

## Molecular detection and characterization of phytoplasma infecting *Celosia argentea* L. plants in Lithuania

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**Abstract.** Symptoms of diseased *Celosia argentea* L. plants included flower phyllody, general yellowing and stunting of plants. Amplification of phytoplasmal 16S rRNA gene sequence, in PCRs containing phytoplasma universal primer pairs P1/P7, R16F2n/R16R2 and template DNA extracted from diseased *C. argentea* plants, confirmed that the plants were infected by phytoplasma. The 1.2 kbp 16S rDNA product of nested PCR, primed by primer pair R16F2n/R16R2 was subjected to single enzyme digestions with 8 restriction endonucleases. RFLP analysis revealed that the plants were infected by a phytoplasma belonging to group 16SrI (aster yellows phytoplasma group), subgroup I–M. Strains in this subgroup have a broad pathogenic potential, since they infect a wide range of plant host species.

**Key words:** *Celosia argentea*, phytoplasma, PCR, RFLP

### INTRODUCTION

Diseases attributed to phytoplasmas have been reported in plant species belonging to more than 90 families worldwide. Molecular methods have been applied to detect them in plants and insect vectors and to construct a system for phytoplasma identification and classification. On the basis of analyses of 16S rDNA, phytoplasmas have been classified into at least 15 groups and over 38 subgroups (Lee et al., 1998; Marcone et al., 2000). Phytoplasmas belonging to six major 16S rRNA gene groups (16SrI, 16SrIII, 16SrV, 16SrX, 16SrXI and 16SrXII) have been reported in Europe (Bertaccini et al., 1993; Seemüller et al., 1994; Lee et al., 1998; 2004; Kamińska, 2000; Marcone et al., 2000). 16SrI (aster yellows) group is the largest, most diverse and widespread phytoplasma group (Marcone et al., 2000; Lee et al., 2004). Molecular investigation of phytoplasmas in Lithuania began recently, and knowledge concerning the genetic and biological diversity is emerging. Phytoplasmas belonging to 16SrI, 16SrIII and 16SrV major phytoplasma groups and 11 subgroups have been detected (Jomantiene et al., 2002; Valiūnas, 2003; Samuitiene et al., 2006).

The objective of this study was to determine possible association of phytoplasma with phyllody disease in the annual ornamental plant *C. argentea* (*Amaranthaceae* Juss. family) to identify and classify the associated phytoplasma.

## MATERIALS AND METHODS

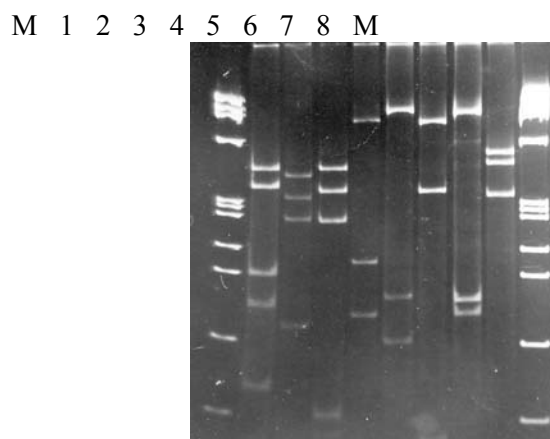
The experimental work was carried out at the Plant Virus Laboratory of the Institute of Botany. Phytoplasma was detected in polymerase chain reactions (PCRs). Nucleic acid, for use as a template in PCR, was extracted from the frozen tissue using the Genomic DNA Purification Kit (MBI Fermentas). Ribosomal (r) DNA was amplified in a nested PCR using two universal primer pairs P1/P7 and R16F2n/R16R2 (Gundersen & Lee, 1996) as described in (Jomantiene et al., 1998 a). Products, from nested PCR primed by R16F2n/R16R2, were analysed by single enzyme digestion, according to manufacturer's instructions with 8 restriction endonucleases (MBI Fermentas). The RFLP profiles of digested DNA were analysed by electrophoresis through 5% polyacrilamide gel. RFLP patterns were compared with previously published (Jomantiene et al., 1998 a,b; Lee et al., 1998; Marcone et al., 2000).

## RESULTS AND DISCUSSION

The diseased *C. argentea* plants exhibiting symptoms of flower phyllody, general yellowing and stunting of plants (Fig. 1) were collected at the botanical garden of Klaipėda's university. Phytoplasma detection was carried out by PCRs. Phytoplasma characteristic 16S rDNA fragments of 1.8 kbp amplified in PCR primed with primers P1/P7 and of 1.2 kbp amplified in nested PCR primed with primers R16F2n/R16R2, confirmed phytoplasmal infection (data not shown). Phytoplasma was named *Celosia phyllody* (CelPh).



**Fig. 1.** *Celosia argentea* plants expressing symptoms of flower phyllody, yellowing and stunting.



**Fig. 2.** RFLP analysis of CelPh phytoplasma 16S rDNA, amplified in n-PCR. Lanes M, PhiX174 DNA HaeIII digest, fragment sizes (bp) from top to bottom: 1353, 1078, 872, 603, 310, 281, 271, 234, 194, 118, 72.1 – AluI, 2 – MseI, 3 – RsaI, 4 – HpaII, 5 – HaeIII, 6 – HinfI, 7 – HhaI, 8 – KpnI

