Fine Mapping of the Dominant Potyvirus Resistance Gene *Pvr7* Reveals a Relationship with *Pvr4* in *Capsicum annuum*

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ABSTRACT

Pepper mottle virus (PepMoV) is the most common potyvirus infection of pepper plants and causes significant yield losses. The *Pvr7* gene from *Capsicum chinense* PI159236 and the *Pvr4* gene from *C. annuum* CM334 both have been reported to confer dominant resistance to PepMoV. The *Pvr7* locus conferring resistance to PepMoV in *C. annuum* '9093' was previously mapped to chromosome 10. To develop a high-resolution map of the *Pvr7* locus in 9093, we constructed an intraspecific F₂ mapping population consisting of 916 individuals by crossing PepMoV-resistant *C. annuum* '9093' and the PepMoV-susceptible *C. annuum* 'Jeju'. To delimit the *Pvr7* target region, single-nucleotide polymorphism (SNP) markers derived from the *Pvr4* region were used for genotyping the F₂

population. Molecular mapping delimited the *Pvr7* locus to a physical interval of 258 kb, which was the same region as *Pvr4* on chromosome 10. Three SNP markers derived from *Pvr4* mapping perfectly cosegregated with PepMoV resistance. Sequencing analyses of the *Pvr7* flanking markers and the *Pvr4*-specific gene indicated that *Pvr7* and *Pvr4* are the same gene. Resistance spectrum analysis of 9093 against pepper potyviruses showed that 9093 has a resistance spectrum similar to that of cultivar CM334. These combined results demonstrate that, unlike previously thought, the dominant PepMoV resistance in 9093 could be derived from *C. annuum* 'CM334', and that *Pvr4* and *Pvr7* should be considered as the same locus.

Potyriviruses are the major group of viruses infecting plants, and are transmitted by aphid vectors (Valkonen et al. 1996). *Pepper mottle virus* (PepMoV), which belongs to the *Potyrivirus* genus, often infects pepper (Abdalla et al. 1991; Dogimont et al. 1996; Lamb et al. 2001; Rialch et al. 2015). Plants infected with PepMoV display a range of symptoms, including mosaic and dark-green vein banding, crinkled leaves, and stunted plant growth (Kim et al. 2010). PepMoV is most prevalent in North America and Asia, and causes serious economic losses in pepper (Kim et al. 2009; Moury et al. 2005; Rodríguez-Alvarado et al. 2002; Warren and Murphy 2003).

Several potyvirus resistance (*R*) genes have been reported in pepper (Caranta et al. 1996; Grube et al. 2000; Kyle and Palloix 1997; Lee et al. 2013). For instance, recessive potyvirus *R* genes encoding eukaryotic translation initiation factors have been identified in *Capsicum* spp. (Hwang et al. 2009; Kang et al. 2005; Murphy and Kyle 1995; Ruffel et al. 2006). The *pvr1* gene, which was identified in *Capsicum chinense* PI159236 and PI152225, confers resistance to *Tobacco etch virus* (TEV) HAT isolate, *Potato virus Y* (PVY) pathotype 0, and PepMoV (Kyle and Palloix 1997; Murphy et al. 1998; Yeam et al. 2005). The gene *pvr1^l* was identified in *C. annuum* 'Yolo Y' and confers resistance to PVY (Kang et al. 2005; Ruffel et al. 2002; Yeam et al. 2005). The *pvr3* gene was reported in *C. annuum* 'Avelar' and confers resistance to PepMoV (Guerini and Murphy 1999; Murphy and Kyle 1995; Parrella et al. 2002). Pepper plants expressing the *pvr6* gene from *C. annuum* 'Perennial' are resistant to *Chilli veinal mottle virus*

(Caranta et al. 1996; Hwang et al. 2009; Ruffel et al. 2006). In addition to these recessive sources of resistance, dominant potyvirus *R* genes also have been reported in pepper. The *Pvr4* gene was identified in *C. annuum* 'CM334' and confers resistance to PepMoV (Caranta et al. 1999; Grube et al. 2000; Kim et al. 2017). *Pvr7* was identified as tightly linked to *Pvr4* in *C. chinense* 'PI159236' (Grube et al. 2000).

