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LETTER TO THE EDITOR

PARTIAL MOLECULAR CHARACTERIZATION OF AN UNUSUAL, RECOMBINANT PLUM POX VIRUS ISOLATE FROM BULGARIA

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Plum pox virus (PPV; the genus *Potyvirus*, the family *Potyviridae*), the causal agent of sharka disease, has been classified into six groups of isolates PPV-M, PPV-D, PPV-Rec, PPV-EA, PPV-C, and PPV-W according to their serological and molecular features (*1–3*). The PPV-Rec group contains viral isolates that emerged from a natural recombination between PPV-D and PPV-M isolates with a recombination breakpoint located in the NIb gene. Later on another recombination point was detected in P3 gene (*2*).

It has been demonstrated that there is a strong correlation among the different techniques used to classify the isolates belonging to PPV-M and PPV-D groups (*1, 4–8*). However, the serological and molecular typing of some PPV isolates gave inconsistent results, when CP protein or gene was examined (*1, 9*).

Present study reported on a PPV isolate (PPV-Troy6) that contained an atypical restriction typing characteristic in genomic region corresponding to the 3'-terminal (Cter) part of the P3, the complete 6K1 and the 5'-terminal (Nter) part of the cylindrical inclusion (CI) genes [P3-6K1].

The isolate PPV-Troy6 was collected from a naturally infected plum tree (*Prunus domestica* L. cv. Hanita) in Troyan (Bulgaria) in 2004 and was sap-transmitted to *Nicotiana benthamiana* DOMIN plants. Partial molecular characterization was done by RT-PCR for amplification of (i) Cter part of the NIb gene, the complete coat protein (CP) gene, and the 3' untranslated region (3'UTR) [(Cter)NIb-CP-3'UTR] using primers PolyT and Poty7941 (*10*); (ii) the P3-6K1 region using the PCI/PP3 set of primers (*8*); (iii) the Cter part of the helper component protease (HC-Pro) and the Nter part of the P3 genes [(Cter)HC-Pro-(Nter)P3] using primers P3-RC and HC-RC (*2*). PCR products were purified and were sequenced either directly (PCI/PP3 and P3-RC/HC-RC amplicons) or after cloning to pBluescript SK$^+$ (Stratagene) (PolyT/Poty7941 amplicon) (Acc. Nos. AM933761, AM260937).

Obtained nucleotide (nt) and deduced amino acid (aa) sequences were compared to other PPV sequences available in GenBank database overlapping the genomic region examined using ClustalW program implemented in the MEGA 3.1 software (*11*). Visual analysis of the nt sequence alignment of the (Cter)NIb-(Nter)CP region of the PPV-Troy6 suggested

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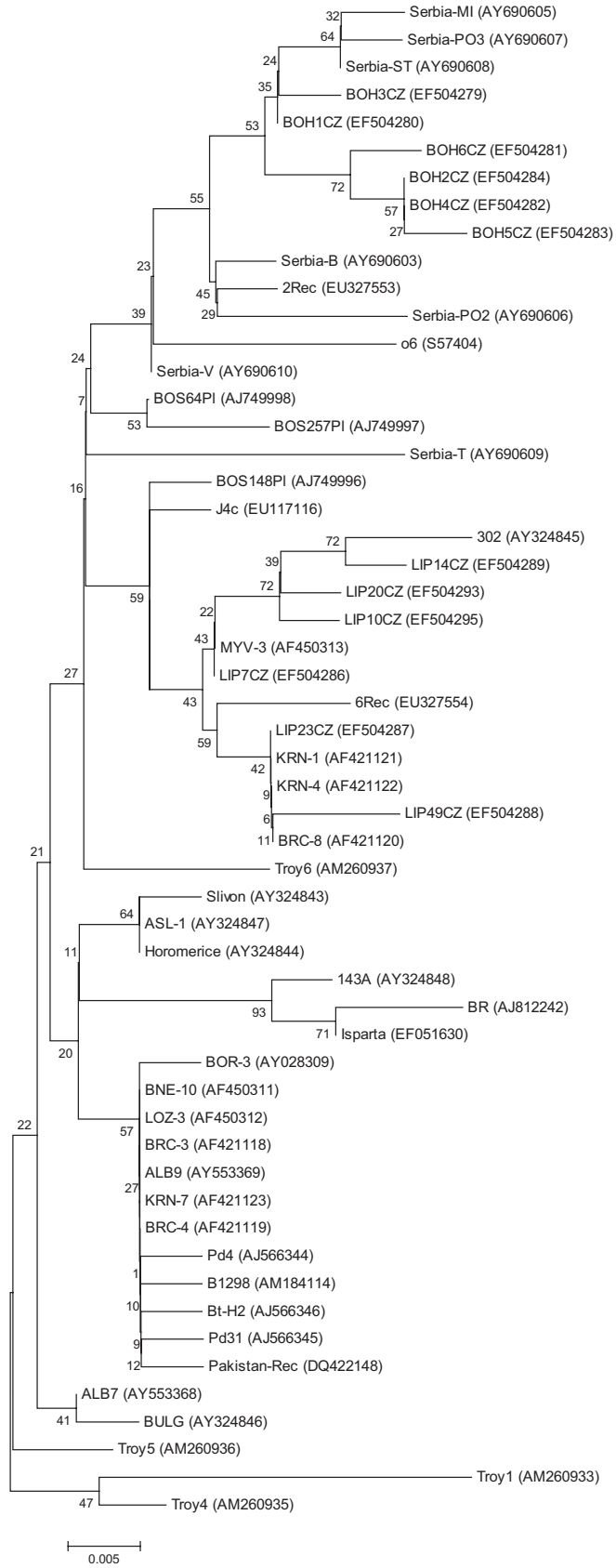
Abbreviations: CI = cylindrical inclusion; CP = coat protein; Cter = 3'-terminal; (Cter)HC-Pro-(Nter)P3 = Cter part of the HC-Pro and the Nter part of the P3 genes; (Cter)NIb-CP-3'UTR = Cter part of the NIb gene, the complete CP gene and the 3'UTR; HC-Pro = helper component protease; Nter = 5'-terminal; P3-6K1 = Cter part of the P3, the complete 6K1 and the Nter part of the CI genes; PPV = Plum pox virus; 3'UTR = 3' untranslated region



