Screening and Field Trials of Virus Resistant Sources in *Capsicum* spp.

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ABSTRACT

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Thirty-seven *Capsicum* accessions containing cultivated and wild species were screened for resistance to *Cucumber mosaic virus* (CMV), and were also investigated for their response to *Tomato aspermy virus* (TAV), *Tomato mosaic virus* (ToMV), *Pepper mild mottle virus* (PMMoV), and *Tomato spotted wilt virus* (TSWV). *C. baccatum* PI 439381-1-3 (PI 439381-1-3), *C. frutescens* LS 1839-2-4 (LS 1839-2-4), and *C. frutescens* cv. Tabasco (cv. Tabasco) showed a hypersensitive reaction against CMV-Y, and thus were not systemically infected. Only inoculated leaves of *C. annuum* cv. Sapporo-oonaga and cv. Nanbu-oonaga were infected with CMV-Y, and viral infection did not spread systemically. These five accessions (PI 439381-1-3, LS 1839-2-4, cv. Tabasco, cv. Sapporo-oonaga, and cv. Nanbu-oonaga) were considered resistant to CMV-Y. These accessions were also resistant to other CMV isolates, but not to the TAV isolate. PI 439381-1-3, LS1839-2-4, cv. Sapporo-oonaga, and cv. Nanbu-oonaga were susceptible to PMMoV, while PI 439381-1-3 and LS1839-2-4 showed systemic necrosis. All CMV-resistant accessions were susceptible to TSWV. Field tests of eight *Capsicum* accessions, including CMV, PMMoV, and/or TSWV-resistant accessions, demonstrated that most of the PI 439381-1-3 plants were not infected with CMV and PMMoV among the virus-infested fields. As occurred with mechanical inoculation, LS 1839-2-4, cv. Tabasco, cv. Sapporo-oonaga, and cv. Nanbu-oonaga were hard to infect with CMV in the field.

Sweet and hot peppers (*Capsicum* spp.) are both important vegetable crops that are cultured worldwide. Commercial pepper production sustains losses from infection by many bacterial, fungal, and viral pathogens. Viral diseases are particularly difficult to control, and therefore, it has become necessary to develop a cultivar with multiple-virus resistance (20,21,28). Several virus-resistance genes in *Capsicum* spp. have been reported, and attempts have been made to transfer these genes to pepper cultivars by classical breeding (5,8,20,30). The *L*, *y^a*, *pvr1*, *pvr3*, *pvr4*, *pvr7*, *et^a*, and *Tsw* genes, which have been identified as resistance genes against viruses in the genera *Tobamovirus*, *Potyvirus*, and *Tospovirus*, have been characterized (1,5,8,9,16,20,30).

The allelic series of the *L* gene, designated *L*¹, *L*², *L*³, and *L*⁴, are dominant resistance genes protective against tobamovirus (8,9). These *L* genes were initially identified in *C. annuum* cv. Verbeterde Glas (*L*¹/*L*¹), *C. frutescens* cv. Tabasco (*L*²/*L*²), *C. chinense* PI 152225 and PI 159236 (*L*³/*L*³), and *C. chacoense* PI 260429

(*L*⁴/*L*⁴) (8,9). Tobamoviruses that are able to overcome *L*¹, *L*², and *L*³ resistance have also been isolated (24,44). These tobamoviruses have been classified into four pathotypes, designated *P*₀, *P*₁, *P*_{1,2}, and *P*_{1,2,3}, according to their pathogenicity to the plants carrying serial *L* resistance genes. The *P*₀ pathotype, such as *Tomato mosaic virus* (ToMV), elicits a hypersensitive reaction (HR) among all serial *L* genes. The *P*₁ pathotype, *Paprika mild mottle virus* (PaMMV), induces an HR on plants expressing the *L*², *L*³, and *L*⁴ genes, and overcomes *L*¹-mediated resistance. The *P*_{1,2} pathotype, such as *Pepper mild mottle virus* (PMMoV)-Spain strain, induces an HR in *L*³ and *L*⁴ plants and systemically infects *L*¹ and *L*² plants. The *P*_{1,2,3} pathotype, such as PMMoV-Italian strain, can overcome the *L*¹, *L*², and *L*³ genes, but induces an HR in *L*⁴ plants (3,8,9,15). These *L* gene-mediated HR responses are elicited by the viral coat protein (3,15). The *L*¹, *L*², and *L*³ genes have been transferred into commercial pepper cultivars by classical hybridization (3,8,9,15).

