

First report of stolbur phytoplasma infecting celery in Serbia

Žarko IVANOVIĆ¹, Nenad TRKULJA¹, Svetlana ŽIVKOVIĆ¹, Erika PFAF DOLOVAC¹, Nenad DOLOVAC¹, Jelena JOVIĆ², Milana MITROVIĆ²

¹Institute for Plant Protection and Environment, Department of Plant Pathology, Teodora Drajzera 9, 11040 Belgrade, Serbia

²Institute for Plant Protection and Environment, Department of Plant Pests, Banatska 33, 11080 Zemun, Serbia

Abstract

During field survey conducted in 2010 in Serbia, symptoms of foliar reddening were observed on celery on the locality Padinska Skela, in the vicinity of Belgrade. Leaf samples from six symptomatic and two asymptomatic plants were collected and tested for phytoplasma presence detection.

Nested polymerase chain reaction analyzes using universal primer pairs P1P7 followed by R16F2n/R2 identified presence of phytoplasmas in all symptomatic plants, while asymptomatic plants were tested negative. Restriction analysis of amplified 16Sr DNA fragments with enzyme *MseI* identified in all positive samples the same pattern as a reference strain of stolbur phytoplasma belonging to the 16SrXII-A ribosomal subgroup. Molecular differentiation of stolbur phytoplasma detected in celery was performed by amplification and RFLP analysis of the elongation factor Tu (*tuf* gene). Digestion of *tuf* gene indicated presence of *tuf*-type b of stolbur phytoplasma in all symptomatic celery plants. This is the first record of stolbur phytoplasma in association with celery expressing symptoms of foliar reddening in Serbia.

Key words: *Apium graveolens*, PCR, RFLP, Serbia, stolbur phytoplasma, *tuf* gene.

Introduction

The stolbur phytoplasma belonging to 16SrXII-A ribosomal subgroup is widely distributed in Europe, associated with severe diseases on many cultivated plants (grapevine, maize, solanaceous crops, potato, carrots, sugarbeet, strawberry).

In celery crops (*Apium graveolens* L.), stolbur phytoplasma infection has been reported in Italy (Carraro *et al.*, 2008), Hungary (Vicizian, 2002) and in Czech Republic (Navratil *et al.*, 2009). Celery was determined as highly susceptible to stolbur phytoplasma infection (Fialova *et al.*, 2009), with symptoms consisting of diffuse yellowing and/or reddening of the leaves and stunting.

In Serbia, stolbur phytoplasma has been associated with maize redness disease of maize (Duduk and Bertaccini, 2006; Jović *et al.*, 2007) and 'bois noir' in grapevine (see in Cvrković, 2010) causing severe yield losses with tendency of growing epidemics, but never in association with celery.

Primary goal of this study was to identify and characterize phytoplasmas in association with celery showing symptoms of foliar reddening.

Materials and methods

In September 2010, a total of six samples of celery with reddish discoloration of leaves (figure 1) were collected on locality Padinska Skela (near Belgrade) and analyzed for phytoplasma presence. In addition, two symptomless plants were collected and used as negative controls.

Nucleic acids were extracted from fresh leaf midribs using CTAB protocol according to Angelini *et al.* (2001). Phytoplasma identification was conducted

through nested PCR amplification of 16S ribosomal RNA gene according to Lee *et al.* (1998), with the universal primer pairs P1/P7 and R16F2n/R2. Restriction fragment length polymorphism (RFLP) analysis of the amplified phytoplasmas 16S rRNA gene fragments was performed with *MseI* enzyme. RFLP profiles of phytoplasma identified in celery were compared with a reference phytoplasma strains.

Molecular differentiation of stolbur phytoplasma detected in celery was performed by amplification and restriction digestion of the elongation factor Tu - *tuf* gene. Amplification was conducted in nested PCR with fTuf1/rTuf1 and fTufAY/rTufAY primers followed by digestion with *HpaII* restriction enzyme, according to Langer and Maixner (2004).



Figure 1. Stolbur infected celery with symptoms of foliar reddening.

(In colour at www.bulletinofinsectology.org)

Results and discussion

Nested PCR analysis with 16S rRNA universal primers detected the presence of phytoplasmas in all celery plants which exhibited symptoms of foliar reddening. All asymptomatic plants tested were negative. Restriction analysis of PCR products with endonuclease *Mse*I showed in all samples the same pattern as the one of the reference strain of the stolbur phytoplasma belonging to the 16SrXII-A subgroup (figure 2A). The *tuf* gene was amplified in all symptomatic samples. Digestion with *Hpa*II endonuclease determined presence of *tuf*-type b stolbur phytoplasma (figure 2B). Identification of stolbur in infected plants represents the first record of this phytoplasma in celery crops in Serbia.

