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DETECTION AND PARTIAL CHARACTERIZATION OF DIFFERENT ISOLATES OF AMERICAN PLUM LINE PATTERN VIRUS IN THE MEDITERRANEAN

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SUMMARY - *American plum line pattern virus* (APLPV) was reported for the first time in the Mediterranean, isolated from Japanese plum in Palestine. Its identification was based on particle morphology, biological, serological and molecular properties. A survey was carried out in Apulia (Italy) and Murcia (Spain), by ELISA and molecular hybridization, to check for the presence of APLPV in plums. Only 4 out of the 671 tested samples turned to be positive for APLPV. These samples originated from a virus collection (Apulia) which in turn was previously collected from Apulia and Sicily (Italy), Albania and Tunisia. The positive samples were collected from symptomatic trees showing ring and line patterns from chlorotic to yellowing. No positives were detected from the Spanish samples.

Key words: Mediterranean, APLPV, Japanese plum, ELISA, molecular hybridisation

RESUME - *L'American plum line pattern virus* (APLPV) a été signalé pour la première fois en Méditerranée et isolé du prunier japonais en Palestine. Son identification a été basée sur l'étude de la morphologie des particules et de leurs propriétés biologiques, sérologiques et moléculaires. Une prospection a été effectuée dans les régions Pouilles (Italie) et Murcie (Espagne), en utilisant la technique ELISA et l'hybridation moléculaire afin de vérifier la présence de l'APLPV chez le prunier. Seuls 4 échantillons sur un total de 671 se sont révélés positifs à l'APLPV. Ces échantillons ont été prélevés d'une collection de virus (dans les Pouilles) originaires des Pouilles et de la Sicile (Italie), de l'Albanie et de la Tunisie. Les échantillons positifs provenaient d'arbres symptomatiques qui montraient des anneaux et des arabesques allant du chlorotique au jaune. Aucun positif n'a été mis en évidence dans les échantillons espagnols.

Mots-clés : Méditerranée, APLPV, prunier japonais, hybridation moléculaire.

INTRODUCTION

Stone fruit trees are affected by different viral diseases and can cause important economic damages. Within the *Ilarvirus* genus, the most important members affecting European and Mediterranean stone fruits are *Apple mosaic virus* (ApMV), *Prunus necrotic ringspot virus* (PNRSV) and *Prune dwarf virus* (PDV). In North America another *Ilarvirus*, *American plum line pattern virus* (APLPV) was reported to infect stone fruits, but it is still very rare in Europe. In Europe and in the Mediterranean, APLPV is included in the Quarantine List A1 of plant pathogens for EPPO Region (Smith *et al.*, 1992), because it was found in France only on imported peach and plum trees coming from the USA (Devignes, 1999).

The symptoms caused by the virus are variable and depend on the cultivar. On plum trees, the leaves developed in springtime may appear with diffuse lines, bands, oak leaf patterns or rings. These symptoms are not appreciated on the leaves developed in the summer. On some Japanese plums, the virus expresses in the form of a thin reticulation or yellowing of the little veins, producing the "golden net" pattern.

APLPV was characterized by Paulsen and Fulton (1968, 1969) who established that the virus was not related to other viruses inducing line pattern symptoms, like *Apple mosaic virus* and *Danish line pattern*. More recently Scott and Zimmerman (2001) reported the complete genomic sequence of APLPV which allowed its detection by molecular hybridization and RT-PCR.

APLPV IN THE MEDITERRANEAN

During the evaluation of the sanitary status of stone fruits in Palestine (Jarrar *et al.*, 2001), a Japanese plum (cv. Beauty) showing peculiar symptoms turned to be negative after the corresponding ELISA test to most of known viruses affecting stone fruits. After sap transmission to different herbaceous hosts a spherical virus could be isolated from *Nicotiana occidentalis* (Alayasa, 2001). Using two degenerated primers consisting of consensus sequences of ilarviruses affecting stone fruit trees (Saade *et al.*, 2000), it was possible to amplify a viral genomic fragment. This fragment was sequenced and the corresponding analysis demonstrated a high homology with the American isolate of APLPV.

This RT-PCR fragment allowed us to obtain the corresponding non isotopic riboprobes to be used for molecular hybridization detection (Pallás *et al.*, 1998) and its comparison to the serological ones (Alayasa, 2001).

Serological and molecular detection methods have recently been used to carry out a survey for APLPV in plums (Myrta *et al.*, 2002). Only 4 out of the 671 tested samples turned to be positive for APLPV. These samples originated from a virus collection which in turn was previously collected from Apulia and Sicily (Italy), Albania and Tunisia this being the first time that this virus is described in these three countries. The positive samples were symptomatic showing ring and line patterns from chlorotic to yellowing (Figs. 1a and b). No positives were detected from the 30 samples, including 23 different plum varieties tested in Spain (M.C. Herranz and V. Pallás, unpublished).

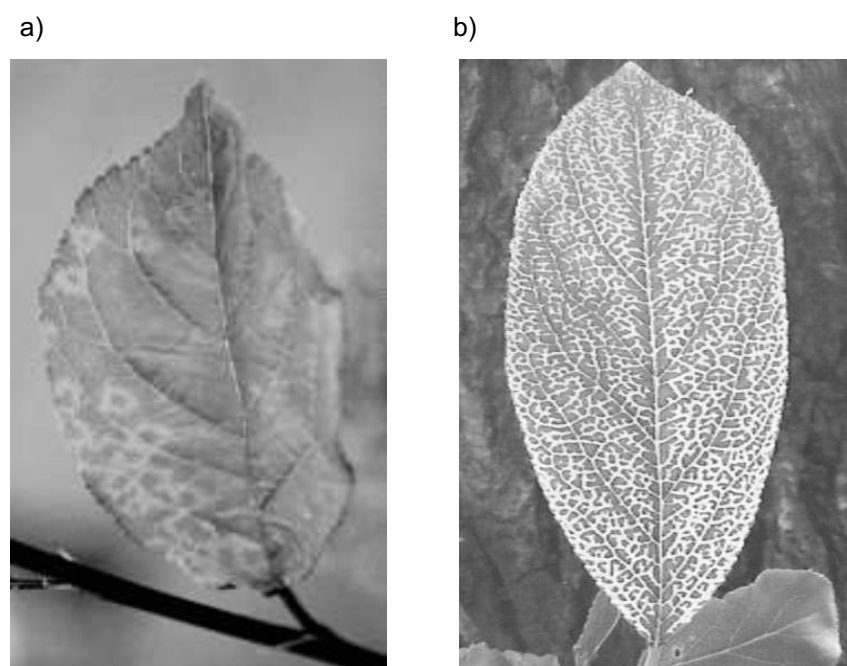


Fig. 1. Symptoms caused by APLPV in Japanese plum, a) early season, b) late spring and summer.

The complete nucleotide sequence was determined of the movement and coat protein genes of these three isolates and those from Palestine (isolate P27) and from USA (isolate 125-04). Sequence comparisons revealed a very low genetic diversity regardless of its different geographic origin, a situation very similar to the one observed for PNRSV (Scott *et al.*, 1998; Aparicio *et al.*, 1999).

The present study can facilitate the application of the new molecular tools in quarantine programs and certification schemes and then to evaluate and to prevent the presence of this virus in the Mediterranean Region.

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