Partial Dominance of Resistance to Potato Virus Y in *Capsicum*

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ABSTRACT

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In pepper plants (*Capsicum annuum* L.) carrying the gene y^a for potato virus resistance, the heterozygous condition $y^{a+}y^a$ can be differentiated from the susceptible $y^{a+}y^{a+}$ by the symptomatology after PVY inoculation. The heterozygous plants, which are more vigorous and contain lower virus titer than the homozygous plants, can be considered partially resistant. Hence, breeding for PVY resistance can be made more efficient by repeated backcrossing of the identified heterozygous plants with the recurrent susceptible cultivars.

Resistance to potato virus Y (PVY) in pepper, *Capsicum annuum* L., was originally thought to be controlled by a single genetic factor (8). Further studies showed, however, that several, mostly recessive alleles at separate loci are involved in the resistance reaction (2–4,7,10), and each of the loci was thought to confer resistance against a different spectrum of PVY strains. Routine breeding work (*unpublished*) based on F_1 and F_2 populations and the local PVY strain complex confirmed the presence of a common locus, y^a, for resistance in the cultivars Yolo-Y, Puerto Rico Wonder, Agronomico-9, and Florida VR-2 (2,4,5,7). Resistance at this locus was considered to be conditioned by multiple recessive alleles, each with its own specific activity (2,4,6,7). Consequently, incorporation of the recessive alleles into susceptible cultivars in a backcrossing program involves crossing, selfing, and progeny testing after PVY inoculation for each backcross generation so that the genotypes carrying the y^a allele can be identified. If heterozygous $y^{a+}y^a$ and homozygous $y^{a+}y^{a+}$ susceptible plants could be differentiated, selfing and progeny testing would be unnecessary.

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MATERIALS AND METHODS

This article relies on Schafer's definition of resistance (9) in which higher resistance is correlated with lower titer of the virus and lower resistance with higher titer.

Maor, a commerical cultivar of the Bell pepper type, is susceptible to PVY after mechanical inoculation but has higher field resistance than some other susceptible cultivars. Florida VR-2 is the most resistant cultivar to local strains of PVY. P-7 is a *Capsicum pendulum* accession resistant to PVY that as a female parent,

can be crossed with *C. annuum*. In addition, the following breeding material was studied: 1) Florida VR-2 × Maor F1, 2) P-7 × Maor F_1, 3) 72 F_2 plants from the cross Florida VR-2 × Maor, and 4) 54 F_3 lines from F_2 plants with mosaic symptoms of the previous population.

Inoculum from a mixture of field isolates of PVY was applied mechanically three times at the cotyledon stage in the greenhouse; 12–15 plants per cultivar or hybrid were inoculated. Symptom development and virus content in cultivars and hybrids were evaluated during a 3-mo period, until flowering of the late cultivar P-7. Among the 72 inoculated F_2 plants, symptomless plants suspected of being resistant $y^a y^a$ were discarded and the remaining plants with mosaic were divided into groups of: 1) plants with rough mosaic and 2) vigorous plants with milder mosaic and the ability to set normal fruits. The final designation into the two groups was made at fruit maturity. From these F_2 plants, F_3 progenies were obtained by selfing in the greenhouse. At least 12 seedlings per F_3 line were inoculated to ensure a high probability (19:1 for 11 plants, based on a 3:1 ratio) of obtaining at least one resistant segregant from heterozygous $y^{a+} y^a$ plants.

