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DETECTION AND CHARACTERISATION OF *PLUM POX VIRUS* (PPV) ISOLATES FROM EASTERN SLOVAKIA REVEALED THE PRESENCE OF THREE MAIN VIRAL STRAINS.

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ABSTRACT

Plum pox virus (PPV), the agent responsible for Sharka disease, is the most important viral pathogen of stone fruit trees world-wide, having an endemic status in Slovakia. To increase knowledge of PPV diversity in Slovakia, a set of 11 isolates, originated from the eastern part of the country, was characterised. The isolates were chip-budded from their original *Prunus* hosts to the susceptible GF305 indicators, exhibiting the symptoms of variable severity. A genomic region encompassing the partial NIb and the hypervariable 5´terminal region of the CP gene was amplified from all 11 isolates in RT-PCR and directly sequenced. The phylogenetic analysis revealed the grouping of the 11 Slovak isolates into 3 distinct clusters, representing the PPV-M (2 isolates), D (7 isolates) and Rec strains (2 isolates). The strain affiliation of isolates was further confirmed by strain-specific RT-PCR, using which the presence of additional mixed infection by minor PPV variants was detected in 2 samples. The results further contribute to the understanding of PPV diversity in Slovakia and confirm the specificity and sensitivity of molecular approaches used for the virus strain determination.

Keywords: *Prunus*; virus; RT-PCR; strain; diversity

INTRODUCTION

Plum pox virus (PPV), belonging to the genus *Potyvirus*, is the causal agent of Sharka, the economically most important viral disease of *Prunus* crops (plum, apricot, peach) as well as wild and ornamental species (**Cambra et al., 2006**). Nowadays, PPV was detected in all continents apart from Australia (**García and Cambra 2007**). Depending on the *Prunus* host and its susceptibility, the PPV-infected trees may exhibit a range of symptoms on leaves (spots, rings and mosaics, vein clearings, chlorosis and/or leaf distortions). Symptoms on plum fruits consist generally of shallow ring or arabesque depressions, sometimes with brown or reddish necrotic flesh and gumming. Infected apricots develop depressed rings on fruits, which may be severely deformed. Symptoms in peach are often less conspicuous and may represent pale rings or diffuse bands on the skin of the fruits. Typically, infected fruits of susceptible *Prunus* genotypes fall prematurely and have considerably decreased organoleptic quality. The symptoms may vary considerably with the age, the temperature and nutrient status of the plant (**Garcia et al., 2013**).

PPV is efficiently transmitted by vegetative propagation of infected material and by aphids in the nonpersistent manner (**Labonne et al., 1995; Garcia et al., 2013**).

The PPV genome consists of a single-stranded positive sense RNA molecule of about 10,000 nucleotides, coding a single open reading frame (ORF) flanked by shorts untranslated regions. The single large polyprotein hydrolyses itself after translation into at least 11 proteins (**Lopez-Moya et al., 2000**).

On the basis of molecular differences, 8 PPV strains are now recognised (referred to as M, D, EA, C, Rec, W, T, CR in the order of their discovery). PPV-M, D and Rec strains are prevalent in Europe and they are considered as major strains (**Glasa and Candresse, 2008; Šubr and Glasa, 2013; Garcia et al., 2013**). RNA viruses are characterised by high mutation rates due to the lack of proofreading activity associated with their viral RNA dependent RNA polymerases (**Desbiez et al., 2011**). Besides the mutations, recombination was found to play an important role in the evolutionary history of PPV (**Glasa et al., 2004; Garcia et al., 2013**). In case of PPV, the genetic diversity might be increased by the fact that *Prunus* trees remain in the field for more years or decades, enabling a development of the heterogeneous viral populations (**Jridi et al., 2006; Predajňa et al., 2012**) or even a mixing of different strains in a single host (**Candresse et al., 1998**).

