Theor Appl Genet (2000) 101:463–472

R. Grumet · E. Kabelka · S. McQueen · T. Wai
R. Humphrey

Characterization of sources of resistance to the watermelon strain of *Papaya ringspot virus* in cucumber: allelism and co-segregation with other potyvirus resistances

Received: 22 July 1999 / Accepted: 2 December 1999

Abstract At least three sources of resistance to the watermelon strain of *Papaya ringspot virus* (PRSV-W) have been identified in cucumber (*Cucumis sativus* L.) including: 'TMG-1', an inbred line derived from the Taiwanese cultivar, 'Taichung Mou Gua'; 'Dina-1', an inbred line derived from the Dutch hybrid 'Dina'; and the South American cultivar 'Surinam'. In this investigation we sought to determine the inheritance of resistance to PRSV-W in 'Dina-1', the allelic relationships among the three sources of PRSV-W resistance, and the relationship between PRSV-W resistance and known resistances to other cucurbit potyviruses. Like 'Surinam' and 'TMG-1', resistance in 'Dina-1' is controlled by a single gene. Despite differences in dominance vs recessive performance and patterns of virus accumulation, all three sources of resistance complemented each other. 'TMG-1' and 'Dina-1' also possess co-segregating, single-gene resistances to *Zucchini yellow mosaic virus* (ZYMV), *Watermelon mosaic virus* and *Moroccan watermelon mosaic virus*. Sequential inoculations and F_3 family analysis indicated that resistance to PRSV-W completely co-segregated with resistance to ZYMV in 'TMG-1'. Although PRSV-W resistances are at the same locus in both 'TMG-1' and 'Surinam', 'Surinam' is only resistant to PRSV-W, and progeny of 'TMG-1' × 'Surinam' were resistant to PRSV-W but susceptible to ZYMV. Susceptibility to ZYMV and resistance to PRSV-W in 'Surinam' was not influenced by co-inoculation or sequential inoculations of the two viruses. Collectively, the co-segregation of resistances to PRSV-W, ZYMV, WMV and MWMV in 'TMG-1' (within 1 cM), allelism of PRSV-W resistances in 'TMG-1' and 'Surinam', and resistance to only PRSV-W in 'Surinam', suggest that multiple potyvirus resistance in cucumber may be due to different alleles of a single potyvirus resistance gene with differing viral specificities, or that the multiple resistances are conferred by a tightly linked cluster of resistance genes, of which 'Surinam' only possesses one member.

Key words Key words: *Cucumis sativus* · Resistance genes · Virus resistance · Gene cluster

Communicated by B.S. Geill

R. Grumet (✉) · E. Kabelka · S. McQueen · T. Wai · R. Humphrey
Department of Horticulture, Michigan State University,
East Lansing MI 48824, USA
e-mail: grumet@pilot.msu.edu
Tel.: +517–353–5568

Introduction

The watermelon strain of *Papaya ringspot virus* (PRSV-W) is one of several potyviruses causing severe damage to the production of cucurbit crops worldwide (Purcifull et al. 1984a). At least three sources of resistance to this virus have been identified in cucumber (*Cucumis sativus* L.). These include: 'Surinam', a cultivar from South America (Wang et al. 1984), 'TMG-1', an inbred line derived from a single plant selection from the Taiwanese cultivar 'Taichung Mou Gua' (Provvidenti 1985), and 'Dina-1' an inbred line derived from self-pollinations of the Dutch hybrid 'Dina' (Kabelka et al. 1997). Resistance in 'Surinam' is conferred by a single recessive gene (Wang et al. 1984), while resistance in 'TMG-1' appears to be conditioned by a single dominant or incompletely dominant gene (Wai and Grumet 1995a). The inheritance of PRSV-W resistance in 'Dina-1' and relationships among the three PRSV-W resistances were not determined.

In addition to PRSV-W, there are several other potyviruses that infect cucurbit crops, including *Zucchini yellow mosaic virus* (ZYMV), *Watermelon mosaic virus* (WMV), the *Moroccan watermelon mosaic virus* (MWMV) and *Zucchini yellow fleck virus* (ZYFV) (Purcifull et al. 1984b; McKern et al. 1993; Gilbert-Albertini et al. 1995; Desbiez and Lecoq 1997). Among these potyviruses, PRSV-W is most closely related to MWMV (McKern et al. 1993). PRSV-W and MWMV share 73% amino-acid identity in their coat proteins; ZYMV and WMV share 79% amino-acid identity, while PRSV-W and MWMW share only approximately 60%

identity with ZYMV or WMW (Lanina and Grumet, unpublished). Isolates of the same virus exhibit greater than 90% amino-acid identity in the coat-protein sequence (Shukla et al. 1994).

'Surinam' is only resistant to PRSV-W, but 'TMG-1' and 'Dina-1' are also resistant to ZYMV, WMV and MWMV (Provvidenti 1985; Gilbert-Albertini et al. 1995; Kabelka et al. 1997). In 'Dina-1' and 'TMG-1,' resistance to ZYMV is conferred by single genes (Provvidenti 1987; Abul-Hayja and Al-Shawan 1991) that are alleles of the same locus (Kabelka et al. 1997). The *zym*^{Dina} allele, which allows for viral spread and distinct veinal chlorosis that is limited to one leaf, is dominant to the *zym*^{TMG} allele which appears to restrict virus accumulation more rapidly; both are recessive to the allele for susceptibility. Resistance to ZYFV (Gilbert-Albertini et al. 1995) and MWMV (Kabelka and Grumet 1997) are also controlled by single recessive genes in 'TMG-1' and 'Dina-1', while resistance to WMV in 'TMG-1' appears to include two resistances, one of which is controlled by a single recessive allele (Wai and Grumet 1995b).

In several cases multiple potyvirus resistances co-segregate. Examples include WMV and *Bean yellow mosaic virus* (BYMV) resistance in pea (Schroeder and Provvidenti 1971), ZYMV and WMV resistance in *Cucurbita moschata* (Gilbert-Albertini et al. 1993), *Potato virus Y* (PVY) and *Pepper mottle virus* (PeMV) resistance in pepper (Dogimont et al. 1996), and the *I* locus in bean (Fisher and Kyle 1994). This also has been observed in cucumber. The gene conferring resistance to ZYMV in 'TMG-1' appears to be the same as, or tightly linked to, genes conferring resistance to WMV and MWMV (Wai et al. 1995b; Kabelka and Grumet 1997); similarly, the resistances to ZYMV and MWMV completely co-segregate in 'Dina-1' (Kabelka et al. 1997).