The *y^a*, *pvr1*, *pvr3*, and *et^a* are recessive resistance genes against potyvirus infection (1,16,30). Among them, *y^a* restricts the cell-to-cell movement of *Potato virus Y*, and *pvr3* restricts phloem-dependent movement of *Pepper mottle virus* (PepMoV), respectively (1,30). The resistance conferred by the *pvr1* and *et^a* genes to *Tobacco etch virus* and/or PepMoV was analyzed precisely at the whole plant and the

cellular level (16,30). Recently, identification and comparative mapping of two dominant potyvirus resistance genes, *pvr4* and *pvr7*, were reported (20).

The *Tsw* gene is a dominant gene identified in *C. chinense* PI 152225, PI 159236, 7204, and Panca. *C. chinense* lines expressing the *Tsw* gene restrict *Tomato spotted wilt virus* (TSWV) infection by inducing necrotic local lesions (4,7,29). The HR responses of these plants have been demonstrated against TSWV, but not against *Tomato chlorotic spot virus* and *Groundnut ringspot virus*, which are serologically distinct from TSWV (6).

In Japan, pepper cultivars are infected by viruses of the genera *Tobamovirus*, *Cucumovirus*, *Tospovirus*, and *Potyvirus* (34). In particular, PMMoV, *Cucumber mosaic virus* (CMV), and TSWV are the most important viruses; they cause severe damage and economic loss to pepper production (28). The resistance gene against TSWV, *Tsw*, has not been incorporated into pepper cultivars. For CMV control, several *Capsicum* accessions have been reported as expressing resistance to CMV (13,21,28, 32,33,38,39,43). The genetic background of CMV resistance is complicated, and a CMV-resistant cultivar has not yet been bred (21,28). Recently, CMV resistance QTL from *C. annuum* 'Perennial' has been described and mapped (2). Therefore, it remains necessary to breed a pepper cultivar with multiple-virus resistance.

In this paper, we screened 37 *Capsicum* spp. accessions for resistance to CMV, and five of them were also screened for ToMV, PMMoV, *Tomato aspermy virus* (TAV), and TSWV resistance. During the course of the present study, we successfully identified several resistant accessions. Furthermore, we assessed the viral resistance of selected accessions in field trials.

MATERIALS AND METHODS

Plant sources. Nine accessions (Table 1) of *Capsicum* spp., including three *C. annuum* (CB 16-111-3-1, cv. Verbeterde Glas, and cv. Shoosuke), one *C. baccatum* (PI 439381-1-3), one *C. chacoense* (PI 260429), two *C. chinense* (PI 152225 and PI 159236), and two *C. frutescens* (cv. Tabasco and LS 1839-2-4), and 28 Japanese pepper cultivars (Wonder-Bell, Eikou, Kyoooyutaka, Kyoonami, Kyoomidori, Newace, Ace, Green-Boy, Houryoku, Sakigake, Housui, Shouro, Shuusui, Oogata-Ise, Shimousa 2, Tosa-Hikari D, New Tosa-Hikari, New-Face, Mihata 1,

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Shishitou, Suishin, Suikou, Housan-Shishitou, Fushimi-amanaga, Yatsufusa, Nikkou-tougarashi, Sapporo-oonaga, and Nanbu-oonaga) were used in this study. These plants were grown in a greenhouse at 20 to 25°C using plastic pots, 10 cm diameter, containing potting soil.

Viruses and methods of inoculation.

