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A strain of phytoplasma related to 16SrII group in *Picris hieracioides* L. in Serbia

Milana MITROVIĆ[1], Ivo TOŠEVSKI[1,2], Oliver KRSTIĆ[1], Tatjana CVRKOVIĆ[1], Slobodan KRNJAJIĆ[1], Jelena JOVIĆ[1]

[1]*Institute for Plant Protection and Environment, Department of Plant Pests, Banatska 33, 11080 Zemun, Serbia*
[2]*CABI Europe - Switzerland, 1 Rue des Grillons, 2800 Delémont, Switzerland*

Abstract

During epidemiological survey for phytoplasmas in association with agricultural crops in Serbia, a new species of common weed has been detected to harbor phytoplasmas in Serbia. In 2010, a total of 38 samples of *Picris hieracioides* (Asteraceae), commonly known as hawkweed oxtongue, were sampled from vineyards in Jasenovik (near Niš, South Serbia) and analyzed for phytoplasma presence. Nested polymerase chain reaction analysis using primers specific to the phytoplasma 16SrDNA gene showed six samples of *Picris hieracioides* to be positive. Digestion of amplified 16SrDNA fragments with endonuclease *Mse*I identified the same pattern as the one of a reference strain of tomato big bud belonging to the 16SrII ribosomal group. Sequence obtained from the PCR product associated with infected *P. hieracioides* was submitted to BLAST analysis which showed a 99% similarity with reference strain of *Picris echioides* phyllody from Italy, belonging to 16SrII-E subgroup. This is the first report of phytoplasma related to 16SrII group infecting *Picris hieracioides*, as well as, the first record on the presence of this group of phytoplasmas in Serbia and South East Europe.

Key words: *Picris hieracioides*, 16SrII, phytoplasma, Serbia, PCR.

Introduction

Phytoplasmas belonging to the peanut witches' broom group (16SrII) have been recorded from weeds and cultivated plants worldwide, causing in significant losses in lime, carrots, alfalfa, potato, and ornamentals. Phytoplasmas of the 16SrII group have been found in the Middle East (Khan *et al.*, 2007), Mediterranean region (Tolu *et al*., 2006), Australia (Aryamanesh *et al*., 2011), Mexico (Hernandez-Perez *et al*., 2009), Israel (Sobolev *et al*., 2007), and Indonesia (Harling *et al*., 2009).

In Europe phytoplasmas of the 16SrII group have so far been detected in several weed species, and also cultivated plants (Tolu *et al*., 2006; Davino *et al*., 2007; Parrella *et al*., 2008). In Sardinia (Italy), phytoplasmas belonging to the 16SrII group have been identified in association with *Calendula arvensis* L., *Solanum nigrum* L. and *Chenopodium* spp., and in central and southern Italy infecting *Picris echioides* L. (Tolu *et al*., 2006).

The main goal of this study was identification and characterization of phytoplasmas associated with *Picris hieracioides* L., (hawkweed oxtongue), a common weed of the family Asteraceae in vineyards in Serbia.

Materials and methods

In July 2010, 38 plants of *P. hieracioides* were collected from vineyards in Jasenovik (near Niš, South Serbia) and analyzed for the presence of phytoplasmas. Weeds were sampled randomly and showed no typical symptoms of phytoplasma infection. Plants were collected with roots, which were later sliced, prepared into 0.2-1.0 gram aliquots, and stored at -20°C until DNA extraction.

Total nucleic acids from *P. hieracioides* plants were extracted using the CTAB protocol described by Angelini *et al.* (2001). Phytoplasmas presence was detected by amplifying the 16S ribosomal RNA gene by nested PCR with universal primer pairs P1/P7 (Deng and Hiruki, 1991; Smart *et al.*, 1996) and R16F2n/R2 (Gundersen and Lee, 1996), followed by RFLP analysis with *Mse*I restriction enzyme. In order to obtain longer fragments for sequencing, 16S rRNA amplicons were obtained in nested PCR assay with the universal primers P1A/P7A with reaction conditions according to Lee *et al*. (2004). Samples of *P. hieracioides* with the same RFLP pattern as a reference phytoplasma strains belonging to 16SrII group were sequenced (BMR Service, Italy) and submitted to BLAST analysis (http://blast.ncbi.nlm. nih.gov/Blast.cgi). Alignment and comparison of phytoplasma sequences were carried out using Clustal*W* program integrated in MEGA4 software (Tamura *et al.*, 2007).

Results and discussion

Specific 16S rRNA fragments of phytoplasmas were amplified from six out of 38 analyzed samples of *P. hieracioides*. Restriction analysis of PCR products with endonuclease *Mse*I showed in all infected plants presence of the same pattern as a reference strain of tomato big bud (TBB) belonging to 16SrII-D subgroup (figure 1). One of the 16S rRNA amplicons was sequenced and submitted to the National Center of Biotechnology Information with the accession number JF799094. BLAST analysis of the 1,447 bp sequence obtained from the Serbian *P. hieracioides* phytoplasma determined it to be 99% identical to reference strain of *Picris echioides* phyllody from Italy (PEP) (Acc. No. Y16393) belonging to 16SrII-E subgroup. Alignment and comparison of 16SrRNA sequences of reference

PEP and Serbian *P. hieracioides* strain showed pairwise distance of 0.6%.

P. hieracioides is a common weed in vineyards in Serbia, however, this plant species has not previously been detected in association with phytoplasma diseases. This is the first report of a phytoplasma from the 16SrII group infecting *P. hieracioides*, as well as the first record of the presence of this group of phytoplasmas in Serbia and South East Europe.

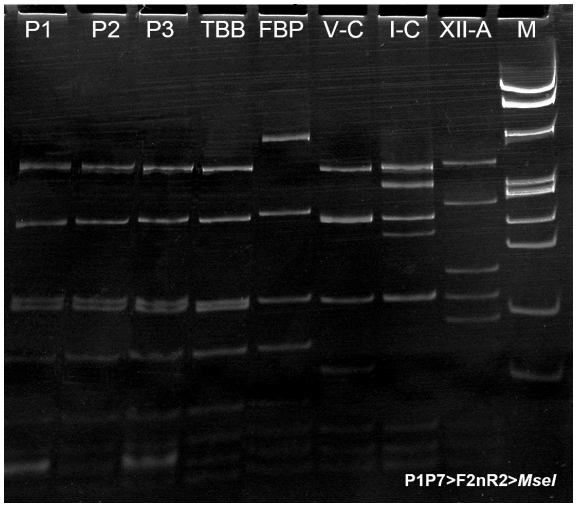

Figure 1. RFLP profiles of 1,447 bp fragment of 16S rRNA gene of 16SrII phytoplasma strain infecting *P. hieracioides* in Serbian vineyard (P1-P3) and reference phytoplasma strains amplified by nested PCR with primers P1/P7 followed by R16F2n/R2 and digested with *Mse*I endonuclease: TBB- tomato big bud (16SrII-D), and FBP- faba bean phyllody (16SrII-C) (provided by A. Bertaccini, Italy); V-C (16SrV-C; FD-C from naturally infected field-growing grapevine from Nišavski region, Serbia); I-C (16SrI-C, provided by Elisabeth Boudon-Padieu, France); XII-A (16SrXII-A, maize redness from naturally infected maize from South Banat region, Serbia); M: molecular weight marker ϕX174/*Hae*III digested (Fermentas, Vilinius, Lithuania).

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Corresponding author: Milana MITROVIĆ (e-mail: milanadesancic@yahoo.co.uk), Institute for Plant Protection and Environment, Banatska 33, 11080 Zemun, Serbia.