Understanding the diversity of plant viruses is the essential step to design efficient management strategies. The Sharka disease has been first described on the territory of Slovakia in the 1950´s (**Králiková, 1964**). The first attempts to characterise the diversity of the PPV isolates spread in Slovakia were based on their symptomatology on the woody and herbaceous indicator plants (**Paulechová, 1981, Glasa et al., 1997**), mobility of their capsid proteins (**Šubr and Glasa, 1999**) and the restriction analysis of the short genome fragment encompassing the capsid protein (CP) gene (**Glasa et al., 1998**). Later, the partial and complete genome characterisation of a number of PPV isolates has permitted to obtain a comprehensive view on their molecular variability on the territory of Slovakia (**Glasa et al., 2004; Predajňa, 2013**). In this work, the picture of PPV diversity was complemented by the biological and partial molecular characterisation of

11 isolates originated from the different *Prunus* species in the Eastern Slovakia.

MATERIAL AND METHODOLOGY

Viral isolates. A set of 11 PPV isolates was collected from the main cultivated and wild *Prunus* hosts from orchards, gardens or wild landscape in Eastern Slovakia in 2012 (Table 1). The isolates were maintained in the chip-budded *Prunus persica* GF305 plants under glasshouse conditions. The symptoms were evaluated visually during two consecutive seasons. In all GF305 plants, the presence of PPV, *Prunus necrotic ringspot virus* (PNRSV) and *Prunus dwarf virus* (PDV) was periodically checked by multiplex reverse-transcriptase polymerase chain reaction (RT -PCR) using the protocol developed by **Jarošová et al. (2008)**.

Strain-specific RT-PCR. Total RNAs were extracted from infected leaves of GF305 using the NucleoSpin RNA Plant kit (Macherey-Nagel). A two-step reverse transcriptase polymerase chain reaction (RT-PCR) protocol was systematically used. The first-strand cDNA was synthesized by reverse transcription of total RNA using random hexamer primers and the *Avian myeloblastosis virus* (AMV) reverse transcriptase (both from Promega Corp.) and subsequently used in the three strain-specific PCRs using the combination of primers mM5 (5'-GCTACAAAGAACTGCTGAGAG-3', forward)/mM3 (5'-CATTTCCATAAACTCCAAAAGAC-3', reverse) for PPV-M detection, mD5 (5'-TATGTCACATAAAG GCGTTCTC-3', forward)/mD3 (5'-GACGTCCCTGTCT CTGTTTG-3', reverse) for PPV-D detection, and mD5/mM3 for PPV-Rec detection **(Šubr et al., 2004)**. PCR was performed using the EmeraldAmp MAX PCR Master Mix (Takara, Bio Inc.) under the following conditions for all combinations: denaturation 98 °C 3 min, 35 cycles of 94 °C 30 sec, 60 °C 20 sec, and 72 °C 30 sec and final elongation 72 °C 5 min.

Partial sequencing and sequence analyses. A 746-bp fragment spanning the 3´-terminal part of the nuclear inclusion b gene (NIb) and the 5´-terminal part of the CP gene (nt 8316–9061, based on the complete sequence of the BOR-3 isolate, AY028309) was amplified using the TaKaRa Ex Taq polymerase (TaKaRa, Bio Inc.) and the primer pair NCuniFor 5′-GAGGCAATTTGTGC TTCAATGG-3´ (sense) and NCuniR 5´-CGCTTAACT CCTTCATACCAAG-3´(antisense) **(Predajňa et al., 2012)** under the following cycling conditions: initial denaturation at 94 °C for 5 min; 25 cycles of 94 °C for 45 s, 54 °C for 45 s, and 72 °C for 1 min; final extension step at 72 °C for 10 min. The RT-PCR products were purified using the Wizard SV Gel and PCR Clean-Up System (Promega Corp.) and directly sequenced on both strands using the same PCR primers, using an automated DNA sequencer (ABI 3130xl Genetic Analyser; Applied Biosystems). Sequence analyses were performed using the Molecular Evolutionary Genetics Analysis software (MEGA v.5; **Tamura et al., 2011**). The obtained nucleotide sequences have been deposited in the GenBank database (http://www.ncbi.nlm.nih.gov/nuccore/) under Acc. Nos. KF840163-KF840173.

