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Identification and comparative mapping of a dominant potyvirus resistance gene cluster in *Capsicum*

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Abstract The dominant gene *Pvr7* from *Capsicum chinense* Jacq. 'PI159236' confers resistance to the pepper mottle potyvirus (PepMoV) Florida (V1182) strain. This gene is tightly linked to the dominant potyvirus resistance gene *Pvr4* with observed recombination frequencies of 0.012 to 0.016. A cleaved amplified polymorphic sequence (CAPS) marker linked to *Pvr4* was used to localize *Pvr4* and, by extension, *Pvr7*, to linkage group 10 on an interspecific map of pepper. Our results indicated that *Pvr4*, *Pvr7*, and *Tsw*, a gene conferring resistance to tomato spotted wilt virus, comprise the first identified cluster of dominant disease resistance genes in *Capsicum* L. This position does not correspond to the locations of dominant potyvirus resistance genes in potato or to the positions of any other mapped solanaceous resistance genes or resistance gene homologues.

Key words PVY · TEV · *Lycopersicon*, *Solanum* · Solanaceae

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Introduction

An anecdotal observation exploited by many breeders who undertake searches for new genetic resources for disease resistance has been the existence of "jackpot" genotypes, typically wild accessions of a cultivated species or sexually compatible related species, that serve as a source of resistance for a number of different diseases. *Capsicum chinense* L. 'PI 159236' has been identified as a source of resistance or tolerance for several major viral pathogens of pepper including potyviruses (Greenleaf 1956; Zitter 1972), tomato spotted wilt tospovirus (Black et al. 1991; Moury et al. 1997), and tobacco mosaic tobamovirus (Boukema 1980), and is the source of the potyvirus resistance locus reported in this paper.

Disease, particularly viral infection, is a major limitation to crop production in pepper (*Capsicum* L.), a genus related to two crops that have been the focus of intense genetic and breeding studies, tomato (*Lycopersicon* Mill.) and potato (*Solanum* L.) (Watterson 1993; Pillen et al. 1996). Comparative maps for the three crops have revealed conservation of marker content and order within rearranged genomic segments, suggesting that comparative mapping may facilitate the transfer of information between Solanaceous genera (Tanksley et al. 1992; Livingstone et al. 1999). Whereas relatively few R genes have been mapped in pepper, disease resistance has been extensively studied in potato and tomato, and numerous tomato R genes have been characterized at the molecular level (Martin et al. 1993; Pillen et al. 1996; Milligan et al. 1998; Thomas et al. 1998).

Many individual members of the Potyviridae, the largest and most economically destructive plant virus family, often infect and damage a single crop species (Provvidenti and Hampton 1992; Shukla et al. 1994). Cosegregation of genetic resistance to related potyviruses has been reported in several species including pea (*Pisum* L.), potato, and bean (*Phaseolus* L.) (Provvidenti 1990; Kyle and Provvidenti 1993; Fisher and Kyle 1994; Hämäläinen et al. 1997; Brigneti et al. 1997). In pepper, cosegregation of resistance to two or more related poty-

viruses, including tobacco etch virus (TEV), pepper mottle virus (PepMoV) and potato virus Y (PVY), has been reported in several genotypes (Cook 1961; Zitter 1972; Zitter and Cook 1973). Each of these genotypes was initially presumed to contain a different broad-spectrum resistance allele at the same locus, designated *et* and/or *y* by different researchers (Greenleaf 1986). The relationships between potyvirus resistance genes and the resistance spectrum of each allele have been difficult to evaluate due to the use of unrelated and uncharacterized sources of resistance and effects of genetic background on the expression of resistance (Greenleaf 1986). Re-examination of the *et*/*y* locus has revealed three unlinked genomic regions containing potyvirus resistance loci (reviewed by Kyle and Palloix 1997). Two loci, *pvr1* and *pvr2*, have been mapped in different populations. While the positions obtained are not comparable due to a lack of shared markers in the two maps, each locus is linked to one of two tomato markers, TG56 (*pvr1*) and CT31 (*pvr2*) (Caranta et al. 1997; Murphy et al. 1998). TG56 and CT31 are located less than 10 cM apart on tomato chromosome 3 (Tanksley et al. 1992), suggesting that *pvr1* and *pvr2* may be linked. A third locus, *pvr3*, has been shown to be unlinked to *pvr1* and *pvr5*; *pvr5* has also been shown to be unlinked to *pvr2* (Dogimont et al. 1996; Murphy et al. 1998; Blauth 1994; Caranta et al. 1999a). Quantitative trait loci for potyvirus resistance have also been mapped, and in some cases coincide with positions of *pvr* loci (Caranta and Palloix 1996; Caranta et al. 1997).

