The First Report of *Tomato spotted wilt virus* in Korea

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Thirteen virus species in the genus *Tospovirus* are reported worldwide so far. *Tomato spotted wilt virus* (TSWV) virions are spherical enveloped particles, about 80-110 nm in diameter and associated with endoplasmic reticulum. Nucleotide sequence and translational analyses show the large (L) genomic RNA of TSWV to be negative-stranded (de Haan *et al.*, 1991) and the other two (S and M) RNAs to be ambisense (de Haan *et al.*, 1990; Kormelink *et al.*, 1992). TSWV transmitted by thrips is common in temperate and subtropical regions throughout the world (Ie, 1970). It has a very broad host range estimated to be up to 900 plant species within 80 families and has a worldwide distribution (Goldbach and Peters, 1994). In peppers, it causes bright yellowing and chlorotic rings on the leaves. The incidence in pepper and Tomato production areas can be as high as 60% (Greenough *et al.*, 1990). Twenty-five weed species are considered important reservoirs of TSWV including *Malva parviflora, Verbescina enceloides, Bidens pilosa* and *Ipomoea congesta* (Cho *et al.*, 1986).

Analyses of purified viral particles show that the envelope is lipidic in nature. It surrounds three types of single-stranded RNA molecules, denoted, in relation to their size, S RNA (2.9 Kb), M RNA (5.4 Kb) and L RNA (8.9 Kb) (van Den Hurk *et al.*, 1977; Mohamed, 1981; de Haan *et al.*, 1990, 1991). Four structural proteins with different molecular masses (28.8, 58, 78 and 331.5 kDa) are found in the viral particles. The 28.8 kDa protein named as nucleocapsid N protein, is tightly associated with the RNA genome, both forming stable pseudopcircular structure, the nucleocapsid. These nucleocapsids with few copies (between 10 and 20) of the 311.4 kDa protein denoted L protein, form the interior of the viral particle. The 78 and 58 kDa proteins named as G1 and G2, respectively, are glycosides. They are located on the viral particle surface forming the spikes observed under the electron microscope (Mohamed *et al.*, 1973; Tas *et al.*, 1977; Mohamed, 1981; van Poelwijk *et al.*, 1993).

The complete nucleotide sequence of the genome of TSWV Brazilian isolate BR01 was recently determined. The L RNA segment has negative polarity (-). Thus the viral

complementary strand (+) has to be copied before the L RNA segment can be read. A promoter sequence not coded by the virus is added in this transcription process. This promoter is necessary to start the translation process. The L RNA viral complementary strand contains a single open reading frame (ORF) (de Haan *et al.*, 1990). This ORF codes for the L protein, which is a RNA-polymerase RNA-dependent (Adkins *et al.*, 1995). Both M and S RNA segments have an ambisense codifying strategy, each of them having two ORF separatedby a central inter-cistonic region rich in A-U links and bent as a curl. The M RNA encodes in the viral sense a non-structural protein called NSm (33.6 kDa) and in the viral complementary sense a precursor of the two glycopoteins. The S RNA encodes in the viral sense a non- structural protein named as NSs (53.4 kDa) and in the viral complementary sense the nucleocapsid N protein. Recently, the genus *Tospovirus*consists of five serogroups based on the serological cross reactivity and sequence homology of the N protein. Serogroup I consists of TSWV (de Haan *et al.*, 1990; Maiss *et al.*, 1991), which reacts weakly with the antibodies to Serogroup II members [TSWV-B; *Groundnut ringspot virus* (GRSV) and *Tomato chlorotic spot virus* (TCSV)] (de Avila *et al.*, 1993; Pang *et al.*, 1993) and do not react with antibodies to INSV, serogroup III members (Law *et al.*, 1990). The serogroup IV members, *Groundnut bud necrosis virus* (GBNV) and *Watermelon silver mottle virus* (WSMoV) are serologically distinct from the members of serogroups I, II and III (Adam *et al.*, 1993). The N protein of TSWV (serogroup I) showed 77-78% sequence identity with GRSV and TCSV (serogroup II) (de Avila *et al.*, 1993), 55% sequence identity with *Impatiens necrotic spot virus* (INSV) (serogroup III) (de Haan *et al.*, 1992) and 33-35% sequence identity with *Peanut bud necrosis virus* (PBNV) and WSMoV (serogroup IV) (Yeh and Chang, 1995).

In this study, A TSWV was isolated from Paprika (*C. annuum* var. grossum) showing malformation on the leaves and necrosis spot on the fruit. The virus could infect systemically on the ten indicator plants including *C. amaranticolor*. TSWV-KP produced necrosis or necrotic ring spots on the inoculated leaves and mosaic, vein necrosis or death on the upper leaves on *D. stramonium*, *N. clevarandii*, *N. rustica* and *N. tabacum* cvs. In ultrastructural studies, thetypical tospovirus particles were observed in the cytoplasm. The virion contained three molecules of genomic RNAs, which were approximately 9.0 kb, 4.9 kb and 3.0 kb. The nucleocapsid (N) protein of the purified virion migrated as a single band with molecular weight of about 29 kDa in SDS-PAGE. The complete nucleotide sequence of the large (L) genome segment of TSWV-KP were

determined, and defective forms of L RNA containing core polymerase region were observed. The 8,917 nucleotides of L RNA contains a single open reading frame (ORF) in the viral complementary (vc) strand, and encodes a protein of 330 kDa. The L protein had high identity in the 'core-polymerase domain' with the corresponding regions of other tospoviruses. The complete nucleotide sequence of TSWV-KP M RNA was 4,768 nucleotides long and indicated the typical tospovirus with the presence of two genes in ambisense arrangement. The vRNA OFR coded for the potential cell-to-cell movement (NSm) protein (34.8 kDa) and the vcRNA ORF for the viral glycoprotein (G1/G2) precursor (128.0 kDa). Multiple sequence alignment of the M RNA showed highest homologies to TSWV-BR01. The amino acid sequence of the TSWV-KP NSm and G1/G2 exhibited 48.7-85.3% and 34.9-96.2% identity, respectively. The TSWV-KP S RNA was 2,991 nucleotides long and had as ambisense coding strategy. The sequence contains two open reading frames (ORFs), one in the viral sense, which encoded a protein with a predicted Mr of 52.4 kDa and another in the viral complementary sense, which encoded the viral nucleocapsid protein of Mr 28.8 kDa. The amino acid sequence of TSWV-KP of S RNA NSs and N exhibited 35.9-87.9% and 19.9-98.4% identity, respectively.