Table 1 List of PPV isolates analysed in this study and their characteristics.

Isolate	Locality (district)	Natural host	Symptomatology		Strain	Accession number
			natural host	GF305	Strain	Accession number
01	Vranov nad Topľov	plum (*P. domestica*), cv. Bystrická	intensive leaf mosaics and rings	+*	D	KF840172
02	Sedliská (Vranov nad Topľou)	plum, cv. Čačanská lepotica	symptomless	++**	D	KF840173
03	Trebišov	peach (*P. persica*), cv. Sunhaven	light rings on the bark of annual shoots	++	M	KF840165
04	Sedliská (Vranov nad Topľou)	plum, cv. Čačanská najbolja	symptomless	+	M	KF840166
05	Sedliská (Vranov nad Topľou)	apricot (*P. armeniaca*), unknown	symptomless	++	D	KF840168
07	Moldava nad Bodvou	plum, cv. President	symptomless	++	D	KF840169
08	Trebišov	blackthorn (*P. spinosa*)	symptomless	++	D	KF840170
09	Trebišov	plum, cv. Bystrická	intensive leaf mosaics and rings	+	Rec	KF840164
10	Trebišov	blackthorn	symptomless	+	Rec	KF840163
11	Vranov nad Topľou	plum, cv. Top five	intensive leaf mosaics and rings	+	D	KF840167
12	Moldava nad Bodvou	myrobalan (*P. cerasifera*), wild type	symptomless	+	D	KF840171

*mild leaf symptoms

**intensive leaf symptoms, mosaics, vein clearings, distorsions

RESULTS AND DISCUSSION

Indexing of GF305 demonstrated a high biological variability of PPV isolates.

The previous studies have revealed an endemic occurrence of PPV in Slovakia (**Glasa et al., 2004; Šubr and Glasa, 2013; Predajňa, 2013**). To widen the knowledge on the variability of PPV in the Eastern Slovakia, 11 isolates were sampled from various *Prunus* hosts showing a different response to natural PPV infection (Table 1).

To evaluate the biological properties, the isolates were transmitted by chip-budding into the widely used peach GF305 indicator. Infection of GF305 was expressed by intensive leaf symptoms, mosaics, vein clearings in case of isolates 02, 03, 05, 07 and 08, contrary to mild symptoms caused by isolates 01, 04, 09, 10, 11 and 12. Only PPV has been repeatedly detected in the infected GF305 (simultaneous RT-PCR tests for the presence of other common fruit tree viruses, PNRSV and PDV, remained negative), thus the observed symptoms are likely to be caused by PPV itself. It is noteworthy for several isolates, that severity of their symptoms scored on the natural hosts did not corresponded to the symptom severity observed on the susceptible GF305 indicator plants (Table 1). It was reported that the use of GF305 for indexing of PPV-Rec isolates can be compromised by the fact, that PPV-Rec isolates usually do not express the symptoms on this indicator, or the symptoms are only mild and/or time-limited (**Glasa et al., 2002, 2004**). Similar situation was observed in case of PPV-Rec isolates 09 and 10 analysed in this study. The observation of a range of symptoms within the isolates of the same strain, e.g. symptomatologically mild PPV-D isolates (01, 11, 12) vs. severe isolates (02, 05, 07, 08) further support the data about the existence of a high intra-strain divergence, limiting the possibility to draw clear-cut strain biological properties (**Glasa et al., 2010**).

Partial sequence analysis assigned the isolates into three main PPV strains.

An informative genomic region encompassing the partial NIb and the hypervariable 5´-terminal region of the CP gene was amplified from all 11 PPV isolates. Uncloned PCR products were sequenced directly to obtain the consensus (master) sequence representative of the PPV population present in the infected sample. After the primer sequences were removed, we have used a portion with a length of 717 nucleotides for further analyses. Comparisons of all obtained sequences showed their complete colinearity. The amino acid conserved Asp-Ala-Gly (DAG) block, which is essential for potyvirus aphid transmission (**Garcia et al., 2013**), was conserved in all deduced Slovak CP aminoacid sequences (Fig. 1).

