Inheritance of Resistance to Melon Mosaic Virus in Cucumbers

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ABSTRACT

The cultivar Kyoto 3 feet (K.3.f.) was used as a source of resistance to melon mosaic virus (MMV) in cucumbers. No symptoms were observed in K.3.f. plants following inoculation with MMV. Moreover, virus concentrations were significantly lower in these plants than in susceptible Bet-Alfa (B.A.) cucumbers. Resistance to MMV was classified according to external symptoms, and was found to be governed by a single dominant gene. Modifying factors affect the degree of susceptibility in the absence of the gene for resistance, and possibly also the relative concentration of virus particles in symptomless resistant plants. Phytopathology 61:253-255.

Additional key words: Chenopodium amaranticolor, melon mosaic symptoms, melon mosaic virus in flower corollas, melon mosaic virus content in leaves.

One of the common diseases of cucurbits in Israel is caused by a virus related to the "Melon Mosaic Virus Group" (2, 4). In many of its characteristics, this virus (MMV) is similar to the watermelon mosaic virus 2 (WMV-2) (6). Since no serological or cross-protection tests could be made between our isolate and the strains of WMV-2, however, there is no clear evidence of their relationship.

During recent years, heavy damage has been caused by this virus in the cucumber fields in Israel. Investigations were therefore carried out in a search for sources of resistance. Many cucumber cultivars of different origin were tested, and several were found to be resistant. Among those, the cultivar Kyoto 3 feet (K.3.f.), originating in Japan, was chosen as the resistant parent in local breeding work (S. Cohen, F.E. Nitzany, E. Galun, & S. Niego, *unpublished data*). It was the purpose of the work presented here to investigate the inheritance of resistance to MMV in this cultivar.

MATERIALS AND METHODS.—The experiments were carried out in an insect-proof greenhouse, which was sprayed weekly with nicotine sulfate or with *0,0*-dimethyl-2,2 dichlorovinylphosphate. The MMV culture was maintained on Bet-Alfa cucumber (B.A.) or on Sihi Lavan squash. The cultures were renewed by mechanical inoculation every 2 to 3 weeks. The MMV culture has been kept in our laboratory since 1958. A description of the culture has been given elsewhere (2).

Homozygous lines of the resistant K.3.f. and the susceptible B.A. cucumbers were used for the hybridizations, and reciprocal crosses were made to obtain seeds of the F_1, F_2, and Bc_1F_1 generations (Table 2).

The seeds of the abovementioned groups were grown in pots and fertilized weekly with a nutrient solution. When the seedlings reached the cotyledonary stage, they were mechanically inoculated after being dusted with Carborundum (500 mesh). Two inoculations were made with undiluted MMV extracts at 2-day intervals.

Observations were continued until the plants reached the 12th true-leaf stage, and the results were analyzed by the chi-square test. The following scale was used in classification of the plants: 0 = No symptoms; 1 = Slight chlorosis near the leaf blade tip on both sides of the midrib; 2 = Chlorotic patches distributed all over the leaf blade and distortion of the leaf; 3 = Symptoms similar to 2, but with chlorosis and distortion more severe and accompanied by the appearance of blisters on the leaf blade.

Differences in the content of infectious MMV particles in the sap of plants of the different crosses were examined on *Chenopodium amaranticolor* Coste & Reyn., which reacts to MMV inoculation with local lesions.

Unless otherwise stated, the inocula used in these tests were diluted with distilled water to 10^{-2}. This MMV concn was found, in preliminary tests, to fall in the straight line section of the dilution curve when B.A. sap was used as the source of inoculum. The inocula were applied with a soft hair brush on Carborundum-dusted leaves of *C. amaranticolor,* using the half-leaf method (1). At least 16 leaves were inoculated in each test. The results were analyzed by the "Student" t-test for related samples.

RESULTS.—*Time interval from inoculation to appearance of symptoms.*—The experiments were carried out using the B.A. susceptible parent and the F_2 of the cross with K.3.f. The seedlings were inoculated as described above; observations on symptom appearance were made daily.

The results summarized in Table 1 shows that at the 7th true-leaf stage, all of the 400 B.A. plants and 22.3% of the 400 F_2 seedlings showed symptoms. The percentage of susceptible plants in this group rose to a max of 24.8% when they reached the 11th leaf stage. Following these results, symptom observations were made in subsequent experiments until the plants reached the 12-leaf stage.

