

## Isolation and Characterization of *Pepper mottle virus* Infecting Tomato in Korea

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A peculiar virus-like disease of tomato showing yellow mosaic and necrotic spots on leaves and necrosis on veins, petioles and stems was observed at the Tomato Experimental Station (TES), Buyeo, Chungcheongnam-do, Korea. The disease incidence at TES fields ranged from 21 to 35% infecting different tomato cultivars. For this reason, to identify the virus infecting tomato and to characterize the virus based on biology, serology, cytology and at molecular level. Here, leaf samples were randomly collected from different infected tomato cultivars at TES fields and greenhouses and tested by ELISA using *Pepper mottle virus* (PepMoV) and *Tomato mosaic virus* (ToMV) antisera. Infected saps were mechanically inoculated in different host plants to test for pathogenicity, symptomatology and host ranges. Infected tissues and ultrathin sections were examined by electron microscopy. Finally, putative coat protein and 3'-untranslated region (CP/3'-UTR) fragment was amplified and cloned for sequence determination and analyzed its genetic relationship to existing PepMoV and PVY sequences at the Genbank. Results showed 69% of the samples were positive with PepMoV, 13% with ToMV and 19% were doubly infected with PepMoV and ToMV. Symptoms greatly varied from different host plants inoculated with tomato leaf sap-infected with PepMoV alone and discussed in detailed in this paper. Electron microscopy from infected tissues showed filamentous particles of 720-750 nm in length, a typical morphology and size of PepMoV. In addition, cylindrical inclusion bodies, pinwheels, scrolls and laminates with masses of fibrillar inclusions were also found in ultrathin sections. Alignment of the sequences of the CP/3'-UTR revealed >96% sequence identity with PepMoV and only <61% with PVY. Taken together, all these evidences presented clearly indicated

that the causal agent infecting tomato at TES was PepMoV and we designated this PepMoV infecting tomato as Tom-sd2 strain in this study.

**Keywords :** inclusion bodies, occurrence, PepMoV, tomato

*Pepper mottle virus* (PepMoV) is a member of the genus *Potyvirus*, the largest genus of plant viruses. PepMoV is characterized by flexuous, filamentous particles of 737 nm in length with a single stranded (ss) RNA genome of approximately 10 kb (Brunt et al., 1996; Nelson et al., 1982; Shukla et al., 1994). PepMoV was first reported in *Capsicum annuum* from Florida, U.S.A. by Zitter (1972). Zitter and Cook (1973) designated the Florida virus as *Pepper mottle virus*, a tentative member of the PVY group. Nelson and Wheeler (1972) reported the occurrence of a similar disease in chili pepper crops in Arizona, which they referred to as Arizona pepper virus. Sequence comparison was the only method by which these viruses could be correctly classified as distinct virus species (Vance et al., 1992; Oruetebarria et al., 2000).

Three isolates of PepMoV have been described in sufficient detail: Arizona (PepMoV-AZ), California (PepMoV-CA) and Florida (PepMoV-FL). Each PepMoV isolate has been partially characterized and compared with the other PepMoV isolates. The Arizona and California isolates were indistinguishable from each other and shown to be closely related to the Florida isolate. However, the Florida isolate was distinguishable from the Arizona and California isolates by host range and symptomatology (Nelson et al., 1972; Nelson et al., 1978; Purcifull et al., 1975; Vance et al., 1992; Zitter T.A., 1972).

Many potyviruses cause economically important yield losses in potato (*Solanum tuberosum* L.), pepper (*Capsicum* spp.) and tomato (*Lycopersicon esculentum* Mill.) crops throughout the world (Benner et al., 1985; Brunt et al.,

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1996; Boonham et al., 1998). Furthermore, the viruses were to some extent serologically related to *Potato virus Y* (PVY) and were initially described as deviant PVY strains but later reclassified as different species (Purcifull et al., 1975). In addition to the biological and serological data, nucleotide sequence identities of coat protein (CP) or 3'-UTR have been increasingly used in recent years to differentiate distinct species from strains (Frankel et al., 1989; Shukla and Ward, 1989). Van der Vlugt et al. (1993) subgrouped the PVY isolates based on the CP and 3'-UTR sequence identities that matched groupings based on biological and/or serological criteria. These PVY strain groups are largely consistent with the phylogenetic groups established on the basis of CP-encoding and 3'-NTR sequences (Boonham et al., 1999; Van der Vlugt et al., 1993), but there is no experimental data to indicate that either CP or 3'-NTR sequences are directly responsible for the symptomatic phenotypes on which the strain group concept is based. Indeed, molecular characterization is an efficient approach by which PVY isolates from different crops may be compared, because many PVY isolates from pepper and tomato do not infect potato cultivars systemically (Gébré-Sélassié et al., 1985).

