PRODUCTION OF A MONOCLONAL ANTIBODY SPECIFIC TO THE EL AMAR STRAIN OF PLUM POX VIRUS

A. MYRTA[1], O. POTERE[1], D. BOSCIA[1], T. CANDRESSE[2], M. CAMBRA[3], V. SAVINO[1]

[1]Dipartimento di Protezione delle Piante dalle Malattie, Università degli Studi and Centro di Studio del CNR sui Virus e le Virosi delle Colture Mediterranee, Bari, Via Amendola 165/A, I-70126, Italy; [2]Station de Pathologie Végétale, INRA, Villenave d'Ornon, France; [3]Instituto Valenciano de Investigaciones Agrarias Moncada, Valencia, Spain

Summary. – Plum pox virus (PPV) isolates are grouped into three clusters differentiated by biological, serological, molecular and epidemiological characteristics: Marcus (M), Dideron (D) and Cherry (C). The El Amar (EA) isolate that does not fit any of the above groups is also known. Monoclonal antibodies (MAbs) that specifically recognize M, D, and C strains of PPV are already available. To complete the set of PPV strain-specific serological reagents, MAbs against the EA isolate were raised by immunizing BALB/c mice and fusing their spleen cells with NS0/1 myeloma cells. After a preliminary characterization by double-antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA), 1 of 13 selected MAbs proved to be EA strain-specific. This MAb (EA24) reacted equally well with a homologous antigen and several PPV isolates from Egyptian apricot trees, supporting the hypothesis of an additional specific PPV group. MAb EA24 did not react either with about a hundred PPV isolates belonging to the D and M groups or with PPV-SwC and PPV-SoC isolates belonging to the C group. The strain specificity of MAb EA24 was confirmed by Western blot analysis and immunoelectron microscopy. We conclude that there is now available a set of MAbs which are highly specific to the four currently known groups of PPV strains.

Key words: plum pox virus; El Amar strain; monoclonal antibodies; Western blot analysis

Introduction

PPV is one of the most injurious pathogens of stone fruit trees as it causes heavy losses to plum, apricot and peach crops in most European countries (Dunez and Šutic, 1988). PPV isolates are grouped into three clusters (groups) differentiated by biological, serological, molecular and epidemiological characteristics: Marcus (M), Dideron (D) and Cherry (C) (Kerlan and Dunez, 1979; Nemchinov *et al.*, 1996). An Egyptian isolate denoted El Amar (EA), which does not fit any of the above groups is also known. Wetzel *et al.* (1991b) differentiated this isolate from PPV-M and PPV-D serotypes on the basis of sequence homology studies. Later it was definitively considered a member of distinct group of PPV strains (Candresse *et al.*, 1994). The differentiation of the EA isolate by restriction fragment length polymorphism (RFLP) and polymerase chain reaction (PCR) analyses (Wetzel *et al.*, 1991a) proved to be difficult because the obtained RFLP pattern was of M-like serotype whereas the PCR with serotype-specific primers gave ambiguous results (Candresse *et al.*, 1998). Hammond *et al.* (1998) performing PCR with primers designed for the NIb gene of PPV and using different enzymes in RFLP analysis were able to differentiate PPV-EA from PPV-M.

To date, available serotype-specific MAbs are those raised to PPV-D (Cambra *et al.*, 1994), PPV-M (Boscia *et al.*, 1997a), and PPV-C (Boscia *et al.*, 1997b). A comparative analysis of amino acid sequence of the coat protein of EA strain (Wetzel *et al.*, 1991b) and representatives of the other three PPV groups showed about 10% heterology, the surface-located N-terminal region being the most heterologous. This suggested that there is a good chance to select isolate-specific antibodies provided MAbs raised against PPV-EA are available. Therefore, to complete the set of strain-specific MAbs, the production of MAbs against PPV-EA was undertaken.

