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MOLECULAR IDENTIFICATION OF AGENTS CAUSING YELLOWS DISEASES IN OATS (*AVENA SATIVA* L.)

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Abstract

Oats (*Avena sativa* L.) are economically important grain crops in Lithuania and also worldwide. The diseased oat plants with typical symptoms of phytoplasmal infection were found in Vilnius, Kaunas and Raseiniai regions. A phytoplasma-characteristic fragment of 16S rDNA primed by phytoplasma universal primers was amplified from diseased oats in nested polymerase chain reactions (PCRs). Restriction fragment length polymorphism (RFLP) analysis of amplified 16S rDNA indicated that diseased oats were infected by phytoplasmas belonging to the group 16SrI and the subgroups: 16SrI-A, 16SrI-B, 16SrI-L.

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

Introduction

Oat (*Avena sativa* L.) is economically important grain crop in Lithuania. Oats, like rye, are usually considered a secondary crop, i. e. derived from a weed of the primary cereal domesticates wheat and barley. As these cereals spread wet wards into cooler, wetter areas, this may have favoured the oat weed component, leading to its eventual domestication /Zhou et al., 1999/. The common oat plant is a species of cereal grain grown for its seed. While oats are suitable for human consumption as oatmeal and rolled oats, one of the most common uses is as livestock feed. Oats make up a large part of the diet of horses and are regularly fed to cattle as well.

Oats are susceptible to a larger number of diseases, many of which can cause damage. Individual oat fields may be damaged severely or even destroyed. Over a period of a year oat diseases annually account for a huge damage by reducing yield and grain quality. They also play a similar, if not more significant, role in the production of oat forage /Wiese, 1987/. Data about fungal and bacterial diseases affecting oat plants are numerous in the literature. But comparatively little is known about phytoplasmal diseases of cereal crops in Europe and other places in the world. Recently molecular methods have been used to detect and identify diverse phytoplasmas associated with diseases of oat plants. Phytoplasmas, associated with diseases of oat, have been identified and classified on the basis of 16S rRNA gene sequence analyses in Lithuania. RFLP analysis of the amplified 16S rDNA indicated that the detected phytoplasmas infecting oats in Lithuania belong to several different subgroups in group 16SrI (aster yellows phytoplasma group). The 16SrI phytoplasma group has been separated into at

fourteen well-defined subgroups according to RFLP patterns and nucleotide sequence of the 16S rDNA /Lee et al., 1998; Marcone et al., 2000/.

Similarly, phytoplasmas associated with diseases of ryegrass (*Lolium multiflorum* Lam.) and smooth bromegrass (*Bromopsis inermis* (Leyss.) Holub) have been identified and classified in aster yellows group and 16SrI-L and 16SrI-B subgroups /Urbanavičienė et al., 2005; Urbanavičienė, 2006/.

Materials and Methods

Plant samples, PCR (polymerase chain reaction)

Oat plants exhibiting symptoms of abnormal proliferation of spikelets, development of numerous short tillers at the plants base, sterile deformed spikes, general stunting and yellowing were found in the fields in Vilnius, Kaunas and Raseiniai regions in July of 2003–2005.

Template DNA was extracted from plant tissues using Genomic DNA Purification Kit (MBI Fermentas, Vilnius, Lithuania) and was used in nested polymerase chain reaction (PCR) for amplification of phytoplasmal 16S rDNA. In nested PCR, the first reaction was primed by phytoplasma-universal primer pair P1/P7 /Deng, Hiruki, 1991/. Products obtained in the first PCR were diluted 1:50 with sterile water and used in the second (nested) PCR primed by primer pair R16F2n/R16R2 (F2n/R2) /Gundersen, Lee, 1996; Lee et al., 1998/. Both amplifications were conducted under the same conditions 94º for 1 min, 55º for 2 min, 72º for 3 min for 35 cycles in Perkin Elmer PCR buffer, 200 pM of each dNTP (2 min for the first time), 0,4µM of each primer, and 1 unit of recombinant Taq polymerase per 50µl of reaction mixture. PCR products were analyzed by electrophoresis through 1% agarose gel, followed by staining with ethidium bromide, and visualization of the DNA bands with a UV transilluminator.

RFLP analysis and phytoplasma classification

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 restriction endonucleases *Alu*I, *Hae*III, *Hha*I, *Hinf*I, *Hpa*II, *Mse*I, *Rsa*I, *Tag*I, *Kpn*I and *Sau*3AI (MBI Fermentas, Vilnius, Lithuania) and electrophoresis through 5% acrylamide gel. DNA bands were stained with ethidium bromide and visualized using UV transilluminator. Phytoplasmas were classified in groups and subgroups, through comparisons of RFLP patterns previously published, in accordance with the classification scheme of Lee et al. (1998) and Marcone et al. (2000).