A dominant allele from *Capsicum annuum* 'Criollo de Morelos 334' (CdM334), *Pvr4*, has been reported to confer dominant resistance to all three pepper pathotypes (0, 1, and 1–2) of PVY (Dogimont et al. 1996). Dominant resistance to PepMoV cosegregated exactly with *Pvr4* in 67 doubled-haploid families (Dogimont et al. 1996) and in 271 F_3 families (C. Caranta, A. Palloix, and A. M. Daubèze, unpublished results), suggesting that *Pvr4* or a tightly linked dominant gene from CdM334 confers resistance to PepMoV. A cleaved amplified polymorphic sequence (CAPS) marker tightly linked to *Pvr4* was developed for marker-assisted selection (Caranta et al. 1999b).

In contrast to pepper, where potyvirus resistance is conferred primarily by recessive genes, all known potyvirus resistance genes in potato are dominant, and two of these have been mapped (Brigneti et al. 1997; Hämäläinen et al. 1997; Kyle and Palloix 1997). The tightly linked or allelic potato virus Y (PVY) resistance genes Ry_{sto} and Ry_{adg} are less than 7 cM from Ra_{adg}, which confers resistance to the closely related potyvirus virus A (PVA) (Brigneti et al. 1997; Hämäläinen et al. 1997). Although polygenic and monogenic recessive potyvirus resistance genes have been described in tomato, none have been mapped to-date, limiting the utility of tomato for comparative analyses (Legnani et al. 1995, 1996). We undertook the present study to determine the comparative genetic basis for dominant potyvirus resistance in pepper and potato (Valkonen et al. 1996) and to define further the genetic resources for potyvirus resistance in *Capsicum*. The primary goal of this paper was to determine the relationship between *Pvr4* and the dominant potyvirus resistance in the "jackpot" genotype *C. chinense* 'PI159236.' A second objective was to further characterize and map *Pvr4* on a comparative map of pepper. Our primary focus has been on PepMoV, rather than PVY or TEV, because of its importance as a North American pathogen of pepper.

Materials and methods

Germplasm and genetic populations

Two potyvirus-susceptible (S) *Capsicum* genotypes, *C. annuum* L. 'NuMex RNaky' (RNaky) (Nakayama and Matta 1985, provided by Dr. F. Loaiza-Figueroa, Asgrow Seed Co., San Juan Bautista, Calif.) and *C. annuum* 'Jupiter' (Novartis Seeds, Inc., provided by Dr. R. Subramanya, Pepper Genetics, Inc., Belle Glade, Fla.) were used as parents and as control genotypes in disease screens. RNaky and *C. chinense* 'PI159234' (provided by Dr. S. Tanksley) were used to create an interspecific F_2 mapping population as described (Livingstone et al. 1999). *C. chinense* 'PI159236–9093' (9093) is a PepMoV-resistant (R) BC_3F_3 line developed from backcrossing a single plant selected from *C. chinense* 'PI159236' to a *C. annuum* recurrent parent. 9093 and *C. annuum* 'Criollo de Morelos 334' (CdM334), the source of *Pvr4*, were obtained from Dr. S. Czaplewski (Rogers Seed Co., Inc., Naples, Fla.). Several individual CdM334 plants were grown and self-pollinated from the original CdM334 population. The resulting families were screened with PVY and PepMoV to confirm homozygosity of *Pvr4* and the factor that confers resistance to PepMoV, if distinct from *Pvr4*. A single CdM334 plant homozygous for resistance to both viruses was used as a parent for inheritance studies. Controlled pollinations were performed in the greenhouse under supplemental lighting.

