Arch Virol (2005) 150: 2051–2060
DOI 10.1007/s00705-005-0548-3

Archives of Virology
Printed in Austria

Analysis of recombinant *Plum pox virus* (PPV) isolates from Serbia confirms genetic homogeneity and supports a regional origin for the PPV-Rec subgroup*

M. Glasa[1], **S. Paunovic**[2], **D. Jevremovic**[2], **A. Myrta**[3], **S. Pittnerová**[1], and **T. Candresse**[4]

[1]Institute of Virology, Department of Plant Virology, Slovak Academy of Sciences, Bratislava, Slovakia
[2]Fruit and Grape Research Centre, Cacak, Serbia and Montenegro
[3]Istituto Agronomico Mediterraneo, Valenzano (BA), Italy
[4]UMR GDPP, INRA et Université Bordeaux 2, Villenave d'Ornon, France

Received January 24, 2005; accepted March 18, 2005
Published online May 23, 2005

Summary. The recent observation of the frequent occurrence of natural recombinant *Plum pox virus* (PPV) isolates has led to the identification of a distinct PPV subgroup, named PPV-Rec. The diversity, origin and geographical spread of the recombinant PPV isolates belonging to this subgroup remain, however, relatively poorly known. In an effort to further our understanding of these isolates, eight PPV isolates from Serbia, the country from which the first such recombinant (PPV-o6) originated, were characterized. Depending on the genomic region targeted by different typing assays, seven of the eight isolates tested presented discrepancies in their typing behavior. Sequence analysis of the (Cter)NIb-(Nter)CP region confirmed the recombinant nature of these seven isolates which all presented an identical recombination breakpoint identical to previously characterized PPV-Rec isolates. Biological indexing and immunoblot analysis provided indications that asymptomatic infection of the GF305 peach indicator and migration of the coat protein as a double-band in immunoblots may represent conserved and discriminating properties of PPV-Rec isolates. The genetic diversity of PPV-Rec isolates from former Yugoslavia (Serbia, Bosnia and Herzegovina) was estimated to be twice as large as that of the PPV-Rec isolates obtained from all other countries to date (Albania, Bulgaria, Czech republic, Germany, Hungary and Slovakia). These last results are consistent with the hypothesis that former Yugoslavia is

*The nucleotide sequences reported in this manuscript have been deposited in the GenBank database under accession numbers AY690603-AY690612.

the center of dispersion of PPV-Rec. Taken together, the results presented here provide further evidence for the wide distribution and temporal genetic stability of these natural PPV recombinant isolates and provide for the first time a possible scenario for their dispersion throughout central and eastern Europe.

Introduction

The Sharka disease poses an important threat to stone-fruit cultivation worldwide. Plum pox virus (PPV), the causal agent of the disease, is a member of the genus *Potyvirus* within the family *Potyviridae*. PPV has a single-stranded plus-sense RNA genome of about 10 kb with a single open reading frame encoding a large polyprotein from which ten functional, mature proteins are released by proteolytic processing [19].

Until recently, the vast majority of the currently identified PPV isolates was assigned to either one of two major subgroups, PPV-M and PPV-D [1, 3]. Recently, a third major subgroup of PPV isolates has been identified and named PPV-Rec [10, 11]. It corresponds to an ensemble of closely related isolates characterized by a homologous ancestral recombination event between PPV-M and PPV-D with a break point located in the 3′ part of the NIb gene, similar to that initially reported for the PPV-o6 isolate [4]. In addition to these major PPV subgroups, two minor subgroups correspond to PPV isolates able to naturally infect cherry (PPV-C) and to the geographically limited El Amar isolates from Egypt (PPV-EA) [17, 27]. A divergent isolate recently discovered on plum in Canada [13] probably represents a third minor subgroup.

Recombination is one of the key evolutionary processes underlying the high genetic variability of plant RNA viruses [8]. While inter-specific or inter-generic recombination seem to have played a significant role in the macro-evolution of viruses through the exchange of functional modules between unrelated taxa [7, 16], intra-specific recombination contributes directly to the intra-specific diversity [2, 24].

