DETECTION AND SEROTYPING OF MEDITERRANEAN PLUM POX VIRUS ISOLATES BY MEANS OF STRAIN-SPECIFIC MONOCLONAL ANTIBODIES

A. MYRTA[1], B. DI TERLIZZI[2*], D. BOSCIA[3], K. ÇAGLAYAN[4], I. GAVRIEL[5], G. GHANEM[6], C. VARVERI[7], V. SAVINO[3]

[1]Ministry of Agriculture and Food, Tirana, Albania; [2]Istituto Agronomico Mediterraneo, Via Ceglie 9, 700 10 Valenzano, (BA), Italy; [3] Dipartimento di Protezione delle Piante dalle Malattie, Università Centro di Studio del CNR sui Virus e le Virosi delle Colture Mediterranee, Bari, Italy; [4]Plant Protection Department, Mustafa Kemal University, Antakya, Turkey; [5]Department of Agriculture, Plant Protection Section, Nicosia, Cyprus; [6]Plant Pathology Department, Cairo University, Giza, Egypt; [7]Benaki Phytopathological Institute, Athens, Greece

Summary. – Plum pox virus (PPV) is a major threat to the expanding Mediterranean stone fruit industry. In order to control the plum pox disease it is of utmost importance to detect early PPV foci and to identify the PPV isolates involved. A survey was therefore carried out in Albania, Cyprus, Egypt, Greece, Italy and Turkey by a double-antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) with the following monoclonal antibodies (MAbs): 5B (universal), 4DG5 (PPV-D-specific), AL (PPV-M-specific), TUV and AC (PPV-C-specific), and EA24 (PPV-El Amar-specific). A hundred and seventy Mediterranean PPV isolates were tested for strain type. PPV-M was detected in Albania, Cyprus, Greece, Italy, and Turkey; PPV-D was detected in Albania and Italy, whereas samples with natural mixtures of both strains were found in a couple of orchards in Albania. Seven PPV isolates from apricots in two Egyptian localities were recognized only by MAb EA24. In conclusion, DAS-ELISA with a combination of the universal MAb5B and the MAbs specific to the four PPV serotypes currently known (M, D, C and El Amar) is an efficient tool for a simple, sensitive and routine detection of PPV and discrimination of its serotypes.

Key words: plum pox virus; Mediterranean isolates; monoclonal antibodies; enzyme-linked immunosorbent assay

Introduction

PPV, one of the main causes of heavy losses to stone fruit production, has been reported from the great majority of Mediterranean countries, where its introduction was relatively recent (Roy and Smith, 1994).

The European isolates of PPV fall in four groups which can be differentiated molecularly and serologically: PPV-Marcus (PPV-M), PPV-Dideron (PPV-D), PPV-Cherry (PPV-C), and PPV-El Amar (PPV-EA). Restriction fragment length polymorphism (RFLP) analysis of PCR-amplified cDNA fragments (Wetzel *et al.*, 1991; Hammond *et al.*, 1998), PCR based on strain-specific primers (Candresse *et al.*, 1995; Nemchinov and Hadidi, 1998) and the electrophoretic mobility of dissociated coat protein (CP) (Bousalem *et al.*, 1994; Pasquini and Barba, 1994) are used to type PPV isolates. However, these techniques are unable to type a PPV isolate in a single test. Furthermore, the above techniques are laborious and inaccessible to many laboratories in the Mediterranean countries. Therefore it is important to dispose of more simple diagnostic tools suitable for both the timely discovery of PPV foci and reliable strain identification. A correct diagnosis is of utmost importance because of differences in the biological and epidemiological behaviour of the virus strains (Quiot *et al.*, 1995) and the threat that their further spread represents for certain crops (e.g. peach).

*Corresponding author.

MAbs with the following serotype-specific reactivity are available: 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). We have used these MAbs in a survey carried out to characterize some Mediterranean PPV isolates.

Materials and Methods

Field surveys for PPV foci in stone fruit orchards were carried out in Albania, Cyprus, Egypt, Greece, Italy, and Turkey. The virus was identified by indirect DAS-ELISA with the PPV-universal MAb 5B (Agritest, Italy). The serotyping of PPV isolates was carried out with the following MAbs: 4DG5 (D-specific), AL (M-specific), TUV and AC (C-specific), and EA24 (EA-specific). Plant tissues were ground in a phosphate-buffered saline – Tween extraction buffer (1/10 w/v) and the obtained extracts were tested by DAS-ELISA in duplicate. The reaction was read after 1 hr in a Titertek Multiskan photometer at 405 nm. Positive samples were those that gave a reading (average from two wells) at least two times higher as compared to healthy control.

