Pod Pepper Vein Yellows Virus, a New Recombinant Polerovirus Infecting Pod Pepper in Yunnan Province, China

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Short report

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Abstract

Pepper vein yellows viruses (PeVYV) are phloem-restricted viruses in the genus *Polerovirus*, family *Luteoviridae*. Typical viral symptoms of PeVYV including interveinal yellowing of leaves and upward leaf curling were observed in pod pepper plants (*Capsicum frutescens*) growing in Wenshan city, Yunnan province, China. The complete genome sequence of a virus from a sample of these plants was determined by next-generation sequencing and RT-PCR. Pod pepper vein yellows virus (PoPeVYV) (MT188667) has a genome of 6015 nucleotides, and the characteristic genome organization of a member of the genus *Polerovirus*. In the 5' half of its genome (encoding P0 to P4), PoPeVYV is most similar (94% nt identity) to PeVYV-3 (*Pepper vein yellows virus 3*) (KP326573) but diverges greatly in the 3'-part encoding P5, where it is most similar (88.8% nt identity) to tobacco vein distorting virus (TVDV, EF529624) suggesting a recombinant origin. Recombination analysis predicted a single recombination event at nucleotide positions 4126 to 5192 nt, with TVDV and PeVYV-3 as the minor and major parents, respectively. A full-length clone of PoPeVYV was constructed and shown to be infectious in *C. frutescens* by RT-PCR and the presence of icosahedral viral particles.

Background

Pepper vein yellows viruses (PeVYV) are phloem-restricted viruses in the genus *Polerovirus*, family *Luteoviridae* and are currently classified into six species (International Committee on Taxonomy of Viruses [ICTV] 2019 release), named *Pepper vein yellows virus 1* to *6* [1-6]. They have 86.23% - 94.63% identity on nucleotides. The genome of *Polerovirus* contains seven open reading frames (ORF0 to ORF5 and ORF3a), putatively encoding proteins P0 to P5 and P3a [6]. Recombination is an important source of genetic variability in viruses, particularly for viruses possessing an RNA genome. PeVYVs have nucleotide identities to tobacco vein distorting virus (TVDV) at 5' half of genome, and are considered to be recombinant from TVDV and other poleroviruses [4, 7, 8]. We here identified a new recombinant of PeVYV that had identity to PeVYV-3 at 5' half of genome and had identity to TVDV at 3' part of genome.

Main Text

Pod pepper (Capsicum frutescens) is widely planted in China, especially around Wenshan city, Yunnan province, and viral diseases have now also become a major threat to pepper production in Yunnan. During July 2019, 89 pepper leaf samples were collected from three different fields in Wenshan. All had typical viral symptoms of interveinal leaf yellowing and fruit discoloration. Total RNAs were extracted from each of three pooled samples of these leaves. The total RNAs were sent for next-generation sequencing. De novo assembly generated 7 contigs, one of which was 5992 nt long and had high identities (>87.5%) to the genome of PeVYV-3 (Pepper vein yellows virus 3) (KP326573;[3]). Primers designed from this sequence were then used to amplify a coat protein (CP) fragment of the virus (PeVYV-CP f: 5'-ATGAATACGGGAGGAGTTAGG -3', PeVYV-CP r: 5'-CTATTTGGGGTTGTGCAGTTG -3'). Using RT-PCR, fragments of the expected size (621bp) were obtained in 58 of the 89 symptomatic samples but not from healthy plants (data not shown). The nucleotide sequences of the amplified fragments were all 93.4% identical to PeVYV-3. 5' and 3'RACE reactions (Invitrogen) were then performed to obtain the complete 5'and 3' terminal sequences of the new virus isolate. To avoid errors in sequence assembly, the whole viral sequence was then amplified using two overlapping sections with the primer pairs PeVYV-1 (PeVYV-1 f: 5'-ACAAAATATACGAAGAGAGAGAG -3'and PeVYV-1 r: 5'-TAACCCATCACTCCTCCCAC-3') and PeVYV-2 (PeVYV-2 f: 5'-GGACAACTGGAATTCTGCTC-3' and PeVYV-2 r: 5'-

