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Naturally occurring recombinant isolate of *Pea seed-borne mosaic virus* – Short Communication

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Abstract: Whole genome sequences of three Czech *Pea seed-borne mosaic virus* isolates belonging to P1 pathotype and causing different symptom intensity were obtained. Using RDP4 analysis the natural recombinant isolate PSB204CZ bearing two breakpoints in nucleotide positions 4053 and 6080 was identified. The isolate was composed of fragment 2028 nt in length partially covering CI and 6K2 regions of the minor parent (PSB262CZ) incorporated into the major parent (PSB194CZ). The results suggest that the observed recombination in CI-6K2 region is responsible for severity of developed symptoms. This observation detected for the first time natural recombination within PSbMV isolates of an important pathogen of leguminous plants.

Keywords: potyvirus; *Pisum sativum* L.; RDP4; P1 pathotype; symptoms

Pea seed-borne mosaic virus (PSbMV), member of the genus *Potyvirus*, is an economically important seed transmitted virus infecting legumes. The economically important host plants are pea, lentil, faba bean, and chickpea. The virus causes various symptoms depending on the host and virus isolate, such as leaf rolling, stunting, mild mosaic, and vein clearing (Šafářová *et al*. 2008). PSbMV was discovered in Czechoslovakia (Musil 1966) and it has recently been reported worldwide, causing serious yield losses and due to its seed-borne transmission it poses a serious phytosanitary risk to both the germplasm maintenance (Alconero *et al*. 1985) and seed production. PSbMV shows the genome structure typical of potyviruses with two ORFs. The main ORF encodes a polyprotein which is finally processed by its three proteases to 9 non-structural proteins and 1 capsid protein (Riechmann *et al*. 1992). An alternative overlapping ORF, PIPO, encodes the next protein that is expressed through polymerase slippage as a fusion product P3N-PIPO (Chung *et al*. 2008; White 2015). The known isolates are distinguished into four pathotypes based on their ability to overcome *sbm* resistance genes present in differential host pea lines. Recessive gene *sbm1* confers resistance to pathotype P1, *sbm2* to pathotype P2, *sbm3* to pathotype P3, and *sbm4* to pathotype P4. Currently, whole genome sequences of four isolates are available in GenBank/NCBI: DPD1 and PSB117CZ isolates representing pathotype 1 (P1), L1 from pathotype P3, and NY from pathotype P4 (Johansen *et al*. 1991, 1996; Olsen & Johansen 2001; Cerna *et al*. 2017).

MATERIAL AND METHODS

Virus isolates. The PSbMV isolates PSB194CZ, PSB204CZ, and PSB262CZ were collected during a long-term survey of pea virus occurrence in the Czech

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Republic (Šafářová *et al*. 2008) and mechanically transmitted and maintained on the pea cultivars Raman and Bohatýr (Cerna *et al*. 2016).

RNA isolation and PSbMV genome amplification. Total RNAs were extracted from 50 mg of fresh pea leaves using a NucleoSpin® RNA isolation kit (Machery-Nagel, Düren, Germany). The reverse transcriptions were done by BioScript™ Reverse Transcriptase (Bioline, London, UK) using Random Primers (Promega, Madison, USA) according to the manufacturer's instructions. Seven overlapping fragments covering the whole genome were obtained with different primer pair combinations (Supplementary Table S1 in EMS) by PCR [pre-denaturation 94°C for 2 min, 35 cycles of amplification (94°C for 1 min, 48–55°C for 1 min, 72°C for 1 min), and a final extension at 72°C for 10 min].

Sequencing. The PCR products were directly sequenced using a BigDye Terminator v. 3.1 Cycle Sequencing Kit using an ABI PRISM 3130 sequencer (both Applied Biosystems, Waltham, USA). The fragments were assembled into the final contigs using the SeqMan program (Lasergene package; DNASTAR, Inc., Madison, USA).

Data analysis. Identity of sequences was checked by the BLASTN program (Altschul *et al*. 1990). Whole genome sequences were aligned with other GenBank available PSbMV genome sequences using the ClustalW algorithm. Genetic diversity of the obtained genome sequences and their parts was calculated by p-distance algorithm and phylogenetic trees were constructed by a neighbour-joining method and visualised in Tree-Explorer, all implemented in MEGA 7.0 (Kumar *et al*. 2016).