In this investigation we sought to further understand the inheritance of multiple potyvirus resistance in cucumber by studying the allelic and dominance relationships among the three sources of PRSV-W resistance, and by examining the relationship between resistance to PRSV-W and the other potyvirus resistances in 'TMG-1' and 'Dina-1'.

Materials and methods

Cucumber genotypes

The inbred potyvirus-resistant cucumber (*Cucumis sativus* L.) lines 'TMG-1' and 'Surinam' were originally provided by Dr. J. Staub (USDA, University of Wisconsin, Madison) and Dr. R. Provvidenti (Cornell University, Geneva, N.Y.), respectively. Self-pollinated progeny of the Dutch hybrid 'Dina', true breeding for resistance to ZYMV ('Dina-1'), were initially provided by Dr. K. Owens (Seminis Seed Co., Woodland, Calif.). All lines were increased by self- or sib-pollinations in the greenhouse. The two susceptible parental genotypes were 'Wisconsin-2757' [WI-2757 (Peterson et al. 1982); provided by Dr. J. Staub] and 'Straight-8' (W. Atlee Burpee and Co., Warminster, Pa.). F_1, F_2 and backcross progeny of the crosses among all genotypes were produced in the greenhouse. F_3 families derived from ZYMV-resistant 'TMG-1' × 'WI-2757' F_2 individuals were produced in the field as described in Kabelka and Grumet (1997).

Virus inocula, inoculation procedures, experimental designs, and symptom scoring

PRSV-W (ATCC PV-380), ZYMV (Connecticut strain), WMV (ATCC PV379), and MWMV (originally provided by Dr. D. Purcifull, University of Florida, Gainesville) were propagated in *Cucurbita pepo* L. cvs 'Black Beauty' (Seed Way Inc., Elizabethtown Pa.) or 'Midas' (Willhite Seed, Poolville Tex.) as described in Kabelka and Grumet (1997). Purity of the virus sources was verified by ELISA and by the use of the differential host, *Phaseolus vulgaris* cv 'Black Turtle 2' (Provvidenti et al. 1984).

Inocula were prepared by grinding young symptomatic zucchini source plant leaves in a 1:10 ratio of leaf tissue: ice-cold 0.02-M sodium phosphate buffer, pH 7.0, and filtering through cheesecloth. Mixed ZYMV and PRSV-W inocula were prepared as for single virus inocula and then combined in equal volumes. Cotyledons (or young leaves where indicated) were lightly dusted carborundum and rub-inoculated with using sponge plugs. Germination, planting, fertilization, and greenhouse growth conditions were as described in Kabelka and Grumet (1997).

Experiments to evaluate F_2 and backcross progeny were performed using cotyledon inoculation. Sixteen rows of ten plants/row were interspersed with five internal control rows consisting of inoculated, mock-inoculated, and non-inoculated parental and F_1 progeny. Similarly tests of F_3 families included ten individuals per family with five control rows evenly distributed along the bench. Sequential inoculation experiments consisted of cotyledon-inoculation of parental and progeny genotypes with one virus followed by true-leaf inoculation of the resistant individuals with the second virus. Additional inoculated, mock-inoculated, and non-inoculated control plants were included for the true-leaf inoculation portion of the experiment to confirm successful virus inoculation. Mixed and sequential inoculations of non-segregating populations (e.g., parental, F_1) were performed using a randomized complete block design with 8–12 replicates per treatment. All experiments included border rows of susceptible 'Straight-8' plants as a further check for any variation in inoculum and/or symptom expression.

In all experiments, plants were visually scored as either resistant (symptom-free) or susceptible at the time of symptom development on the susceptible genotypes (approximately 14-days post-inoculation with PRSV-W and 7-days post-inoculation with ZYMV). In some cases, a 0–4 symptom severity scale was used with 0 = no symptoms; 1–2 = mild symptoms and an absence of symptoms in the youngest leaves; 3–4 = moderate to severe symptoms including the emerging leaves. Segregation ratios were analyzed by chi-square analysis; if only two classes were predicted, the Yate's correction factor was applied.

ELISA analyses

ELISA was performed using leaf-disk samples as described in Wai and Grumet (1995b). ZYMV was detected with anti-ZYMV (Ct strain) polyclonal rabbit IgG antibody (Hammar and Grumet, unpublished); anti-PRSV-W antibody was purchased from Agdia (Elkhart, Ind.). Experiments were performed in a randomized complete block design with five replicates per treatment. Data were analyzed by analysis of variance.

Results

To further understand multiple potyvirus resistances in cucumber we addressed the following questions: (1) what is the relationship among the different sources of PRSV–W resistance, i.e., are all sources allelic, what are

Table 1 Potyvirus resistances in the cucumber genotypes 'TMG-1', 'Dina-1' and 'Surinam'

Source		PRSV-W	ZYMV	WMV	MWMV
TMG-1	Response	Resistant	Resistant	Resistant	Resistant
	Inheritance	One incompletely dominant (Wai and Grumet 1995a)	One recessive (Provvidenti 1987)	Complex (Wai and Grumet 1995b)	One recessive (Kabelka and Grumet 1997)
Dina-1	Response	Resistant (Kabelka et al. 1997)	Resistant	Resistant (Kabelka et al. 1997)	Resistant
	Inheritance	Not determined	One recessive (Abul Hayja and Al-Shawan 1991)	Not determined	One recessive (Kabelka et al. 1997)
Surinam	Response	Resistant	Susceptible	Susceptible	Susceptible
	Inheritance	One recessive (Wang et al. 1984)			

