR:R[a]	S:MR:R[a]
1	*Pc1 Pc2*	*Pc1 Pc2*	R	R	0:0:1	0:3:13
2	*Pc1 Pc2* Noncomplementary	*Pc4 Pc5*	MR	R	1:224:31	0:3:13
3	*Pc1 Pc2* Complementary	*Pc4 Pc5*	R	R	1:92:163	0:3:13
4	*Pc1 Pc2* Noncomplementary	*Pc1 Pc4*	MR	R	0:9:7	0:3:13
5	*Pc1 Pc2* Complementary	*Pc1 Pc4*	R	R	0:5:11	0:3:13

[a] R = resistant; MR = moderately resistant; S = susceptible.

is not allelic with one of the pairs of resistance genes in PI 124111F. As expected, the BC progeny plants of F_1 (PI 124112 × PI 124111F) to either PI 124112 or PI 124111F all were highly resistant to *P. cubensis* (Table 2).

DISCUSSION

The symbols *Pc1* and *Pc2* were assigned to the two incompletely dominant genes conferring resistance against *P. cubensis* in the *C. melo* breeding line PI 124111F (4,11). MR-1, which is very closely related to PI 124111F, also carries these genes (17,19).

In this report, we showed that PI 124112 is different from PI 124111F in one gene for resistance it carries against *P. cubensis*. The lack of identity of the resistance gene in these two closely related germ plasm lines means breeders can improve the level of downy mildew resistance in newly developed material.

We found that PI 124112 has two incompletely dominant genes for resistance against downy mildew. This was evident from F_1, F_2, and BC progenies made with the susceptible cultivar AY. In this respect, PI 124112 did not differ from PI 124111F or MR-1.

The F_2 progeny between these two resistant parents segregated 13:3 resistant/moderately resistant. This would resemble epistasis between two dominant resistance genes. However, the F_2 population did not yield any susceptible individuals, nor did the testcross progeny between their F_1 and the susceptible AY. Thus, with the fact that both parents carry partially dominant genes for resistance (Table 2 [11,19]), we suggest that one resistance gene in both parents is allelic and the other is a distinct resistance gene weakly linked to the first.

Table 3 presents the possible segregation ratios that could have resulted from the following five hypothetical models for the genes carried in PI 124112: 1) *Pc1* and *Pc2*, or two genes fully allelic with them; 2) two genes for resistance, *Pc4* and *Pc5*, which are distinct and noncomplementary with *Pc1* and *Pc2*; 3) two nonallelic, distinct genes, *Pc4* and *Pc5*, which are complementary with *Pc1* and *Pc2*; 4) two genes, one fully allelic with *Pc1* (or *Pc2*) and a second, distinct gene, *Pc4*, which is noncomplementary with *Pc2* (or *Pc1*); and 5) two genes, one fully allelic with *Pc1* (or *Pc2*) and a second, distinct gene, *Pc4*, which is complementary with *Pc2* (or *Pc1*).

The fact that F_1 plants from the cross PI 124112 × PI 124111F are all resistant is inconsistent with models 2 and 4. Model 1, which would result in all-resistant progeny in the F_2, also is dismissed because of the appearance of moderately resistant individuals in our F_2 progenies. Of the two remaining models, 3 and 5, the latter is closer to our progeny data because it allows no susceptible (RT 11) segregants, results in a segregation ratio of 11:5 (resistant/moderately resistant), which is close to our segregation data (13:3 resistant/moderately resistant), and does not allow for individuals with RTs 12, 13, and 22 as we obtained.

Hence, we suggest that PI 124112 carries two incompletely dominant genes for resistance against *P. cubensis*, one fully allelic with *Pc1* (or *Pc2*) and another, *Pc4*, which is weakly linked to *Pc1* (or *Pc2*). The linkage hypothesis is based on the fact that 81% resistant individuals were obtained in the F_2 of PI 124112 × PI 124111F compared with 69% that should have been obtained with no such linkage.

We are currently investigating whether resistance to downy mildew in PI 124112 is associated with similar structural and histochemical responses in the host (7), is temperature-dependent (1), or is associated with a 45-kDa constitutive protein (2) as it is in PI 124111F. Such a comparative study may shed light on the nature of resistance in these two important genotypes of muskmelon.

