

SHORT COMMUNICATION

FIRST REPORT OF '*CANDIDATUS PHYTOPLASMA PRUNORUM*'
INFECTING APRICOTS IN TUNISIAM. Ben Khalifa¹, M. Aldaghi², H. Hacheche¹, J. Kummert², M. Marrakchi¹ and H. Fakhfakh¹¹ *Laboratoire de Génétique Moléculaire Immunologie et Biotechnologie, Faculté des Sciences de Tunis, Campus Universitaire, Tunis El-Manar, 2092 Tunis, Tunisia*² *Unité de Phytopathologie, Département de Biochimie et de Biologie Appliquées-FUSAGX Passage Des Déportés 2, 5030 Gembloux, Belgium*

SUMMARY

'*Candidatus* Phytoplasma prunorum' was detected for the first time in Tunisia in apricot trees that showed early leaf reddening in autumn, off-season growth in winter followed by dieback, and bore small and tasteless fruits. Phytoplasma was detected by nested PCR using the universal phytoplasma primer pairs R16mF2/R1 and R16F2n/R2. An amplification product of the expected size (1.2 kbp) was obtained from samples collected from symptomatic but not from symptomless apricot trees. Amplicons subjected to RFLP analysis after digestion with endonucleases *MseI* and *RsaI*, gave patterns similar to those reported for the European stone fruit yellows phytoplasma (ESFY, 16SrX-B). Identification was further confirmed by PCR using ESFY-specific primer pairs (ECA1/ECA2).

Key words: apricot, ESFY, '*Candidatus* Phytoplasma prunorum', PCR, RFLP, diagnosis.

Phytoplasmas cause a range of persistent diseases to many fruit trees in the world (Ogawa *et al.*, 1995). In Europe, many economically important decline diseases of stone fruit species have been associated with the presence of European stone fruit yellows (ESFY) phytoplasma (Jarusch *et al.*, 1998), a member of subgroup B of the apple proliferation group (16SrX-B) (Lee *et al.*, 1998), denoted as '*Candidatus* Phytoplasma prunorum' by Seemüller and Schneider (2004). The most common hosts of ESFY phytoplasma include apricot (Morvan, 1977), plum (Giunchedi *et al.*, 1982), peach (Poggi Pollini *et al.*, 2001), and almond (Seemüller *et al.*, 1998).

Apricot (*Prunus armeniaca* L.) is one of the most common stone fruit trees grown in Tunisia, its production accounting for 18.5% of the global stone fruit yield. Most of the crop is destined for local consumption and only 1% is exported, mainly to neighboring countries (Anonymous, 2009).

Disease symptoms resembling those caused by ESFY phytoplasma were recently observed on apricots in northern Tunisia, where 17% of the apricot orchards are located (Anonymous, 2009). As reported in the present paper, the identification of the putative phytoplasma associated with the apricot syndrome was carried out by PCR using universal and ESFY-specific primer pairs followed and RFLP analysis.

Samples were collected during autumn and winter 2008 from apricot trees of the local cv. Arengi growing in the Ras Jebel area (northern Tunisia). Symptomatic plants showed early leaf reddening in autumn and off-season growth in winter in addition to sudden death of infected branches or of the entire canopy. These symptoms are similar to those previously reported by Morvan (1977). Infected trees bore small and tasteless fruits, which resulted in heavy yield losses.

A total of nine trees were sampled from the same apricot orchard, six of which showed ESFY-like symptoms whereas three were symptomless. Phytoplasma reference strains, i.e. apple proliferation (AP, 16SrX-A), European stone fruit yellows (ESFY, 16SrX-B) and pear decline (PD, 16SrX-C) phytoplasma (Lee *et al.*, 1998) were maintained in the experimental host *Catharanthus roseus* (periwinkle) and used as positive controls.

Total DNA, used as template in PCR reactions, was extracted from *ca.* 1 g of freshly collected bark tissue. DNA from control strains was obtained from petioles and midribs of infected *C. roseus* plants. Nucleic acids were isolated according to the phytoplasma enrichment procedure described by Ahrens and Seemüller (1992).

