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Phytoplasmas in Lebanon: characterization of '*Candidatus* Phytoplasma pyri' and stolbur phytoplasma respectively associated with pear decline and grapevine "bois noir" diseases

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Abstract

Surveys of phytoplasma diseases were conducted since 2000 as part of a phytosanitation program in Lebanon. Several phytoplasmas have been detected: '*Candidatus* Phytoplasma phoenicium' associated with a lethal disease of almond trees, '*Ca*. P. trifolii' associated with diseases of solanaceous crops and a phytoplasma of the 16SrII ribosomal group associated with shoot proliferation of an ornamental cactus. More recently, phytoplasmas were detected in diseased grapevines and declining pear trees collected in the central part of the country. Both diseases are respectively associated with the stolbur phytoplasma of the 16SrXII-A group and '*Ca.* P. pyri' of the group 16SrX-C. Using the stol1H10 PCR-RFLP typing tool, we demonstrate that the Lebanese "bois noir" phytoplasma strains are genetically identical to the stolbur subtype P7, a strain found in 2001 in Lebanon on yellowing periwinkle. Sequences of part of the 16SrDNA and *ace*F genes confirmed the presence of specific strains of '*Ca.* P. pyri' in Lebanese declining pears. This is the first report of the pear decline disease presence in Lebanon.

Key words: Phytoplasma, pear decline, "bois noir", Lebanon.

Introduction

In Lebanon, phytoplasmas were first detected in 1999-2000. '*Candidatus* Phytoplasma phoenicium' was associated with a witches' broom disease killing almond trees (Choueiri *et al*., 2001; Abou-Jawdah *et al*., 2002; Abou-Jawdah *et al*., 2003; Verdin *et al*., 2003). The presence of stolbur phytoplasma (Stolp) associated with "bois noir" (BN) disease of grapevine (Daire *et al*., 1993; Maixner et *al*., 1994; Choueiri *et al*., 2002); of a 16SrII group phytoplasma associated with shoot proliferation of ornamental *Opuntia monacantha* (Choueiri *et al*., 2005) and of '*Ca.* P. trifolii' in diseased tomatoes and peppers (Choueiri *et al.*, 2007) were also reported. In 2005 and 2006, during surveys of Lebanese fruit tree orchards, symptoms typical of pear decline, induced by '*Ca.* P. pyri' member of the 16SrX-C ribosomal subgroup (Seemüller and Schneider, 2004) were observed. The geographical distribution and the preliminary molecular typing of '*Ca*. P. pyri' and stolbur phytoplasmas detected in Lebanon is presented.

Materials and methods

Nucleic acids were extracted from 0.5 g of leaf midribs of symptomatic and symptomless grapevine and pear plants, according to the CTAB method (Maixner *et al*., 1995). DNA from periwinkle plants infected with twelve strains of Stolp or various strains of the 16SrXII-A ribosomal subgroup were used as controls and references. The 16SrDNA PCR detection was performed with universal phytoplasma primers pairs P1/P7 (Deng and Hiruki, 1991; Smart *et al*., 1996) and nested primers pairs fU5/rU3 (Seemüller *et al.*, 1994).

Non-ribosomal specific PCR tests were carried out with primers pairs: Stol1H10F1/R1 and the nested TypH10F/R (unpublished) for typing Stolp strains, AcefF1/AcefR1 and the nested AcefF2/AcefR2 for typing '*Ca.* P. pyri' strains (Danet *et al*., 2007). For RFLP typing, the fU5/rU3 PCR products were digested with *Alu*I and *Rsa*I, and TypH10F/R PCR products were digested with *Rsa*I, according to standard procedures. Resulting restriction fragments were analysed on 1% agarose gels or 8% polyacrylamide gel electrophoresis. Sequences of fU5/rU3 and AcefF2/R2 amplified products were carried out on ABI-PRISM. Sequence alignments and phylogenetic analyses were performed using MEGA3 software using adequate sets of sequences.

Results

In vineyards, BN is present in both Caza Zahle and Caza West Bekaa where most of Lebanon's vineyards are located. During the late summers and autumns of 2005 and 2006, 133 diseased grapevine samples were collected in North Bekaa, Central Bekaa and West Bekaa from different cultivars: 94 samples of Chardonnay, 17 samples of Gamay, 10 samples of Cabernet-Sauvignon, 9 samples of Syrah and 3 samples of Marselan. Except in North Bekaa, more than half of the samples from diseased grapevines tested positive for phytoplasma. No PCR product was obtained in symptomless plants. Regarding the cultivars, Chardonnay with 56 positives of 93 samples tested and Gamay with 6 positives of 17 samples tested were the mostly affected. On the contrary, Cabernet Sauvignon, Syrah and Marselan

never tested positive despite presence of symptoms on the grapes. All fU5-rU3 amplified products gave stolp profiles when digested with *Alu*I and *Rsa*I and compared to control phytoplasmas. Stolp positives DNA samples were submitted to stol1H10 PCR-RFLP typing. The resulting 1.4 kbp products displayed a *Rsa*I digestion profile identical to that of Stolp detected in an ornamental periwinkle (strain P7) collected in Tyr-Lebanon in 2001 and different from all the other Stolp reference strains tested.

Thirty one declining pears of two cultivars grown in Lebanon (cv. California and Coscia) were sampled (table 1). The majority of the samples collected in west and central Bekaa were positive using the fU5/rU3 primers but a sample collected near Baalbeck was negative. Both cultivars were found infected in the same proportion. Sequencing of fU5/rU3 PCR products confirmed the detected phytoplasma as strains of '*Ca.* P. pyri'. Sequence typing of the *ace*F amplified product AcefF2/AcefR2 of 0.9 kbp was performed for 3 samples collected in Tanayl and in Terboul (table 1). All pear samples gave an identical *ace*F sequence presenting one single nucleotide polymorphism as compared to the corresponding sequence of European '*Ca.* P. pyri' strains, and therefore constituting a specific Lebanese type.

Discussion

The Stolp strain detected in Lebanese BN affected grapevines was specific to Lebanon and identical to the Stolp isolate found in a clump of periwinkles (isolate P7). It is likely that this Stolp strain is locally transmitted by insects, and does not represent a new transmission from young vines imported from Europe.

Table 1. Phytoplasma typing and distribution.

Location (Région-Caza)	Year	Positive/ tested	Phytoplasma
Grapevine			
North Bekaa-Caza Hermel	2006	0/8	-
North Bekaa-Caza Baalbeck	2006	0/9	-
Central Bekaa-Caza Zahle	2005	40/70	Stolp (P7)
West Bekaa-Caza W. Bekaa	2005	22/42	Stolp (P7)
West Bekaa-Caza W. Bekaa	2006	3/3	Stolp (P7)
Pear			
Central Bekaa-Caza Zahle	2005	7/11	'*Ca*. P. pyri' (LEB)
Central Bekaa-Caza Zahle	2006	6/12	'*Ca*. P. pyri' (LEB)
West Bekaa-Caza West Bekaa	2006	3/7	'*Ca*. P. pyri' (LEB)
North Bekaa-Caza Baalbeck	2006	0/1	-

'*Ca* P. pyri' identified in Lebanon is genetically different from the European strains and may be specific to the Eastern part of the Mediterranean basin. At the moment, we do not know if there are local transmissions by pear psyllids, but imported plant material must be tested.

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