An Iwate isolate of PMMoV (L^2 gene resistance-breaking type, pathotype $P_{1,2}$) was propagated in *Nicotiana tabacum* cv. Samsun or *N. benthamiana* and purified as described (19). The subgroup I isolates of CMV (Y, UD8, UN3, US49, and E5) were maintained in tobacco plants (*N. tabacum* cv. Samsun), and purification was previously described (17). Purified virus preparations (25 to 50 µg/ml) of PMMoV and the CMV subgroup I isolates were used as an inoculum. ToMV (pathotype P_0), CMV-EU (subgroup II), and TAV were maintained in Samsun tobacco. The prairie gentian isolate of TSWV (26) was maintained in *N. rustica*. Inoculum of TSWV, ToMV, CMV-EU, and TAV were prepared by grinding 1 g of infected leaves in 10 ml of 0.1 M phosphate buffer (pH 7.0). All test plants were inoculated on the three intermediate leaves in the stage of six to eight true leaves. Test plants were dusted with 600-mesh Carborundum and inoculated by rubbing the leaves with inoculum. After inoculation, plants were kept in a growth chamber maintained at 25°C during the day and 20°C at night with a 14 h photoperiod. Symptoms were observed until 2 to 3 weeks postinoculation. For each genotype, at least 5 to 10 plants were inoculated with each virus. Viral infection and replication were confirmed by enzyme-linked immunosorbent assay (ELISA) (12,25).

Assay of viral antigen. Detection of the viral antigens was performed by double-antibody sandwich ELISA (DAS-ELISA) or by indirect ELISA (I-ELISA) by a

slightly modified method described (12,25). DAS-ELISA for TSWV and ToMV detection was performed using the DAS-ELISA kit supplied by the Japan Plant Protection Association (Tokyo, Japan). Polystyrene microtiter plates were coated with an IgG (1.6 µg/ml) in CBN (0.05 M sodium carbonate buffer, pH 9.6, containing 0.02% NaN_3) and then were washed with PBSNT (0.02 M phosphate-buffered saline, pH 7.4, containing 0.05% Tween 20 and 0.02% NaN_3). The 200 µl of sample extracts, prepared by grinding 0.1 g of leaf tissue in 1 ml of CBN, were added to the wells and incubated for 2 h at 37°C or overnight at 4°C. After incubation, the plates were washed with PBSNT, and 200 µl of alkaline phosphatase-conjugated IgG at a dilution of 1:500 was placed in each well. Following incubation for 3 h at 37°C, the plates were washed with PBSNT, and then 200 µl of alkaline phosphatase substrate (1 mg/ml) was added to the wells. The enzyme reaction was allowed to develop at room temperature for 30 min, and absorbance values were measured at 405 nm by a microplate reader model 3550-UV (Bio-Rad Laboratories, Inc., Hercules, CA).

I-ELISA was used for detection of CMV, TAV, and PMMoV. Plant extracts were prepared by homogenizing 0.1 g of leaf tissue in 1 ml of CBN, and 200 µl of extracts were added to each well of the microtiter plate. Three hours after incubation at 37°C, the wells were washed with PBSNT and then were reacted with first antibody (0.5 µg/ml anti-virus antibody in PBSNT) for 2 h at 37°C. The plates were washed with PBSNT, and then 200 µl of alkaline phosphatase-conjugated second antibody (Bio-Rad) at a dilution of 1:2,000 was added to the wells. Enzyme reactions and absorbance measurements were as described above. All samples were assayed

in duplicate wells. Purified samples of known concentration were added to plates to allow a standardized reaction from one test to another. Tests were considered positive when the absorbance value of each sample was at least two times greater than that of the healthy control plant.

Reverse transcription polymerase chain reaction (RT-PCR).