The PPV-Rec isolates have recently been reported to represent a significant fraction of PPV isolates in central and eastern Europe [10, 11]. The evolutionary history and geographical dispersion of these isolates is, however, still unclear. The first such PPV-Rec isolate to be analyzed was PPV-o6 which was isolated in former Yugoslavia (Serbia) and characterized in the eighties and early nineties [4]. The aim of the present work was to evaluate the presence and the variability of PPV-Rec isolates from Serbia using a combination of biological, serological and molecular techniques. The results obtained provide further evidence for the frequent occurrence of PPV-Rec isolates and tentatively identify former Yugoslavia as the original center of dispersion of these isolates.

Material and methods

Virus isolates

PPV isolates were collected from naturally infected plum trees (*Prunus domestica*) in the summer of 2003, in various locations in Serbia. Two PPV isolates, collected in 2002, from

plum and nanking cherry (*P. tomentosa)*, were maintained by chip-budding on *P. tomentosa* indicator plants (Table 1). The presence of PPV in the various sources was confirmed by DAS-ELISA. All samples were tested in parallel by DAS-ELISA to detect the presence of the Ilarviruses *Prunus necrotic ringspot virus* (PNRSV) and *Prune dwarf virus* (PDV) and of the Trichovirus *Apple chlorotic leafspot virus* (ACLSV) using commercial kits (Loewe Biochemica).

Typing of PPV isolates

Typing of PPV isolates was performed as described previously [9, 10] using the RT-PCR/RFLP tests targeting the CP and P3-6K1 genome parts. In addition, RT-PCR with subgroup specific primers enabling direct discrimination of PPV-M, D and Rec isolates was carried out as described [22].

Serological typing was also performed using monoclonal antibodies (MAbs) specific to the PPV-M or the PPV-D groups [3] in triple antibody sandwich (TAS)-ELISA assays as recommended by the suppliers. For immunoblot analysis, the standardized procedure [1, 21] was used.

Partial sequencing of PPV isolates and analysis of the sequences

Total RNAs were extracted using the RNA Easy Mini kit (Qiagen). The first strand cDNA was synthesized using oligo(dT) or pdN6 random primers and the *Avian myeloblastis virus* reverse transcriptase (Promega). For all PPV isolates, the cDNA fragment spanning the (Cter)NIb-(Nter)CP region was amplified and sequenced using PCR primer P4 (5′-TGCCTTCAAA CGTGGCACTG-3′$^{8893-8912}$) in combination with either mD5 (5′-TATGTCACATAAAGG CGTTCTC-3′$^{8207-8228}$) for PPV-Rec amplification or mM5 (5′-GCTACAAAGAACTGCT GAGAG-3′$^{8350-8370}$) for PPV-M amplification.

The 3′ terminal part of the genome spanning (Cter)NIb-CP-3′UTR of Serbia-MI and Serbia-T isolates were determined from two overlapping PCR fragments amplified using mD5/P1 and P2/oligo(dT) primers sets (see text above and [26] for the sequence the primers). In addition, the sequence of a (Cter)HC-(Nter)P3 fragment of these isolates was determined using HC-RC/P3-RC primers as described previously [11]. All PCR amplifications were performed using the proofreading TaKaRa Ex Taq^{TM} polymerase (Takara Bio Inc). PCR products were gel-purified (QIAprep® Spin Miniprep Kit, Qiagen) before being directly sequenced.

PPV sequences used for comparisons were retrieved from the Genbank database (www.ncbi.nlm.nih.gov). The unreleased sequences of three Bosnian PPV isolates [15] were also included in the analysis. Multiple sequence alignments and phylogenetic reconstructions (neighbor-joining with bootstrap analysis) were performed using the ClustalX program [23]. The trees were visualized using the Treeview v.1.6.1 program [18]. The recombinant sequences and the location of recombination breakpoints were detected using PHYLPRO v. 1.0 [25]. MEGA2 [14] and DnaSP version 4.00.5 [20] were used to estimate average intra-group and inter-group nucleotide divergence values.

Results

Analysis of Serbian PPV isolates using various typing assays

Eight PPV isolates collected from naturally infected *Prunus* trees from distinct locations in Serbia (Table 1) were analyzed in this study. All isolates reacted positively with a polyclonal antiserum raised against PPV. Based on additional serological tests, the Serbia-MI isolate was found to occur in mixed infection with

Table 1. Origin of the PPV isolates used in this study and accession numbers of the partial sequences determined