Inheritance of resistance to MMV.—In the reciprocal and back-cross experiments, only plants remaining symptomless were classified as resistant, while plants with various symptoms were counted as susceptible. The results of the first experiment are summarized in Table 2, and indicate that a single dominant factor is

TABLE 1. Leaf stage at which melon mosaic virus (MMV) symptoms appeared in cucumbers of the cultivar Bet Alfa (B.A.) and in the F_2 of the cross B.A. × Kyoto 3 feet (K.3.f.)

Stage (leaf no.)	Cumulative percentage of plants developing symptoms[a]	
	B.A.	F_2 (B.A. × K.3.f.)
Cotyledons	0	0
1	0	0
2	9.5	1.0
3	42.5	4.2
4	88.1	11.8
5	97.3	12.9
6	98.9	20.2
7	100	22.3
8		23.5
9		24.4
10		24.7
11		24.8
12		24.8

[a] 400 plants of each cultivar inoculated at the cotyledonary stage.

responsible for resistance. Similar results were obtained in the second experiment. The above explanation, however, does not account for the different degrees of susceptibility found in segregating populations. In the F_2, the average percentages of plants of resistance classes 0, 1, 2, and 3 in the two experiments were 74.7%, 14.4%, 6.0%, and 4.9%, respectively. The appropriate figures for the Bc_1F_1 to the susceptible parent were 48.3%, 27.4%, 15.7%, and 8.6%. As such varying degrees of susceptibility were not found in P_1, P_2 and F_1-generations, nor in the Bc_1F_1 to the resistant parent, the action of modifying factors was indicated. As far as visible symptoms are concerned, modifying factors were expressed only in absence of the major resistance gene.

The occurrence among the F_2 plants of several degrees of symptom severity aroused our interest as to possible differences in the MMV content of the different groups of plants.

Dilution and infectivity of leaf extracts of parent lines.—To determine if inhibitors (1) associated with extraction of inoculum were present, six equal groups of B.A. and of K.3.f. seedlings (65/group) were inoculated. When the plants reached the 12-leaf stage, the 2nd leaf from the top of every plant in each group was collected and used as inoculum. The inocula were diluted as required and inoculated on *C. amaranticolor*. The results (Table 3) show that at all dilutions tested, the number of local lesions produced on the half-leaves inoculated with the sap extracted from B.A. was significantly higher than the number produced on the half-leaf side inoculated with the sap from K.3.f. These data suggest that the differences between the virus concn in B.A. and K.3.f. were not a result of differences in the content of inhibitors formed during the extraction of the plants.

The rate of infectivity of MMV in extracts of flower corollas.—Since flower corollas were found to contain less inhibitory material than leaves (5), their infectious MMV content was examined.

The corollas of flowers of each of the groups of plants which were tested in the previous experiment were collected. In six tests on *C. amaranticolor*, a total of 1,265 local lesions were produced on the half-leaf sides inoculated with the sap from B.A., while only 779 local lesions were produced on the half-leaf sides inoculated with the sap from K.3.f.; the differences were significant in all six tests.

These data, as well as those presented in Table 3, indicate that a difference may exist in the MMV content of the two varieties.

The content of infectious MMV in the sap of plants.—The MMV contents of plants of different generations and resistance classes were compared in a series of experiments. The leaves for the preparation of inocula were collected from plants at the 12-leaf stage and the extracts were diluted to 10^{-2}. The results (Table 4) show that less infectious MMV could be recovered from plants of the resistant parent than from the ap-

TABLE 2. The resistance to melon mosaic virus in reciprocal F_1, F_2, and backcross populations of a cross between the cucumber cultivars Bet Alfa (B.A., susceptible) and Kyoto 3 feet (K.3.f., resistant)

Generation	Cultivar or cross	Number of plants			χ^{2a}	P
		Resistant	Susceptible	Total		
P_1	Bet Alfa	0	316	316		
P_2	Kyoto 3 feet	331	0	331		
F_1	K.3.f. × B.A.	364	0	364		
F_1	B.A. × K.3.f.	387	1	388		
Bc_1F_1	K.3.f. × F_1	303	0	303		
Bc_1F_1	F_1 × K.3.f.	316	1	317		
Bc_1F_1	B.A. × F_1	171	164	335	0.15	.70
Bc_1F_1	F_1 × B.A.	188	198	386	0.26	.50-.70
Bc_1F_1	Pooled	359	362	721	0.01	.90-.95
F_2	F_1 × F_1	455	152	607	0.001	.95-.98

[a] Expected ratio of resistant:susceptible is 3:1 for F_2 and 1:1 for backcross to susceptible parent.