In Korea, the distribution of PepMoV is correlated with the widespread occurrence of pepper (*C. annuum* L. var. *grossum*). Virus diseases of pepper had an average disease incidence of about 2%, but infection rate and kind of viruses in each province was different (Ko et al., 2003). So far, viruses known to infect tomato are: *Cucumber mosaic virus* (CMV), *Tomato mosaic virus* (ToMV), *Tobacco mosaic virus* (TMV) and PVY and these viruses observed various symptoms described previously (Choi et al., 1997; Choi et al., 2001). In this paper we report the identification and isolation of PepMoV infecting tomato in Korea and its characterization based on biological, serological and 3'-UTR sequence properties.

## Materials and Methods

**Disease monitoring and sample collection.** The disease incidence of unidentified virus causing peculiar symptoms in tomato plant was monitored in the entire fields and greenhouses at Tomato Experiment Station (TES), Buyeo, Korea in 1997. Investigation was conducted by walking through the entire fields and greenhouses and leaves with symptoms showing with unidentified virus were collected. Disease incidence was calculated by the number of plants showing symptoms relative to the total number of plants collected in an area. Percent infection of the disease incidence was obtained from 8 tomato cultivars (Fig. 1).

**Virus isolation, source and propagation.** From the collected

samples, 1-3 plants per variety with distinct symptoms were selected and tested by ELISA using *Pepper mottle virus* (PepMoV) and *Tomato mosaic virus* (ToMV) antisera. The highest number of samples found reacting positively to single antiserum was suspected to be causal agent of the virus disease spreading at TES. Sap was extracted from infected tomato tissues using 0.1 M phosphate buffer, pH 7.0 and was mechanically inoculated on carborundum-predusted leaves of *Nicotiana benthamiana*. Inoculated plants were kept in an insect-free greenhouse maintained at 20-25°C with 12 h light period. The re-isolated virus was designated as Tom-sd2 isolate and served as virus source in this study.

**Symptomatology.** The symptoms expressed from the collected samples in the field and greenhouses were described as well as the symptoms of the plants inoculated with Tom-sd2 isolate. Percent symptom occurrence per cultivar was determined in the field and distinct symptoms of each cultivar in the field were judged by the highest percent type(s) of symptom occurrence in each cultivar.

**Pathogenicity and host ranges.** A total of 12 tomato cultivars, 8 *chili pepper cultivars* and 17 indicator host plants were used to investigate the pathogenicity and host ranges of the Tm-sd 2 isolate. Five to 10 plants seedlings of each of the species were mechanically inoculated at the 3 - 5 leaf stage. Disease symptoms were recorded 3 times a week for 30 days. The symptoms of the inoculated lower leaf and the upper leaf systemic infection were recorded and described.

**Serology.** Direct-antibody coated enzyme linked immunosorbent assay (DAC-ELISA) was conducted essentially as described by Clark & Bar-Joseph (1984). PepMoV and ToMV specific monoclonal antibodies (MAb) (Adgen, United Kingdom) were used for virus detection. The MAbs and conjugate were both diluted 1:200 and all incubations were carried out at 37°C for 2 h except for the substrate which was incubated for 30 min. Quantitative measurements of generated *p*-nitrophenol were made by determining absorbance at 405 nm ( $A_{405}$ ) in an EL312e EIA model spectrophotometer (Bio-Tek Instruments Inc., USA). An absorbance value reading higher than the twice of the mean of negative control was considered as positive value.

**Electron microscopy.** Dip preparations were prepared by grinding a small piece of infected *N. benthamiana* in 2-3 drops of 2% phosphotungstic acid, pH 7.0. Ultrathin sections were conducted as described by Choi et al. (1993). For interpretation of results, the sections were viewed under electron microscope LEO 912AB (Carl Zeiss, Germany) at 80 kV.