Isolate	Original host	Locality	Subgroup	Sequenced region(s)	GenBank accession numbers
Serbia-B	*P. domestica*	Central Serbia (Cacak)	Rec	(Cter)NIb-(Nter)CP	AY690603
Serbia-MI	*P. domestica* cv. Cacanska rodna[a]	Central Serbia (Milatovici)	Rec	(Cter)NIb-CP-5′UTR (Cter)HC-(Nter)P3	AY690605, AY690611
Serbia-PO2	*P. domestica* cv. Pozegaca	Central Serbia (Cacak)	Rec	(Cter)NIb-(Nter)CP	AY690606
Serbia-PO3	*P. domestica* cv. Pozegaca	Central Serbia (Cacak)	Rec	(Cter)NIb-(Nter)CP	AY690607
Serbia-ST	*P. domestica* cv. Stanley	Central Serbia (Cacak)	Rec	(Cter)NIb-(Nter)CP	AY690608
Serbia-T	*P. tomentosa*[a]	Central Serbia (Cacak)	Rec	(Cter)NIb-CP-5′UTR (Cter)HC-(Nter)P3	AY690609, AY690612
Serbia-V	*P. domestica*	Central Serbia (Cacak)	Rec	(Cter)NIb-(Nter)CP	AY690610
Serbia-K	*P. domestica*	Eastern Serbia	M	(Cter)NIb-(Nter)CP	AY690604

[a]Maintained under controled greenhouse condition on *P. tomentosa* indicator since 2002

PNRSV while the Serbia-B and Serbia-K isolates were in mixed infection with PDV and with ACLSV, respectively.

Based on the CP typing using subgroup-specific MAbs [3] and *Rsa*I RFLP analysis of the RT-PCR amplified part of the CP gene [1], all eight Serbian isolates were classified as PPV-M. However, seven of these isolates typed as PPV-D (PCR products were cleaved by *Eco*RI and *Dde*I) when the RFLP typing assay targeting the P3-6K1 genomic region [9] was performed.

As expected from these initial results, inspection of their (Cter)NIb-(Nter)CP sequence and PHYLPRO analysis confirmed that these seven isolates were recombinants between PPV-M and PPV-D and had the same cross-over site around nt position 8450 as the previously characterized PPV-Rec isolates [11]. The only isolate analyzed having a typical PPV-M sequence was the Serbia-K isolate (Table 1), which also typed as PPV-M in all three typing assays performed.

Analysis of partial sequences of the Serbian PPV isolates

The phylogenetic tree presented in Fig. 1, reconstructed from the sequence of the (Cter)NIb-(Nter)CP region (nt 8384–8858) clearly showed the clustering of the seven Serbian recombinant isolates with all other previously characterized recombinant isolates [11]. Although the branch lengths were short and the boostrap support values relatively low, one of the recombinant isolates, Serbia-T, clustered away from the other Serbian isolates. The only PPV-M isolate analyzed in this study, Serbia-K, clustered as expected with the classical PPV-M isolates such as PS and SK-68.

The average pairwise genetic distances (genetic diversity) were calculated in the same genomic region for all major PPV subgroups detected during this

