МОЛЕКУЛЯРНА ДИАГНОСТИКА НА ФИТОПЛАЗМЕНАТА БОЛЕСТ СТОЛБУР ПО ЦЕЛИНАТА *APIUM GRAVEOLENS* L.
MOLECULAR DIAGNOSTICS OF STOLBUR PHYTOPLASMA DISEASE IN CELERY *APIUM GRAVEOLENS* L.

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Abstract

In a field survey, conducted during 2012-2014 in Bulgaria, symptoms of foliar reddening were observed on celery in the locality of the village of *Stryama*, near Plovdiv. Leaf samples from 9 symptomatic and 5 asymptomatic plants were collected and tested for phytoplasma presence detection.

Nested polymerase chain reaction (PCR) analyses using universal primer pairs P1/P7 followed by R16F2n/R2 identified the presence of phytoplasmas in all symptomatic plants, while asymptomatic plants were tested negative. Restriction analysis of amplified 16S rRNA fragments with MseI enzyme identified in all positive samples the same pattern as a reference strain of stolbur phytoplasma belonging to the 16SrXII-A ribosomal subgroup.

Key words: *Apium graveolens* L., PCR, RFLP, stolbur phytoplasma, Bulgaria.

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, carrots, strawberry, etc.).

In celery crops (*Apium graveolens* L.), stolbur phytoplasma infection has been reported in Italy (Carraro *et a*l., 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.

Aster yellows symptoms and severity can be highly variable depending on the strain of the pathogen, age of plant when infected, and other factors.

Celery plants are usually severely stunted and yellowed. Inner petioles are characteristically short, yellow to white in color, and moderately to severely curved and twisted. On older plants, petioles become brittle in texture and the epidermis and underlying tissues can crack and peel.

In later stages of the disease, the inner heart of the plant turns brown and can decay.

Aster yellows is transmitted to crops by leafhopper insect vectors.

Overwintering leafhoppers can harbor the phytoplasma, or leafhoppers can obtein the pathogen while feeding on infected plants.

Important weed hosts include dandelion, plantains, pineapple-weed, sow thistle, wild lettuce, wild chicory, horseweed, and wild asters.

When leafhoppers migrate from pasture or non crop land to vegetable fields, or when drying vegetation drives leafhoppers from foothills and other areas, the insects encounter celery and other crops and transmit the phytoplasma during feeding.

Therefore, significant aster yellows outbreaks almost always occur in fields near pastures, rivers, ditch banks, foothills, and weedy non crop land. Because of fluctuations in the populations and flight patterns of leafhoppers, and fluctuations in the populations of infected reservoir plants, aster yellows incidence varies greatly from year to year. Overall impact on celery is generally low.

Aster yellows is difficult to control, in part, because of the extensive host range of the phytoplasma. Over 300 species of food, forage, ornamental, and weed plants are susceptible.

While weed management should be practiced, this will have little effect on aster yellows.

There are no chemical controls for the aster yellows phytoplasma.

Insecticides will have little effect on leafhopper transmission of the pathogen and are therefore not recommended.

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

MATERIALS AND METHODS

Celery (*Apium graveolens* L.) is important vegetable crop in Bulgaria.

In August, September 2012-2014, a total of 10 samples of celery with reddish discoloration of leaves (Fig. 1, 2, 3, 4) were collected from locality village Stryama, near Plovdiv and analyzed for phytoplasma presence. In addition, two symptomless plants were collected and used as negative controls.

Samples were tested for phytoplasma with the polymerase chain reaction (PCR) method using universal primer pairs, fP1 (5‘ AAG AGT TTG ATC CTG GCT CAG GAT 3‘) (Deng and Hiruki, 1991) and rP7 (5‘ CGT CCT TCA TCG GCT CTT 3‘) (Schneider et al. 1995) that amplified parts of the ribosomal operon comprising the 16S rRNA gene, the spacer region (SR) and the start of the 23S rRNA gene.

