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Note

Outbreak of *Cucumber mosaic virus* and *Tomato spotted wilt virus* on Bell Pepper Grown in Jeonnam Province in Korea

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In August 2006, a severe disease incidence showing mosaic and/or necrotic symptoms on two bell pepper varieties including red-colored 'Special' and yellow-colored 'Fiesta' was observed in a greenhouse located in Gwangyang, Jeonnam province, Korea. To identify causal viruses, total RNAs were extracted from 11 fruit samples with and without symptoms. Specific oligonucleotide primers for *Cucumber mosaic virus* (CMV), *Pepper mottle virus* (PepMoV), *Tomato spotted wilt virus* (TSWV) and *Pepper mild mottle virus* (PMMoV) were designed based on the sequences available on GenBank. Database comparisons of the deduced amino acid sequences of each sequence produced 100% and 98% matches with nucleocapsid protein gene of TSWV (Acc. No. ABE11605) and coat protein gene of CMV (Acc. No. DQ018289), respectively, suggesting that the symptoms on bell pepper fruits might be caused by the infection of CMV and TSWV. To our knowledge this is the first report of necrotic as well as mosaic virus disease on bell pepper fruits by the infection of CMV and TSWV in Jeonnam province, Korea.

Keywords **:** bell pepper, CMV, Jeonnam province, necrotic symptom, SWV, virus infection

Bell pepper (paprika) is a sweet-to-mildly hot cultivar of the chilli pepper (*Capsicum annuum*). Paprika has become more popular as an important vegetable in Korea since its first cultivation in Jeju Island in 1994. Thus, the crop cultivation area has increased steadily over the past decade. However, virus diseases are currently causing yield and quality losses of paprika fruit. In this study, we describe the natural infection of two paprika cultivars by CMV and TSWV in Korea. As shown in Fig. 1, a severe disease incidence showing mosaic and/or necrotic symptoms on two bell pepper varieties including red-colored Special and yellow-colored Fiesta was observed in the greenhouse located in Gwangyang, Jeonnam province, in 2006. We obtained 11 different fruit samples, that showing differences of symptom development including mosaic, severe mosaic, and necrosis. Mosaic symptoms are similar to those described for virus disease caused by *Cucumber mosaic virus* (CMV), *Pepper mottle virus* (PepMoV), and *Pepper mild mottle virus* (PMMoV) infection reported in Korea (Yoon et al., 2005). Severe mosaic and necrotic symptoms looked associated with *Tomato spotted wilt virus* (TSWV), a *Tospovirus* recently found in *Capsicum annum* species (Cho et al., 1989; Warrwn and Murphy, 2003).

To identify the causal viruses, specific oligonucleotide primers were designed for CMV, PepMoV, TSWV, and PMMoV based upon viral sequences obtained from the GenBank of the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov). The designed primer sequences, Tm values and expected amplified fragment sizes of each primer set are listed in Table 1. Total RNAs were extracted from unknown virus-infected paprika fruits using Trizol reagent (Molecular Research Center, Inc., USA). cDNA was synthesized as described by previously (Choi et al., 2007). Briefly, first strand cDNA synthesis was carried out in a 20 μl reaction volume using 1st Strand cDNA Synthesis Kit for RT-PCR (Boehringer-Mannheim, Germany) according to the manufacture's instructions with 10 μg total RNAs from fruit samples as a template using specific primer complementary to CMV, PepMoV, TSWV, and PMMoV RNAs. The designed sequences are as follows: CMV forward 5'-CTCATGGA-TGCTTCTCCGC-3' and reverse 5'-CAACTCAGATCC-CGCCACAGA-3'; PepMoV forward 5'-CCATAACAC-TATTCATGCTTACC-3' and reverse 5'-CAACATACTC-TTTCCATACGCC-3'; TSWV forward 5'-GAGATTCTC-AGAATTCCCAGT-3' and reverse 5'-AGAGCAATCGT-GTCAATTTTATTC-3'; and PMMoV forward 5'-TAAAT-GGCGTTAGTAGTCAAGGA-3' and reverse 5'-TGGGCC-GCTACCCGCGGTTCGGGG-3'.

