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Multiple alleles for zucchini yellow mosaic virus resistance at the *zym* locus in cucumber

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Abstract Sources of resistance to several potyviruses have been identified and characterized within the cucumber (*Cucumis sativus* L.) germplasm. Resistance to zucchini yellow mosaic virus (ZYMV) is present in inbred lines derived from the Dutch hybrid Dina (Dina-1) and from the Chinese cultivar 'Taichung Mou Gua' (TMG-1). Tests of allelism indicated that the genes for resistance to ZYMV in TMG-1 and Dina-1 are at the same locus; however, the two genotypes exhibited different phenotypes in response to cotyledon inoculation with ZYMV. Dina-1 exhibited a distinct veinal chlorosis and accumulation of virus limited to the first and/or second true leaves, while TMG-1 remained symptom-free and did not accumulate virus. The distinct veinal chlorosis phenotype in Dina-1 was dominant to the symptom-free phenotype in TMG-1 and was shown not to be due to a separate gene. These results indicate that a series of alleles differing in effectiveness and dominance relationships occurs at the *zym* locus such that $Zym > zym^{Dina} > zym^{TMG\text{-}1}$. In addition to ZYMV resistance, TMG-1 is also resistant to watermelon mosaic virus (WMV), the watermelon strain of papaya ringspot virus (PRSV-W) and the Moroccan watermelon mosaic virus (MWMV); the WMV and MWMV resistances are at the same locus, or tightly linked to the *zym* locus. Dina-1 also was found to be resistant to PRSV-W and MWMV. The gene for MWMV resistance in Dina-1 appeared to be at the same locus or tightly linked (<1% recombination) to the gene for ZYMV resistance. In contrast to the response to ZYMV inoculation, Dina-1 does not exhibit distinct veinal chlorosis when inoculated with PRSV-W or MWMV. Collectively, these observations suggest that the gene(s) conferring resistance to ZYMV, WMV, and MWMV may be part of a gene cluster for potyvirus resistance in cucumber.

Key words *Cucumis sativus* · Gene cluster · Moroccan watermelon mosaic virus · Papaya ringspot virus · Potyvirus

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Introduction

Cucurbit crops including cucumber (*Cucumis sativus* L.) are subject to severe losses due to an array of distinct potyviruses, including zucchini yellow mosaic virus (ZYMV), watermelon mosaic virus (WMV), the watermelon strain of papaya ringspot virus (PRSV-W), zucchini yellow fleck virus (ZYFV) and the Moroccan watermelon mosaic virus (MWMV) (Lisa and Lecoq 1984; Purcifull et al. 1984a,b; McKern et al. 1993; Gilbert-Albertini et al. 1995). Sources of resistance to several of these viruses have been identified and characterized in cucumber. Inheritance of resistance to ZYMV has been characterized in an inbred line derived from the Chinese cultivar 'Taichung Mou Gua' (TMG-1) (Provvidenti 1985, 1987) and also in the Dutch hybrid Dina (Abul Hayja and Al-Shahwan 1991). The relationship between these ZYMV resistances is not known. Lines derived from TMG (TMG-1 and TMG-2) also have been shown to be resistant to WMV, ZYFV, PRSV-W and MWMV (Provvidenti 1985; Gilbert-Albertini et al. 1995; Kabelka and Grumet 1997).

Resistance to ZYMV in Dina is conferred by a single recessive gene (Abul Hayja and Al-Shahwan 1991). The TMG-derived resistances to ZYMV, ZYFV and MWMV (Provvidenti 1987; Gilbert-Albertini et al.

1995; Kabelka and Grumet 1997, respectively) are each conferred by single recessive genes, while resistance to PRSV-W is due to a single dominant gene (Wai and Grumet 1995a). Resistance to WMV involves two types of resistances under separate genetic control (Wai and Grumet 1995b). 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 and Grumet 1995b; Kabelka and Grumet 1997).