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).

Results and Discussion

The analysis of 170 PPV isolates from apricot (87) peach (46), plum (37) coming from 6 Mediterranean countries proved that the isolates belong to the following serotypes: M (139), D (20), EA (7), and (M + D), natural mixture of M and D strains (4) (Table 1).

Albania. Besides 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 three plum and one apricot samples coming from two orchards, mixed infections of M + D serotype were found. PPV-M was present in all sampled orchards, while PPV-D was found only in a single one.

Cyprus. All the 21 samples of apricot (5), peach (9) and plum (7) from different orchards were classified as PPV-M. The same strain was recorded earlier from a single source (Wetzel *et al.*, 1991). A more extensive survey carried out recently confirmed the extensive presence of PPV-M in the country.

Egypt. Seven samples from apricot trees from two distant localities in the El Amar County were recognized by MAb EA24 only. The presence of several isolates belonging to the EA serotype seems to confirm the existence of the EA serotype group as suggested by Candresse *et al.* (1994).

Table 1. Characterization of Mediterranean isolates of PPV with serotype-specific MAbs

Origin	Host species	No. of tested isolates	MAbs						Serotype
			5B (Univ)	AL (M)	4DG5 (D)	AC (C)	TUV (C)	EA24 (EA)	
Italy	Plum	10	+	+	–	–	–	–	M
"	Peach	5	+	+	–	–	–	–	M
"	Apricot	1	+	+	–	–	–	–	M
"	Plum	5	+	–	+	–	–	–	D
"	Apricot	8	+	–	+	–	–	–	D
"	Peach	1	+	–	+	–	–	–	D
Albania	Plum	6	+	+	–	–	–	–	M
"	Peach	1	+	+	–	–	–	–	M
"	Plum	6	+	–	+	–	–	–	D
"	Plum	3	+	+	+	–	–	–	M+D
"	Apricot	1	+	+	+	–	–	–	M+D
Turkey	Apricot	15	+	+	+	–	–	–	M*
Egypt	Apricot	7	+	–	–	–	–	+	EA
Cyprus	Apricot	5	+	+	–	–	–	–	M
"	Peach	9	+	+	–	–	–	–	M
"	Plum	7	+	+	–	–	–	–	M
Greece	Apricot	50	+	+	–	–	–	–	M
"	Peach	30	+	+	–	–	–	–	M
Total		170							

*M isolates that contain also D-specific epitope.

Greece. All 50 apricot and 30 peach samples coming from different localities reacted only with the PPV-M-specific MAb AL. This result confirms previous findings on the wide spread of this strain in Greece (Varveri and Boutsika, 1998).

Italy. PPV-D and PPV-M were identified in peach, plum and apricot from different Italian regions, thus confirming the presence of PPV-D in several areas (Pasquini and Barba, 1994) and the recent spread of PPV-M in the north-eastern Italy (Poggi Pollini *et al.*, 1996; Frisinghelli *et al.*, 1997). A single PPV-infected apricot tree in the central Italy was found to be of the PPV-M serotype, while only PPV-D was found in the south-east areas.

Turkey. Fifteen apricot samples reacted with both the MAbs 4DG5 and AL which made their typing difficult. In further investigation performed by PCR and RFLP analyses (Wetzel *et al.*, 1991), the typing yielded the M serotype. A similarly ambiguous result (the presence of both M- and D-specific epitopes in the same isolate) was reported by Candresse *et al.* (1998) on a Turkish PPV peach isolate. The unusual behaviour of all the Turkish PPV isolates so far examined regardless of the host (apricot or peach) and geographical location (Ankara or Yalova) opens a hypothesis on the existence of an additional atypical PPV group; however, it deserves further investigation.

This is the first report on a large-scale serotyping of PPV isolates from several Mediterranean countries. The combined use of one universal and four serotype-specific MAbs in DAS-ELISA proved to be an efficient tool for a simple, sensitive and routine detection and typing of PPV isolates.

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