Results and Discussion

During the summer of 2003–2005 years, twenty samples from oat plants exhibiting symptoms of possible phytoplasma infection (stunting, proliferation, sterility and yellowing of spikes) were collected under field conditions. Total DNA extracted from the samples was analyzed 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 the diseased oats. Among the 20 samples tested, 7 were found infected by phytoplasmas. The phytoplasma strains were classified on the basis of RFLP analysis of 16S rDNA amplified in PCR primed by primer pair F2n/R2 /Lee et al., 1998/. Comparison of RFLP patterns of the amplified 16S r DNA with patterns

previously published for 16S rDNA from other phytoplasmas /Lee et al., 1998; Marcone et al., 2000/ revealed that phytoplasmas detected in oats belong to group 16SrI (aster yellows phytoplasma group) and represent three different phytoplasma subgroups.

Subgroup 16SrI-A (tomato big bud phytoplasma subgroup)

In 2002, diseased plants of oat exhibiting abnormal proliferation of spikelets were observed in the field in Raseiniai (Figure 1). Phytoplasmal rDNA was amplified in the nested PCR, indicating that the plants contained a phytoplasma. Based on the disease symptoms in plant hosts, phytoplasma was designated as oat proliferation (OatP).

Figure 1. *Avena sativa* infected with OatP phytoplasma; Healthy spike is on the left

On the basis of collective RFLP patterns of the 16S rDNA, the OatP phytoplasma was classified as a member of group 16SrI (group I, aster yellows phytoplasma group). The RFLP patterns of the 16S rDNA were indistinguishable from those of tomato big bud (BB) phytoplasma and other phytoplasmas classified in group I, subgroup A. Phytoplasmas classified in subgroup I-A have previously been reported in a broad range of plant species in North America and Europe, although there are no previous definitive reports of oat as host of as a for subgroup I-A phytoplasmas. Subgroup I-A phytoplasma strains are geographically widespread and have been found in numerous plant species /Marcone et al., 2000; Valiūnas, 2003; Samuitienė et al., 2006/. In Siberia mycoplasma-like organisms (phytoplasmas) were observed in diseased oat by electron microscopy, but the identity of phytoplasma remains unknown /Fedotina, 1977/.

Subgroup 16SrI-B (aster yellows phytoplasma subgroup)

In the years 2004 and 2005, the presence of aster yellows phytoplasmas belonging to subgroup 16Sr-B were identified for three oat plant samples out of 20 found in the fields in Vilnius region after nested PCR and RFLP analyses. Symptoms of infected oat (*A. sativa* L.) plants were stunting, sterile deformed and yellow spikes (Figure 3). The disease was termed oat stunt (OatSt).

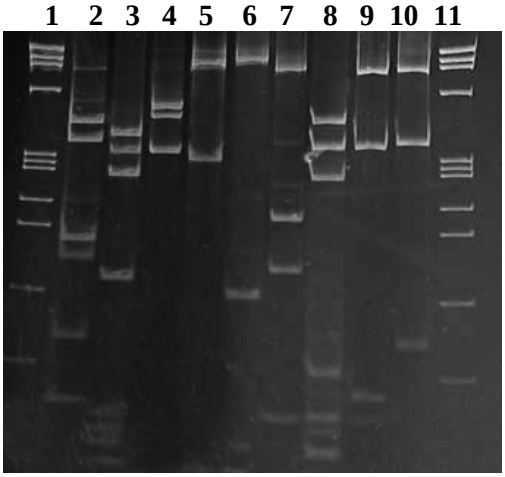

Figure 2. RFLP patterns of 16S rDNA (F2n/R2 PCR product) from the subgroup 16SrI-A from infected oat. Lanes: 1, 11-*Ø*X174/*Hae*III digest DNA standard; the fragment sizes are (from top to bottom) 1353, 1078,872, 692, 310, 281, 271, 234, 194, 118, 72 bp; 2-*Alu*I, 3-*Mse*I,4-*Kpn*I, 5-*Hha*I, 6- *Hae*III, 7-*Hpa*II, 8- *Rsa*I, 9-*Hinf*I, 10- *Taq*I

Figure 3. *Avena sativa* infected with OatSt phytoplasma; Healthy spike is on the left

The collective RFLP patterns of rDNA were indistinguishable from previously published patterns of this subgroup /Lee et al., 1998/. Based on RFLP profiles with 8 restriction enzymes, the phytoplasma associated with disease in oat plants was classified as a I-B subgroup phytoplasma, 16Sr-I group (aster yellows, AY) (Figure 4). Previously, in our country subgroup 16SrI-B phytoplasma strains were detected in plant families: *Salicaceae, Rosaceae, Valerianaceae* /Valiūnas, 2003/, *Caryophyllaceae, Crassulaceae, Ranunculaceae,* solanaceae /Samuitienė et al., 2006/, *Poaceae* /Urbanavičienė, 2005;

Urbanavičienė, Jomantienė, 2005/. Phytoplasmas belonging to subgroup 16SrI-B are spread worldwide, mostly in herbaceous plants / Lee et al., 1998; Marcone et al., 2000; / but have also been reported in woody plants /Marcone et al., 2000/.