In this study described here we sought to examine the relationship between the genes conferring resistance to ZYMV in lines derived from TMG and Dina (TMG-1 and Dina-1) and to test for possible differences in the performance of the resistance alleles. In addition, since multiple potyvirus resistances appear to be controlled by the same or tightly linked genes in TMG-1 (Wai and Grumet 1995b; Kabelka and Grumet 1997), we tested Dina-1 for resistance to other cucurbit potyviruses.

Materials and methods

Germplasm used

The inbred cucumber (*Cucumis sativus* L.) line TMG-1, resistant to ZYMV, WMV, PRSV-W and MWMV (Provvidenti 1985; Kabelka and Grumet 1997), was provided by Dr. J. Staub (USDA, University of Wisconsin at Madison). Self-pollinated progeny of the Dutch hybrid Dina, true-breeding for resistance to ZYMV (Dina-1), were initially provided by Dr. K. Owens (Seminis Peto Seed Company, Woodland, Calif.) and then increased by self- or sib-pollination in the greenhouse. The susceptible parental genotype used in this investigation was the open-pollinated cucumber cultivar 'Straight-8' (ST-8; W. Atlee Burpee & Company, Warminster, Pa.). Progeny of the crosses between Dina-1 and TMG-1, Dina-1 and ST-8 and ST-8 and TMG-1 were produced in the greenhouse. The F_1 progeny of each cross were either self- or sib-pollinated to produce the F_2 generations; backcrosses were made to both parents.

Viral inocula and inoculation procedures

The majority of experiments were performed with the Connecticut isolate of ZYMV (ZYMV-CT); additional isolates were tested that varied in place of origin and in symptom expression and severity. These included: Taiwan 1, Taiwan 2, Florida and California (provided by Dr. R. Provvidenti, Cornell University, Geneva, N.Y.), and a non-aphid transmissible isolate from Israel (NAT) (provided by Dr. B. Raccah, The Volcani Institute, Bet Dagan, Israel). Two additional potyviruses used in this investigation were MWMV, provided by Dr. D. Purcifull (University of Florida, Gainesville, Fla.), and PRSV-W (PV-380; American Type Culture Collection, Rockville, Md.). All viruses were propagated in *Cucurbita pepo* L. cvs 'Black Beauty' (SeedWay, Elizabethtown, Pa.) or 'Midas' (Willhite Seed, Poolville, Tex.) and maintained in a growth chamber (16-h day, 24°C constant temperature, approx. 300 μmol photons $m^{-2} s^{-1}$). Purity of the virus source was verified by ELISA as described by Wai and Grumet (1995a), and by the use of the differential host, *Phaseolus vulgaris* cv 'Black Turtle 2' (Provvidenti 1983).

Young symptomatic leaves were harvested 2–4 weeks post-inoculation and macerated in ice-cold 20 m*M* sodium phosphate buffer, pH 7.0, in a pre-chilled mortar and pestle. Cotyledons of 7- to 10-day-old, seedlings, or the upper surface of the fully expanded fourth and half expanded fifth true leaves (sequential inoculation experiments), were lightly dusted with 320-grit Carborundum (Fisher Scientific, Pittsburgh, Pa.) and rub-inoculated with virus-infected sap (approximately 1 : 4 dilution leaf material : buffer) using sponge plugs. Mock-inoculated control plants were included in each experiment. All non-biological materials were sterilized prior to use.

Experimental designs and data analysis

Seed for all experiments were pregerminated in an incubator kept at 30°C for 24 h then sown in the greenhouse into 10.2-cm-diameter clay pots filled with Baccto growing medium (Professional Planting Mix, Michigan Peat Company, Houston, Tex.). Upon emergence of the cotyledons, a fertilization regimen of 300 ppm N-P-K (Peters Professional All-Purpose Fertilizer, 20-20-20, Grace-Sierra Horticultural Products, Milpitas, Calif.) was applied three times per week. Greenhouse temperatures ranged from 25°C to 35°C throughout the year.

Experiments to evaluate the F_2 and backcross populations 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. Sequential inoculation experiments consisted of cotyledon inoculation of parental genotypes and their progeny with ZYMV followed by true leaf inoculation of the resistant individuals with the MWMV. 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. All experiments included border rows of ST-8 (susceptible genotype) as a further check for any possible variation in the inoculation technique and/or expression of viral symptoms.