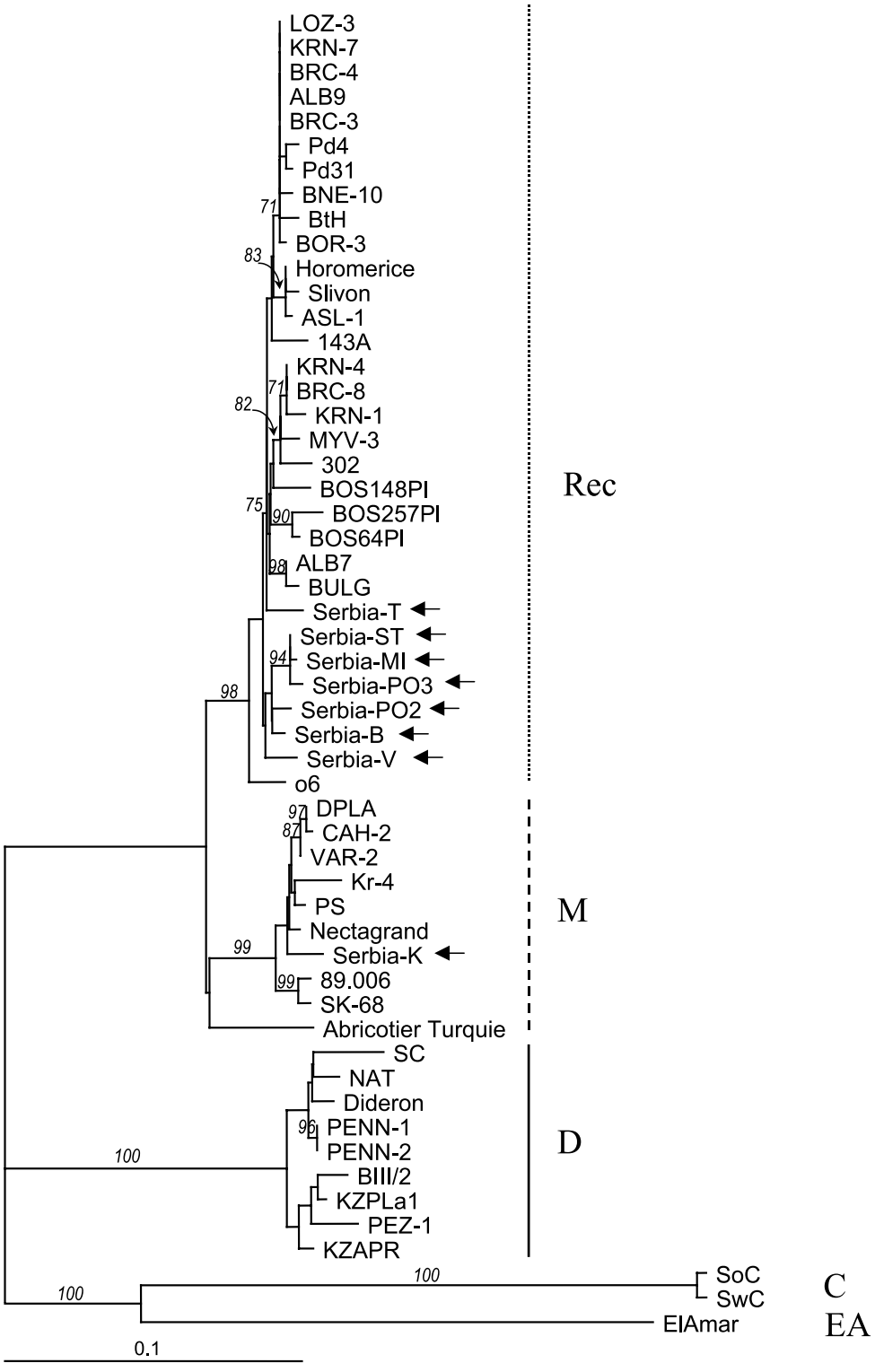

Fig. 1. Phylogenetic tree of PPV isolates reconstructed from the 475 nt long (Cter)NIb-(Nter)CP region (nt 8384–8858). The scale bar represents a distance of 0.1 substitutions per site. Only bootstrap values >70% are shown. The divergent PPV-SoC, SwC and ElAmar were used to root the tree. Serbian isolates are highlighted by arrows

Table 2. Average genetic distances calculated within various groups of PPV isolates

Subgroup[a]	Average divergence		Average NG distance	
	nt identity	aa identity	dNS	dS
PPV-D (9 isolates)	0.024 ± 0.003	0.035 ± 0.010	0.015 ± 0.003	0.049 ± 0.012
PPV-M[b] (8 isolates)	0.016 ± 0.003	0.022 ± 0.007	0.011 ± 0.004	0.034 ± 0.010
PPV-Rec (32 isolates)	0.017 ± 0.002	0.024 ± 0.004	0.011 ± 0.002	0.036 ± 0.007
PPV-Rec (former Yugoslavia, 11 isolates)	0.022 ± 0.003	0.035 ± 0.007	0.016 ± 0.003	0.042 ± 0.010
PPV-Rec (other countries, 21 isolates)	0.012 ± 0.002	0.015 ± 0.003	0.007 ± 0.001	0.028 ± 0.008

Using a dataset corresponding to the (Cter)NIb-(Nter)CP (positions 8341–8858), the average divergence level calculated on nucleotide and amino acid sequence identities are shown, together with the Nei Gojobori (NG) average rates of non-synonymous (dNS) and synonymous (dS) nucleotide substitutions. Average values ± standard deviations are given

[a]The number of PPV isolates in each analysed PPV subgroup is given in parentheses