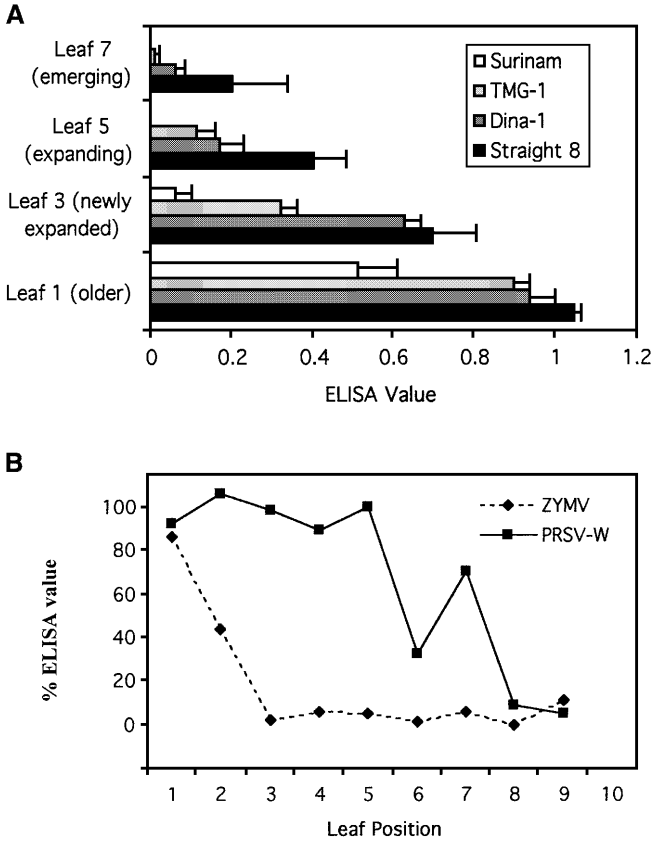

Fig. 1 A PRSV-W accumulation in emerging, expanding, newly expanded and older leaves of 'Straight 8' (susceptible), 'Surinam', 'TMG-1' and 'Dina-1' at 3-weeks post-inoculation. Each point is the mean of five replicate plants. **B** PRSV-W or ZYMV accumulation in 'TMG-1' leaves as a percentage of accumulation in susceptible 'WI-2757' leaves 6-weeks post-inoculation. Each data point is the ratio of the ELISA value for a given leaf position in TMG / ELISA value in Straight 8; each ELISA value used was the mean of five replicate plants

the dominance relationships among the sources, do all the resistances appear to have a similar mechanism; and (2) what is the relationship of PRSV-W resistances to other cucurbit potyvirus resistances, i.e., do they co-segregate, is response to one virus influenced by another? As a first step, the three genotypes possessing resistance to PRSV-W, 'TMG-1' (Provvidenti 1985), 'Surinam' (Wang et al. 1984) and 'Dina-1' (Kabelka et al. 1997), were directly compared for their response to inoculation with PRSV-W, ZYMV, WMV, and MWMV. The observed resistances, which confirmed and expanded previous reports, are summarized in Table 1. 'Surinam' differs from 'TMG-1' and 'Dina-1' in that it is only resistant to PRSV-W.

The three genotypes were also examined for virus accumulation using ELISA (Fig. 1A). As might be expected, virus accumulated most rapidly and to the greatest extent in the susceptible 'Straight 8' plants. While virus titers were barely or not detectable in the emerging leaves (1/4–1/3 expanded) of the three resistant genotypes, measurable virus accumulation was present in the emerging 'Straight 8' leaves. Despite the lack of symptom expression, virus eventually accumulated in the more-developed leaves of the resistant genotypes, but to a lesser extent and at a slower rate in 'Surinam' than 'TMG-1' or 'Dina-1'. There was very little or no virus accumulation in the young expanding or newly expanded 'Surinam' leaves. Analagous results were obtained at 3- and 4-weeks post-inoculation (the data shown are from week 3).

Relationship among the three sources of PRSV-W resistance in cucumber

Segregation ratios for symptom development in the progeny of 'Dina-1' × 'Straight 8' (susceptible) fit a single-gene model for inheritance of PRSV-W resistance in 'Dina-1' (Table 2). F_1 progeny of 'Dina-1' × 'Straight 8' were characterized by an intermediate phenotype of reduced symptom severity and rate of spread. When scored on a scale of 0–4 (experiments 3 and 4), the majority of the F_1 progeny fell into a reduced symptom class with ratings of 1–2 (symptoms were mild and were only present on older leaves, but not young emerging leaves). The number of individuals exhibiting the intermediate phenotype in the F_2 and backcross generations was consistent with the numbers expected for the heterozygous classes. These results suggest that a single copy of the resistance allele from 'Dina-1' delayed and reduced, but did not prevent, symptom development.

When hybrid combinations of the three resistant genotypes, 'TMG-1' × 'Dina-1', 'TMG-1' × 'Surinam',

Table 2. Inheritance of PRSV-W resistance in 'Dina-1'

Genotype	Experiment	Response to inoculation with PRSV-W[a]			Expected ratio (R:S)	χ^2
		Res	Susceptible			
		(0)	Int (1–2)	Mod./sev.(≥3)		
Dina-1	1,2	20	–	0		
	3,4	30	0	0		
Straight 8	1,2	0	–	20		
	3,4	0	0	30		
F_1	1,2	2	–	19		
	3,4	2	24	8		
F_2	1	32	–	87	1:3	0.28 ns[c]
	3	23	35	31	1:2:1	5.49 ns
	1,3 pooled[b]	55	–	154	1:3	0.09 ns
BC (F_1 × Dina)	1,2	52	–	49	1:1	0.23 ns (expl); 0 ns (exp2)
	3,4	65	68	4	1:1	0.28 ns (exp3); 0.01 ns (exp4)
	1–4 pooled	117	–	121	1:1	0.04 ns
BC (F_1 × St8)	1	0	–	100		
	3,4	1	67	69	1:1	0.16 ns (exp3); 0.05 ns (exp4)
	1–4 pooled	1	–	236		

[a] In experiments 1 and 2 plants were only scored as resistant or susceptible. In experiments 3 and 4 plants were rated on a 0–4 scale. 0 = no symptoms; 1–2 = mild symptoms and an absence of symptoms in the youngest leaves; 3–4 = moderate to severe symptoms including the emerging leaves.

[b] Pooled data were classified as either resistant or susceptible.

[c] ns, χ^2 value not significant $P \leq 0.05$

Table 3 Tests for allelism among PRSV-W resistances in 'TMG-1', 'Surinam' and 'Dina-1'

Parent or progeny		Number of plants	
		Resistant	Susceptible
TMG-1		56	0
Surinam		34	0
Dina-1		60	0
Straight 8		0	86
F_1	TMG × Surinam	16[a]	0
F_2	TMG × Surinam	320	0
BC1	F_1 × TMG	80	0
BC2	F_1 × Surinam	200	0
F_1	TMG × Dina	30[a]	0
F_2	TMG × Dina	318	0
BC1	F_1 × TMG	120	0
BC2	F_1 × Dina	159	0
F_1	Dina × Surinam	20	0
F_2	Dina × Surinam	157	0
BC1	F_1 × Dina	80	0
BC2	F_1 × Surinam	78	0

[a] Data for each pair of parents are pooled from two experiments

and 'Dina–1' × 'Surinam' were examined for response to inoculation with PRSV-W, all individuals of the various F_1, F_2 and backcross generations remained symptom free (Table 3), indicating that the resistance alleles are at the same locus in all three genotypes. Although the alleles from all three genotypes complemented each other, the allele from 'Surinam' is reported to be recessive (Wang et al. 1984), while the alleles from 'TMG-1' and 'Dina-1' appeared to be incompletely dominant or dominant (Table 2; Wai and Grumet 1995a). Each characterization, however, was performed at different times and with different susceptible parents. To clarify their relative performances, 'TMG-1' and 'Surinam' were each crossed to a common susceptible genotype, 'Straight 8', and their progeny tested concurrently. In addition, since inheritance of resistance to PRSV-W in 'TMG-1' was initially characterized using 'WI-2757', progeny of 'WI-2757' × 'TMG-1' were included for comparison.