In all experiments, plants were visually scored as either resistant (symptom-free) or susceptible (evidence of virus) when symptom development was optimal (approximately 7 days post-inoculation for ZYMV-induced mosaic and approximately 14 days post-inoculation for ZYMV-induced veinal chlorosis and for MWMV and PRSV symptoms). Segregation ratios were analyzed by chi-square analysis.

The relationship between symptom expression and virus levels was examined by enzyme-linked immunosorbent assay (ELISA) as described by Wai and Grumet (1995a). ELISA data were analyzed by analysis of variance of a randomized complete block design with five replicates; individual comparisons were analyzed by orthogonal contrasts.

Results

Relationship between ZYMV resistance in TMG-1 and Dina

To determine if the resistance genes in TMG-1 and Dina-1 are at the same locus, we inoculated progeny of this cross with ZYMV-CT at the cotyledon stage. Seven-to ten-days post-inoculation, the susceptible genotype ST-8 developed a systemic mosaic pattern of the foliage, while TMG-1, Dina-1 and all progeny appeared symptom-free (Table 1).

Approximately 14 days post-inoculation, however, the two resistant genotypes, TMG-1 and Dina-1, exhibited strikingly different phenotypes (Fig. 1). Dina-1 responded with a distinct veinal chlorosis limited to the

Table 1 Response of TMG-1, Dina-1 and their progeny to ZYMV inoculation (*ns* non-significant χ^2 value)

Parent or progeny	Number of plants			Ratio of veinal chlorosis : symptom-free[c]	χ^2	Probability
	Susceptible	Resistant[a]				
		Distinct veinal chlorosis[b]	Symptom-free			
TMG-1 (resistant)	0	0	32	0:1	–	–
Dina-1 (resistant)	0	31	0	1:0	–	–
Straight-8 (susceptible)	32	0	0	–	–	–
F_1(Dina-1 × TMG-1) (TMG × Dina-1)	0	64	0	1:0		
F_2(Dina-1 × TMG-1)[d] (TMG × Dina-1)	0	230	90	3:1	1.51	0.22 ns
BC(F_1 × TMG-1)[e] (TMG-1 × F_1)	0	88	72	1:1	1.40	0.24 ns
BC(F_1 × Dina-1)[f] (Dina-1 × F_1)	0	157	3	1:0	0.04	0.86 ns

[a] Plants remain either symptom-free or exhibit an occasional sparse veinal chlorosis
[b] Symptom expression of the distinct veinal chlorosis was confined to one or two true leaves with subsequent leaves symptom-free
[c] Expected ratios based on the distinct veinal chlorosis phenotype dominant to the symptom-free phenotype
[d–f] Data pooled from two independent experiments. Each experiment fits the predicted ratios.
[d] χ^2 exp1 = 1.41 ns; χ^2, exp2 = 0.21 ns; χ^2 homogeneity = 0.32, df = 1
[e] χ^2 exp1 = 2.82 ns; χ^2 exp2 = 0.00 ns; χ^2 homogeneity = 2.13, df = 1
[f] χ^2 exp1 = 0.08 ns; χ^2 exp2 = 0.00 ns; χ^2 homogeneity = 0.28, df = 1

Fig. 1 Phenotypic response of Dina-1 (*right*) and TMG-1 (*left*) 14 days post-cotyledon inoculation with ZYMV. Dina responds with a distinct veinal chlorosis limited to the first and/or second true leaves with subsequent leaves symptom-free. In TMG-1, this distinct veinal chlorosis phenotype is not observed.

first and/or second true leaves. Subsequent leaves in Dina-1 remained symptom-free. In TMG-1, the distinct veinal chlorosis phenotype was not observed. TMG-1 remained either symptom-free throughout the plant or occasionally would exhibit sparse veinal chlorosis. For simplicity, the response to ZYMV inoculation in Dina-1 will be referred to as veinal chlorosis and the response in TMG-1 as symptom-free. The distinct veinal chlorosis in Dina-1 only occurred when cotyledons were inoculated with ZYMV. When true leaves (first, second or third) were inoculated, there was no veinal chlorosis on either the inoculated or subsequent leaves (data not shown).