LITERATURE CITED

1. Ballas, M., Cohen, Y., and Bar-Joseph, M. 1989. Temperature-dependent resistance in downy mildew-infected muskmelons. Pages 160-165 in: Proceedings of Cucurbitaceae 89: Evaluation and Enhancement of Cucurbit Germplasm. C. E. Thomas, ed. U.S. Dep. Agric. Agric. Res. Serv., Charleston, SC.
2. Ballas, M., Cohen, Y., and Bar-Joseph, M. 1990. Isolation of a 45 KD protein band associated with resistance to downy mildew in PI 124111F. (Abstr.) Int. Symp. Mol. Genet. Plant-Microbe Interact., 5th. Mikrobiol. Inst., Zurich. 226 pp.
3. Cohen, Y. 1981. Downy mildew of cucurbits. Pages 342-354 in: The Downy Mildews. D. M. Spencer, ed. Academic Press, New York.
4. Cohen, Y., Cohen, S., Eyal, H., and Thomas, C. E. 1985. Inheritance of resistance to downy mildew in *Cucumis melo* PI 124111. Cucurbits Genet. Coop. Rep. 8:36.
5. Cohen, Y., and Eyal, H. 1983. Preliminary observations on resistance of *Cucumis melo* to *Sphaerotheca fuliginea* and *Pseudoperonospora cubensis*. (Abstr.) Phytoparasitica 11:224.
6. Cohen, Y., and Eyal, H. 1987. Downy mildew-powdery mildew- and Fusarium wilt-resistant muskmelon breeding line PI 124111F. Phytoparasitica 15:187-195.
7. Cohen, Y., Eyal, H., Hanania, J., and Malik, Z. 1989. Ultrastructure of *Pseudoperonospora cubensis* in muskmelon genotypes susceptible and resistant to downy mildew. Physiol. Mol. Plant Pathol. 34:27-40.
8. Cohen, Y., Eyal, H., and Kenigsbuch, D. 1988. Genetics, nature and utilization of resistance to downy mildew and powdery mildew in muskmelon. Eucarpia Meet. Cucurbitaceae 88:45-49.
9. Epinat, C., and Pitrat, M. 1989. Inheritance of resistance of three lines of muskmelon (*Cucumis melo*) to downy mildew (*Pseudoperonospora cubensis*). Pages 133-135 in: Proceedings of Cucurbitaceae 89: Evaluation and Enhancement of Cucurbit Germplasm. C. E. Thomas, ed. U.S. Dep. Agric. Agric. Res. Serv. Charleston, SC.
10. Ivanoff, S. C. 1944. Resistance of cantaloupes to downy mildew and the melon aphid. J. Hered. 35:35-39.
11. Kenigsbuch, D., and Cohen, Y. 1989. Inheritance of resistance to downy mildew in a gynoecious muskmelon. Plant Dis. 73:994-996.
12. Palti, H., and Cohen, Y. 1980. Downy mildew of cucurbits (*Pseudoperonospora cubensis*). The fungus and its hosts, distribution, epidemiology and control. Phytoparasitica 8:109-147.
13. Pitrat, M. 1990. Gene list for *Cucumis melo* L. Cucurbits Genet. Coop. Rep. 13:58-70.
14. Pitrat, M., Blancard, D., and Epinat, C. 1989. A study on the variability of downy mildew and looking for sources of resistance in *Cucumis melo*. Pages 137-139 in: Proceedings of Cucurbitaceae 89: Evaluation and Enhancement of Cucurbit Germplasm. C. E. Thomas, ed. U.S. Dep. Agric. Agric. Res. Serv. Charleston, SC.
15. Samoucha, Y., and Cohen, Y. 1985. Occurrence of metalaxyl-resistant isolates of *Pseudoperonospora cubensis* in Israel: A five-year survey. Eur. Plant Prot. Org. Bull. 15:419-422.
16. Thomas, C. E. 1982. Resistance to downy mildew in *Cucumis melo* plant introductions and American cultivars. Plant Dis. 66:500-502.
17. Thomas, C. E. 1986. Downy and powdery mildew resistant muskmelon breeding line MR-1. HortScience 21:329.
18. Thomas, C. E., Cohen, Y., Jourdain, E. L., and Eyal, H. 1987. Use of reaction types to identify downy mildew resistance in muskmelons. HortScience 22:638-640.
19. Thomas, C. E., Cohen, Y., McCreight, J. D., Jourdain, E. L., and Cohen, S. 1988. Inheritance of resistance to downy mildew in *Cucumis melo*. Plant Dis. 72:33-35.
20. Thomas, C. E., Inaba, T., and Cohen, Y. 1987. Physiological specialization in *Pseudoperonospora cubensis*. Phytopathology 77:1621-1624.