The resistant 9093 parent was crossed with Jupiter and RNaky to generate F_1, F_2, and backcross (BC_R and BC_S) populations for inheritance studies. For all inheritance experiments, data from reciprocal crosses were bulked after chi-square tests of homogeneity were performed and chi-square goodness-of-fit tests were used to examine genetic hypotheses. To determine the genetic relationship between PepMoV resistances in 9093 and CdM334, 9093 was crossed with CdM334 and the resulting F_1 was testcrossed to Jupiter and to RNaky. The (F_1×Jupiter) and (F_1×RNaky) testcrosses were each made twice with different parental plants to minimize the possibility that the observed susceptible plants were the result of seedlot contamination. One seedlot was used for screens I, II and III, and a second seedlot was used for screen IV. To determine whether PepMoV and PVY resistances from 9093 segregated independently, survivors from PepMoV and PVY screens of a (Jupiter×9093) F_2 population were self-pollinated to generate F_3 families. For each of the 29 F_3 families from which sufficient seed was obtained, 18 F_3 plants/family were screened with each virus.

Viral cultures

All potyvirus cultures were maintained on TMV-resistant *Nicotiana tabacum* (cv Petit Havana) plants, and were transferred every 4–8 weeks. PepMoV strain V1182 (Florida Strain) was used for all PepMoV experiments and was provided by Dr. T. A. Zitter (Cornell University, Ithaca, N.Y.). TEV strain V47, provided by Dr. P. Himmel (Seminis Vegetable Seeds Inc., San Juan Bautista, Calif.), was used for all TEV experiments. Isolates of PVY are classified into pathotypes, determined by differential responses observed when pepper genotypes containing known alleles at the *pvr2* locus are inoculated. PVY strains P-27–81 (pathotype 0), P-62–81 (pathotype

1), and P-22–88 (pathotype 1–2), originally isolated from pepper fields in Spain, were provided by Dr. M. Luis Arteaga (Servicio de Investigación Agroalimentaria, Zaragoza, Spain). Parents were screened with all PVY pathotypes; however, only the most virulent pathotype, PVY(1–2), was used for inheritance studies.

Inoculation procedures

For all potyviral inoculations, infected tobacco tissue was ground in chilled 0.05 M potassium phosphate buffer, pH 8.0 (approximately 1 g tissue: 5 ml buffer), mixed with a pinch of 320-grit carborundum, and rubbed manually on the one or two youngest fully expanded leaves. Plants were grown either in the greenhouse with supplementary artificial lighting or in growth rooms. For inheritance studies, the third pair of leaves on 4- to 6-week-old plants were inoculated, and symptoms were checked every 2 to 3 days for 5 weeks. For experiments confirming the resistant phenotypes, plants were inoculated once on the distal portion of a young fully expanded leaf, usually the 6th true leaf. Plants were checked for viral symptoms up to 30 days post-inoculation (dpi) and ELISA tests were performed at 6, 12, and 30 dpi.

Indirect enzyme-linked immunosorbent assay (indirect-ELISA)

For inheritance studies, two uninoculated partially expanded young leaves from 5 or 10 plants from each population were assayed by ELISA 28 dpi. For experiments confirming the resistance phenotype by assessing the presence of virus in different plant portions, 5–10 plants of several genotypes were sampled for ELISA at each time point (6, 12, and 30 dpi). Four individual samples (each consisting of one half-leaf) were taken from each plant at each time point: the inoculated portion of the inoculated leaf; the portion of the inoculated leaf proximal to the plant; the proximal and distal portions of the youngest uninoculated fully expanded leaf. Samples were ground in coating buffer to a final dilution of 0.1 g tissue : 1.0 ml buffer. Immunoglobulin (Ig) was purified by ammonium sulfate-precipitation from antisera to PepMoV, TEV, and PVY (American Type Culture Collection, Rockville, Md.). Ig was then passed through a DEAE Sephacel (Sigma Immunochemicals, St. Louis, Mo.) column for selective purification of IgG. Indirect ELISA was used to detect of viral coat protein antigens (PepMoV, TEV, or PVY CP) according to Voller et al. (1976) with the modifications described in Grube (1999). Samples were declared positive for the presence of viral CP if their absorbance (405 nm) was significantly higher than a threshold value, determined from the mean absorbance of samples from phosphate buffer-inoculated leaves plus 3 standard deviations.

