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First Report on *Tomato bushy stunt virus* Infecting Tomato in Korea

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A new virus-like disease of tomato showing chlorotic spots, malformation and necrosis on leaves, and chlorotic blotching, rings, and necrosis on fruits was observed around Sacheon, Gyeongsangnam-do, Korea in 2004. Host range analysis could not differentiate 4 field isolates collected from tomatoes showing different symptoms but identified them as *Tomato bushy stunt virus* (TBSV). TBSV-tsf2 isolate induced symptoms in indicator plants similar to those caused by the TBSV-C, -S and -Nf. As the isolate could not systemically infect *Chenopodium quinoa*, the isolate might belong to the previously described TBSV-S isolate. TBSV-tsf2 isolate caused similar cytological alterations that were similar to that generally caused by previously reported TBSV isolates. TBSV-tsf2 isolate, however, could be considered to belong to new strain of TBSV because masses of small electron-dense patches that were not observed from the previously described TBSV. The complete nucleotide sequences of the genomic RNA of 4739 nt excluding non-translated sequences at both termini have been determined and compared to sequences of other TBSV strains. The complete nucleotide sequence identity among TBSV isolates was 98.9% to 99.7%, and to the other tombusviruses ranged from 80.8% to 94.9%. Comparison of the amino acid sequences all five ORFs with those of other TBSV strains shows a similar genomic organization, and high percentage of amino acid sequence homology with TBSV-Nf than TBSV-S isolate. Since the TBSV symptoms were only observed in Sacheon fields where imported seeds from Japan were planted, the TBSV incidence probably caused by the planting contaminated tomato seeds and thus require more through quarantine procedure to prevent settlement of TBSV in Korea. Altogether, these results support that the Korean isolate of TBSV infecting tomato might be new strain.

Keywords : TBSV incidence, tomato, virus identification, virus characterization

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Tomato bushy stunt virus (TBSV), a type species of the genus *Tombusvirus*, has spherical particles of 30 nm in length containing a positive-sense single-stranded RNA genome of approximately 4.8 kb that encodes five major open reading frames (ORFs) (Brunt et al., 1996). An ORF1 and a ORF2 are required for viral replication (Scholthof et al., 1995a). The ORF3 encodes coat protein while ORF4 encodes viral movement protein that is necessary for cell-to-cell movement and symptom determination on certain host plants (Russo et al., 1994; Scholthof et al., 1993, 1995b). Product of ORF5 has a role in the induction of necrotic symptoms and in the long-distance spread of the virus, depending on the host.

TBSV was first isolated from tomato with viral symptoms in Ireland, and subsequently reported in North and South America, Europe, Africa, and Japan (Borges et al., 1979; Cherif and Spire, 1983; Fischer and Lockhart, 1977; Koenig and Avgelis, 1983; Pontis et al., 1968; Smith, 1935). However, no natural outbreaks by the type strain of TBSV have been reported. Instead all new outbreak caused by TBSV strain BS3 (Gerik et al., 1990). Recently, TBSV caused epidemic outbreaks in tomato and eggplant crops in southeastern Spain and has spreading with the expansion of nipplefruit cultivation in Japan (Luis-Arteaga et al., 1996). TBSV is readily transmitted by mechanical inoculation to a wide range of host plants, while natural transmission of the virus is occurred mainly through seed and soil, apparently not involving vector. Severe yield losses associated with TBSV have been reported in peanut, tobacco, tomato, pepper and potato as well as in some ornamental crops (Martelli et al., 2001). The virus causes stunting and bushy

growth, chlorotic spots, crinkling, deformation and necrosis of leaves of tomato and pepper. Fruit from the infected commercial solanaceous crops develop necrosis and chlorotic blotching, resulting in serious economic damage such as yield loss and deterioration in the quality in both greenhouses and fields.

TBSV has been divided on the basis of serological reactivity into three major strains, type strain, BS3 strain (TBSV-BS3) and cherry strain (TBSV-Ch). The type and the BS3 strains usually infect herbaceous hosts, whereas TBSV-Ch has the tendency to infect woody plants. Because of serological reactivity and of the high sequence homology in their genomes, it was also proposed that *Petunia asteroid mosaic virus* and *Artichoke mottled crinkle virus* (AMCV) are also strains of TBSV (Luis-Arteaga et al., 1996). Cytopathologically, the intracellular hallmark of TBSV infection is the occurrence of multivesicular bodies that consist of a main body surrounded by many spherical to ovoid vesicles 80-150 nm in diameter originating as invaginations of the boundary membrane of peroxisomes.

