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'*Candidatus* Phytoplasma phoenicium'-related strains infecting almond, peach and nectarine in Lebanon

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Abstract

Genetic diversity among '*Candidatus* Phytoplasma phoenicium'-related strains infecting almond, peach and nectarine plants in diverse geographic regions of Lebanon was investigated by virtual restriction fragment length polymorphism (RFLP) analysis of 16S rDNA nucleotide sequences. Calculation of virtual restriction similarity coefficients indicates the presence of two new subgroups, -F and -G, in group 16SrIX. Obtained results open new opportunities for in-depth studies on the distribution of '*Ca.* P. phoenicium' strains in plant hosts and insect vector populations from different geographic areas of Lebanon.

Key words: single nucleotide polymorphisms, genetic diversity, phytoplasma classification, restriction fragment length polymorphism.

Introduction

'*Candidatus* Phytoplasma phoenicium' strains, belonging to subgroups 16SrIX-B and -D, are associated with a lethal devastating disease of almond trees (almond witches broom, AlmWB) in Lebanon (Abou-Jawdah *et al.*, 2002). By the year 2002, more than 100,000 almond trees had died by AlmWB in Lebanon; in 2009, '*Ca.* P. phoenicium' was identified also in association with a severe disease of peach and nectarine in southern Lebanon (Abou-Jawdah *et al.*, 2009).

The rapid spread of '*Ca.* P. phoenicium' over large geographical areas in North Lebanon suggested the presence of an efficient vector (Abou-Jawdah *et al.*, 2009). However, this vector has not been identified yet. In order to have a better understanding of the disease epidemiology and achieve an effective disease management, a development project financed by Italian Cooperation is being implemented by AVSI (Association of Volunteers in International Service) Foundation in Lebanon. In the present study, data on genetic diversity among '*Ca.* P. phoenicium' strains infecting almond, nectarine and peach plants from diverse Lebanese regions are reported.

Materials and methods

Leaf samples were collected in 15 orchards from 24 plants (table 1) showing symptoms such as witches' broom, phyllody, virescence and chromatic alterations. Total DNA was extracted from 100 mg of leaf veins and used for phytoplasma detection by 16S rDNA amplification in nested PCRs primed by phytoplasma-universal primer pairs P1/P7 and R16F2n/R16R2 (Gundersen and Lee, 1996).

Amplicons from nested PCRs were sequenced, assembled, and compared with the GenBank database with the aim of searching possible identity. A total of 37 16S rDNA sequences of phytoplasma group 16SrIX (13 from GenBank and 24 obtained in this work), plus sequences from phytoplasma strains representative of known 16Sr subgroups, were analyzed through an automated *in silico* restriction assay, as described by Wei *et al*. (2007).

Results and discussion

Primer pair R16F2n/R16R2 primed amplification of DNA from templates derived from all samples studied. Phytoplasma strains identified here shared a 99-100% of sequence identity with '*Ca*. P. phoenicium' (accession number AF515636). Visualization and comparison of virtual gel plotted images revealed three different RFLP patterns (table 1). One pattern, indistinguishable from that characteristic of strains classified in the subgroup 16SrIX-D, was exhibited by DNAs from 15 '*Ca.* P. phoenicium' strains (figure 1). The remaining two virtual RFLP patterns differed from that of the previously described subgroup IX-D (figure 1), and shared similarity coefficients of 93 to 97%, confirming their affiliation with group 16SrIX. Actual RFLP analyses confirmed the recognition of two new subgroups in group 16SrIX. Prior to this work, five subgroups had been described in the group 16SrIX; the results of this study add two new, confirmed by real RFLP subgroups -F (two strains) and -G (seven strains) from almond, nectarine and peach plant hosts. The data evidenced extensive diversity of '*Ca*. P. phoenicium' in Lebanon, particularly in Sarada regions, where three 16SrIX subgroups (-D, -F, and -G) co-exist and infect nectarine plants.

Table 1. '*Ca.* P. phoenicium' strains, belonging to distinct 16SrIX subgroups, in orchards of Lebanon regions.

