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Disease Notes



First Report of 16SrXII-A Subgroup Phytoplasma (Stolbur) Associated with Reddening of *Oenothera biennis* in Serbia

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Abstract

Evening primrose (*Oenothera biennis* L.) is a biennial medicinal, edible, and ornamental plant species. It has attracted great interest for its seed oil that contains gamma linolenic acid, thus distinguishing this plant as a main commercial source of this essential fatty acid (4). This species has been grown as a permanent member of a medicinal plant collection established near Backi Petrovac (northern Serbia) for 22 years. The first disease symptoms were recognized as red spots on leaf rosette in July 2011, spreading gradually during vegetative growth and covering 1/3 to 1/2 of the leaf surface. Symptoms, observed on 16% of the plants (32 of 200) in the second half of May 2012 and on 23% (69 of 300) at the beginning of May 2013, appeared as reddening of

lower leaves of flower-bearing stems. Affected plants exhibited stunted growth, while reddening spread over other leaves of flower-bearing stems. In severely affected plants, the flower-bearing stems were poorly developed, frequently forming witches' brooms. For that reason, 30 reddened and 20 symptomless leaves (2 leaves per plant) were sampled in both July 2012 and 2013 and total nucleic acids were extracted. Direct PCR assays were performed using phytoplasma universal primer pair P1/P7 (2) to amplify 1,800-bp fragments (the 16S rRNA gene, the 16S-23S intergenic spacer region, and a part of the 5' region of the 23S rRNA gene). PCR products were used in nested PCR with primers R16F2n/R2 (2) to amplify 1,200-bp fragments. The identification of phytoplasmas was done using RFLP (restriction fragments length polymorphisms) analyses of R16F2n/R2 amplicons digested with *AluI*, *Kpn I*, *HpaII*, *TruI*, or *HhaI* endonucleases (Thermo Scientific, Lithuania) (2). RFLP patterns were identical to that of STOL reference strain of the 16SrXII-A subgroup, indicating that symptomatic plants were infected with phytoplasma (2). The 16S rDNA nucleotide sequence of representative strain E7 was deposited in GenBank under accession number KF850526. The BLASTn search showed 100% homology to an Iranian strain (KF263684.1) from peach and Serbian strains JQ730742.1 and JQ730750 from valerian and corn, respectively, all belonging to '*Candidatus* Phytoplasma solani' (Stolbur). Sequencing data confirmed the association of Stolbur phytoplasma with affected *O. biennis* plants. It has already been reported that phytoplasma infection caused yellows disease of *O. biennis* (1). Also, the virescence of *O. hookeri* was associated with phytoplasma strain OAY from aster yellows (AY) group (subgroups 16SrI-B), and selected as the reference strain for the novel taxon '*Ca. P. asteris*' (3). Here we provide the first report of naturally occurring Stolbur phytoplasma disease of *O. biennis* in Serbia.

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