Consistent with the previous studies of 'Surinam' (Wang et al. 1984), segregation ratios indicated a recessive allele. The F_1 and susceptible F_2 and backcross progeny of 'Straight 8' × 'Surinam' exhibited PRSV-W symptoms throughout the plant that were as severe as those of the susceptible parent (Table 4). The F_1 progeny of 'TMG-1', however, exhibited an intermediate phenotype. The young leaves and growing points were symptom free, while older leaves exhibited variable degrees of rugosity and/or silver banding ranging from nearly symptom free to moderately severe. Segregating 'TMG-1' progeny showed the expected phenotypic ratios for the intermediate phenotype. Symptom severity of the intermediate phenotype did not appear to be affected by the different susceptible parents; when tested concurrently, the response of 'TMG-1' × 'Straight 8' and 'TMG-1' × 'WI-2757' was equivalent.

Accumulation of PRSV-W in newly expanded leaves of resistant 'TMG-1' had been observed previously and was contrasted with the lack of measurable virus accumulation in comparable leaves of ZYMV-inoculated 'TMG-1' plants (Wai and Grumet 1995a). It was suggested that accumulation of PRSV-W, but not ZYMV, in the 'TMG-1' plants reflected dominant or incompletely dominant vs recessive performance of the PRSV-W and

Table 4 Response of 'TMG-1', 'Surinam', 'Straight-8', 'WI-2757' and their progeny to inoculation with PRSV-W

Parent or Progeny		Number of plants			Expected ratios (R:I:S)	χ^2
		Resistant[a]	Susceptible			
			Intermediate[b]	Mod/severe[c]		
TMG-1		15	0	0		
Surinam		15	0	0		
Straight 8		0	0	15		
WI-2757		0	0	15		
F_1	Straight 8 × Surinam	0	0	40		
F_2	Straight 8 × Surinam	33	0	86	1:3	0.34 ns[d]
BC1	F_1 × Surinam	32	0	28	1:1	0.15 ns
BC2	F_1 × Straight 8	0	0	60		
F_1	Straight 8 × TMG	0	20	0		
F_2	Straight 8 × TMG	33	55	32	1:2:1	0.85 ns
BC1	F_1 × TMG	28	32	0	1:1	0.15 ns
BC2	F_1 × Straight 8	0	29	31	1:1	0.02 ns
F_1	WI-2757 × TMG	0	20	0		
F_2	WI-2757 × TMG	14	29	15	1:2:1	0.03 ns
BC1	F_1 × TMG	21	19	0	1:1	0.03 ns
BC2	F_1 × WI-2757	0	17	23	1:1	0.63 ns

[a] Plants are symptom-free, vigorous and healthy
[b] Young leaves and growing points remain symptom free, but older leaves exhibit rugosity and/or silver banding
[c] Systemic rugosity and/or silver banding throughout the plant, similar to that exhibited by either susceptible parent
[d] ns, $P \leq 0.05$

ZYMV resistances, respectively. A similar correlation between presence or absence of virus accumulation and dominant vs recessive allele performance might be made if only newly expanded leaves were examined for PRSV-W in 'TMG-1', 'Dina-1' and 'Surinam'. However, when viewing the entire plant (Fig. 1A), it was evident that the older 'Surinam' leaves also eventually accumulated PRSV-W.

Re-examining 'TMG-1' plants for the presence of ZYMV in older leaves gave results similar to those observed for PRSV-W in 'Surinam'. ZYMV was absent in younger leaves of 'TMG-1', but eventually accumulated in the oldest leaves (Fig. 1B). To compare ZYMV and PRSV-W accumulation in 'TMG-1', virus levels were plotted as a percentage of the amount present in systemically infected leaves of the susceptible genotype 'WI-2757'. PRSV-W was detected in most of the leaves, including young expanded leaves.

Relationship between resistances to PRSV-W and ZYMV

Previous studies have shown that the alleles conferring resistance to ZYMV, WMV and MWMV in 'TMG-1', and ZYMV and MWMV resistance in 'Dina-1' appear to be at the same locus or very tightly linked loci (< 1 cM apart) (Wai and Grumet 1995b; Kabelka et al. 1997). The relationship of PRSV-W resistance to the other potyvirus resistances, however, had not been determined.

Unlike 'TMG-1' and 'Dina-1', 'Surinam' is susceptible to ZYMV, WMV and MWMV. This separation of PRSV-W resistance from other potyvirus resistances and the range of expression observed for the PRSV-W resistances (recessive, incompletely dominant, dominant) vs recessive only for ZYMV, WMV and MWMV, suggested that resistance to PRSV-W might be unique relative to the other resistances. Possible explanations for these observations include: PRSV-W resistance is at a distinct location in the genome, multiple potyvirus resistance is due to a cluster of genes and 'Surinam' only has the gene for PRSV-W resistance, or there are varying alleles of a single potyvirus resistance gene with differing specificities and effectiveness relative to the different viruses.

Relationship between response to PRSV-W and ZYMV in 'TMG-1'

The relationship between ZYMV and PRSV-W resistances in 'TMG-1' was examined by sequential inoculations and F_3-family analysis. For the sequential inoculations, cotyledons of backcross progeny of ('Straight 8' × 'TMG-1') × 'TMG-1' were either inoculated with PRSV-W followed by true leaf inoculation of the resistant individuals with ZYMV, or vice versa (Table 5 A,B). In all experiments, additional control plants were included to verify successful inoculation at the true leaf stage. Although symptoms of ZYMV or PRSV-W developed on the susceptible 'Straight 8' and F_1 progeny following either cotyledon- or true leaf-inoculation, in all cases, those backcross individuals that were resistant to cotyledon inoculation with the first virus remained symptom free upon true leaf inoculation with the second virus.