Strain	Origin	Orchard No.	Host	Subgroup 16SrIX
SarN1-2	Sarada	1	nectarine	-G
SarN5	Sarada	1	nectarine	-F
SarN8-1	Sarada	2	nectarine	-D
SarN9-7	Sarada	1	nectarine	-D
SarN10-8	Sarada	3	nectarine	-D
SarP10(297)	Sarada	4	peach	-D
MarN13-1	Marjayoun	5	nectarine	-D
MarN14-1	Marjayoun	6	nectarine	-D
MarN27-2	Marjayoun	7	nectarine	-F
MarN28-1	Marjayoun	7	nectarine	-D
FegA1-1	Feghal	8	almond	-G
FegA11-4	Feghal	9	almond	-D
FegA13-1	Feghal	9	almond	-G
FegA16-4	Feghal	8	almond	-D
FegA18-1	Feghal	10	almond	-G
FegP1-2	Feghal	11	peach	-D
FegP2-6	Feghal	11	peach	-D
FegP3-1	Feghal	11	peach	-G
FegPL3-1	Feghal	11	almond	-D
FegA3	Feghal	12	almond	-G
FegA4	Feghal	13	almond	-G
KKN18-1	Kerbet Kanafar	14	nectarine	-D
KKN19-1	Kerbet Kanafar	14	nectarine	-D
KKN29-1	Kerbet Kanafar	15	nectarine	-D

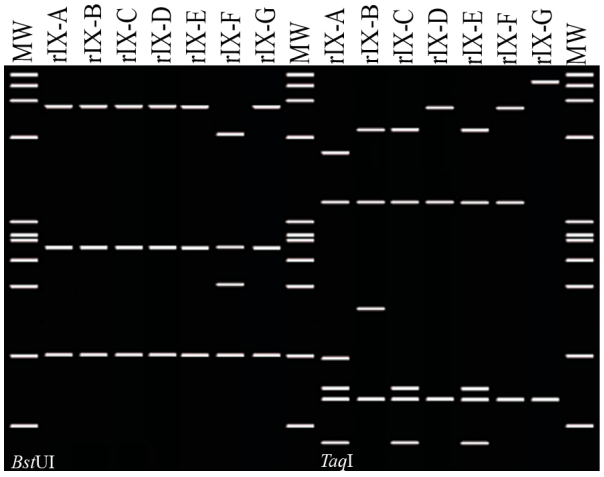

Figure 1. Virtual R16F2/nR2 RFLP patterns by key enzymes *Bst*UI and *Taq*I for distinguishing among 16SrIX subgroups.

Conclusions

The broad genetic diversity among '*Ca*. P. phoenicium'-related strains suggests possible influence of different ecological and/or climatic niches on phytoplasma population composition. In particular, it would be interesting to investigate whether particular '*Ca*. P. phoenicium' subgroup(s) could be correlated with certain biological properties and different species of insect vector. These investigations will be crucial for a better understanding of the disease epidemiology and achieving an effective disease management.

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References

ABOU-JAWDAH Y., KARAKASHIAN A., SOBH H., MARTINI M., LEE I-M., 2002.- An epidemic of almond witches' broom in Lebanon: classification and phylogenetic relationship of the associated phytoplasma.- *Plant Disease,* 86: 477-484.

ABOU-JAWDAH Y., SOBH H., AKKARY M., 2009.- First report of almond witches' broom phytoplasma ('*Candidatus* Phytoplasma phoenicium') causing a severe disease on nectarine and peach trees in Lebanon.- *Bulletin OEPP/EPPO*, 39: 94-98.

GUNDERSEN D. E., LEE I-M., 1996.- Ultrasensitive detection of phytoplasmas by nested-PCR assays using two universal primer pairs.- *Phytopathologia Mediterranea*, 35: 144-151.

WEI W., DAVIS R. E., LEE I-M., ZHAO Y., 2007.- Computer-simulated RFLP analysis of 16S rRNA genes: identification of ten new phytoplasma groups.- *International Journal of Systematic and Evolutionary Microbiology*, 57: 1855-1867.

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