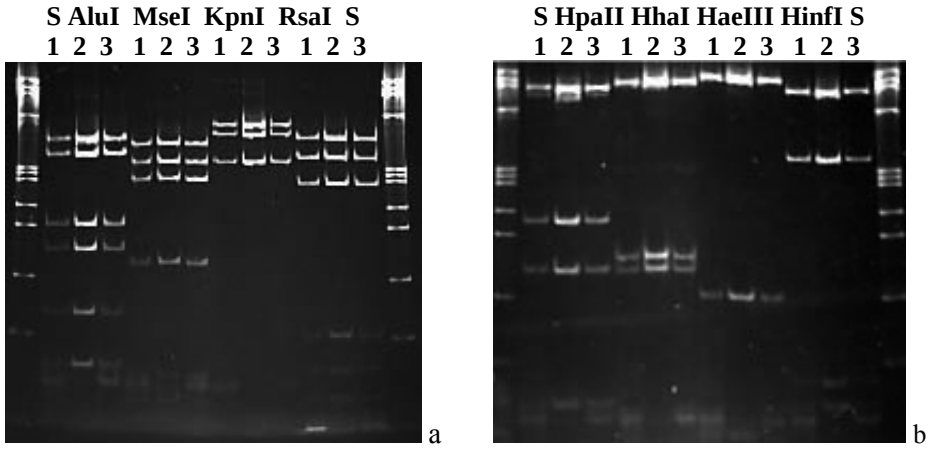

Figure 4. RFLP analysis of 1.2 kb 16S rDNAs from phytoplasma strains detected in 1, 2, 3 *Avena sativa* plant samples. DNA products from the nested PCR, primed by R16F2n/R16R2, were digested with restriction endonucleases (left) *AluI, MseI, KpnI, RsaI and (right) HpaII, HhaI, HaeIII, HinfI*. Lanes S – 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.

Subgroup 16SrI-L

Subgroup 16SrI-L were identified in two oat samples out of 20 examined symptomatic plants found in Vilnius and Kaunas region fields (Figure 5). Phytoplasma infected oat plants showed stunting, development of numerous short tillers at the plants base, and sterile deformed spikes.

Further a phytoplasma-characteristic 1.2 kbp rDNA fragment was amplified in nested PCR primed by phytoplasma universal primers and containing DNA template extracted from diseased oats found in Vilnius region. The collective RFLP patterns of amplified rDNA were indistinguishable from characteristic RFLP patterns previously published for rDNA from group 16SrI subgroup B phytoplasmas /Lee et al., 1998/ except for the *Hinf*I RFLP pattern (Figure 2). The sum of the sizes of rDNA fragments in the *Hinf*I RFLP pattern exceeded the size of 1.2 kbp. We interpreted this pattern to indicate the presence of two sequence heterogeneous 16S rRNA genes in the genome of the phytoplasma. A similar *Hinf*I RFLP pattern was previously published for rDNA of a phytoplasma classified in subgroup 16SrI-L and found in primrose and aster plants in Germany /Marcone et al., 2000/. On this basis the phytoplasma present in oats was classified in group 16SrI, subgroup L and named OatY (oat yellows) phytoplasma.

Figure 5. *Avena sativa* L. plant infected by OatY phytoplasma

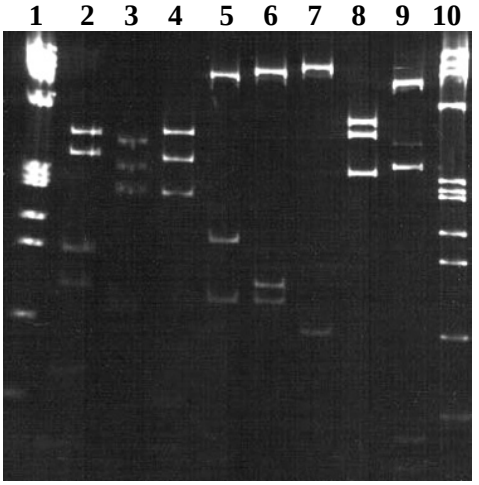

Figure 6. RFLP patterns of 16S rDNA (F2n/R2 PCR product) from the subgroup 16SrI-L phytoplasma from infected oat. Lanes: 1, 10-*Ø*X174/*Hae*III digest, size standard; 2-*Alu*I; 3-*Mse*I; 4-*Rsa*I; 5-*Hpa*II; 6-*Hha*I; 7-*Hae*III; 8-*Kpn*I; 9-*Hinf*I

Previously, subgroup 16SrI-L phytoplasma strains had been detected in *Hiacinthus orientalis* /Alminaitė et al., 2001/, *Brassica napus* /Valiūnas, 2003/, *Grosheimia macrocephala*, *Lunaria annua*, *Armeria alliaceae*, *Aconitum napellus* /Samuitienė et al., 2007/ plants in Lithuania.

Conclusions

1. RFLP analysis of amplified 16S rDNA from diseased oats (*Avena sativa* L.) indicated that the oats grown in Vilnius, Kaunas and Raseiniai regions were infected by phytoplasma strains belonging to the group 16SrI (aster yellows group).

2. Based on analyses of 16S rDNA the identified phytoplasmas were classified in 16SrI phytoplasma group and defined as members of 16SrI-A, 16SrI-B and 16SrI-L phytoplasma subgroups.

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