Although Dina-1 and TMG-1 had different phenotypes on the first and/or second true leaves, the resistance alleles appeared to be at the same locus (Table 1). No susceptible individuals were observed among the F_1, F_2 and backcross progeny of TMG-1 × Dina-1; from the third true leaf on, all plants remained symptom-free, vigorous and healthy.

Performance of the resistance alleles in Dina-1 and TMG-1

Although progeny from the cross between Dina-1 and TMG-1 were all resistant, there was segregation for the veinal chlorosis phenotype. The veinal chlorosis appeared to be due to a single gene with the veinal chlorosis phenotype dominant to the symptom-free phenotype (Table 1). F_1 progeny exhibited the veinal chlorosis phenotype; F_2 progeny segregated 3 : 1 (veinal chlorosis : symptom free); and progeny of the backcross TMG-1 segregated 1 : 1 veinal chlorosis : symptom-free. Progeny of the backcrosses to Dina-1 exhibited the veinal chlorosis phenotype limited to the first and/or second true leaves.

Possible explanations for the observed inheritance of the veinal chlorosis phenotype are: (1) Dina-1 and TMG-1 have different alleles for resistance at the same locus, one of which causes the veinal chlorosis, or (2) there is a separate locus responsible for the veinal chlorosis phenotype. To distinguish between these possibilities, we crossed TMG-1 and Dina-1 separately to a common susceptible genotype, ST-8, and tested their progeny for response to cotyledon inoculation with ZYMV (Table 2). The parental genotypes responded as

Table 2 Responses of TMG-1, Dina-1, 'Straight-8' and their progeny to inoculation with ZYMV

Parent or progeny	Number of plants		
	Susceptible[a] Mosaic	Resistant	
		Symptom-free	Distinct veinal chlorosis
TMG-1 (resistant)	0	40	0
Dina-1 (resistant)	0	0	40
Straight-8 (ST-8) (susceptible)	40	0	0
F_1 (ST-8 × TMG)	40	0	0
F_1(Dina-1 × ST-8)	40	0	0
F_2(ST-8 × TMG)	120	38	2
F_2(Dina-1 × ST-8)	245	1	73
BC (ST-8 × TMG) × ST-8	80	0	0
BC (Dina-1 × ST-8) × ST-8	80	0	0
BC (ST-8 × TMG) × TMG	42	38	0
BC (Dina-1 × ST-8) × Dina-1	37	0	43

[a] Note that susceptible (mosaic; *Zym*) is dominant to both of the resistance phenotypes (symptom-free and veinal chlorosis); veinal chlorosis can only be seen in the absence of the *Zym* allele

expected. TMG-1 remained symptom-free; Dina-1 responded with a veinal chlorosis of the first and/or second true leaves with subsequent leaves symptom-free; and the susceptible genotype, ST-8, exhibited a systemic mosaic pattern throughout. Consistent with published results (Provvidenti 1987; Abul Hayja and Al-Shahwan 1991), segregation ratios of resistant versus susceptible indicate that resistance to ZYMV in both Dina-1 and TMG-1 is conferred by a single recessive gene. All of the F_1 progeny, three-quarters of the F_2 progeny, one-half of the backcross to either TMG-1 or Dina-1 and all of the backcross progeny to ST-8 were susceptible.

Within the resistant category, however, different responses were observed depending on the resistant parent. Fourteen days post-inoculation, resistant F_2 or backcross individuals derived from the Dina-1 and ST-8 cross developed the veinal chlorosis phenotype, while individuals derived from the ST-8 and TMG-1 cross remained symptom-free. If a separate gene was responsible for veinal chlorosis, we would expect segregating progeny within a cross to show all three phenotypes of mosaic, veinal chlorosis and symptom-free. With very few exceptions this was not observed; the progeny from a given cross exhibited only one type of resistance phenotype. Thus, segregation ratios for the veinal chlorosis phenotype support a model in which the resistance alleles in TMG-1 and Dina-1 are performing differently; an unlinked or loosely linked locus is not responsible for the veinal chlorosis phenotype. Alternatively, the few exceptions observed in the F_2 population might suggest tight linkage of a modifier gene.

