RESTRICTION FRAGMENT LENGTH POLYMORPHISM DIFFERENTIATION OF PLUM POX VIRUS ISOLATES

Z. PONCAROVÁ, P. KOMÍNEK

Research Institute of Crop Production, Drnovská 507, 161 06 Prague, Czech Republic

Summary. – Reverse transcription-polymerase chain reaction (RT-PCR) technique and restriction fragment length polymorphism (RFLP) analysis were used to analyse six isolates of plum pox virus (PPV). Whole coat protein (CP) gene was amplified in four isolates using the unipoty-polyT primer pair. PPV-D was identified by RFLP analysis using *Sfu*I and *Dra*I enzymes in two of the isolates. Two isolates of PPV-M strain yielded RT-PCR products which could not be digested by the two enzymes. Other isolates were subjected to RT-PCR using P1-P2 primers. The specificity of the RT-PCR products was confirmed by *Alu*I digestion, while *Rsa*I digestion enabled strain differentiation. No mixed infection was found.

Key words: plum pox virus; PPV-D strain; PPV-M strain; coat protein; restriction fragment length polymorphism; reverse transcription; polymerase chain reaction

Introduction

PPV is most harmful virus of stone fruit in the Czech Republic widely distributed in plum, myrobalan and apricot trees but affecting also peaches and blackthorns (Polák, 1997). Two prevalent serotypes, PPV-M and PPV-D strains showing different pathogenicity in various plant species are present in Europe (Candresse *et al.*, 1998).

Currently, the most accurate method used in the research of PPV strains is rerverse transcription and amplification of a specific region of viral RNA combined with restriction analysis of the obtained product (Wetzel *et al.*, 1991; Candresse *et al.*, 1998).

Materials and Methods

PPV isolates were maintained on *Nicotiana clevelandi x glutinosa*, *N. benthamiana*, *Prunus persica* GF 305, and *P. persica* Siberian C (Komínek *et al.*, 1997). Samples of PPV-W isolate were taken from *Nicotiana clevelandi x glutinosa*. PPV isolate from plum (PPV-plum Horomerice) originated from Horomerice, PPV isolate from apricot cv. Vegama (PPV-Vegama) and PPV isolate from peach cv. Nectagrand (PPV-Nectagrand) were taken from *N. benthamiana*. Naturally infected trees were source of infected seeds of apricot cv. Vegama (PPV-seed), flowers and leaves of plum from Ruzyne (PPV-plum Ruzyne), and leaves of plum from Horomerice (PPV-plum Horomerice).

Isolation of viral RNA. Total RNA was isolated from about 50 mg of plant tissue using RNeasy Plant Mini Kit (Qiagen) according to manufacturer's instructions.

RT-PCR. Total RNA was added to a reaction mixture consisting of AMV buffer (Promega), 1 mmol/l dNTPs, 40 U of RNase inhibitor, 10 U of AMV reverse transcriptase (Promega) and 0.2 μmoles of polyT (Deborré *et al.*, 1995) or P1 (Candresse *et al.*, 1995) reverse primers. The reaction was performed at 42°C for 45 mins. The RT product was added to the PCR mixture containing PCR buffer (Promega), 3 mmol/l $MgCl_2$, 0.5 mmol/l dNTPs, 0.5 U of Taq polymerase (Promega) and 0.1 μmoles of unipoty-polyT (Deborré *et al.*, 1995) or P1-P2 primers (Candresse *et al.*, 1995).

RFLP analysis The RT-PCR products (10 μl aliquots) amplified with unipoty-polyT primer pair were subjected to digestion with *Dra*I and *Sfu*I restriction nucleases (Boehringer Mannheim) and those amplified with P1-P2 primer pair were digested with *Alu*I and *Rsa*I (Boehringer Mannheim). Overnight digestion at 37°C was followed by electrophoresis of the digests on 1.5% agarose or 6% polyacrylamide gels.

Results and Discussion

PPV-Nectagrand, PPV-Vegama, PPV-W and PPV-plum Horomerice isolates were subjected to RT-PCR with unipoty-polyT primer pair amplifying the CP gene. The reac-