In Korea, number of viruses including *Cucumber mosaic virus* (CMV), *Tomato mosaic virus* (ToMV), *Tobacco mosaic virus* (TMV), *Pepper mottle virus* (PepMoV) and *Potato virus* Y (PVY) were identified from tomato plants (Choi et al., 2001). In this study, we found a new virus-like disease from tomato showing chlorotic spots, malformation and necrosis on leaves, and chlorotic blotching, rings, and necrosis on fruits in Sacheon, Gyeongsangnam-do and characterized it as TBSV based on biological, serological, cytopathological and molecular properties. To our knowledge, this is the first report of TBSV infecting tomato in Korea.

Materials and Methods

Sample collection and host range studies. A survey of TBSV on tomato plants was carried out around Sacheon, Gyeongsangnam-do, Korea, in 2004. Typical viral symptoms including chlorotic blotching, rings and necrosis were observed on several tomato houses where imported seeds from Japan were planted. To determinate the infectivity of virus isolates and the symptoms induced on the test plant, leaves and fruits of tomato with typical viral symptoms were used to prepare sap for inoculum with 0.01 M of sodium phosphate buffer (pH 7.0). Single local lesion isolates from *Gomphrena globosa* were maintained on *Nicotiana clevelandii*. Virus isolates was inoculated on 26 herbaceous hosts, kept in an insect-free greenhouse maintained at 20-25°C with 12 h light period, and observed for symptom expression for up to four weeks. Both symptomatic and non-symptomatic plants were verified for TBSV infection by DAS-ELISA.

Virus purification. Each virus isolate was infected onto *N. clevelandii*. Infected leaves were collected at 7 days after inoculation, homogenized with 3 vol. of 0.1 M sodium phosphate buffer (pH 5.5) containing 0.2% 2-mercaptoethanol and 0.1% thioglycolic acid, and filtered through cheesecloth. Filtrates were kept on ice for 2 h and centrifuged at 8,000 rpm for 20 min. Eight % PEG (mol. wt 8,000) was added to the supernatant fluid and subjected to two cycles of differential centrifugation. The crude virus was precipitated by ultracentrifugation at 30,000 rpm for 2 h and then suspended with 10 mM of sodium phosphate buffer (pH 5.5) for subsequent study.

DAS-ELISA. Double antibody sandwich enzyme linked immunosorbent assay (DAS-ELISA) was conducted essentially as described by Clark and Bar-Joseph (1984) using TBSV specific monoclonal antibodies (Agdia, USA). 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). A positive result was taken as twice the mean of the corresponding negative control an absorbance after incubation at room temperature for 30 min.

Electron microscopy. Purified virus preparations were negatively stained with 2% uranyl acetate, pH 7.0. For thin sectioning, infected *N. clevelandii* leaves were fixed in 2.5% glutaraldehyde in Millonig's phosphate buffer, post-fixed 2% osmium tetraoxide, stained in 1.0% uranyl acetate, dehydration with graded ethanol series and embedded in Spurr's resin, ultra-thin sections and staining were prepared from as described by Choi et al. (2005). For interpretation of results, the sections were observed and photographed with a LEO 912AB electron microscope.

RT-PCR, Cloning and Sequencing. Viral RNA was extracted from purified virus preparations as described by Choi et al. (2005). To amplify cDNA fragments of TBSV genomic RNA, five pairs of primer sets were designed by using previously reported sequences of TBSV (Table 3). End sequences of the genomes were obtained with the 5'/3' rapid amplification of cDNA ends (RACE) protocol (Boehringer Mannheim, Mannheim, Germany). cDNA clones containing the 5' end of the genomes were obtained using oligo dC primers and 5tbsvR (5'-GGCCAAATCA-TCCTCTTAATGGTCTCCA-3'), while cDNA clones containing the 3' end were obtained using 3tbsvF (5'-CTACCGGACAACCGGAACATTGCA-3') and oligo dT primers (Fig. 3). The amplified PCR products were cloned

in pGEM-T vector (Promega, USA) and recombinant plasmids containing cloned cDNAs were purified with a plasmid kits according to the manufacturer's protocol. Nucleotide and amino acid sequence analyses were aligned and pairwise distance matrixes were calculated with the program DNAMAN version 4.0 (Lynnon Biosoft, Canada). Sequence comparison was done using BLAST program in the NCBI-GenBank database. Phylogenetic trees were