Potential recombination events were assessed in RDP4 program v. 4.85 using RDP, GeneConv, Bootscan, MaxChi, Chimaera, SiScan and 3Seq methods (Martin *et al*. 2015). Default settings and the Bonferroni corrected *P*-value 0.05 were applied throughout analyses; the potential recombination events detected by at least five methods were considered as significant. The recombination breakpoint locations were determined based on the highest consensus recombination scores (> 0.5) and the least average *P*-values obtained for the various algorithms used.

RESULTS AND DISCUSSION

The three PSbMV isolates collected and pathotyped during the previous survey, PSB194CZ inducing mild mosaic on infected pea plants of cvs Raman and Bohatýr, PSB204CZ, and PSB262CZ inducing mosaic and leaf rolling, were studied in detail. The nearly full-length genome sequence of each isolate was obtained using Sanger sequencing strategy through seven overlapping segments (Supplementary Table S1 in ESM). All of them were deposited in GenBank under the accessions MK116871-3 for isolates PSB194CZ, PSB204CZ, and PSB262CZ, respectively.

The genome of all three PSbMV isolates showed the same structure, it was 9919 nt in size excluding 3' poly(A) tail and contained two typical potyvirus ORFs: one large ORF at position 144–9 764 nt encoding the polyprotein of 3 206 amino acids in length. The polyprotein is predicted to give rise to ten potyvirus proteins: P1, HC-Pro, P3, 6K1, CI, 6K2, VPg, NIa-Pro, NIb, and CP. The deduced second small ORF at nt position 3 197–3 433 and 78 amino acids in size encodes the C proximal part of P3N-PIPO.

The BLASTN analysis of genomic sequences revealed their highest identity with PSbMV reference isolate DPD1 representing pathotype P1 (Acc. No. NC_001671). It was 96% for PSB194CZ isolate, 97% for PSB204CZ isolate, and 99% for PSB262CZ isolate. In agreement with previous analysis when the mutually compared isolates PSB194CZ and PSB204CZ showed 99% identity, PSB262CZ isolate showed 96% identity with PSB194CZ isolate, and 97% identity with PSB204CZ isolate.

Phylogenetic analysis based on the nearly complete genome sequences showed the distribution of Czech isolates into 2 clusters of pathotype P1 branch (Figure 2A). The PSB194CZ and PSB204CZ isolates formed a separate cluster significantly distant from PSB262CZ isolate that clustered with the Czech isolate PSB117CZ and reference isolate DPD1.

The complete genome sequences of the PSbMV isolates aligned in previous analyses were screened for the presence of possible recombination events by RDP4 program. The only recombinant isolate PSB-204CZ was detected. Two highly significant recombination breakpoints were localised at 4 053 nt position (90% confidence interval from 3 972 to 4 198 nts) and 6 080 nt position (90% confidence interval from 5 862 to 6 175 nts). Both of these breakpoints were detected and evaluated as highly significant by all seven methods implemented in RDP4 (Supplementary Table S2 in ESM). For this event, PSB194CZ isolate was predicted as a major parent and PSB262CZ isolate as a minor parent. The recombinant isolate PSB204CZ was composed of fragment 2028 nt (676 aa) in length partially covering CI and 6K2 regions of

