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Detection and serotyping of plum pox virus (PPV) isolates by monoclonal antibodies

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SUMMARY - A study was started to detect the presence of PPV foci and characterise Mediterranean isolates of the virus by means of universal and strain-specific monoclonal antibodies (MAbs). This survey was carried out in Albania, Cyprus, Egypt, Greece, Italy, Lebanon, Malta and Turkey. No PPV was found in Lebanon and Malta, confirming previous reports. Detailed antigenic analyses among 170 PPV isolates from apricot, peach and plum originating from six Mediterranean countries, proved that they belong respectively to isolates M (139), D (20), EA (7) and (4) a mixture of strains (M + D). PPV-M was detected in Albania,

Cyprus, Greece, Italy, and Turkey; PPV-D in Albania and Italy, amd the strain mixture in two orchards in Albania. Several PPV-infected apricots from two Egyptian localities were recognised only by the MAb specific to El Amar serotype.This is the first report of a large-scale serotyping of PPV isolates in several Mediterranean countries.

Key word: stone fruits, PPV strain, serological diagnosis, monoclonal antibodies, Mediterranean countries.

RESUME *- Une étude a été entreprise pour détecter la présence de foyers de PPV et pour caractériser les isolats méditerranéens de ce virus à travers des anticorps monoclonaux universels et spécifiques. L'enquête a été conduite en Albanie, à Chypre, en Egypte, en Grèce, en Italie, au Liban, à Malte et en Turquie. Au Liban et à Malte, on n'a pas trouvé de PPV, ce qui confirme les données précédemment rapportées. L'analyse de 170 isolats de PPV à partir de matériel d'abricotier, pêcher et prunier, provenant de six pays méditerranéens, a montré qu'ils appartiennent aux isolats M (139), D (20), EA (7) et aux mélanges naturels de souches (M+D) (4), respectivement. La souche M du PPV a été mise en évidence en Albanie, à Chypre, en Grèce, Italie et Turquie ; la souche D a été signalée en Albanie et Italie, alors que des échantillons contenant des mélanges naturels des deux souches ont été repérés dans quelques vergers en Albanie. Plusieurs échantillons d'abricotier infectés par le PPV, originaires de deux localités égyptiennes, n'ont été reconnus que par l'anticorps monoclonal spécifique pour le sérotype El Amar. C'est la première fois que, sur une grande échelle, on regroupe en sérotypes des isolats du PPV provenant de plusieurs pays méditerranéens.*

Mots-clés: *essences à noyaux, souche de PPV, diagnostic sérologique, anticorps monoclonaux, Pays Méditerranéens.*

Plum pox virus (PPV), causing heavy losses in stone fruit production, was detected in several Mediterranean countries, where its introduction was relatively recent (Roy and Smith, 1994).

The European isolates of PPV fall in four groups, differentiated molecularly and serologically as PPV-Marcus (PPV-M), PPV-Dideron (PPV-D), PPV-Cherry (PPV-C) and PPV-El Amar (PPV-EA). RFLP analyses of the PCR-amplified cDNA fragment (Wetzel *et al.*, 1991; Hammond *et al.*, 1998), RT-PCR assay based on strain-specific primers (Candresse *et al.*, 1995; Nemchinov and Hadidi, 1997), and the differences in electrophoretic mobility of dissociated coat protein (CP) (Bousalem *et al.*, 1994; Pasquini and Barba, 1994) were used to type PPV isolates. It is important to use a simpler diagnostic assay suitable for both the timely discovery of PPV foci and reliable strain identification. Monoclonal antibodies (MAbs) with serotype-specific reactivity were already available for this purpose: PPV-D (Cambra *et al.*, 1994), PPV-M (Boscia *et al.*, 1997), PPV-C (Boscia *et al.*, 1998) and PPV-EA (Myrta *et al.*, 1998).