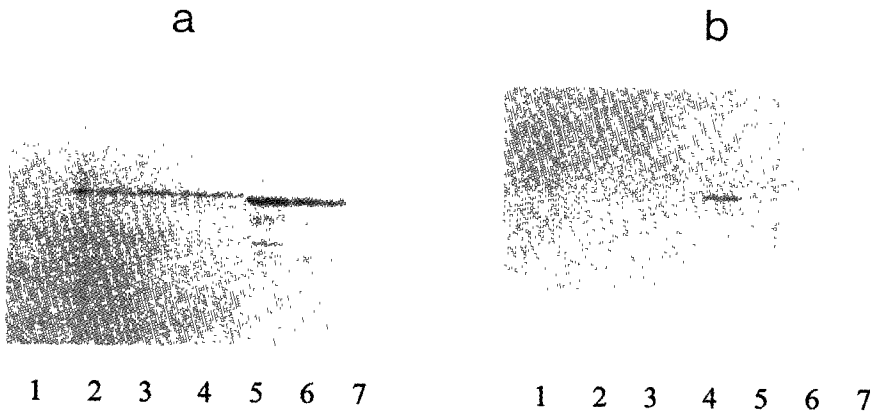

Fig. 1
Western blot analysis of PPV CP preparations with polyclonal antibody (a) and MAb EA24 (b)
Size marker (lane 1); PPV-SwC (lane 2); PPV-SoC (lane 3); PPV-EA (lane 4), PPV-M (lane 5); PPV-D (lane 6); PPV-D (lane 6); healthy control (lane 7).

Materials and Methods

PPV-EA purified from *Nicotiana benthamiana* (Van Osten, 1972) was used for immunization of BALB/c mice (Harlan Nossan, Correzzana). The fusion of immunized splenocytes and NSO/1 myeloma cells (American type Culture Collection, Rockville) was carried out as described by Boscia *et al.* (1992). Screening of cell culture supernatants for hybridomas secreting specific MAbs was done by DAS-ELISA (Cambra *et al.*, 1994). Plates were coated with IgGs from a polyclonal antiserum (Myrta *et al.*, 1996) and extracts of healthy and PPV-EA-infected *N benthamiana* plants were used as antigens.

Characterization of MAbs by DAS-ELISA. The obtained MAbs were first tested against the homologous antigen (PPV-EA), a French isolate of PPV-M, an Italian isolate of PPV-D (ISPAVE 31), a sweet cherry PPV-C isolate (PPV-SwC), and a sour cherry PPV-C isolate (PPV-SoC). Selected MAbs were subjected to further controls to ascertain their strain-specificity by extensive indirect DAS-ELISA against 119 characterized PPV isolates belonging to the M, D, C and EA strains. These tests were carried out in comparison with other MAbs, namely 5B universal for PPV isolates, 4DG5 specific to PPV-D (Cambra *et al*, 1994), AL specific to PPV-M (Boscia *et al.*, 1997a), AC and TUV, both specific to PPV-C (Boscia *et al.*, 1997b).

Western blot analysis was described by Hu *et al.* (1990). Dissociated CPs from crude sap of plants infected with PPV-EA, PPV-M, PPV-D, PPV-SoC and PPV-SwC isolates were blotted onto two nitrocelluloses membranes and incubated separately with MAb EA24 and IgGs from a polyclonal antiserum in 2% non-fat milk in phosphate-buffered saline. The membranes were washed, incubated in anti-mouse and anti-rabbit IgG solutions, respectively, and developed with Sigma Fast Kit. The MW-SDS-70 Kit (Sigma) for molecular weights of 10,000 – 70,000 was used as reference marker.

Electron microscopy. MAb EA24 in the form of diluted ascitic fluid was used for decoration of particles of the homologous virus and PPV-SwC (Milne, 1993).

Results and Discussion

Of the 420 hybridoma cultures obtained 13 reacted positively in DAS-ELISA with PPV-EA but not with healthy plant extracts. These lines continued to secrete antibodies after transferring, cloning and successive cycles of freezing and thawing in liquid nitrogen.

In preliminary DAS-ELISA tests, MAb EA24 recognized only the homologous antigen (PPV-EA) but not PPV-M, PPV-D, PPV-SwC, and PPV-SoC. Later, this MAb reacted equally well with the homologous antigen and seven PPV isolates from Egyptian apricot trees. The strain-specificity of MAb EA24 was confirmed by the behaviour of the other serotype-specific MAbs. In particular, MAb 5B reacted with all the PPV isolates, while all isolates that were not recognized by MAb EA24 were recognized by the PPV-D-specific MAb 4DG5 (35), PPV-M-specific MAb AL (75), PPV-C-specific MAbs TUV and AC (2) (Table 1).

In Western blot analysis (Fig. 1), MAb EA24 recognized specifically only the CP of PPV-EA but did not react with extracts from plants of *N. benthamiana* infected with PPV-M, PPV-D, PPV-SwC, and PPV-SoC isolates. The polyclonal antiserum recognized all the PPV isolates tested.

In immunoelectron microscopy, MAb EA24 decorated homologous particles but not those of PPV-SwC.

In conclusion, a newly produced MAb (EA24) proved to be PPV-EA strain-specific. It reacted also with several PPV isolates from Egyptian apricot trees not belonging to any of the traditional PPV groups This finding supports a hypothesis that the EA strain is an additional PPV serotype. A set of MAbs that are highly specific for the recognition of the four currently known groups of PPV serotypes is now available.

