

THESIS OF PhD DISSERTATION

**CHARACTERIZATION OF DIFFERENT NATURAL
AND GENETICALLY ENGINEERED RECOMBINANT
*PLUM POX VIRUS (PPV) ISOLATES***

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1. INTRODUCTION AND THE AIMS OF THE RESEARCH

Economical production of stone fruits is affected by several factors all over the world, from which the most important elements are the ever-increasing plant diseases, especially viral infections causing serious losses in *Prunus* orchards.

Plum pox virus (PPV, sharka virus) is the most important viral pathogen of stone fruit trees in Europe and also in Hungary. Sharka disease, caused by PPV, was first described in Bulgaria around 1917–1918, since then through international trade of propagation material PPV has spread throughout Europe. Nowadays it is present nearly all over the world, and is considered the most devastating viral pathogen of plum, apricot and peach trees. PPV causes serious decrease in fruit quality and substantial yield losses. Infected fruits are not marketable and can not be used for industrial purposes because of its altered sugar-acid ratio. In the case of sensitive plum varieties the dropping of infected fruits can reach even 100%.

The most effective way for the protection against viral diseases is prevention. For prevention, it is crucial to safely detect virus isolates causing serious epidemics in the plant tissues, especially in the propagating materials is essential.

During the analysis of a large number of PPV isolates over the last 30 years three major (PPV-D, PPV-M, PPV-Rec) and three minor (PPV-EA, PPV-C, PPV-W) groups of isolates were identified, which differ in their pathogenicity, host range, aphid transmissibility and geographical distribution. Furthermore PPV isolates emerging after natural recombination and mutation events can possess altered biological, serological, molecular and epidemiological features, which may prevent reliable detection. Thus molecular and serological identification and characterization of these recombinant and/or mutant PPV isolates, as well as determination of the genetic variability of the viral genome is essential for designing effective sharka disease-control strategies and for accurate detection.

Regarding to the high economic importance of PPV and the requirement of safe detection of virus infection the aims of the research covered the followings:

1. Molecular characterization of PPV isolates originated from Bulgaria from plum, and from Hungary from apricot and plum. Determination of their genetic variability and identification of their specific isolate groups;
2. Determination of the phylogenetic relationships among the analysed Bulgarian and Hungarian PPV isolates, as well as their relationships to other PPV isolates;

3. Identification of location and stretch of the deletion located in the genome of a PPV isolate (PPV-B1298) emerging after natural mutation and bearing a truncated coat protein (CP) gene;
4. Analyses of pathogenicity, virions and aphid transmissibility of the truncated CP gene bearing virus;
5. Testing of immuno-detectability of the PPV-B1298 isolate, since accurate identification of virus infection is essential for prevention;
6. Determination of the effect of location and stretch of the deletion on the accumulation of the natural mutant virus, as well as on the structure of the CP.

2. MATERIALS AND METHODS

2.1. Location and duration

Researches were done at Agricultural Biotechnology Center of Gödöllő and Corvinus University of Budapest, Faculty of Horticultural Sciences, Department of Plant Pathology, between 2003 and 2008.

2.2. Materials

Leaves of different plum and apricot varieties showing typical symptoms of PPV infection were collected from different locations. Leaf samples were taken from four Hungarian locations: Vilyvitány, Sóskút, Gödöllő and the experimental apricot orchard of Corvinus University of Budapest, Department of Genetics and Plant Breeding in Szigetcsép, and from Bulgaria (Troyan): the experimental plum orchard of Research Institute of Mountain Stockbreeding and Agriculture (RIMSA).

2.3. Methods

2.3.1. Mechanical inoculation and total nucleic acid (TNA) extraction

Isolates were sap-transmitted to *Nicotiana benthamiana* Domin and *N. clevelandii* Gray. test plants. Total nucleic acids were extracted from systemically infected *N. benthamiana* leaves by the method of White and Kaper (1989) with a few modifications.

2.3.2. RT-PCR

First-strand complementary DNAs (cDNAs) were synthesized by reverse transcription (RT) of TNAs using the appropriate antisense primers, then cDNAs were amplified by polymerase chain reaction (PCR). Applied primer sequences are summarized in Table 1. Three different genomic regions were amplified by RT-PCR. The 3' part of the nuclear inclusion "b" protein (NIb) gene, the complete CP gene and the 3' untranslated region (3'UTR) (3'NIb-CP-3'UTR), the fragment that contained the 3' recombination point, were amplified using primers PolyT₂ and Poty7941. The 3' part of the third protein (P3), the complete first 6 kilodalton protein (6K₁) and the 5' cylindrical inclusion protein (CI) genes (3'P3-6K₁-5'CI) were amplified by the PCI/PP3 set of primers. For the analysis of the 5' recombination point amplification of the 3' part of the helper component protease (HC-Pro) and the 5' part of the P3 genes (3'HC-Pro-5'P3) were done by primers PPV-P3-RC and PPV-HC-RC.