Candidate genes for *Pvr4* have been identified by Kim et al. (2017) using a map-based cloning approach. Transient coexpression analyses of the putative candidate genes in *Nicotiana benthamiana* showed that a nucleotide-binding leucine-rich repeat (NB-LRR)-type disease *R* gene (*CaNBARC322*) is responsible for hypersensitive-response-mediated resistance (Kim et al. 2017). The *Pvr4* gene consists of seven exons encoding a typical coiled-coil NB-LRR family protein, whereas the susceptible allele *pvr4* from *C. annuum* 'ECW' consists of six exons (Kim et al. 2017).

Pvr4 and *Pvr7* were originally reported as tightly linked but nonallelic due to the apparent identification of a small number of putative recombinant plants in a segregating population (Grube et al. 2000). In that study, *Pvr4* and *Pvr7* were localized on the lower arm of chromosome 10, approximately 15 centimorgans (cM) from the random amplified polymorphic DNA marker Q6, which is tightly linked to *Tomato spotted wilt virus* (TSWV) resistance gene *Tsw* (Grube et al. 2000; Jahn et al. 2000). Although previous research showed that *Pvr7* is located on chromosome 10, the precise location of *Pvr7* in the pepper genome and the gene encoding *Pvr7* have not previously been identified. The widely used 9093 line carrying *Pvr7* was derived from repeated backcrossing of a bell-type *C. annuum* recurrent parent line to *C. chinense* 'PI159236' (Grube et al. 2000). Genetic fine mapping of the *Pvr7* locus promises to broaden our molecular and genetic understanding of the dominant inheritance mechanism, and could facilitate the breeding of PepMoV-resistant varieties.

In this study, we performed fine mapping of the *Pvr7* locus using 916 9093 × Jeju F₂ plants derived from a cross between the PepMoV-resistant parental line *C. annuum* '9093' and the susceptible parental

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line *C. annuum* 'Jeju'. Our fine mapping localized the *Pvr7* locus to a physical interval of 258 kb flanked by the single-nucleotide polymorphism (SNP) markers SNP-H2.3 and SNP-H1.7 on chromosome 10, in which *Pvr4* also was localized (Kim et al. 2017). To determine the relationship between *Pvr4* and *Pvr7*, we investigated the resistance spectrum of 9093 using other pepper viruses, including TSWV and TEV. The results of viral resistance spectrum analysis, genetic fine mapping, and sequence characterization of the *Pvr4* gene-specific insertion and deletion (InDel) and the *Pvr7* flanking markers all indicate that *Pvr7* and *Pvr4* are, in fact, the same gene; hence, we suggest the revision of the *Pvr7* designation to be *Pvr4*.

MATERIALS AND METHODS

Plant materials and construction of mapping population.

Five *Capsicum* accessions were used for viral resistance spectrum and fine-mapping analysis, including *C. chinense* 'PI159236' and 'PI152225' and *C. annuum* 'ECW', '9093', and 'Jeju'. PI159236 and PI152225 harbor resistance to PepMoV (Grube et al. 2000), whereas ECW is susceptible to PepMoV (Kim et al. 2011). To perform a genetic analysis and fine mapping of the *Pvr7* gene, F₁ and F₂ populations were developed by crossing the PepMoV-resistant *C. annuum* '9093' to the PepMoV-susceptible *C. annuum* 'Jeju'. In total, 916 9093 × Jeju F₂ plants were used to map the *Pvr7* locus. *C. annuum* 'CM334' and 'B2110-1A' and commercial F₁ hybrid IPH10023 were kindly provided by Bob Heisey (United Genetics Seeds Co., Hollister, CA) and used to validate the identity of the *Pvr7* gene.