PCR amplifications of phytoplasma 16S rDNA were carried out with universal primers R16mF2/R1 (Gundersen and Lee, 1996) and R16F2n/R2 (Lee *et al.*, 1995). Non ribosomal primers ECA1/ECA2 (Jarusch *et al.*, 1998) were also used for ESFY-specific detection. PCR was performed in 25 µl reaction volumes containing 20 ng DNA, 0.4 mM of each primer, 0.25 mM of each dNTP, 1.5 mM MgCl₂ and 0.5 units of HotGold-Star DNA polymerase (Eurogentec, Belgium) with the manufacturer's supplied buffer. Thermocycling was performed with a Peltier thermocycler (Hybaid, UK) for 35 cycles with 1 min each for denaturation, annealing and extension, followed by a 10 min final extension. For

nested PCR, DNA amplified in the cycle primed by R16mF2/R1 was diluted 1:25 with sterile distilled water and used as template for an additional PCR run primed by R16F2n/R2. PCR products were separated on 1.5 % agarose gels containing 0.5 $\mu\text{g ml}^{-1}$ ethidium bromide and visualised using a UV transilluminator. Size of the PCR products was determined by comparison with 100 bp ladder (Fermentas, Lithuania).

For RFLP analysis, nested PCR products (10-12 μl) were digested with *MseI*, and *RsaI* endonucleases (Fermentas, Lithuania) according to the manufacturer's instructions. *MseI* restriction products were separated in 5% polyacrylamide gel electrophoresis and stained by ethidium bromide. *RsaI*-digested fragments were separated in 2% agarose gel containing 0.5 $\mu\text{g ml}^{-1}$ ethidium bromide. DNA bands were visualised under UV light. Size of the PCR products was determined by comparison with 100 bp ladder (Fermentas, Lithuania). RFLP patterns were compared with those obtained from the phytoplasma reference strains and the RFLP patterns previously published (Lee *et al.*, 1998).

Whereas nested PCR assays using the primer pairs R16mF2/R1 and R16F2n/R2 yielded a characteristic band of approximately 1.2 kb from all symptomatic apricot samples tested and from reference phytoplasma strains (AP, ESFY and PD) used as positive controls, no amplification was obtained from any of the symptomless trees.

As shown in Fig. 1 and 2, RFLP patterns obtained from diseased apricot trees were indistinguishable from

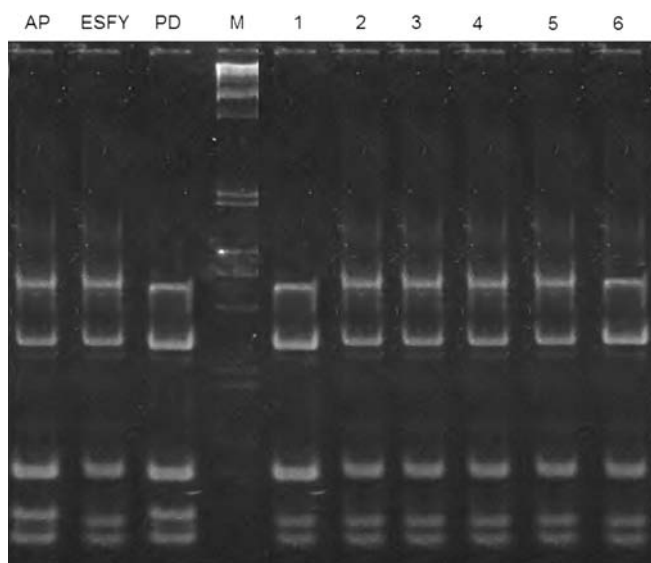


Fig. 1. *MseI* restriction profiles of phytoplasma ribosomal DNA amplified using the universal primer pairs R16mF2/R1 and R16F2n/R2. Template DNA was from symptomatic apricots cv. Arengi (1-6) or from periwinkle plants infected with the following phytoplasma reference strains: AP, apple proliferation; ESFY, European stone fruit yellows; PD, Pear decline. M, marker.

