Inheritance of Resistance to Downy Mildew in *Cucumis melo*

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ABSTRACT

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The F_1, F_2, and BC_1 from reciprocal crosses of the downy mildew-resistant, inbred muskmelon line MR-1, developed from *Cucumis melo* PI 124111, and the susceptible cultivar Ananas Yokneam were used to determine inheritance of resistance to downy mildew incited by *Pseudoperonospora cubensis*. The reactions of the parental lines and progenies to sporangial inoculation with *P. cubensis* support the hypothesis that the resistance of line MR-1 is conferred by two incompletely dominant genes designated *Pc*1 and *Pc*2. F_2 phenotypic ratios were 6 susceptible:9 moderately resistant:1 resistant. The BC_1 to the resistant parent had a phenotypic ratio of 3 moderately resistant:1 resistant and the BC_1 to the susceptible parent segregated 3 susceptible:1 moderately resistant. The resistance to downy mildew in line MR-1 is expressed as the production of reaction type 4 lesions in response to challenge by *P. cubensis*.

Downy mildew incited by *Pseudoperonospora cubensis* (Berk. & Curt.) Rostow. is a devastating foliar disease of cucurbits in humid growing areas of the world (11). Important economic hosts include *Cucumis sativus* L. (cucumber), *C. melo* L. (muskmelon), *Citrullus lanatus* (Thunb.) Matsum & Nakai (watermelon), and *Cucurbita* spp. (squash and pumpkin) (19). Breeding for resistance to downy mildew in both cucumber and muskmelon has been extensive, and some studies on inheritance of these resistances have been conducted.

In cucumber, Jenkins (9) found that resistance of the cultivars Chinese Long and Puerto Rico 37 was controlled by multiple genes. Barnes and Epps (1) reported that resistance from another source, PI 197087, appeared multigenic and that inheritance was probably controlled by one or two major and one or more minor genes. McFerson (10) used GY 14A, which had been developed using PI 197087 as the source of resistance, as the resistant parent in inheritance studies and concluded that resistance was polygenic. Van Vliet and Meysing (18) demonstrated that resistance in Poinsett, which derived its resistance from PI 197087, was conditioned by a single recessive gene that was linked or pleiotropic to a powdery mildew resistance gene.

In muskmelon, the genetics of downy mildew resistance has not been fully investigated (2,13). In 1944, Ivanoff (8) reported that four muskmelon cultivars of West Indian origin were resistant to downy mildew under south Texas conditions. In a field plot study, he concluded that resistance appeared to be partially dominant. The most resistant parent in his study was the cultivar Smith's Perfect. Thomas (14) found that although Smith's Perfect was the most resistant cultivar that he tested against one pathotype of *P. cubensis*, it was highly susceptible to another pathotype. In his study, an inbred of *C. melo* PI 124111 exhibited a high level of resistance to downy mildew. This inbred line of PI 124111 had been developed for powdery mildew resistance at the U.S. Horticultural Field Station, La Jolla, CA, from 1938 to 1948 and designated 90319 (16). However, the first three generations in the development of line 90319 had also been selected for downy mildew resistance. Muskmelon line MR-1 was developed from 90319 through an inbreeding and selection protocol that was designed to stabilize the high level of downy mildew resistance characterized by reaction type 4 (5) on both young and older leaves while maintaining the already high level of powdery mildew resistance in that line.

The objective of the research reported in this paper was to determine the inheritance of resistance to downy mildew from muskmelon breeding line MR-1.

MATERIALS AND METHODS

Muskmelon breeding line MR-1 was used as the highly resistant parent in crosses with the susceptible cultivar Ananas Yokneam (AY). The reactions of these parents to downy mildew had been determined from numerous field and greenhouse studies. Parental lines were crossed in the greenhouse using standard techniques for muskmelon (19), except AY was emasculated 2 days before anthesis because of early dehiscence of its anthers and MR-1, which is monoecious, did not require emasculation. Seeds of the parental, F_1, F_2, and BC_1 generations to both parents were produced.

The isolate of *P. cubensis* used in this study was collected by Edward Cox, Texas A&M University Agricultural Experiment Station, Weslaco, from *Cucurbita pepo* L. in 1983. It was maintained and sporangia for inoculum were produced on cotyledons of the susceptible muskmelon cultivar Perlita. Individual plants to be inoculated were grown in Jiffy-7s (Jiffy Products Co. of America, West Chicago, IL) peat pellets in the greenhouse. Before inoculation, each plant was labeled with a string marking tag and plants were completely randomized among and within planting trays.