Table 1. Names, nucleotide sequences and orientations of the primers used and their application fields

Names of primers	Sequences 5'–3'	Orientation	Application fields
PPV-P3-RC	CAAGGATCCTGCGATTCCAAGATTGCA	antisense	RT-PCR, sequencing
PPV-HC-RC	ATACCGCGGATCACTGTCAACTGGAATGC'	sense	RT-PCR, sequencing
PCI	TTGAGTCAAATGG(AG)ACAGTTGG	antisense	RT-PCR, sequencing
PP3	TTATCTCCAGGA(AG)TTGGAGC	sense	RT-PCR, sequencing
PolyT ₂	CGGGGATCCTCGAGAAGCTTTTTTTTTTTTTTTTTT	antisense	RT-PCR, sequencing
Poty7941	GGAATTCCCGCGG(AGCT)AA(CT)AA(CT)AG(CT)GG(AGCT)CA(AG)CC	sense	RT-PCR, sequencing
Unipoty	GGAATTCCCGCGGAAAAGCCCCGTACATTGC	sense	sequencing
PPV-CP5	CACTACACTCCCCTCACACCGAGG	antisense	sequencing
PPV-CP4	CTACATCAGATACAAGGGCCTGTG	antisense	sequencing

2.3.3. Restriction analysis of the PCR products

PCR products amplified from the 3'P3–6K₁–5'CI region were cleaved by such restriction endonucleases [DdeI (*Hpy*F3I), EcoRI], which recognized only cDNA fragments synthesized from genomic RNAs of PPV isolates that have D-type genome in this analysed region (members of PPV-D and PPV-Rec groups).

2.3.4. Cloning of PCR products and sequence analysis

PCR fragments in appropriate size were ligated into pBluescript II SK (+) (Stratagene) or pGEM-T Easy vectors (Promega), and the genetically modified (recombinant) plasmids were transformed into *Escherichia coli* DH5 α competent cells. Isolation of recombinant plasmids from bacterium cells were done by minipreparation based on alkaline lysis.

Nucleotide (nt) sequences of the cloned fragments were determined using ABI PRISM 3100 Genetic Analyzer automated DNS sequencer. Analyses and comparisons of the obtained nucleotide and deduced amino acid sequences were performed by the Wisconsin Package version 10.0 Genetic Computer Group (GCG) sequence analysis software (Devereux et al., 1984) and the EMBOSS-Align (Rice et al., 2000) program using the corresponding data of EMBL/NCBI/DDBJ databases. Multiple alignments were created by the CLUSTAL X program (Thompson et al., 1997) and the MEGA version 3.1 (Kumar et al., 2004). Phylogenetic analyses were conducted using the CLUSTAL X program. The phylogenetic trees were visualized using the program TREEVIEW version 1.6.6 (Page, 1996).

2.3.5. Construction of the B1298 truncated CP gene bearing cDNA clone and determination of pathogenicity

The cDNA fragment corresponding to the CP gene of the PPV-B1298 isolate was introduced into the pPPV-SK68 full-length infectious cDNA clone. The obtained clone (pPPV-SK68CPB1298) contained full-length PPV cDNA sequence under the control of the 35S promoter of the *Cauliflower mosaic virus* (CaMV), but in the CP gene it represents the PPV-B1298 isolate. *N. benthamiana* plants were mechanically inoculated with the chimeric, pPPV-SK68CPB1298 clone. Infectivity was determined by visual observation of systemic symptoms and by RT-PCR using primers PolyT₂ and Poty7941.

2.3.6. Analysis of the virions of the B1298 truncated CP gene bearing virus

Particle formation of the pPPV-SK68CPB1298 progeny and morphological analysis of their virions were investigated by electron microscopy using Zeiss EM910 transmission electron microscope with negative staining method. Virions of pPPV-SK68 progeny was also analysed as a control.

2.3.7. Aphid transmissibility of the B1298 truncated CP gene bearing virus

For aphid transmission test non-viruliferous *Myzus persicae* Sulz. aphids were used. After a 2-hour starvation period aphids were transferred to *N. benthamiana* plant systemically infected with the pPPV-SK68CPB1298 progeny. Aphids can feed on donor plant for 5 min, then 10 aphids were placed on young, virus-free *N. benthamiana* plants. Efficiency of transmission was determined by visual observation of systemic symptoms and by RT-PCR using primers PolyT₂ and Poty7941 after a 3-week incubation period.