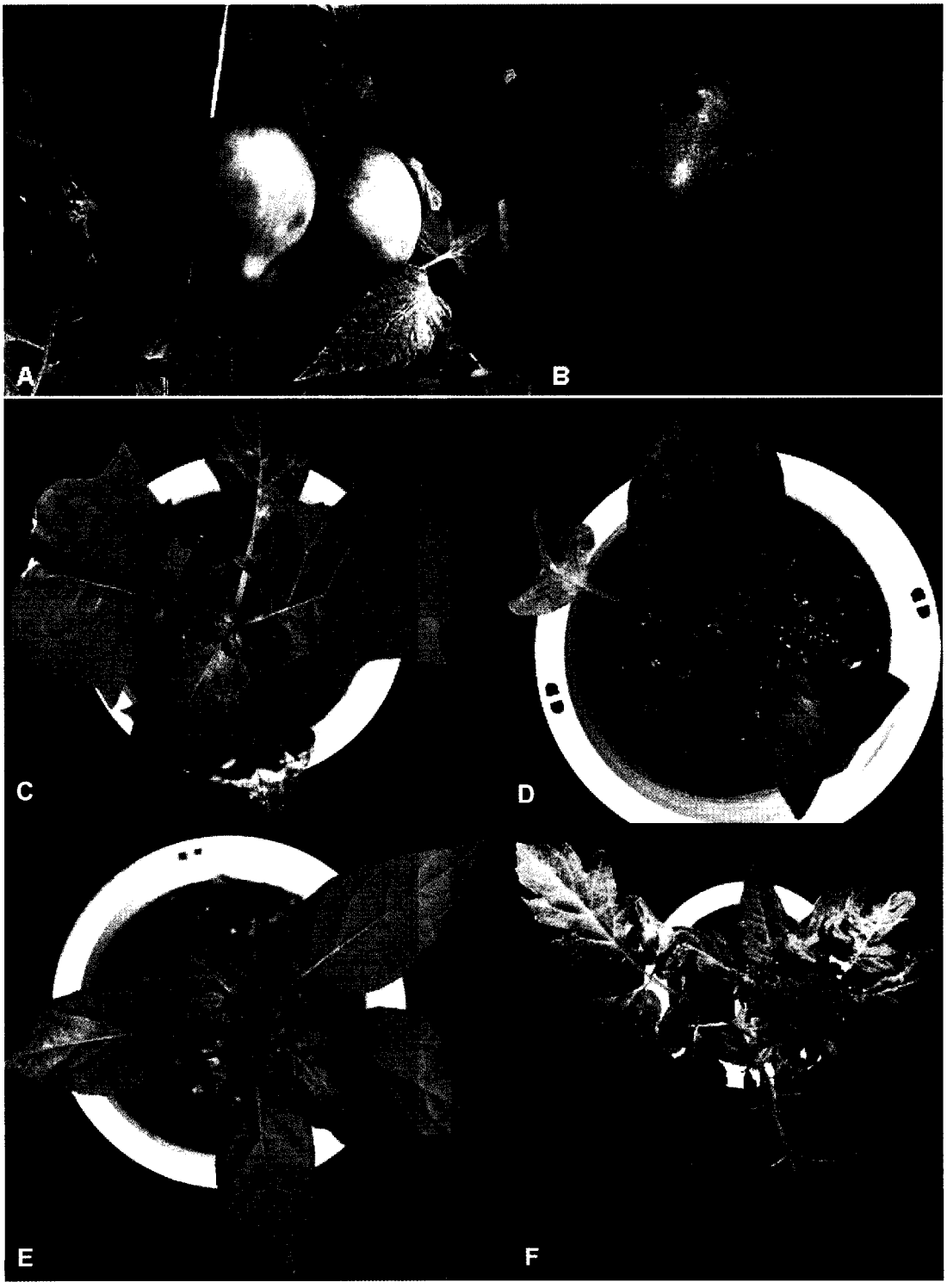

Fig. 1. TBSV infected tomato fruits showing (A and B) chlorotic blotching, rings and necrosis symptoms in the field and symptom expression in *D. stramonium, S. melongena, C. annuum* and *S. lycopersicum*, respectively (C, D, E and F).

calculated according to the neighbor-joining method options of Saitou and Nei (1987) with 100 bootstrap replications (Felsenstein, 1985).

Results and Discussion

Virus isolates and host ranges. A new virus-like disease of tomato was observed around Sacheon, Gyeongsangnam-do, Korea in 2004 and virus disease incidence of tomato cultivating fields were about 20% in a cultivated area. A total of 4 tomato leaf and fruit were collected and analyzed. The tsf2 isolate out of eight TBSV-isolates was representatively used depending on indicator host species. Main symptoms of the disease were chlorotic spots, malformation and bushy growth of the young leaves. In addition, necrosis of lower leaves was sometimes observed in greenhouse fields (Fig. 1-A), Fruits were smaller than normal and showed chlorotic blotching, rings and necrosis (Fig. 1-B).

