First report of tomato spotted wilt virus in *Lycium chinense*

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Abstract

Tospovirus transmitted by thrips can cause serious diseases in many economically important plants. Based on molecular and biological assays, a virus infecting boxthorn plants in Korea was identified as tomato spotted wilt virus (TSWV). To the best of our knowledge, this is the first report of TSWV in *L. chinense*.

Keywords Boxthorn · Tospovirus · Thrips

Boxthorn (*Lycium chinense*) is a deciduous shrub belonging to the Solanaceae family and is a well-known medicinal plant in Asia (Kim and Chung 2009; Qian et al. 2004). In Korea, production and consumption of this plant have gradually increased due to its medicinal and nutritional value. In May 2018, virus-like symptoms, such as chlorotic and necrotic spots and mosaic on the leaves, necrosis on the stems, and stunting were observed on boxthorn plants in open fields and greenhouses in the Cheongyang area of South Korea (Fig. 1). Disease incidence was greater than 60% in approximately 1000 m² of cultivated land. Tomato spotted wilt virus (TSWV) has recently been found to be problematic in chili plants in this important chili cultivation region of South Korea (Kwon et al., 2018).

TSWV, as a member of the genus *Orthotospovirus* of family Tospoviridae, is transmitted by thrips and causes a serious disease in many economically important plants, including pepper

and tomato (Parrella et al. 2003). TSWV has been reported to infect other *Lycium* spp., such as *Lycium ferocissimum* and *Lycium procissimum* (Edwardson and Christie, 1997).

We collected ten symptomatic boxthorn plant samples from two greenhouses and open fields, and then performed reverse transcription (RT)-PCR for TSWV, broad bean wilt virus 2, and cucumber mosaic virus, belonging to major pepper-infecting viruses in Korea. As a result, they were found to be infected with TSWV using specific primer pairs (TSWV-5F: 5'-GCACAACACACAGAAAGCAAA-3', TSWV-6R: 5'-AGAGCAATCGTGTCAATTTTATTC-3') designed to amplify 1065 bp including the N gene based on reported nucleotide sequences of S segments of TSWV isolates in GenBank (Supplementary Table S1). Amplified fragments were obtained from all the leaf samples and directly sequenced. The nucleotide sequences among the ten isolates showed 98 to 100% identity to several pepper-infecting isolates from South Korea, including TSWV-YY (Genbank accession MF159073) and TSWV-CY (MF159063).

In order to isolate TSWV from boxthorn, sap from symptomatic leaves was inoculated mechanically to *Nicotiana tabacum* cv. 'Samsun' via three single-lesion passages. This procedure was followed by propagation in *N. rustica*, and the isolate was named TSWV_CY-LC. To investigate the host range of TSWV_CY-LC, sap from infected *N. rustica* was inoculated on 34 indicator plants, including boxthorn (Table 1). TSWV_CY-LC induced the following symptoms in 19 indicator plants (Fig. 2 & supplementary fig. S4): chlorotic ring spots, necrotic local lesions, and mosaic in *N. rustica* (Fig. 2a); necrotic local lesions and mosaic in *N. clevelandii*, *N. glutinosa*, and *N. occidentalis*; mosaic and distortion in *Capsicum annuum* (Fig. 2b); mosaic in *N. benthamiana* and *Solanum lycopersicum*; necrotic local lesions in

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Fig. 1 Symptoms of TSWV on leaves of boxthorn. (a) Necrotic ring spots and (b) chlorosis on the leaves of naturally infected boxthorn plants

Chenopodium quinoa, *N. debneyi*, *N. tabacum* cv. ‘Xanthi-nc’, and *Physalis angulata* L.; necrotic local lesions in inoculated leaves of *Petunia hybrida*, *Vicia faba*, *Cucurbita moschata*, and *Gomphrena globosa*; and pinpoint local lesions in inoculated leaves of *Chenopodium amaranticolor*.

Table 1 Symptoms observed on indicator plants mechanically inoculated with TSWV_CY-LC isolate from boxthorn