ACATCATAGACCAGGGGGGGGTATCTATAC -3') (Fig. 1A and B). RT-PCR products (expected sizes ~4600bp and 2800bp) were obtained using the methods of KOD-plus-Neo (Toyobo) and the two products (which overlapped by 1.4kb) were cloned into the pGEM-T Easy Vector (Promega) and sequenced. The complete sequence was 6015 nt long (GenBank accession number: MT188667). Because of its distinctive features it was named pod pepper vein yellows virus (PoPeVYV). PoPeVYV has a genome organization characteristic of members of the genus Polerovirus, with seven predicted genes encoding proteins P0 to P5 and P3a [6] (Fig. 1A). Over its entire genome our isolate is most closely related (85.3% nt identity) to Tobacco vein distorting virus (TVDV, accession EF529624) and had 78.9% identity to the (Chinese) PeVYV-3 (accession KP300822). However, if the different genes are compared separately, the new isolate is slightly more closely related to all the other PeVY viruses than to TVDV over most of the genome but diverges from them strongly in the 3'-end (encoding the coat protein readthrough P5 domain). The CP-RT P5 region (nts 4252-5790) of PoPeVYV has only 43.7% nt identity to the corresponding region of PeVYV-3, but 91.1% identity to that of TVDV. This is reflected in the phylogenetic analysis of the amino acid sequences of the separate gene products [9]: the P0 of PoPeVYV is most similar to PeVYV-1/4, P1/P2/P3/P4 are closely related to other PeVYVs but the P5/RTD is most similar to TVDV (Fig. 2). Poleroviruses are prone to recombination among themselves or with viruses belonging to other genera and the relationships between PoPeVYV, the previously described PeVYV isolates and TVDV suggests that there has been a recombination event affecting the 3'-end of the genome. This was confirmed using a variety of methods on the RDP4 recombinant platform [10]. A single recombination event affecting nucleotide positions 4126-5952, with TVDV and PeVYV-3 as the respective minor and major parents was consistently identified using GENECONV (P value of 1.035 E-93), RDP (8.410 E-85), BootScan (7.726 E-80), MaxChi (4.294 E-35), Chimera (7.719 E-05), SiScan (3.341 E-68), and Phylpro (2.331 E-15) (Fig. 1C). Alignment of amino acid sequences of the PoPeVYV proteins with those of the six species of PeVYVs and TVDV also indicated a recombination event (Table. 1). Proteins encoded by the 5' half of the genome (P0 to P4) had the highest identity to those of PeVYV-3 and PeVYV-6, whereas proteins translated from the 3' half (P5/RTD) were more closely related to those of TVDV. These results suggest that PoPeVYV is a new recombinant polerovirus. An infectious clone of the virus was constructed for further investigation. The full-length (6015 bp) cDNA was assembled and cloned into the 35S promoter of binary vector pCB301 using two overlapping fragments (from nucleotides 1-4615 and 4596-6015) and the CloneExpress MultiS One Step Cloning Kit (Vazyme). This was transformed into Agrobacterium tumefaciens which was then delivered to C. frutescens plantlets by infiltration. There was mild upward leaf curling 45 days after inoculation (Fig. 3A), and RT-PCR using primers to detect the coat protein gene in the newly-emerged non-inoculated leaves showed that viral RNA was present and had spread systemically in all the inoculated plants (12/12) but not in the controls (Fig. 3B). Virions were purified from C. frutescens leaves using the method described previously [11]. Isometric particles about 25nm in diameter were observed in the purified preparation from the inoculated plants (Fig. 3C) but not from the controls. These results demonstrated the infectivity of full-length PoPeVYV to C. frutescens. Recombination is an important source of genetic variability in viruses, particularly for viruses possessing an RNA genome. PeVYVs have higher identities to TVDV at 5' half of genome and are considered to be recombinant from TVDV and other poleroviruses [4, 7, 8]. The new recombinant identified here has higher identity to TVDV at 3' part of genome, indicating a different recombinant event of PeVYV. Recombination poses a problem for classification. The currently-recommended species demarcation criteria in the family Luteoviridae suggest that different species should have >10% difference in amino acid sequence identity in any gene product from their closest relative. The P0 and minor capsid (P3-P5) proteins of PoPeVYV have respectively 14.9-22.5% and 36.8-56.2% difference in amino acid identity to those of PeVYV1-6 (Table. 1). Also, all the proteins (except the small P3a) of PoPeVYV have >10% difference in amino acid identity

to those of TVDV (Table. 1). If applied here, those criteria suggest that PoPeVYV is representative of a distinct species.

