MOLECULAR VARIABILITY OF CZECH PLUM POX VIRUS ISOLATES

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Summary. – Seventy-two Czech plum pox virus (PPV) isolates from different hosts were tested by double-antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA). In addition, the coat protein mobility and the *RsaI* restriction fragment length polymorphism (RFLP) pattern of reverse transcription-polymerase chain reaction- (RT-PCR) amplified coat protein (CP) gene fragment of the isolates were analysed. Both PPV-D and PPV-M serotypes were found in the Czech Republic. The results obtained by these different methods were in accord with exception of a few cases probably caused by mutation or recombination.

Key words: plum pox virus; coat protein; enzyme-linked immunosorbent assay; Western blot analysis; reverse transcription; polymerase chain reaction; restriction fragment length polymorphism

Introduction

Recently, four groups of PPV have been distinguished on the basis of serological characteristic, PCR, restriction fragment length polymorphism (RFLP) and coat protein mobility (Cambra *et al.*, 1994; Candresse *et al.*, 1995). In the Czech Republic, the PPV-M and PPV-D serotypes have been identified using strain-specific monoclonal antibodies (MAbs) (Navrátil *et al.*, 1992; Hilgert *et al.*, 1993; Navrátil *et al.*, 1998). The aim of this work was to characterise some Czech PPV isolates on molecular basis with respect to the fact that their epidemiological properties seem to correlate with the PPV grouping.

Materials and Methods

Virus isolates. Seventy-two Czech PPV isolates including the Vegama and Slivoň isolates obtained from the Research Institute of Crop Production, Prague, were studied. The presence of the virus was confirmed by DAS-ELISA using polyclonal antibodies raised against the PPV-W isolate (the Rankovic necrotic strain). Selected isolates were mechanically transmitted and maintained on *Nicotiana benthamiana* plants. For mechanical transmission, 1 g of infected leaf tissue was grinded with 2 ml of 10 mmol/l phosphate buffer pH 8.0 supplemented with 1% activated charcoal and "Celite" and the obtained homogenate was rubbed onto the leaves of experimental plants.The Dideron and Marcus isolates kindly provided by INRA, Bordeaux, France, were maintained on peach seedlings and used as a control.

DAS-ELISA. Serological variability of PPV isolates was determined by an indirect DAS-ELISA (Cambtra *et al*, 1994). Four PPV-D serotype-specific MAbs (4DG5 from IVIA, Moncada, Spain; 03, 06, and 08 from the Palacky University, Olomouc, Czech Republic) and one PPV-M serotype-specific MAb (M from the University of Bari, Bari, Italy) were used as intermediate antibodies in the assay.

Western blot analysis. Sap was pressed from leaves, mixed (1:3, v/v) with a denaturation solution (17.5 ml of 0.5 mol/l Tris-HCl pH 8.0, 2 g of sodium dodecyl sulfate, 5 ml of 2-mercaptoethanol, 10 ml of glycerine, and 0.1 ml of Bromophenol Blue solution) and boiled for 5 mins. Proteins were resolved by polyacrylamide gel electrophoresis in the presence of 10% SDS (SDS-PAGE) under denaturing conditions (Laemmli, 1970) and electroblotted onto nitrocellulose membrane. Blots were reacted with universal MAb 05 and group-specific MAbs to PPV-M and PPV-DA. A goat anti-mouse alkaline phosphatase conjugate was used as the secondary antibody. Nitro Blue Tetrazolium and 5-bromo-4-chloro-3-indolylphosphate (both from Serva) were used for staining as recommended by the supplier.