Indicator plant		Symptoms ^a	TSWV incidence ^b
Solanaceae	<i>Lycium chinense</i> Mill	cs, ns/cl, m	3/3
	<i>Nicotiana rustica</i>	nl,m,rs/nl,m,rs	3/3
	<i>Nicotiana clevelandii</i>	nl/nl,m	3/3
	<i>Nicotiana glutinosa</i>	nl/nl,m	3/3
	<i>Nicotiana occidentalis</i>	nl/nl,m	3/3
	<i>Nicotiana debneyi</i>	nl/nl	3/3
	<i>Nicotiana tabacum</i> cv. ‘Xanthi-nc’	nl/nl	3/3
	<i>Nicotiana benthamiana</i>	nl/m	3/3
	<i>Solanum lycopersicum</i>	–/m	2/3
	<i>Capsicum annuum</i>	nl/m,distortion	3/3
Leguminosae	<i>Physalis angulata</i> L.	nl/nl	3/3
	<i>Petunia hybrida</i>	ns/–	3/3
	<i>Vicia faba</i>	nl/–	3/3
	<i>Pisum sativum</i>	nl/nl	3/3
	<i>Phaseolus vulgaris</i>	–/–	0/3
	<i>Vigna unguiculata</i>	–/–	0/3
	<i>Vigna radiata</i>	–/–	0/3
Chenopodiaceae	<i>Vigna angularis</i>	–/–	0/3
	<i>Chenopodium amaranticolor</i>	pp, nl/–	3/3
	<i>Chenopodium quinoa</i>	nl/nl	3/3
Cucurbitaceae	<i>Cucurbita moschata</i>	nl/–	2/3
	<i>Cucumis melo</i>	–/–	0/3
	<i>Cucumis sativus</i>	–/–	0/3
	<i>Citrullus lanatus</i>	–/–	0/3
Amaranthaceae	<i>Gomphrena globosa</i>	nl/–	3/3
Brassicaceae	<i>Brassica campestris</i>	–/–	0/3
	<i>Brassica juncea</i>	–/–	0/3
	<i>Raphanus sativus</i>	–/–	0/3
Malvaceae	<i>Malva verticillata</i>	–/–	0/2
	<i>Alcea rosea</i>	–/–	0/2
	<i>Abelmoschus esculentus</i>	–/–	0/2
	<i>Hibiscus rosa-sinensis</i>	–/–	0/2
Aizoaceae	<i>Hibiscus mutabilis</i>	–/–	0/2
	<i>Tetragonia tetragonioides</i>	–/–	0/3

^a cl, chlorotic local lesions; cs, chlorotic spots; nl, necrotic local lesions; ns, necrotic spots; m, mosaic;

rs, ring spots; pp., pinpoint; –, no symptom; ^b No. of plant infected / No. of plant inoculated

On inoculated boxthorn plants, chlorotic and necrotic spots (Fig. 2c) and mosaic symptoms (Fig. 2d) were reproduced, which were similar to those in the original isolate. No symptoms were observed in an additional 15 indicator plants tested.

To genetically characterize TSWV_CY-LC, the complete genome sequence of TSWV_CY-LC was obtained using RT-PCR using the specific primers described in the supplementary Table S1 (Lee et al., 2011). Total RNA was extracted from the infected leaf samples using an Easy-spin Total RNA Extraction Kit (Intron Biotech., Seongnam, Korea), following the manufacturer’s instructions. Two-step RT-PCR was carried out using AMV reverse transcriptase (Promega, Madison, WI, USA) and high-fidelity LA Taq polymerase (Takara, Tokyo, Japan) for full-length genome sequencing. Tripartite full-length genomes of TSWV were amplified using virus-specific primer pairs. The sizes of each amplified fragment ranged from 0.8 to 1.2 kb, and each fragment overlapped with its neighboring fragments by at least 100 nucleotides. Terminal sequences were obtained following the protocol for 5′- and 3′- rapid amplification of cDNA ends (RACE; Boehringer Mannheim, Germany). cDNA clones containing the 5′-end of the genomes were obtained using Xec primer (5′-

Fig. 2 Symptoms on indicator plants inoculated mechanically with TSWV isolate CY-LC. (a) *Nicotiana rustica*, (b) *Capsicum annuum*, (c) *Lycium chinense* Mill (Hasoo cultivar), and (d) *Lycium chinense* Mill (Cheonghong cultivar)

AAAGAATCCCCCCCCCCCCC-3') and TSWV_5'-race-R primer complementary to the nucleotides of TSWV-L, -M, and -S RNAs. In addition, cDNA clones containing the 3'-end of the genomes were obtained using TSWV 3'-race-F primer complementary to the nucleotides of TSWV-L, -M, and -S RNAs and an anchor primer (5'-GACCACGCGTATCG ATGTCGACTTTTTTTTTTTTTTTTV-3'). Each PCR fragment was purified using a MEGA Quick-spin™ Kit (Intron, Korea) and cloned into the pGEM-T easy vector (Promega, Madison, WI, USA), according to the manufacturer's instructions, and transformed into *Escherichia coli* DH5α. Sequencing of purified recombinant cloned DNAs was performed by a commercial company (Bionics, Daejeon, Korea). The sequences obtained were assembled using DNA Star v. 5.02 (Lasergene, USA) and the assembled full-length sequences of L, M, and S segments were 8914, 4772, and 2972 nucleotides in length, respectively, and these were deposited in GenBank under the accessions MN064722, MN064723, and MN064724, respectively. The full-length sequences of each segment were aligned with those of previously reported isolates (Supplementary Table S2) using 'Geneious alignment' program in Geneious Pro 10 software. The phylogenetic trees based on full-length sequences of each segment were analyzed by the maximum likelihood (ML) method in MEGA 7 (Kumar et al. 2016). Phylogenetic analysis revealed that the sequences of each segment were clustered, respectively, with TSWV-L, -M, and -S RNAs of TSWV-YY (MF159049, MF159061, and MF159073) and TSWV-CY (MF1590393, MF159051, and MF159063) isolated from peppers in Korea with the highest nucleotide identities of 99%.

TSWV has been problematic in various crops in Korea since its first detection in 2003 (Kim et al. 2004). As boxthorn

may serve as an intermediate host of TSWV for other crops, such as pepper, the identification of TSWV infection in boxthorn can help prevent and control the disease in boxthorn and other crops susceptible to TSWV in Korea.

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