Conclusions

A full length of Pod pepper vein yellows virus (MT188667) was determined. Alignment and recombination analysis predicted a single recombination event at nucleotide positions 4126 to 5192 nt, with TVDV and PeVYV-3 as the minor and major parents, respectively. PoPeVYV is a new recombinant *Polerovirus* infecting Pod Pepper in Yunnan province, China.

Abbreviations

aa: Amino acid; nt: Nucleotide; RACE: Rapid amplification of cDNA ends; RT-PCR: Reverse transcription polymerase chain reaction; PoPeVYV: Pod Pepper vein yellows virus; PeVYV: Pepper vein yellows virus; TVDV: Tobacco vein distorting virus

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

The complete genome sequences of PoPeVYV were submitted to the GenBank (http://

www.ncbi.nlm.nih.gov/genbank/) and the accession number is MT188667.

Competing interests

The authors declare that they have no conflict of interest.

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Authors' contributions

SX, EP, LL collected the samples. XH, HY, HR and JJ conceived and designed the experiments. KJ, YY, LL and MY performed the experiments. JJ, YW and JP analyzed the data. YW, JJ and FY wrote the paper. All authors read and approved the final manuscript.

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Tables

Table 1 Sequence comparisons between PoPeVYV and its most closely-related poleroviruses

Virus species	Accession Number	Amino acid identity (%) in the encoded proteins									Whole genome
		P0	P1	P2	P1-P2	P3a	P3	P4	P3-P5	P5/RTD	(% nt)
PoPeVYV	MT188667	100	100	100	100	100	100	100	100	100	100
PeVYV-1	AB594828	83.5	89.8	93.4	92.2	97.8	94.2	89.7	44.2	24.2	78.86
PeVYV-2	HM439608	83.9	89.9	94.7	92.8	91.1	91.8	85.3	63.2	50.8	82.95
PeVYV-3	KP326573	87.2	90.8	93.7	92.7	100	94.2	90.4	44.5	23.8	78.88
PeVYV-4	KU999109	85.1	90.1	94.4	92.8	95.6	92.7	91.0	43.8	24.2	78.05
PeVYV-5	KY523072	77.5	87.2	95.0	91.5	95.6	94.2	93.0	45.4	25.9	78.86
PeVYV-6	LT559483	85.1	90.5	95.0	92.8	95.6	94.7	92.3	45.7	26.9	80.06
TVDV	EF529624	76.3	76.8	89.4	81.6	91.5	89.3	84.6	88.9	88.8	85.33

