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Detection of aster yellows group (subgroup 16SrI-B) phytoplasma in oats based on nested PCR and RFLP in Lithuania

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Abstract. Phytoplasma strains were detected in oats (*Avena sativa* L.) in Lithuania exhibiting disease symptoms of stunting, sterile, deformed and yellow spikes. A phytoplasma-characteristic 1.8 kb and 1.2 kb rDNA PCR products were amplified from DNAs of all diseased but not healthy oat plants tested, using phytoplasma universal primer pairs P1/P7 and R16F2n/R16R2 confirming that symptomatic plants were infected by phytoplasma. Phytoplasma was termed oat stunt (OatSt). Restriction fragment length polymorphism (RFLP) analysis of amplified 16S rDNA indicated that diseased oats were infected by phytoplasma belonging to the group 16SrI (aster yellows, AY, group) and subgroup 16SrI-B. This is the first report of phytoplasma belonging to 16SrI (aster yellows) group and 16SrI-B phytoplasma subgroup identified in oat plants.

Key words: *Avena sativa*, phytoplasma, PCR, RFLP, 16S rRNA gene

INTRODUCTION

Oat (*Avena sativa* L.) is the most widely grown and the most economically important grain crop in Lithuania. Considering that cereal crops and forage feeds are important in Lithuania, great attention was paid to the prevalence of phytoplasmal diseases in these crops. However, there is no literature offering molecular data about phytoplasmas affecting plants of *Poaceae* family in the Baltic States. Recently molecular methods have been used to detect and identify diverse phytoplasmas associated with diseases of cereal crops. Phytoplasmas, associated with diseases of oat, common meadow-grass, barley, *Triticosecale*, ryegrass, smooth bromegrass and tall fescues have been identified and classified on the basis of 16S rRNA gene sequence analyses in Lithuania (Jomantienė et al., 2002; Valiūnas, 2003; Urbanavičienė et al., 2004; Urbanavičienė et al., 2005; Urbanavičienė, 2005; Urbanavičienė & Valiūnas, in publication). RFLP analysis of the amplified 16S rDNA indicated that the detected phytoplasmas infecting cereal crops in Lithuania belong to several different subgroups in group 16SrI (aster yellows phytoplasma group). The 16SrI phytoplasma group has been separated into at least six well-defined subgroups according to RFLP patterns and nucleotide sequence of the 16S rDNA (Jomantienė et al., 1998; Lee et al., 1998; Marcone et al., 2000). Our previous investigations revealed phytoplasmas of 16SrI-A and 16SrI-L subgroups in oats (Jomantienė et al., 2002; Urbanavičienė et al., 2004).

The aim of the present study was to identify and classify the phytoplasma associated with stunted oats in Lithuania.

MATERIALS AND METHODS

The diseased oat plants with typical symptoms of phytoplasmal infection (stunting, bushiness, sterility and yellows of spikes) were found in the fields in the Vilnius region. Template DNAs were extracted from one asymptomatic and three symptomatic plants tissue using the Genomic DNA Purification Kit (MBI 'Fermentas') and were used in polymerase chain reactions (PCR) for amplification of phytoplasma 16S rDNA. In nested PCR, the first reaction was primed by phytoplasma-universal primer pair P1/P7 (Deng & Hiruki, 1991; Schneider et al., 1995) and the second (nested) PCR primed by primer pair R16F2n/R16R2 (F2n/R2) (Gundersen & Lee, 1996). Both amplifications were conducted under the same conditions as described previously (Lee et al., 1998). Healthy tissue and water were used as negative controls. The final amplification products (1.2 kbp) of the nested PCR, primed by primer pair F2n/R2, were subjected to enzymatic restriction fragment length polymorphism (RFLP) analysis using 8 different restriction endonucleases (MBI 'Fermentas'). The RFLP profiles of digested DNA were analysed by electrophoresis through 5% acrylamide gel. Standard fragment size of DNA bands was ØX174 DNA/BsuRI (HaeIII) (MBI 'Fermentas'). RFLP patterns were compared with those previously published, in accordance with the classification scheme of Lee et al. (1998).

