

## Molecular detection of potato stolbur phytoplasma in Serbia

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### Abstract

In August 2010 potato plants expressing symptoms of phytoplasma infection were collected from three localities in Vojvodina province of Serbia and analyzed for phytoplasma presence. Phytoplasma detection was performed by PCR/RFLP analyzes of 16S rRNA gene and by stolbur-specific PCR amplification with Stol11 primers. Stolbur phytoplasma was detected in all symptomatic potato plants analyzed. This is the first report of molecular detection of stolbur phytoplasma infecting potato in Serbia.

**Key words:** detection, stolbur phytoplasma, potato, aerial tubers, leaf discoloration, PCR, RFLP.

### Introduction

Phytoplasmas are wall-less, non-culturable, phloem-limited, insect-transmitted plant pathogens from the class *Mollicutes*. They are associated with diseases in several hundred species of plants, many of which are economically important, including potato (*Solanum tuberosum* L.). Phytoplasma diseases of potato have become increasingly important in recent years (reviewed in Ember *et al.*, 2011), due to the epidemic appearance and geographic spread of the diseases, as well as significant yield losses in potato production and low quality of produced tubers. Different phytoplasma 16Sr groups are infecting potato worldwide, however many are causing similar symptoms. In Europe, potato stolbur phytoplasma is the most common, and by impact the most significant phytoplasma infecting potato and has a quarantine status in the European Union (EPPO/CABI, 1996).

In southeast Europe, including Serbia, presence of potato phytoplasma disease was for a long time, diagnosed solely on the basis of visual symptoms and/or presence of tentative insects-vectors (Ember *et al.*, 2011). First record of potato stolbur in Serbia is dating from the 1950's (Panjan, 1950), in time when the knowledge on stolbur "virus" was limited to descriptive records of symptoms expression on infected potatoes and general assumption that *Hyalesthes obsoletus* Signoret is the main cixiid vector of the disease. Since then, no attempt has been made on molecular characterization of phytoplasmas associated with symptomatic potato plants in Serbia.

Presence of symptoms typical for potato stolbur phytoplasma including reddening and upward rolling of the top leaves, shorten internodes and aerial tubers were observed in recent years in several localities in potato growing regions of Serbia. The main aim of this study was to identify phytoplasmas infecting potato in Serbia by employing PCR/RFLP methods of detection.

### Materials and methods

During August 2010, potato growing areas of Bačka and Srem regions in Vojvodina province of Serbia were surveyed for the presence of potato phytoplasma. Samples of potato plants expressing symptoms of leaf yellowing, reddish or purplish discoloration, rolling of the top leaves and presence of aerial tubers (figure 1) were collected from three localities: Bački Petrovac, Titel and Zemun. Six symptomatic potato plants were sampled per site. From every plant leaves with petioles, stems and aerial tubers were collected and stored at -20°C before being processed. Leaves of two asymptomatic potatoes from each site were used as negative controls.

Total nucleic acids were extracted from potato plants using the CTAB method (Angelini *et al.*, 2001). Initial phytoplasmas identification was conducted on 16S rRNA gene using nested PCR procedure with the P1/P7 and R16F2n/R2 primers according to Lee *et al.* (1998). Obtained amplicons of the expected size (approximately 1,200 bp) were subjected to restriction analyses with *MseI* endonuclease, in order to identify 16S rRNA subgroup of detected phytoplasmas.

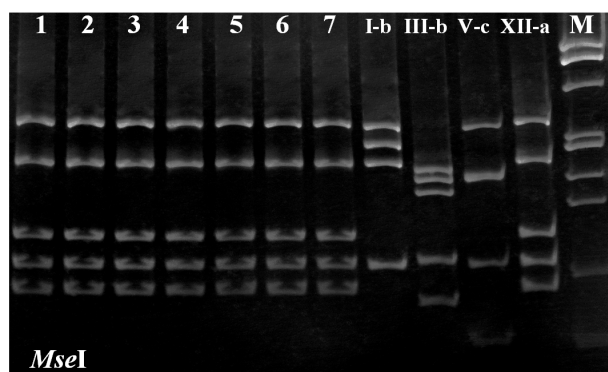


**Figure 1.** Symptoms of potato stolbur phytoplasma. Aerial tubers, yellowing and upward rolling of the top leaves in stolbur-infected potato plant.

To confirm the presence of stolbur phytoplasma, potato samples that were tested positive with the universal phytoplasma primers were also submitted to nested PCR with the stolbur-specific Stoll1 primers (Clair *et al.*, 2003) according to previously described protocol (Radonjić *et al.*, 2009).

## Results and discussion

Molecular analyses of phytoplasma 16S rRNA PCR products from symptomatic potato plants revealed the presence of stolbur phytoplasma (16SrXII-A group) in all 18 samples analyzed, from all the three localities. Restriction digestion patterns of 16S gene fragment PCR products with *MseI* for all samples were identical to each other and to the maize redness (MR) sample from Serbia (figure 2). None of the symptomless plants were positive for the presence of phytoplasmas. Analyses with stolbur specific Stoll1 primers confirmed that all tested symptomatic potatoes were infected with stolbur phytoplasma (data not shown).



**Figure 2.** RFLP analyses of the 1,200 bp 16S rRNA gene fragment of stolbur phytoplasma infecting potato amplified by nested PCR with P1/P7 and F2nR2 primers, digested with *MseI* and separated by electrophoresis through 13% polyacrylamide gels. 1-7: symptomatic potato plants; I-b (16SrI-B, provided by E. Angelini, Conegliano); III-b (16SrIII-B, naturally infected *Cirsium arvense* with symptoms of multiple inflorescence disease, Serbia); V-C (FD-C, field growing infected grapevine from Central Serbia); XII-a (MR, field growing infected maize from South Banat region, Serbia); M: molecular weight marker *φX174/HaeIII* digested (Fermentas, Vilnius, Lithuania).

Although presence of potato phytoplasma is generally considered to be common in Serbia, our results represent the first detection of stolbur phytoplasma associated with previously observed and reported symptoms on potato. Frequency of infected plants and distribution of the disease in the territory of Serbia is yet not known, and will be determined in future studies. Attention should be given to the economic importance of potato production, especially considering recent report of stolbur induced yield losses of potato in Russia and

Romania (Ember *et al.*, 2011), as well as stolbur phytoplasma epidemics on maize in Serbia (Jović *et al.*, 2009). Due to widespread appearance of stolbur phytoplasma in Europe it is of great importance to determine epidemiological cycle and subsequently define proper management practice to control potato stolbur disease. Accurate identification of phytoplasmas by applying molecular tools, as well as identification of insect vector(s) and natural plant reservoirs of potato stolbur phytoplasma will be crucial to meet these demands.

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