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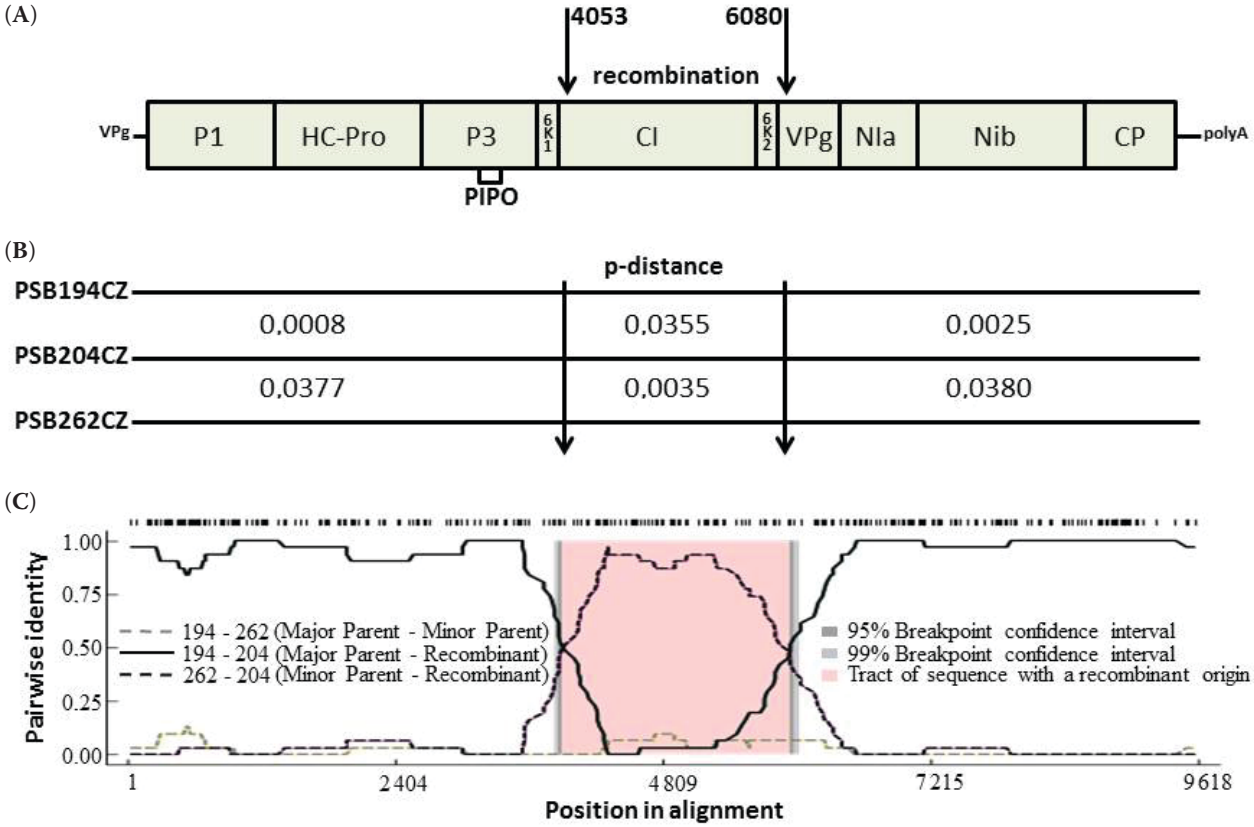

Figure 1. Schematic diagram of genome organization of recombinant PSbMV isolate: (**A**) Schematic genome organization of recombinant PSB204CZ isolate showing detected recombination points marked by arrows; (**B**) p-distances of PSbMV isolates in targeted genomic segments calculated in MEGA7.0; (**C**) RDP plot showing recombination events with PSB194CZ as a major parent and PSB262CZ as a minor parent

the minor parent, incorporated into the major parent (Figure 1).

Comparisons between the nucleotide (nt) and deduced appropriate amino acid (aa) sequences of the recombinant and potential minor parent (PSB204CZ × PSB262CZ) showed their 3.77% nt-diversity and 3.30% aa-diversity in the 5'end large segment (at nt position 1–4 052), and/or 3.80% nt-distance and 7.40% aa-distance in the 3' end large segment (at nt position 6 081–9 924). The distance of the small segment (at nt position 4 053–6 080) was 0.35% for nt- and 1.33% for aa-sequence. Similar sequence analyses of the recombinant and major parent isolates (PSB204CZ × PSB194CZ) showed that they differed by 0.08% for nt- and 0.23% for aa-sequences in the 5' end large segment, and/or by 0.22 and 1.19% in the 3' end large segment, and 3.55% nt-diversity and 1.19% aa-diversity for the small segment.

The recombinant event was confirmed in the detailed phylogenetic analyses based on the genomic parts involved in the recombination (i.e. CI and 6K2). The recombinant segment of PSB204CZ isolate (at nt position 4 053–6 080) clustered with the minor parent PSB262CZ (Figure 2B). The concatenated sequences of the recombinant isolate PSB204CZ (at nt positions 1–4 052 and 6 081–9 924) clustered together with the major parent, PSB194CZ isolate, and the phylogenetic tree showed the same topology as the tree reconstructed on nearly full-length sequences (data not shown).