The phylogenetic analysis clearly showed the grouping of the 11 Slovak isolates into 3 distinct clusters, representing the PPV-M, D and Rec strains (Fig. 2). On the contrary, the presence of other PPV strains was not detected. Especially, strains PPV-W and CR, recently identified in the eastern part of Europe (**Glasa et al., 2011; Mavrodieva et al., 2013; Glasa et al., 2013**) could have a potential emergence potential.

The intra-strain pairwise analysis of nucleotide sequences revealed that the 7 sequenced Slovak PPV-D isolates shared 96.0 - 99.3% nucleotide identity (the most distant being 01 and 08 isolates), the identity between 2 PPV-M isolates reached 99.6% and 2 PPV-Rec isolates were 100% identical. Similar results were obtained by the analysis of a more broad range of Slovak PPV isolates (**Predajňa, 2013**), indicating that the PPV-D strain has accumulated the highest molecular divergence, which can be indicative of its more ancient introduction in Slovakia as compared to other PPV strains. Blast search (http://blast.ncbi.nlm.nih.gov/Blast.cgi) showed that isolates 09 and 10 are the most closely related to the previously characterised PPV-Rec isolates from Slovakia (AY028309) and Hungary (AJ566344, AJ566345).

PPV-M isolates from this study (03, 04) showed the highest identity with Serbian (AJ2433957) and Slovak isolate (AY324837), indicating much lower level of divergence within these strains.

Strain-specific RT-PCR typing confirmed the sequence data, but revealed an additional mixed infection.

A substantial molecular distinctiveness between PPV-D and M isolates in the NIb-CP region of the PPV genome enabled to develop a RT-PCR tool, specifically detecting both virus strains.

Moreover, this region spans the recombination hotspot identified in the natural recombinant PPV isolates (forming the strain PPV-Rec, **Glasa et al., 2004**), allowing also its specific detection (**Šubr et al., 2004**).

Using this method, we could specifically detect the isolates of all three strains (Fig. 3) in the leaves of infected GF305, fully confirming the results previously obtained from the sequence analysis. In addition, the presence of mixed infection was detected in two samples (no. 05 and 10). In both cases, PPV-M has been detected in mixed infection with PPV-D and Rec, respectively (Fig. 3). Although not quantified, the intensity of amplified products indicates that in both samples, the detected PPV-M represent only a minor viral population. This is supported also by the fact, that in both cases, only dominant PPV-D (sample no. 05) or PPV-Rec sequences (sample no. 10) have been revealed by the direct sequencing of the PCR products. This result shows that a single *Prunus* host can be infected by two or more PPV strains, as previously found in the field conditions (**Candresse et al., 1998**).

However, it has been observed that competitiveness between different strain isolates in a single tree might lead over time to a displacement of less aggressive ones leaving the tree infected by a single strain isolate (**Predajňa et al., 2012**).