The 1.2 kbp product was subjected to single digestions with restriction endonucleases: AluI, MseI, RsaI, HpaII, HaeIII, HinfI, HhaI, and KpnI. The RFLP patterns of CelPh phytoplasma 16S rDNA (Fig. 2) were similar to 16S rDNA from phytoplasmas classified to group 16SrI, and subgroup 16SrI-B, except for the HaeIII RFLP pattern. The sum of sizes of the CelPh rDNA fragments exceeded the size of 1.2 kbp expected for the product of PCR analysed, indicating the presence of two heterogeneous 16S rRNA genes in CelPh phytoplasma. Indistinguishable HaeIII patterns were published for a phytoplasma (strain AVUT) belonging to subgroup 16SrI-M (Marcone et al., 2000). On this basis CelPh phytoplasma was classified to 16SrI-M subgroup. Among 16SrI group phytoplasmas in Europe, the subgroup 16SrI-B strains have the widest plant host range including ornamental species (Schneider et al., 1993; Marcone et al., 2000; Kamińska, 2000). In Lithuania after the latter years of intensive phytoplasma screening, phytoplasmas of 16SrI-M subgroup seem to be more frequently detected and widespread than 16SrI-B. Phytoplasmas belonging to subgroup 16SrI-M were detected in 19 plant species of 13 plant families (Valiūnas, 2003; Samuitienė et al., 2004; Navalinskienė et al., 2005; Samuitiene et al., 2006). Subgroup 16SrI-M phytoplasma strains are characterized by rRNA sequence heterogeneity (Marcone et al., 2000; Valiūnas, 2003). Strains in this subgroup have a broad pathogenic potential, since they infect a wide range of plant host species.

## CONCLUSIONS

1. *Celosia* phyllody disease is associated with a phytoplasma belonging to the 16SrI (aster yellows) phytoplasma group and 16SrI-M subgroup, classified on the basis of RFLP analyses of 16S rDNA sequences.
2. Subgroup 16SrI-M phytoplasma strains have a broad pathogenic potential, since they infect a wide range of plant host species and are widespread in Lithuania.

## REFERENCES

- Bertaccini, A., Vibio, M., Davis, R. E. & Lee, I.-M. 1993. Molecular characterization of some mycoplasma-like organisms infecting plants in Italy. *Petria* **3**, 9.
- Gundersen, D. E. & Lee, I.-M. 1996. Ultrasensitive detection of phytoplasmas by nested PCR assays using two universal primer pairs. *Phytopathol. Mediterr.* **35**, 144–151.
- Jomantiene, R., Davis, R. E., Dally, E. L. & Maas, J. L. 1998a. The distinctive morphology of *Fragaria multicipita* is due to phytoplasma. *HortScience* **33**, 1069–1072.
- Jomantiene, R., Davis, R. E., Maas, J. L. & Dally, E. L. 1998b. Classification of new phytoplasmas associated with diseases of strawberry in Florida, based on analysis of 16 rRNA and ribosomal protein gene operon sequences. *Int. J. System. Bacteriol.* **48**, 269–277.
- Jomantiene, R., Davis, R.E., Valiunas, D., & Alminaitė, A. 2002. New group 16SrIII phytoplasma lineages in Lithuania exhibit rRNA interoperon sequence heterogeneity. *Eur. J. Plant Pathol.* **108**, 507–517.
- Kamińska, M., 2000. Phytoplasma diseases of ornamental plants in Poland. *Zeszyty Naukowe Instytutu Sadownictwa i Kwiaciarstwa* **7**, 79–86.
- Lee, I.-M., Gundersen-Rindal, D. E., Davis, R.E., & Bartoszyk, I. M. 1998. Revised classification scheme of phytoplasmas based on RFLP analyses of 16rRNA and ribosomal protein gene sequences. *Int. J. System. Bacteriol.* **48**, 1153–1169.
- Lee, I.-M., Gundersen-Rindal, D. E., Davis, R. E., Bottner, K. D., Marcone, C., & Seemüller, E. 2004. ‘*Candidatus Phytoplasma asteris*’ a novel phytoplasma taxon associated with aster yellows and related diseases. *Int. J. System. Evol. Microbiol.* **54**, 1–12.
- Marcone, C., Lee, I.-M., Davis, R. E., Ragozzino, A., & Seemüller, E. 2000. Classification of aster yellows–group phytoplasmas based on combined analyses of RNA and *tuf* gene sequences. *Int. J. System. Evol. Microbiol.* **50**, 1703–1713.
- Navalinskienė, M., Samuitienė, M., & Jomantiene, R. 2005. Molecular detection and characterization of phytoplasma infecting *Callistephus chinensis* plants in Lithuania. *Phytopathol. Pol.* **35**, 109–112.
- Samuitiene, M., Navalinskiene, M., Jomantiene, R. & Davis, R. E. 2004. Molecular characterization of phytoplasmas infecting columbine (*Aquilegia L.*) plants. *Biologija* **2**, 15–17.
- Samuitiene, M., Navalinskiene, M., Davis, R. E., & Jomantiene, R. 2006. Molecular characterization of phytoplasmas of subgroup 16SrI–A, 16SrI–B, 16SrI–L, and 16SrI–M infecting ornamental plants in Lithuania. *Bulletin OEPP/EPPO* **36** (in press).
- Schneider, B., Ahrens, U., Kirkpatrick, B. C., & Seemüller, E. 1993. Classification of plant-pathogenic mycoplasma-like organisms using restriction-site analysis of PCR-amplified 16S rDNA. *J. Gen. Microbiol.* **139**, 519–527.
- Seemüller, E., Schneider, B., Maurer, R. & 7 other authors. 1994. Phylogenetic classification of phytopathogenic mollicutes by sequence analysis of 16S ribosomal DNA. *Int. J. System. Bacteriol.* **44**, 440–446.
- Valiūnas, D. 2003. *Identification of phytoplasmas in Lithuania and estimation of their biodiversity and molecular evolutionary relationships. Summary of doctoral thesis.* Institute of Botany, Vilnius, 36 pp.