Inoculum preparation and plant inoculation. PepMoV strain Vb1 (hereafter PepMoV) and TEV were maintained in *N. benthamiana*. TSWV was maintained in *N. rustica*. Approximately 1 g of infected leaf tissue was ground on ice in 5 ml of ice-cold 0.1 M potassium phosphate buffer (pH 7.0) mixed with 400-grit carborundum, manually rubbed on fully expanded leaves of experimental plants, allowed to stand for 20 min, and then washed off with distilled water.

Either the first or second pair of true leaves of 3- to 4-week-old pepper plants were inoculated. Inoculated plants were grown in a growth chamber maintained at 23 and 21°C (day and night, respectively) with 16 h of light provided by cool fluorescent lighting. Inoculated pepper plants were monitored for development of viral symptoms up to 21 days postinoculation (dpi). In total, 10 each of PI159236, PI152225, 9093, Jeju, and 9093 × Jeju F₁ plants were inoculated with TSWV and TEV to investigate the virus-resistance spectrum of *Pvr7*.

Double-antibody sandwich enzyme-linked immunosorbent assay. Enzyme-linked immunosorbent assay (ELISA) tests were performed at 7, 8, and 13 dpi to detect the viral coat proteins of TSWV, TEV, and PepMoV. ELISA tests were performed according to the manufacturer's instructions (Agdia, Elkhart, IN). Samples were collected from inoculated and noninoculated upper leaves. The ELISA tests were performed three times.

Monitoring viral infection using PepMoV tagged with green fluorescent protein. PepMoV constructs fused to green fluorescent protein (GFP) were used to monitor the systemic spread of PepMoV. GFP was visualized in infected plants with the MultiDoc-It imaging System (UVP, LLC, Upland, CA).

Genomic DNA extraction. Total genomic DNA was extracted from fully expanded cotyledon tissue of each plant using the cetyltrimethylammonium bromide method (Kang et al. 2010). DNA concentration and quality were analyzed using the NanoDrop spectrophotometer (NanoDrop Technologies, Inc., Wilmington, DE).

SNP marker development and genotyping. We used 35 previously reported SNP markers on chromosome 10 (Kang et al. 2014) to localize the *Pvr7* locus. The linkage between SNP marker and phenotype was analyzed by comparing marker genotype and

resistance phenotype using CarthaGene software (Schiex and Gaspin 1997). To develop additional SNP markers, polymerase chain reaction (PCR) primers were designed based on the sequence information of the *C. annuum* 'CM334' genome sequence (Kim et al. 2014). Amplified PCR products were sequenced by the National Instrumentation Center for Environmental Management (Seoul National University, Korea). Newly identified SNP markers were tested for polymorphism between susceptible and resistant parents, and polymorphic SNP were converted into high-resolution melting (HRM) markers. In total, 916 F₂ plants were genotyped by HRM analysis using the Rotor-Gene 6000 Real-Time Rotary Analyzer (Corbett Research, Sydney, Australia).

Fine genetic mapping and identification of candidate genes. Map distances were estimated by determining the frequency of susceptible recombinants among the mapping population. The map was drawn using the MapChart program. SNP markers linked to the *Pvr7* locus were used to identify a corresponding pepper genome scaffold sequence. Scaffold A was obtained from the pepper genome database (<http://passport.pepper.snu.ac.kr/?t=PGD>) by BLASTN search against *C. annuum* scaffold version 1.5. Candidate genes were predicted in the target region using the FGENESH tool (Solovyev et al. 2006).

Comparison of *Pvr4* and *Pvr7*. The *Pvr4* and *Pvr7* genes were compared using *Pvr7* flanking markers and a locus-specific *Pvr4* InDel marker (*Pvr4*-EI-F 5'-CCTGGAAAAGCTATATATC TATTCGGACAT-3' and *Pvr4*-EI-R 5'-TGGGGGAAGGTAAAA GGAAGCTAA-3'), which was developed in the present study. Marker sequences were PCR amplified from the pepper lines and sequenced by Macrogen (Seoul, Korea Republic). The following pepper lines were screened for polymorphisms: PI159236, CM334, B2110-1A, the commercial heterozygous pepper line IPH10023, and the susceptible cultivar ECW.