The three parental genotypes also were examined to determine whether the different phenotypes reflected differences in virus accumulation (Fig. 2). Virus titers were measured from the first through the third true leaves post-inoculation with ZYMV. TMG-1 remained symptom-free and showed no significant virus accumulation above background ELISA values (analysis of variance). Dina-1 exhibited significant virus levels, but only in leaves showing the veinal chlorosis phenotype. As expected, the susceptible ST-8 had high levels of virus accumulation.

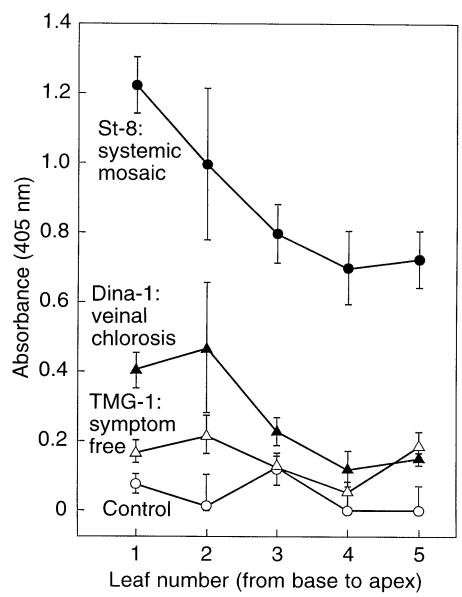

Fig. 2 Virus accumulation in TMG-1, Dina-1 and 'Straight-8' 2 weeks post-inoculation with ZYMV. Each point is the mean of five replicate plants ± SE. Virus levels were measured by ELISA

Dina-1 and TMG-1 were also examined for differences in response to various ZYMV isolates. None of the isolates tested (Connecticut, Taiwan 1, Taiwan 2,

Florida, NAT and California) overcame the resistance in either TMG-1 or Dina-1. The control ST-8 plants were infected by all isolates, while TMG-1 remained symptom-free in response to all isolates. Dina-1 exhibited veinal chlorosis on the first and/or second true leaves in response to all isolates except the mild California isolate; in that case no symptoms were observed.

Inoculation of Dina-1 with other potyviruses

Previous studies indicated that lines derived from TMG are resistant to ZYMV, WMV, PRSV-W and MWMV (Provvidenti 1985; Kabelka and Grumet 1997). In addition, the TMG-1 resistance gene for ZYMV is the same as, or tightly linked to, the genes for resistance to WMV and MWMV (Wai and Grumet 1995b; Kabelka and Grumet 1997). Since Dina-1 possesses resistance to ZYMV, we sought to determine if it is also resistant to other potyviruses and, if so, to examine whether a similar genetic relationship exists among these resistances. When Dina-1 cotyledons were inoculated with PRSV-W and MWMV, Dina-1 remained symptom-free and, interestingly, exhibited no veinal chlorosis on the first and/or second true leaves as seen with ZYMV inoculation.

The resistance to MWMV in Dina-1 was further examined by characterizing the mode of inheritance and determining its relationship to the ZYMV resistance gene. Following rub inoculation with MWMV, the susceptible genotype, ST-8, showed a mild systemic rugosity and silvering of the true leaves 14 days post-inoculation (Table 3). Symptom intensity of MWMV varied during the course of plant growth; the rugosity and silvering of the true leaves would fade occasionally but then resurge to full expression on subsequent leaves. Dina-1 remained symptom-free. The F_1 progeny of Dina-1 and ST-8 were susceptible, the F_2 progeny segregated 1 : 3 [resistant(R) : susceptible(S)], backcross to the resistant parent segregated 1 : 1 (R : S) and progeny of backcrosses to the susceptible parent were all susceptible. These segregation ratios support a model in which inheritance of resistance to MWMV in Dina-1 is conferred by a single recessive gene.