Phylogenetic tree of PPV-Rec isolates based on (Cter)NIb-(Nter)CP region

Bootstrap values are represented next to tree nodes. The bar represents 0.005 nucleotide substitution per site.

a recombination event between PPV-D and PPV-M isolates in the NIb gene in a similar position as in all previously characterized PPV-Rec isolates (*2, 12–14*). In the phylogenetic trees reconstructed from the (Cter)NIb-(Nter)CP (nt 8466–8795) and from the (Cter)HC-Pro-(Nter)P3 (nt 2387–2907) region using the minimum evolution method of the MEGA 3.1 with 1000 bootstrap replicates, the PPV-Troy6 clustered with previously published PPV-Rec isolates. Next, we constructed another phylogenetic tree using the same region of the PPV-Rec isolates in order to highlight the distinction of the PPV-Troy6 isolate (figure; Acc. No. of PPV isolate is in brackets after the name of isolate). Multiple aa sequence alignment of the (Nter)CP region showed that there occurred 3 aa changes (A2838, V2848, R2902) in the sequence of PPV-Troy6 that were not present in previously analyzed isolates.

Restriction analysis of the PCR product of the P3-6K1 genomic region using *Eco*RI and *Dde*I (*Hpy*F3I) restriction enzymes (Fermentas) resulted in an unexpected restriction pattern. The PCR product could be cleaved by *Eco*RI, what is a typical feature of PPV-D and PPV-Rec isolates. On the other hand, it could not be cleaved by *Dde*I, what was the typical attribute of PPV-M isolates. Thus, depending on the restriction enzyme used, the isolate PPV-Troy6 behaved either as a member of PPV-D or a member of PPV-M group. Sequence analysis revealed that the lack of the *Dde*I cleavage site resulted from a point mutation (A3102 to C3102) in this site as compared to the PPV-D sequences.

Phylogenetic analyses demonstrated that the PPV-Troy6 isolate belonged to the PPV-Rec group. However, out of the 5 aa (K2814, I2848, T2852, I2868, T2878) that are highly conserved among PPV-Rec sequences only 4 aa could be find in the CP gene of the PPV-Troy6 isolate (*2*). The fifth aa was mutated to V2848. Another point mutation resulted in lacking of a *Dde*I site in the P3-6K1 region of the PPV-Troy6. This restriction site is specifically conserved among PPV-D and PPV-Rec sequences (*8*). Therefore, *Dde*I digestion of the P3-6K1 fragment combined with techniques focusing on other genomic regions is widely used for typing of PPV isolates (*3, 13, 14*). However, some PPV-Rec isolates have been characterized that carried an additional *Dde*I site in this region (*8, 13*).

Obtained data indicate some limitation of restriction enzyme mapping for accurate classification of PPV isolates and support the recommendation of multiple approaches used for identification of PPV isolates. Point mutations are common among PPV isolates, but no PPV-Rec or PPV-D isolates have been detected to date that possess point mutation in the conserved *Dde*I restriction site of the P3-6K1 region.

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