Localization of *Pvr4* on a comparative pepper map

A *Pvr4*-linked cleaved amplified polymorphic sequence (CAPS) marker (Caranta et al. 1999b) was amplified according to the protocol of Caranta et al. (1999b) from DNA samples of mapping parents and the available 48 of 75 (*C. annuum* 'RNaky'×*C. chinense* PI159234) F_2 individuals used to construct a comparative genetic map of pepper (Livingstone et al. 1999). Potato genomic clones GP125 and GP259 were provided by C. Gebhardt (Max-Planck Institut für Züchtungforschung, Germany), and were hybridized to mapping filters as described in Livingstone et al. (1999). Genotypes were obtained for 70 and 71 out of 75 mapping individuals for GP125 and GP259, respectively. All three markers were scored and map positions were ascertained using MapMaker/Exp v3.0b (Lincoln et al. 1993) by the method described in Livingstone et al. (1999).

Results

Response of 9093 to PepMoV

By 12 dpi, the susceptible genotypes Jupiter and RNaky uniformly developed bright systemic mosaic symptoms characteristic of PepMoV on newly emerged uninoculated leaves, and no symptoms were observed in inoculated leaves. Uninoculated leaves on 9093 remained asymptomatic, although occasionally (0/6 plants at 6 dpi; 1/9 plants at 12 dpi), a single large necrotic local lesion (approximately 5–6 mm in diameter) appeared on the inoculated leaf. CdM334 also occasionally (2/8 plants at 6 dpi; 1/8 plants at 12 dpi) exhibited one or two smaller local necrotic lesions (2–4 mm in diameter) on the inoculated leaf but never developed systemic symptoms. By 30 dpi, inoculated leaves of all genotypes had usually abscised.

Most of the susceptible RNaky and Jupiter plants contained PepMoV CP levels above the threshold throughout the plant within 6 dpi (Fig. 1). By 12 dpi, all RNaky and Jupiter plants were systemically infected; therefore, this time point was selected for comparisons between genotypes (Fig. 1). At 12 dpi, inoculated leaves of 9093, on average, did not contain viral antigen at levels above the experimental threshold, although the inoculated leaf of 1/8 plants did contain PepMoV CP. Antigen levels in the inoculated leaves of CdM334 were more often above threshold, in 3/8 plants tested. Viral antigen was never detected in uninoculated tissue of either 9093 or CdM334 and virus could not be recovered by back-inoculation to susceptible tobacco plants (data not shown). Based on these results, both 9093 and CdM334 were classified as resistant to PepMoV because the virus failed to spread systemically.

Inheritance of PepMoV resistance from 9093

Responses of (9093×RNaky) and (9093×Jupiter) F_1, F_2, BC_R and BC_S populations to inoculation with PepMoV demonstrated segregation ratios consistent with monogenic dominant inheritance of resistance (Table 1). Chi-square tests of homogeneity indicated that reciprocal crosses responded similarly to PepMoV, providing no evidence of maternal inheritance. Mean absorbance values from indirect ELISA confirmed visual symptoms in all populations tested (Table 1). When phenotypes and ELISA values of uninoculated leaves were considered, the F_1 appeared completely resistant, suggesting complete dominance of resistance. When phenotypes of inoculated leaves are considered, however, there is some indication that PepMoV resistance in 9093 is incompletely dominant. While uninoculated leaves of (Jupiter×9093) and (RNaky×9093) F_1 plants remained asymptomatic, slightly more viral antigen was detected on average in the inoculated leaves of heterozygotes than in the homozygous 9093 plants (Fig. 1). Further, 5 out of 14 (Jupiter×9093) and (RNaky×9093) F_1 plants accumulated significant levels of viral antigen in the inoculated leaf, in contrast to only 1 out of 8 9093 plants.