RT-PCR and RFLP analysis. The P1 and P2 primers (Wetzel *et al.*, 1991) and serotype-specific primers PM and PD (provided by Dr. T. Candresse, INRA, Bordeaux, France) in conjunction with P1 primer were used throughout the study. The immunocapture PCR protocol by Candresse *et al.* (1995) was used with the following modification of the cycling scheme: 45 mins at 42°C (RT reaction), 5 mins at 92°C (denaturation of template), 40 cycles of

Table 1. D/M serotype classification of Czech PPV isolates by different techniques

PPV isolate	Serotype	DAS-ELISA	PCR*	RFLP	Coat protein mobility
Diderón, W, CH1, Ag50, Š13, V10/8	D	D	D	D	D (38 K)
Š3	D	D	D	D	D (33 K)
Š10	D	D	D	M	D (38 K)
Vegama	M	M	M	D	M (40 K)
Marcus, 289	M	M	M	M	M (40 K)
Slivoň, 288, 294, 295, 299, 300, 302	M	M	M	M	M (41 5 K; 39.5 K)

*Serotype-specific PCR using PM/P1 primer pairs

amplification: 30 secs at 92°C , 30 secs at 62°C, 60 secs at 72°C. Ten µl of PCR product was digested with restriction endonuclease *Rsa*I (Promega). Restriction patterns were evaluated after electrophoresis in 1.8% agarose gel and staining with ethidium bromide under UV light.

Results and Discussion

There was an overall accordance in the results obtained in PPV D/M typing by the different methods used (Table 1). In this study, 52 Czech PPV isolates were recognized by PPV-D serotype-specific MAbs, 20 PPV isolates reacted with serotype-specific MAb-M, and one case of mixed infection was found.

Four PPV-D serotype-specific MAbs indicated variability of the CP within the PPV-D group. CPs of the serotypes of concern showed different electrophoretic mobilities. CP of the PPV-M group members was slightly larger than that of the PPV-D group. The Western blot pattern of PPV-M group isolates except Marcus, 289 and Vegama isolates was represented by a "double band" recognized by the universal MAb as well as PPV-M-specific MAb but never by the PPV-D-specific MAbs. The upper band is considered an intact CP, while the lower one probably represents a product of CP degeneration (Pasquini *et al.*, 1995).

RT-PCR-based assays allow a direct detection and differentiation of PPV isolates (Candresse *et al.*, 1998). Our results of the serotype-specific RT-PCR using PM/P1 and PD/P1 primer pairs confirmed the D/M serotype determined by DAS-ELISA and Western blot analysis. In RFLP analysis, the isolates were identified according to the presence of the *Rsa*I restriction site that is attributed to the D serotype. However, using the *Rsa*I restriction pattern of P1/P2-amplified fragment, the distinguishing of the Vegama and Š10 isolates failed.

We can conclude that these results show an overall correlation between the results obtained by DAS-ELISA using PPV-D and PPV-M serotype-specific MAbs and those obtained by specific RT-PCR assay, RFLP analysis of RT-PCR fragments or Western blot evaluation of CP mobility. However, in a few cases, the results obtained with these techniques diverged. This variability may be explained by mutation of the epitopes and/or the restriction site of concern. With regard to the PPV-M and PPV-D serotype occurrence in the same locality, a recombination event between the two serotypes can be taken into account. Further study is needed for obtaining more information about possible recombinant PPV types and their importance in plum pox disease epidemiology.

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References

Cambra M, Asensio M, Gorris MT, Perez 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, Quiot-Douin L, Quiot JB, Dunez J (1995): Analysis of plum pox virus variability and development of strain-specific PCR assays. *Acta Hortic.* **386**, 375-369.

Candresse T, Cambra M, Dallot S, Lanneau M, Asensio M, Gorris MT, Reves 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 the D and M serotypes of plum pox potyvirus. *Phytopathology* **88**, 198-204.

Hilgert I, Cikánek D, Krištofová H, Karešová R, Navrátil M (1993): Monoclonal antibodies against plum pox virus determination. *Hybridoma* **12(a)**, 215-220.

Laemmli UK (1970): Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **227**, 680-685.

Navrátil M, Cikánek D, Hilgert I, Krištofová H, Karešová R (1992): Monoclonal antibodies against plum pox virus. *Acta Hortic.* **309**, 169-173.

Navrátil M, Šimonová V, Paprštein F, Karešová R (1998): Detection and serological identification of plum pox virus isolates in Czech Republic. *Acta Hortic.* (in press).

Pasquini G, Mazzei M, Pilotti M, Barba M (1995): Characterization of PPV isolates using monoclonal antibodies. *Acta Hortic.* **386**, 346-353.

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