RESULTS AND DISCUSSION

Three samples from naturally infected oat (*Avena sativa* L.) plants exhibiting stunting, sterile, deformed and yellows spikes were collected in fields in the Vilnius region, Lithuania (Fig. 1). The disease was termed oat stunt (OatSt). Total DNA extracted from the samples was analysed by nested PCRs using two phytoplasma universal primer pairs P1/P7 and F2n/R2. A phytoplasma characteristic 1.2 kbp 16S rDNA PCR products were amplified from the total DNA of all 3 diseased samples, but not from negative controls with healthy oat plants and water, indicating that the plants were infected by phytoplasma. The phytoplasma strains were classified on the basis of RFLP analysis of 16S rDNA amplified in PCR primed by primer pair F2n/R2, according to the classification scheme established by Lee et al. (1998). Based on RFLP profiles with 8 restriction enzymes, the phytoplasma associated with disease in oat plants was clasified as a B subgroup phytoplasma, 16Sr-I group (aster yellows, AY) (Fig. 2, a, b). Previously in our country, subgroup 16SrI-B phytoplasma strains were detected in willow (*Salix* L.), common pear-tree (*Pyrus communis* L.), common valerian (*Valeriana officinalis* L.) (Valiūnas, 2003), barley (*Hordeum vulgare* L.), *Triticosecale,* smooth bromegrass (*Bromopsis inermis* (Leyss.) Holub) (Urbanavičienė et al., 2004; Urbanavičienė 2005), and delphinium (*Delphinium* L.) (Navalinskienė et al., 2004). Phytoplasmas belonging to subgroup 16SrI-B are spread worldwide, mostly in herbaceous plants (Marcone et al., 2000; Lee et al., 1998) but have also been reported in woody plants (Marcone et al., 2000).

Fig. 1. *Avena sativa* infected with phytoplasma; healthy spike is on the left.

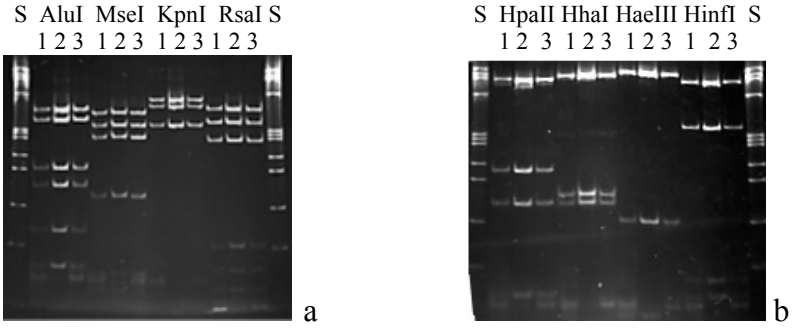

Fig. 2. RFLP analysis of 1.2 kb16S rDNAs from phytoplasma strains detected in 1, 2, 3 *Avena sativa* plant samples. DNA products from the nested PCR, primed by R16F2n/R16R2, were digested with restricted endonucleases (left) AluI, MseI, KpnI, RsaI and (right) HpaII, HhaI, HaeIII, HinfI. Lanes S – PhiX174/HaeIII digest standard; the fragment sizes are (from top to bottom) 1353, 1078, 872, 602, 310, 281, 271, 234, 194, 118, and 72 bp.

CONCLUSIONS

1. RFLP analysis of amplified 16S rDNA from diseased oats (*Avena sativa* L.) established that the oats grown in the Vilnius region were infected by a phytoplasma strain belonging to the group 16SrI (aster yellows group) and 16SrI-B subgroup.
2. 16SrI-B phytoplasma strains are widespread in cereal crops and forage grass grown in Lithuania.

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