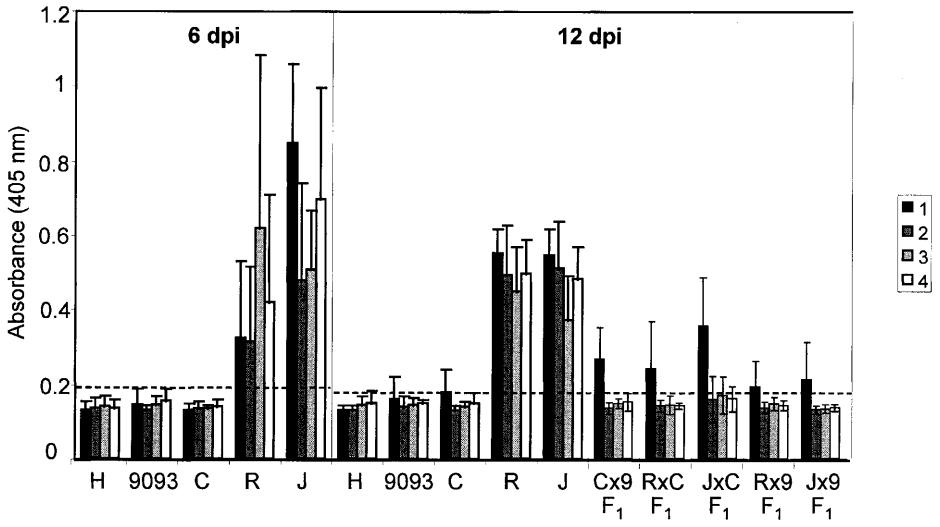

Fig. 1 Detection of PepMoV in genotypes 6, 12, and 30 days post-inoculation. Leaf samples *1*, *2*, *3*, and *4* represent the inoculated portion of the inoculated leaf, the uninoculated portion of the inoculated leaf, the proximal (to the stem) portion of a young uninoculated leaf, and the distal portion of the same young leaf, respectively. Genotype codes: *H*=healthy tissue, *C*=Criollo de Morelos 334, *R*=NuMex RNaky, *J*=Jupiter. *Error bars* represent one standard deviation above the mean absorbance value. The *dotted line* designates the cutoff for a statistically significant difference from the absorbance of mock-inoculated healthy leaves (mean absorbance plus three standard deviations)

Test for independent segregation of PepMoV resistance genes from 9093 and Criollo de Morelos 334

PepMoV resistance in CdM334 was confirmed to be conferred by a single dominant gene (Grube 1999). If the PepMoV resistance genes from CdM334 (R1) and 9093 (R2) are distinct and segregating independently, approximately 25% susceptible offspring should be recovered in an (R1×R2)×S testcross population. If the two genes are allelic, no susceptible individuals should be recovered. In four separate experiments, five susceptible plants were identified among 826 RNaky-derived testcross plants and seven susceptible plants were recovered from 905 Jupiter-derived testcross plants (Table 2). Fruit morphologies of susceptible plants were intermediate between the F_1 and the susceptible parent used, ruling out seed contamination by the susceptible parent. All surviving susceptible testcross plants gave rise to 100% susceptible progeny, ruling out the possibility that a low proportion of testcross individuals failed to express resistance as a result of incomplete penetrance of either resistance gene. The possibility that unequal crossing-over led to the deletion of both alleles at a single locus is unlikely given the observed frequency of susceptible individuals. The number of observed susceptible individuals

Table 1 Inheritance of PepMoV resistance in *C. chinense* '9093'

Genotype[a]	Resistant		Susceptible		Expected ratio (R:S)	χ^{2c}	P^d
	No.	ELISA (N)	No.	ELISA (N)			
9093	12	0.3318 (5)[e]	0	–	1:0	–	–
Jupiter (J)	0	–	15	**0.6436** (5)[e]	0:1	–	–
NuMex RNaky (R)	NA[f]	–	–	–	0:1	–	–
(J×9093) F_1	19	0.1956 (5)	0	–	1:0	–	–
J×(J×9093) F_1	10	0.1818 (5)	13	**0.6446** (5)	1:1	0.39	0.53
9093×(J×9093) F_1	40	0.1824 (10)	0	–	1:0	–	–
(J×9093) F_2	65	0.1756 (5)	21	**1.1236** (5)	3:1	0.02	0.90
(R×9093) F_1	17	0.1876 (5)	0	–	1:0	–	–
R×(R×9093) F_1	19	0.1670 (10)	26	**0.7699** (10)	1:1	1.09	0.30
9093×(R×9093) F_1	44	0.1695 (10)	0	–	1:0	–	–
(R×9093) F_2	72	0.1916 (5)	20	**0.7762** (5)	3:1	0.52	0.47