2.3.8. Analysis of the structure of the B1298 truncated CP

The tertiary structure of the B1298 CP was predicted using the Plotstructure and Peptidestructure programs of Wisconsin Package version 10.0 GCG software.

2.3.9. Testing of immuno-detectability of the B1298 truncated CP gene bearing virus

Immuno-detectability of the PPV-B1298 isolate in comparison with PPV-SK68 (PPV-M group) and PPV-Pd4 (PPV-Rec group) was analysed by Western blot using different polyclonal and monoclonal antibodies from different commercial sources.

The following Kits were used for the analysis: Bioreba PPV ELISA Kit, DSMZ PPV ELISA Kit, Real Durviz PPV ELISA Kit, Sanofi Planttest PPV ELISA Kit.

2.3.10. Competition experiments

The advantage or disadvantage of the natural deletion in the CP gene of the PPV-B1298 isolate was evaluated in competition experiments, in which virus accumulation was analysed. *N. benthamiana* test plants were mechanically inoculated together with the B1298 truncated CP gene bearing, chimeric virus and the full-length CP gene containing, wild-type virus. Two weeks post-inoculation viruses from mixed inoculated *N. benthamiana* plant were sap-transmitted to healthy *N. benthamiana*. After this first passage, five more successive passages were performed at 2-week intervals. The accumulation of the wild-type and the chimeric viruses were tested at time of all passages by Western blot using the Real Durviz 5B PPV-specific monoclonal antibody.

3. RESULTS

3.1. Molecular characterization of Bulgarian and Hungarian PPV isolates

Nine PPV isolates, 5 Bulgarian from plum (PPV-Troy1, PPV-Troy2, PPV-Troy4, PPV-Troy5, PPV-Troy6), 3 Hungarian from apricot (PPV-Sóskút1, PPV-Gödöllő2, PPV-Szigetcsép1) and 1 Hungarian from plum (PPV-B1298), were characterized during the research at levels of nucleotides and amino acids.

The cDNA fragment corresponding to the 3'NIb-CP-3'UTR genomic region was amplified and investigated in the case of all 9 isolates. The DAG motif, which is essential for aphid transmission, was found in all sequences in the N-terminal region of the deduced CP. CPs of 7 isolates (PPV-Troy1, PPV-Troy2, PPV-Troy4, PPV-Troy5, PPV-Troy6, PPV-Sóskút1, PPV-Szigetcsép1) were identical in size (330-amino acid long), while in the case of 2 Hungarian isolates (PPV-B1298, PPV-Gödöllő2) the CP proved to be shorter. In the case of the PPV-B1298 isolated from plum and the PPV-Gödöllő2 isolated from apricot in frame deletions were detected in the CP genes during the sequence analysis of the RT-PCR amplified cDNA fragments corresponding to this region. The PPV-B1298 and the PPV-Gödöllő2 isolates bearing a 135- and a 33-nucleotide deletion, respectively, which correspond to a 45-, and an 11-amino acid deletion in the N-terminal region of the CP.

On the bases of nucleotide and deduced amino acid sequence similarities and phylogenetic analyses generated using sequences of the RT-PCR amplified cDNA fragments corresponding to CP gene (8577–9566. nt position) 6 isolates (PPV-Troy1, PPV-Troy4, PPV-Troy5, PPV-Troy6, PPV-B1298, PPV-Gödöllő2) out of the 9 analysed belonged to the PPV-Rec group, while the remaining 3 (PPV-Troy2, PPV-Sóskút1, PPV-Szigetcsép1) could be classified as members of the PPV-D group. To confirm recombination event phylogenetic analysis was performed using nucleotide sequence data corresponding to the 3'NIb-5'CP region (8050–8902. nt position), and nucleotide sequences located upstream (8050–8449. nt position) and downstream (8450–8902. nt position) to the 3'recombination point previously identified in the genomes of recombinant PPV isolates were also analysed separately. Analysing the sequences corresponding to the 8050–8449. or the 8450–8902. regions separately PPV-Troy1, PPV-Troy4, PPV-Troy5, PPV-Troy6, PPV-B1298 and PPV-Gödöllő2 isolates – as expected –clustered with PPV-D or PPV-M isolates, respectively, similar to other PPV-Rec isolates. According to the phylogenetic analysis performed using the latter region the PPV-B1298 and the PPV-Gödöllő2 clustered away from the analysed recombinant isolates. PPV-Troy2, PPV-Sóskút1 and PPV-Szigetcsép1 isolates on the bases of the nucleotide sequences corresponding to both of the two analysed regions clustered with the PPV-D isolates. Although the PPV-Sóskút1 and the PPV-Szigetcsép1 according to nucleotide sequences of the

region located upstream to the 3' recombination point clustered on the sidebranch of PPV-Rec isolates, in an intermediate position between the recombinant and the typical PPV-D type isolates.