Figures

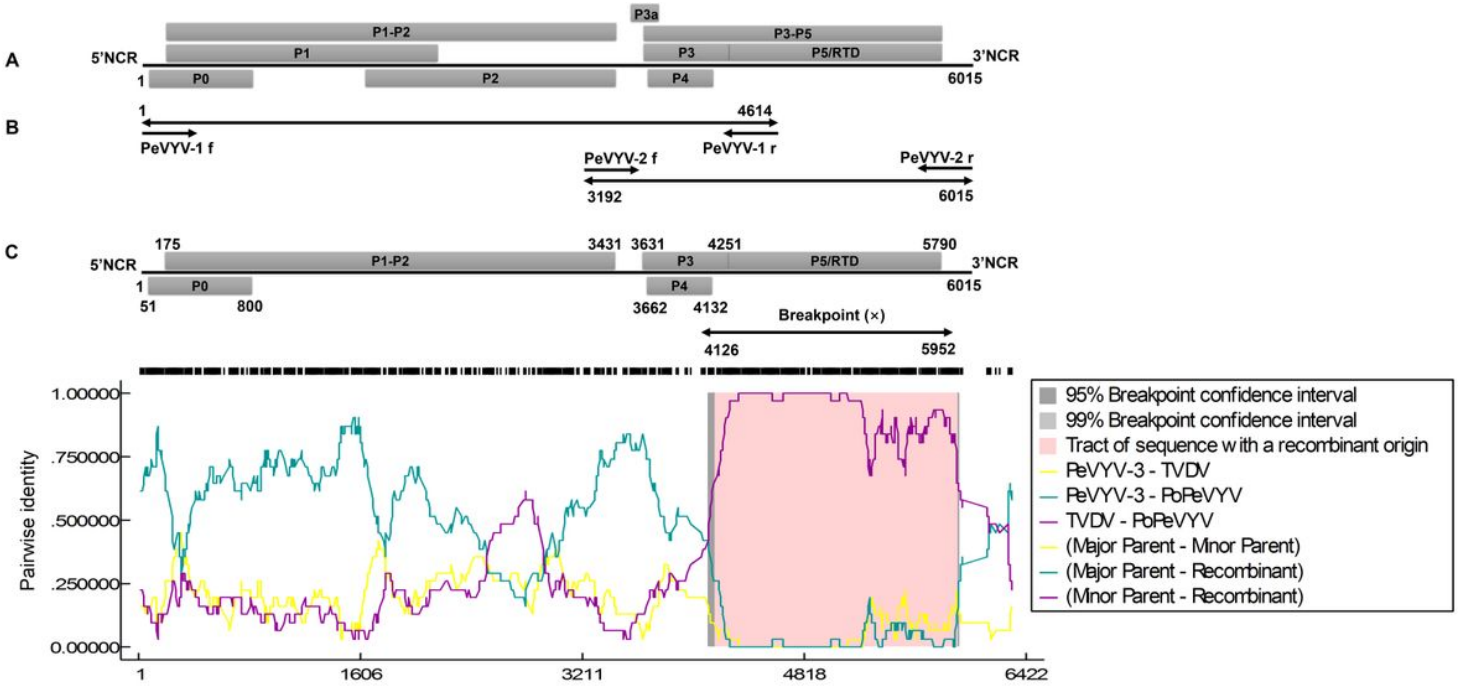
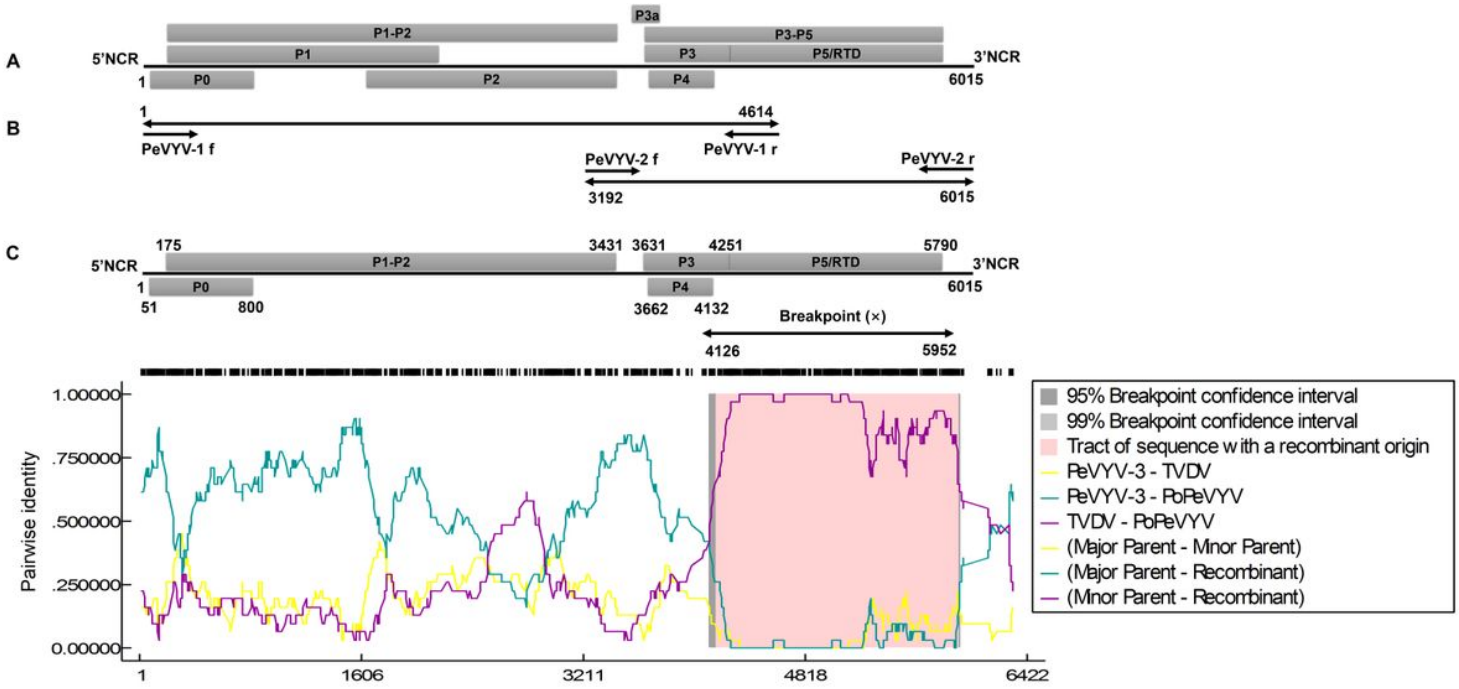


