Inheritance of Resistance to Pepper Veinal Mottle Virus in Chilli

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ABSTRACT

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Classical Mendelian analyses of the results of a greenhouse test using an isolate of pepper veinal mottle virus (PVMV) and of a field test with natural infection gave strong indication of a recessive gene conditioning resistance to veinal mottle virus in chilli. Additive effects also played some role in the expression of PVMV resistance and its heritability estimate was high. Results of systemic virus assays suggested that the resistance gene functioned by partially inhibiting virus multiplication.

Additional key words: Capsicum annuum, veinal mottle virus resistance, virus inhibition.

Veinal mottle is the most serious disease of chilli in Malaysia and invariably occurs wherever chilli is grown. The symptoms vary from leaf mottling, leaf distortion and narrowing, to fruit distortion and plant stunting. The severity of the disease depends on the cultivar, the management, the environment, and the stage of growth of the crop at which infection occurs.

Tobacco mosaic virus (TMV), cucumber mosaic virus (CMV) and a third virus distinct from the first two and very similar in its properties to pepper veinal mottle virus (PVMV) (2, 6), of which the last is the most prevalent, are known to occur in local chillies (8).

One of the authors (K. M. G.) observed that some of his chilli collections from Chiangmai, Thailand, showed only mild mottling under field conditions. This observation led to a systematic study of the inheritance and nature of this resistance with the objective of developing veinal mottle-resistant commercial chilli cultivars.

MATERIALS AND METHODS

The six bulked genetic populations P_1, P_2, F_1, F_2, B_1, and B_2 of a cross between a resistant parent (accession 7.4) and a highly susceptible parent (accession C.2), both from Thailand, were grown in small polyethylene bags in the aphid-proof greenhouse. The greenhouse temperature was 19-32 C and the relative humidity 48-80%. A randomized complete block design of four replicates, with approximately 140 plants per replicate, was used to avoid bias in inoculation and in location.

Both parents had been selfed once. Accession 7.4 was chosen as the resistant parent because it has yellow fruits. Because yellow fruit color is recessive to red, it served as a genetic marker to check the purity of the resistant parent and the legitimacy of the crosses.

The source of the inoculum was leaves infected with an isolate of PVMV. As there were no apparent local lesion hosts (8), no attempt was made to purify the inoculum. The crude sap virus preparation was a dilution of one part crude sap (filtered through double layer of cheesecloth) with ten parts 0.05M phosphate buffer (pH 7) containing 0.1% $NaSO_3$ and 0.05M $MgCl_2$ as additives. Four rounds of inoculation on two consecutive days were made on the four- to six-leaved seedlings by the Carborundum [22-μm (600-mesh)] leaf-rubbing method.

The plants were scored weekly for leaf symptom expression on an arbitrary scale of 0-5: 0 = no symptoms; 1 = chlorosis; 2 = indistinct mottling; 3 = distinct mottling with slight leaf distortion; 4 = severe leaf distortion; 5 = severe leaf narrowing/filiform leaves. The scores at 5 weeks were used in the analyses.

An experiment identical to the greenhouse test was laid out in the field with 7-week-old seedlings. The plants were spaced 1.0×0.5 m and each plant was fertilized with 150 g of a mixed fertilizer (7% N, 6% P_2O_5, 1.5% K_2O) divided into six monthly applications. Disease scorings were made at flowering and at fruiting. The latter scores were used in the analyses.

Plants with different disease ratings 5 weeks after inoculation in the greenhouse test were assayed for virus content. All the young infected leaves of plants in each symptom class from 1 to 3 and of classes 4 + 5 (combined) in each population were ground, squeezed through a double layer of cheesecloth, and a dilution series with phosphate buffer was prepared based on the results of a preliminary assay. Dilutions $10^{-0.5}$, $10^{-1.5}$, $10^{-2.0}$, and $10^{-2.5}$ were used for disease ratings 1 and 2, whereas dilutions $10^{-1.5}$, $10^{-2.5}$, $10^{-3.0}$, and $10^{-3.5}$ were used for ratings 3 and 4

+ 5. To reduce the work load and to achieve valid results for comparative purposes, assays of populations P_1, P_2, F_1 and B_2 were performed on the same day and assays of the segregating F_2 and B_1 were on the following two other consecutive days. Twenty seedlings (three- to five-leaved) of a uniformly susceptible host (accession C.2) in small polyethylene bags were inoculated with each of the dilutions. After 3 weeks, the percentage of plants infected by each dilution was recorded. Virus concentrations were estimated by the weighted average $\log_{10}u$ systemic assay method (1).

Narrow- and broad-sense heritability values (Table 3) were computed by the variance component analysis method (7).

RESULTS

Table 1 indicates that if the line of division between resistant and susceptible classes is drawn at disease rating 2, the distribution of the observed frequencies of diseased plants in all the six generations conformed to the expected frequencies on the assumption of a single recessive gene controlling resistance to PVMV disease in the greenhouse as well as in the field. Complete dominance of susceptibility was indicated. No other simple genetic models seemed consistent with the data.

