PPV distribution on Prunus : experiences of diagnosis with ELISA

Myrta A., Potere O., Ismaeil F., Boscia D.

in

Myrta A. (ed.), Di Terlizzi B. (ed.), Savino V. (ed.).
Virus and virus-like diseases of stone fruits, with particular reference to the Mediterranean region

Bari : CIHEAM
Options Méditerranéennes : Série B. Etudes et Recherches; n. 45

2003
pages 107-110

PPV DISTRIBUTION ON *PRUNUS*: EXPERIENCES OF DIAGNOSIS WITH ELISA

A. Myrta[1], O. Potere[2], F. Ismaeil[1] and D. Boscia[2]
[1]Istituto Agronomico Mediterraneo, Via Ceglie 9, 70010 Valenzano (BA) (Italy)
[2]Dipartimento di Protezione delle Piante e Microbiologia Applicata, Università degli Studi and Istituto di Virologia Vegetale, Sezione di Bari, Via Amendola 165/A, 70126 Bari (Italy)

Summary - The results of ELISA tests done on leaves, with or without symptoms, sampled in different areas of the canopy of trees affected by Sharka are reported. The results demonstrate that ELISA is not reliable for *Plum pox virus* (PPV) detection when asymptomatic leaves are used. Comparative analysis of different areas of the leaf blade indicates the possibility of improving the efficiency of the test. However, the definition of a more efficient sampling methodology needs further studies.

Key words: PPV, ELISA, diagnosis, apricot

RESUME *- Dans le présent travail, on illustre les résultats des tests ELISA effectués sur des feuilles, symptomatiques et non, prélevées de différentes parties du feuillage des arbres atteints de Sharka. Les résultats ont montré que l'ELISA n'est pas fiable pour la détection du Plum pox virus (PPV), si on utilise des feuilles asymptomatiques. Une analyse comparative des différentes parties du limbe foliaire indique la possibilité d'améliorer l'efficacité de ce test. Toutefois, on devrait approfondir les études afin de mettre au point une méthodologie d'échantillonnage plus efficace.*

Mots-clés : *PPV, ELISA, diagnostic, abricotier*

INTRODUCTION

Plum pox (sharka), caused by the *Plum pox virus* (PPV), is the most severe stone fruit disease in Europe and in the Mediterranean. It has moreover been reported in South America (Roy and Smith, 1994), India (Bwardhaj, 1995), USA (Levy *et al*., 2000) and Canada (Thompson *et al*., 2001) proving the high potential of its global expansion over the last few years.

The availability of fast and reliable diagnostic tools is imperative to implement a timely and effective control of the disease. ELISA, which is presently widely used in virus diagnosis, is often unable to detect the virus in asymptomatic leaves of infected plants (Adams, 1978; Knapp *et al.*, 1995). Results of assays reported here were made in an attempt to develop more precise indications as to the reliability of PPV diagnosis in asymptomatic leaves.

MATERIALS AND METHODS

Leaf samples were taken from one apricot trees identified in a commercial orchard and from GF 305 plants infected with PPV at a collection of the University of Bari. All tested plants and healthy controls were visually inspected to identify symptoms consisting (extended chlorotic areas or spotting along secondary veining, and ring spots).

PPV detection was achieved by DASI-ELISA using the universal monoclonal antibody MAb 5B (Cambra *et al*., 1994) while strain determination was by strain-specific MAb (Boscia *et al*., 1998) concomitantly. Immuno-enzymatic assays were performed, to diagnose the possible presence in the plants of *Prune dwarf virus* (PDV), *Prunus* necrotic ring spot virus (PNRSV), *Apple mosaic virus* (ApMV) and *Apple chlorotic leaf spot virus* (ACLSV).

A PPV-infected tree of cv. Tyrinthos, was identified in the area of Ugento (LE) during May, in the framework of the programme called “Monitoring of stone fruit and citrus quarantine diseases” organised by Apulian Regional Government, from which fully developed leaves were collected near to fruit ripening.