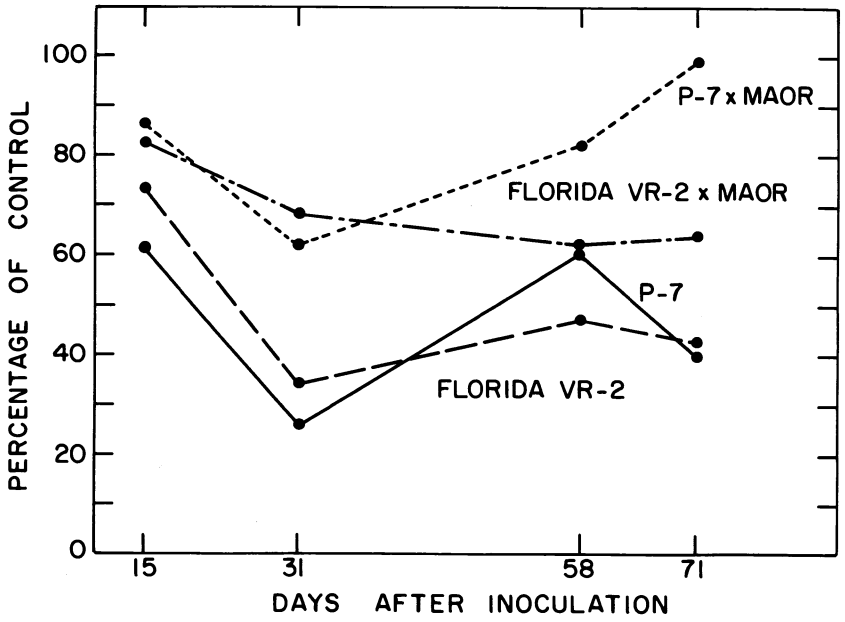

Fig. 1. Virus titer in different cultivars and lines as percentage from the susceptible control cultivar Maor.

Table 1. Response of F_3 lines from different F_2 plants with mosaic after inoculation with potato virus Y

Suspected heterozygous $y^{a+}y^a$ F_2 plants (77-135)	No. of F_3 plants		Suspected homozygous $y^{a+}y^{a+}$ F_2 plants (77-135)	No. of F_3 plants	
	Mosaic	Symptomless		Mosaic	Symptomless
1	9	4	33[b]	9	2
2	8	4	34	12	0
3	9	4	35	12	0
4	9	3	36	12	0
5	6	3	36	12	0
6	9	4	37	12	0
7	9	3	38	12	0
8	9	4	39	12	0
9	8	5	40[b]	7	4
10	9	3	41[b]	11	2
11	9	3	42	12	0
12	8	3	43	12	0
13	9	2	44	12	0
14	10	3	46[b]	10	2
15	8	4	48	4	0
16	10	2	49	13	0
17	7	4	50	7	0
18	10	4	51[b]	10	1
19[a]	0	10	52	12	0
20	10	2	53	12	0
21	7	5	54	12	0
22	7	5	55	12	0
23	9	4	56	12	0
24[a]	13	0	57	12	0
25	8	5			
26	8	3			
28	7	5			
29	9	3			
30	9	3			
31	9	3			
32	7	4			

[a] Plants erroneously classified as $y^{a+}y^a$.
[b] Plants erroneously classified as $y^{a+}y^{a+}$.

Enzyme linked immunosorbent assay (ELISA) tests were made as described by Clark and Adams (1). Leaf discs (6 mm in diameter, about 5 mg) were homogenized in phosphate-buffered saline solution containing 0.05% Tween 20 and 2% polyvinyl-pyrrolidone (PBS buffer) and tested in microtiter plates (Linbro Scientific Co., Hamden, CT). The dilution of the coating γ-globulin was 1:1,000 and that of the conjugated γ-globulin was 1:2,000.

Color intensity, which is proportional to the virus titer (1), was measured with a spectrophotometer. Discs were taken from young, intermediate, and old leaves of the cultivars, hybrids, and 20 selected old F_2 plants. Similarly, control discs were taken from healthy plants for ELISA measurements.

The F_2 generation included 10 plants that were representative, by symptomatology, of the expected susceptible $y^{a+}y^{a+}$, and 10 of the expected partially resistant $y^{a+}y^a$ genotype. The 20 plants were topped after fruit ripened, and their regenerated foliage was tested.

RESULTS

Florida VR-2 remained symptomless, but mild mosaic symptoms appeared in P-7 toward the end of the experiment. Although mosaic symptoms developed on Maor 2 wk after the first inoculation, symptoms developed on both F_1s, ie, Florida VR-2 × Maor and P-7 × Maor, only after 6 wk. The ELISA results showed the virus in all lines and cultivars but in different titers. At four dates of measurements (Fig. 1), the resistant parents demonstrated fluctuating virus levels but within 26–73% of those of the susceptible cultivar Maor (Fig. 1). Similarly, with one exception (71 days after inoculation), the F_1s demonstrated lower PVY levels than those of Maor, with a range of 62–99% of the levels of the control cultivar Maor. In addition, virus content of the F_1s seems to be intermediate between their resistant and

