CHARACTERIZATION OF PLUM POX VIRUS ISOLATES FROM SLOVAKIA

M. GLASA, J. MATISOVÁ, O. KÚDELA

Institute of Virology, Slovak Academy of Sciences, Dúbravská cesta 9, 842 46 Bratislava, Slovak Republic

Summary. – Plum pox virus (PPV) isolates from stone-fruit trees (plums, myrobalans, apricots and peaches) from orchards and gardens were characterized. To characterize their biological properties, several PPV isolates were transmitted by chip budding to GF 305 seedlings and mechanically to selected herbaceous test plants. The isolates differed in severity of infection, host range and symptomatology. A subgroup differentiation of 43 isolates from 22 localities of western and middle Slovakia was accomplished using reverse transcription-polymerase chain reaction (RT-PCR), immunocapture RT-PCR (IC-RT-PCR) and restriction analysis. These assays confirmed the presence of isolates belonging to PPV-M and PPV-D subgroups. PPV-M and PPV-D isolates were almost equally represented in tested samples. Tests of subgroup variability of PPV isolates from infected tolerant plum cultivars showed great predominance of PPV-M isolates.

Key words: plum pox virus isolates; enzyme-linked immunosorbent assay; polymerase chain reaction; restriction analysis; tolerant plum cultivars

Introduction

As in some other European countries, PPV is the most important pathogen of stone fruit trees in Slovakia too. On the territory of Slovakia, the PPV-caused sharka disease was for the first time identified by symptomatology around 1950 (Králiková, 1962). Later on, 55 PPV isolates from the former Czechoslovakia (36 isolates from Slovakia) were compared regards their interaction with *Chenopodium foetidum* (Paulechová, 1981). All these isolates produced chlorotic lesions on this indicator plant.

Recently, a more detailed analysis of several PPV isolates from western Slovakia has been published (Glasa *et al.*, 1997; Kúdela *et al.*, 1998).

This report presents a more complete analysis of PPV occurrence on the territory of Slovakia and a more detailed characterization of PPV isolates using indicator plants and molecular-biological methods.

Materials and Methods

Source of virus PPV isolates were obtained from naturally infected plum, myrobalan, apricot and peach trees from commercial orchards and private gardens in Slovakia. The Marcus and Dideron isolates kindly provided by Dr. Quiot from INRA, Montpellier, France, were used as positive controls. The negative controls (healthy plum and PNRSV-infected apricot) were provided by Prof. Fuchs from the Martin Luther University, Halle, Germany.

Biological assays. The isolates were transmitted by chip-budding to GF 305 seedlings (Glasa *et al.*, 1997). Five isolates were mechanically transmitted to a set of herbaceous test plants (*Ch. foetidum* Schrad., *Nicotiana benthamiana* Domin., *N. clevelandii* Gray, *N. clevelandii* x *glutinosa* hybrid, *Pisum sativum* L.cv. Colmo) for symptom evaluation.

Double-antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) (Clark and Adams, 1977) was performed using monoclonal antibodies (MAbs) provided by Dr. Navrátil from the Palacky University, Olomouc, Czech Republic.

Polymerase chain reaction (PCR). Leaf tissues of infected trees and/or test plants were tested by RT-PCR and IC-RT-PCR according to Wetzel *et al.* (1991, 1992). Two procedures for viral RNA preparation were used. (1) For RT-PCR, a leaf tissue was ground in sterile TE buffer pH 8.0 and centrifuged. The total nucleic acids were extracted from the supernatant by phenol-chloroform extraction and precipitated by ethanol. After precipitation, the nucleic acids were resuspended in RNase-free water and an aliquot was processed in the test. (2) For IC-RT-PCR, samples were prepared as described previously (Kúdela *et al.*, 1998).

Restriction analysis. PCR products of expected size (243 bp) were analysed by cleaving with *Alu*I and *Rsa*I restriction endonucleases. The obtained restriction fragments were electrophoresed in an agarose gel by a standard procedure.

Results and Discussion

Ten PPV isolates were transmitted to and maintained in GF 305 indicator plants. The isolates were found to differ in their severity of infection and symptomatology. Three types of leaf symptoms on GF 305 (tissue clearings, distortions and atypical small chlorotic spots) were observed (Table 1).