TABLE 3. The total number of melon mosaic virus local lesions on *Chenopodium amaranticolor* half-leaves inoculated with extracts of Bet Alfa (B.A.) and Kyoto 3 feet (K.3.f.) cucumbers[a]

Extract dilution	Total no. of local lesions[b] produced by the sap	
	B.A.	K.3.f.
10^{-1}	1,482	599
5×10^{-2}	1,232	597
10^{-2}	815	273
5×10^{-3}	658	249
10^{-3}	195	67
5×10^{-4}	102	62
10^{-4}	89	42
5×10^{-5}	85	37
10^{-5}	56	12

[a] Six tests for each extract dilution.
[b] Significant differences ($P = .05$) in all six tests, except for the 10^{-3} and 5×10^{-4} dilutions, where five of the tests showed significant differences ($P = .05$).

parently resistant symptomless F_1. Similarly, the local lesions produced were fewer in number with inoculum from the F_1 than from the susceptible parent. The respective backcrosses behaved according to the same pattern. The relative recovery of infectious MMV material from F_2 plants was related to severity of the disease symptoms observed.

DISCUSSION.—Plants of parent lines of F_1, F_2, and backcross populations were classified as resistant or as susceptible according to visible disease symptoms. Resistance was found to be governed mainly by a single dominant gene (Table 2). But the susceptible group in F_2 and in backcross populations was not uniform. Different degrees of susceptibility were observed and found to be correlated with the relative amt of infectious MMV particles which could be recovered. This indicated the existence of modifying factors, expressed in the absence of the major resistance gene. As the classification of susceptible plants into definite groups was arbitrary, no conclusions could be drawn as to the number and nature of the factors modifying resistance.

In further experiments (Table 4), it was found that less infectious MMV could be recovered from plants of the resistant cultivar than from similarly symptomless F_1 plants. This again may have been due to modifying factors, assuming that resistance in K.3.f. is based on one major dominant gene plus modifiers. The present material does not, however, eliminate the possibility of incomplete dominance of the resistance factor as far as the relative amount of infectious MMV particles is concerned.

The lack of symptoms in the K.3.f. plants following MMV inoculation is not in itself sufficient to make this cultivar suitable as a source of resistance. Kooistra (3) demonstrated that concn of Cucumis virus 2 in infected symptomless cucumber plants may reach the same level as in plants of a susceptible cultivar. Moreover, there were no significant differences in yield between the two infected cultivars. In the present work, however, it was found that the MMV concn in resistant, symptomless K.3.f. plants was significantly lower than in the susceptible B.A. plants (Table 3), and therefore the K.3.f. cultivar may serve as a valuable source of resistance in breeding work.

The evaluation of MMV concn in the plants was sufficiently accurate for differences to be detected between MMV concn in symptomless F_1, Bc_1F_1, and K.3.f. plants. This procedure might be employed as a tool in the selection of highly resistant lines containing a minimum of infectious particles.

TABLE 4. The total number of melon mosaic virus (MMV) local lesions on *Chenopodium amaranticolor* half-leaves inoculated with extracts of cucumbers of different generations and resistance classes

MMV sources		Total no. local lesions from six tests	No. tests which differ significantly[a]
Bet Alfa		1,766	6
F_1		968	
Kyoto 3 feet		680	6
F_1		1,012	
F_1		905	5
Bet Alfa $\times$ F_1		1,155	
F_1		1,182	5
Kyoto 3 feet $\times$ F_1		983	
Kyoto 3 feet		656	3
$F_1 \times$ Kyoto 3 feet		733	
F_2	0[b]	590	5
	1	685	
F_2	1	710	6
	2	1,002	
F_2	2	762	6
	3	1,243	

[a] $P = .05$. In tests in which no significant differences were found, the tendency was as in those which were significantly different.
[b] Resistance classes: 0 = no symptoms; 1 = slight chlorosis; 2 = chlorosis and leaf distortion; 3 = severe chlorosis and leaf distortion.

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