[b]The divergent Abricotier Turquie isolate [12] was excluded from the PPV-M subgroup

phylogenetic analysis (PPV-D, PPV-M and PPV-Rec). In addition, in the case of PPV-Rec, the diversities were also calculated on two groups based on the geographical origin of the isolates, separating isolates originating from former Yugoslavia (Serbian and Bosnian isolates) from isolates obtained from all other countries. The results of these analyses are provided in Table 2. Two results can be pointed out: first, when excluding the divergent Abricotier Turquie isolate from the PPV-M subgroup, PPV-M and PPV-Rec appeared to have a similar level of genetic diversity, which was somewhat lower than that observed for PPV-D isolates. Second, the genetic diversity measured for PPV isolates from former Yugoslavia (Serbia, Bosnia and Herzegovina; 11 isolates) was roughly twice as high as that of all other PPV-Rec isolates (0.022 vs. 0.012) despite the fact that these 21 isolates were obtained from six different European countries (Albania, Bulgaria, Czech republic, Germany, Hungary and Slovakia). The statistical significance of this difference was validated ($P < 0.0001$) in a Mann-Whitney-Wilcoxon non-parametric test (result not shown).

Further analysis of the Serbia-MI and Serbia-T isolates

Two isolates, Serbia-MI and Serbia-T, recovered from different original *Prunus* hosts (Table 1), were successfully transmitted by chip-budding to *P. tomentosa* and *P. persica* GF305 indicator plants. Although both isolates exhibited characteristic symptoms on *P. tomentosa* – chlorotic mottling, vein chlorosis, leaf malformation [6], they did not produce symptoms on *P. persica* GF305 seedlings.

The capsid protein of the Serbia-MI and Serbia-T isolates migrated as a double band in the SDS-PAGE as revealed by an immunoblot analysis (Fig. 2). In order to avoid the possibility that this unusual migration pattern was due to the presence of

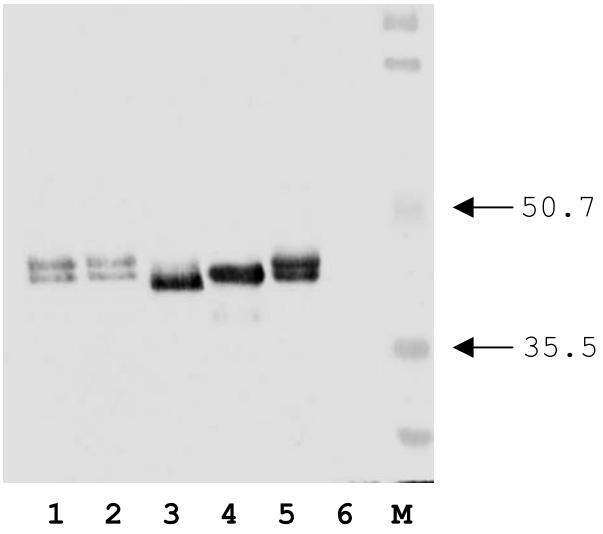

Fig. 2. Immunoblot analysis of PPV coat proteins from crude plant sap of infected *N. benthamiana* using anti-PPV polyclonal antibodies. Serbia-T (*1*), Serbia-MI (*2*) and control PPV isolates BOJ-3 (PPV-D), CAH-2 (PPV-M), BOR-3 (PPV-Rec) (*3–5*, respectively) were analyzed, together with healthy *N. benthamiana* (*6*) as a control. *M* – Prestained SDS-PAGE Standards (Bio-Rad Laboratories). Arrows indicate the molecular mass of proteins in kDa

a mixed infection by isolates belonging to different PPV subgroups, the samples used were re-tested by subgroup specific RT-PCR [22], which failed to provide evidence for any mixed infection (results not shown).

Sequence analyses of Serbia-MI and Serbia-T isolates were extended to the ca. 1500 nt region encompassing (Cter)NIb-CP-3′UTR and to the (Cter)HC-(Nter)P3 region (ca. 600 nt). Complete CP gene comparisons revealed that nucleotide identity between Serbia-MI and Serbia-T reached 97.9% (aa identity was 97.6% with a total of eight amino acid differences located mainly in the N terminus of CP). Comparisons with other PPV-Rec isolates showed complete co-linearity of the various sequences. The average intra-subgroup divergence reached 1.3%, which was close to the 1.2% value observed for the PPV-D subgroup (n = 31 isolates). A reliable calculation for PPV-M was not performed, because when excluding the highly divergent Abricotier Turquie, only two complete CP sequences of PPV-M isolates were available in the databases.