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 16SrRNA gene fragments was performed with MseI enzyme. RFLP profiles of phytoplasma identified in celery were compared with a reference phytoplasma strains.

RESULTS AND DISCUSSION

Phytoplasmas are phloem-limited disease agents of a great number of plant species (McCoy et al., 1989) including vegetables, ornamentals, fruit and timber trees, and wild plants. In nature, they are transmittted by sapsucking insect such as leafhoppers, planthoppers and psyllids (Weintraub and Beanland, 2006).

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Fig. 1. *Stolbur infected celery with symptoms of foliar reddening and size reduction of root*

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Fig. 2. *Stolbur infected celery with symptoms of foliar reddening*

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Fig. 3. *Stolbur infected celery with symptoms of foliar reddening*

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Fig. 4. *Stolbur infected celery with symptoms of foliar reddening*

Phytoplasma diseases are considered typically epidemic because, under particularly favourable conditions, they can spread quickly with a high incidence. The success of a phytoplasma disease in an ecosystem depends on several factors, including the contemporary presence of the pathogen, the susceptible host plant(s) and the vector(s). So, in a given ecosystem, a phytoplasma disease can be particularly severe and widespread.

Among phytoplasmas, those belonging to the stolbur group (16SrXII), subgroup A, are present in Europe and cause diseases in different host plants (Lee et al., 2000).

Recently, the name '*Candidatus* Phytoplasma solani' was proposed for the reference strain of this '*Candidatus*' species (IRPCM, 2004).

Stolbur was reported several years ago in eastern Europe (Valenta et al., 1961), where also the vector *Hyalesthes obsoletus* Signoret was identified (Aleksic et al., 1967). After the introduction of molecular-based methods for the diagnosis of phytoplasmas, stolbur has been found in several naturally infected plants species (Marzachì et al., 2000) and the list continues to grow (Duduk and Bertaccini, 2006).

The present work describes and analyzes the exceptionally high epidemicity of stolbur phytoplasma on celery in a restricted area of village Stryama, near Plovdiv characterised by intensive horticulture.

Cultivated and wild plants showing phytoplasma-like symptoms such as yellowing, stunting, proliferation, virescence and phyllody, and symptomless plants of the same species were collected during the surveys.

Nested PCR analysis with 16SrRNA universal primers detected the presence of phytoplasmas in all celery plants which exhibited symptoms of foliar reddening. All asymptomatic plants tested were negative (Fig. 5). Restriction analyses 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 (Fig. 6). Identification of stolbur in infected plants represents the first record of this phytoplasma in celery crops in Bulgaria.

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 Bulgaria, 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.

