

## DISEASE NOTE

**MELOCHIA CORCHORIFOLIA, A NEW HOST OF 16SRI-B PHYTOPLASMA IN CHINA**

W. Chen, Y. Li, F. Liu, L.Y. Zeng, L. Ren, L. Xu,  
R.K. Chen and X.P. Fang

*Oil Crops Research Institute of Chinese Academy of Agricultural Sciences, Key Laboratory of Biotechnology and Genetics Improvement of Oil Crops, Ministry of Agriculture, Wuban 430062, Hubei, China*

*Melochia corchorifolia* L. is a herb and weed commonly found in China. In August 2015, three *M. corchorifolia* plants displaying symptoms of phytoplasma infection such as witches'-broom, virescence and phyllody were found in two wastelands in Haikou, Hainan province, China. All symptomatic and three asymptomatic plants were collected for phytoplasma detection and classification (Dickinson and Hodgetts, 2013). Phytoplasma diagnosis was performed by nested PCR using phytoplasma universal primers P1/P7 and R16F2n/R16R2. Fragments *ca.* 1.8 (P1/P7) and 1.2 kb (R16F2n/R16R2) in size were only observed in symptomatic samples. For phytoplasma classification, gene fragments were amplified using R16F2n/R16R2 primers for 16S *rRNA*, rp(I)F1A/rp(I)R1A for *rp*, and fTufu/rTufu (Schneider *et al.*, 1997) for *tuf* genes, respectively; three clones of each were sequenced. The consensus sequences were submitted to GenBank with the accession numbers KX150461 (16S *rRNA*), KX158198 (*rp*) and KX158199 (*tuf*). A phylogenetic tree based on the partial 16S *rRNA* gene sequences (1246 nt) from *M. corchorifolia* phytoplasma (Mcp) and other phytoplasma group representatives was built by MEGA 5.0 using neighbor-joining method. It showed that Mcp clustered with 'Candidatus Phytoplasma asteris' (16SrI group) phytoplasmas. Furthermore, virtual RFLP analyses were performed by *iPhyClassifier* and Vector NTI. 16S *rRNA*, *rp* and *tuf* gene RFLP profiles of Mcp were consistent with the RFLP profiles of 16SrI-B, rpI-B and tuf-B subgroup phytoplasmas. To our knowledge, this is the first report on *M. corchorifolia* phytoplasma worldwide.

Dickinson M., Hodgetts J., 2013. *Phytoplasma Methods and Protocols*. Humana press Inc, Totowa, USA.

Schneider B., Gibb K.S., Seemuller E., 1997. Sequence and RFLP analysis of the elongation factor Tu gene used in differentiation and classification of phytoplasmas. *Microbiology* **143**: 3381-3389.

Corresponding author: X.P. Fang  
E-mail: xpfang2008@163.com

Received September 19, 2016  
Accepted December 7, 2016

## DISEASE NOTE

**FIRST REPORT OF TOMATO SPOTTED WILT VIRUS IN HOT PEPPER IN PAKISTAN**

M. Ashfaq and A. Ahmad

*Department of Plant Pathology, PMAS-Arid Agriculture University Rawalpindi-46300, Pakistan*

*Tomato spotted wilt virus* (TSWV, genus *Tospovirus*) is one of the most devastating plant viruses which is known to infect several plant species in more than 84 different plant families (Parrella *et al.*, 2003). In June 2014, five hot pepper (*Capsicum annuum* L.) leaf samples showing chlorosis, chlorotic spots and tip necrosis were collected from Islamabad district. All symptomatic samples were tested positive for TSWV by using TSWV-specific AgriStrip assay (Bioreba AG, Switzerland) whereas two leaf samples from healthy plants were negative. The presence of TSWV in pepper samples was further confirmed by RT-PCR using TSWV-specific primers CP5-Bam and CP3-Pst (Antignus *et al.*, 1997). All five PCR amplicons were purified using QIAquick® PCR purification kit (Qiagen) and subsequently sequenced in both orientations. A total of 777 nucleotides of nucleocapsid protein (N) gene were obtained from each amplicon. All five sequences were 100% identical and the sequence of TSWV isolate hot pepper (AAICPK) from Pakistan was submitted to GenBank as Accession No. KX121046. BLAST analysis revealed 99% sequence identity with TSWV pepper isolates from France (FR693046), Hungary (KJ649612), Italy (GU369717, GU369722, DQ431238) and South Korea (HQ260982, HQ267713). This destructive virus has been previously reported to infect tomato crops in Pakistan (Hassan, 1995). To the best of our knowledge, this is the first confirmed report of TSWV in hot pepper crops in Pakistan.

Antignus Y., Lapidot M., Ganaim N., Cohen J., Lachman O., Pearlman M., Raccach B., Gera A., 1997. Biological and Molecular Characterization of *Tomato spotted wilt virus* in Israel. *Phytoparasitica* **25**: 319-330.

Hassan S., 1995. Investigations on virus diseases of tomato in Malakand, Pakistan. 5<sup>th</sup> National Conference of Plant Scientists, NARC, Islamabad, Pakistan. March 28-30, 1995: 115-116.

Parrella G., Gognalons P., Gebre-Selassie K., Vovlas C., Marchoux G., 2003. An update of the host range of *Tomato spotted wilt virus*. *Journal of Plant Pathology* **85**: 227-264.

Corresponding author: M. Ashfaq  
E-mail: mashfaq1642@gmail.com

Received September 23, 2016  
Accepted November 29, 2016