On the phylogenetic tree constructed using the nucleotide sequence data corresponding to the 3'Nib-5'CP (8466-8795. nt position) region of analysed isolates proved to be recombinants and all PPV-Rec isolates available in the databases the PPV-Troy6 Bulgarian recombinant isolate – although with low bootstrap value – clustered away from the other Bulgarian recombinants.

The 3'P3-6K₁-5'CI (2976-3696. nt position) region was also amplified by RT-PCR in the case of 8 analysed isolates (PPV-Troy1, PPV-Troy2, PPV-Troy4, PPV-Troy5, PPV-Troy6, PPV-Sóskút1, PPV-Gödöllő2, PPV-Szigetcsép1) in order to determine the type of this region. PCR products were subjected to restriction analysis using such endonucleases (*EcoRI* and *DdeI*, respectively) that cleave cDNA fragments amplified from isolates having D-type genome in the analysed region (PPV-D and PPV-Rec isolates), while not recognize cDNAs derived from isolates having M-type genome in this region (PPV-M isolates). RFLP analyses using D-type sequence-specific enzymes resulted in an atypical restriction pattern in the case of PPV-Troy6 and PPV-Sóskút1 isolates. The PCR fragment amplified in the case of the PPV-Troy2 could be cleaved only by *EcoRI*, but not by *DdeI*, while the PCR product obtained in the case of the PPV-Sóskút1 could be recognized only by *DdeI*, but not by *EcoRI*, although these isolates were determined as PPV-Rec and PPV-D type according to the 3'Nib-CP region, respectively. Such unusual typing behaviour could arise from point mutations affecting the recognition sites but may also indicate possible recombination events between PPV-D and PPV-M. In order to clarify these points the PCR products were sequenced and the exact positions of *DdeI* and *EcoRI* sites were mapped. Sequence analysis revealed that despite lacking of those restriction sites both the PPV-Troy6 and the PPV-Sóskút1 isolates have D-type genome in the 3'P3-6K₁-5'CI region. Lack of the recognition sites resulted from point mutations in the cleavage sites as compared to D-type sequences, and these unusual restriction properties are not consequences of recombination events targeted this region. In the sequence of the PPV-Troy6 the fourth nucleotide of the *DdeI* site (A₃₁₀₂C, CTNAG>CTNCG), while in the case of the PPV-Sóskút1 the sixth base of the *EcoRI* site (C₃₄₁₀T, GAATTC>GAATTT) have been changed.

For further investigation of the PPV-Troy6 Bulgarian isolate its 3'HC-Pro-5'P3 (2387-2907. nt position) region was also amplified by RT-PCR, that contained the recombination point located at the 5' part of the genome. The phylogenetic tree reconstructed from the nucleotide data of the analysed region showed the clustering of the PPV-Troy6 isolate with PPV-Rec isolates available from the databases.

3.2. Analysis of the B1298 truncated CP gene bearing virus

For more detailed characterization of the PPV-B1298 isolate and to prove that the PCR product corresponding to the B1298 CP gene was not a PCR artifact, but it is shorter indeed, the cDNA fragment derived from the CP gene of the PPV-B1298 isolate was cloned into the pPPV-SK68 infectious clone. The integration of the truncated CP gene had no effect on infectivity and symptomatology. Symptoms appeared on *N. benthamiana* test plants infected with the progeny virus of the pPPV-SK68CPB1298 clone or the PPV-B1298 original isolate were as intense as those which developed on indicator plants infected with the progeny virus of the control pPPV-SK68 clone.

The deletion has no effect on particle formation. The truncated CP gene bearing chimeric virus formed virus particles morphologically similar to those of other PPV isolates, but the average diameter of the virions was smaller than of those produced by the progeny virus of the pPPV-SK68 clone bearing complete CP gene.

Due to the natural deletion, the CP of the PPV-B1298 isolate had been shortened by 13.64%, while focusing solely on the N-terminal region, the reduction was 48.91%. This deletion did not cause structural changes in the CP.

The B1298 CP gene bearing virus was experimentally aphid-transmissible to *N. benthamiana* plants. 3 weeks post-inoculation systemic mosaic symptoms – which are typical features of PPV infection – appeared on *N. benthamiana* acceptor plants, which proved to be infected with the virus by the RT-PCR analysis as well.

Immuno-detectability of the truncated CP gene bearing virus varied according to the PPV CP-specific antibody used, available from commercial sources. The Real Durviz PPV M-specific monoclonal antibody, called AL, failed to react with the CP of the PPV-B1298 isolate, and the Sanofi PPV M + D-specific, AP-conjugated monoclonal antibody was neither sensitive enough at detecting the PPV-B1298 recombinant isolate. In contrast, the general PPV-specific monoclonal antibody, called 5B, gave similar high sensitivity with the deletion recombinant PPV-B1298 as with the control, non-deletion viruses.