Five isolates (BIII/2, BOJ-3, BOR-3, CAH-2, and VAR) were mechanically transmitted from GF 305 to *N. benthamiana*. Around 20 days p.i., the isolates developed systemic chlorotic spots and dark green flecs on this plant. Later on, leaf puckering and dark green flecks around the veins could be identified too. The isolates transmitted to *N. benthamiana* were further

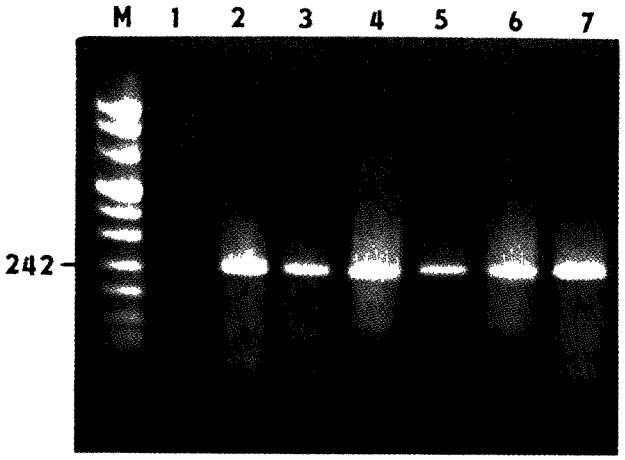

Fig. 1
Agarose gel electrophoresis of RT-PCR products
DNA size markers (Boehringer) (lane M); healthy plum (lane 1); PPV Marcus, positive control (lane 2); infected *Prunus* trees (lanes 3-7).

Table 1. List of Slovak PPV isolates maintained in GF 305 seedlings

PPV isolate	Locality	Origin	Symptomatology on GF 305		PPV subgroup specification
			Symptoms	Severity of infection	
BBR-1	Veselé pri Piešťanoch	Apricot, cv. M-VA-2	TC	+	D
BIII/2	Nitra	Plum, cv. Bystrická	TC	+	D
BOJ-1	Bojnice	Plum, cv. Pamjat Vavilova	TC, D	+	D
BOJ-3	Bojnice	Plum, cv. BO-4-75	TC, D	+	D
BOR-1	Borovce	Plum, wild type	TC, D	++	D
BOR-3	Borovce	Apricot, cv. 123/9	SC	+	M
CAH-2	Čachtice	Apricot, cv Maďarská	TC, D	++	M
TG	Trnava	Plum, cv. Tulcu Gras	TC	+	D
VAR	Bratislava-Vajnory	Peach, cv. Harland	TC, D	++	M
VAR-2	Bratislava-Vajnory	Peach, cv. Tenira	TC, D	++	M

TC = tissue clearing along the veins, D = leaf distortion, SC = small chlorotic spots, (+) = mild infection, (++) = severe infection.

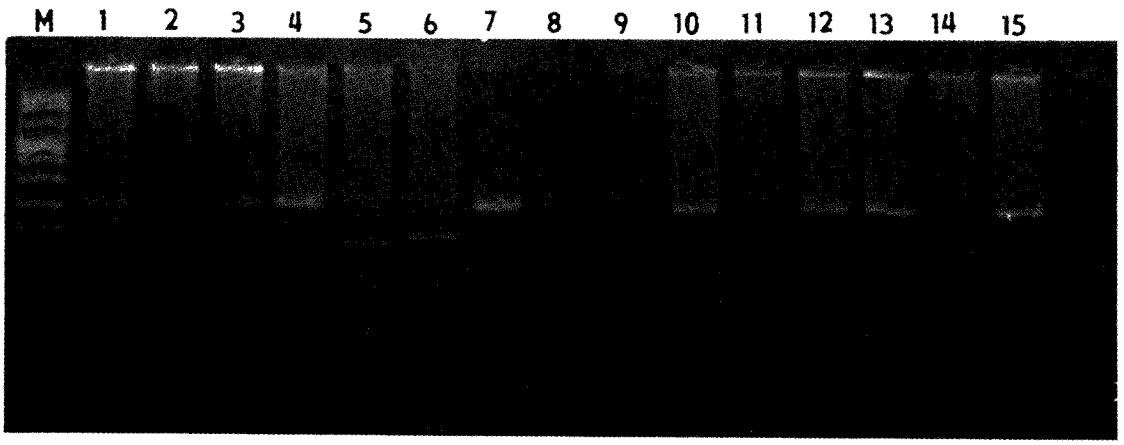

Fig. 2
Agarose gel electrophoresis of *Alu*I- and *Rsa*I-digested PCR products
DNA size markers (Boehringer) (lane M); undigested RT-PCR products (lanes 1, 4, 7, 10 and 13); *Alu*I-digested RT-PCR products (lanes 2, 5, 8, 11 and 14); *Rsa*I-digested RT-PCR products (lanes 3, 6, 9, 12 and 15).