[a] Data from standard and reciprocal crosses were pooled

[b] ELISA data are represented by mean absorbance value with the number of individuals randomly sampled indicated in parentheses. The critical value was determined by the mean of healthy tissue plus three standard deviations. This threshold was 0.2843 except for the sample marked[e], for which the critical value was 0.4863. Values in bold are statistically significantly positive

[c, d] The χ^2 and *p*-values resulting from a chi-square test of fit for the the data to a single dominant gene model (3R:1S-F_2, 1R:1S-BC_S)

[f] NuMex RNaky data not available from this experiment; see Fig. 1 for representative NuMex RNaky performance

in (R1×R2)×S testcross populations is equal to 1/2 of the actual number of recombinant individuals. The calculated recombination frequency between the resistance loci is 2×(5/826)=0.0121 in RNaky derived populations and 2×(7/905)=0.0155 in Jupiter-derived populations. PepMoV resistance from CdM334 and 9093 is therefore controlled by different genes, linked at <2.0 cM. We have designated the PepMoV resistance gene from 9093 *Pvr7*, for *P*otyvirus *r*esistance locus *7*, according to the accepted proposal for potyvirus resistance gene nomenclature in pepper (Kyle and Palloix 1997).

Table 2 Testcross to assess allelism of PepMoV resistance genes in *C. chinense* '9093' and *C. annuum* 'Criollo de Morelos 334' (CdM334)

Screen no.	(R1×R2)×S population	PepMoV susceptible/total no. plants screened
I	(9093×CdM334) F_1×Jupiter	1/104
II	(9093×CdM334) F_1×Jupiter	1/248
II	(9093×CdM334) F_1×NuMex RNaky	1/279
III	(9093×CdM334) F_1×NuMex RNaky	0/284
IV	(9093×CdM334) F_1×NuMex RNaky	4/263
IV	(9093×CdM334) F_1×Jupiter	5/553
Total		12/1731

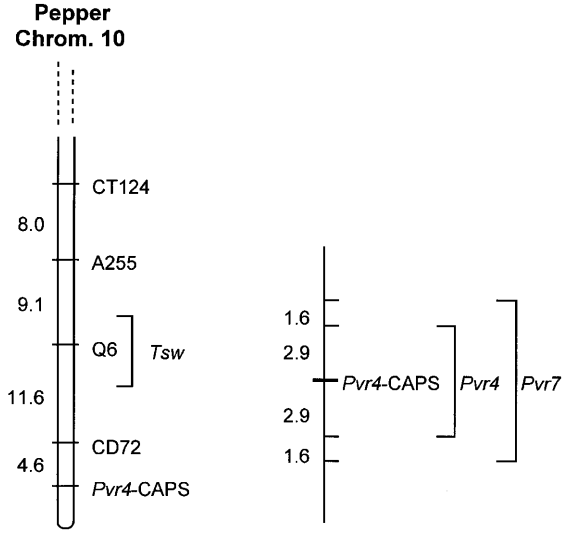

Fig. 2 Position of *Pvr4*-linked CAPS marker (Caranta et al. 1999b) on a comparative pepper map (Livingstone et al. 1999) and the relative positions of *Pvr4*, the *Pvr4*-linked CAPS marker and *Pvr7*. Intervals containing the indicated genes are denoted by *brackets*. *Numerals* to the left of chromosome define genetic distances between tick marks in cM. Markers included are CT124, a tomato cDNA clone; A255, an AFLP marker; Q6, a RAPD marker tightly linked to *Tsw*; and CD72, a tomato cDNA clone. All were obtained, or generated, and mapped as described by Livingstone et al. (1999)