Figure 1

(A) Genome organization PoPeVYV. (B) The strategy for assembling the full-length sequence by overlap extension RT-PCR. (C) Recombination events among related viruses predicted by RDP4; the recombinant region is highlighted in pink.



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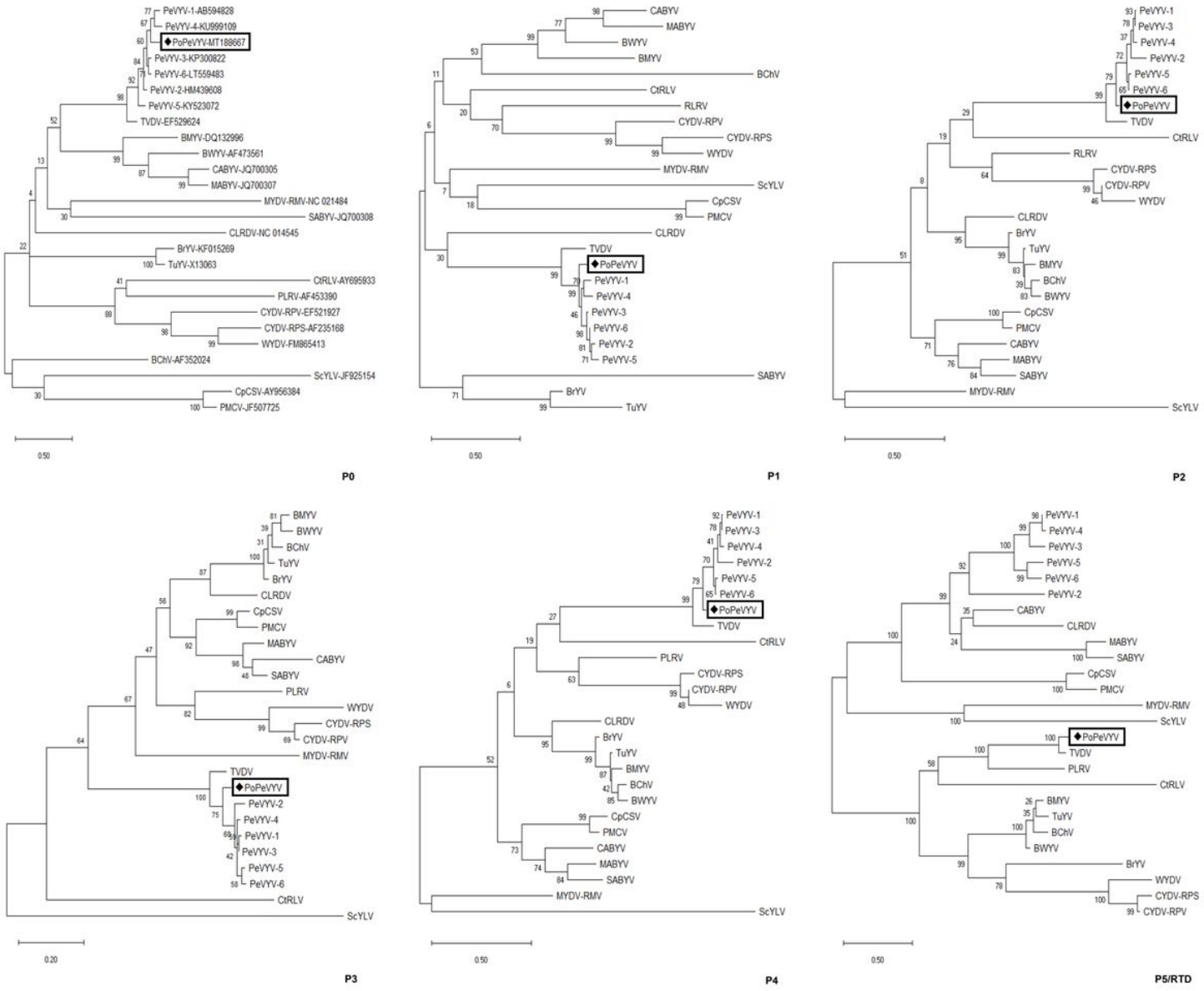