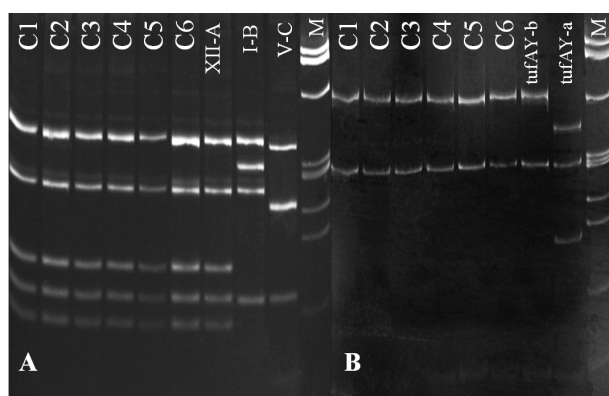


Figure 2. RFLP profiles of: (A) 16S rRNA fragments amplified by nested PCR with primer pairs P1/P7 and R16F2n/R2, followed by digestion with *Mse*I; (B) *tuf* gene fragments amplified with primers fTuf1/rTuf1 and fTufAY/rTufAY and digested with *Hpa*II; C1-C6: stolbur infected celery, XII-A (16SrXII-A, from naturally infected maize from South Banat region, Serbia), I-B (16SrI-B, provided by E. Angelini, Italy), V-C (16SrV-C, FD-C from naturally infected field-growing grapevine from Nišavski region, Serbia), tufAY-b: stolbur type tufAY-b from the Mosel region of Germany (provided by M. Maixner, Germany); tufAY-a: stolbur tuf-type a, from Middle-Rhine region of Germany (provided by M. Maixner, Germany); M: molecular weight marker ϕ X174/*Hae*III digested (Fermentas).

Stolbur phytoplasma mostly originates from the naturally infected plants, from which it is transmitted to cultivated plants by polyphagous planthoppers of the Cixiidae family. It is known that celery is a very susceptible host to stolbur phytoplasma infection (Fialova *et al.*, 2009), which implicates that cultivation of this crop can be seriously compromised when the pathogen occurring in natural reservoirs is transmitted by active vectors to cultivated plants.

Celery is important vegetable crop in Serbia, thus, it is of particular importance, besides incidence and impact of the disease, to study the epidemiology of stolbur

appearance in correlation with movement of potential vectors from wild plants to vegetable crops during the growing season. These studies are key points for elucidating the epidemiological cycle of stolbur disease in association with celery and relevant point for further pest management strategy of this phytoplasma.

Acknowledgements

We thank the Ministry of Education and Science of the Republic of Serbia for financial support during this study through grants TR31018 and III43001.

References

- ANGELINI E., CLAIR D., BORGIO M., BERTACCINI A., BOUDON-PADIEU E., 2001.- Flavescence dorée in France and Italy - Occurrence of closely related phytoplasma isolates and their near relationships to Palatinate grapevine yellows and an alder phytoplasma.- *Vitis*, 40: 79-86.
- CARRARO L., FERRINI F., MARTINI M., ERMACORA P., LOI N., 2008.- A serious epidemic of stolbur on celery.- *Journal of Plant Pathology*, 90(1): 131-135.
- CVRKOVIĆ T., 2010.- Diversity of Auchenorrhyncha species in Serbian vineyards and their role in 'Bois noir' transmission.- PhD thesis, 1-103 pp. University of Belgrade.
- DUDUK B., BERTACCINI A., 2006.- Corn with symptoms of reddening: new host of stolbur phytoplasma.- *Plant Disease*, 90: 1313-1319.
- FIALOVA R., VALOVA P., BALAKISHIYEVA G., DANET J. L., SAFAROVA D., FOISSAC X., NAVRATIL M., 2009.- Genetic variability of stolbur phytoplasma in annual crop and wild plant species in South Moravia.- *Journal of Plant Pathology*, 91(2): 411-416.
- JOVIĆ J., CVRKOVIĆ T., MITROVIĆ M., KRNIJAČIĆ S., REDINBAUGH M. G., PRATT R. C., GINGERY R. E., HOGENHOUT S. A., TOŠEVSKI I., 2007.- Roles of stolbur phytoplasma and *Reptalus panzeri* (Cixiinae, Auchenorrhyncha) in the epidemiology of Maize redness in Serbia.- *European Journal of Plant Pathology*, 118: 85-89.
- LANGER M., MAIXNER M., 2004.- Molecular characterization of grapevine yellows associated phytoplasmas of the stolbur-group based on RFLP-analysis of non-ribosomal DNA.- *Vitis*, 43: 191-199.
- 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.
- NAVRATIL M., VALOVA P., FIALOVA R., LAUTERER P., SAFAROVA D., STARY M., 2009.- The incidence of stolbur disease and associated yield losses in vegetable crops in South Moravia (Czech Republic).- *Crop Protection*, 28: 898-904.
- VICZIÁN O., 2002.- Identification and investigation of phytoplasmas occurring in Hungary by molecular methods.- PhD thesis, Szent Istvan University The faculty of horticulture.

Corresponding author: Milana MITROVIĆ (e-mail: milanadesancic@yahoo.co.uk), Institute for Plant Protection and Environment, Department of Plant Pests, Banatska 33, 11080 Zemun, Serbia.