**RT-PCR, cloning and sequencing.** Total RNA was extracted from infected leaf samples as described by Prescott and Martin (1987). The 3' terminal region comprising part of the coat protein (CP) gene and 3'-untranslated region (UTR) of the tomato isolate was amplified following the RT-PCR procedure described by Pappu et al. (1993). The amplified PCR products were cloned in pGEM-T vector (Promega, USA) and sequenced by a commercial company (Green Gene Biotechnology, Korea). The CP/3'-UTR sequence has been deposited in GenBank under accession number AY748920.

**Phylogenetic analysis.** The CP/3'-UTR sequence of Tomsd2 isolate determined in this study was phylogenetically compared to those of other PepMoV /PVY (GenBank and EMBL) using the multiple sequence alignment application of DNAMAN version 4.0 (Lynnon Biosoft, Quebec, Canada) full optimal sequence alignments and neighbor-joining method options of Saitou and Nei (1987) with 1000 bootstrap (Felstein, 1985) replications. Percent nucleotide (nt) and ORF amino acid sequence identities between virus isolates were calculated using the distance between all pairs of sequences in the multiple alignments. PepMoV/PVY (CP/3'-UTR) sequences used for comparison and their database accession numbers were as follows: AJ223592 (PVY-N854 isolate), AJ223593 (PVY-O768 isolate), AJ390285 (PVY-N-RB isolate), AJ390289 (PVY-v942490 isolate), AJ390295 (PVYN-S-NTN isolate), AJ390309 (PVY-TU619 isolate), D12539 (PVY-O isolate), D12570

(necrotic-PVY-T isolate), M22470 (PVY-N isolate), M95491 (PVY-Hungarian isolate), U09508 (PVY-N27-92 isolate), U09509 (PVY<sup>0</sup>-Canadian isolate), U06789 (PVY-VN Korean isolate), U10378 (PVY-Hungary pepper isolate), X97895 (PVY-N605 isolate), X14136 (PVY-Yo Argentina isolate), Z70237 (PVY-Nysa isolate), AB098560 (PepMoV-JKK isolate), AF227728 (PepMoV-NC165 isolate), AF501591 (PepMoV- Florida isolate), M96425 (PepMoV-California isolate).

## Results

**Field disease incidence and distinct symptoms.** A total

**Table 1.** Percent infection of different tomato cultivars infected with unknown virus by visual inspection at Tomato Experimental Station (TES), Buyeo, Chungcheongnam-do, Korea, 1997

Cultivar	No. of plants		% infection
	examined	infected	
Minicarol	167	56	33.5
Momotaro	164	44	26.8
Popi	94	25	26.6
Koko	91	25	27.5
Paepae	94	32	35.5
Supersun	96	23	24.0
Sunextra	95	20	21.1
Dadagi	89	31	34.8
Total	890	256	28.8 (Ave)

**Table 2.** Percent symptom expression on different cherry tomato cultivars in the field and greenhouses at Tomato Experimental Station, Buyeo, Chungcheongnam-do, Korea

Symptom <sup>a</sup>	% symptom in cultivar of							
	Momotaro	Minicarol	Popi	Sunextra	Supersun	Dadagi	Koko	Paepae
M	0.0	0.0	16.0	15.0	<b>43.5</b>	<b>41.9</b>	32.0	9.1
YM	<b>54.6</b>	0.0	24.0	30.0	21.7	32.3	<b>56.0</b>	0.0
M, NS	0.0	3.6	4.0	5.0	17.4	3.2	0.0	15.2
YM, NS	11.4	23.2	8.0	10.0	0.0	3.2	0.0	18.2
NS, VN	6.8	1.8	0.0	5.0	0.0	12.9	0.0	3.0
M, CS	2.3	<b>39.3</b>	0.0	0.0	0.0	0.0	0.0	<b>30.3</b>
YM, CS	15.9	32.1	0.0	0.0	0.0	0.0	0.0	24.2
YM, CS, VN	2.3	0.0	<b>48.0</b>	0.0	0.0	0.0	0.0	0.0
YM, NS, VN	0.0	0.0	0.0	<b>30.0</b>	17.4	6.5	0.0	0.0
CS	2.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0
NS	0.0	0.0	0.0	5.0	0.0	0.0	0.0	0.0
CS, VN	2.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CS, NS, YM	2.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0
VN	0.0	0.0	0.0	0.0	0.0	0.0	8.0	0.0
VN, BN	0.0	0.0	0.0	0.0	0.0	0.0	4.0	0.0