RT-PCR and subsequent nested PCR were carried out for PMMoV detection. All PCR primers were designed on the basis of the nucleotide sequence data of PMMoV-J (GenBank accession no. AB000709). Total RNA was extracted from either soil or leaf tissue using a FastRNA Kit-GREEN (BIO101, Inc., Vista, CA), and then RT was performed with a random hexamer and a M-MuLV reverse transcriptase (Amersham Biosciences Co., Piscataway, NJ). The cDNA was then amplified by PCR using *Ex Taq* DNA polymerase (Takara Shuzo Co., Otsu, Japan) and forward primer PMSPF1 (5'-AGAAGCTCGGAGTCATCG-GAC-3') and reverse primer PMSPR1 (5'-GAGTTATCGTACTCGCCACG-3'), which amplified a 576-bp cDNA corresponding to the 5642 to 6217 nucleotide sequence region of PMMoV-J. PCR cycling was performed for 5 min at 94°C followed by 30 cycles of 30 s at 94°C, 20 s at 55°C, and 30 s at 72°C, and a final extension at 72°C for 5 min.

The nested PCR was performed following the first PCR amplification. The first PCR product was added to the nested PCR mixture with primers PMCPF (5'-CCG-AGCTCATGGCTTACACAGTTTCCAG-3') and PMCPR (5'-CCGAGCTCC-TAAGAGTTGTAGCCCAGG-3'), both of which contain an additional *SacI* recognition sequence at the 5' proximal site. The nested PCR amplified a 490-bp fragment corresponding to the coding region of the coat protein. Thermal cycling conditions were 94°C for 5 min, 30 cycles of 94°C for 30 s, 50°C for 30 s, and 72°C for 30 s, followed by 72°C for 5 min. After the reaction, PCR products were separated by electrophoresis in a 0.8% agarose gel and visualized by ethidium bromide staining. The nested PCR fragment was cloned into *SacI* site of pUC18 and sequenced with an automated DNA sequencer (Model 377; Applied Biosystems, Inc., CA).

Field trials. *Capsicum* spp. accessions were tested from 26 May to 31 October 1999 in the test farm at Iwate Agricultural Research Center in Kitakami City, and from 26 May to 30 September 2000 in a plastic greenhouse located in the town of Ishidoriya in Iwate Prefecture. Accessions were planted together within two replicates of the experimental block, which was composed of 10 or 15 plants. The experimental block of the same accession was arranged separately. In both trials, plants were grown according to the general cultivation procedure. All plants were checked for viral infection by DAS-ELISA or I-

Table 1. Response of *Capsicum* spp. accessions to inoculation with plant viruses

Accession	Inoculated virus ^x			
	CMV-Y	ToMV	PMMoV	TSWV
<i>Capsicum annuum</i>				
CB 16-111-3-1	C/M ^y	-/M	-/M	C/M
cv. Nanbu-oonaga	C/-	-/M	-/M	C/M
cv. Sapporo-oonaga	C/-	NS, LA/-	-/M	C/M
cv. Shoosuke (L^+) ^z	C/M	-/M	-/M	C/M
cv. Verbeterde Glas (L^1)	C/M	NS, LA/-	-/M	C/M
<i>C. baccatum</i>				
PI 439381-1-3	NS, LA/-	NS, LA/-	NS, LA/NS	C/M, N
<i>C. chacoense</i>				
PI 260429 (L^4)	C/M	NS, LA/-	NS, LA/-	C/M
<i>C. chinense</i>				
PI 152225 (L^3 , Tsw)	C/M	NS, LA/-	NS/-	NS, LA/-
PI 159236 (L^3 , Tsw)	C/M	NS, LA/-	NS/-	NS, LA/-
<i>C. frutescens</i>				
cv. Tabasco (L^2)	NS, LA/-	NS, LA/-	-/M	C/M, N
LS 1839-2-4	NS, LA/-	NS, LA/-	N, LA/M, N	C/M, N

^x CMV-Y = *Cucumber mosaic virus* strain Y, ToMV = *Tomato mosaic virus*, PMMoV = *Pepper mild mottle virus*, TSWV = *Tomato spotted wilt virus*.

^y Symptoms are inscribed as inoculated leaves/upper leaves. C = chlorosis, LA = leaf abscission, M = mosaic, N = necrosis, NS = necrotic spot, - = symptomless.

^z Abbreviations in parentheses indicate resistance genes carried as previously reported.

ELISA at 2-week intervals. Data analysis was carried out according to Duncan's new multiple range test.

