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Stolbur phytoplasma infection of kale crops (*Brassica oleracea* var. *gemmifera* L.) in Serbia

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Abstract

Kale plants (*Brassica oleracea* var. *gemmifera* L.) showing symptoms of reddening on leaf and petioles and overall stunting were found on locality Pančevo (south Banat) during inspection on vegetable crops carried out in 2010 in Serbia. The phytoplasma associated with the disease was detected through nested PCR with primer pairs P1/P7 and R16F2n/R2 followed by RFLP analysis with restriction enzyme *Mse*I. A total of 10 symptomatic and 4 asymptomatic plants were collected and analyzed for the presence of phytoplasma. Nested PCR amplified 16S ribosomal RNA fragments of phytoplasma in all samples of kale with symptoms, while all asymptomatic plants tested negative. Digestion with *Mse*I identified in infected kale the same pattern as a reference strain of stolbur phytoplasma belonging to 16SrXII-A group. Amplification of the elongation factor *tuf* gene and digestion with *Hpa*II restriction enzyme was performed for genotype differentiation of stolbur phytoplasmas detected in kale. Digestion of *tuf* gene indicated presence of tuf-type b of stolbur phytoplasma in all symptomatic kale plants. This is the first report of stolbur phytoplasma infecting kale crops in Serbia.

Key words: stolbur phytoplasma, Serbia, *tuf* gene, *Brassica oleraceae* var. *gemmifera.*

Introduction

Phytoplasmas of the stolbur group (16SrXII-A) are widely distributed in Europe, infecting a wide range of cultivated and wild plants. In past 10 years, increasing incidence of stolbur phytoplasma was registered in different crops (grapevine, maize, sugar beet, potato, vegetable crops), suggesting progressive spread of diseases associated with this phytoplasma.

In the vegetable crops, severe yield losses caused by stolbur phytoplasma have been recorded in solanaceous crops (tomato, potato, pepper) and celery (Viczian, 2002; Carraro *et al*., 2008; Navratil *et al*., 2009; Fialova *et al*., 2009). Among phytoplasma associated diseases affecting kale crops, previous studies identified a phyllody of kale caused by phytoplasma belonging to aster yellows group (16SrI-B) (Marcone *et al*., 2000).

Primary goal of this study was to identify and characterize phytoplasma in association with kale plants showing symptoms typical of phytoplasmas-associated diseases such as reddening and stunting.

Materials and methods

A total of 10 stunted plants of kale with red leaves and petioles (figure 1) were collected in 2010 in locality Pančevo (south Banat) and submitted to nested PCR analysis to identify presence of phytoplasma. Additional 4 asymptomatic plants were collected from the same locality and used as negative controls.

Nucleic acids were extracted from leaves and petioles using CTAB protocol previously described by Angelini *et al.* (2001). Phytoplasma presence was detected by amplifying 16S ribosomal RNA gene through nested PCR analysis with universal primer pairs P1/P7 and R16F2n/R2 (Lee *et al*., 1998). Restriction fragment length polymorphism (RFLP) analysis of the amplified DNA fragments from positive samples of kale was performed with *Mse*I restriction enzyme. Furhter stolbur characterization was performed by amplifying the elongation factor *tuf* gene with fTuf1/rTuf1 and fTufAY/rTufAY primers followed by digestion with *Hpa*II restriction enzyme (Langer and Maixner, 2004).

Results and discussion

Presence of phytoplasmas was detected in all kale plants exhibiting symptoms of reddening and stunting, while symptomless plants tested negative. Digestion of nested PCR products of 16S rRNA gene with endonuclease *Mse*I determined in all samples the same RFLP profile as reference strain of the stolbur phytoplasma belonging to 16SrXII-A subgroup (Lee *et al.*, 1998).

The *tuf* gene was amplified in all samples positive for stolbur presence. Digestion of TufAY PCR products with *Hpa*II restriction enzyme detected the presence of tuf-type b of stolbur phytoplasma in infected kale (figure 2). Identification of stolbur phytoplasma represents the first record on presence of this group of phytoplasma in association with kale crops in Serbia and South East Europe.

Diseases caused by the stolbur phytoplasma are considered typically epidemic because, under favourable conditions, they can spread quickly with a high incidence. The ability of phytoplasma to infect numerous wild and cultivated plants and its transmission by polyphagous planthoppers plays a key role in the spread of stolbur disease. Each of the three

stolbur genotypes differentiated based on *tuf* gene (tuf-types a, b, c) had been associated with different host plants of the main stolbur phytoplasma vector *Hyalesthes obsoletus* Signoret (Langer and Maixner, 2004). All strains of the tuf-type b genotype are known to be associated with bindweed *Convolvulus arvensis* L in German vineyards, however, this type of stolbur has been later detected in vegetable crops such as celery, pepper, potato, tomato and in diverse weed plants sampled from the vegetable plots (Fialova *et al*., 2009).

In Serbia, naturally growing weeds can often be found along the borders and inside vegetable plots, presenting a real threat by continuously harboring stolbur phytoplasma or hosting planthoppers as potential vectors of phytoplasma from wild to cultivated plants.

Further study of stolbur disease in association with kale crops in Serbia are required, such as inspection of common weeds in and around vegetable plots, and identification of potential insect vectors.

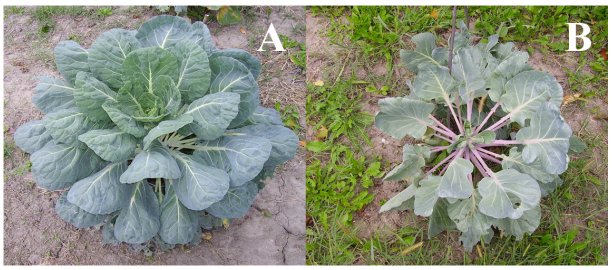

Figure 1. A) healthy kale, and B) kale infected with stolbur phytoplasma expressing symptoms of petioles reddening and overall stunting.
(In colour at www.bulletinofinsectology.org)

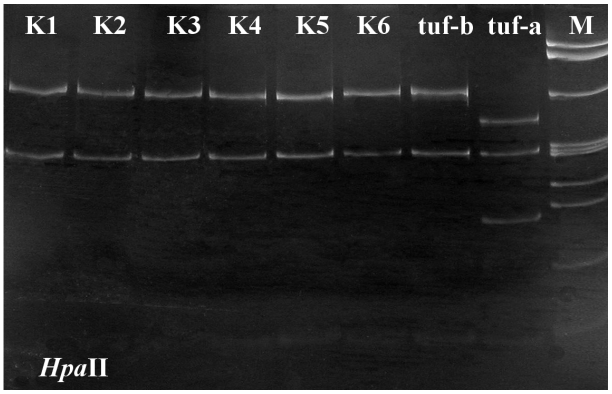

Figure 2. Polyacrilamide gel showing the *Hpa*II RFLP patterns of *tuf* gene of stolbur phytoplasmas from kale obtained with fTuf1/rTuf1 and fTufAY/rTufAY primers; K1-K6 – stolbur infected kale; tuf-b (stolbur tuf-type b from the Mosel region of Germany); tuf-a (stolbur tuf-type a, from Middle-Rhine region of Germany, provided by M. Maixner, Bernkastel-Kues); M: molecular weight marker ϕX174/*Hae*III digested (Fermentas, Vilinius, Lithuania).

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