Statistical analysis. Statistical analyses were computed and graphs were generated using Microsoft Excel 2013 (Microsoft, Redmond, WA). The χ^2 test was performed using GraphPad Prism, version 7.0. $P < 0.05$ was considered as statistically significant.

RESULTS

PepMoV resistance in 9093. PepMoV resistance was tested in 9093, F₁ plants of 9093 × Jeju, and the control PI159236, Jeju, and ECW plants by challenging with PepMoV. The Jeju and ECW susceptible controls uniformly developed bright systemic mosaic symptoms of PepMoV on newly emerged uninoculated and inoculated leaves, whereas no symptoms were observed in the resistant PI159236 and 9093 controls or in 9093 × Jeju F₁ plants at 10 dpi (Fig. 1A).

Systemic spreading of PepMoV was confirmed in susceptible Jeju and ECW plants by visualizing green fluorescence under UV light, whereas green fluorescence was not detected in 9093, PI159236, or 9093 × Jeju F₁ plants (Fig. 1A). Systemic infection was further confirmed by ELISA. Viral coat protein accumulated to high levels in inoculated and uninoculated upper leaves of the susceptible Jeju and ECW control plants (Fig. 1B), whereas viral coat protein was not detected in inoculated and uninoculated leaves of 9093, PI159236, and 9093 × Jeju F₁ plants (Fig. 1B). These results confirm that PI159236, 9093, and 9093 × Jeju F₁ plants are resistant to PepMoV (Table 1).

Spectrum of *Pvr7*-mediated PepMoV resistance in 9093. The *Pvr7* locus (derived from PI159236) was reported to confer dominant resistance to PepMoV in 9093 (Grube et al. 2000), and PI159236 showed resistance to TEV and TSWV (Dogimont et al. 1996). PepMoV resistance in 9093 was considered to be introgressed from PI159236; therefore, 9093 may display resistance to TEV and TSWV. We performed viral-resistance spectrum analysis to confirm resistance to TEV and TSWV in 9093 (Fig. 2; Table 2).

PI159236 carrying the TSWV resistance gene *Tsw* displayed resistance to TSWV infection (as did PI152225); PI159236 did not

display any visual symptoms of TSWV infection at 8 dpi. If 9093 was derived from PI159236 (Grube et al. 2000), 9093 also might exhibit resistance to TSWV because *Tsw* and *Pvr7* loci are tightly linked. Inoculated leaves of either 9093 or 9093 × Jeju F₁ plants displayed typical symptoms of TSWV infection in uninoculated upper leaves (Fig. 2A). ELISA results confirmed that TSWV coat protein accumulated to high levels in 9093, 9093 × Jeju F₁, and the susceptible Jeju control (Fig. 2B). These results suggest that 9093 likely does not carry the TSWV resistance gene *Tsw*. In contrast to the earlier reports of Grube et al. (2000), the present results indicate that 9093 carries only *Pvr7*.

TEV belongs to the *Potyvirus* genus. *C. chinense* ‘PI159236’ and ‘PI152225’ express known resistance to TEV. These accessions carry the recessive resistance gene *pvr1*, which is located on chromosome 4. If 9093 was derived from PI159236, 9093 also would be expected to carry *pvr1*. To investigate whether 9093 expresses *pvr1*, homozygous 9093 and control plants were inoculated with TEV (Fig. 2). The resistant lines PI159236 and PI152225 did not develop viral symptoms in inoculated and upper uninoculated leaves by 7 dpi. By contrast, 9093, 9093 × Jeju F₁, and Jeju plants displayed clear TEV symptoms in uninoculated upper leaves (Fig. 2A). TEV coat protein did not accumulate in inoculated or uninoculated upper leaves of PI159236 and PI152225. By contrast, TEV coat protein was highly accumulated in both the inoculated and uninoculated upper leaves of 9093 and susceptible control plants (Fig. 2B). These results indicate that 9093 is susceptible to TEV and

does not carry *pvr1*. These combined results confirm that 9093 is susceptible to TSWV and TEV viruses (Table 2), showing virus-resistance spectra similar to those of CM334 (Janzac et al. 2009; Moury et al. 2005).