Relationship between resistance to ZYMV and resistance to MWMV in Dina

By analogy to ZYMV resistance in TMG-1, one would predict that the gene for MWMV resistance in Dina-1 is the same as, or tightly linked to, the gene for ZYMV resistance. This hypothesis was tested in two ways: (1) sequential inoculation of Dina-1, ST-8 and their progeny with ZYMV and MWMV and (2) tests for segregation of MWMV resistance in the progeny of Dina-1 and TMG-1.

The sequential inoculation procedure consisted of cotyledon inoculation of Dina-1, ST-8 and their progeny with ZYMV followed by true leaf inoculation of the resistant individuals with MWMV (Table 4). Symptoms of ZYMV, a systemic mosaic pattern, developed on the susceptible ST-8 and Dina-1 $\times$ ST-8 F_1 progeny plants approximately 7 days post-cotyledon inoculation. The F_2 and backcross progeny segregated as expected for a single gene recessive trait. Fourteen days post-cotyledon inoculation with ZYMV, all resistant individuals developed veinal chlorosis of the first and/or second true leaves with subsequent leaves symptom-free. The resistant individuals were then sequentially inoculated on the asymptomatic fourth and fifth true leaves with MWMV (18 days post-cotyledon inoculation with ZYMV). Symptoms of MWMV, mild rugosity and silvering of the true leaves developed approximately 14 days post-true leaf inoculation on the susceptible ST-8 and F_1 progeny plants. The symptoms of MWMV were clearly distinguishable from ZYMV. If the genes were segregating independently, three-quarters of the ZYMV resistant progeny should be susceptible to MWMV. In all cases, those individuals resistant to cotyledon inoculation with ZYMV, although initially responding with a veinal chlorosis limited to the first and/or second true leaves, remained free of symptoms upon true leaf inoculation with MWMV. The failure to observe segregation of the two resistances implies that they are either at the same locus or at two tightly linked loci ($<1\%$ recombination; product ratio method).

The second approach tested for segregation of MWMV resistance among the progeny of Dina-1 and

Table 3 Response of Dina-1, 'Straight-8' and their progeny to inoculation with MWMV (*ns* non-significant χ^2 value)

Parent or progeny	Number of plants		Expected ratio (R : S)[a]	χ^2	Probability
	Resistant	Susceptible			
Dina-1	20	0	–	–	
Straight-8 (ST-8)	0	20	–	–	
F_1 (Dina-1 $\times$ ST-8)	0	20	0 : 1	–	
F_2(Dina-1 $\times$ ST-8)	35	92	1 : 3	0.317	0.59 ns
BC (F_1 $\times$ Dina-1)	65	73	1 : 1	0.356	0.54 ns
BC(F_1 $\times$ ST-8)	0	20	0 : 1	–	

[a] Expected ratios based on a single recessive gene model; R = resistant, S = susceptible

Table 4 Response of Dina-1, 'Straight-8' and their progeny to cotyledon inoculation with ZYMV followed by true leaf inoculation of resistant individuals with MWMV

Parent or progeny	Total plants	Cotyledon inoculation with ZYMV		Sequential inoculation of ZYMV resistant individuals with MWMV at true leaf stage	
		Number of plants		Number of plants	
		Susceptible	Resistant	Susceptible	Resistant
Dina-1	20	0	20	0	20
Straight-8 (ST-8)[a]	10	10	0	–	–
Straight-8 (ST-8)[b]	10	–	–	10	0
F_1(Dina-1 × ST-8)[a]	10	10	0	–	–
F_1(Dina-1 × ST-8)[b]	10	–	–	10	0
F_2(Dina-1 × ST-8)[c]	320	245	75	0	75
BC(Dina-1 × ST-8) × Dina-1[d]	80	42	38	0	38