Single local lesion isolates from *G globosa* were maintained on *N. clevelandii.* Of the 25 plants tested, only *N. clevelandii, Solanum lycopersicum, Capsicum annuum* and *Solanum melongena* were susceptible to TBSV-tsf2 isolate (Table 1 and Fig. 1-D). The following plants developed necrotic local lesions with TBSV-tsf2 isolate: *Chenopodium amaranticolor*; *C. quinoa*; *N. tabacum* cvs. Xanthi-nc, Bright Yellow, Samsun NN, and 'KY-57'; *N. glutinosa*; *Physalis floridana*; *Vicia unguiculate*; *Zinnia elegans*; *G globosa*; *Tetragonia expensa.* However, no symptom was observed on *Petunia* spp., *Vicia fava, Pisum sativum, Cucumis sativus, Cucumis melo, Cucubita pepo, Cucubita moshata, Brassica campestris* and *Raphanus sativus* (Table 1 and Fig. 1-C). Interestingly all varieties of *S. lycopersicum* and *C. annuum* were not found to be equally susceptible to

Table 1. Symptoms developed on indicator plants inoculated with TBSV-tsf2 isolate

Indicator plants	Reactions of Tom-tsf2	
	Symptom	ELISA
Chenopodium amaranticolor	nl/–	Nt
C. quina	nl/–	Nt
Nicotiana tabacum cv. Xanthi-nc	nl/–	Nt
N. tabacum 'Bright Yellow'	nl/–	Nt
N. tabacum 'Samsun NN'	nl/–	Nt
N. tabacum 'KY-57'	nl/–	Nt
N. glutinosa	nl/–	Nt
N. clevelandii	nl/ns, st, n	+
Capsicum annuum	crl/crl	+
Solanum lycopersicum	crl/nl, bu, st, mal	+
Solanum melongena	–/cl, m	Nt
Physalis floridana	nl/–	Nt
Petunia spp.	–/–	Nt
Vicia fava	–/–	Nt
Pisum sativum	–/–	Nt
Vicia unguiculate	pp/-	Nt
Cucumis sativus	–/–	Nt
Cucumis melo	–/–	Nt
Cucubita pepo	–/–	Nt
Cucubita moshata	–/–	Nt
Datura stramonium	crl/–	Nt
Zinnia elegans	cl/–	Nt
Brassica campestris	–/–	Nt
Raphanus sativus	–/–	Nt
Gomphrena globosa	rnl/–	+
Tetragonia expensa	crl/–	Nt

[a]cl, chlorotic local; nl, necrotic local; crl, chlorotic ring local; nrl, necrotic ring local; m, mosaic; bu, bushy; mal, malformation; st, stunt; n, necrosis; inoculated leaves/upper leaves; Nt, not tested; –, no symptoms.

Table 2. Infectivity of TBSV-fp2 isolate in tomato and redpepper cultivars

Indicator plants		Reactions of TBSV-tsf2			
		Inoculated		Upper	
Solanum lycopersicum	'Pungsang'	crl/[a]	12/12	crl, vn, st	1/12
	'Hongyoung'	crl/	12/12	crl	1/12
	'Juok'	crl, nrl	12/12	cl	1/12
	'Alchan'	crl	12/12	–	0/12
	'Dadagi'	nrl	12/12	–	0/12
	'bbobbo'	crl, nrl	6/6	–	0/6
	'Dotaerang'	crl, vn	12/12	–	0/12
	'Gangsu'	nrl	12/12	cl	1/12
	'Ggoggo'	crl, vn	12/12	–	0/12
	'Seokang'	crl	6/6	crl, vn, bu, st	1/6
	'Sunmung'	crl	6/6	crl, vn, bu, st	1/6
	'Rokkusanmaru'	nrl	12/12	crl, vn, bu, st	1/12
Casicum annuum	'Kumtop'	crl	12/12	cl	3/12
	'Gusung'	crl	12/12	–	0/12
	'Dongbang'	crl	6/6	crl	0/6
	'Dabokgun'	crl	12/12	crl, nrl	2/12
	'Nokgwang'	crl	12/12	crl	3/12
	'Choyang'	crl	12/12	crl	4/12
	'Buchon'	crl	12/12	–	0/12
	'Daemung'	crl	12/12	cl	1/12
	'Bugang'	crl	12/12	cl	1/12
	Pochungchun	crl	6/6	–	0/6
	Daewang	crl	12/12	cl	1/12
	Kumdang	crl	12/12	–	0/12
	Pungchon	crl	12/12	crl	3/12

[a]cl, chlrorotic local; crl, chlrorotic ring local; nl, necrotic local; nrl, necrotic ring local; vn, vein necrosis; bu, bushy; st, stunt; infected plants/test plants; -, no symptoms.