The results of the competition experiments showed that this natural deletion beneficial for the virus. The truncated CP gene bearing virus has detectable advantage in virus accumulation over the wild-type PPV, without any changes in symptom development on *N. benthamiana*.

3.3. New scientific results

1. Partial molecular characterization of 9 (5 Bulgarian and 4 Hungarian) PPV isolates, determination of their specific isolate groups and their phylogenetic relationships have been done. Obtained sequences have been deposited into the international database.
2. PPV-Rec and PPV-D type isolates having point mutations in the conserved *DdeI* and *EcoRI* restriction sites in the RT-PCR amplified part of the 3'P3–6K₁–5'CI genomic region have been identified firstly. Obtained data indicate some limitation of *DdeI* and *EcoRI* restriction enzyme mapping of the PCR product corresponding to the 3'P3–6K₁–5'CI region for accurate classification of PPV isolates, although restriction analyses of the 3'P3–6K₁–5'CI PCR fragments using *DdeI* and *EcoRI* enzymes is widely used for typing of PPV isolates.
3. Two PPV isolates (PPV-B1298 and PPV-Gödöllő2) bearing natural deletion in the CP gene have been identified, and the exact location and stretch of the deletions have been determined. The PPV-B1298 isolate had a 45-amino acid deletion, the PPV-Gödöllő2 an 11-amino acid deletion in the N-terminal part of the CP. The PPV-B1298 isolate is the largest deletion bearing isolate among the known natural CP deletion mutant PPV isolates.
4. An infectious PPV cDNA clone containing the truncated B1298 CP gene has been constructed.
5. Pathogenicity and aphid transmissibility of the truncated CP gene bearing virus, as well as the diameter of its virions and the tertiary structure of the mutant CP have been determined. The deletion neither affected pathogenicity or aphid transmissibility of the mutant virus in *N. benthamiana* plants, nor did it cause structural changes in the CP, but diameter of the virions was reduced compared to particles of the wild type virus bearing complete CP gene.
6. Immuno-detectability of the shorter CP gene carrying virus has been determined. The PPV-B1298 isolate was recognized with different efficiency by the antibodies tested. Not all antibodies could detect the mutant virus.
7. Effect of the natural deletion occurred in the CP gene of the PPV-B1298 isolate on virus accumulation has been ascertained. The truncated CP gene bearing virus has detectable advantage in virus accumulation over the wild-type CP expressing virus on *N. benthamiana* plants.

4. DISCUSSION AND CONCLUSION

4.1. Molecular characterization of Bulgarian and Hungarian PPV isolates

On the bases of nucleotide and deduced amino acid sequence similarities and phylogenetic analyses generated using sequences of the RT-PCR amplified cDNA fragments corresponding to genomic regions under focus (3'N1b-CP, 3'P3-6K₁-5'CI, 3'HC-Pro-5'P3), as well as RFLP analyses of the PCR fragments derived from the 3'P3-6K₁-5'CI region 6 isolates out of the 9 (PPV-Troy1, PPV-Troy4, PPV-Troy5, PPV-Troy6, PPV-B1298, PPV-Gödöllő2), two third of the analysed isolates, belonged to the PPV-Rec group, while the remaining three (PPV-Troy2, PPV-Sóskút1, PPV-Szigetcsép1) could be classified as members of the PPV-D group. All of the isolates identified as recombinants possessed recombination breakpoint at the 3' end of the PPV genome. In the case of the PPV-Troy6 isolate, of which nucleotide and deduced amino acid sequences derived from the 3'P3-6K₁-5'CI region were also analysed, a recombination event had also occurred at the 5' end of the genome. Despite the fact that a relatively small number of isolates were tested our results support the previous observation that recombinant PPV isolates are represent large fraction of the PPV populations in Europe (Glasa et al., 2004).

In the case of two Hungarian PPV-Rec isolates (PPV-B1298, PPV-Gödöllő2) in frame deletions were detected in the CP gene during the sequence analyses of the RT-PCR amplified cDNA fragments corresponding to this region. There were only three known examples for natural CP deletion mutant PPV isolates to date (PPV-NAT, PPV-SH, PPV-KAZ) (Maiss et al., 1989; Deborré et al., 1995; Spiegel et al., 2004). In all three cases, deletions were located in the N-terminal, hypervariable region of the CP similar to PPV-B1298 and PPV-Gödöllő2, but were much smaller than that of the PPV-B1298 isolate. It is known that deletions in the CPs of PPV-NAT and PPV-SH isolates affect DAG amino acid motif, which is involved in aphid transmissibility, while DAG motif in the CPs of PPV-B1298 and PPV-Gödöllő2 isolates are not affected by the deletions.