The association between ZYMV and PRSV-W resistance was also examined by screening 51 F_3 families pro-

Table 5 Response of 'TMG-1', 'Straight 8', F_1 and backcross progeny to sequential inoculation with ZYMV and PRSV-W

A

Genotype	Total plants	Cotyledon inoculation with PRSV-W			Leaf inoculation of PRSV–W resistant	
		Number of plants			Number of plants	
		Res[a]	Int	Sus	Res	Sus
TMG-1	10	10	0	0	10	0
Straight 8[b]	10	0	0	10	–	–
Straight 8[c]	5	–	–	–	0	5
F_1[b]	10	0	10	0	–	–
F_1[c]	5	–	–	–	0	5
BC (F_1 X TMG)[d]	160	77	83	0	77	0

B

Genotype	Total plants	Cotyledon inoculation with ZYMV		Leaf inoculation of ZYMV resistant		
		Number of plants		Number of plants		
		Res[a]	Sus	Res	Int	Sus
TMG-1	10	10	0	10	0	0
Straight 8[b]	10	0	10	–	–	–
Straight 8[c]	5	–	–	0	0	5
F_1[b]	10	0	10	–	–	–
F_1[c]	5	–	–	0	5	0
BC ($F_1 \times$ TMG)[d]	160	78	82	78	0	0

[a] Res., Int., Sus. are resistant, intermediate, and susceptible, respectively
[b] Control plants to verify successful inoculation at the cotyledon stage
[c] Control plants to verify successful inoculation at the true leaf stage
[d] Data fit the predicted segregation ratios based on resistance to PRSV-W conferred by an incompletely dominant gene ($\chi^2 = 0.16$ ns) and ZYMV by a single recessive gene ($\chi^2 = 0.06$ ns)

duced by the self-pollination of ZYMV-resistant F_2 individuals from the 'TMG-1' × 'WI-2757' cross. As expected, based on recessive resistance to ZYMV, all progeny were resistant to ZYMV. If the two resistances had been segregating independently, 9/16th of the F_3 families should be susceptible, or segregating for susceptibility, to PRSV-W. There was, however, no segregation for susceptibility to PRSV-W either within or among the F_3 families screened. The failure to segregate resistance to PRSV-W from ZYMV indicates that resistance to the two viruses is conferred by the same, or two tightly linked, genes (less than 1 cM apart; product-ratio method). Since all the sources of PRSV-W resistance are allelic, the resistance in 'Surinam' also resides at this location.

Relationship between response to PRSV-W and ZYMV in 'Surinam'

The separation between ZYMV and PRSV-W resistance in 'Surinam' was further examined in segregating progeny of 'TMG-1' × 'Surinam' (Table 6). The F_1 progeny were resistant to PRSV-W (Tables 3, 7A), but susceptible to ZYMV (Table 6). However, unlike the recessive inheritance of ZYMV resistance in crosses between 'TMG-1' and the susceptible 'Straight 8' or 'WI-2757' lines (Table 7A; Wai and Grumet 1995b; Kabelka et al. 1997), the F_1 progeny of 'TMG-1' × 'Surinam' exhibited a distinct intermediate phenotype of delayed symptom development and reduced symptom severity. The 'Surinam' plants all exhibited strong mosaic symptoms on the first true leaf within 5–7 days post-inoculation of the cotyledons; in F_1 plants the symptoms did not appear until approximately 7–9 days post-inoculation and there was a delay in the rate of spread and the symptom severity on the subsequent systemic leaves. The intermediate phenotype also was clearly observed in the segregating F_2 and backcross progeny of 'TMG-1' and 'Surinam'.

'Surinam' plants also were tested to determine whether the differential response to ZYMV and PRSV-W observed in single inoculations was affected by exposure to the other virus using either mixed or sequential inoculations. When cotyledons of 'Surinam' plants were inoculated with both viruses, the systemic leaves only exhibited ZYMV symptoms that occurred at the same time as, and were as severe as, those inoculated with ZYMV alone (Table 7A). Similarly, 'TMG-1' × 'Surinam' progeny developed only ZYMV symptoms, but they showed the reduced severity observed earlier for response to ZYMV inoculation alone. Finally, when 'Surinam' plants were first inoculated with PRSV and then inoculated with ZYMV 1 or 2 weeks later (Table 7B, treatment 4), they developed obvious ZYMV symptoms at the same frequency as those that had not been pre-inoculated with PRSV-W (Table 7B,

Table 6 Response of progeny of 'TMG-1' and 'Surinam' to inoculation with ZYMV. Data are pooled from three experiments

Parent or progeny	Number of plants			Expected ratio	χ^2
	Resistant	Susceptible			
		Intermediate	Mod./Severe		
TMG-1	59	0	0		
Surnam	0	0	56		
F_1	0	56	0		
F_2	139	223	111	1:2:1	4.93 ns[a]
$F_1 \times$ TMG	117	112	0	1:1	0.07 ns
$F_1 \times$ Surinam	0	122	100	1:1	1.99 ns

[a] ns $P \leq 0.05$

Table 7. Sequential and mixed inoculation with PRSV-W and ZYMV

A. Mixed inoculations. Data are polled from two experiments

Genotype	Inoculum			
			ZYMV+PRSV	
	ZYMV	PRSV	ZYMV	PRSV
Straight 8	20/20[a]	19/19	19/19	18/18[b]
Surinam	17/17	0/19	17/18	0/18
TMG-1	0/20	0/19	0/19	0/18
Straight 8 x Surinam F_1	19/19	18/19	19/19	18/19
TMG x Straight 8 F_1	20/20	18/19	19/19	18/19
TMG x Surinam F_1	19/20[c]	0/19	19/19[c]	0/19

[a] Number with symptoms / total number inoculated
[b] PRSV-W symptoms appeared faster when inoculated separately than in combination
[c] Symptoms were milder and slower to develop than on the other susceptible genotypes

B. Sequential inoculations. Data are pooled from four experiments

Treatment	Genotype	Number with symptoms/total	
		PRSV	ZYMV
1. ZYMV, cotyledon[a]	Straight 8	–	39/39
	Surinam	–	34/35
2. ZYMV, true leaf	Straight 8	–	27/43
	Surinam	–	29/42
3. PRSV-W, cotyledon	Straight 8	43/45	–
	Surinam	0/43	–
4. PRSV-W, cotyledon; ZYMV true leaf	Straight 8	29/29	–
	Surinam	0/42	12/16

[a] Cotyledon inoculations were performed at 6–7 days post planting. True leaf inoculations were performed at 21-days post-planting (exps. 1,2) or 12 or 14 days post-planting (exps. 3,4). For 21 day inoculations, PRSV-W symptoms were clearly visible on the susceptible 'Straight-8' plants

treatment 2). Thus prior inoculation with PRSV-W did not induce a subsequent resistance to ZYMV infection, nor did co-inoculation with ZYMV and PRSV-W result in resistance to ZYMV or susceptibility to PRSV-W.

Discussion

Inheritance studies of PRSV-W resistance in 'Dina-1' indicated that, like the resistances in 'TMG-1' and 'Surinam', resistance in 'Dina-1' is controlled at a single locus. Crosses among the three resistant genotypes demonstrated that the three PRSV-W resistances are all at the same locus. PRSV-W resistance in 'Surinam', however, differed from the other two sources in that it was not associated with other potyvirus resistances, and it appeared to be recessive, whereas the resistances from 'TMG-1' and 'Dina-1' appeared to be incompletely dominant.

