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Pepino mosaic virus, a first report of a virus infecting tomato in Syria

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Summary. This is the first report of *Pepino mosaic virus* (PepMV) occurring in tomato plants grown in plastic greenhouses in a Mediterranean city in Syria. One tomato fruit from sixty samples tested positive for this virus by DAS-ELISA. Biotest assay, RT-PCR, and sequencing confirmed the presence of PepMV. The highest sequence identity of the Syrian isolate was with the EU-tomato strains of PepMV.

Key words: *Solanum lycopersicum*, viral symptoms, DAS-ELISA, sequencing, plastic greenhouses.

Pepino mosaic virus (PepMV) is currently one of the most important viral pathogens of tomato (*Solanum lycopersicum* L.) causing loss of fruit quality and yield worldwide. The virus induces various symptoms on the leaves, such as mosaic, mottling, bubbling, distortion, and spiky or nettle-like heads. The fruits show discoloured yellow spots, stripes or marbling. Thus far, four strains of PepMV (PE, EU, US1/Ch1, Ch2) have been identified (Ling, 2007; Hanssen *et al*., 2008; Hasiów *et al*., 2008; van der Vlugt, 2009).

Tomato is one of the fastest growing crops in Syrian horticulture. Due to its high productivity and relatively low cost, tomatoes are widely cultivated in open fields. Increasing production in glasshouses and tunnels provides an indispensable source of year-round income for many farmers as tomato is the most important exported vegetable in Syria (FAO Statistics division, 2006).

In winter 2007/2008, tomato fruits were collected from the main markets in different cities in Syria covering the South (Damascus), North (Aleppo and Idlib), East (Deir Ezzor), and West (Latakia and Tartous).

Tomato fruit samples from Latakia and Tartous were tunnel-produced vegetables, and these two cities are situated near the Mediterranean Sea. Due to the climate of this part of the country farmers produce large amounts of different vegetables in winter because only little heating of greenhouse tunnels is necessary and vegetable prices are high. Samples obtained in winter from the other Syrian cities consist of tomatoes that are collected at the end of summer and are kept in refrigerators to get higher prices.

Sixty samples were tested using double-antibody sandwich DAS-ELISA with specific antisera AS-0632 (DSMZ GmbH, Germany), according to manufacturer's instructions.

DAS-ELISA revealed that one fruit sample from Latakia was PepMV-infected. The mean absorbance values for the positive sample and the negative control were 0.31 and 0.09 respectively.

The fruit extract containing celite was mechanically inoculated onto the leaves of three indicator plants (*Nicotiana benthamiana* L.). Inoculated plants and mock-inoculated controls

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were maintained in the greenhouse (conditions: 18–24°C, under a long day; RH 65%) and monitored for symptom development.

Three weeks after inoculation the indicator plants showed mosaic symptoms and PepMV infection was confirmed by DAS-ELISA and the reverse transcription polymerase chain reaction (RT-PCR), applying two PepMV-specific primer sets. For RT-PCR, total RNA was extracted with the NucleoSpin RNA plant kit (Macherey and Nagel, Düren, Germany) according to manufacturer's instructions. From 2 μg of total plant RNA, cDNA was generated with 100 units of M-MuLV-RTase (Fermentas, St. Leon-Rot, Germany) primed with 100 pmol of the PepMV-specific reverse primers PepMV-UTR-R and Pep4. Using primers PepMV-TGB-F/PepMV-UTR-R (Mumford and Metcalfe 2001), 846 bp of the genome including the coat protein (CP) sequence (714 bp) was amplified in PCR. The Pep3/Pep4-generated fragment (625 bp) covered parts of the RNA-dependent RNA polymerase- and triple-gene-block I-genes (Pagán *et al*., 2006). PCR-products were ligated into pBluescript II SK(-) based T-vectors and transformed into chemocompetent *E. coli* (XL1-Blue MRF´, Agilent Technologies, Waldbronn, Germany) using standard protocols (Sambrook *et al*. 1989). Plasmids from two clones of each vector construct were purified and sequenced from both directions by applying a BigDye® Terminator v1.1 Ready Reaction Cycle Sequencing Kit using standard protocols and an ABI PRISM® 310 Genetic Analyzer from Applied Biosystems (Foster City, CA, USA). Sequences were assembled in BioEdit 7.0.9.0 (Hall, 1999), and submitted to the European Molecular Biology Laboratory (EMBL, Heidelberg, Germany) database. Sequence alignments and comparisons with selected PepMV sequences retrieved from BLAST search (Altschul *et al*., 1997) at the National Center for Biotechnology Information (NCBI, Bethesda, MD, USA) were done with ClustalX 1.83 (Thompson *et al*., 1997).

The PepMV isolate from Syria revealed a nucleotide identity of between 99.4 and 99.8% of the CP-coding region (GenBank Accession No. FN178521) with EU genotypes of PepMV (Accessions No. AJ438767, AJ606360, AF484251, AM113795, AM113829, AM930243). The identity with other genotypes was lower, with 96.6% to the PE genotype (Accession No. AJ606361), 83.0% to the US1 genotype (Accession No. AY509926), and 78.2% to the Ch2 genotype (Accession No. DQ000985). The finding that the Syrian PepMV-isolate represented an EU genotype was supported by the second fragment (Accession No. FN178522) which showed a maximum nucleotide identity of 99.1% with the EU genotypes (Accessions No. EF408822, AJ438767), and an identity below 96% with the isolates of the other genotypes. Although PepMV infection was confirmed in only one tomato fruit sample from Latakia, the virus nevertheless poses a threat to the tomato growing industry in Syria.

Further studies are needed to determine the frequency of PepMV in Syrian tomatoes and the occurrence of the strains, which can easily spread through the country.

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