RESULTS AND DISCUSSION

Response of *Capsicum* accessions to CMV-Y. We used *C. annuum* cv. Shoosuke as susceptible control. After being inoculated with CMV-Y, cv. Shoosuke formed chlorotic spots on inoculated leaves 3 to 4 days postinoculation. At 7 to 10 days after inoculation, severe mosaic symptoms were observed on upper uninoculated leaves. All *Capsicum* spp. lines were infected with CMV-Y in the inoculated leaves; however, some infected plants did not show any symptoms on the upper uninoculated leaves (Table 1). *C. baccatum* PI 439381-1-3 formed necrotic spots on inoculated leaves 2 to 3 days after inoculation, and in those plants, abscission of the inoculated leaves occurred and the virus did not spread systemically to the upper leaves. *C. frutescens* LS 1839-2-4 and cv. Tabasco also showed a similar response, which differed from that of *C. baccatum* only in terms of lesion phenotype. In LS 1839-2-4 and Tabasco, chlorotic spots formed on inoculated leaves 3 to 4 days postinoculation and then developed into necrotic spots 1 to 2 days later. The necrotic spots of *C. baccatum* were 1 to 2 mm in diameter, whereas those of *C. frutescens* were somewhat larger and irregular in shape. The number of necrotic spots increased proportionally with the concentration of virus inoculum (data not shown). In these cases, the viral antigen was detected in the local lesions, but not in stems and upper leaves. This suggests that the response of these accessions was an HR.

C. annuum CB 16-111-3-1 showed susceptibility, although the symptoms on the upper, uninoculated leaves were extremely mild. I-ELISA was used to estimate viral accumulation on upper leaves, and similar absorbance values were obtained for *C. annuum* CB 16-111-3-1 to those obtained in the susceptible cultivar Shoosuke. These results suggest that a type of tolerant response occurred in *C. annuum* CB 16-111-3-1.

C. annuum cv. Sapporo-oonaga and cv. Nanbu-oonaga were infected with CMV-Y, and the inoculated leaves showed symp-

toms similar to those of other susceptible plants; however, there were no systemic symptoms. The absorbance value of the inoculated leaves demonstrated that CMV-Y had accumulated in these cultivars to a similar degree as in cv. Shoosuke, a cultivar susceptible to all virus species tested. ELISA of the upper leaves detected no accumulation of virus, suggesting that the plants allowed CMV-Y to replicate and move cell-to-cell in inoculated leaves but not in uninoculated leaves. Other *Capsicum* accessions and 26 of the Japanese pepper cultivars tested were infected with CMV-Y systemically and showed susceptible reactions.

Response of *Capsicum* accessions to other cucumoviruses. Similar responses were observed when inoculations consisted of other CMV isolates and a TAV isolate (Table 2). *C. annuum* cv. Sapporo-oonaga and cv. Nanbu-oonaga were infected with all isolates and showed chlorosis on inoculated leaves; these symptoms were similar to those observed in the case of cv. Shoosuke. The isolates UN3, E5, and EU infected inoculated leaves, but systemic spread was suppressed, showing that the resistance response was exhibited not only against the CMV subgroup I isolates, but also against the subgroup II isolates. CMV-UD8 and CMV-US49 induced a severe mosaic symptom on upper leaves, indicating that resistance was not effective against all isolates of CMV. All tested CMV isolates elicited necrotic spots on inoculated leaves of *C. frutescens* LS 1839-2-4 and *C. baccatum* PI 439381-1-3. In particular, isolates UD8, UN3, and US49 also induced necrosis on stems and upper uninoculated leaves. ELISA demonstrated viral antigen from the necrotic lesions, not only on inoculated leaves, but also on upper leaves.

TAV systemically infected *C. annuum* cv. Sapporo-oonaga, cv. Nanbu-oonaga, and *C. baccatum* PI 439381-1-3. The reactions on the inoculated leaves of *C. baccatum* PI 439381-1-3 against TAV were also similar to the reactions against CMV isolates.

