First report of stolbur phytoplasma infecting celery in Serbia

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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 (Viczian, 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 MseI 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 HpaII 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.

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.

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