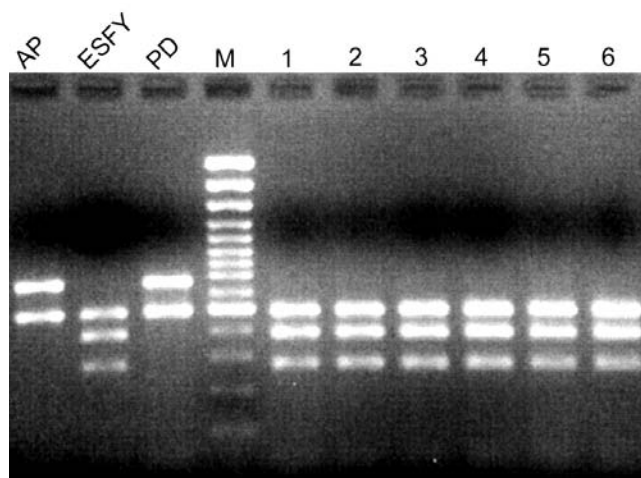


Fig. 2. *RsaI* restriction profiles of phytoplasma ribosomal DNA amplified using the universal primer pairs R16mF2/R1 and R16F2n/R2. Template DNA was from symptomatic apricots cv. Arengi (1-6) or from periwinkle plants infected with the following phytoplasma reference strains: AP, apple proliferation; ESFY, European stone fruit yellows; PD, Pear decline. M, marker.

each other and were similar to those previously reported for the ESFY phytoplasma (Lee *et al.*, 1998).

PCR analysis using ESFY-specific primer pair ECA1/ECA2 yielded an amplification product of 273 bp from all apricot samples tested and from ESFY phytoplasma used as positive control, whereas no amplification was obtained from AP and PD controls (Fig. 3).

These results were taken as evidence that all symptomatic apricot trees of cv. Arengi tested were infected by '*Ca. Phytoplasma prunorum*', a new record for Tunisia.

Notwithstanding the uprooting of infected apricot

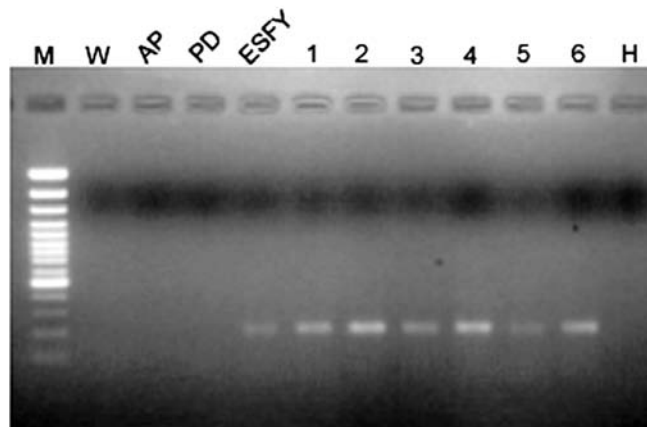


Fig. 3. Detection of ESFY phytoplasma using ESFY specific primer pair ECA1/ECA2. Template DNA was obtained from asymptomatic apricots (H), symptomatic apricots (1-6) or from periwinkle plants infected with the following phytoplasma reference strains: AP, apple proliferation; ESFY, European stone fruit Yellows; PD, Pear decline. W, water control; M, marker.

trees, there was no apparent effect on disease spreading. Since this is the first report on the occurrence of '*Ca. Phytoplasma prunorum*' in apricot in Tunisia, investigations on its epidemiology must be undertaken. Moreover, the alleged pathogenicity of this phytoplasma to different stone fruit species (Giunchedi *et al.*, 1982; Morvan, 1977; Poggi Pollini *et al.*, 2001; Seemüller *et al.*, 1998), calls for investigations for assessing its possible presence and distribution in peach and almond which, along with apricot, represent the most common stone fruit trees grown in the country.

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