Restrictotyping of the 3'P3-6K₁-5'CI region of the analysed PPV isolates using *EcoRI* and *DdeI* D-type sequence-specific restriction enzymes resulted an atypical restriction pattern in the case of the recombinant PPV-Troy6 and PPV-D type PPV-Sóskút1 isolates. PCR fragments could be cleaved only by one of the two enzymes used. Obtained data indicate some limitation of restriction enzyme mapping for accurate classification of PPV isolates and support the recommendation of multiple approaches used for proper identification. Point mutations are common among PPV isolates, but this is the first report on such PPV-Rec or PPV-D isolates that possess point mutation in the conserved *DdeI* or *EcoRI* restriction sites of the 3'P3-6K₁-5'CI region.

4.2. Analysis of the B1298 truncated CP gene bearing virus

Deletions observed in the genome of PPV-B1298 and PPV-Gödöllő2 isolates were located in the highly immunogenic N-terminal region of the CP. CP is a multifunctional protein. It is involved in a number of processes during the virus life-cycle. The main function of the CP is to encapsidate the viral RNA. Deletion of nearly the one half of the N-terminal region of the B1298 CP did not affect particle formation in virus infected *N. benthamiana* plants. Our result is in good correlation with those obtained from deletion mutation analyses of *Tobacco etch virus* (TEV), that the N-terminal domain of the CP is not required for encapsidation or assembly of the CP subunits (Dolja et al., 1994).

The exact role of the N-terminal CP domain in viral long-distance movement is still unclear. Mutation and deletion studies made on distinct potyviruses gave contradicting results. Although almost the half of the B1298 CP N-terminal region was naturally deleted, the virus was able to move systemically *in vivo*, suggesting either that (i) sequence motifs required for such function located on other parts of the PPV CP N-terminal domain or (ii) not the amino acid sequence (original sequence) is essential but other features, such as CP N-terminal net charge can be important for long-distance transport of PPV as it have been concluded for the ZYMV (Kimalov et al., 2004).

Different forms of ELISA technique are widely used in almost all quarantine laboratories worldwide for detection of plant viruses since decades. ELISA based on the detection of the viral CP, using antibodies produced usually against the N-terminal region. Thus a deletion affecting the N-terminal domain should have a significant role in virus detection. Immuno-detectability of the shorter CP gene carrying virus varied according to the PPV CP-specific antibody used. A PPV M-specific monoclonal antibody (AL) failed to recognize the PPV-B1298 isolate. The PPV-B1298 isolate is a recombinant isolate, and it is known that CPs of PPV-Rec isolates correspond to PPV-M due to the recombination point is located upstream to the CP gene, in the 3' part of the N1b gene (Glasa et al., 2004). The recombination causes amino acid changes in the sequence of the CP, however, the failure of this antibody in detection of PPV-B1298 is not attribute to these changes since among the antibodies used AL had the highest sensibility against the control recombinant PPV-Pd4 isolate, and regarding all types of isolates tested this antibody was the most efficient in detection of the recombinant type. These results suggested that the detectable epitope is located in the deleted region. Thus occurrence of deletions in the highly immunogenic regions could result in failure of detection in spite of using well-characterized and widely used serological diagnostic reagents for plant virus identification, which could be dangerous with regard to safe detection of PPV infection.

The shorter B1298 CP gene bearing virus has detectable advantage in virus accumulation over the wild-type, full length CP expressing PPV on *N. benthamiana*. If the accumulation rates are similar in stone fruit trees in the nature, this type of PPV variant could become a dominant isolate of the virus populations in the near future.