One hundred asymptomatic leaves were collected from branches having symptomatic leaves, choosing those that developed nearest to symptomatic ones. Four disks (1.5 cm in diameter) per leaf sections (Fig. 1) were excised and extracted in extraction buffer and submitted to ELISA.

At the same time, 100 symptomatic leaves were sampled and they were submitted to visual examination to identify the localisation of symptoms on the leaf blade, in particular on the four sections obtained when drawing a St. Andrew's cross (Fig. 2). Among symptomatic leaves, 40 were selected which did not show symptoms at the basal quadrant (area 1) and ELISA tests were conducted with symptomatic and asymptomatic portions.

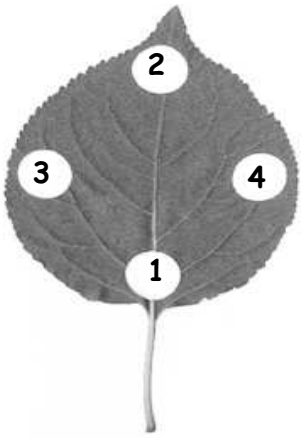

Fig. 1. Position of the leaf disks tested

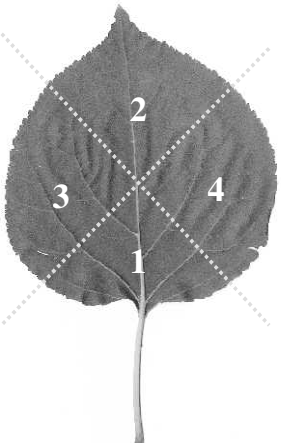

Fig. 2. Subdivision of the leaf blade adopted to report and locate symptoms

At the beginning of June, 20 symptomatic and 20 asymptomatic leaves (taken off symptomatic twigs) were collected from two potted plants of GF 305 inoculated with strain PPV-M. The collected leaves were submitted to testing. Also in mid-October, 40 asymptomatic leaves (from asymptomatic branches) and 4 symptomatic leaves were collected from two potted plants of GF 305 plants infected with PPV-M, and submitted to testing.

RESULTS AND DISCUSSION

Apricot tree cv. Tyrinthos was reported free from PDV, PNRSV, ApMV and ACLSV and infected by PPV-D. In the assay performed on the four disks obtained from each of the asymptomatic leaves, the result was positive for 56 leaves in at least one of four sections considered. Moreover, comparing the outcome of assays carried out on the four disks, it was reported that 46 were positive in disk 1, near to the petiole, and a much lower percentage shows infections in the other three disks (Tab. 1).

Table 1. Results of ELISA assay on the various sections of the 100 asymptomatic leaves

Leaf section	N° of positive sections	Incidence to tested samples (%)	Incidence to positive samples (%)
1	46	46	82,1
2	16	16	28,6
3	18	18	32,1
4	11	11	19,6
1+2+3+4	56	56	100

On each of the 100 symptomatic leaves the distribution of symptoms in the four sections considered per leaf was reported: only in 6% of cases symptoms spread to the whole leaf blade, while generally they were confined to one or two sectors of the blade. Surprisingly section 1, which was giving ELISA positives more often in asymptomatic leaves, turned out to be the less involved in the onset of symptoms (26 samples only) (Tab. 2).

ELISA assay done on 40 leaves, which were symptomless in the basal section and showed symptoms in at least one of the other three sections, was positive for all symptomatic sections, while 8 asymptomatic basal sections (20% of the total) were negative. The analysis of the intensity of those reactions based on photometric readings highlighted a correlation between absorbance values and symptoms visibility: high values in the readings correspond to visible symptoms and average-low values correspond to their absence.

The assay carried out in June on GF 305 grown in pot confirmed a full matching between the presence of symptoms and a positive result in all the 20 symptomatic leaves, but did not report any positive sample among the 20 asymptomatic leaves from the same plants. It is important to underline that, differently from May assay on cv. Tyrinthos, where asymptomatic leaves were chosen near to symptomatic ones, in this case the opposite criterion was followed (maximum distance from symptomatic leaves).