The differences in performance may be due to different PRSV-W resistance alleles. A series of alleles with differing phenotypes and descending dominance occurs at the *zym* locus where *zym*^{Dina}, which results in a veinal chlorosis phenotype, is dominant to symptomless *zym*^{TMG} but recessive to the susceptible allele (Kabelka et al. 1997). However, there is not a distinct phenotype to distinguish between the PRSV-W resistances. Genetic background and/or environmental factors may also influence the apparent relative effectiveness of the resistance genes. Environmental effects on dominance classifications have been reported in other virus resistance systems (e.g., Kyle and Provvidenti 1993; Lewellen 1973) and also have been observed to influence symptom presence and severity in our experiments (these results; Wai and Grumet 1995a). However, the resistances from 'TMG-1' and 'Surinam' still performed differently when compared in the same environment and crossed with the same susceptible parent. This suggests a genetic component that may either be due to the allele itself and/or other modifying factors, including possible association with other tightly linked potyvirus resistance loci in 'TMG-1'.

The lack of ZYMV accumulation in young expanded leaves of 'TMG-1' had suggested that the mode of resistance to PRSV-W might differ from that of ZYMV (Wai and Grumet 1995a). However, more complete analysis of the whole plant over time suggested that a similar response might exist for both viruses, but with a difference in the kinetics of the resistance phenotype. A possible reason for high virus titers without symptom expression is that sufficient virus titers do not accumulate in the leaf at the critical time for symptom development. A variety of mechanisms of resistance to potyviruses have been observed including hypersensitivity [e.g., the *I* gene in bean (Fisher and Kyle 1994) and the *Pvr4* locus in pepper (Dogimont et al. 1996)], inhibition of viral replication observed at the level of protoplasts [e.g., the *pvr1* and *pvr2*^{2} loci in pepper (Deom et al. 1997; Murphy et

al. 1998)], and restriction of viral spread [e.g., the *pvr2*^{1} and *pvr3* loci in pepper (Murphy and Kyle 1995; Arroyo et al. 1996)]. The fact that measurable titers of both PRSV-W and ZYMV eventually accumulated, suggests that the resistances limit the rate of viral spread, but do not prevent viral replication.

Earlier studies have shown that both 'TMG-1' and 'Dina-1' are resistant to multiple potyviruses including PRSV-W, ZYMV, WMW and MWMV, and that the alleles for ZYMV, WMV and MWMV completely co-segregate. In this study we also identified a tight association between resistance to PRSV-W and ZYMV in 'TMG-1'. Sequential inoculation of ZYMV-resistant backcross progeny with PRSV-W (or PRSV-resistant backcross progeny with ZYMV) and analysis of F_3 families derived from F_2 individuals selected for resistance to ZYMV, indicate that both resistances are conferred by the same locus, or tightly linked loci. Population sizes were not sufficiently large to observe crossing-over within 1 cM. Since both ZYMV and PRSV-W resistances have been shown to be at the same locus in 'Dina-1' as 'TMG-1' (Kabelka et al. 1997; this paper) this conclusion also should hold for 'Dina-1'. These results are also consistent with analyses indicating that both PRSV-W and ZYMV resistances are linked to the *bi* locus for bitterfree cotyledons (Wang et al. 1987; Wai et al. 1997), and observations that have been made in cucumber breeding programs (Kyle and Provvidenti 1993).

The existence of simply inherited genes, or clusters of separate tightly linked genes, that confer resistance to two or more distinct potyviruses has been described previously in other cucurbit, legume and Solanacious species. In *C. moschata* a single dominant gene confers resistance to both ZYMV and WMV (Gilbert-Albertini et al. 1993), while in *P. vulgaris* the possibility of a single gene, or cluster of tightly linked genes co-segregating as a unit with the *I* gene, conditions resistance and/or lethal necrosis to a set of nine potyviruses (Kyle and Dickson 1988; Fisher and Kyle 1994). In *Pisum sativum*, well-defined clusters of tightly linked loci conferring resistance to a total of 11 potyviruses are located on two chromosomes; chromosome 2 contains resistance to seven potyviruses, while chromosome 6 includes resistance to three more potyviruses, two of which overlap with those on chromosome 2 (Provvidenti and Hampton 1993; Provvidenti and Niblett 1994). In potato, a single dominant gene, or a tightly linked cluster, introgressed from *Solanum stoloniferum* confers resistance to three potyviruses, *Potato virus Y, Potato virus A* and *Potato virus V* (Barker 1997), and in pepper an allele from CM334, confers resistance to PVY^{0}, PVY^{1}, PVY^{12} and PeMV (Dogimont et al. 1996). There are also in examples in potato and pepper of additional genes conferring resistance to a single potyvirus, e.g., *Ra* for PVA in potato and *Pvr5* for PVY^{0} in pepper (Barker 1996; Dogimont et al. 1996).

Despite the inability to break the linkage among the resistances to PRSV-W, ZYMV, WMV and MWMV in 'TMG-1', 'Surinam' is only resistant to PRSV-W and prior inoculation of 'Surinam' plants with PRSV-W did not induce resistance to ZYMV. Similarly, the presence of PRSV-W in mixed inoculations did not induce resistance to ZYMV, nor did infection by ZYMV overcome resistance to PRSV-W. This lack of association of resistances was also observed in the progeny of 'Surinam' × 'TMG-1'. Although all progeny are resistant to PRSV-W, they segregated for susceptibility to ZYMV. There are examples where co-inoculation of viruses can lead to a loss of resistance; infection by the cucumovirus *Cucumber mosaic virus* overcame the movement-based resistance of the *pvr2*^{1} gene for PeMV in pepper (Murphy and Kyle 1995). In this case, however, where both viruses were potyviruses, the PRSV-W resistance from 'Surinam' was not overcome, suggesting a specific interaction between PRSV-W and the resistance allele.

A somewhat analogous example occurs in pepper (*Capsicum annum*). Resistances of PVY and *Tobacco etch virus* (TEV) in the genotype SC46252 completely co-segregated, leading to the conclusion that a single recessive gene, *ey*^{a} (current nomenclature, *pvr2*; Kyle and Palloix 1997), conferred resistance to both viruses (Cook 1960). The subsequent discovery of a pepper line YRP10 with resistance to PVY that was allelic to the resistance in SC4625, but did not possess resistance to TEV, led to the conclusion that two tightly linked genes *et*^{a} and *y*^{a} were responsible for the two resistances (Cook 1961). Although it remained possible that there were different alleles with different viral specificities, there is now evidence indicating that *pvr2* is a complex locus, and that *et*^{a} (*prv2*^{2}) and *y*^{a} (*prv2*^{1}) are separate genes that confer resistance by different mechanisms (Kyle and Palloix 1997). TEV was unable to replicate in protoplasts from plants with the *et*^{a} gene (Deom et al. 1997) whereas the *y*^{a} gene allows for viral replication but interferes with subsequent cell to cell movement (Arroyo et al. 1996).