Fine mapping of *Pvr7*. To determine *Pvr7* inheritance in 9093, 916 9093 × Jeju F₂ individuals were screened for PepMoV resistance by visualizing GFP fluorescence. ELISA tests were performed to confirm the visual phenotypes. Phenotype analysis of the 9093 × Jeju F₂ population showed that 688 individuals were resistant and 228 individuals were susceptible to PepMoV, which fit a 3:1 segregation ratio (Table 1). These results confirm that

TABLE 1. Inheritance of *Pepper mottle virus* (PepMoV) resistance in the 9093 × Jeju F₂ population

Population ^a	Number of plants ^b			Expected ratio (R:S)	χ^2	P value
	Total	R	S			
Jeju	10	0	10	0:1	—	—
ECW	10	0	10	0:1	—	—
PI159236	10	10	0	1:0	—	—
9093	10	10	0	1:0	—	—
9093 × Jeju F ₁	5	5	0	1:0	—	—
9093 × Jeju F ₂	916	688	228	3:1	0.0058	0.94

^a Parental lines and populations.
^b R = resistant and S = susceptible.

Fig. 1. Visual symptoms and accumulation of *Pepper mottle virus* (PepMoV) in pepper lines at 10 days postinoculation (dpi). **A**, No green fluorescent protein (GFP) fluorescence was observed in PI159236, 9093, and 9093 × Jeju F₁ resistant plants in both inoculated and uninoculated upper leaves under UV light. Susceptible plants Jeju and ECW displayed GFP fluorescence in both inoculated and uninoculated leaves. **B**, Enzyme-linked immunosorbent assay tests were performed at 13 dpi. Data represent an average of three independent experiments. Error bars represent standard deviation of the mean absorbance value.

PepMoV resistance in 9093 is controlled by a single dominant *Pvr7* gene (Table 1).

The previous study (Grube et al. 2000) mapped the *Pvr7* locus onto chromosome 10 within 4.5 cM on either side of the *Pvr4*-SCAR marker, and showed that *Pvr7* is less than 2 cM away from *Pvr4* (Fig. 3A). In the present study, we used 35 SNP markers that are evenly distributed on pepper chromosome 10 (Kang et al. 2014) to perform fine mapping of the *Pvr7* locus in the F₂ mapping population. In all, 4 of the 35 SNP markers showing clear polymorphism between parental lines and distinguishing PepMoV resistance and susceptibility were converted into the codominant HRM markers. Initial genotyping and segregation analysis revealed that these four SNP markers (SNP-H1.5, SNP-H1.6, SNP-H1.7, and SNP-H1.8) were linked to the *Pvr7* locus (Fig. 3B). Additional markers around the *Pvr7* locus were developed using genomic information from *Capsicum* spp. For direct sequencing, PCR primers around the *Pvr7* region in chromosome 10 were designed based on the CM334 genome sequence information (Kim et al. 2014). We also used direct sequencing information to develop four additional HRM SNP markers (SNP-H2.1, SNP-H2.2, SNP-H2.3, and SNP-H2.4), which showed clear polymorphisms (Supplementary Fig. S1). In

summary, eight polymorphic codominant markers were used for genotyping the F₂ individuals (Table 3).

Fine mapping of the *Pvr7* locus resulted in the identification of 17 recombinant plants: 7, 5, and 3 recombinants for SNP-H2.1, SNP-H2.2, and SNP-H2.3, respectively, and 1 recombinant each for SNP-H1.7 and SNP-H1.8 (Fig. 3C). These recombinant plants were susceptible to PepMoV. Three of the eight SNP markers (SNP-H2.4, SNP-H1.5, and SNP-H1.6) perfectly cosegregated with the PepMoV-resistant phenotype (Fig. 3C). The *Pvr7* locus was

TABLE 2. Summary of resistance response of 9093

Virus ^a	Pepper accessions ^b				
	PI159236 (R:S)	PI152225 (R:S)	9093 (R:S)	Jeju (R:S)	9093 × Jeju F ₁ (R:S)
TSWV	10:0	10:0	0:10	0:10	0:10
TEV	10:0	10:0	0:10	0:10	0:10

^a *Tomato spotted wilt virus* (TSWV) and *Tobacco etch virus* (TEV).