Table 1. Characterization of MAb EA24 in DAS-ELISA with different PPV isolates in comparison with PPV-universal MAb 5B and MAbs specific to Marcus (AL), Dideron (4DG5) and Cherry serotypes (AC and TUV)

No. of isolates	Strain	MAbs					
		5B	EA24	AL	4DG5	AC	TUV
75	M	+	–	+	–	–	–
35	D	+	–	–	+	–	–
2	C	+	–	–	–	+	+
7	EA	+	+	–	–	–	–

Acknowledgements. Our thanks are due to Prof. M.A. Castellano for the help in electron microscopy and Dr. A. Hadidi for kind supply of PPV-SoC.

References

Boscia D, Aslouj E, Elicio V, Savino V, Castellano MA, Martelli GP (1992): Production, characterization and use of monoclonal antibodies to grapevine virus A. *Arch. Virol.* **127**, 185-194.

Boscia D, Zeramdini H, Cambra M, Potere O, Gorris MT, Myrta A, Di Terlizzi B, Savino V (1997a): Production and characterization of a monoclonal antibody specific to the M serotype of plum pox potyvirus. *Eur. J. Plant Pathol.* **103**, 477-480.

Boscia D, Crescenzi A, Myrta A, Potere O, Nuzzaci M (1997b): Produzione di un anticorpo monoclonale specifico per il ceppo del virus della vaiolatura del susino isolato da ciliegio dolce (PPV-SwC). *Atti del Convegno Nazionale del Ciliegio*, Valenzano (in press).

Cambra M, Asensio M, Gorris MT, Pérez E, Camarasa E, Garcia JA, Moya JJ, Lopez-Abella D, Vela C, Sanz A (1994): Detection of plum pox potyvirus using monoclonal antibodies to structural and non-structural proteins. *EPPO Bull.* **24**, 569-577.

Candresse T, Macquaire G, Lanneau M, Bousalem M, Wetzel T, Quiot-Douine L, Quiot JB, Dunez J (1994): Detection of plum pox potyvirus and analysis of its molecular variability using immunocapture-PCR. *EPPO Bull.* **24**, 585-594.

Candresse T, Cambra M, Dallot S, Lanneau M, Asensio M, Gorris MT, Revers F, Macquaire G, Olmos A, Boscia D, Quiot JB, Dunez J (1998): Comparison of Monoclonal Antibodies and Polymerase Chain Reaction Assays for the Typing of Isolates Belonging to D and M Serotypes of Plum Pox Potyvirus. *Phytopathology* **88**, 198-203.

Dunez J, Šutic D (1988): Plum pox virus (PPV). In Smith IM, Dunez J, Lelliott RA, Philips DH, Archer SA (Eds): *European Handbook of Plant Diseases*. Blackwell Scientific Publications, Oxford, pp. 44-46.

Hammond J, Puhringer H, da Câmara Machado A, Laimer da Câmara Machado M (1998): Combined RT-PCR and RFLP analysis to detect and differentiate field isolates of plum pox potyvirus. *Acta Hortic.* (in press).

Hu JS, Gonsalves D, Boscia D, Namba S (1990): Use of monoclonal antibodies to characterize grapevine leafroll associated closteroviruses. *Phytopathology* **80**, 920-925

Kerlan C, Dunez J (1979): Différenciation biologique et sérologique de souches du virus de la Sharka. *Ann. Phytopathol.* **11**, 241-250.

Milne RG (1993): Electron microscopy of *in vitro* preparations. In Matthews REF (Ed.): *Diagnosis of Plant Virus Diseases* CRC Press, Boca Raton, pp. 215-251.

Myrta A, Di Terlizzi B, Digiaro M, Savino V (1996): Viruses of stone fruits in Albania. *EPPO Bull.* **26**, 141-146.

Nemchinov L, Hadidi A, Maiss E, Cambra M, Candresse T, Damsteegt V (1996): Sour cherry strain of Plum Pox Potyvirus (PPV): Molecular and Serological Evidence for a New Subgroup of PPV strains. *Phytopathology* **86**, 1215-1221.

Van Osten HJ (1972): Purification of plum pox (sharka) virus with the use of Triton X-100. *Neth. J. Plant Pathol.* **78**, 33-44.

Wetzel T, Candresse T, Ravelonandro M, Dunez J (1991a): A polymerase chain reaction assay adapted to plum pox potyvirus detection. *J. Virol. Methods* **33**, 355-365

Wetzel T, Candresse T, Ravelonandro M, Delbos RP, Mazyad H, Aboul-Ata AE, Dunez J (1991b): Nucleotide sequence of the 3'-terminal region of the RNA of the El Amar strain of plum pox potyvirus. *J. Gen Virol.* **72**, 1741-1746.