<sup>a</sup>CS, chlorotic spot; NS, necrotic spot; M, mosaic; YM, yellow mosaic; VN, vein necrosis; BN, bud necrosis. Highlighted figures indicate distinct symptoms in each cultivar.



of 890 tomato plants (consist of 8 tomato cultivars) infected with suspicious virus were collected and symptoms were examined. Percent infection of collected samples in the field and greenhouses ranged from 21 to 35%. Out of 8 examined cultivars 3 such as Paepae, Dagdagi and Minicarol obtained the highest infection of 35.5, 34.8 and 33.5%, respectively. While lowest percent infection was obtained with cultivar Sunextra (Table 1).

The most common symptom observed on the youngest leaves was yellow mosaic (Table 2). However, distinct symptoms by natural infection were relatively varied among cultivars (Table 2). Like for instance, yellow mosaic were distinct in Momotaro and Koko, both showed >50% yellow mosaic symptom occurrence. However, Koko showed additional but unique symptoms such as vein necrosis (8.0%) and bud necrosis (4%). Although, occurred at low percentage but these symptoms were only expressed in Koko cultivar. Mosaic was more in Supersun and Dagdagi (>40%). The common symptoms in Minicarol and Paepae were combination of mosaic and chlorotic spot (>30%). Combinations of symptoms were also distinct in Popi with yellow mosaic, chlorotic spot and vein necrosis (48%) and Sunextra had yellow mosaic, necrotic spot and vein necrosis (30%).

**Virus detection.** A total of 16 leaf samples from different cultivars possessing distinct symptoms in each cultivar were collected and tested by DAC-ELISA using PepMoV and ToMV antiserum. Surprisingly, ELISA test showed that 69% of the samples tested were infected with PepMoV,

13% with ToMV and 19% had a mixed infection of both viruses (Table 3). Most of the symptoms observed include: yellow mosaic, necrotic spot, vein necrosis and a combination of chlorotic/necrotic spot and yellow mosaic. However, in Momotaro cultivar plants showing yellow mosaic symptoms were infected with PepMoV, whereas plants with a combination of chlorotic/necrotic spot and yellow mosaic had a mixed infection of the two viruses. It is interesting to note that although 2 of the Sunextra cultivar samples showing symptoms of yellow mosaic, one was infected with PepMoV while the other was infected with ToMV. In the case of cultivar Koko showing vein and bud necrosis symptoms had a double infection of PepMoV and ToMV (Table 3).

**Pathogenicity and host ranges.** All the 12 *L. esculentum* and 8 *C. annuum* plants tested were all infected indicating that the family *Solanaceae* was susceptible to Tom-sd2 isolate or the tomato-PepMoV isolate (Table 4). The reactions of Tom-sd2 to these plants however varied on the manner of its infection (whether localized or systemic). All *C. annuum* plants tested showed only systemic infection and no differences in symptoms were observed (Table 5). In the case of *L. esculentum*, 5 species showed systemic only and the other 7 species showed both localized and systemic infection. The latter developed more severe symptoms indicating that these were more susceptible than the former

**Table 3.** Symptoms in different cherry tomato cultivars by natural infection and viruses detected by ELISA

No.	Variety	Symptom <sup>a</sup>	Virus detected by ELISA
1	Momotaro	ym	PepMoV
2	Momotaro	ym	PepMoV
3	Momotaro	cs, ns, ym	PepMoV+ToMV
4	Minicarol	ns, ym	PepMoV
5	Popi	m	ToMV
6	Popi	ns, ym	PepMoV
7	Sunextra	ym	ToMV
8	Sunextra	ym	PepMoV
9	Sunextra	ns, ym, vn	PepMoV
10	Supersun	ns, m	PepMoV
11	Supersun	ns, ym, vn	PepMoV
12	Dagdagi	ns, vn	PepMoV
13	Dagdagi	ns, ym, vn	PepMoV
14	Koko	vn	PepMoV+ToMV
15	Koko	vn, bn	PepMoV+ToMV
16	Paepae	ns, ym	PepMoV

<sup>a</sup>cs, chlorotic spot; ns, necrotic spot; m, mosaic; ym, yellow mosaic; vn, vein necrosis; bn, bud necrosis.