Comparative mapping of dominant potyvirus resistance loci in *Capsicum*

Caranta et al. (1999b) described the development of a CAPS marker linked at a distance of 2.1±0.8 cM from *Pvr4*. We obtained a map position for this marker using an interspecific (*C. chinense* 'PI159234'× *C. annuum* RNaky) F_2 mapping population that is not segregating for either *Pvr4* or *Pvr7*. The *Pvr4*-associated allele of the *Pvr4*-linked CAPS marker was present in PI159234 and absent in RNaky. This marker was located 4.6 cM from CD72, the last framework marker on pepper chromosome 10 (LOD=1.9, Fig. 2). *Pvr4* was estimated to be 4.6±2.9 cM from CD72 and, by extension, *Pvr7* is 4.6±4.5 cM from CD72. Due to lack of polymorphism for the CAPS marker in populations segregating for *Pvr7*, the linkage between *Pvr7* and the *Pvr4*-linked CAPS marker has not been directly confirmed. Resistance gene analogs (RGAs) that share sequence similarity with cloned R genes have also been amplified and analyzed in populations segregating for *Pvr7*, and none were demonstrated to cosegregate with *Pvr7* (Grube 1999).

GP125 and GP259 were mapped to pepper chromosome 11 (LOD >3.0), establishing colinearity with the region of potato chromosome XI containing Ry_{sto}/Ry_{adg} (*Ry*) and Ra_{adg} (*Ra*) (Brigneti et al. 1997; Hämäläinen et al. 1997); however, no pepper disease resistance loci are mapped in this region. Similarly, the marker content and order near *Pvr4* and *Pvr7* (pepper chromosome 10) was maintained on potato chromosome X (Tanksley et al. 1992; Livingstone et al. 1999), which contains no mapped potato disease resistance loci, suggesting that the *Ry*/*Ra* and *Pvr4*/*Pvr7* gene clusters are not orthologous.

Responses of Criollo de Morelos 334 and 9093 to PVY and TEV

CdM334 and 9093 remained asymptomatic after inoculation with pepper PVY strains representing the three pepper pathotypes, 0, 1, and 1–2. Jupiter and RNaky

Table 3 Inheritance of PVY resistance in *C. chinense* '9093'

Genotype[a]	No. resistant	No. susceptible	Expected ratio (R:S)	χ^{2b}	P^c
9093	13	0	1:0	–	–
Jupiter (J)	0	18	0:1	–	–
(J×9093) F_1	14	0	1:0	–	–
J×(J × 9093) F_1	23	32	1:1	1.47	0.23
9093×(J × 9093) F_1	24	0	1:0	–	–
(J×9093) F_2	42	9	3:1	1.47	0.23

[a] Data from standard and reciprocal crosses were pooled
[b, c] The χ^2 and *P*-values resulting from a chi-square test of fit of the data to a single dominant gene model (3R:1S-F_2, 1R:1S-BC_S)

plants uniformly developed systemic veinal necrosis, mild mosaic, and chlorosis on leaves, and Jupiter plants occasionally died. ELISA results for PVY(1–2) confirmed phenotypic results in all populations tested (data not shown). The responses of (9093×Jupiter) F_1, F_2, BC_R, and BC_S populations were consistent with those expected for monogenic dominant inheritance of resistance (Table 3).

In contrast to results shown for PepMoV and PVY, 9093 was completely susceptible to TEV-V47, showing chlorosis and systemic mottling in all plants tested. Jupiter and RNaky also uniformly developed a systemic mottle in response to inoculation with TEV-V47. Some inbred CdM334 lines that were homozygous for *Pvr4* segregated for TEV symptoms, while other lines were entirely asymptomatic. These data suggest that the CdM334 population may contain resistance to TEV-V47, but that this resistance is not conferred by *Pvr4*.

Cosegregation of PepMoV and PVY resistances in 9093

To determine whether dominant resistances to PepMoV and PVY in 9093 are linked, 29 (Jupiter×9093) F_3 families were inoculated with PVY (1–2) and with PepMoV. All 29 families showed complete agreement between PepMoV and PVY resistance genotypes, demonstrating that the genes conferring these resistances do not segregate independently. Either two linked genes or a single allele therefore control PVY and PepMoV resistances in 9093.