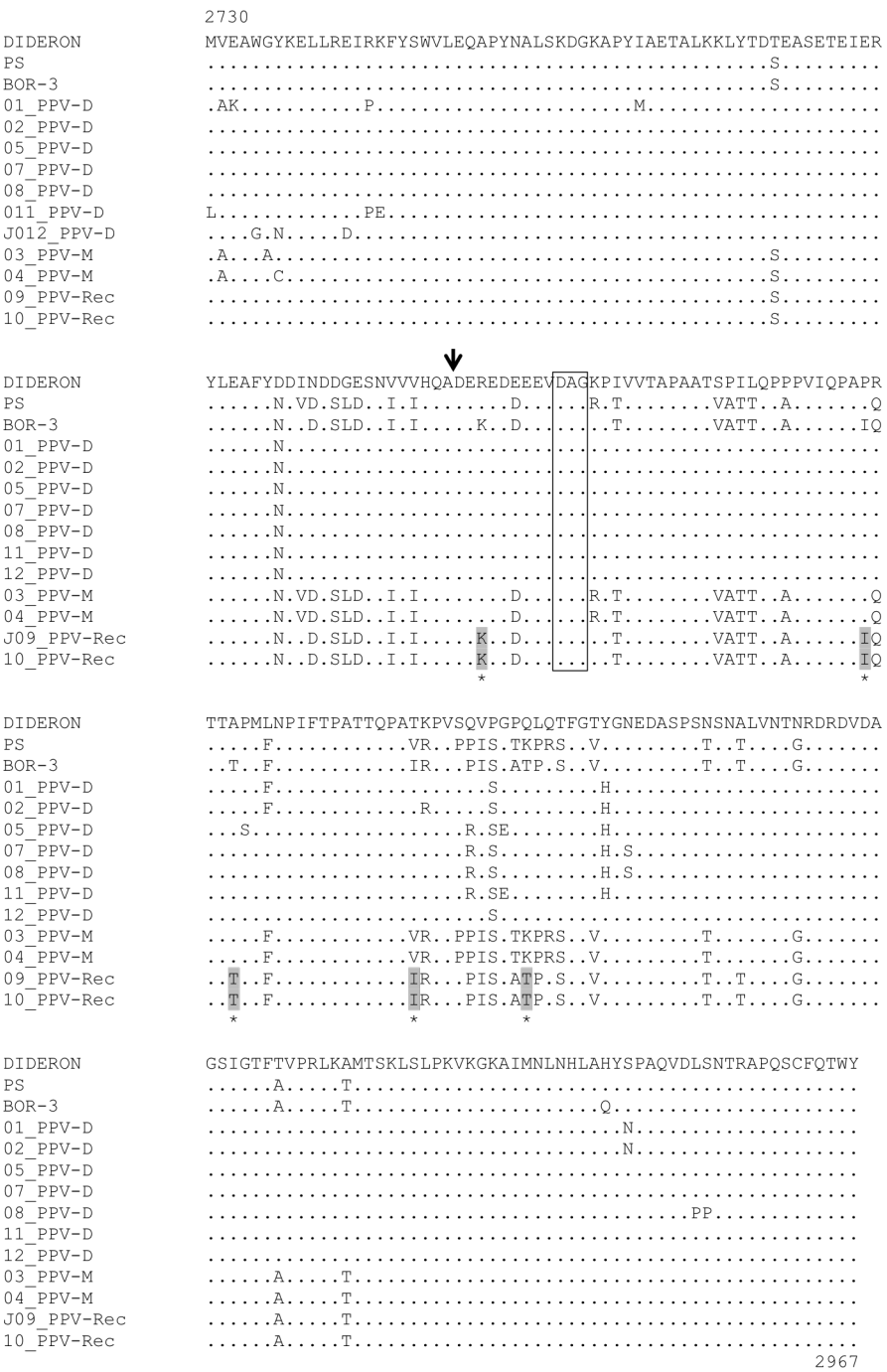

Figure 1 Multiple alignment of the deduced amino acid sequences of the C-terminal part of NIb and the N-terminal part of the capsid protein of PPV isolates analysed in this study (amino acid positions 2730-2967, numbered according to the BOR-3 polyprotein). The isolates PS (PPV-M, GenBank Acc. no. AJ243957), Dideron (PPV-D, X16415) and BOR-3 (PPV-Rec, AY028309) were included to the analysis. Identical amino acids to those of the Dideron isolate, used as the reference, are indicated by dots. The DAG motif associated with aphid transmission is boxed. The cleavage site between NIb and CP are indicated by an arrow. Specific amino acid positions in the CP N-terminus of PPV-Rec isolates (**Glasa et al., 2002**) are marked by asterisks and grey-shaded.