At the two-expanded-leaf stage, plants were inoculated with a suspension of 5×10^3 sporangia of *P. cubensis* per milliliter. The adaxial surfaces of leaves 1 and 2 on each plant were sprayed to incipient runoff with a Paasche Type-H airbrush (Paasche Airbrush Co., Chicago, IL) at 275 kPa. Inoculated plants were placed in the dark in a dew chamber at 20 C for 20 hr. Plants were then removed from the dew chamber and placed in a 20 C growth chamber with a 12-hr photoperiod (631 $\mu E\ m^{-2}\ s^{-1}$). For the sixth night after inoculation, plants were returned to the 20 C dew chamber for 20 hr. Upon removal from the dew chamber, disease reactions of leaves 1 and 2 on each plant were evaluated separately and assigned a reaction type (RT) on a scale of 1–4, where 1 = 10–15 mm, irregular, chlorotic lesions with abundant sporulation that may extend beyond the apparent margins of the lesions; 2 = RT 1 lesions mixed with RT 3 lesions; 3 = 3–4 mm, irregular to circular, chlorotic lesions with water-

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soaked margins beneath and sparse sporulation; and 4 = 1 mm, circular, chlorotic lesions with necrotic centers and water-soaked margins beneath, and limited or no apparent sporulation. Assignment of a separate RT to each leaf resulted in a two-digit numerical rating in which the first digit represented the RT for the older leaf 1 and the second digit represented the RT for the younger leaf 2. The reaction types encountered were classified as: 11, 12, and 13 = susceptible (S); 22, 23, 24, and 33 = moderately resistant (MR); and 34, 44 = resistant (R). Chi-square tests were used to determine goodness of fit of observed to hypothetical segregation ratios in the F_2 and BC_1 populations. Because the growth chambers could not hold all the plants necessary for genetic analysis, the data represent the compilation of three tests. Each generation was included in all three tests, except the F_2 was not included in the third test.

RESULTS

Segregation data for resistance to downy mildew as expressed by the combined reactions of leaves 1 and 2 are presented in Table 1. The parents reacted as expected; all plants of the susceptible AY exhibited RT 11 and all plants of MR-1 exhibited RT 44. The F_1 was moderately resistant, and there was no difference in RTs between the two reciprocal F_1 families. The F_2 generation segregated in a 6S:9MR:1R ratio. The BC (F_1 × susceptible parent) generation segregated 3S:1MR. The BC (F_1 × resistant parent) segregated 3MR:1R as the theoretical model predicted. Homogeneity chi-square indicates that the differences between families in each respective generation are not greater than expected due to chance alone.

The segregation ratios observed in the F_2 and two BC_1 generations are expected from the action of two incompletely dominant genes that are mutually epistatic to each other. In this model, the homozygous recessive genotype at one locus conditions susceptibility when the second locus is homozygous dominant or heterozygous, and the homozygous recessive genotype at the second locus conditions susceptibility when the first locus is heterozygous. If the homozygous recessive at the second locus were also epistatic to the homozygous dominant at the first locus, a 7S:8MR:1R ratio would be expected in the F_2 generation. Although there is little difference in the expected F_2 ratios between these two models, the observed data do not fit the 7S:8MR:1R model (Table 1). The two models have the same expected segregation ratios in the BC_1 generation with each parent.

DISCUSSION

In an earlier report (3) on the inheritance of resistance to downy mildew from muskmelon PI 124111, some deviations from the expected ratios did not support the hypothesis of two incompletely dominant genes. These deviations were probably contributed to, in part, by two factors. The first of these is that in the previous study, an F_5 inbred developed from the mass lot of PI 124111 as obtained from the U.S. PI collection was used as the resistant parent. In the present study, the resistant parent, MR-1, is an F_6 inbred developed from line 90319, which is an F_{10} inbred developed from PI 124111 (16) and may represent a more homogeneous source of resistance. The second contributing factor was probably the use of the cultivar Hemed as the susceptible parent in some F_2 crosses that were presented in that study. On the basis of additional information (Y. Cohen and C. E. Thomas, *unpublished*), we now believe that Hemed, even though at one time considered to be as fully susceptible as AY, has a very low level of resistance that is not easily recognized.

We propose that the two incompletely dominant genes that condition downy mildew resistance in muskmelon breeding line MR-1 be designated *Pc*-1 and *Pc*-2. Therefore, the genotype for resistance in MR-1 would be *Pc*-1*Pc*-1 *Pc*-2*Pc*-2. Likewise, the genotype of the fully susceptible AY would be *pc*-1*pc*-1 *pc*-2*pc*-2.

The genes act in an additive manner and are mutually epistatic to each other. We hypothesize that one gene affects lesion expansion and the other affects sporulation. Support for this hypothesis is found in the expression of RT 4, representing the double homozygous dominant genotype (*Pc*-1*Pc*-1 *Pc*-2*Pc*-2), in which lesion expansion is minimal and resultant sporulation is extremely limited or not apparent. Conversely, in RT 1, representative of the double homozygous recessive genotype (*pc*-1*pc*-1 *pc*-2*pc*-2), lesion expansion is maximal with accompanying profuse sporulation. Lesion expansion is a necessary precedent to subsequent sporulation, and sporulation rate is proportional to infected leaf area. Therefore, if lesion expansion is inhibited through the dominant expression of one gene, then sporulation is also inhibited regardless of the condition of the second gene, the dominant expression of which is also inhibition of sporulation. Conversely, the full phenotypic expression of the second gene in the inhibition of sporulation may be partially masked in genotypes in which the first gene is heterozygous. Under these conditions, even though sporulation is inhibited, it may appear more abundant to the unaided eye because of the uninhibited expansion of lesions, i.e., sporulation per unit of infected leaf area may be lower, but the area over which sporulation occurs is greater.