^b R = resistant and S = susceptible plants.

Fig. 2. Visual symptoms and accumulation of *Tomato spotted wilt virus* (TSWV) and *Tobacco etch virus* (TEV) in *Capsicum* spp. **A**, PI159236 and PI152225 inoculated with TSWV and TEV displayed resistant phenotypes, whereas 9093, Jeju, and 9093 × Jeju F₁ plants displayed systemic infection in uninoculated upper leaves. **B**, Enzyme-linked immunosorbent assay tests were performed at 8 and 7 days postinoculation (dpi) for TSWV and TEV, respectively. Bars A = positive control, B = negative control, C = PI159236, D = PI152225, E = 9093, F = Jeju, and G = 9093 × Jeju F₁. Data represent an average of three independent experiments. Error bars represent standard deviation of the mean absorbance value.

delimited to a genetic interval between the SNP-H2.3 and SNP-H1.7 markers on chromosome 10 (Fig. 3C). The pepper genome sequence information of *C. annuum* ‘CM334’ (Kim et al. 2014) enabled us to determine that the *Pvr7* locus was located within the 258-kb region of scaffold A (Fig. 3C). Gene prediction analysis identified nine putative NB-LRR-type *R* genes within the *Pvr7* region (Fig. 3C).

Comparison between *Pvr4* and *Pvr7*. Molecular mapping analysis localized the *Pvr7* locus in a genomic region where the *Pvr4* locus was recently mapped in the CM334 genome (Kim et al. 2017). This prompted us to investigate the genetic relationship between *Pvr4*- and *Pvr7*-mediated PepMoV resistance. Therefore, we genotyped pepper plants using the SNP markers flanking the *Pvr7* locus (SNP-H2.3 and SNP-H1.7). Sequence analysis of the SNP-H2.3 marker showed no polymorphism among the pepper lines CM334, 9093, IPH10023, and B2110-1A (source of *Pvr7*). By contrast, SNP-H1.7 was polymorphic, and genotypes of 9093 and

B2110-1A were found to be the same as that of CM334 (Table 4), suggesting that *Pvr7* could be derived from CM334.

The specific InDel marker developed for the *Pvr4* locus in the present study was used for genotyping pepper lines (Supplementary Fig. S2). *Pvr4*-specific InDel marker amplification was observed in 9093, B2110-1A, and the commercial F₁ hybrid (IPH10023), which are known to carry the *Pvr7* locus. However, no amplification was observed in ECW or PI159236, which was reported as the source of *Pvr7* (Grube et al. 2000). These results further confirm that PI159236 is not a source of so-called *Pvr7*-mediated PepMoV resistance. The actual source of resistance to PepMoV in 9093 could be introgressed from *C. annuum*, probably via CM334, which is known to carry *Pvr4*, a dominant resistance gene (Kim et al. 2017; Kyle and Palloix 1997). Therefore, these combined results suggest that *Pvr4* and *Pvr7* should be considered as the same locus derived from *C. annuum* ‘CM334’.

Fig. 3. Fine mapping of the potyvirus resistance gene *Pvr7*. **A**, Linkage map of the *Pvr7* locus. **B**, Linkage map of the *Pvr7* locus that was developed based on the 9093 × Jeju F₂ population in the present study. Numbers above the chromosome indicate genetic distances in centimorgans. Single-nucleotide polymorphism (SNP) markers linked to the *Pvr7* locus are indicated below the chromosome. **C**, Fine mapping of the *Pvr7* locus. The *Pvr7* locus was delimited to a 258-kb region between the SNP-H2.3 and SNP-H1.7 markers on the *Capsicum annuum* ‘CM334’ scaffold A. Numbers in parentheses indicate the numbers of recombinant plants identified. A to I indicate the predicted nucleotide-binding leucine-rich repeat (NB-LRR) candidate genes. The candidate gene corresponding to the *Pvr7* gene is indicated in the light-shaded box.