Two genes conferring resistance against CMV were identified in *Vigna unguiculata* and *Arabidopsis thaliana* ecotype C24, and these genes are referred to as *CRY* and *RCY1*, respectively (31,42). Several CMV-

resistant *Capsicum* accessions have been screened (13,21,28,32,33,38,39,43), although to date, a CMV-resistant cultivar has not been bred. Recently, CMV resistance QTL from *C. annuum* 'Perennial' have been described and mapped (2). The existence of potential CMV resistance genes was evaluated in several *Capsicum* lines in the present study. *C. frutescens* LS 1839-2-4, cv. Tabasco, and *C. baccatum* PI 439381-1-3 showed an HR-like response to CMV infection, whereas *C. annuum* cv. Sapporo-oonaga and cv. Nanbu-oonaga resisted systemic infection of CMV. These five accessions were resistant to some CMV isolates and thus could be useful as sources for breeding CMV-resistant cultivars. However, we also identified CMV isolates that could overcome the resistance stated above.

Reaction of *Capsicum* accessions to tobamoviruses. Five *Capsicum* accessions carrying *L* genes, six CMV-resistant accessions screened in this study (one of which is CMV-tolerant), and one virus-susceptible control cultivar were subjected to subsequent inoculation study (Table 1). After being inoculated with ToMV (pathotype P₀) and PMMoV (pathotype P_{1,2}), a susceptible control cv. Shoosuke formed a mosaic symptom on upper uninoculated leaves. Virus accumulation in the inoculated leaves was detected by ELISA, whereas no symptom was observed on the inoculated leaves. An HR was elicited by ToMV and PMMoV infection in the *Capsicum* accessions in which serial *L* genes had initially been identified (3,8,9,15). Necrotic local lesions were formed 2 to 3 days postinoculation on inoculated leaves, and there were no systemic symptoms in the incompatible combinations.

C. baccatum PI 439381-1-3, *C. frutescens* LS 1839-2-4, and *C. annuum* cv. Sapporo-oonaga plants showed necrotic spots on inoculated leaves 2 to 3 days after inoculation with ToMV and were resistant to systemic infection. But these accessions became systemically infected with PMMoV, indicating that these accessions contained the *L*¹ or *L*² resistance gene. Responses against the *L*¹ resistance-breaking tobamovirus, PaMMV (18), will be necessary to determine whether the *L*¹

Table 2. Response of *Capsicum* spp. accessions to inoculation with several *Cucumber mosaic virus* (CMV) isolates and *Tomato aspermy virus* (TAV) isolate

Accession	CMV subgroup I				CMV subgroup II	TAV
	UD8	UN3	E5	US49	EU	
<i>Capsicum annuum</i>						
cv. Nanbu-oonaga	C/M ^z	C/–	C/–	C/M	C/–	C/M
cv. Sapporo-oonaga	C/M	C/–	C/–	C/M	C/–	C/M
cv. Shoosuke	C/M	C/M	C/M	C/M	C/M	C/M
<i>C. baccatum</i>						
PI 439381-1-3	NS, LA/N, LA	NS, LA/N, LA	NS, LA/–	NS, LA/N, LA	NS, LA/–	NS, LA/N, LA
<i>C. frutescens</i>						
LS 1839-2-4	NS, LA/N, LA	NS, LA/N, LA	NS, LA/–	NS, LA/N, LA	NT	NT

^z Symptoms are inscribed as inoculated leaves/upper leaves. C = chlorosis, LA = leaf abscission, M = mosaic, N = necrosis, NS = necrotic spot, – = symptomless, NT = not tested.

or L^2 gene is present in these plants. *C. annuum* CB16-111-3-1 and cv. Nanbu-oonaga were susceptible to ToMV and PMMoV. *C. baccatum* PI 439381-1-3 and *C. frutescens* LS 1839-2-4 were systemically infected with PMMoV. Plants developed necrosis or necrotic spots on inoculated leaves, stems, and upper uninoculated leaves. These responses, systemic necrosis, were thought to be of an HR type, but differed from those caused by L genes, because necrotic spot formation on inoculated leaves in *C. baccatum* PI 439381-1-3 and *C. frutescens* LS 1839-2-4 was generally slower than that mediated by L genes. We consider that systemic necrosis of these two accessions might be the result of a special type of HR that is expressed too slowly to localize the virus in the inoculated leaves.

