*Bulletin of Insectology* ***60*** *(2): 127-128, 2007*
ISSN 1721-8861

# Molecular identification of 16SrI-A, 16SrI-B, 16SrI-C, and 16SrI-L subgroups of phytoplasmas in gramineous plants in Lithuania

**Laima URBANAVIČIENĖ[1], Rasa JOMANTIENĖ[1], Deividas VALIŪNAS[1], Robert E. DAVIS[2]**
[1]*Institute of Botany, Vilnius, Lithuania*
[2]*USDA-Agricultural Research Service, Beltsville, USA*

## Abstract

Diseased plants of family *Poaceae* (*Gramineae*) were observed in cereal crop and forage feed plant fields in the Vilnius, Kėdainiai and Raseiniai regions of Lithuania. Disease symptoms included yellowing of leaves and spikes, general stunting, sterility and deformation of spikes, dwarfed spikes, and twisted awns, indicating possible phytoplasmal infections. A phytoplasma-characteristic fragment of 16S rDNA was amplified from all the symptomatic plants that were tested in nested polymerase chain reaction (PCR), using phytoplasma-specific primers. RFLP analysis of the amplified 16S rDNA indicated that detected phytoplasmas infecting gramineous plants in Lithuania belong to several different subgroups in group 16SrI (aster yellows phytoplasma group): 16SrI-A (in *Avena sativa*); 16SrI-B (in *A. sativa*, *Hordeum vulgare*, *Triticosecale* and *Bromopsis inermis*); 16SrI- L (in *A. sativa* and *Lolium multiflorum*) and 16SrI-C (in *Poa pratensis* and *Festuca arundinaceae*).

**Key words:** gramineous plants, phytoplasma, 16S rRNR gene, PCR, RFLP.

## Introduction

Considering that cereal crops and forage feeds are important in Lithuania, a great attention was paid to the presence of phytoplasmal diseases in these crops. The observation of gramineous plants in several locations in Lithuania during seven years indicated widespread presence of phytoplasmas belonging to group 16Sr-I (table 1). Comparatively little is known about phytoplasmal diseases of cereal crops in Europe and other places in the world. This work summarizes data of molecular evidence indicating that phytoplasmas could damage yields of gramineous plants, causing economic losses.

## Materials and methods

Samples of diseased plants exhibiting yellowing of leaves and spikes, general stunting, sterility and deformation of spikes, dwarfed spikes, and twisted awns were collected in fields in Vilnius, Kėdainiai and Raseiniai districts in Lithuania. Template DNA was extracted from the tissues using Genomic DNA Purification Kit (MBI Fermentas, Vilnius, Lithuania) and used in 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 and Hiruki, 1991) and the second (nested) PCR primed by primer pair R16F2n/R16R2 (F2n/R2) (Gundersen and Lee, 1996). PCR products were analyzed by electrophoresis through 1% agarose gel, stained with ethidium bromide, and DNA bands vizualized using a UV transilluminator.

Products (1,2 kbp) of the nested PCR, obtained with primer pair R16F2n/R16R2, were subjected to enzymatic restriction fragment 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) and electrophoresis through 5% acrylamide gel, staining with ethidium bromide, and visualization using a 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

Phytoplasma characteristic 1.2 kbp 16S rDNA PCR products were amplified from the total DNA of all symptomatic plants, but not from healthy plants, indicating that these plants were infected by phytoplasmas. The phytoplasmas strains were classified on the basis of RFLP analysis of 16S rDNA amplified in PCR, according to the classification scheme established by Lee *et al*. (1998) and Marcone *et al*. (2000). RFLP analysis of the amplified 16S rDNA indicated that the detected phytoplasmas infecting gramineous plants in Lithuania belong to several different ribosomal subgroups in group 16SrI (aster yellows phytoplasma group, AY): 16SrI-A (in *Avena sativa* L.); 16SrI-B [in *A. sativa*, *Hordeum vulgare* L., *Triticosecale* Wittm. ex A. Camus (*Triticum* L. × *Secale* L.), and *Bromopsis inermis* (Leyss.) Holub.]; 16SrI-L (in *A. sativa* and *Lolium multiflorum* Lam.) and 16SrI-C (in *Poa pratensis* L. and *Festuca arundinaceae* Schreb.) (table l).

## Discussion

The symptomatic gramineous plants were infected by phytoplasmas belonging to group 16SrI (AY phytoplasma group) (Lee *et al*., 1998; Marcone *et al*., 2000).

**Table 1.** Diseases of gramineous plants associated with infection by phytoplasma strains belonging to diverse subgroups of 16S rDNA ribosomal group 16SrI in Lithuania.

| Disease (Phytoplasma strain) | Natural host plant | 16S rDNA Group-subgroup | Reference |
|---|---|---|---|
| Oat proliferation (OatP) | *Avena sativa* L. | I-A | Jomantienė *et al*., 2002 |
| Oat stunt (OatSt) | *Avena sativa* L. | I-B | Urbanavičienė *et al*., 2006 |
| Oat yellows (Oat Y) | *Avena sativa* L. | I-L | Urbanavičienė *et al*., 2004 |
| Smooth bromegrass stunt (BrS) | *Bromopsis inermis* (Leyss.) | I-B | Urbanavičienė, 2005 |
| Fescue yellows (FesY) | *Festuca arundinaceae* Schreb. | I-C | Valiūnas *et al*., 2007 |
| Barley deformation (BaDef) | *Hordeum vulgare* L. | I-B | Urbanavičienė *et al*., 2004 |
| Ryegrass yellows (RgY) | *Lolium multiflorum* Lam. | I-L | Urbanavičienė *et al*., 2005 |
| Poa stunt (PoaS) | *Poa pratensis* L. | I-C | Valiūnas *et al*., 2007 |
| *Triticosecale* stunt (TrSt) | *Triticosecale* Wittm. ex A. Camus (*Triticum* L. × *Secale* L.) | I-B | Urbanavičienė *et al*., 2004 |