5. LITERATURE CITED

- Deborré, G., Jelkman[n], W., Maiss, E. (1995): Biological and molecular biological investigations of several plum pox virus (PPV) isolates. *Acta Horticulturae*, 386: 253–262. p.
- Devereux, J., Haeberli, P., Smithies, O. (1984): A comprehensive set of sequence analysis program for the VAX. *Nucleic Acids Research*, 12 (1/1): 387–395. p.
- Dolja, V. V., Haldeman, R., Robertson, N. L., Dougherty, W. G., Carrington, J. C. (1994): Distinct functions of capsid protein in assembly and movement of tobacco etch potyvirus in plants. *EMBO Journal*, 13 (6): 1482–1491. p.
- 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. *Journal of General Virology*, 85 (9): 2671–2681. p.
- Kimalov, B., Gal-On, A., Stav, R., Belausov, E., Arazi, T. (2004): Maintenance of coat protein N-terminal net charge and not primary sequence is essential for zucchini yellow mosaic virus systemic infectivity. *Journal of General Virology*, 85 (11): 3421–3430. p.
- Kumar, S., Tamura, K., Nei, M. (2004): MEGA3: Integrated software for Molecular Evolutionary Genetics Analysis and sequence alignment. *Briefings in Bioinformatics*, 5 (2): 150–163. p.
- Maiss, E., Timpe, U., Brisske, A., Jelkmann, W., Casper, R., Himmler, G., Mattanovich, D., Katinger, H. W. D. (1989): The complete nucleotide sequence of plum pox virus RNA. *Journal of General Virology*, 70 (3): 513–524. p.
- Page, R. D. M. (1996): TreeView: An application to display phylogenetic trees on personal computers. *Computer Applications in the Biosciences*, 12 (4): 357–358. p.
- Rice, P., Longden, I., Bleasby, A. (2000): EMBOSS: The European Molecular Open Software Suite. *Trends in Genetics*, 16 (6): 276–277. p.
- Spiegel, S., Kovalenko, E. M., Varga, A., James, D. (2004): Detection and partial molecular characterization of two *Plum pox virus* isolates from plum and wild apricot in Southeast Kazakhstan. *Plant Disease*, 88 (9): 973–979. p.
- Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F., Higgins, D. G. (1997): The Clustal_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research*, 25 (24): 4876–4882. p.
- White, J. L., Kaper, J. M. (1989): A simple method for detection of viral satellite RNAs in small tissue samples. *Journal of Virological Methods*, 23 (2): 83–93. p.

6. PUBLICATIONS OF THE AUTHOR IN THE TOPIC OF THE THESIS

1. ARTICLES IN JOURNALS

Journals with IF

Szathmáry, E., Novák Nádudvari, J., Szabó, L., Tóbiás, I., Balázs, E., Palkovics, L. (2009): Characterization of a natural Plum pox virus isolate bearing truncated coat protein. *Archives of Virology*, 154: 141–145. p. IF:1,839 (2007).

Szathmáry, E., Tóbiás, I., Dragoyski, K., Palkovics, L. (2009): Partial molecular characterization of an unusual, recombinant Plum pox virus isolate from Bulgaria. *Acta Virologica*, 53 (1): 65–67. p. IF: 0,56 (2007).

Journals without IF

Szathmáry, E., Dragoyski, K., Hristova, D., Stoev, A., Tóbiás, I., Palkovics, L. (2006): Molecular characterization of some *Plum pox virus* isolates originated from Troyan region, Bulgaria. *Plant Science*, 43 (5): 407–412. p.

Szathmáry E., Tóbiás I., Palkovics L. (2007): Bulgáriai szilva himlő vírus (*Plum pox virus*) izolátumok jellemzése. *Növényvédelem*, 43 (12): 583–589. p.

Other papers in journals

Palkovics L., **Szathmáry E.** (2006): Szilva himlő vírus – a csonthéjasok legfontosabb víruseredetű kórokozója. *Agroinform*, 15 (10): 16. p.

2. CONFERENCE PAPERS

Hungarian conferences (abstracts)

Szathmáry E., Tóbiás I., Palkovics L. (2004): Egy rövidebb köpenyfehérjét tartalmazó természetes szilvahimlő vírus (*Plum pox virus*) izolátum jellemzése. A Magyar Mikrobiológiai Társaság 2004. évi Nagygyűlése és a X. Fermentációs Kollokvium, 2004. október 7-9., Keszthely, Előadás kivonatok, 115–116. p.

Szathmáry E., Nádudvariné Novák J., Tóbiás I., Palkovics L. (2005): Egy rekombináns és köpenyfehérjében deléciót hordozó szilva himlő vírus izolátum jellemzése. 51. Növényvédelmi Tudományos Napok, 2005. február 22-23., Budapest, Növényvédelmi Tudományos Napok 2005, 36. p.

Szathmáry E., Tóbiás I., Palkovics L. (2007): Egy különös, Bulgáriából származó rekombináns szilva himlő vírus (*Plum pox virus*) izolátum jellemzése. 53. Növényvédelmi Tudományos Napok, 2007. február 20-21., Budapest, Növényvédelmi Tudományos Napok 2007, 27. p.

Szathmáry E., Palkovics L. (2009): Magyarországi, kajsziról származó szilva himlő vírus (*Plum pox virus*) izolátumok jellemzése. 55. Növényvédelmi Tudományos Napok, 2009. február 23-24., Budapest, Növényvédelmi Tudományos Napok 2009, 34. p.

International conferences (full papers)

Szathmáry, E., Tóbiás, I., Palkovics, L. (2006): Analysis of some recombinant *Plum pox virus* (PPV) isolates from Bulgaria, the country where PPV was first recorded. 4th International Plant Protection Symposium at Debrecen University (11th Trans-Tisza Plant Protection Forum), Recent developments of IPM, 18-19 October, 2006, Debrecen, Hungary, Proceedings, 70–77. p.