Table 2. Representation of M and D isolates in tested *Prunus* trees

	PPV-M	PPV-D
Plum	12	15
Apricot	4	3
Myrobalan	3	3
Peach	3	0
Almond	1	0
Total	23	21

Table 3. List of evaluated PPV isolates from plums

Isolate	Locality	Host plant	Subgroup (*Rsa*I site)
BIII/1	Nitra	Plum, cv. Bystrická	M
BIII/2	Nitra	Plum, cv. Bystrická	D
BOJ-1	Bojnice	Plum, cv. Pamjat Vavilova	D
BOJ-3	Bojnice	Plum, cv. BO-4-75	D
BOR-1	Borovce	Plum, cv. Bystrická	D
BOR-11	Borovce	Plum, wild type	M
CFR-1	Cífer	Plum, wild type	D
CFR-2	Cífer	Plum, cv. Undetermined	M
DVZ	Dvory nad Žitavou	Plum, cv. Undetermined	M
IVD-1	Ivánka pri Dunaji	Plum, cv. Bystrická	D
IVD-2	Ivánka pri Dunaji	Plum, wild type	D
KRA-70	Krajné	Plum, wild type	D
MT	Martin	Plum, cv. Bystrická	M
NM	N. Mesto n/Váhom	Plum, wild type	M
NR-1	Nitra	Plum, cv. Undetermined	M
NR-2	Nitra	Plum, cv. Undetermined	D
PL-6	Dolné Plachtince	Plum, cv. Tarana	M
PL-7	Dolné Plachtince	Plum, cv. Herman	D
SEN-1	Šenkvice	Plum, cv. Undetermined	M
TG II	Trnava	Plum, cv. Undetermined	D
TG	Trnava	Plum, Tuleu Gras	D
TTE-1	Trenč. Teplá	Plum, cv. Undetermined	M
TTE-2	Trenč. Teplá	Plum, cv. Undetermined	D
TTE-3	Trenč. Teplá	Plum, cv. Undetermined	M
VR-1	Veľké Ripňany	Plum, cv. Undetermined	D
VR-2	Veľké Ripňany	Plum, cv. Nitrian. Bystrička	D
X	Krajné	Plum, wild type	M

tested on *N. clevelandii*, *N. clevelandii* x *glutinosa* hybrid and *P. sativum* cv. Colmo. On tobaccos, the isolates caused systemic chlorotic spots (CAH-2 and VAR) or growth reduction and latent infection (BIII/2, BOJ-3, and BOR-3). In case of latent infection the DAS-ELISA was positive. The mechanical infection of the pea cv. Colmo caused ligth green mosaic.

Biological properties of PPV isolates were further tested on *Ch. foetidum*. When a sap from infected GF 305 was used as inoculum, all the isolates developed local chlorotic spots. However, character of symptoms of some isolates was changed when *Ch. foetidum* plants were infected with a sap from *N. benthamiana*. We could identify local yellow spots

Table 4. List of evaluated PPV isolates from apricots

Isolate	Locality	Host plant	Subgroup (*Rsa*I site)
BBR-1	Veselé	Apricot, cv. M-VA-2	D
BOR-3	Borovce	Apricot, cv. VS 123/9	M
CAH-2	Čachtice	Apricot, cv. Maďarská	M
CFR-3	Cífer	Apricot, cv. Rakovského	M
NZ-1	Nové Zámky	Apricot, cv. Leala	D
PL-2	Dolné Plachtince	Apricot, cv. Vegama	M
RAK 2/9	Rakovice	Apricot, cv. Ligeti Orias	D

Table 5. List of evaluated PPV isolates from myrobalans

Isolate	Locality	Host plant	Subgroup (*Rsa*I site)
CFR-4	Cífer	Myrobalan, yellow type	M
MOD-1	Modra	Myrobalan, wild type	D
PL-1	Dolné Plachtince	Myrobalan, cv. Klasik	M
PZK	Pezinok	Myrobalan, undetermined	D
RCA	Bratislava-Rača	Myrobalan, yellow type	M
SEN-3	Šenkvice	Myrobalan, red form	D