TBSV-tsf2 isolate (Table 2). In *S. lycopersicum*, the isolate caused chlorotic ring local lesions on inoculated leaves 5 to 7 days post-inoculation, followed by bushy growth symptoms in addition to chlorotic ring spots, vein necrosis and stunt (Fig. 1-F). Latent systemic infection was also observed in an additional week. However, the main symptom on *C. annuum* was always chlorotic ring local lesion on inoculated leaves, chlorotic ring spots and necrotic ring spots was sometimes observed on new leaves (Fig. 1-E). TBSV-tsf2 isolate induced symptoms in indicator plants similar to those caused by the TBSV-C, -S and -Nf. However, in *C. quinoa*, TBSV-tsf2 and TBSV-S isolates could not systemically infect but TBSV-Ch and TBSV-Nf isolates induced mosaic or stem necrosis symptoms. Also, TBSV-tsf2 and TBSV-S isolates caused chlorotic local and mosaic on *S. melongena* but TBSV-Ch and TBSV-Nf isolates induced only chlorotic local symptoms. Therefore, this characterization of TBSV isolates at the biological level is probably may be due to different TBSV strains.

In addition, it is worth noting that TBSV-tsf2 isolate failed to infect 5 of the 13 varieties of *C. annuum* tested, whereas 5 of the 12 varieties of *S. lycopersicum* tested (Table 2). These results were consistent with the finding of Fischer and Lockhart (1977) that TBSV isolated from tomato produced only mild mosaic symptoms on 8 of the 10 varieties of *C. annuum*, and caused infrequently severe stunting, necrosis and foliar deformation in the 10 varieties of *S. lycopersicum* (Table 2). In spite of virus disease incidence of tomato cultivating fields were about 20% in a cultivated area, why virus transmission rate by mechanical inoculation is low is not clear. Therefore, the cause and