The strains of this phytoplasma group are spread worldwide, however little is known about phytoplasma infections in plants of family *Poaceae*. Some data show, for example, that AY phytoplasma disease may cause severe losses in barley and wheat (Chiykowski, 1963). At least six species of grass have been reported to belong to the host range of AY phytoplasma in the United States and Canada (Banttari, 1966). In Finland, AY phytoplasma was readily transmitted by *Macrosteles laevis* (Rib.) to and from graminaceous plants (Murtomaa, 1966). The literature contains only few reports of phytoplasmas possibly infecting oats. In some early reports, only phytoplasma-characteristic disease symptoms were reported. Interestingly, phytoplasma strains belonging to subgroup 16SrI-C were detected in common meadow-grass and tall fescues (Valiūnas *et al*., 2007). Previously, *Poa annua* was identified as host of a subgroup 16SrXIV-A phytoplasma in Italy (Lee *et al*., 1997). The detection of phytoplasmas infecting gramineous plants in Lithuania provides new insights on threatening diseases of grass species, including important grains, in Europe.

## Acknowledgements

This work was partly supported by Lithuanian State Science and Studies Foundation. We are grateful to MBI Fermentas for financial support of participation by L.U., R.J., and D.V. in First IPWG Meeting.

## References

BANTTARI E. E., 1966.- Grass hosts of aster yellows virus.- *Plant Disease Reporter*, 50 (1): 17-21.

CHIYKOWSKI L. N., 1963.- Endria inimiga (say), a new leafhopper vector of a celery-infecting strain of aster-yellows virus in barley and wheat.- *Canadian Journal of Botany*, 41: 669-672.

DENG S., HIRUKI C., 1991.- Amplification of 16S rRNA genes from culturable and non-culturable mollicutes.- *Journal Microbiology Methods*, 14: 53-61.

GUNDERSEN D. E., LEE I.-M., 1996.- Ultrasensitive detection of phytoplasmas by nested-PCR assays using two universal primer pairs.- *Phytopathologia Mediterranean*, 35: 144-151.

JOMANTIENE R., DAVIS R. E., VALIUNAS D., JASINSKAITE R., 2002.- First report of oat (*Avena sativa* L.) as host of a phytoplasma belonging to group 16SrI, subgroup A.- *Plant Disease*, 86: 443.

LEE I.-M., PASTORE M., VIBIO M., DANIELLI A., ATTATHOM S., DAVIS R. E., BERTACCINI A., 1997.- Detection and characterization of phytoplasma associated with annual blue grass (*Poa annua*) white leaf in southern Italy.- *European Journal of Plant Pathology*, 103: 251-254.

LEE I.-M., GUNDERSEN-RINDAL D. E., DAVIS R. E., BARTOSZYK I. M., 1998.- Revised classification scheme of phytoplasmas based on RFLP analyses of 16S rRNA and ribosomal protein gene sequences.- *International Journal of Systematic Bacteriology*, 48: 1153-1169.

MARCONE C., LEE I.-M., DAVIS R. E., RAGOZZINO A., SEEMÜLLER E., 2000.- Classification of aster yellows-group phytoplasmas based on combined analyses of rRNA and *tuf* gene sequences.- *International Journal of Systematic and Evolutionary Microbiology*, 50: 1703-1713.

MURTOMAA A., 1966.- Aster yellows-type virus infecting grasses in Finland.- *Annales Agriculture Fenniae*, 5: 324-333.

URBANAVIČIENĖ L., JOMANTIENĖ R., VALIŪNAS D., DAVIS R. E., 2004.- Molecular detection of phytoplasmas in oats, barley, and *Triticosecale* and their classification based on 16S rRNA gene polymorphisms.- *Žemės ūkio mokslai* (Vilnius), 3: 15-19.

URBANAVIČIENĖ L., VALIŪNAS D., JOMANTIENĖ R., 2005.- Molecular detection and identification of subgroup 16SrI-L phytoplasma in ryegrass (*Lolium multiflorum* Lam.).- *Phytopathologia Polonica*, 35: 121-124.

URBANAVIČIENĖ L., 2005.- Molecular detection and identification of phytoplasma affecting smooth bromegrass (*Bromopsis inermis*).- *Botanica Lithuanica*, 11 (3): 191-194.

VALIŪNAS D., URBANAVIČIENĖ L., JOMANTIENĖ R., DAVIS R. E., 2007.- Molecular detection, classification, and phylogenetic analysis of subgroup 16SrI-C phytoplasmas detected in diseased *Poa* and *Festuca* in Lithuania.- *Biologija*, 18 (2): 63-66.

**Corresponding author:** Laima URBANAVIČIENĖ (e-mail: laima.urbanaviciene@botanika.lt), Institute of Botany, Žaliųjų Ežerų 49, Vilnius, Lithuania.