1. Detection of PPV foci

In several countries where detailed information on PPV was lacking e.g. Cyprus, Lebanon, Malta and Turkey, surveys were done and DASI-ELISA was performed using MAb5B (universal) to detect all strains ofPPV.

Cyprus. One hundred thirty-three of 672 samples from stone fruit trees (peach, almond, apricot, cherry and plum), were virus-infected. Incidence of PPV was 16% in plum, 12,6% in apricot, and 9,8% in peach trees. No ne of the 249 almond and 224 cherry samples tested was positive by ELISA.

Lebanon. Field inspections were carried out in commercial orchards and home gardens. ELISA tests were performed in 193 peach, cherry and plum trees. All the samples tested were negative for PPV. The data confirms an earlier report (Jawhar *et al.*,1996).

Malta. This study was performed on mother trees and in a varietal collection. Surveys were also carried out in commercial orchards. All tested samples (peaches, apricots, plums, and different rootstocks) were negative, confirming the sharka-free status of the Maltese islands (Gatt *et al.*, 1998).

Turkey. None of the randomly selected: 226 plum, 218 cherry, 215 apricot, 201 peach, and 65 sour cherry trees sampled from East Mediterranean and East Anatolia Region was positive by ELISA.

2. Serotyping of PPV isolates

A study was conducted to characterise Mediterranean PPV isolates with monoclonal antibodies (MAbs) in DASI-ELISA. The monoclonals included: MAb5B (universal), MAb4DG5 (PPV-D specific), MAbAL (PPV-M specific), MAbTUV and MAbAC (PPV-C specific), and MAbEA24 (PPV-El Amar specific).

PPV-infected samples of apricot, peach and plum to be tested for serotype identification were collected in different areas of the following countries: Albania (17), Cyprus (21), Egypt (7), Greece (80), Italy (30) and Turkey (15). The analysis of 170 PPV isolates from apricot (87) peach (46), plum (37) coming from the above six Mediterranean countries proved that they belong to isolates M (139), D (20), EA (7) and to natural mixtures of strains M + D (4).

Albania. Beside the PPV-M serotype, previously reported from Albania by Myrta *et al.* (1996), PPV-D was identified in several plum trees from the same orchard. In one apricot and thee plum samples from two orchards, mixed infections of M and D serotypes were found. PPV-M was present in all orchards, whereas PPV-D was found only in one.

Cyprus. Twenty-one PPV-positive samples from apricot (5), peach (9) and plum (7) from different orchards were infected with PPV-M. The same strain was previously recorded from a single source (Wetzel *et al.*,1991)..

Egypt. Seven samples from apricot trees from El Amar County in two distant localities were recognized only by MAbEA24. This confirms the existence of the El Amar strain as suggested by Candresse *et al.* (1994).

Greece. All 50 apricot and 30 peach samples from different localities were positive for PPV-M strain. This confirms previous findings on the widespread presence of this strain in Greece (Varveri and Boutsika, 1998).

Italy. The strains PPV-D and PPV-M were identified in peach, plum and apricot from different Italian regions, thus confirming reports of PPV-D occurrence in several areas (Pasquini and Barba, 1994) and recent spread of M strain in the North-Eastern area (Poggi Pollini *et al.*, 1996; Frisinghelli *et al.*, 1997). A single diseased apricot was infected by PPV-M in central Italy. Only PPV-D strain was found in the South-East area.

Turkey. Fifteen PPV-positive apricot samples from Central Anatolia and Marmara Region reacted with both MAb4DG5 and MAbAL which made their serotyping difficult. In further investigations the isolates were retested in RT-PCR+RFLP as described by Wetzel *et al.*, 1991, and typed out serotype M. A similar ambiguous result (presence of both M- and D-specific epitopes in the same isolate) was reported by Candresse *et al.* (1998) with a Turkish peach isolate of PPV.

The combined use in DASI-ELISA of one universal and four serotype-specific MAbs proved to be an efficient tool for the simple, rapid, routine detection and serotyping of PPV isolates.

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