Resistance involving HR against tobamoviruses has been observed in two *Nicotiana* species (N and N' genes) and in several *Capsicum* species (L genes) (8,9,14,23,35,37). The elicitor of N gene-mediated resistance was investigated through comparative study of overcoming strain TMV-Ob (23) and a reversional mutant in which the replicase gene has been identified (35). The HR mediated by N' , L^2 , and L^3 gene was elicited by the coat protein of tobamoviruses (3,14,15,37). In this study, existence of the gene(s) that elicits necrosis against PMMoV infection was evaluated in *C. baccatum* PI 439381-1-3 and *C. frutescens* LS 1839-2-4. Genetic analysis will be necessary to identify a gene that could account for the difference between the response elicited by that observed in this study with the L genes.

Reaction of *Capsicum* accessions to TSWV. *C. chinense* PI 152225 and PI 159236 are resistant to TSWV infection, and a Tsw resistance gene has been identified in those genotypes (5). These plants

showed necrotic spots on inoculated leaves 3 to 4 days after inoculation and were resistant to systemic infection with TSWV. The virus was not detected in the upper leaves. *C. baccatum* PI 439381-1-3, *C. frutescens* LS 1839-2-4 and cv. Tabasco, and *C. annuum* cv. Sapporo-oonaga and Nanbu-oonaga, which were resistant to CMV-Y in the present study, were susceptible to TSWV. Other *Capsicum* accessions tested were systemically infected with TSWV (Table 1).

In *Solanaceae* plants, TSWV resistance in tomato (*Lycopersicon esculentum*) and other *Lycopersicon* species has been well investigated (10,11,36,40,41). In particular, $Sw-5$ gene confers high resistance to a broad range of TSWV isolates. The $Sw-5$ gene is a single dominant gene that has recently been genetically mapped, cloned, and analyzed (10,11,40,41), and has been used for breeding (36). On the other hand, resistance-breaking isolates of TSWV have been recovered and have been used for the mapping analysis of $Sw-5$ resistance-breaking determinants (22,27). This analysis indicated that the M RNA segment of TSWV was associated with the $Sw-5$ mediated resistance. In *Capsicum*, only the Tsw gene has been identified; however, the gene has not yet been used in pepper breeding, and no TSWV-resistant cultivar has been bred.

Field testing of *Capsicum* accessions screened in terms of viral resistance. Eight *Capsicum* accessions, including the CMV-resistant accessions screened in this study, were subjected to field trials to evaluate resistance to viruses under natural conditions. Before the field trials in both years, PMMoV detection was undertaken in the soil using RT-PCR, demonstrating that no specific DNA fragment was amplified from soil samples in 1999; however, the field soil used in 2000 carried the L^2

gene breaking - PMMoV population (data not shown).

In the 1999 field trial, CMV and TSWV were detected by ELISA (Table 3), but no PMMoV infection was observed (data not shown). CMV symptoms were observed and increased on susceptible accessions (cv. Shoosuke, *C. chinense* PI 152225, and PI 159236) at later cultivation stages. On the other hand, few or no plants of the accessions considered CMV-resistant (*C. annuum* cv. Sapporo-oonaga and cv. Nanbu-oonaga, *C. baccatum* PI 439381-1-3, and *C. frutescens* LS 1839-2-4 and cv. Tabasco) were infected with CMV (Table 3). Severe, systemic symptoms were observed on susceptible plants, whereas those on resistant plants were observed only on a portion of the twigs. Furthermore, necrotic symptoms and leaf abscission were also observed in *C. frutescens* LS 1839-2-4 and cv. Tabasco plants. TSWV infection occurred immediately after planting and gradually spread from plant to plant. However, *C. chinense* PI 152225 and PI 159236 were not infected with TSWV during the cultivation period.