Table 6. List of evaluated PPV isolates from almonds and peaches

Isolate	Locality	Host plant	Subgroup (*Rsa*I site)
NZ-2	Nové Zámky	Peach, cv. Redhaven	M
NZ-4	Nové Zámky	Almond, cv. Rekord Tetenyi	M
VAR	Vajnory	Peach, cv. Harland	M
VAR-2	Vajnory	Peach, cv. Tenira	M

Table 7. List of evaluated PPV isolates from tolerant plum cultivars

Isolate	Locality	Cultivar	Subgroup (*Rsa*I site)
DV-20	Dvory nad Žitavou	Cacanska Lepotica	D
DV-25	Dvory nad Žitavou	Cacanska Rodna	M
DV-27	Dvory nad Žitavou	Cacanska Rodna	M
DV-29	Dvory nad Žitavou	Cacanska Rodna	M
DV-36	Dvory nad Žitavou	Cacanska Rana	M
DV-37	Dvory nad Žitavou	Cacanska Rana	M
DV-39	Dvory nad Žitavou	Cacanska Rana	M
DV-40	Dvory nad Žitavou	Cacanska Rana	M
DV-46	Dvory nad Žitavou	Stanley	M
KRA-64	Krajné	Cacanska Lepotica	M
KRA-65	Krajné	Cacanska Lepotica	M
KRA-66	Krajné	Cacanska Lepotica	M
PL-3	Dolné Plachtince	Cacanska Najbolja	M
PL-4	Dolné Plachtince	Cacanska Rana	M
PL-5	Dolné Plachtince	Stanley	M

(BIII/2 and BOR-3), yellow spots with necrotic centres (VAR) and necrotic lesions (CAH-2). Repeated transmission of BOJ-3 isolate was negative.

The strain variability was analysed in 44 samples (DAS-ELISA-positive) from 22 localities of western and middle Slovakia.

In PCR, a 243 bp fragment was obtained from all the tested samples (Fig. 1). The restriction analysis confirmed that all the PCR fragments contained *Alu*I site (Fig. 2). According to *Rsa*I polymorphism, the presence of isolates belonging to PPV-M and PPV-D subgroups could be identified. No relation between the symptomatology and subgroup specification of isolates was found.

First results of subgroup specification of Slovak PPV isolates (Kúdela *et al.*, 1998) showed a mild predominance of PPV-M isolates, however, with the increasing number of tested samples the proportion of M and D isolates was found to be approximately equal (Table 2). 23 isolates belonged to PPV-M subgroup and 21 to PPV-D. The evaluated isolates are listed in Tables 3, 4, 5, and 6.

Separately, the PPV subgroup variability was tested in samples from 3 orchards of tolerant plum cultivars (Cacanska Lepotica, C. Najbolja, C. Rodna, C. Rana, and Stanley). The tests showed a great prevalence of PPV-M isolates in tested samples (Table 7). These results indicate that plum trees of tolerant cultivars widely imported for plantation in 80es from the former Yugoslavia could be an important source of PPV-M spread in Slovakia.

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References

Clark MF, Adams AN (1977): Characterization of the microplate method of enzyme-linked immunosorbent assay (ELISA) for detection of plant viruses. *J. Gen. Virol.* **34**, 475-483.

Glasa M, Matisová J, Hričovský I, Kúdela O (1997): Susceptibility of peach GF 305 seedlings and selected herbaceous plants to plum pox virus isolates from western Slovakia. *Acta Virol.* **41**, 341-344.

Králiková K (1962): Survey on the investigation of plum pox disease in Slovakia. *Proc. 5th Conf. Czechoslovak Plant Virol.*, Prague, pp. 346-351.

Kúdela O, Glasa M, Fuchs E, Kúdelová M (1998): Strain variability of plum pox virus isolates from western Slovakia. *Acta Virol.* **42**, 71-74.

Paulechová K (1981): Comparative study on plum sharka virus isolates from Slovakia. *Biológia* (Bratislava) **36**, 225-229.

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

Wetzel T, Candresse T, MacQuaire G, Ravelonandro M, Dunez J (1992): A highly sensitive immunocapture polymerase chain reaction method for plum pox potyvirus detection. *J. Virol. Methods* **39**, 27-37.