

## DISEASE NOTE

**MIXED INFECTION BY *EGGPLANT MOTTLED DWARF VIRUS* AND AN OPHIOVIRUS SPECIES IN JAPANESE PITTOSPORUM**

**M. Morelli<sup>1</sup>, A. De Stradis<sup>1</sup>, A. Minafra<sup>1</sup>, P. Saldarelli<sup>1</sup> and G.P. Martelli<sup>2</sup>**

<sup>1</sup>CNR Istituto per la Protezione Sostenibile delle Piante (IPSP), UOS Bari, 70126 Bari, Italy

<sup>2</sup>Dipartimento di Scienze del Suolo, della Pianta e degli Alimenti, Università degli Studi di Bari Aldo Moro, 70126 Bari, Italy

In spring of 2014, an outstanding vein yellowing sometimes expanding to the interveinal tissues, was observed on the foliage of a Japanese pittosporum (*Pittosporum tobira*) plant growing in a private garden at Bari (Apulia, Italy). These symptoms resembled very much those induced by *Eggplant mottled dwarf virus* (EMDV), a nucleorhabdovirus endemic in the Mediterranean region that affects also *P. tobira* (Martelli *et al.*, 2011). Electron microscope observations of ultrathin sections from symptomatic pittosporum leaves showed the consistent presence of enveloped bacilliform or bullet-shaped particles accumulating in dilations of the nuclear envelope, whereas leaf dips revealed the presence of coiled filaments *ca.* 3 nm in diameter resembling ophiiovirus-like virions. A next generation sequencing analysis of small RNA libraries from the symptomatic pittosporum plant yielded a number of contigs matching the genome of both EMDV and of the ophiiovirus species *Ranunculus white mottle virus* (RWMV) genome (Vaira *et al.*, 1997). Two primer sets were therefore designed on the assembled contigs and used for RT-PCR amplification. Products of the expected size (426 and 400 bp for EMDV and RWMV, respectively) were amplified and sequenced. BLAST analyses showed that the cloned sequences, deposited in GenBank under the accession Nos. KT211397 and KT211398, shared *ca.* 96% identity at the nucleotide level with the sequence of an EMDV isolate from *Agapanthus* sp. (KJ082087) and of 91% with that of a RWMV isolate (AY542957). To our knowledge, this seems to be the first record of a mixed infection by a rhabdo- and an ophiiovirus in *P. tobira*.

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Corresponding author: M. Morelli  
E-mail: massimiliano.morelli@ipsp.cnr.it

Received June 28, 2015  
Accepted June 29, 2015

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**FIRST REPORT OF A NEW “*CANDIDATUS PHYTOPLASMA AUSTRALASIA*”-RELATED STRAIN IN *CAPSICUM ANNUUM* IN INDIA**

**A. Sharma, S. Sharma and S. Kang**

Department of Plant Pathology, Department of Vegetable Science Punjab Agricultural University, Ludhiana- 141 004, Punjab

Plants of *Capsicum annuum* from an experimental plot at Punjab Agricultural University (Ludhaina, India) showing phyllogly, abnormal flower development, yellowing, reduced leaf size and short internodes in 2014 were investigated for the presence of phytoplasmas. DNA was extracted from symptomatic and symptomless plants and used in PCR with universal phytoplasma primers P1/P7 (Deng and Hiruki, 1991; Smart *et al.*, 1996) and in nested PCR with primers R16F2n/R16R2 primers (Lee *et al.*, 1993). Results showed the presence of phytoplasmas in all symptomatic plants tested. Three samples were cloned and their F2nR2 region was sequenced (GenBank accession No. KP120878). This sequence clustered with the 16SrII (Peanut witches' broom) group members in BLAST search. For subgroup characterization, *in silico* restriction fragment length polymorphism (RFLP) profiles were generated using the iPhyClassifier program, resulting in the identification of a strain related to the 16Sr II-D reference strain '*Candidatus Phytoplasma australasia*' (Y10097). *In silico* analysis of the sequence obtained from the chilli phytoplasma showed a pattern different from those of established 16Sr group and subgroup strains. The reference pattern of the 16Sr group II, subgroup D (Y10097) shared a similarity coefficient of 0.95, which placed it in a new subgroup, 16Sr II-N (Zhao *et al.*, 2009). Further comparison of *in silico* RFLP patterns within subgroup II strains identified *HbaI* and *MseI* to distinguish the new subgroup pattern. Restriction digestion of the 1.8 kb fragment with *HbaI* and *MseI* confirmed the expected *in silico* RFLP pattern. To our knowledge, this is the first report of '*Ca. P. australasia*'-related strain associated with chilli phyllogly.

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Corresponding author : S. Sharma  
E-mail : shikhasharma-coapp@pau.edu

Received June 28, 2015  
Accepted June 29, 2015