International conferences (abstracts)

Palkovics, L., Novák Nádudvari, J., **Szathmáry, E.**, Tóbiás, I., Balázs, E. (2004): Characterization of an altered isolate of *Plum pox virus* containing shorter coat protein gene. European Meeting '04 on Plum Pox, 1-4 September, 2004, Rogów-Skierniewice, Poland, Programme, Book of Abstracts and List of Participants, 20. p.

Palkovics, L., Nádudvari Novák, J., **Szathmáry, E.**, Balázs, E. (2004): *Plum pox virus*, the devastating disease agent in the European stone fruit orchards. 6th Annual Meeting of Research Centre for Plant Growth and Development, 18-19 November, 2004, University of KwaZulu-Natal, Pietermaritzburg, South Africa, 39–40. p.

Palkovics, L., Novák Nádudvari, J., Szabó, L., **Szathmáry, E.**, Tóbiás, I., Balázs, E. (2005): Characterization of a *Plum pox virus* isolate with truncated coat protein. Joint Meeting of the 3 Divisions of the International Union of Microbiological Societies 2005, XIII International Congress of Virology, 23-27 July, 2005, San Francisco, California, USA, Abstracts of the International Congress of Virology, V-486.

7. OTHER PUBLICATIONS OF THE AUTHOR

1. ARTICLES IN JOURNALS

Journals without IF

Karsa, D., Haltrich, A., **Szathmáry, E.** (2004): Two new species in the Hungarian fauna: *Stomaphis mordvilkoii* Hille Ris Lambers and *Stomaphis juglandis* Petrovič (Lachnidae, Homoptera). *Acta Phytopathologica et Entomologica Hungarica*, 39 (4): 405–407. p.

Szathmáry, E., Haltrich, A., Tartally, A. (2005): Data to the knowledge of the lachnid fauna (*Homoptera: Lachnidae*) of Hungary. *Acta Phytopathologica et Entomologica Hungarica*, 40 (3-4): 403–408. p.

Szathmáry E., Salamon P., Palkovics L. (2008): Egy hazai tarlórépa mozaik vírus (Turnip mosaic virus, TuMV) izolátum molekuláris jellemzése. *Növényvédelem*, 44 (11): 553–557. p.

2. CONFERENCE PAPERS

Hungarian conferences (full papers)

Szathmáry E. (2003): A kéregtetvek (*Lachnidae, Homoptera*) hazai kutatottsága és a jövő tennivalói. Magyar Biológiai Társaság, III. Kárpát-medencei Biológiai Szimpózium, 2003. október 28-30., Budapest, Előadások összefoglalói, 167–171. p.

Hungarian conferences (abstracts)

Szathmáry E. (2003): Magyarországi kéregtetvek (*Homoptera, Lachnidae*) elterjedésének és populációdinamikájának vizsgálata. 49. Növényvédelmi Tudományos Napok, 2003. február 25-26., Budapest, Növényvédelmi Tudományos Napok 2003, 74. p.

Szathmáry E., Haltrich A. (2003): A magyarországi fásszárú növényeken élő kéregtetvek (*Lachnidae, Homoptera*) revíziója. [Revision of Lachnidae species (*Lachnidae, Homoptera*) feeding on woody plants in Hungary.] Lippay János-Ormos Imre-Vas Károly Tudományos Ülésszak, 2003. november 6-7., Budapest, Összefoglalók kertészettudomány, 452–453. p.

Salamon P., **Szathmáry E.**, Paksi A. M., Palkovics L. (2008): Potyvirus járvány görögdinnyén (*Citrullus lanatus*), sárgadinnyén (*Cucumis melon*) és kiwanon (*Cucumis metuliferus*): A *Watermelon mosaic virus* (WMV) újra előtérben? 54. Növényvédelmi Tudományos Napok, 2008. február 27-28., Budapest, Növényvédelmi Tudományos Napok 2008, 44. p.

International conferences (abstracts)

Szathmáry, E., Tóbiás, I., Palkovics, L., (2008): Only one amino acid change responsible for an interspecific hybrid potyvirus viability. Meetings of the Three Divisions of the International Union of Microbiological Societies 2008, XIV International Congress of Virology, 10-15 August, 2008, Istanbul, Turkey, Abstract Book, VP-321.

Szathmáry, E., Salamon, P., Paksy A. M., Palkovics, L., (2008): Characterization of some *Watermelon mosaic virus* (WMV) isolates originated from Hungary. 3rd Conference of the International Working Group on Legume and Vegetable Viruses (IWGLVV), 20-23 August, 2008, Ljubjana, Slovenia, Book of Abstract, 69. p.