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Phytoplasmas in apricot, peach and sour cherry orchards in East Bohemia, Czech Republic

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Abstract

Symptoms of prematurely yellowing and leaf roll in the summer were observed in apricot, peach and sour cherry orchards in the East Bohemia (Czech Republic) during 2009-2010. Samples from selected symptomatic trees were tested by PCR assays followed by RFLP analyses. Detected phytoplasmas were classified in the apple proliferation group, subgroup 16SrX-B, which representative strains are apricot chlorotic leaf roll and European stone fruit yellows. Although the majority of symptomatic trees was observed in apricot orchards, out of 54 apricot trees examined by PCR/RFLP assays, only 10 plants were positive (18.5%) to phytoplasma presence. In the peach orchards, phytoplasmas were detected in 4 trees out of 14 plants tested (28.6%). Total number of trees examined from sour cherry orchards was 25, out of these 9 plants revealed positive reaction (36%). DNA sequencing of phytoplasma strain from one sour cherry tree confirmed the RFLP identification as '*Candidatus* Phytoplasma prunorum'. Experiments for transmission of '*Ca*. P. prunorum' by double budding from positive trees to indicator plants were carried out in open field during 2009 and 2010. No symptoms typical for phytoplasma infection were observed to date.

Key words: '*Candidatus* Phytoplasma prunorum', PCR/RFLP, biological indexing, apricot, peach and sour cherry orchards.

Introduction

'*Candidatus* Phytoplasma prunorum' associated with of European stone fruit yellows (ESFY) disease is the quarantine phytoplasma infecting trees of the genus *Prunus* worldwide (Seemüller and Schneider, 2004). This pathogen can be transmitted by insect vector of psyllid species, *Cacopsylla pruni* (Scopoli) (Carraro *et al.*, 1998). Typical symptoms for ESFY are yellowing and leaf roll in summer. The disease is very dangerous, because infected plants die within one or two years. The first report of ESFY identification in Czech Republic was from declining apricot trees (Navrátil *et al.*, 1998). We report here the results of a survey on phytoplasmas infecting apricot, peach and cherry trees in East Bohemia during 2009-2010.

Materials and methods

The observation of phytoplasma disease symptoms was carried out in 2 apricot, 1 peach and 2 sour cherry orchards in East Bohemia during 2009-2010. Samples were collected from symptomatic branches during late summer and autumn. Altogether, 54 apricot trees, 14 peach trees and 25 sour cherry trees were examined.

DNA was extracted from phloem tissues of apricot and peach branches according to the modified method of Ahrens and Seemüller (1992), and from phloem tissues of leaves and branches of sour cherry using a phenol/chloroform method. The polymerase chain reaction (PCR) assay was carried out with two different primer pairs combinations. To amplify region that includes the 16S rRNA gene, the spacer region, and the start of 23S rRNA gene of the phytoplasmas, the primer pair P1/P7 (Deng and Hiruki, 1991; Schneider *et al.*, 1995) was used in direct PCR. PCR products were diluted with sterile distilled water (1: 29) prior to amplification by nested PCR using R16F2n/R2 (Gundersen and Lee, 1996). Similarly, R16F2n/R2 was used in direct PCR as outer primer pair and fU5/rU3 (Lorenz *et al*., 1995) was used as inner primer pair in further nested PCR. About 20 ng of each DNA preparation in water were added to the PCR mix (Schaff *et al*., 1992) in a final reaction mixture volume of 25µl. Approximately 200 ng of DNA of each positive PCR product were separately digested with 2.5 U of *Mse*I, *Ssp*I and *Rsa*I, *Bfm*I restriction enzymes (Fermentas, Lithuania) after R16F2n/R2 and fU5/rU3 amplification, respectively. Restriction patterns obtained were compared with those of positive controls and patterns described in the literature.

DNA isolated from one sour cherry tree cv. Morela pozdní was used for sequencing. A set of overlapping PCR products was generated by amplification with primers R16P1/U3 (position 2-1,230), R16F2n/R2 (position 152-1,379), R16Pc399/Pc1694 (position 399-1,694), R16F1/R0 (position 130-1,503) and 17RF758/P7 (position 758-1,818). PCR products were sequenced in both directions using the BIG DYE sequencing terminator kit (PE Biosystems, Warrington, UK). Sequencing was performed in ABI PRISM 310 sequencer (PE Applied Biosystems, Foster City, CA, USA). The sequences were aligned with those of phytoplasmas available in the GenBank using World Wide Web service BLAST (http://www.ncbi.nlm.nih.gov).

The buds from phytoplasma positive trees were picked up in August 2009 and 2010. Indicator plants suitable for climatic condition of Czech Republic were inoculated on the virus-free rootstocks St. Julien for apricot and peach, Colt for sour cherry. The buds from infected trees were inoculated under indicator buds after 10-14 days. Indicators used were *Prunus persica* cv. GF-305 for apricot and peach trees, *P. avium* cv. Sam, *P. avium* cv. Bing, *P. avium* cv. F12/1 and *P. avium* cv. Canindex

for sour cherry trees. Indicators maintained in the field (3-5 plants for each) are visually inspected twice during the growing season for at least 2 years.

Results

Although 92 trees examined showed premature leaf yellowing and leaf roll symptoms mainly in the end of summer and in the beginning of autumn, as well as smaller size of leaves, sparse foliage and smaller underripened fruits, only 22 plants were positive for phytoplasma presence. Unexpectedly, one tree of sour cherry, from which specimens were collected as negative control, revealed also positive reaction in PCR. RFLP with *Mse*I, *Ssp*I and *Rsa*I, *Bfm*I endonucleases of the DNA sequences amplified by PCR with primer pair R16F2n/R2 and fU5/rU3, respectively, showed that phytoplasma from apricot, peach and sour cherrry trees had a pattern identical to each other and to the phytoplasmas belonging to apple proliferation ribosomal group, subgroup 16SrX-B.

Phytoplasmas were identified in 10 apricot trees (18.5%), in 4 peach trees (28.6%) and in 9 sour cherry trees (36.0%). Moreover, to confirm the RFLP results, DNA amplified from 1 sour cherry tree cv. Morela pozdní was used for sequencing. Comparison of the 840 bp sequence obtained confirmed the RFLP classification and indicating 99% identity with '*Ca.* P. prunorum', strain ESFY-142 (accession No. AJ575108) from apricot from Spain. The results of biological indexing were not successful, the typical symptoms were not observed on the indicator plants till this time.

Discussion

Visual inspection of apricot, peach and sour cherry orchards in East Bohemia (Czech Republic) revealed frequent presence of plants with symptoms recalling phytoplasma infection. The PCR/RFLP results indicated the presence of phytoplasmas in 22 out of 92 examined symptomatic trees. Therefore, on the bases of up to now investigation, we can not consistently demonstrate the association of phytoplasmas with symptoms and the presence of other pathogens, like viruses, fungi or other bacteria can not be excluded. Moreover, one asymptomatic sour cherry plant was also positive in PCR/RFLP analyses for phytoplasma presence. Phytoplasmas attributed to subgroup 16SrX-B were identified in all PCR positive trees. Moreover, DNA sequence obtained from phytoplasma detected in sour cherry tree revealed the closest relationship with '*Ca.* P. prunorum', strain ESFY-142.

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