**Table 4.** Pathogenicity, host ranges and symptoms on infected indicator plants inoculated with Tom-sd2 isolate

Indicator plants	Symptomatic reactions
<i>Chenopodium quinoa</i>	—/— <sup>a</sup>
<i>C. amaranticolor</i>	—/—
<i>Gomphrena globosa</i>	—/—
<i>Nicotiana glutinosa</i>	nl/vc, m
<i>N. rustica</i>	nl/vc, m
<i>N. tabacum</i> 'Ky-57'	cr/vc, m
<i>N. tabacum</i> 'Bright yellow'	nl, vn/m, mal
<i>N. tabacum</i> 'Xanthi-nc'	nl/cl, m
<i>N. tabacum</i> 'Samsun'	nl, vn/cl, m
<i>Datura stramonium</i>	—/—
<i>Tetragonia expansa</i>	cr/—
<i>Physalis floridana</i>	cl/cl, m, mal
<i>Cucumis sativus</i>	—/—
<i>Vigna unguiculata</i>	—/—
<i>V. sesquipedalis</i>	—/—
<i>Brassica campestris</i>	—/—
<i>Raphanus sativus</i>	—/—

<sup>a</sup>cl, chlorotic local; nl, necrotic local; vc, vein clearing; m, mosaic; vn, vein necrosis; mal, malformation; inoculated lower leaves/upper systemic leaves; —, no symptoms.

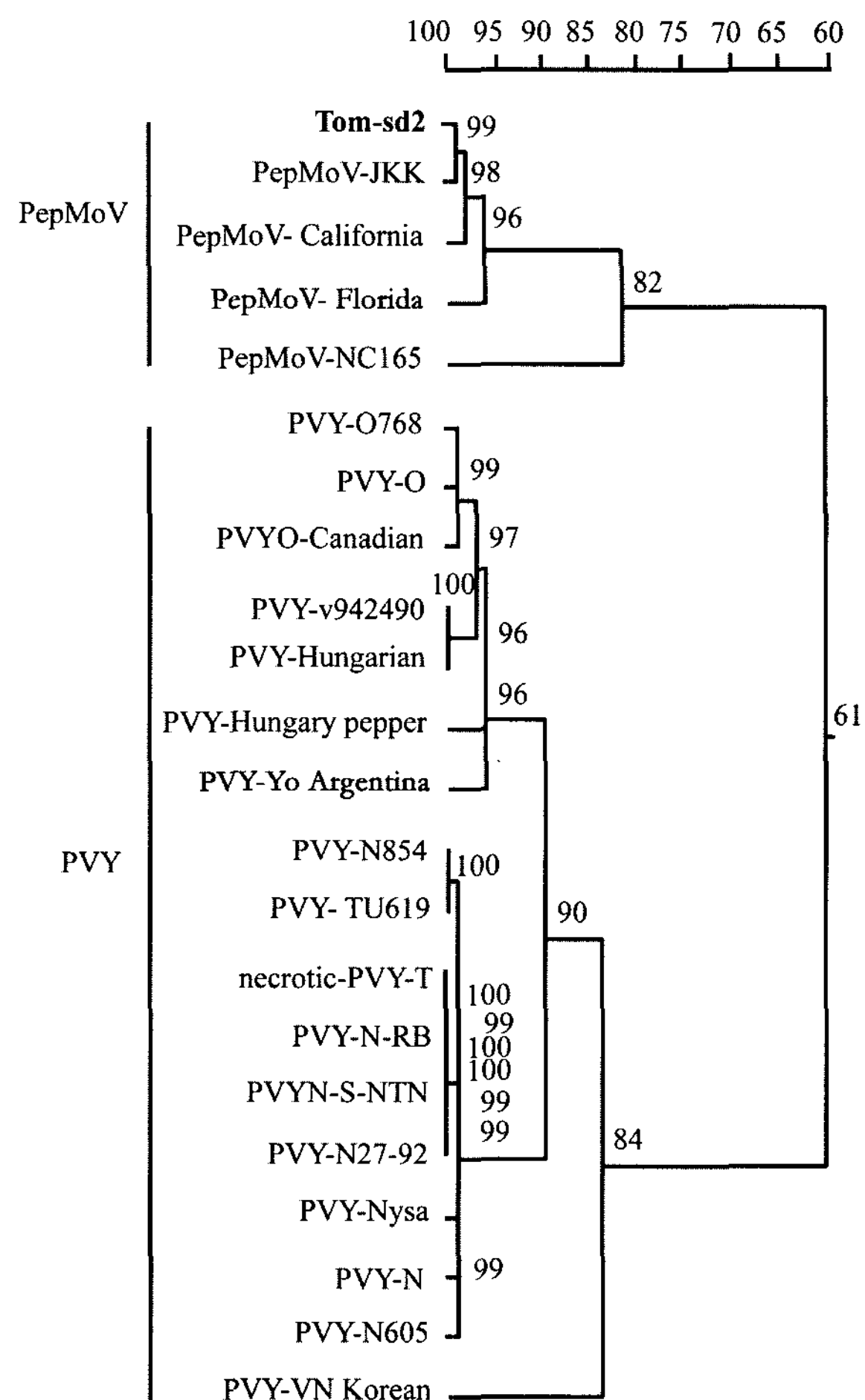
**Table 5.** Pathogenicity, host ranges and symptoms on infected tomato and pepper cultivars inoculated with Tom-sd2 isolate

