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# A PHYTOPLASMA DISEASE OF ALMOND IN LEBANON

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**SUMMARY** - During a survey conducted to establish the sanitary status of stone fruits in Lebanon, almond trees with symptoms of leaf yellowing, shoot proliferation and dieback were observed in the Bekaa region. Witches'-brooms on affected trees was the most characteristic symptom. Because such symptoms are often seen on plants infected by phytoplasma, samples were collected and analyzed by PCR. All almond tissues with witches'-brooms gave positive PCR reactions, but not the healthy or symptomless samples. The amplified DNA of the phytoplasma was analyzed by RFLP, and the restriction profiles were different from those published for other phytoplasmas infecting almond trees in Western Europe. Sequence analysis of the amplified DNA showed that it is closely related to the Pigeon pea Witches'-brooms cluster (PPWB). In Bekaa Valley, the disease is presently diffused in different localities of the center and east parts. Recent surveys showed the disease to spread rapidly in the north of Lebanon resulting a serious outbreak and high severity levels of infection. The most severely affected areas were those of Raskifa and Dair Amar.

**Key words:** Lebanon, almond, phytoplasma, PPWB, diagnosis, PCR

**RESUME** - Au cours d'une prospection effectuée pour évaluer l'état sanitaire des espèces fruitières à noyau au Liban, des amandiers affichant des symptômes de jaunissement foliaire, prolifération des pousses et dépérissement ont été observés dans la région de la Bekaa. La manifestation la plus caractéristique sur les arbres infectés était représentée par les balais de sorcière. Etant donné que de tels symptômes sont aussi visibles chez des plantes infectées par les phytoplasmes, des échantillons ont été prélevés et analysés par PCR. Tous les tissus d'amandier présentant des balais de sorcière ont réagi positivement en PCR, à l'opposé des échantillons sains ou sans symptômes. Le DNA amplifié du phytoplasme a été analysé par la RFLP et les profils de restriction obtenus se sont révélés différents par rapport à ceux publiés pour d'autres phytoplasmes qui infectent les amandiers dans l'Europe occidentale. L'analyse des séquences du DNA amplifié a montré une corrélation étroite avec le groupe du Pigeon pea Witches'brooms. Dans la vallée de la Bekaa, la maladie est actuellement répandue dans différentes localités du centre et de l'est. Des prospections récentes ont indiqué une diffusion rapide de la maladie dans le nord du Liban, où les attaques sont très graves et les niveaux d'infection très significatifs. Les zones les plus atteintes sont Raskifa et Dair Amar.

**Mots-clés:** Liban, amandier, phytoplasme, PPWB, diagnostic, PCR

## INTRODUCTION

Almond (*Prunus amygdalus*) which has been cultivated in Lebanon since several years, is the most important and widespread fruit crop among stone fruit trees, distributed throughout the country on a surface of about 6.500 ha with a production of 28,600 T (Anonymous, 2000).

Almonds are grown in the Bekaa Valley, in the areas of Byblos and Batroun located north of Beirut, in Abde areas and in the hills of Akkar Casa in the north, and to lesser extent in the south around Saïda. Investigations on stone fruit tree virus diseases started about 5 years ago in Lebanon (Jawhar *et al.*, 1996) and viruses known at present in the country were, for the most part, detected during that period with some new data in 2001 (Choueiri *et al.*, 2001). However, no research work was undertaken to study the presence of phytoplasma diseases in Lebanon.

Phytoplasmas, formerly termed mycoplasma-like organisms, were discovered and described more than three decades ago by a group of Japanese scientists (Doi *et al.*, 1967). Phytoplasmas are nonhelical mollicutes associated with diseases of several hundred plant species (McCoy *et al.*, 1989). New disease

outbreaks occur from time to time in various geographic regions, so numerous new phytoplasma strains has been identified in the last 5 years (Marcone *et al.*, 1995; Zreik *et al.*, 1995; Zhu *et al.*, 1996; Seemüller *et al.*, 1998).

## MATERIALS AND METHODS

In October 1999, during a survey conducted to establish the sanitary status of stone fruit in Lebanon, almond trees with symptoms of leaf yellowing, shoot proliferation and dieback were observed in the Bekaa region. In spring 2000, almond growers witnessed severe decline of almond trees.

Samples (cuttings and leaves) were collected for serological testing for the presence of *Prunus necrotic ringspot virus* (PNRSV), *Apple mosaic virus* (ApMV), *Prune dwarf virus* (PDV), *Apple chlorotic leaf spot virus* (ACLSV), *Cherry leaf roll virus* (CLRV), *Plum pox virus* (PPV), *Tomato ringspot virus* (ToRSV) and Strawberry latent ring spot virus (SLRSV), but the results were negative.

Because such symptoms are often associated with phytoplasmas, samples were collected and analyzed by PCR at the Molecular Laboratory of INRA-Bordeaux using universal primers for amplification of phytoplasma ribosomal RNA genes (Schneider *et al.*, 1995). DNA was extracted from the leaf midveins and/or bark phloem tissue from symptomatic trees (Maixner *et al.*, 1995).

## RESULTS AND DISCUSSION

Witches'-brooms on affected trees was the most characteristic symptom. All symptomatic samples tested by ELISA for the above mentioned viruses were negative.

All almond tissues with witches'-brooms tested gave strong positive PCR reactions, but not the healthy or symptomless samples. The amplified DNA of the phytoplasma was analyzed by RFLP, and the restriction profiles were different from those published for other phytoplasmas infecting almond trees in Western Europe (Jaraush *et al.*, 1998). Sequence analysis of the amplified DNA showed that it closely related to the Pigeon pea Witches'-brooms cluster (PPWB) (Schneider *et al.*, 1995).

In Bekaa Valley, the disease is presently diffused in different localities of the center and east parts. Recently, two surveys done by the Department of Plant Protection-ARIL (April 2001) and the Molecular Laboratory-INRA Bordeaux (July 2001) showed phytoplasma disease of almond to spread rapidly in the north of Lebanon resulting a serious outbreak and high severity levels of infection. The most severely affected areas were those of Raskifa and Dair Amar. The last one had the highest number of affected trees. The most typical symptom of the disease is the appearance of witches'-broom, small leaves and deformed fruits. The trees died within two years after first symptoms appearance. In some orchards such trees were uprooted during the same year of the disease outbreak. The rapid spread of the disease and its phytoplasma etiology suggest that it may have an insect vector.

PCR positive shoots taken from infected plants were individually bud-grafted on almond seedlings and other woody host plants under quarantine conditions. Experimental transmission by dodder to periwinkle, electron microscopy, molecular studies and epidemiology of the disease were carried out to characterize the phytoplasma disease of almond and its vector in order to reduce the incidence of the disease and adopt an integrated control.

This is the first report of a phytoplasma infection in Lebanon and the first report for a PPWB group phytoplasmas in almond trees. The results of field surveys and laboratory assays revealed that this phytoplasma is one of the most lethal disease of almond.

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