In the case of ZYMV and PRSV-W resistance in cucumber it is not clear that there are distinct mechanisms; however, noticeable differences in response to PRSV-W and ZYMV were observed in each of the PRSV-W resistant genotypes. In 'TMG-1' the resistance to PRSV-W appeared incompletely dominant, while resistance to ZYMV appeared recessive. Furthermore, high levels of PRSV-W were detected in young, symptomless 'TMG-1' leaves, but ZYMV was not detected in equivalent leaves. In 'Dina-1', which is resistant to ZYMV, PRSV-W and MWMV, inoculation with ZYMV resulted in a distinct veinal-chlorosis phenotype limited to the first and second true leaves, while no symptoms were observed with PRSV-W or MWMV inoculation (Kabelka et al. 1997). It is possible that these differences in response to ZYMV and PRSV-W in 'TMG-1' and 'Dina-1' may be due to varying effectiveness of one gene against different potyviruses, but it is also possible that they are due to two tightly linked genes with differing specificities. Finally, although 'Surinam' is resistant to PRSV-W, it is susceptible to ZYMV, WMV and MWMV; the PRSV-W resistance was not influenced by prior or co-inoculation with ZYMV. This may be due to different responses of one

gene to different potyviruses, or it may be that 'Surinam' only possesses one member of a gene cluster.

In several well-studied disease resistance loci, such as the *Rp1* locus of maize (Sudupak et al. 1993), the *Xa21* locus in rice (Song et al. 1997), the *Dm3* locus in lettuce (Meyers et al. 1998) and the *RPP* loci in *Arabidopsis* (Botella et al. 1998; McDowell et al. 1998), multigene families clustered at a single locus have been observed. Molecular evidence suggests that the multiple resistances have evolved via unequal crossing-over, gene-duplication events and the subsequent evolution of varying specificities. Analagous, well-defined clusters of virus resistance genes have not been molecularly characterized yet, but resistance gene-like fragments have been amplified from potato and shown to co-segregate with resistance to PVY (Hamalamen et al. 1998). This region of potato chromosome XI also contains a resistance gene for PVA within approximately 7 cM, and corresponds to the region of the tobacco genome including the *N* gene for hypersenstitive resistance to *Tobacco mosaic virus*. Most of the currently described resistance genes share certain molecular features such as a nucleotide-binding site and leucine-rich repeats, and confer a hypersensitive response (Bent 1996). It remains to be determined whether other sorts of resistance genes, such as those limiting the rates of viral spread, show similar features.

In conclusion, although we have not been able to break the linkage associations among the resistances to PRSV-W, ZYMV, WMV and MWMV in 'TMG-1', varying responses to the different viruses with regard to dominance relationships, resistance mechanisms, symptom expression, and the specific viruses protected against, support the possibility that multiple potyvirus resistance in cucumber is conferred by a tightly linked cluster of resistance genes.

Acknowledgements We thank Dr. R. Provvidenti (Cornell University) for providing 'Surinam' seed, Sue A. Hammar for assistance with ELISA analyses, Holly Kuhnle for greenhouse assistance, and Drs. J. Hancock and J. Kelly for helpful reviews of the manuscript. This work was in part supported by the office of USAID Cairo AgrA under Cooperative Agreement No. 263–0152-A-00–3036–00, and by the Michigan Agriculture Experiment Station and by a Michigan State University Undergraduate Professorial Assistantship to S. McQueen. Experiments were performed in compliance with current laws of the United States.

References

Abul-Hayja Z, Al-Shahwan IM (1991) Inheritance of resistance to zucchini yellow mosaic virus in cucumber. J Plant Dis Protection 98:301–304

Arroyo R, Soto MJ, Martinez-Zapater JM, Ponz F (1996) Impaired cell-to-cell movement of potato virus Y in pepper plants carrying the y^a ($pr2^1$) resistance gene. Mol Plant-Microbe Interact 9:314–318

Barker H (1996) Inheritance of resistance to potato viruses Y and A in progeny obtained from potato cultivars containing the gene *Ry*: evidence for a new gene for extreme resistance to PVA. Theor Appl Genet 93:710–716

Barker H (1997) Extreme resistance to potato virus V in clones of *Solanum tuberosum* that are also resistant to potato viruses Y and A: evidence for a locus conferring broad-spectrum potyvirus resistance. Theor Appl Genet 95:1258–1262

Bent AF (1996) Plant disease resistance genes: function meets structure. Plant Cell 8:1757–1771

Botella MK, Parker JE, Frost LN, Bittner-Eddy PD, Beynon JL, Daniels MJ, Holub EB, Jones JDG (1998) Three genes of the *Arabidopsis RPP1* complex resistance locus recognize distinct *Peronospora parasitica* avirulence determinants. Plant Cell 10: 1847–1860

Cook AA (1960) Genetics of resistance in *Capsicum annum* to two virus diseases. Phytopathology 50:364–367

Cook AA (1961) A mutation for resistance to potato virus Y in pepper. Phytopathology 51:550–552

Deom CM, Murphy JF, Paguio OR (1997) Resistance to tobacco etch virus in *Capsicum annum*: inhibition of virus RNA accumulation. Mol Plant Microbe Interact 10:917–921

Desbiez C, Lecoq H (1997) Zucchini yellow mosaic virus. Plant Pathol 46:809–829

Dogimont C, Palloix A, Daubze AM, Marchoux G, Selassie KG, Pochard E (1996) Genetic analysis of broad spectrum resistance to potyviruses using doubled-haploid lines of pepper (*Capsicum annum* L.). Euphytica 88:231–239

Fisher ML, Kyle MM (1994) Inheritance of resistance to potyviruses in *Phaseolus vulgaris* L. III. Co-segregation of phenotypically similar dominant responses to nine potyviruses. Theor Appl Genet 89:818–823

Gilbert-Albertini F, Lecoq H, Pitrat M, Nicolet JL (1993) Resistance of *Cucurbita moschata* to watermelon mosaic virus type 2 and its genetic relation to resistance to zucchini yellow mosaic virus. Euphytica 69:231–237

