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Occurrence of two '*Candidatus* Phytoplasma asteris'-related phytoplasmas in poplar trees in Serbia

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Abstract

During a survey carried out in Serbia in black poplar trees, typical symptoms of phytoplasma presence were observed such as yellowing and undersize of the leaves, witches' broom and decline. Survey for phytoplasma presence in Belgrade and in its surrounding allow to identify phytoplasmas belonging to '*Candidatus* Phytoplasma asteris' group, in particular to two 16SrI subgroups 16SrI-P and 16SrI-A. Blast search of obtained strain in subgroup 16SrI-P of the 16SrDNA sequence showed 100% homology with aster yellows phytoplasma strain from poplar from Croatia.

Key words: '*Candidatus* Phytoplasma asteris', black poplar, PCR-RFLP.

Introduction

Populus nigra L. 'Italica' is one of several populus species that are known to be affected by phytoplasma diseases (Berges *et al*., 1997). The witches' broom (PopWB) disease of black poplar (*Populus nigra* L. 'Italica') and *P. canadensis* was observed for the first time in 1973 in Bulgaria by Atanasoff. Van der Meer (1980, 1981) observed the disease on white poplar (*P. alba*), grey poplar (*P. canescens*) and black poplar in the Netherlands. The electron microscopic detection of phytoplasmas in white poplar was reported in France (Sharma and Cousin, 1986) and in *P. tremula* in Germany (Seemüller and Lederer, 1988). It was then demonstrated using PCR/RFLP analyses, that the PopWB phytoplasma in France and Germany belongs to the aster yellows group, AY, (Maurer *et al*., 1994; Cousin, 1997). Later AY phytoplasmas were reported in black poplar also in Croatia (Šeruga *et al*., 2002).

Black poplar, like other poplar species, is one of the common ornamental trees grown in Serbia: they are grown in city parks, street avenues, and in other horticultural areas, and are often present by the river coasts. In previous reports, the presence of phytoplasmas in black poplar was associated with witches' broom symptoms although, in some cases, less specific syndromes were reported such as undersized leaves, yellowing, sparse foliage, stunting and dieback (Sharma and Cousin 1986; Cousin 1996; Berges *et al*., 1997).

On black poplar trees, on Belgrade street avenue, typical symptoms of phytoplasma presence were observed: yellowing and undersize of the leaves, witches' broom and decline. Survey for phytoplasma presence in poplar trees in Belgrade (Serbia) and in its surrounding was therefore performed.

Materials and methods

To verify the presence and determine the identity of phytoplasma, molecular assays were performed on 10 DNA extracts from symptomatic black poplar leaf midribs. Total DNA extraction was performed using CTAB protocol described by Angelini *et al*., 2001. Polymerase chain reaction (PCR) was performed for amplification of phytoplasma 16S rRNA gene, spacer region and part of 23S rRNA gene, using phytoplasma-universal primer pair P1/P7 (Deng and Hiruki, 1991; Schneider *et al*., 1995).

For identification, nested PCR on P1/P7 amplicons was performed using R16F2/R2 primer pair under reported conditions (Lee *et al*., 1995). Products amplified by PCR assays were visualised and positive samples were subjected to the restriction fragment length polymorphism (RFLP) analysis. Three restriction endonucleases were used, *Tru1*I, *Taq*I and *Hha*I (Fermentas, Vilnius, Lithuania), according to the manufacturer's instructions. Reference phytoplasma strain used were: chrysanthemum yellows 16SrI-A (CHRYM), primula green yellows 16SrI-B (PrG) and carrot yellows 16SrI-C (CA). The P1/P7-amplified product of a poplar sample, was purified using Qiagen PCR purification kit (Qiagen GmbH, Hilden, Germany) and sequenced in both directions with two forward primers P1 and R16F2 (Lee *et al*., 1995) and one reverse primer P7, using the BIG DYE sequencing terminator kit (PE Biosystems, Warrington, UK). The sequence was assembled using Pregap4 from the Staden program package (Staden *et al*., 2000) and compared with 16S ribosomal sequences of phytoplasmas in the GenBank database using blast (version Blast N 2.2.18) at the National Center for Biotechnology Information.

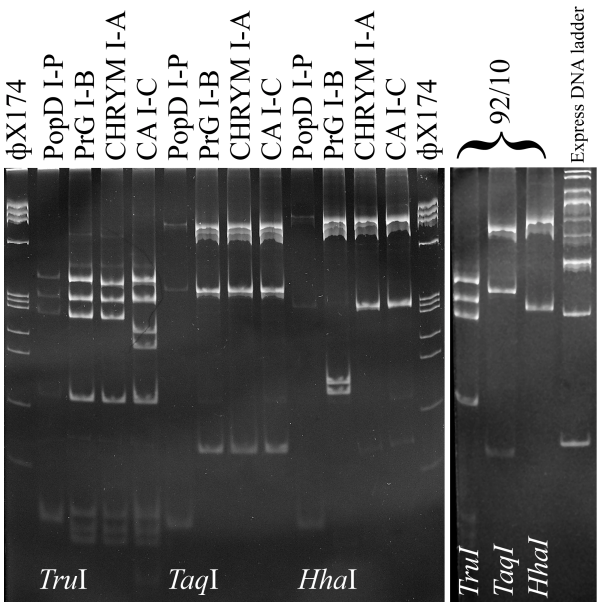

Figure 1. Differential RFLP profiles obtained from R16F2/R2 amplicons of two phytoplasmas from poplar trees and reference strains with different restriction enzymes.

Results and discussion

Among the 10 plant tested only two (PopD and 92/10) showed phytoplasma presence, both resulting positive also in direct PCR with P1/P7. Collectively, restriction profiles on R16F2/R2 amplicons for phytoplasma identification showed that phytoplasmas from Serbian black poplar trees belong to the '*Candidatus* Phytoplasma asteris' group and to two diverse 16SrI subgroups (figure 1). The strain PopD belongs to 16SrI-P, while strain 92/10 belongs to 16SrI-A subgroup. Blast search of obtained PopD phytoplasma 16SrDNA sequence (1,494 bp) showed 100% homology with aster yellows phytoplasma strain from poplar from Croatia (AF503568). This is the first report of this phytoplasma in black poplar in Serbia, and indicates that this strain is present in larger areas than previously reported (Šeruga *et al*., 2002). On the other hand the presence of 16SrI-A phytoplasma in poplar tree was never reported before.

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