[a] Control plants to verify successful inoculation at cotyledon stage with ZYMV
[b] Control plants to verify successful inoculation at true leaf stage with MWMV
[c] Data fit predicted segregation ratios based on resistance to ZYMV conferred by a single recessive gene. $\chi^2 = 0.33$ ns
[d] Data fit predicted segregation ratios based on resistance to ZYMV conferred by a single recessive gene. $\chi^2 = 0.12$ ns

Table 5 Segregation for resistance to MWMV in the progeny derived from the cucumber lines TMG-1 and Dina-1

Parent or progeny	Number of plants	
	Resistant	Susceptible
TMG-1 (resistant)	20	0
Dina-1 (resistant)	20	0
Straight-8 (susceptible)	0	32
F_1(TMG-1 × Dina-1) (Dina-1 × TMG-1)	20	0
F_2(TMG-1 × Dina-1) (Dina-1 × TMG-1)	140	0
BC(F_1 × TMG-1) (TMG-1 × F_1)	80	0
BC(F_1 × Dina-1) (Dina-1 × F_1)	80	0

TMG-1 (Table 5). Fourteen days post-inoculation the susceptible genotype, ST-8, developed a mild rugosity and silvering of the true leaves while the resistant genotypes TMG-1 and Dina remained symptom-free throughout. Evaluation of segregation for resistance and susceptibility in the progeny of TMG-1 and Dina-1 indicates that the two MWMV resistance alleles are located at the same locus as all individuals of the F_1, F_2 and backcross generations remained symptom-free.

Discussion

Segregation ratios among the F_1, F_2 and backcross progeny of TMG-1 and Dina-1 indicated that the ZYMV resistance alleles are at the same locus. However, the two sources of ZYMV resistance performed differently. TMG-1 remained symptom free while Dina-1 responded to ZYMV cotyledon inoculation with a distinct veinal chlorosis on the first and/or second true leaves with subsequent leaves symptom-free. The progeny of TMG-1 and Dina-1 segregated for the veinal chlorosis phenotype with veinal chlorosis dominant to symptom-free. An alternate possibility, that an additional unlinked or loosely linked factor was responsible for the distinct veinal chlorosis phenotype, was ruled out based on evaluation of the segregating progeny of TMG-1 and Dina-1 crossed to a common susceptible background.

The symptoms in Dina-1 also appeared to reflect virus replication and movement. Consistent with previous studies (Wai and Grumet 1995a; Al-Shahwan et al. 1995), detectable virus titer, limited to the first and second true leaves, was observed in Dina-1 while no virus accumulation was observed in TMG-1. The distinct veinal chlorosis phenotype in Dina-1 only occurred when cotyledons were inoculated with ZYMV. When first, second or third leaves were inoculated with ZYMV, no distinct veinal chlorosis on the inoculated leaves or subsequent leaves was seen. This suggests a tissue-specific or developmental expression of the resistance (cotyledon vs. true leaf) to ZYMV in Dina-1. Tissue-specific expression of potyvirus resistance has been observed previously in cucurbits. In TMG-1, resistance to WMV is conferred by two independently segregating factors; one resistance is expressed in the cotyledons and throughout the plant, while the second resistance is expressed only in true leaf tissue (Wai and

Grumet 1995b). Tissue specificity is also observed in muskmelon cultivars resistant to PRSV-W (Gibb et al. 1994). The hybrid progeny of the resistant cultivar 'Cinco' crossed with the susceptible cultivar 'Planter's Jumbo' exhibited mild systemic symptoms upon cotyledon inoculation but remained symptom-free upon true leaf inoculation.

From the data presented, there appears to be a series of alleles at the *zym* locus that differ in effectiveness and dominance relationships. Both of the resistance alleles are recessive to the wild-type, susceptible allele (*Zym*). Veinal chlorosis is only observed in the absence of the susceptible *Zym* allele. The veinal chlorosis phenotype associated with the resistance allele from Dina-1 (zym^D) is dominant to the symptom-free phenotype associated with the resistance allele from TMG-1 (zym^T). These results suggest descending dominance at the *zym* locus, such that $Zym > zym^D > zym^T$. Of the two resistances examined, however, the allele from TMG-1 would represent a more effective resistance because neither veinal chlorosis nor viral accumulation was seen. Thus, the relative dominance of the different alleles at the *zym* locus appears to be inversely related to the extent by which they reduce virus multiplication and/or prevent movement.