Gilbert-Albertini F, Pitrat M, Lecoq H (1995) Inheritance of resistance to zucchini yellow fleck virus in *Cucumis sativus* L. HortScience 30:336–337

Gray SM, Moyer JW, Kennedy GG (1988) Resistance in *Cucumis melo* to watermelon mosaic 2 correlated with reduced virus movement within leaves. Phytopathology 78:1043–1047

Hamalainen JH, Sorri VA, Watanabe KN, Gebhardt C, Valkomen JPT (1998) Molecular examination of a chromosome region that controls resistance to potato Y and A potyviruses in potato. Theor Appl Genet 96:1036–1043

Kabelka E, Grumet R (1997) Inheritance of resistance to the Moroccan watermelon mosaic virus in the cucumber line TMG-1 and cosegregation with zucchini yellow mosaic virus resistance. Euphytica 95:237–242

Kabelka E, Ullah Z, Grumet R (1997) Multiple alleles for zucchini yellow mosaic virus resistance at the *zym* locus in cucumber. Theor Appl Genet 95:997–1004

Kyle MM, Dickson MH (1988) Linkage of hypersensitivity to five potyviruses with the *B* locus for seed color in *Phaseolus vulgaris* L. J Hered 79:308—311

Kyle MM, Palloix A (1997) Proposed revision of nomenclature for potyvirus resistance genes in *Capsicum*. Euphytica 97: 183–188

Kyle MM, Provvidenti R (1993) Genetics of broad spectrum viral resistance in bean and pea. In: Kyle MM (ed) Resistance to viral diseases of vegetables: genetics and breeding. Timber Press, Portland, Oregon, pp 153–166

Lewellen RT (1973) Inheritance of beet mosaic virus resistance in sugar beet. Phytopathology 63:877–881

Lisa V, Lecoq H (1984) Zucchini yellow mosaic virus. Descriptions of Plant Viruses No. 282. Commonwealth Mycol Inst/Assocn Appl Biol, Kew, Surrey, England

McDowell JM, Dhandaydham M, Long TA, Aarts MGM, Goff S, Holub EB, Dangl JL (1998) Intragenic recombination and diversifying selection contribute to the evolution of downy mildew resistance at the *RPR8* locus of *Arabidopsis*. Plant Cell 10:1861–1874

McKern NM, Strike PM, Barnett OW, Ward CM, Shukla DD (1993) Watermelon mosaic virus-Morocco is a distinct potyvirus. Arch Virol 131:467–473

Murphy JF, Kyle MM (1995) Alleviation of restricted systemic spread of pepper mottle potyvirus in *Capsicum annum* cv

Avelar by coinfection with a cucumovirus. Phytopathology 85:561–566
Murphy JF, Blauth JR, Livingston KD, Lackney VK, Jahn MK (1998) Genetic mapping of the pvr1 locus in Capsicum spp, and evidence that distinct potyvins resistance loci control responses that differ at the whole plant and cellular levels. Molec Plant Microbe Interact 11:943–951
Myers BC, Shen KA, Rohani P, Gant BS, Michelmore RW (1998) Receptor-like genes in the major resistance locus of lettuce are subject to divergent selection. Plant Cell 11:1833–1846
Peterson, CE, Williams, PH, Palmer, M, Louward, P (1982) Wisconsin-2757 cucumber. HortScience 25:605–615
Provvidenti R (1985) Sources of resistances to viruses in two accessions of *Cucumis sativus*. Cucurbit Genet Coop Rep 8:12
Provvidenti R (1987) Inheritance of resistance to a strain of zucchini yellow mosaic virus in cucumber. HortScience 22: 102–103
Provvidenti R, Hampton RO (1993) Inheritance of resistance to white lupin mosaic virus in common pea. HortScience 28: 836–837
Provvidenti R, Niblett CL (1994) Inheritance of resistance to a strain of passionfruit woodiness virus in pea (*Pisum sativum* L.) HortScience 29:901–902
Provvidenti R, Gonsalves D, Humaydan HS (1984) Occurrence of zucchini yellow mosaic in cucurbits from Connecticut, New York, Florida, and California. Plant Dis 66:443–446
Purcifull D, Edwardson J, Hiebert E, Gonsalves D (1984a) Papaya ringspot virus. Descriptions of Plant Viruses No. 292. Commonwealth Mycol Inst/Assocn Appl Biol, Kew, Surrey, England
Purcifull D, Hiebert E, Edwardson J (1984b) Watermelon mosaic virus 2. Descriptions of Plant Viruses No. 293. Commonwealth Mycol Inst/Assocn Appl Biol, Kew, Surrey, England
Schroeder WT, Provvidenti R (1971) A common gene for resistance to bean yellow mosaic virus 2 in *Pisum sativum*. Phytopathology 61:846–848
Shukla DD, Ward CW, Brunt AA (1994) The Potyviridae. CAB International, Wallingford, UK
Song WY, Pi LY, Wang GL, Gardner J, Holsten T, Ronald RC (1997) Evolution of the rice *Xa21* disease resistance gene family. Plant Cell 9:1279–1287
Sudupak MA, Bennetzen JL, Hulbert SH (1993) Unequal exchange and meiotic instability of disease resistance genes in the *Rp1* region of maize. Genetics 133:119–125
Wai T, Grumet R (1995a) Inheritance of resistance to watermelon strain of papaya ringspot virus in the cucumber line 'TMG-1'. HortScience 30:338–340
Wai T, Grumet R (1995b) Inheritance of resistance to the watermelon mosaic virus in the cucumber line 'TMG-1': tissue-specific expression and relationship to zucchini yellow mosaic virus resistance. Theor Appl Genet 91:699–706
Wai T, Staub JE, Kabelka E, Grumet R (1997) Linkage analysis of potyvirus resistance alleles in cucumber. J Hered 88:454–458
Wang HL, Gonsalves D, Provvidenti R, Lecoq HL (1991) Effectiveness of cross protection by a mild strain of zucchini yellow mosaic virus in cucumber, melon, and squash. Plant Dis 75: 203–207
Wang HL, Ye SD, Chiu RJ, Gonsalves D (1987) Effectiveness of cross-protection by mild mutants of papaya ringspot virus for control of papaya in Taiwan. Plant Dis 71:491–497
Wang YJ, Provvidenti R, Robinson RW (1984) Inheritance of resistance to watermelon mosaic 1 virus in cucumber. HortScience 19:587–588
Wang YJ, Robinson RW, Provvidenti R (1987) Linkage relationships of watermelon mosaic 1 virus in cucumber. Cucurbit Genet Coop Rep 6:92
Yeh SD, Gonsalves D (1984) Evaluation of induced mutants of papaya ringspot virus for control by cross protection. Phytopathology 74:1086—1091