PCR products were amplified by PCR using the virus-

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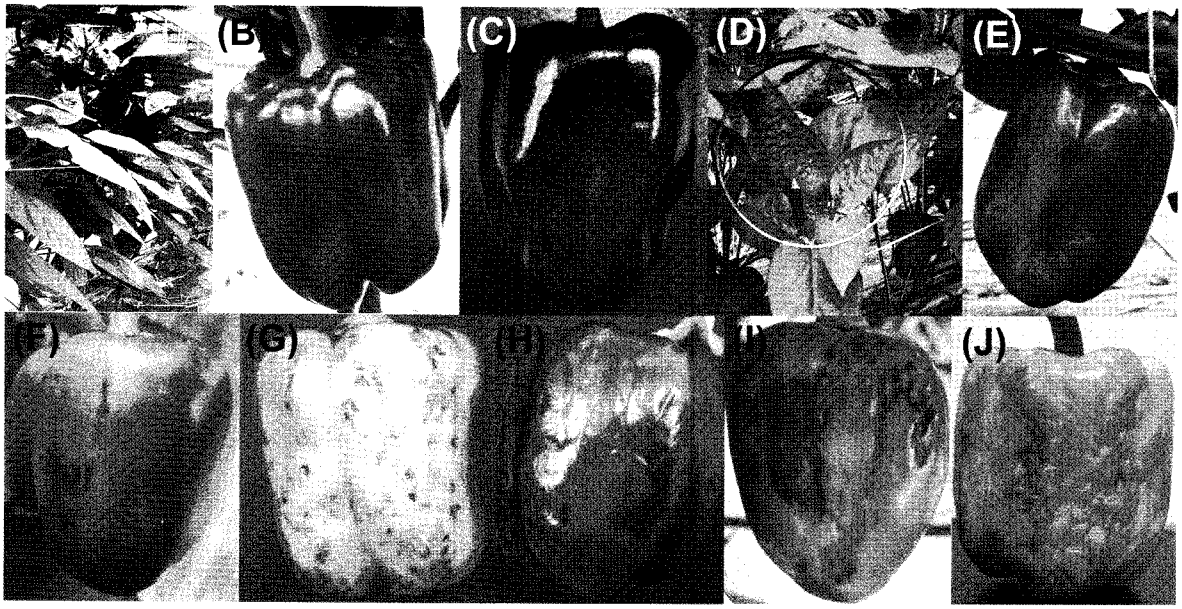

Fig. 1. Bell pepper fruit naturally infected by CMV and TSWV. (A) Bell pepper plant cultivated in a greenhouse, (B and C) healthy fruits of bell pepper; (D-I) CMV symptoms showing a typical symptom of leaf mosaic and leaf veinal necrosis (D) and different fruit necrosis (E-I), and (J) typical spotted wilt caused by TSWV.

Table 1. Nucleotide sequences of oligonucleotide primers used in reverse transcription-polymerase chain reaction assays

Primer	Sequence	Tm (°C)	Length (bp)
CMV-CP 5'	5'-CTCATGGATGCTTCTCCGC-3'	55°C	203
CMV-CP 3'	5'-CAACTCAGATCCCGCCACAGA-3'	59°C	
PepMoV-CP 5'	5'-CCATAACACTATTCATGCTTACC-3'	50°C	1,134
PepMoV-CP 3'	5'-CAACATACTCTTTCCATACGCC-3'	53°C	
TSWV-CP 5'	5'-GAGATTCTCAGAATTCCCAGT-3'	49°C	492
TSWV-CP 3'	5'-AGAGCAATCGTGTCAATTTTATTC-3'	53°C	
PPMoV-CP 5'	5'-TAAATGGCGTTAGTAGTCAAGGA-3'	53°C	473
PPMoV-CP 3'	5'-TGGGCCGCTACCCGCGGTTCGGGG-3'	79°C	

specific primer sets with reaction condition consisting of initial denaturation at 94°C for 5 min; 30 cycles, each consisting of denaturation at 94°C for 30s, annealing at 59°C (CMV), 51°C (PepMoV), 52°C (TSWV), and 58°C (PMMoV) for 3 min; and final extension at 72°C for 10 min. The PCR product was purified and cloned into pGEM-T EASY vector (Promega, USA). The plasmid that contained cDNA inserts of the correct sizes (203, 1134, 492, and 473 bps for CMV, PepMoV, TSWV, and PMMoV, respectively; Table 1) was selected for nucleotide sequencing. All clones were sequenced in both orientations at least two times. The fragments sequenced using the ABI prism-TM Terminator Cycle Sequencing Ready Reaction kit and ABI Prism 3700 Genetic Analyzer (Perkin Elmer, USA) located at the NICEM, Seoul National University according to the manufacture's instructions. Sequence analysis was carried out using the Clustal W algorism of LaserGeneTM program (DNASTAR Inc.).

