Diseases Caused by Viruses

First Report of a Resistance-Breaking Strain of Tomato Spotted Wilt Orthotospovirus Infecting *Capsicum annuum* Carrying the *Tsw* Resistance Gene in South Korea

J. Y. Yoon,¹ N. H. Her,² I. S. Cho,¹ B. N. Chung,¹ and S. K. Choi^{3,†}

¹ Department of Horticultural and Herbal Environment, National Institute of Horticultural and Herbal Science, RDA, Wanju, Jeollabuk-do, 55365, South Korea

² Department of Plant Pathology, The Kiban Institute, Anseong-si, Gyeonggi-do, 17513, South Korea

³ Department of Research Planning and Coordination, Rural Development Administration, Jeonju, Jeollabuk-do, 55365, South Korea

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Tomato spotted wilt orthotospovirus (TSWV) was first reported in 2004 from paprika in South Korea (Kim et al. 2004), where it is currently widespread. TSWV infections were reported in chili pepper, tomato, weeds, and ornamental plant species in South Korea (Choi and Choi 2015; Choi et al. 2014; Yoon et al. 2017, 2018, 2019). One of the best strategies for TSWV management is planting resistant cultivars containing the *Tsw* gene. In 2019 virus-like symptoms were observed in chili pepper (*Capsicum annuum*) plants bearing the *Tsw* gene in Anseong-si, South Korea. The infected chili peppers showed mosaic and wilting followed by necrosis on leaves and fruits in the field. To identify the causal virus, symptomatic leaf samples were analyzed using ImmunoStrip kits (Agdia, U.S.A.); we detected three pepper-infecting viruses: pepper mild mottle virus, cucumber mosaic virus, and TSWV. TSWV was only detected from 40 naturally infected chili pepper plants exhibiting virus-like symptoms. To further confirm the presence of TSWV (named TSWV-P1), we amplified reverse-transcription polymerase chain reaction (RT-PCR) products for L, M, and S RNA segments using tospovirus-specific and TSWV-specific primers (Batuman et al. 2014). Expected fragments of 445, 868, and 777 bp in length were amplified and sequenced. The complete genome sequences of TSWV-P1 from a symptomatic chili pepper plant were also determined using TSWV-specific primers (Choi et al. 2014; Lian et al. 2013). The complete genome sequences of TSWV-P1 were

deposited to GenBank (LC549179, LC549180, and LC549181). The sequences of each fragment were identical to a consensus sequence, showing 99.1, 98.5, and 98.6% identity with TSWV-L, M, and S RNA (KP008132, AY744492, and KP008134), respectively. These results clearly showed only a single TSWV infection among the naturally infected chili pepper plants, without reassortment between TSWV and another tospovirus. To confirm whether TSWV-P1 is a resistance-breaking (RB) strain, *Nicotiana rustica* was mechanically inoculated with sap from leaves of the infected pepper samples to propagate TSWV-P1. A non-RB TSWV isolate (TSWV-Kor-lisianthus) from lisianthus was used as a control (Yoon et al. 2017). Two resistant (with *Tsw*) and two susceptible chili pepper cultivars (20 plants per cultivar) were mechanically inoculated with sap from leaves of the TSWV-infected *N. rustica*. The incidence rates of disease caused by TSWV-P1 were 90 to 100% for resistant and 95 to 100% for susceptible cultivars. In contrast, TSWV-Kor-lisianthus caused symptoms only in the susceptible pepper cultivars (90 to 100% incidence). TSWV infection in representative plants was confirmed using the TSWV-ImmunoStrip kit and RT-PCR. The NSs gene of TSWV-P1 consists of 1,404 nucleotides (468 amino acids); sequence analysis of the TSWV-P1 NSs gene showed high nucleotide (99.7%) and amino acid identities (99.8%) with the NSs sequences of two TSWV isolates (FR693035, CBX24121). Protein sequence analysis of TSWV-P1 NSs revealed that no amino acid mutation was associated with those of a representative TSWV RB strain, as previously described (Almási et al. 2017), suggesting that TSWV-P1 is an RB strain. Because this TSWV-P1 can overcome resistance conferred by the *Tsw* gene in commercially grown chili pepper cultivars, it represents a potential threat to pepper production in South Korea.

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[†]Indicates the corresponding author.
S. K. Choi; viroid73@gmail.com