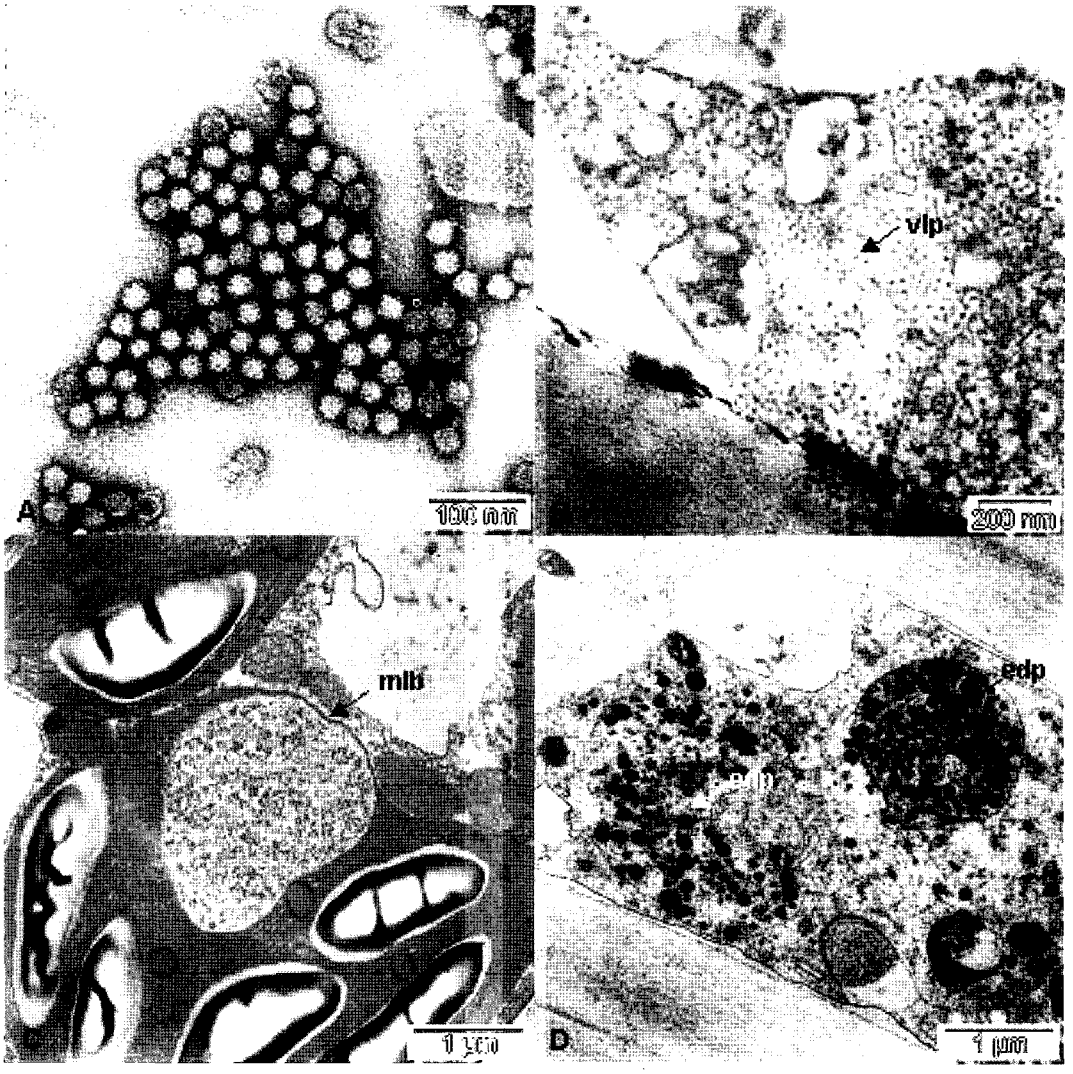

Fig. 2. Electron micrograph of virus particles in purified viruses (A) and of virus-like particles, multivesicular-like bodies (B and C) and small electron-dense patches (D) in ultrathin sections of TBSV infected *C. annuum* and *D. stramonium* leaf. vlp, virus-like particles; mlb, multivesicular-like bodies; edp, electron-dense patches.

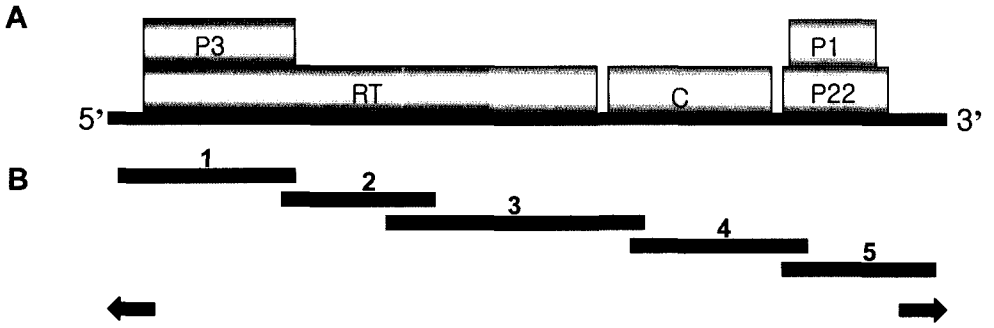

Fig. 3. Schematic representation of the genomic organization of TBSV isolates (A) and the sequencing strategy (B).

Table 3. The sense and antisense primer pairs used for TBSV in RT-PCR

Primer name	Locus	Sequence	Expected size
TBSV 1-F	10-29	5' CCAGGATTTCTCGACCTAGT 3'	1102bp
TBSV 1-R	1112-1093	5' ATGGGATATCTCGATTGATC 3'	
TBSV 2-F	938-958	5' AGGTATGTTGACAGGGATGTC 3'	898bp
TBSV 2-R	1836-1816	5' TTGCCAGGGTACATGGCCCTG 3'	
TBSV 3-F	1634-1654	5' AAAGCTGTTGATGGCGTGTTC 3'	1415bp
TBSV 3-R	3049-3031	5' GTAACAAATTGCCGACAAT 3'	
TBSV 4-F	3018-3035	5' AATGGGGGTATTGTCGGC 3'	1002bp
TBSV 4-R	4020-4001	5' TAGCCGCCACTCAGTCCAAC 3'	
TBSV 5-F	3841-3860	5' TGAACAAGACCAGTTCATGG 3'	923bp
TBSV 5-R	4764-4739	5' TGCAATGTTCCGGTTGTCCGGTAGTG 3'	

mechanism for causing systemic symptoms of TBSV-sd2 isolate will remain for further investigation. In this regard it might worth mentioning that the TBSV incidence on tomato in 2004 was observed only on some houses/fields where imported seeds from Japan were planted and was not observed elsewhere. Since the transmission rate by mechanical inoculation is very low and the other significant TBSV incidence, except Sacheon area in 2004, was not observed since 2004, the thorough quarantine procedure of the imported seeds may be a successful strategy to prevent the settlement of TBSV in Korea.