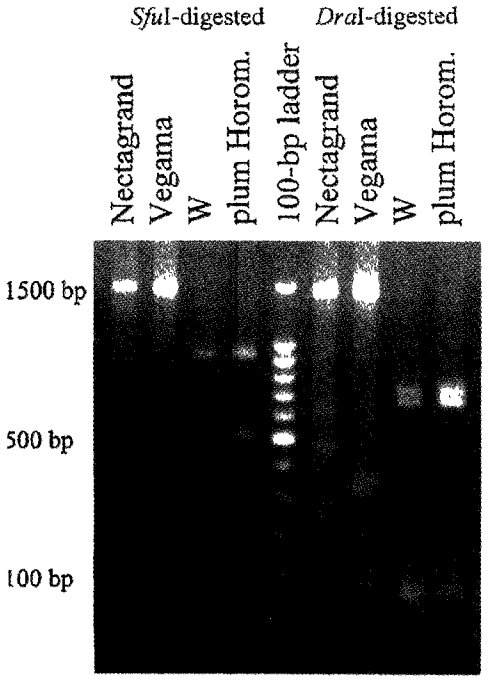

Fig. 1
Agarose gel electrophoresis of *Dra*I- and *Sfu*I-digested RT-PCR products amplified with unipoty-polyT primer pair

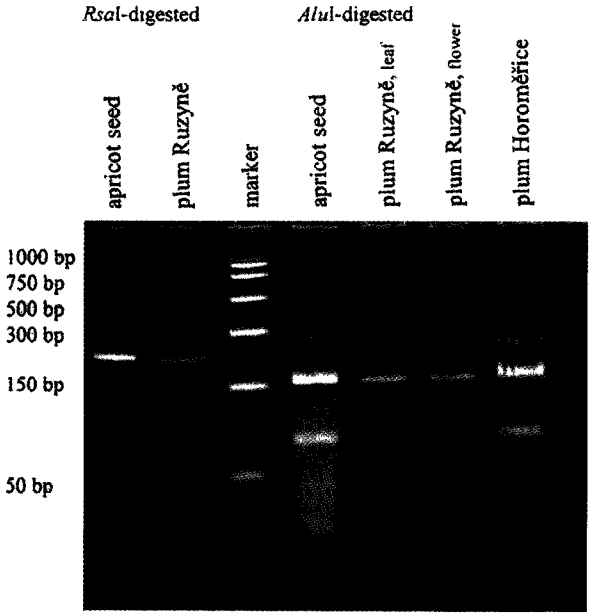

Fig. 2
Polyacrylamide gel electrophoresis of *Rsa*I- and *Alu*I-digested RT-PCR products amplified with P1-P2 primer pair

tion resulted in product of about 1400 bp. Its subsequent digestion by *Dra*I and *Sfu*I enzymes (Deborré *et al.*, 1995) classified PPV-W and PPV-plum Horomerice isolates as PPV-D strain. As the same enzymes did not digest PPV-Nectagrand and PPV-Vegama isolates, they were classified as PPV-M strain (Fig. 1). These results confirmed our previous serological strain differentiation (Komínek *et al.*, 1997, 1998).

PPV isolates from naturaly infected trees (PPV-plum Horomerice, PPV-plum Ruzyne, and PPV-seed) were analysed by RT-PCR with P1-P2 primer pair. The reaction resulted in product of about 240 bp. Its specifity was confirmed by digestion with *Alu*I enzyme (Candresse *et al.*, 1995). The presence of D-specific *Rsa*I restriction site was determined in PPV-plum Horomerice (data not shown). As PPV-seed and PPV-plum Ruzyne isolates were not digested with *Rsa*I enzyme, they were characterised as PPV-M strain (Fig. 2).

In the experiments reported here, no indication of a mixed infection with the two strains was observed.

Acknowledgement. We wish to thank Miss P. Čápová for excellent technical assistance. This work has been partly supported by grant No. 522/96/0406 of the Grant Agency of the Czech Republic and by the project No. RE 5535 of the National Agency for Agricultural Research.

References

Candresse T, MacQuaire G, Lanneau M, Bousalem M, Quiot-Douine L, Quiot JB, Dunez J (1995): Analysis of plum pox virus variability and development of strain-specific PCR assay. *Acta Hortic.* **386**, 357-369.

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 the D and M serotypes of plum pox potyvirus. *Phytopathology* **88**, 198-204.

Deborré G, Maiss E, Jelkmann W (1995): Biological and molecular investigations of several plum pox virus (PPV) isolates. *Acta Hortic.* **386**, 253-262.

Komínek P, Bittóová M, Polák J (1997): To the differentiation of plum pox virus isolates in the Czech Republic. *Proc. Middle Eur. Meet. '96 Plum Pox*, Budapest, 1996, pp. 131-136.

Komínek P, Hák R, Polák J (1998): Characterisation of three plum pox virus isolates in the Czech Republic. *Proc. 7th Int. Congr. Plant Pathol.*, Edinburgh, 1998 (in press).

Polák J (1997): On the epidemiology of plum pox virus in the Czech Republic. *Ochr. Rostl.* **33**, 81-88.

Wetzel T, Candresse T, Ravelonandro M, Dunez J (1991): A polymerase chain reaction adapted to plum pox virus detection. *J. Virol. Methods* **33**, 355-366.