A similar trial was done in a plastic greenhouse during the next year. The field had been planted with sweet pepper for the past several years, and PMMoV was detected in the soil samples. CMV and TSWV were detected in only a small number of susceptible plants, because the insect vectors (aphids and thrips) might be well-controlled. One month after planting, initial symptoms of PMMoV were observed on *C. annuum* cv. Shoosuke, and the number of infected plants gradually increased. The disease caused by PMMoV was also observed on *C. frutescens* (LS 1839-2-4 and cv. Tabasco), which showed necrosis and mosaic symptoms at later cultivation stages (Table 3). Sequencing analyses of the coat protein gene demonstrated that the PMMoV in infected plants had identical sequences to those of the RT-PCR fragment amplified from the soil samples. Therefore, it was concluded that PMMoV in the soil had infected the plants in this experiment. No *C. chinense* plant carrying both the L^3 and Tsw genes was infected with any of the PMMoV or TSWV. Interestingly, no *C. baccatum* plant was infected with PMMoV; however, *C. baccatum* was susceptible to PMMoV and showed systemic necrosis after artificial inoculation (Tables 1 and 3). We suggest that *C. baccatum* PI 439381-1-3 has partial resistance, which might protect a plant from the PMMoV infection in the soil under field conditions, but not in artificial inoculation to the leaves. However, it is not known at present why another accession, *C. frutescens* LS 1839-2-4, which showed systemic necrosis after artificial inoculation to PMMoV, resulted in systemic infection in the field trial.

In this study, we screened the sources of viral resistance in *Capsicum* spp. acces-

Table 3. Occurrence of Cucumber mosaic virus (CMV), Pepper mild mottle virus (PMMoV), and Tomato spotted wilt virus (TSWV) in field tested *Capsicum* lines^w

Accession	Field		Plastic greenhouse		
	CMV	TSWV	CMV	PMMoV	TSWV
<i>Capsicum annuum</i>					
cv. Sapporo-oonaga	2/30 ^x a ^y	4/30 ab	NT ^z	NT	NT
cv. Nanbu-oonaga	2/30 a	4/30 ab	NT	NT	NT
cv. Shoosuke	14/30 b	8/30 b	1/20 a	11/20 a	2/20 a
<i>C. baccatum</i>					
PI 439381-1-3	0/30 a	1/30 a	0/20 a	0/20 b	0/20 a
<i>C. chinense</i>					
PI 152225	4/30 a	0/30 a	NT	NT	NT a
PI 159236	8/30 ab	0/30 a	0/20 a	0/20 b	0/20 a
<i>C. frutescens</i>					
LS 1839-2-4	2/30 a	2/30 ab	0/20 a	3/20 ab	2/20 a
cv. Tabasco	5/30 a	2/30 ab	0/20 a	4/20 ab	3/20 a

^wThe tests were run from 26 May to 31 October 1999 (field test), or 26 May to 30 September 2000 (greenhouse test).

^x Infected plants/total plants. Test plant, whether infected or not, was judged by enzyme-linked immunosorbent assay (ELISA) value compared with that of healthy plant. The ELISA were performed on 24 September 1999 (field test) or 3 August 2000 (greenhouse test).

^y Number of infected plants followed by different letters within each column are significantly different ($P < 0.05$) according to Duncan's new multiple range test.

^z NT = not tested.

sions and subjected these accessions to field trials. In general, *C. baccatum* PI 439381-1-3, *C. frutescens* LS 1839-2-4, cv. Tabasco, *C. annuum* cv. Sapporo-onnaga, and cv. Nanbu-onnaga failed to show CMV symptoms, and they were negative in ELISA, thus confirming that these accessions are resistant to CMV infection in nature. In addition, *C. baccatum* PI 439381-1-3 was not infected with PMMoV. Further investigation is needed to determine the mechanism and the inheritance of CMV and/or PMMoV resistance in these accessions. The efficiency of *L*³ and *Tsw* gene-mediated viral resistance under field conditions was also verified in the present study. Inheritance analysis and introduction to pepper cultivars of the putative CMV and other virus-resistance genes should provide more effective control of viral diseases in pepper production.

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