In the systemic assay experiment (Table 2), negligible virus was assayed in the P_1 while P_2, F_1, and B_2 which had 4 + 5 disease ratings had very similar virus concentrations. In the segregating populations F_2 and B_1, there was a distinct difference between the virus content of grouped ratings 1 and 2 and ratings 3 and 4 + 5, and there was no significant difference in virus content between rating 3 and ratings 4 + 5. A similar picture was obtained in a preliminary assay: 1+2 (0.98 ± 0.16); 3(2.12 ± 0.08); 4+5(1.98 ± 0.08).

Narrow- and broad-sense heritability values were high, 62% and 83%, respectively (Table 3).

DISCUSSION

The assumption of disease rating 2 as a resistant reaction seemed logical for the following reasons: (i) it

TABLE 1. Frequency distribution of pepper veinal mottle virus disease ratings in greenhouse and field tests of chilli

		Disease reaction								
		Resistant ratings			Susceptible ratings			Expected ratio	Chi-	
Population	Test	0	1	2	3	4	5	(Res:Susc.)	square	*P*
P_1	G[a]		101	2				1:0		
	F[b]			60				1:0		
P_2	G						77	0:1		
	F						55	0:1		
F_1	G					1	11	0:1		
	F						19	0:1		
B_1	G		36	18	27		30	1:1	0.08	0.70-0.80
	F			35	9	5	33	1:1	1.76	0.10-0.20
B_2	G						82	0:1		
	F					1	106	0:1		
F_2	G		28	10	16	4	91	1:3	0.02	0.98-0.99
	F			35	1		106	1:3	0.01	0.99

[a]G = Greenhouse test.
[b]F = Field test.

TABLE 2. Estimated mean pepper veinal mottle virus ($\log_{10}u$) concentrations and their standard errors in the leaves of inoculated chilli plants

	Disease rating[a]			
	Resistant		Susceptible	
Population	1	2	3	4 + 5
P_1	0.00			
P_2				1.20 ± 0.12
F_1				0.90 ± 0.19
B_2				1.11 ± 0.15
B_1	0.02 ± 0.11	1.25 ± 0.08	1.78 ± 0.08	1.66 ± 0.08
F_2	0.00	1.06 ± 0.08	1.47 ± 0.11	1.29 ± 0.10

[a]Disease rating scale: 1 = chlorosis; 2 = indistinct mottling; 3 = distinct mottling with slight leaf distortion; 4 = severe leaf distortion; 5 = severe leaf narrow/filiform leaves.

TABLE 3. Calculation of narrow(h^2_N)- and broad(h^2_B)-sense heritabilities of pepper veinal mottle virus resistance from the variances of the six genetic populations in chilli

Variances						Components of variation			Heritability (%)	
P_1	P_2	F_1	B_1	B_2	F_2	1/2D[a]	1/4H[b]	E[c]	h^2_N[d]	h^2_B[e]
0.00	0.00	0.00	1.90	0.01	1.39	0.86	0.53	0.00	62.00	83.00

[a] $D/2 = 2V_{F_2} - (V_{B_1} + V_{B_2})$

[b] $H/4 = V_{F_2} - D/2 - E$

[c] $$E = \frac{V_{F_1} + V_{P_1} + V_{P_2}}{3}$$

in which: V = variance
D/2 = additive variance
H/4 = dominance variance
E = environmental variance

[d] $$h^2_N = \frac{D/2}{V_{F_2}}$$

[e] $$h^2_B = \frac{(D/2 + H/4)}{V_{F_2}}$$

was manifested only in the P_1 plants in the nonsegregating populations in the greenhouse test; (ii) it was the only disease expression of P_1 plants in the field test; and (iii) it was distinctly different in symptom expression from ratings 3, 4, and 5 (10).

Admittedly, contamination by other strains of the virus and/or other viruses might have occurred with the use of unpurified inoculum and field infection. However, because a consistent discrete simple inheritance pattern was obtained and the heritability estimate was high, it can be inferred that such contamination, if present at all, was minimal. Different ratings in resistant and susceptible plants might be caused by modifying genes or environmental modification of the disease reaction expression. Narrow-sense heritability (62%) indicated that additive effects, which presumably arose from the cumulative effects of minor genes, played a significant role in the expression of PVMV resistance and susceptibility.

The elucidation of the major gene control of PVMV resistance here is consistent with the findings by most workers on chilli virus resistance, especially those of Greenleaf (4), Cook and Anderson (3), and Zitter and Cook (11), who also reported single recessive gene control of resistance to TEV, PVY and pepper mottle virus, respectively, in *Capsicum*.

In bulking plants for assaying the relative virus concentrations, inherent differences within classes and misclassification of a few plants might have occurred. As such, the results of the virus assays can only suggest that the resistance operates by partially suppressing virus multiplication. This type of resistance, a widespread phenomenon in virus-infected crops (9), is generally termed tolerance (5) and is presumably the type reported by Greenleaf (4) and Zitter and Cook (11).

The relative stability of this resistance under local conditions suggests that the resistant parent (accession 7.4) is of practical use in plant breeding. However, accession 7.4 is late-bearing, tall, leafy, and produces commercially unacceptable fruits. Simple backcrossing alternating with selfing should incorporate this pair of resistant alleles into susceptible but commercially acceptable cultivars fairly rapidly. Furthermore, the presence of additive effects suggests that higher resistance may be achieved by selection among the resistant progenies.

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