Molecular Identification of a Phytoplasma Naturally Infecting *Populus nigra* L. cv. Italica Trees in Croatia

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Abstract

Leaf and branch samples of 10 *Populus nigra* L. cv. Italica trees were collected from the urban Zagreb area in late summer/autumn 2001. One of the trees exhibited leaf yellowing, overall sparse foliage, stunting and decline. Phytoplasma 16S rDNA was amplified in direct and nested PCR assays using universal and specific phytoplasma primer pairs, from nucleic acids extracted by two different procedures. Strong amplification signals were observed in samples from symptomatic Lombardy poplar as well as in samples from 4 of the asymptomatic trees. RFLP analyses of amplicons showed patterns characteristic of the phytoplasmas belonging to the Aster yellows group (16SrI). This is the first report of a phytoplasma naturally infecting poplar in Croatia.

Keywords: aster yellows phytoplasma; lombardy poplar; PCR; RFLP; 16S rDNA

INTRODUCTION

Several *Populus* species are known to be affected by phytoplasma diseases and among them is *P. nigra* L. cv. Italica (Lombardy poplar) (BERGES *et al.* 1997). Phytoplasmoses of Lombardy poplar are mainly associated with witches' broom (COUSIN 1996), but some less specific symptoms are also possible, such as: yellowing, sparse foliage, stunting and dieback (BERGES *et al.* 1997). A Lombardy poplar tree displaying these unspecific symptoms was found in the urban area of Zagreb. Molecular assays were performed on symptomatic and asymptomatic poplar samples to verify the presence and to determine the identity of phytoplasmas.

MATERIALS AND METHODS

In late summer and autumn of year 2001, leaf and branch samples were taken from 1 symptomatic and 9 apparently asymptomatic Lombardy poplar trees in different urban areas of Zagreb. In order to compare their applicability, two different procedures were used for extraction of total nucleic acids (TNA) (PRINCE *et al.* 1993; DAIRE *et al.* 1997).

In direct PCR assays, amplification of phytoplasma 16S rDNA was performed by using R16F1/R0 (LEE *et al.* 1995) or P1/P7 (SCHNEIDER *et al.* 1997) universal phytoplasma primer pair. The PCR products were diluted and reamplified in a nested PCR with primers R16F2/R2 (LEE *et al.* 1993). Diluted amplification products from nested PCR were used as templates in two separate PCR experiments using universal primers $16R_{738f}/R_{1232r}$ (GIBB *et al.* 1995) or group-specific primers R16(I)F1/R1 (LEE *et al.* 1994). Each reaction was performed and PCR products were analysed as previously described by LEE *et al.* (1993).

DNA products from PCR assays primed by R16F2/R2, R16(I)F1/R1 and $16R_{738f}/R_{1232r}$ were digested individually with restriction endonucleases *Mse*I (= *Tru*9I), *Alu*I and *Kpn*I. Digestion products were separated in 5% polyacrylamide gels and stained with ethidium bromide.

RESULTS

In the first nested PCR experiment using phytoplasma universal R16F2/R2 primer pair, 1.2 kbp long products were amplified from symptomatic

P. nigra Italica samples and from two samples in the asymptomatic group of trees. This result was confirmed in the subsequent amplification performed with R16(I)F1/R1 primers where fragments of about 1.1 kbp from 2 additional asymptomatic tree samples were also obtained. Another set of PCR experiments starting from the TNA prepared according to DAIRE *et al*. (1997) yielded amplification products from the symptomatic and only from one of the asymptomatic samples that tested positive in the previous procedure started with the extracts according to PRINCE *et al*. (1993). The detection was achieved with the same primers employed above.

All DNA fragments of 16S rRNA gene amplified from Lombardy poplar samples had RFLP patterns characteristic of the phytoplasmas belonging to the 16SrI group, subgroup-B (Figure 1).

DISCUSSION

The majority of Lombardy poplar trees visually inspected in the Zagreb urban area appeared symptomless at the time of sampling and in the following season.

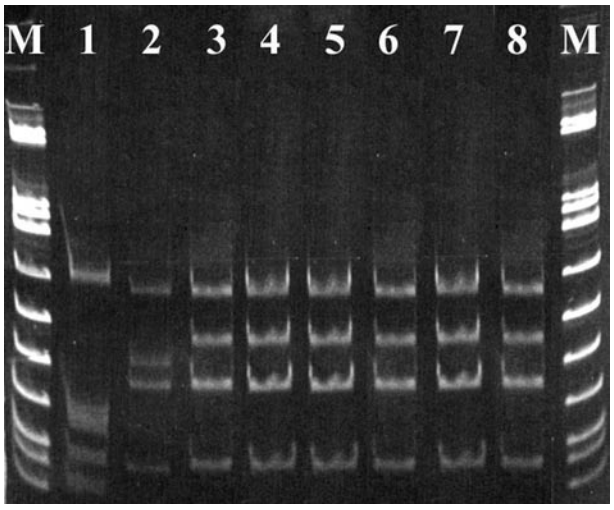

Figure 1. Polyacrylamide gel (5%) showing the RFLP patterns of phytoplasma detected in Croatian *Populus nigra* Italica samples and 3 phytoplasma reference strains. Phytoplasma 16S rDNA fragments of 1.1 kbp, amplified using R16(I)F1/R1, were digested with *Mse*I (= *Tru*9I)

M – molecular weight marker VIII (Roche), fragment sizes in base pairs from top to bottom: 1 114, 900, 692, 501, 489, 404, 320, 242, 190, 147, 124, 110. Phytoplasma reference strains: 1 – PG3 (subgroup 16SrXII-A), 2 – PPT (subgroup 16SrI-C), 3 – HydB (subgroup 16SrI-B). Symptomatic (4) and asymptomatic (5–8) Lombardy poplar samples

The symptomatic *P. nigra* cv. Italica tree included in this survey exhibited symptoms similar to those previously described in Germany (BERGES *et al*. 1997) and France (COUSIN 1996). Phytoplasma 16S rDNA was amplified from the symptomatic and 4 asymptomatic Lombardy poplar trees. The maximum sensitivity of detection was reached with R16(I)F1/R1 primers starting with nucleic acids extracted using method of PRINCE *et al*. (1993). The PCR results obtained with the templates from the other, much shorter extraction procedure (DAIRE *et al*. 1997), proved to be less sensitive for phytoplasma detection in this survey. The phytoplasma detected in *P. nigra* cv. Italica trees from Croatia has been classified as a member of the 16SrI group (Aster yellows), on the basis of RFLP analysis of 16S rRNA gene. Although phytoplasmas have been investigated at the molecular level in the Croatian grapevines (ŠERUGA *et al*. 2000), this is the first record of the Aster yellows for Croatia as well as the first record of any phytoplasma in forest/ornamental trees in the country.

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