CMV and TSWV specific DNA fragment containing

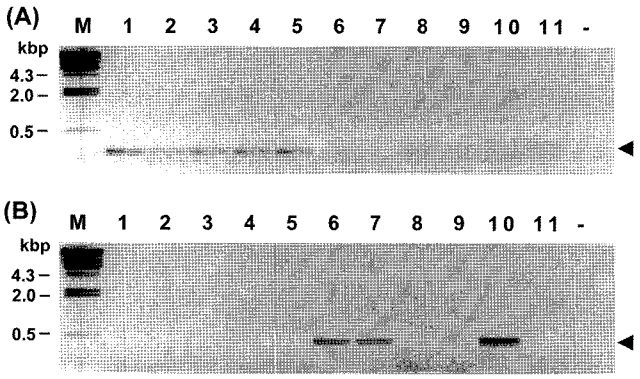

Fig. 2. RT-PCR detection of CMV and TSWV using virus-specific primer sets. (A) CMV detection. dsDNA band corresponding CMV CP region was amplified in samples 1, 2, 3, 4, 5 and 8. (B) TSWV detection. TSWV was detected in samples 6, 7 and 10. Total RNAs were extracted from healthy and symptomatic bell pepper fruits and subjected to RT-PCR analysis using both CMV and TSWV specific primers. Position of amplified dsDNA is indicated by arrowhead.

capsid protein (CP) gene was successfully amplified by RT-PCR using each specific primer set. Total RNAs from 9 paprika fruits produced a PCR fragment of the expected size (203 and 492 bps for CMV and TSWV, respectively; Fig. 2). Two independent clones were generated for each selected sample. The CP gene sequences of CMV and TSWV were determined and used for sequence analyses. No differences in nucleotide sequences were found between independent clones representing the same strain. Not surprisingly CMV and TSWV displayed 100% and 98% matches with nucleocapsid protein gene of TSWV (Acc. No. ABE11605) and CP gene of CMV (Acc. No. DQ018289), respectively. No signal was observed with either primer for PepMoV and PMMoV.

It has been reported that the average disease incidence of paprika virus disease is around 4.5-17.5% and is caused by PepMoV, *Broad bean wilt virus 2* (BBWV2), PMMoV, CMV and some other unknown viruses in Jeonnam province, but infection rate and kind of viruses were different upon the paprika cultivating provinces (Ko, S.-J., personal communication). Recently, *Potato virus Y* (PVY) was also isolated from paprika and its characteristics to the other PVY strains was compared using biological, serological, and cytological properties (Choi et al., 2005b). They also reported that virus diseases were more severe on the vinyl house cultivation than on the green house cultivation. CMV is characterized by an extremely broad host range and high variability. Hundreds of isolates have been identified from a number of plant sources. In 1999, a CMV was isolated from paprika fruit showing malformation in a plastic house in Pyungchang, alpine area of Korea (Kim et al., 2002). In a survey on virus diseases of paprika in 2003 in Yesan, Korea, a TSWV isolate causing fruit malformation and necrotic spots in leaves was identified (Kim et al., 2004). In this study, we identified natural infection of paprika by CMV and TSWV based on the symptoms on bell pepper fruits showing necrotic and mosaic virus disease on bell pepper fruits in Gwangyang, Jeonnam province, in 2006. These results suggest that CMV and TSWV transmitted to the southern part, major paprika production area, in Korea and might have changed to produce more severe symptom causing necrosis along with malformation on fruits since their first report in Pyungchang and Yesan in 1999 and 2003, respectively.

Earliest efforts in identifying viruses were based on its biological and physico-chemical properties such as disease symptoms, transmission characteristics, serology, and EM observation. However, in some host different viruses could cause very similar symptoms while similar strains of one virus could cause very different symptoms (Matthews, 1991). Recently, RT-PCR and subsequent sequencing of amplified nucleotide using virus or strain specific primers have been used for detection and strain identification of many viruses to overcome these difficulties (Hadidi et al., 1995; Kim, 1999). RT-PCR analysis using specific oligonucleotide primers proved to be useful for the detection of TSWV and CMV in infected paprika fruits. It remains, however, to be determined how much variation was introduced in TSWV genome since 2003 and how it moved to southern parts in Korea. In this regard, it is worth to mentioning the result of a survey on pepper, a close relative of paprika, virus diseases in 31 regions including Jeonnam province in Korea from November 2001 to December 2004 (Choi et al., 2005a). Among 1,056 samples collected, 343 (32.5%) were infected with CMV, 209 (19.8%) with PepMoV, 141 (13.4%) with PMMoV, and 5 (0.5%) with TSWV. It is possible that CMV and TSWV as well as the other unidentified viruses infecting paprika might come from pepper plants cultivating nearby. Full genome analysis of virus isolates and more extensive analysis on virus disease incidences caused by CMV and TSWV as well as the other viruses will shed light on further understanding on RNA virus variation and will provide more information on paprika virus disease epidemic in Korea.

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