Furthermore, the expression of these two genes is also apparently conditioned by leaf age. In two-leaf-stage plants, the RT of leaf 1 is always equal to or greater in susceptibility than the RT of leaf 2. This is also true of older plants in the field in which the RT of older leaves is always equal to or greater in susceptibility than the RT of younger leaves (5). Therefore, the complementary nature of these two genes combined with the influence of leaf age on their expression results in the

Table 1. Segregation for resistance to downy mildew in parental and reciprocal F_1, F_2, and BC_1 generations for crosses of susceptible (S) Ananas Yokneam and resistant (R) MR-1[a]

Generation	Pedigree	Observed (S:MR:R)	Expected ratio (S:MR:R)	Chi-square	df	*P*
S	Parent	119:0:0	All S	···	···	···
R	Parent	0:0:103	All R	···	···	···
F_1	S × R	0:153:0	All MR	···	···	···
	R × S	0:65:0	All MR	···	···	···
F_2	(S × R) × (S × R)	51:107:11	6:9:1	3.94	2	0.15
			7:8:1	13.12	2	0.023
	(R × S) × (R × S)	57:96:7	6:9:1	1.45	2	0.49
			7:8:1	6.51	2	0.041
	Combined	108:203:18	6:9:1	3.97	2	0.14
			7:8:1	18.30	2	<0.001
	Homogeneity	···	6:9:1	1.41	2	0.49
		···	7:8:1	1.33	2	0.25
BC_1	S × (S × R)	68:27:0	3:1:0	0.5929	1	0.46
	S × (R × S)	65:25:0	3:1:0	0.3704	1	0.56
	Combined	133:52:0	3:1:0	0.953	1	0.34
	Homogeneity	···	···	0.01	1	0.92
	R × (S × R)	0:67:28	0:3:1	1.01	1	0.32
	R × (R × S)	0:56:22	0:3:1	0.43	1	0.52
	Combined	0:123:50	0:3:1	1.40	1	0.24
	Homogeneity	···	···	0.0367	1	0.86

[a] Plants inoculated at two-leaf-stage with 5×10^3 sporangia per milliliter, placed in dew chambers at 20 C in the dark for 20 hr, then removed to 20 C growth chamber with 12-hr photoperiod for 5 days, returned to 20 C dew chamber for sixth night for 20 hr, and ratings made on seventh day. Ratings: RT 11, 12, and 13 = S; 22, 23, 24, and 33 = MR; and 34 and 44 = R.

phenotypic segregation ratios that indicate that resistance to downy mildew in line MR-1 is conferred by two incompletely dominant genes.

Sitterly (12,13) listed sources of resistance to downy mildew in muskmelon as PI 124112 and Seminole. He presented a range of resistance among cultivars as immune Seminole, resistant Edisto 47, moderately resistant Georgia 47, tolerant Smith's Perfect, and susceptible Hale's Best. Thomas (*unpublished*) has found that an inbred line of PI 124112, 92398, developed in parallel with the inbred line 90319 of PI 124111 at the former U.S. Horticultural Field Station, also exhibits RT 4 when challenged with *P. cubensis*. When he conducted his earlier resistance evaluations (14), 92398 was not included. PI 124112 was represented by the mass lot as obtained from the U.S. plant introduction station. Cohen and Eyal (4) have confirmed that a high level of resistance can be obtained from PI 124112. An examination of the pedigrees of the cultivars in Sitterly's ranking (12,13) reveals that PI 124112 is in the background of Seminole, Georgia 47, and thus Edisto 47 (2,7,17,20). Sitterly probably did not list PI 124111 because it was rarely used in breeding programs in the southeastern United States; however, PI 124112 was often used as a source of powdery mildew resistance in these programs. This made possible the utilization of the downy mildew resistance that was also present in this PI. On the other hand, PI 124111 was often used as a source of powdery mildew resistance in breeding programs in the western United States. Because downy mildew occurrence is very rare in that area, resistance to it would easily go unrecognized. The ranking of Seminole as "immune" was not substantiated in later tests (14).

Muskmelon genotypes such as MR-1 that exhibit RT 4 against *P. cubensis* are valuable sources of resistance to plant breeders for three reasons. First, the sporulation rate on RT 4 genotypes is $< 1\%$ of that on susceptible commercial cultivars (15). This reduction in inoculum potential (6) would greatly reduce the spread of the disease. Second, RT 4 lesions do not expand and coalesce with the resultant leaf collapse (15) so that the foliage is retained to shade the developing fruits from sunscald. Third, RT4 is expressed in line MR-1 and PI 124111 against challenge from different pathotypes of *P. cubensis* (Y. Cohen, *unpublished*; 14,16), which may make them sources of more durable resistance to downy mildew. Knowledge of the inheritance of downy mildew resistance expressed as RT 4 from breeding line MR-1 should substantially enhance the usefulness of this germ plasm to breeding programs.

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