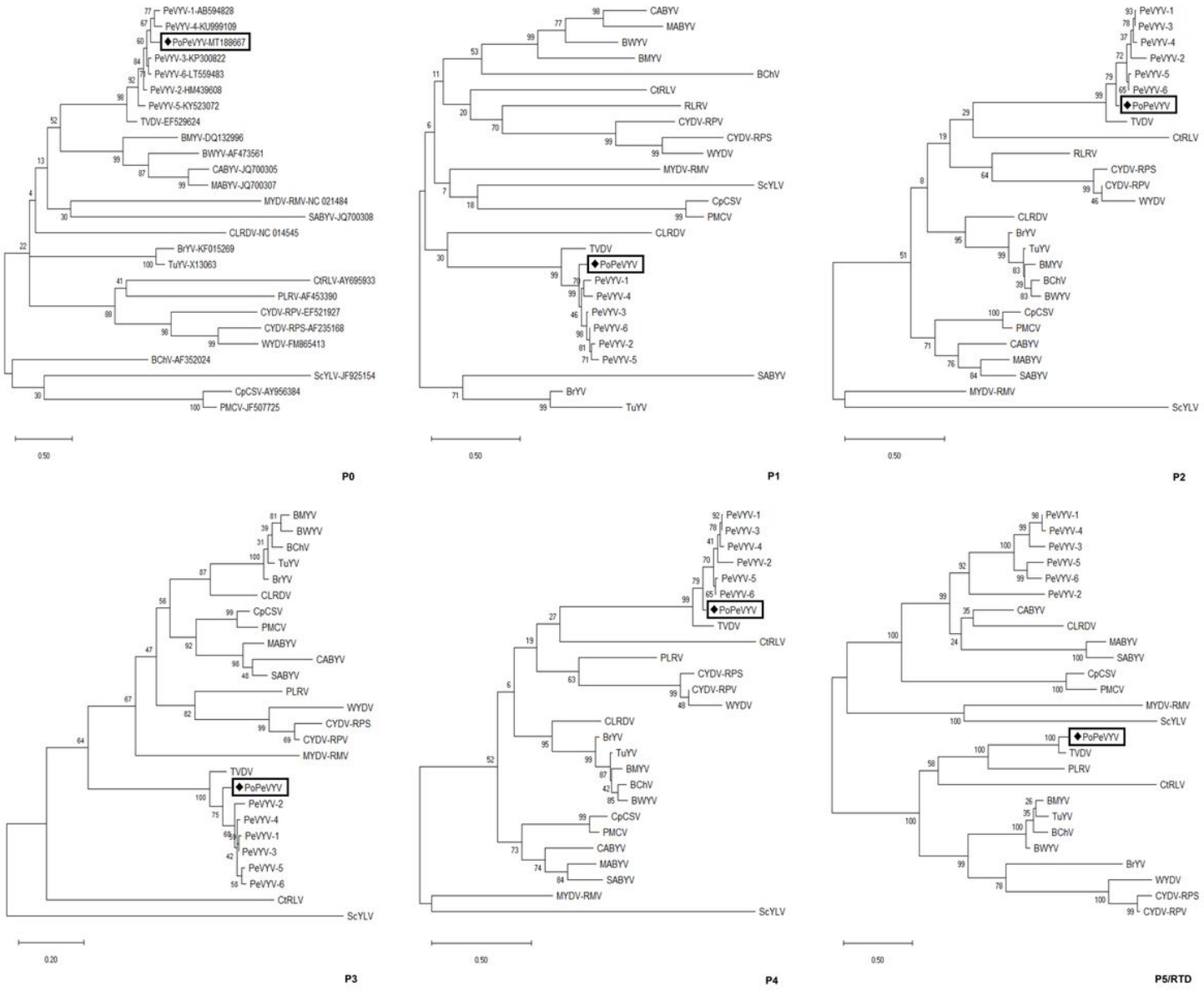


Figure 2

Maximum Likelihood phylogenetic trees constructed using MEGA X, showing the relationship of PoPeVYV to other members of the family Luteoviridae using amino acid sequences of the different gene products. Numbers on branches are bootstrap support values (1,000 replicates).