As expected from the analysis of other PPV-Rec isolates [11], high sequence conservation was observed between Serbia-MI, Serbia-T and previously sequenced PPV-Rec isolates in the highly conserved region spanning the end of HC and the start of P3 (nt 2388–2933), with nucleotide identity levels reaching 98–99.8%.

Discussion

In the case of PPV, the existence of recombinant isolates has for a long time been overlooked due to the sole use of typing assays targeting the capsid protein or its

gene. The identification of recombinant isolates resulting from the recombination event in the C terminus of NIb between PPV-M and PPV-D was facilitated by an approach supplementing conventional CP-based typing techniques with methods targeting other genomic regions upstream of CP, namely P3-6K1 and CI [9, 10]. In this study, analysis of a limited number of Serbian PPV isolates showed that with the exception of the Serbia-K isolate identified as belonging to PPV-M, all other isolates could be classified as belonging to the PPV-Rec subgroup.

Despite reproducible positive ELISA results, the Serbia-MI and Serbia-T isolates did not induce symptoms on peach GF305, which is a generally accepted woody indicator for PPV. Similar asymptomatic reactions of this indicator to other recombinant PPV isolates have been reported [10, 15] and this may thus represent a particular biological feature of this group of isolates. As a consequence, the effectiveness of GF305 as a woody indicator for the biological indexing of PPV-Rec may need to be evaluated further.

It has been shown, in immunoblot assays, that the electrophoretic mobility of the coat protein can potentially be used to distinguish between PPV-M and D isolates ([1, 5], see also Fig. 2, lanes 3 and 4). As has previously been observed for other recombinant isolates [21, and our unpublished results], the CP of the Serbia-MI and Serbia-T isolates migrated as a double band in the SDS-PAGE. This particular CP mobility pattern of recombinant isolates, possibly due to some post-translational CP modification, therefore appears to be a typical feature of PPV-Rec isolates which could be used to discriminate them from PPV-D or PPV-M isolates.

One interesting finding of this study is the observation that PPV-Rec isolates from former Yugoslavia show a molecular diversity that is close to two-fold larger than that of all other PPV-Rec isolates analyzed together. In theory, older populations are expected to have accumulated more genetic diversity than younger ones [8]. Although other effects (genetic bottlenecks, bias in populations sampling . . .) might mask this trend under some circumstances, the results reported here are consistent with the hypothesis that former Yugoslavia is the original center of dispersion of the PPV-Rec isolates. This hypothesis is also consistent with the fact that the first recombinant isolate, PPV-o6, which was characterized over a decade ago [4] also originated from this region. Further confirmation of this hypothesis would unfortunately probably require access to a broad range of "old" PPV isolates from a number of countries. Although in some cases relatively ancient virus isolates have been retrieved from herbarium samples, the feasibility of such an approach remains to be evaluated for *Plum pox virus*.

Acknowledgements

The authors gratefully thank Dr. S. Matic for making available the partial sequences of 3 PPV isolates from Bosnia and Herzegovina prior to publication. The authors thank Drs. J.B. Quiot and G. Labonne for the generous gift of ELISA reagents derived from the V.196 PPV antiserum and Kathryn Mayo for help with the English of the manuscript. This work was supported by the grants APVT-51-011802 and APVT-51-001305 from the Slovak Science and Technology Assistance Agency (APVT).