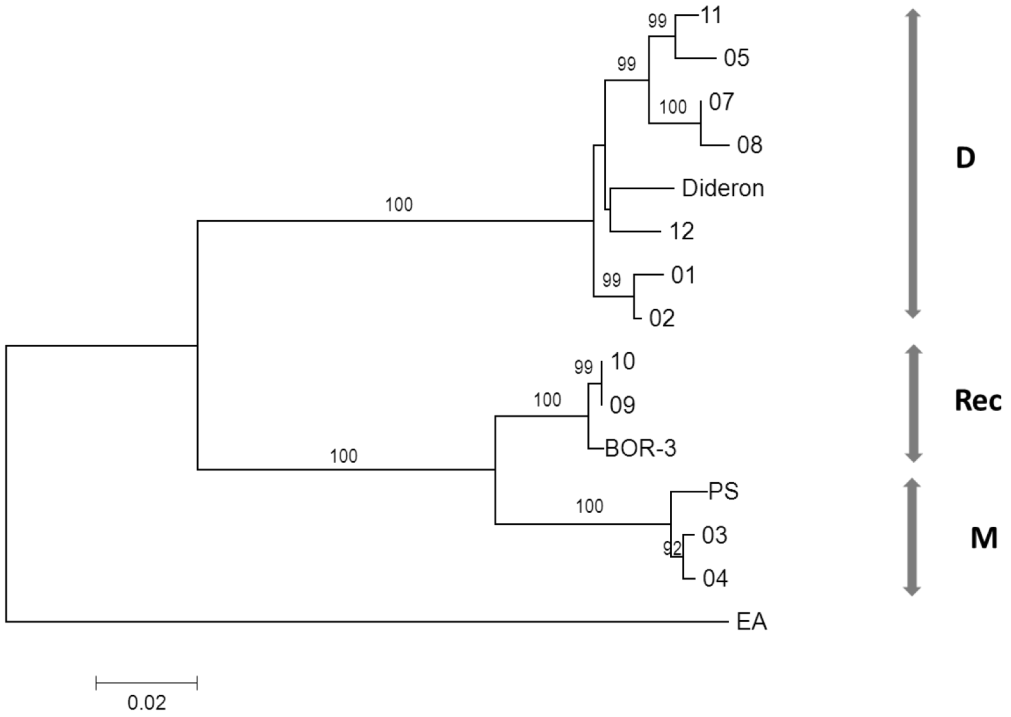

Figure 2 Phylogenetic tree of characterised PPV isolates and the representatives of the main PPV strains, generated from the NIb-CP sequences (nt 8332-9048, numbered according to the BOR-3 genome). Strain affiliation of the isolates is marked in the right margin. The divergent El Amar isolate (DQ431465) was used as an outgroup. The scale bar indicates a genetic distance of 0.02. Bootstrap values >70 (1000 bootstrap resamplings) are indicated as percentages on the branches. The tree was constructed by the neighbour-joining method, using the p-distance model.