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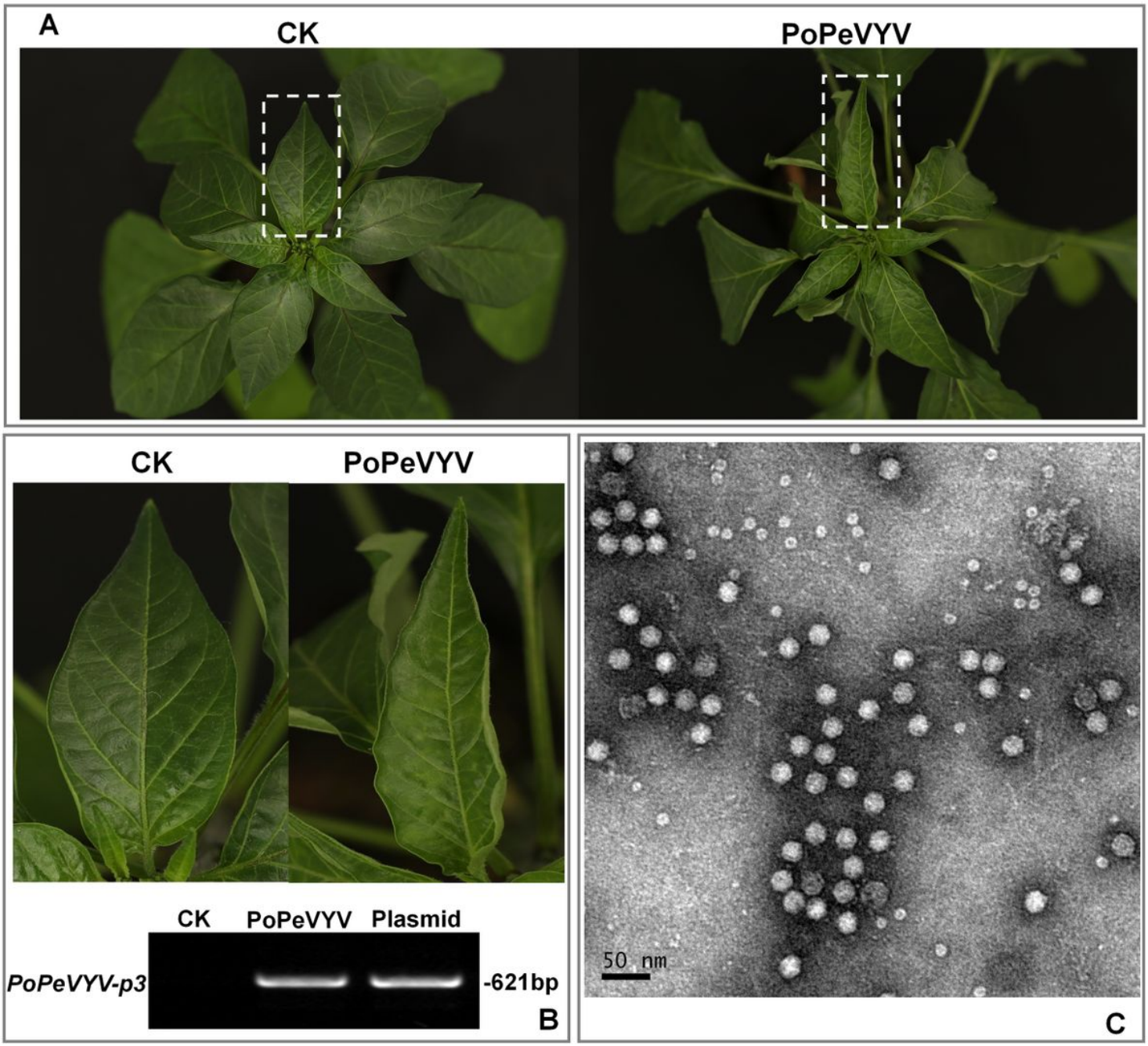
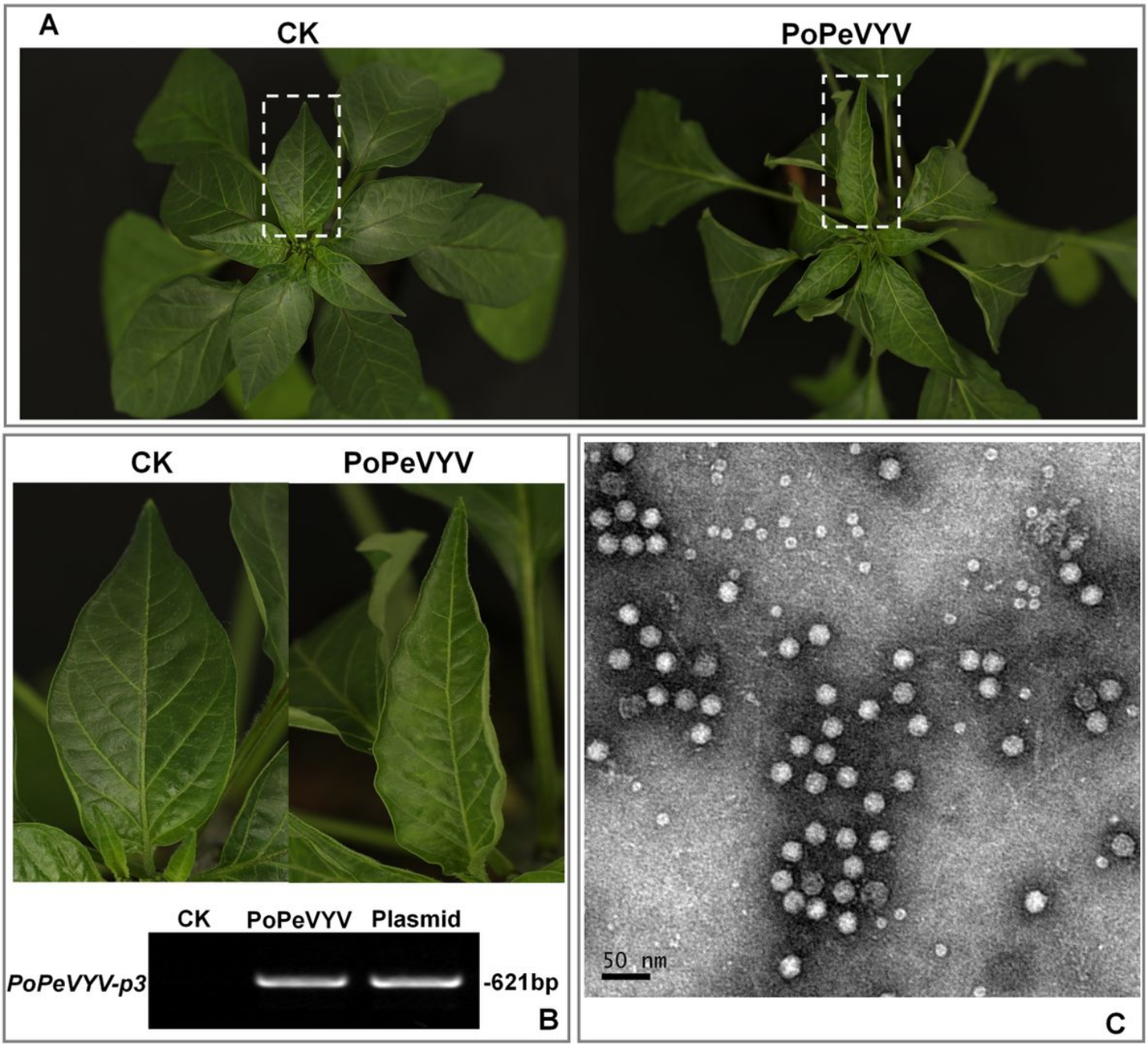


Figure 3

Symptoms caused by PoPeVYV infectious clone in C. frutescens (A) Phenotype of C. frutescens plants agroinfiltrated with PoPeVYV infectious clone or empty agrobacterium (CK) 45 days post infiltration. (B) RT-PCR confirming the presence of viral RNA in systemic leaves of inoculated plants. A 621-bp fragment of the p3 was amplified, with a plasmid containing the p3 as a positive control. (C) Virions purified from leaves infected by the PoPeVYV infectious clone and observed by TEM. Bars represent 100nm.



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Supplementary Files

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- 7.PoPeVYVMT188667.txt
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