Assay hosts	Symptomatic reactions
<i>Lycopersicon esculentum</i> 'Minicarol'	nl, vn/cl, m <sup>a</sup>
<i>L. esculentum</i> 'Momotaro'	nl, vn/cl, m
<i>L. esculentum</i> 'Paepae'	nl, vn/m, r
<i>L. esculentum</i> 'Sunextra'	nl, vn/cl, m
<i>L. esculentum</i> 'Dadagi'	nl/m, r
<i>L. esculentum</i> 'Jok'	nl/m, r
<i>L. esculentum</i> 'Pungsang'	cl/cl, vc
<i>L. esculentum</i> 'Seokang'	–/vc
<i>L. esculentum</i> 'Kangsu'	–/vc
<i>L. esculentum</i> 'Hongyoung'	–/vc
<i>L. esculentum</i> 'Kangmung'	–/vc
<i>L. esculentum</i> 'Alchan'	–/vc
<i>Casicum annuum</i> 'Kumtop'	–/m, r
<i>C. annuum</i> 'Gusung'	–/m, r
<i>C. annuum</i> 'Dongbang'	–/m, r
<i>C. annuum</i> 'Dabok'	–/m, r
<i>C. annuum</i> 'Umsung'	–/sm, mal
<i>C. annuum</i> 'Choyang'	–/m, r
<i>C. annuum</i> 'Kari'	–/m, r
<i>C. annuum</i> 'Newace'	–/m, r

<sup>a</sup>cl, chlorotic local; nl, necrotic local; vc, vein clearing; m, mosaic; sm, severe mosaic; r, rugose; vn, vein necrosis; mal, malformation; inoculated leaves/upper leaves; –, no symptoms.

**Fig. 1.** PepMoV-infected cherry tomato plants showing (A) yellow mosaic and (B) vein necrosis symptoms in the field. (C) Electron micrographs showing inclusion bodies. Pinwheels (p) and masses of fibrillar inclusions (mfi) are shown with arrows.

group of tomato species. On the other hand, Tom-sd2 isolate was only pathogenic to some indicators hosts tested in this study. Eight out of 17 indicator hosts were infected and these were: all 6 *Nicotiana* spp., caused vein mosaic

**Fig. 2.** Phylogenetic tree constructed from nucleotide sequence alignments of the CP/3'-UTR fragments of PepMoV/PVY strains.

symptoms in addition to vein clearing; 1 *Tetragonia expansa* and 1 *Physalis floridana*. However the remaining 9 indicator hosts plants (Table 4) failed to induce symptoms or not infected indicating that they were non-host of the Tom-sd2 isolate. Among infected indicator hosts, *T. expansa* only showed localized infection.

**Virus particle and inclusion bodies.** Electron microscopy examination of crude sap extracts revealed long flexuous particles 720–750 nm in length. PepMoV isolated from tomato plants induced typical cytoplasmic inclusions bodies like pinwheels, scrolls and laminated aggregates in cells of *C. annuum*, *N. tabacum* 'Bright Yellow' and *P. floridana* (Fig. 1). In addition, masses of fibrillar inclusions were found in the cytoplasm of cells infected with PepMoV. Virus was easily detected by ELISA and no false positive or negative were found with this method.