Discussion

A newly designated gene from *C. chinense* 'PI159236–9093', *Pvr7*, confers dominant resistance to PepMoV strain V1182 and is less than 2 cM from another locus conferring dominant PepMoV resistance from *C. annuum* 'Criollo de Morelos 334'. PepMoV CP is occasionally detected in inoculated leaves of both 9093 and CdM334 several days post-inoculation, although uninoculated tissue of both resistant genotypes remain free of symptoms and viral CP cannot be detected by indirect ELISA and is not infectious in biological recovery assays. Therefore, both *Pvr7* and the PepMoV resistance gene from CdM334 appear to affect local and/or long-distance viral movement in the plant.

Dominant resistance to PVY pathotype 1–2 in 9093 did not segregate independently from *Pvr7* when F_3 progeny from segregating F_2 populations were screened with both viruses. A similar observation for PVY and PepMoV resistance was made for *Pvr4* (Dogimont et al. 1996; C. Caranta, A. Palloix, and A. M. Daubèze, unpublished results). In the case of both *Pvr7* and *Pvr4*, the resistance genes may have dual specificity; however, in view of the small population sizes tested, particularly for *Pvr7*, the presence of two tightly linked dominant alleles in each genotype, each conferring resistance to a single virus, could also account for our observations. Thus, dominant PVY resistance in 9093 could be due to *Pvr4*, *Pvr7*, or a third unnamed linked gene. The total number of potyvirus resistance loci at this position in the genome and their resistance spectra therefore remain unknown.

Pvr4 and *Pvr7* are similar in both inheritance and resistance phenotype to a cluster of two or more potyvirus resistance loci in *Solanum* (Ry_{sto}/Ry_{adg}, Ra_{adg}); however, comparative mapping results suggest that these clusters are not orthologous. There is no indication to date that *Pvr4*/*Pvr7* orthologues, if present, have maintained any resistance function in potato because no known potato R genes have been mapped to this region. A QTL conferring resistance to *Clavibacter michiganensis* in tomato in one population has been located near the centromere of chromosome 10, but the positions of *Pvr4* and *Pvr7* relative to this locus cannot be determined precisely (Sandbrink et al. 1995). These results are consistent with a trend emerging for R loci. To the limited extent that they have been examined, R loci from different solanaceous genera that confer resistance to similar or identical pathogens do not appear to occur in colinear genomic regions (Ohmori et al. 1998; Grube et al. 2000.

Pvr4 and *Pvr7* are located on pepper chromosome 10, approximately 15 cM from a random amplified polymorphic DNA (RAPD) marker tightly linked to *Tsw*, a dominant allele that confers resistance to tomato spotted wilt virus (Jahn et al. 2000). Pairwise estimates of linkage between *Pvr4*, *Pvr7*, and *Tsw* have yet to be established in a single population; however, the identification of *Pvr7* and *Tsw* from a common accession (PI159236) suggests that genotypes containing both genes in the coupling linkage phase should exist and will allow improved estimates of genetic distance. These genes are well within the 30 cM interval typical of R gene clusters in many other plants; therefore, this grouping of two or more potyvirus R genes and *Tsw* define the first such cluster in pepper (Michelmore and Meyers 1998). Two additional R loci identified in PI159236, *L* (for tobacco mosaic virus resistance) and *pvr1* (for PepMoV and TEV resistance), occur on the small linkage group B and at the bottom of pepper chromosome 11, respectively (Lefebvre et al. 1995; Murphy et al. 1998). These and similar results from studies of a *S. tuberosum* accession that has been a source of many R loci (Van der Voort et al. 1999) suggest that there may be no single unifying basis for the existence of "jackpot" genotypes, which may contain a mixture of linked clusters of possibly related loci as well as unrelated alleles that have simply been lost during crop domestication. Understanding the organization of useful loci within available genetic resources will facilitate future identification and utilization of beneficial traits, and reveal the extent to which results from comparative mapping may streamline gene discovery.

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