DISCUSSION

Two dominant potyvirus *R* genes, *Pvr4* and *Pvr7*, are reported to be tightly linked. They are believed to have originated from different sources, with *Pvr4* originally reported to confer PVY and PepMoV resistance from *C. annuum* ‘CM334’ and *Pvr7* reported for resistance to PepMoV from *C. chinense* ‘PI159236’ (Grube et al. 2000; Kyle and Palloix 1997). The *Pvr7* locus was mapped to a genetic interval of 9 cM on chromosome 10 (Grube et al. 2000). A previous study reported that *Pvr4* and *Pvr7* have the same inheritance mechanism but are not allelic due to the recovery of a small number of fully susceptible plants in an R × R segregating population (Grube et al. 2000). The present study aimed to perform fine mapping of the *Pvr7* locus and elucidate the precise genetic relationship between *Pvr4* and *Pvr7*.

Fine mapping of the *Pvr7* locus using a segregating population of 916 F₂ plants from the 9093 × Jeju cross delimited the *Pvr7* locus to a 258-kb region between two flanking SNP markers (SNP-H2.3 and SNP-H1.7) on chromosome 10. This localized the *Pvr7* locus in the same genomic interval that was previously mapped for the *Pvr4* locus in the CM334 genome (Kim et al. 2017). The *Pvr4* mapping results of Kim et al. (2017) also point to a cluster of multiple NB-LRR genes (NBARC-LRR A to I) that were predicted from the *Pvr7* target region.

Pvr7-mediated PepMoV resistance in 9093 was reported to be derived from a repeated backcross of *C. chinense* PI159236 with an unknown recurrent bell pepper parent (*C. annuum*) (Grube et al. 2000), and the donor of the PepMoV resistance was considered to be *C. chinense* PI159236. However, the fine-mapping results for *Pvr7* and the viral-resistance spectrum of 9093 clearly suggest that PepMoV resistance in 9093 might be derived from an unknown parental line rather than *C. chinense* ‘PI159236’. If 9093 is derived from PI159236, then TSWV resistance also would be expected in 9093 because PI159236 carries the *Tsw* locus (Grube et al. 2000; Kim et al. 2017), which is very tightly linked to *Pvr7*. However, 9093 and 9093 × Jeju F₁ plants were susceptible to TSWV. Previous genetic analyses of PepMoV resistance in pepper varieties (Murphy et al. 1998; Subramanya 1982) have also demonstrated the recessive

resistance of PI159236 against PepMoV. Thus, all these studies indicate the mischaracterization of the dominant PepMoV resistance in 9093.

If, as we conclude, the PepMoV *R* genes *Pvr4* and *Pvr7* from CM334 and 9093 are the same gene, then no susceptible individuals would be expected in the test-cross populations derived from a cross between 9093 × CM334 F₁ and Jupiter or RNaky (Grube et al. 2000). Genetic analyses of these test-cross populations recovered five and seven susceptible plants among 826 RNaky- and 905 Jupiter-derived test-cross plants, with recombination frequencies of 0.0121 and 0.0155, respectively (Grube et al. 2000). Therefore, Grube et al. (2000) proposed that PepMoV resistance from CM334 and 9093 is conditioned by two different genes, *Pvr4* and *Pvr7*, linked at less than 2 cM. However, it was not conclusive whether *Pvr7* and *Pvr4* are allelic or the same locus because the linkage between *Pvr7* and *Pvr4* loci has not been directly confirmed. Those unexpected recombinants in test-cross populations may be caused by unequal crossing-over following pairing between duplicated sequences, similar to the case in the maize complex resistance gene locus *Rp1*, which consists of multiple genes (Hulbert and Bennetzen 1991; Hulbert et al. 1993), or simply breakdown of resistance in otherwise genetically resistant individuals in the disease screen. It was suggested that unequal exchange events would result in the mapping of genes to different relative positions even if the genes were identical or allelic (Hulbert et al. 1993), and could lead to misidentification of the alleles or genes. To avoid such problems, the sequences of the flanking markers or the gene itself should be verified in addition to performing genetic linkage analysis to unravel the genetic relationship between closely linked genes or multiple allelic variants.