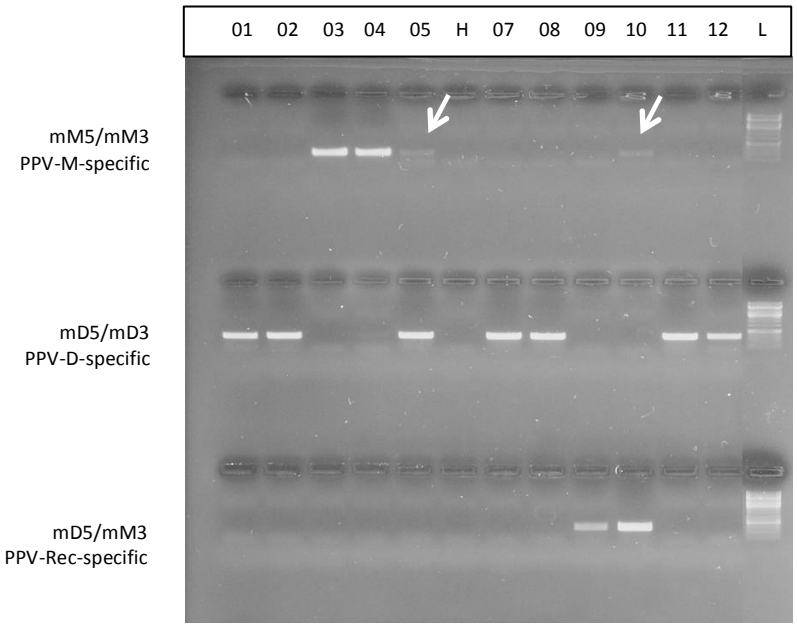

Figure 3 Agarose gel electrophoretic analysis of the strain-specific detection of PPV-M, D and Rec sequences in the NIb-CP genomic region according to **Subr et al. (2004).** Combination of primers and their specificity is indicated on the left. L = GeneRuler 1-kb DNA Ladder (Fermentas); H = healthy control; lanes 01 - 12 indicate the analysed Slovak isolates. Arrows highlight the presence of PPV-M in the samples 05 and 10.

CONCLUSION

The presence of PPV isolates belonging to the PPV-D, M and Rec strains was confirmed in Eastern Slovakia by their partial sequence analysis targeting the NIb-CP genomic region. The application of strain-specific RT-PCR has approuved the strain affiliation of isolates, and in addition, has permitted the detection of minor PPV variants in 2 samples, revealing the mixed PPV infection in these cases. The results further confirm the high incidence of PPV in the *Prunus* orchards and complement the global picture of virus diversity in Slovakia.

REFERENCES

Cambra, M., Capote, N., Myrta, A., Llacer, G. 2006. Plum pox virus and the estimated costs associated with Sharka disease. *EPPO Bull*etin. vol. 36, no. 2, p. 202-204. http://dx.doi.org/10.1111/j.1365-2338.2006.01027.x

Candresse, T., Cambra, M., Dallot, S., Lanneau, M., Asensio, M., Gorris, M. T., Revers, F., Macquaire, G., Olmos, A., Boscia, D., Quiot, J. B. 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.* vol. 88, no. 3, p. 198-204. http://dx.doi.org/10.1094/PHYTO.1998.88.3.198 PMid: 18944965

Desbiez, C., Moury, B., Lecoq, H. 2011. The hallmarks of "green" viruses: Do plant viruses evolve differently from the others? *Infection, Genet. and Evol.* vol. 11, no. 5, p. 812-824. http://dx.doi.org/10.1016/j.meegid.2011.02.020 PMid: 21382520

Garcia, J. A., Glasa, M., Cambra, M., Candresse, T. 2013. Plum pox virus and sharka: a model potyvirus and a major disease. *Molecular Plant Patho*logy, in press, http://dx.doi.org/10.1111/mpp.12083 PMid: 24102673

García, J. A. Cambra, M. 2007. Plum pox virus and sharka disease. *Plant Viruses* vol. 1, p. 69-79.

Glasa, M., Marie-Jeanne, V., Labonne, G., Šubr, Z., Kúdela, O., Quiot, J. B. 2002. A natural population of recombinant Plum pox virus is viable and competitive under field conditions. *Eur. J. Plant Pathol*. vol. 108, p. 843-853. http://dx.doi.org/10.1023/A:1021294221878

Glasa, M., Matisová, J., Kúdela, O. 1998. Characterization of Plum pox virus isolates from Slovakia. *Acta Virol*. Vol. 42, no. 4, p. 226-229. PMid: 10073223

Glasa M., Predajňa, L., Šubr, Z. W. 2010. Competitiveness of different plum pox virus isolates in experimental mixed infection reveals rather isolate- than strain-specific behaviour. *J. Plant Pathol.* vol. 92, p. 267-271. http://dx.doi.org/10.4454/jpp.v92i1.42

Glasa, M., Candresse, T. 2008. Plum Pox Virus. *Encyclopedia of Virology, Third Edition,* vol. 4, p. 238-242.