It was repeatedly demonstrated for example for *Bean yellow mosaic virus* (Wylie & Jones 2009), *Lettuce mosaic virus* (Krause-Sakate *et al.* 2004), *Plum pox virus* (Glasa *et al.* 2004), *Potato virus Y* (Green *et al.* 2017), and *Turnip mosaic virus* (TuMV) and it is generally accepted that intraspecies recombination plays an important role in the evolution of potyviruses. Our observation extends a list of recombinant events within this viral group describing for the first time intraspecies recombination in the case of *Pea seed-borne mosaic virus*.

It seems that this recombination could be associated with the severity of induced symptoms, as the recombinant isolate PSB204CZ manifested similar

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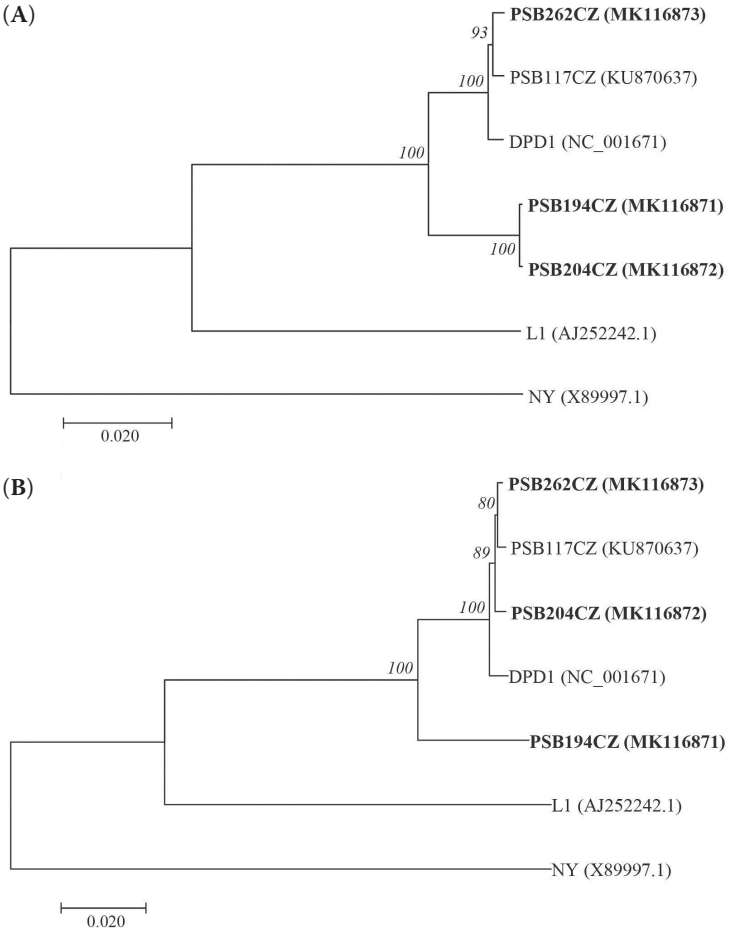

Figure 2. Neighbour-joining phylogenetic tree of PSbMV isolates reconstructed on nucleotide sequences of the nearly full-length sequences (**A**) and recombinant segment [nt positions 4 053–6 080] detected in PSB204CZ isolate (**B**)

The virus isolates are described by the name and GenBank accession number in parenthesis; bootstrap values ≥ 70% obtained from 1 000 replicates are shown; bar represents 2% nt-sequence divergence

severe symptoms of mosaics and leaf rolling as the minor parent (PSB262CZ), donor of CI-6K2 fragment (Supplementary Figure S1 in ESM). It is in agreement with the biological significance of recombined genome part and encoded proteins. Potyviral CI protein is a multifunctional protein involved in viral replication, in cell to cell and long-distance movement, in symptom development, and it is considered as an avirulence factor (Abdul-Razzak *et al*. 2009; Sorel *et al*. 2014; Deng *et al*. 2015). Similarly, 6K2 protein acts in symptom induction and long-distance movement (Spetz & Valkonen 2004).

The observed natural recombination event is important from the epidemiological view as it shows an open possibility of the evolution of new recombinant PSbMV isolates and potentially new PSbMV pathotypes overcoming the resistance of recently cultivated pea cultivars.

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