Table 2. Distribution of symptoms on the 100 symptomatic leaves of apricot

Frequency	Leaf sections			
(%)	1	2	3	4
17	-	+	+	-
14	-	-	+	+
10	-	-	+	-
9	-	+	-	+
9	-	-	-	+
8	-	+	+	+
7	-	+	-	-
6	+	+	+	+
5	+	+	-	+
5	+	+	+	-
3	+	-	-	+
3	+	-	+	-
2	+	-	+	+
1	+	-	-	-
1	+	+	-	-
100	26	58	65	56

+ symptomatic ; - asymptomatic

Finally, the assay carried out in October on two plants of GF 305 from the same collection, besides the positive reaction of the 4 control symptomatic leaves, confirmed the difficulty in reporting PPV in asymptomatic leaves, which gave a positive reaction only in 30% of cases with considerable differences between the two plants (Tab. 3).

Table 3. Results of ELISA assay on asymptomatic leaves sampled from two plants of GF 305 in October

	Positive (%)	Negative (%)
Plant 1	9 (45%)	11 (55%)
Plant 2	3 (15%)	17 (85%)
TOTAL	12 (30%)	28 (70%)

CONCLUSIONS

ELISA assay on apricot trees and GF 305 plants, infected respectively by PPV-D and PPV-M, confirmed the reliability of the assay for the identification of the virus on symptomatic leaves. However, the analysis of asymptomatic leaves from the same plants highlighted the high risk to obtain false negatives. The technique of assays on different sections of the leaf blade, although in need of further support from a larger number of isolates and species and/or varieties, seems to suggest that the use of the section near to the petiole reduces significantly, although still insufficiently, the risk of obtaining false negatives. The scarce reliability of the assay on asymptomatic leaves of infected plants showing symptoms suggests that this situation is even more distinct in already infected but still asymptomatic plants. These observations therefore lead to express a serious doubt as to the usefulness to apply ELISA to the control of PPV - free Status of source material (C.A.C. category), which is asymptomatic by definition.

REFERENCES

Adams, A.N. (1978). The detection of plum pox virus in *Prunus* species by enzyme-linked immunosorbent assay (ELISA). *Ann. Appl. Biol.* 90: 215-221.

Boscia, D., Myrta, A., Potere, O., Cambra, M., Crescenzi, A., Di Terlizzi, B., Candresse, T. and Savino, V. (1998). Impiego di anticorpi monoclonali per la identificazione dei diversi ceppi del virus della vaiolatura delle drupacee (PPV). *Notiziario sulla Protezione delle Piante* 9: (Nuova Serie) 207-212.

Bwardhaj, S.V., Thakur, P.D., Kohosla, K. and Sharma, D.R. (1995) Detection of Plum pox virus in India. *Acta Hort.* 386: 237-240.

Cambra, M., Asensio, M., Gorris, M.T., Pérez, E., Camarasa, E., Garcia, J.A., Moya, J.J., Lòpez-Abella, D., Vela, C. and Sanz, A. (1994). Detection of plum pox potyvirus using monoclonal antibodies to structural and non-structural proteins. *EPPO Bull.* 24: 569-579.

Knapp, E., da Camara Machado, A., Puhringer, H., Wang, Q., Hanzer, V. , Weiss, H., Weiss, B., Katinger, H. and Laimer da Camara Machado, M. (1995). Localization of fruit tree viruses by immuno-tissue printing in infected shoots of *Malus* sp. and *Prunus* sp. *J. Virol. Methods* 55: 157-173.

Levy, L., Damsteegt, V. and Welliver R. (2000). First report of plum pox virus (Sharka Disease) in *Prunus persica* in the United States. *Plant Dis.* 84: pp. 202. (disease note)

Pasquini G. and Barba M. (1997). Plum pox potyvirus strains: an overview. *In*: Proceedings of the Middle European Meeting '96 on Plum Pox. Budapest, 2nd - 4th October 1996, 168-171.

Roy, A. and Smith I. (1994). Plum pox situation in Europe. *EPPO Bull.* 24: 515-523.

Thompson, D., McCann, M., Mac Leod, M., Lye, D., Green, M. and James D. (2001). First report of Plum pox potyvirus in Ontario, Canada. *Plant Dis.* 85: pp. 97 (disease note).