**CP/3'-UTR sequence analysis.** The 3' terminal 721 nt sequence comprising 455 nt of the CP and 266 nt UTR with

a poly (A) tail were retrieved (Fig. 2). Alignment of CP-UTR nucleotide (nt) sequences revealed a varying degree of sequence identity grouping. A high degree of sequence homology (>96%) was evident between the Tom-sd2 isolate, the Japan and USA isolates of PepMoV. However, when compared to other PepMoV and 17 distinct PVY isolates, this isolate showed 82% homology with PepMoV-NC165 and only <61% with PVY strain. Interestingly, PepMoV isolate from the same geographical location have a high percentage of nt identity. For instance, PepMoV isolate from cherry tomato shared >99% nt sequences with the Japanese isolate, <98% with those from California and <96% with those from Florida, while only 82% identical with isolate PepMoV-NC165.

## Discussion

Previous reports have indicated that several potyviruses exist in Korea (Kim et al., 1995; Ko et al., 2003; Yoon et al., 1998) and since all these potyviruses are transmitted by the same aphid (*Myzus persicae*) which is polyphagous in nature, a possibility that multiple virus infection is most likely to occur. This has been further confirmed in this paper that 19% of the samples tested had a mixed infection of PepMoV and ToMV. Under such circumstances (mixed infection) the possibility of recombination is very high. Mixed infections occur commonly in nature and may result in a range of effects on the host as well as on the levels of accumulation and degrees of movement of either of the viruses involved (Carr and Kim, 1983; Murphy and Kyle, 1995). Sherwood et al. (1988) found that synergistic interaction occurred between PVY and Tobacco mosaic virus (TMV) in Bahamian hot chile. There have been numerous reports of PVY and TMV infecting pepper (Benner et al., 1985; Sherwood et al., 1988; Villalon, 1975). Indeed, the chance of recombination of the two viruses in single plant is high but in the case of mixed infection of PepMoV and ToMV warrants further investigation.

In this paper, we report the identification of a virus infecting cherry tomato occurring in the fields and greenhouses of TES, Buyeo, Korea. The causal virus belongs to *Potyvirus*, specifically PepMoV. Although, we have been observing these symptoms since 1997 in tomato fields at TES but had been ignored and initially thought to be PVY or other common virus infecting tomato. However, as time progresses, the said symptoms became so prevalent that prompted us to closely examine and we confirmed in this study that it was caused by PepMoV which common host is *C. annuum*. Since it took a while before the confirmation of the said disease, here based to our knowledge this is the first report of PepMoV found to infect tomato plant in

Korea. Although Verhoeven et al. (2002) also reported the first PepMoV infecting tomato from a sample in Guatemala in 2002. In their paper also indicated that the host ranges of PepMoV infecting tomato were mostly *Nicotiana* spp. which also supports our findings in this study.

Another striking feature of PepMoV infecting tomato is the appearance of inclusion bodies in the host cells. Recently, Han et al. (2006) in their cytological study on PepMoV-SNU1 infecting chili pepper has shown inclusion bodies but these structures were only observed in tissues doubly infected with *Pepper mild mottle virus* (PMMoV). Unlike PepMoV infecting tomato, these unique structures were detected in *C. annuum*, *N. tabacum* 'Bright Yellow' and *P. floridana* cells infected with PepMoV only. However, it was not indicated in the PepMoV infecting tomato in Guatemala also showed these unique structures in ultrathin sections. Consequently, this paper also reports the first findings of these structures associated in PepMoV infecting tomato. The induction of these ultrastructures in infected host cells however is not yet understood if it is induced by the host or virus.

Phylogenetic analysis of PepMoV CP/3'-UTR classified Tom-sd2 isolate in the same cluster with other PepMoV and very close to PepMoV-JKK implying that two isolates are very close to one another and could be strains of the same virus (Fig. 2). However, alignment of CP/3'-UTR nucleotide sequence between Korean and Guatemala (accession nos. AY748920 and AF440801, respectively) PepMoV-infected tomato strains showed only 84% homology (Data not shown). These 2 strains could be regarded as geographical strains. Conclusively, our results on biological, serological, cytological and molecular analyses indicate that Tom-sd2 is another strain of PepMoV. The complete genome of this strain and other tomato infected with PepMoV collected in other provinces in Korea were determined (Kim et al., unpublished). This will pave way for further studies and in understanding thoroughly the PepMoV-infecting tomato in Korea towards achieving the ultimate goal in controlling the disease.

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