References

1. Bousalem M, Candresse T, Quiot-Douine L, Quiot JB (1994) Comparison of three methods for assessing plum pox virus variability: further evidence for the existence of two major groups of isolates. J Phytopathol 142: 163–172
2. Bousalem M, Douzery EJ, Fargette D (2000) High genetic diversity, distant phylogenetic relationships and intraspecies recombination events among natural populations of Yam mosaic virus: a contribution to understanding potyvirus evolution. J Gen Virol 81: 243–255
3. 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 M and D serotypes of plum pox potyvirus. Phytopathology 88: 198–204
4. Cervera MT, Riechmann JL, Martin MT, Garcia JA (1993) 3′ terminal sequence of the plum pox virus PS and o6 isolates: evidence for RNA recombination within the potyvirus group. J Gen Virol 74: 329–334
5. Dallot S, Labonne G, Quiot-Douine L, Boeglin M, Candresse T, Quiot JB (1998) Peculiar plum pox virus D-populations are epidemics in peach trees. Acta Hortic 472: 355–365
6. Damsteegt VD, Waterworth HE, Mink GI, Howell WE, Levy L (1997) Prunus tomentosa as a diagnostic host for detection of Plum pox virus and other Prunus viruses. Plant Dis 81: 329–332
7. Desbiez C, Lecoq H (2004) The nucleotide sequence of Watermelon mosaic virus (WMV, Potyvirus) reveals interspecific recombination between two related potyviruses in the 5′ part of the genome. Arch Virol 149: 1619–1632
8. Garcia-Arenal F, Fraile A, Malpica JM (2001) Variability and genetic structure of plant virus populations. Annu Rev Phytopathol 39: 157–186
9. Glasa M, Marie-Jeanne V, Moury B, Kúdela O, Quiot JB (2002a) Molecular variability of the P3-6K1 genomic region among geographically and biologically distinct isolates of Plum pox virus. Arch Virol 147: 563–575
10. Glasa M, Marie-Jeanne V, Labonne G, Šubr Z, Kúdela O, Quiot JB (2002b) A natural population of recombinant Plum pox virus is stable and competitive under field conditions. Eur J Plant Pathol 108: 843–853
11. Glasa M, Palkovics L, Komínek P, Labonne G, Pittnerová S, Kúdela O, Candresse T, Šubr Z (2004) Geographically and temporally distant natural recombinant isolates of Plum pox virus are genetically very similar and form a unique PPV subgroup. J Gen Virol 85: 2671–2681
12. Glasa M, Candresse T (2005) Partial sequence analysis of an atypical Turkish isolate provides further information on the evolutionary history of Plum pox virus (PPV). Virus Res 108: 199–206
13. James D, Varga A, Thompson D, Hayes S (2003) Detection of a new and unusual isolate of plum pox potyvirus in plum (Prunus domestica). Plant Dis 87: 1119–1124
14. Kumar S, Tamura K, Ingrid BJ, Nei M (2001) MEGA2: molecular evolutionary genetics analysis software. Bioinformatics 17: 1244–1245
15. Matic S, Al-Rwahnih M, Myrta A (2004) Typing of Plum pox virus isolates in Bosnia and Herzegovina. In: Eur Meeting '04 on Plum pox, Rogów, Poland, Abstr. p 16
16. Moonan F, Molina J, Mirkov TE (2000) Sugarcane yellow leaf virus: an emerging virus that has evolved by recombination between Luteoviral and Poleroviral ancestors. Virology 269: 156–171
17. Nemchinov L, Crescenzi A, Hadidi A, Piazzolla P, Verderevskaya T (1998) Present status of the new cherry subgroup of Plum pox virus (PPV-C). In: Hadidi A, Khetarpal

RK, Koganezawa H (eds) Plant virus disease control, pp 629–638. APS Press St Paul, Minnesota
18. Page DR (1996) TreeView: an application to display phylogenetic trees on personal computer. Comp Appl Biosci 12: 357–358
19. Riechmann JL, Lain S, Garcia JA (1992) Highlights and prospects of potyvirus molecular biology. J Gen Virol 73: 1–16
20. Rozas J, Sánchez-DelBarrio JC, Messeguer X, Rozas R (2003) DnaSP, DNA polymorphism analyses by the coalescent and other methods. Bioinformatics 19: 2496–2497
21. Šubr Z, Glasa M (1999) Plum pox virus capsid protein mobility in SDS-polyacrylamide gel electrophoresis. Acta Virol 43: 259–262
22. Šubr Z, Pittnerová S, Glasa M (2004) A simplified RT-PCR-based detection of recombinant Plum pox virus isolates. Acta Virol 48: 173–176
23. Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG (1997) The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res 24: 4876–4882
24. Tomimura K, Gibbs AJ, Jenner CE, Walsh JA, Ohshima K (2003) The phylogeny of Turnip mosaic virus; comparisons of 38 genomic sequences reveal a Eurasian origin and a recent 'emergence' in east Asia. Mol Ecol 12: 2099–2111
25. Weiller GF (1998) Phylogenetic profiles: a graphical method for detecting genetic recombinations in homologous sequences. Mol Biol Evol 15: 326–335
26. 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
27. 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

Author's address: Miroslav Glasa, Institute of Virology, Department of Plant Virology, Slovak Academy of Sciences, Dúbravská cesta 9, 84505 Bratislava, Slovakia; e-mail: virumig@savba.sk