Electron microscopy and serological relationships. Electron microscopic examination of purified viruses revealed spherical virus particles with a diameter of about 30 nm (Fig. 2-A). In ultrathin sections of TBSV-tsf2 infected *C. annuum* and *D. stramonium* leaf, many virus particles were spotted around the cytoplasm that was similar to that generally reported for TBSV isolates. Virus-like particles and multivesicular-like bodies were occasionally found in the cytoplasm to form amorphous or crystalline inclusions (Fig. 2-B). Multivesicular-like bodies may arise from deranged peroxisomes and mitochondria, but not from chloroplasts (Fig. 2-C). Peculiarly, masses of small electron-dense patches were accumulated in the central vacuole and cell organelles (Fig. 2-D). This feature was not reported for any of the previously described tombusviruses infections. Virus was easily detected by ELISA and no false positive or negative were found with this method.

Nucleotide sequence of TBSV isolates. The complete genomes of five TBSV isolates were 4782 nt in length, including some non-translated sequences at both termini. The nucleotide sequence identity among TBSV isolates was 98.9% to 99.7%, and with the other tombusviruses ranged from 80.8% to 94.9% (data not shown). Analysis of putative ORF shows five ORFs (Fig. 3) which is typical tombusvirus genome organization (Hearne et al., 1990). The first ORF starts from an ATG at nts 159 and terminates with an amber stop codon TAG at nts 1051. Readthrough of the stop codon of ORF1 would extend the frame up to another stop codon at nt 2618 (ORF2). ORF3 was separated from the upstream ORFs by an intergenic region of 24 nt. ORF3 initiates at nt 2642 ending with a TAA at nt 3808 and identified as the viral coat protein (CP). ORF4 from nt 3844 to nt 4413. ORF4 and ORF5 followed, being separated by intergenic regions 35 nt and 67 nt in size, respectively. ORF4 extended from nt 3844 to nt 4413, whereas ORF5 from nt 3876 to nt 4394 (Fig. 3). In addition, the deduced

Table 4. Pairwise amino acid sequence comparison of protein encoded by the genome of TBSV isolates

Isolate	Virus[a]	Amino acid identity (%)				
		p33	rt	cp	p22	p19
Tsl1	TBSV-S	97.6	97.9	97.9	96.8	92.4
	TBSV-Nf	96.3	98.2	97.9	98.4	95.9
	TBSV-C	95.3	96.7	74.2	98.9	88.4
	GALV	95.6	96.6	46.5	88.9	70.3
	AMCV	93.9	95.2	70.8	98.9	88.4
Tsf2	TBSV-S	97.0	97.4	97.7	96.8	92.4
	TBSV-Nf	95.9	97.8	97.7	97.4	95.9
	TBSV-C	94.9	96.3	73.9	97.9	88.4
	GALV	95.3	96.2	46.8	87.8	70.3
	AMCV	93.6	94.9	70.5	97.9	88.4
Tsf3-1	TBSV-S	98.0	98.2	97.4	96.8	91.9
	TBSV-Nf	96.6	98.4	97.4	98.4	95.3
	TBSV-C	95.6	96.9	73.6	98.9	87.8
	GALV	95.9	96.8	46.8	88.9	69.8
	AMCV	94.3	95.5	70.3	98.9	87.8
Tsf3-2	TBSV-S	98.0	97.9	97.9	96.8	92.4
	TBSV-Nf	96.6	98.0	97.9	98.4	95.9
	TBSV-C	95.6	96.6	74.4	98.9	88.4
	GALV	95.9	96.5	46.8	88.9	70.3
	AMCV	94.3	95.1	71.1	98.9	88.4
Tsf4	TBSV-S	98.0	98.2	97.7	96.8	92.4
	TBSV-Nf	96.6	98.4	97.7	98.4	95.9
	TBSV-C	95.6	96.9	73.9	98.9	88.4
	GALV	95.9	96.8	47.1	88.9	70.3
	AMCV	94.3	95.5	70.5	98.9	88.4

[a]TBSV-S, *Tomato bushy stunt virus-statice* (AJ249740); TBSV-Nf, *Tomato bushy stunt virus-nipple fruit* (AY579432); TBSV-C, *Tomato bushy stunt virus-cherry* (M21958, *Petunia asteroid mosaic virus*); GALV, *Grapevine Algerian latent virus* (AF540885); AMCV, *Artichoke Mottled Crinkle Virus* (X62493)

amino acid sequences of P19, P22, P33 and P92 had high sequence identity (>98.5%), while CP contained low sequence identities between TBSV isolates and the other TBSV isolates (Table 5). The putative amino acid sequence of TBSV isolates was compared to the corresponding ORFs