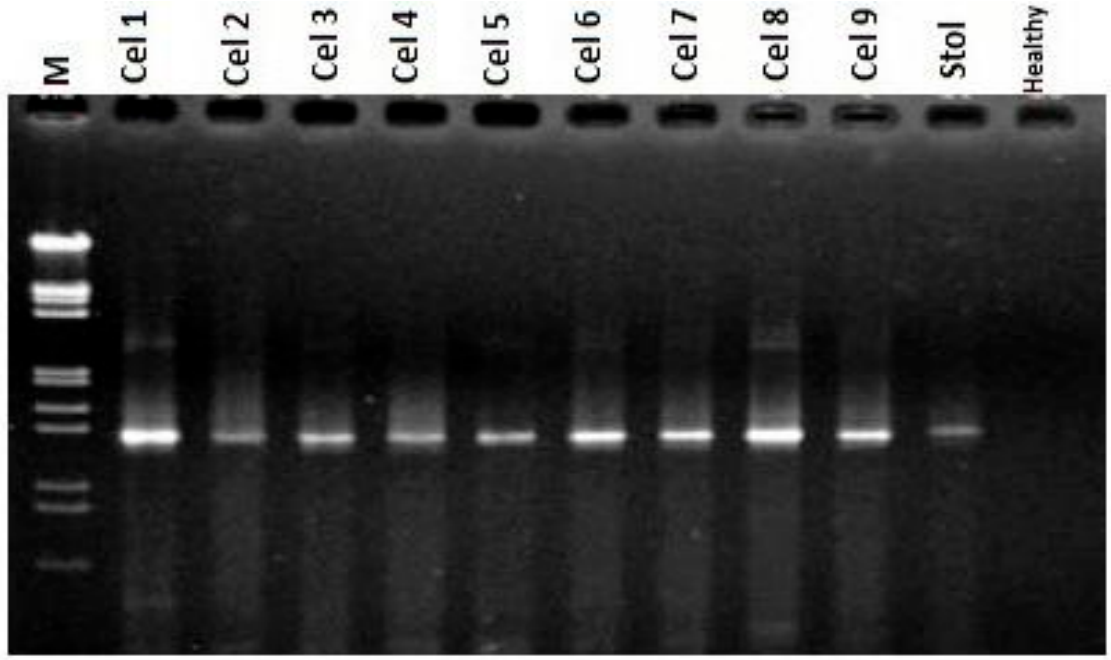

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Fig. 5. *Results of nested PCR analysis detected the presence of phytoplasmas in all celery plants with symptoms of foliar reddening*

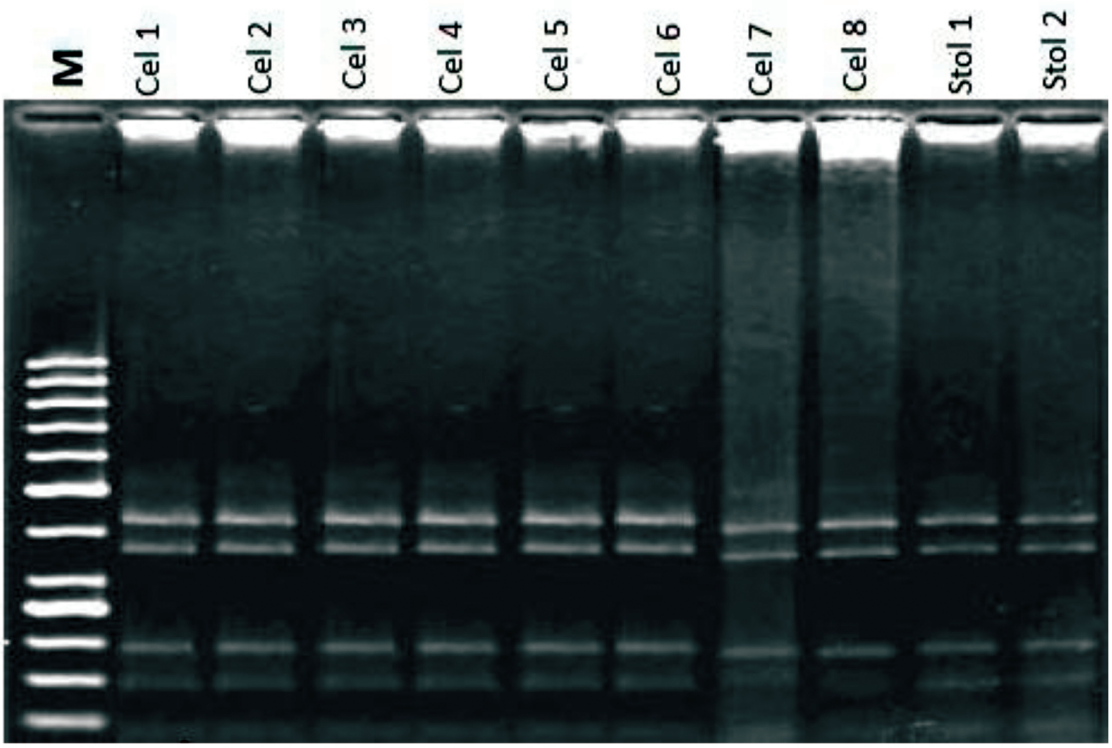

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Fig. 6. *RFLP profiles of 16SrRNA fragments amplified by nested PCR with pairs P1/P7 and R16F2n/R2, followed by digestion with MseI*

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