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# Identification of phytoplasmas belonging to aster yellows ribosomal group in vegetables in Serbia

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## Abstract

Leaf and root samples of carrot, and flower samples of broccoli with symptoms referable to phytoplasma presence were collected and tested for phytoplasma presence. Detection, identification and molecular characterization were performed on 16S rDNA, *Tuf*, *rpS3*, putative aminoacid kinase and putative DNA helicase phytoplasma genes. Analyses of all five DNA fragments showed that carrot was infected with aster yellows phytoplasmas belonging to ribosomal subgroups 16SrI-A and 16SrI-B and broccoli only with phytoplasmas belonging to ribosomal subgroup 16SrI-B.

**Key words:** Aster yellows phytoplasmas, carrot, cabbage, molecular identification.

## Introduction

During vegetable crops field surveys, carrot and broccoli plants with symptoms referable to phytoplasma presence were observed. On both species presence of symptoms was sporadic, but carrot was more severely affected. Symptoms on carrots included leaf reddening, purpling and yellowing, formation of chlorotic adventitious shoots, and reduction of size and quality of taproots, while on broccoli flower proliferation and phyllody were present. Diseases with similar symptoms were reported in several vegetable growing areas worldwide. Recently, diverse prokaryotes with almost undistinguishable symptoms were described in carrot in North America i.e. phytoplasmas belonging to 16SrVI-A, 16SrI-A and 16SrI-B ribosomal subgroups, and *Spiroplasma citri* (Lee *et al*., 2006). Aster yellows phytoplasmas belonging to ribosomal subgroup 16SrI-B were reported in cabbage in North Italy and North America (Bertaccini *et al*., 1992; Lee *et al*., 2003), while aster yellows phytoplasma was detected in broccoli in South Italy (Marcone and Ragozzino, 1995) and in carrot in Israel (Orenstein *et al*., 1999).

Verification of phytoplasma presence, together with their identification, was performed in order to determine the agent(s) of these diseases in Serbia.

## Materials and methods

Samples of carrot and broccoli showing the symptoms described above were collected during October 2006, in the South Bačka region of Serbia. Total nucleic acids were extracted from 1 g of carrot leaf and root tissue, and from broccoli flowers following the protocol described by Angelini *et al*. (2001), dissolved in TE buffer, and maintained at -20 °C. Before performing polymerase chain reaction (PCR) tests nucleic acids were quantified and diluted in sterile distilled water, to a final concentration of 20 ng/µl.

Reference strains (Bertaccini, 2003) employed for phytoplasma identification and characterization were: *Chrysanthemum* yellows (CHRY, 16SrI-A), European aster yellows (EAY, 16SrI-B), *Catharanthus* virescence (CVB, 16SrI-F), carrot yellows (CA, 16SrI-C), primula yellows (PRIVA, 16rI-L), and stolbur from pepper from Serbia (STOL, 16SrXII-A). Direct PCR reactions were carried out respectively with P1/P7 (Deng and Hiruki, 1991; Schneider *et al*., 1995) universal phytoplasma primer pair, and with R16(I)F1/R1 (Lee *et al*., 1994) ribosomal group I, II and XII specific primers. Reaction mix and PCR conditions were as described (Schaff *et al*., 1992). PCR products separated in 1% agarose gels and stained with ethidium bromide were visualized with a UV transilluminator. Phytoplasma identification was done by RFLP analyses with *Hha*I and *Tsp509*I restriction enzymes. Visualization of RFLP products was performed in a 5% polyacrylamide gel.

Further molecular characterization on rpS3 gene was performed by direct PCR using rp(I)F1A/rp(I)R1A primer pair with reaction mix and PCR conditions as described (Lee *et al*., 2003). Molecular analyses were also performed using BB88F1/R1 to amplify genes coding putative aminoacid kinase plus ribosome recycling factor (Gundersen *et al*., 1996; Botti and Bertaccini, 2003) and TufAYf/r (Schneider *et al*., 1997) to amplify the *tuf* gene. For both primer pairs 35 PCR cycles were performed under the following conditions: 30 sec for denaturation step at 95 °C, 30 sec for annealing at 55 °C, and 1 min for primer extension at 72 °C. RFLP analyses of all obtained PCR products were performed with restriction enzymes *Tsp*509I and *Tru*I. Visualization of PCR and RFLP products was performed as described above.

G35p/m primer pair using conditions as described (Davis *et al*., 1992) was also used to amplify a phytoplasma putative DNA helicase (Duduk and Bertaccini, 2006).

## Results

PCR reactions with P1/P7 and R16(I)F1/R1 primer pairs resulted in expected length fragment amplification, (about 1,800 bp and 1,100 bp, respectively) from the symptomatic carrot and broccoli samples tested. The RFLP profiles obtained with carrot sample amplicons show 2 different groups: i) referable to reference strain CHRY, 16SrI-A ribosomal subgroup, and ii) referable to strain EAY, 16SrI-B ribosomal subgroup. Restriction profiles obtained with all amplicons of broccoli samples were referable to strain EAY.

PCR reactions with rp primers resulted in expected length fragment amplification (about 1,200 bp) from all the symptomatic carrot and broccoli samples tested. RFLP analyses with *Tru*I and *Tsp509*I produced two different groups of profiles in agreement to those described above. Also BB88F1/R1 and TufAYf/r primer pairs amplified expected length fragments (about 740 and 1,200 bp respectively) from all the symptomatic carrot and broccoli samples tested, and RFLP analyses confirmed the two groups of profiles.

PCR reactions with G35p/m primer pair produced expected length fragment amplification (about 1,200 bp) only from some carrots and cabbage samples that resulted without polymorphisms in RFLP analyses.

## Discussion

RFLP analyses of 16S rDNA and of 16S rDNA plus spacer regions confirmed phytoplasma presence in both symptomatic carrot and broccoli samples. Phytoplasmas in carrot were identified as belonging to ribosomal subgroups 16SrI-A and 16SrI-B and phytoplasmas in broccoli as belonging to ribosomal subgroup 16SrI-B. Phytoplasmas 16SrI-A and 16SrI-B were detected in the same carrot field but never in mixed infection. Collective RFLP profiles on putative aminoacid kinase plus ribosome recycling factor, *tuf* and *rpS3* genes distinguished phytoplasmas belonging to the two 16SrI subgroups confirming results on 16S ribosomal gene.

Lack of amplification of putative DNA helicase gene of some carrot and broccoli infected samples is in agreement with published data indicating that G35p/m primers failed to amplify 16SrI-A phytoplasmas as well as a number of 16SrI-B strains (Botti and Bertaccini, 2003). Since primers were developed from 16SrXII-A phytoplasmas (Davis *et al*., 1992), variability of this region could be a reason for inconsistent amplification of aster yellows-related phytoplasma strains.

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