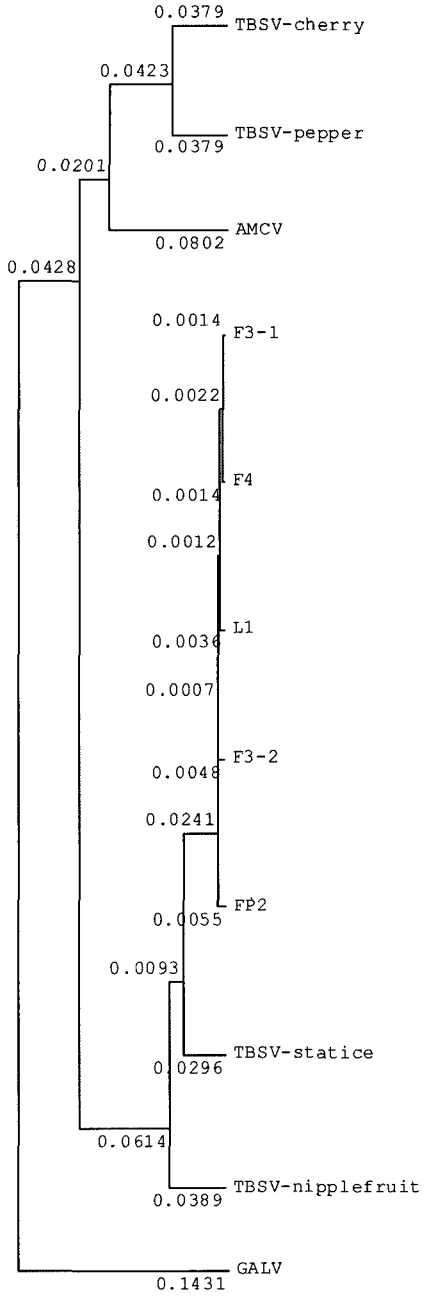

Fig. 4. Phylogenetic analysis of the complete genome of TBSV isolates with aligned nucleotide sequences.

Table 5. Pairwise amino acid sequence comparison of protein encoded by the genome of TBSV isolates

	P19				P22				P33				P92				CP			
	Tsf2	Tsf 3-1	Tsf 3-2	Tsf4	Tsf2	Tsf 3-1	Tsf 3-2	Tsf4	Tsf2	Tsf 3-1	Tsf 3-2	Tsf4	Tsf2	Tsf 3-1	Tsf 3-2	Tsf4	Tsf2	Tsf 3-1	Tsf 3-2	Tsf4
Tsl1	100[a]	99.4	100	100	98.9	100	100	100	98.6	99.7	99.7	99.7	98.8	99.5	99.3	99.8	99.2	99.0	99.5	99.2
Tsf2		99.4	100	100		98.9	98.9	98.9		99.0	99.0	99.0		99.0	98.5	99.0		97.4	97.9	99.0
Tsf3-1			99.4	99.4			100	100			100	100			99.3	99.8			99.0	99.2
Tsf3-2				100				100				100				99.5				99.2

[a]Numbers represent amino acid identity (%) between each isolate.

of the nipple fruit isolate (TBSV-Nf, AY579432), the cherry isolate (TBSV-Ch, M21958) and the statice isolate (TBSV-S, AJ249740). The pairwise amino acid sequence identity of each ORF among TBSV strains was over 88.9%, while showing >70.3% and <47.1% homology with AMCV (X62493) and with *Grapevine Algerian latent virus* (GALV, AF540885), respectively (Table 4). The overall sequence identity among the complete genomes of five TBSV isolates revealed that TBSV isolates have the closest relationship to TBSV-S. However, based on amino acid sequence analyses of all five ORFs, the TBSV isolates shows that the higher degree of homology occurred TBSV-Nf than TBSV-S isolate. Interestingly, the corresponding ORFs including ORF2 (RNA-dependent RNA polymerase), ORF4 (cell-to-cell movement) and ORF5 (symptoms and long-distance movement) except for ORF1 (duplication) showed close relationship with TBSV-Nf isolate than TBSV-S isolate. Further studies involving construction of infectious clone of TBSV are required in order to detect and identify the viral function of TBSV. This would help in developing an effective strategy for managing the viruses. This possibly indicates the existence of variation in TBSV-Nf and TBSV-S isolate. Altogether, our results clearly support that the infection of TBSV in tomato plants and the name TBSV-ts is supposed for the strain based upon host ranges, cytopathology as well as sequence analyses.

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