Glasa, M., Malinowski, T., Predajňa, L., Pupola, N., Dekena, D., Michalczuk, L., Candresse, T. 2011. Sequence variability, recombination analysis, and specific detection of the W strain of Plum pox virus. *Phytopathology*, vol. 101, no. 8, p. 980-985. http://dx.doi.org/10.1094/PHYTO-12-10-0334 PMid: 21425932

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*. vol. 41, p. 341-344. PMid: 9607094

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 (PPV) are genetically very similar and form a unique PPV subgroup. *J. Gen. Virol*. vol. 85, no. 9, p. 2671-2681. http://dx.doi.org/10.1099/vir.0.80206-0 PMid: 15302961

Glasa, M., Prikhodko, Y., Predajna, L., Nagyova, A., Shneyder, Y., Zhivaeva, T., Subr, Z., Cambra, M., Candresse, T. 2013. Characterization of sour cherry isolates of Plum pox virus from the Volga basin in Russia reveals a new cherry strain of the virus. *Phytopathology*. vol. 103, no. 9, p. 972-979. http://dx.doi.org/10.1094/PHYTO-11-12-0285-R PMid: 23581702

Jarošová, J., Polák, J., Kumar, J 2008. Molecular detection of stone fruits virus methodology by RT-PCR and multiplex - RT-PCR (in Czech). Praha: Výzkumný ústav rostlinné výroby, v.v.i., p. 26. ISBN 978-80-87011-86-7.

Jridi, C., Martin, J. F., Marie-Jeanne, V., Labonne, G., Blanc, S. 2006. Distinct viral populations differentiate and evolve independently in a single perennial host plant. *J. Virol.* vol. 80, no. 5, p. 2349-2357. http://dx.doi.org/10.1128/JVI.80.5.2349-2357.2006 PMid: 16474141

Králiková, K. 1964. Survey on the investigation of plum pox disease in Slovakia. *Plant Virology*: 5th Conference of Czechoslovak Plant Virologists, p. 346-351.

Labonne, G., Yvon, M., Quiot, J. B., Avinent, L., Llacer, G. 1995. Aphids as potential vectors of Plum pox virus: comparison of methods of testing and epidemiological consequensec. *Acta Hortic. vol.* 386, p. 207-218.

López-Moya, J. J., Fernandez-Fernandez, M. R., Cambra, M., García, J.A. 2000. Biotechnological aspects of Plum pox virus. *Journal of Biotechnology. vol.* 76, no. 2-3, p. 121-136. http://dx.doi.org/10.1016/S0168-1656(99)00196-0

Mavrodieva, V., James, D., Williams, K., Negi, S., Varga, A., Mock, R., Levy, L. 2013. Molecular analysis of a Plum pox virus W isolate in plum germplasm hand carried into the USA from the Ukraine shows a close relationship to a Latvian isolate. *Plant Disease.* vol. 97, no. 1, p. 44-52. http://dx.doi.org/10.1094/PDIS-01-12-0104-RE

Paulechová, K. 1981. Comparison of plum pox virus isolates from Czechoslovakia. *Biologia* vol. 36, p. 225-229.

Predajňa, L. 2013. *Molekulárna diverzita a epidemiológia vírusu šarky slivky (PPV)*. Disertation theses, PriFUK Bratislava, p. 140.

Predajňa, L., Šubr, Z., Candresse, T., Glasa, M. 2012. Evaluation of the genetic diversity of Plum pox virus in a single plum tree. *Virus Research*. vol. 167, no. 1, p. 112-117. http://dx.doi.org/10.1016/j.virusres.2012.04.002 PMid: 22521553

Šubr, Z., Glasa, M. 2013. Unfolding the secrets of plum pox virus: from epidemiology to genomics. *Acta Virol*. vol. 57, no. 2, p. 217-228. http://dx.doi.org/10.4149/av_2013_02_217 PMid: 23600878

Šubr, Z., Glasa, M. 1999. Plum pox virus capsid protein mobility in SDS-polyacrylamide gel electrophoresis. *Acta Virol*. vol. 43, no. 4, p. 259-262. PMid: 10749373

Šubr, Z., Pittnerová, S., Glasa, M. 2004. A simplified RT-PCR-based detection of recombinant Plum pox virus isolates. *Acta Virol*. vol. 48, no. 3, p. 173-176. PMid: 15595211

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., Kumar, S. 2011. MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology and Evol*ution. vol. 28, no. 10, p. 2731-2739. http://dx.doi.org/10.1093/molbev/msr121 PMid: 21546353

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