Series of alleles occurring at a specific locus but differing in performance have been described previously. A well-characterized example is the *Tm-2* locus in tomato; two allelic factors, *Tm-2* and $Tm\text{-}2^2$, confer resistance to tobacco mosaic virus (TMV) (Hall 1980). Although both *Tm-2* and $Tm\text{-}2^2$ resistances can be overcome by mutations in the TMV 30-kDa movement protein gene, strains overcoming $Tm\text{-}2^2$ occur much less frequently (Meshi et al. 1989; Weber et al. 1993). Location of the mutations within the movement protein suggests that *Tm-2* interferes with movement, while $Tm\text{-}2^2$ may interfere with host-pathogen recognition events.

As mentioned earlier, previous studies indicated that TMG-1 is also resistant to several potyviruses including ZYMV, WMV, PRSV-W and MWMV (Provvidenti 1985; Kabelka and Grumet 1997). In addition, the resistance gene for ZYMV in TMG-1 appears to be the same as, or tightly linked to, the genes for resistance to WMV and MWMV (Wai and Grumet 1995b; Kabelka and Grumet 1997). Our studies reveal that, like TMG-1, Dina-1 also possesses resistance to multiple potyviruses, but upon inoculation with PRSV-W or MWMV, Dina-1 remains symptom-free and does not exhibit the veinal chlorosis phenotype as seen with several isolates of ZYMV except the California isolate. Although MWMV, PRSV-W and ZYMV-California are distinct potyviruses (Lisa and Lecoq 1984; Purcifull et al. 1984a,b; McKern et al. 1993), one shared feature is the time interval preceding observable symptoms in the susceptible genotype. While symptoms of most ZYMV isolates developed approximately 7–10 days post-inoculation, symptoms of MWMV, PRSV-W and ZYMV-California did not become evident until approximately 14–21 days post-inoculation. This difference in time of onset may represent a slower rate of virus multiplication and/or movement resulting in lack of veinal chlorosis in Dina-1. As virus accumulation was observed in the first and second true leaves of Dina-1 post-inoculation with ZYMV-Connecticut, it would be interesting to determine if the lack of veinal chlorosis corresponds with a lack of virus accumulation. It is possible, however, to have virus accumulation in the absence of symptoms (i.e. tolerance). Previous studies with TMG-1 and PRSV-W (in contrast to ZYMV) have indicated that although TMG-1 remains symptom-free after inoculation with PRSV-W, there is PRSV-W accumulation (Wai and Grumet 1995a).

The resistance to MWMV in Dina-1 was further examined by characterizing the mode of inheritance and determining its relationship to the ZYMV resistance gene. Resistance to MWMV in Dina-1, like resistance to ZYMV, is conferred by a single recessive gene. Sequential inoculation of progeny possessing resistance to ZYMV followed by MWMV suggests that both resistances are conferred by the same gene, or two tightly linked genes. In addition, tests for segregation of MWMV resistance in the progeny of Dina-1 and TMG-1 indicate the gene for MWMV resistance is at the same locus in both sources.

Collectively, analysis of data suggests that one gene, or two tightly linked genes, confer resistance to ZYMV and MWMV in both TMG-1 and Dina-1. Tests for allelism suggest that these genes are at the same locus in TMG-1 and Dina-1. However, as evidenced by Dina-1's strikingly different response to inoculation with ZYMV, both with regard to symptom expression, virus accumulation and dominance relationships, the ZYMV resistance alleles in TMG-1 and Dina-1 are likely to be different indicating that a series of alleles of descending dominance exist at the *zym* locus with $Zym > zym^D > zym^T$. Since the differences in response were observed only for ZYMV and not MWMV, it is possible that rather than a single gene conferring resistance to all three potyviruses, the MWMV, WMV and ZYMV genes may be part of a gene cluster for potyvirus resistance in cucumber.

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