susceptible parents (Fig. 1). The 72 F_2 plants from the cross Florida VR-2 × Maor segregated into 19 symptomless plants and 53 showing mosaic. Of the 53, 21 plants with severe symptoms were thought to be susceptible $y^{a+}y^{a+}$ genotypes and 32 with milder symptoms were thought to be heterozygous $y^{a+}y^{a}$ genotypes. The F_3 lines demonstrated two patterns of inheritance: homozygous susceptible lines and lines that segregated into symptomless plants and those showing mosaic, in a ratio of approximately 1:3, respectively (Table 1). An exceptional F_3 line that had only resistant plants was obtained from a resistant F_2 plant (No. 19, Table 1) that had mild mosaic symptoms.

Based on symptomatology in F_2 plants, good fit was found between suspected heterozygous F_2 individuals and their F_3 progenies. Only one of 30 F_2 plants (No. 24, Table 1) could have been erroneously classified as heterozygous $y^{a+}y^{a}$. In the second F_2 group, five of 23 plants were erroneously classified as homozygous $y^{a+}y^{a+}$ (Nos. 33,40,41,46, and 51, Table 1).

The 20 F_2 plants expected to represent the homozygous susceptible and the heterozygous partially resistant genotypes performed both genetically (Table 1) and in virus titer (Table 2) as expected from previous information (Fig. 1). Although the virus titer in the two groups was significantly diffferent, some overlapping in virus titers occurred between the two groups (Table 2).

Table 2. Absorbance (A_{405}) after enzyme linked immunosorbent assay of suspected heterozygous and homozygous F_2 plants

Suspected heterozygotes (partially resistant)		Suspected homozygotes (susceptible)	
Plant no.	A_{405}[a]	Plant no.	A_{405}[a]
3	0.138	38	0.208
4	0.181	42	0.151
5	0.073	44	0.290
10	0.095	48	0.208
11	0.083	49	0.225
12	0.108	53	0.165
16	0.172	54	0.213
17	0.119	55	0.190
19	0.024	56	0.205
20	0.120	57	0.218
$\bar{X}$[b]	0.121 ± 0.012		0.207 ± 0.011
Range[b]	0.73–0.181		0.151–0.290

[a] Results do not include the absorbance of the appropriate control discs.
[b] Exceptional homozygous $y^{a}y^{a}$ resistant plant 19, with lowest virus titer, was not included in the comparison.

DISCUSSION

Demonstrated partial dominance of the y^{a} allele permits its faster incorporation into desired recurrent parents. The ELISA technique also revealed partial dominance in the cross P-7 × Maor, ie, with a P-7 parent less resistant than Florida VR-2. Thus, partial dominance may be general and identification of heterozygotes for breeding purposes may be generally possible.

The relatively low virus titer in heterozygous $y^{a+}y^{a}$ genotypes may have an advantage in pepper hybrids produced from susceptible and resistant parents. Although ELISA demonstrates average differences in virus titer between heterozygous and homozygous groups (Fig. 1, Table 2), the technique is unsatisfactory for comparisons of individual plants (Table 2). Symptomatology should be considered as the most reliable criterion for selecting heterozygous individuals. The low error value (3%, one of 30 plants) in identifying the heterozygous F_2 plants and the higher error (21%, five of 23 plants) in the homozygous group was obtained after continual selective pressure against individuals with strong mosaic symptoms. The high probability of identifying the heterozygotes can facilitate incorporating PVY resistance into susceptible cultivars. Backcrossing PVY resistance, like incorporating a single recessive gene, has depended so far on selfing and progeny tests for identifying individuals carrying the y^{a} allele. In each generation at least seven plants were backcrossed to the recurrent parent and selfed to achieve a 99% probability of at least one $y^{a+}y^{a}$ plant. The selfed progenies of the seven plants were inoculated with PVY to identify their parental genotypes. With the present information, the same seven seedlings expected to be $y^{a+}y^{a}$ and $y^{a+}y^{a+}$ in a 1:1 ratio could be inoculated, the homozygotes discarded, the heterozygotes backcrossed, and the progeny seedlings bulked for the next cycle of inoculation, selection, and backcrossing.

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