To reveal the genetic relationship between *Pvr4* and *Pvr7*, we compared *Pvr7* flanking marker sequences with the *Pvr4* gene using pepper lines that were known sources of *Pvr7* and *Pvr4*. First, if 9093 carried the *Pvr7*-introgressed region from PI159236, no *Pvr4*-specific InDel marker amplification would be expected. Second, the genotype of *Pvr7* flanking markers should be similar to that of *C. chinense* ‘PI159236’. However, *Pvr4*-specific InDel marker amplification was observed only in 9093, B2110-1A, and the commercial F₁ hybrid (IPH10023) that carries the *Pvr7* locus. The genotype of the SNP-H1.7 flanking marker was similar to that of CM334. These results combined with *Pvr7* fine mapping and viral-resistance spectrum analysis of 9093 clearly demonstrate that the actual source of PepMoV resistance in 9093 could be derived from CM334, which is known to carry the dominant *Pvr4* resistance gene (Kim et al. 2017; Kyle and Palloix 1997).

Analysis of genetic relationships between potyvirus *R* genes and their resistance spectrum has been complicated because of the use of unrelated or uncharacterized resistance sources and the existence of complex loci. In the present study, viral-resistance spectrum analysis of 9093, which carries *Pvr7*, revealed resistance responses similar to those of CM334 against known pepper viruses. The *Pvr7* locus was fine mapped in the same genomic interval of chromosome 10 in CM334 in which *Pvr4* was localized. Genotyping of flanking markers and gene-specific InDel markers of *Pvr4* or *Pvr7* revealed identical genotypes for 9093 and CM334. Thus, this work confirmed that *Pvr4* and *Pvr7* are the same gene, with both originating from CM334. These combined results demonstrate the importance and utility of the flanking markers and gene-specific markers in the genetic dissection of complex loci.

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TABLE 3. Markers used for fine mapping of the *Pvr7* locus

Marker ^a	Sequence
SNP-H2.1-F	GCTTCTCAACTCTGCCACAA
SNP-H2.1-R	TCTCATTCGAACGTGAATTTGTT
SNP-H2.2-F	AGCCCATGAGGAATGAGGTG
SNP-H2.2-R	CTGGGTCCGCTTGTATGAT
SNP-H2.3-F	GGTCCGCTGATCAATTTTGG
SNP-H2.3-R	GTTGTCCCAAGGAGTTGGT
SNP-H1.7-F	TTGGCCCAAAGTCCATTTCTT
SNP-H1.7-R	TCAGTGAACACGTCAGGCA
SNP-H1.8-F	GCATGTACTTTGATTAAATTCACGG
SNP-H1.8-R	CAAGCTAACCCAAAGTAAATTAAAGCAA

^a Single-nucleotide polymorphism (SNP) markers, SNP-H2.4, SNP-H1.5, and SNP-H1.6 correspond to the SNP markers 575, 61786, and 17918 from Kim et al. (2017).

TABLE 4. Sequence analysis of the single-nucleotide polymorphism (SNP) markers, SNP-H1.7 and SNP-H2.3

Plant	SNP-H1.7 ^a					SNP-H2.3
	1	2	3	4	5	
IPH10023 (Hetero)	G/T	C/T	T/C	C/T	G/A	T
B2110-1A (Homo)	G	C	T	C	G	T
CM334	G	C	T	C	G	T
9093	G	C	T	C	G	T

^a SNP positions within the high-resolution melting marker are indicated from 1 to 5.

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