

Two novel poty-like viruses identified from the transcriptome data of purple witchweed (*Striga hermonthica*)

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Summary. – Potyvirids (the family *Potyviridae*) are the largest family of plant RNA viruses. Two novel potyvirid viruses, Striga-associated poty-like virus 1 (SaPIV1) and Striga-associated poty-like virus 2 (SaPIV2), were identified from the transcriptome data of purple witchweed (*Striga hermonthica*). SaPIV1 was most closely related to bellflower veinal mottle virus (BVMoV), the only member of the genus *Bevemovirus*, and then to macluraviruses (the genus *Macluravirus*). The SaPIV1 genome encodes a 2462-amino acid (aa) polyprotein that may be cleaved into nine mature peptides. The cleavage sites of SaPIV1, BVMoV, and macluravirus polyproteins shared strong sequence similarities. SaPIV2 was most closely related to celery latent virus, the sole species of the genus *Celavirus*, which is the most divergent potyvirid genus. The SaPIV2 polyprotein contained 3329 aa and it may be cleaved into at least seven or eight mature peptides. Phylogenetic analysis suggested that SaPIV1 and SaPIV2 may be novel species of the genera *Bevemovirus* and *Celavirus*, respectively. The genome sequences of SaPIV1 and SaPIV2 are useful resources for studying the genome evolution of potyvirids.

Keywords: Striga-associated poty-like virus 1; Striga-associated poty-like virus 2; Potyviridae; *Bevemovirus*; *Celavirus*; purple witchweed; *Striga hermonthica*

Introduction

Potyviridae is the largest family of plant-infecting RNA viruses containing 235 species approved by the International Committee on Taxonomy of Viruses (ICTV) (<https://talk.ictvonline.org>; ICTV Master Species List #36, March 2021) (Gibbs *et al.*, 2020; Palani *et al.*, 2021). Potyvirids (members of the family *Potyviridae*) are classified into 12 genera: *Arepavirus*, *Bevemovirus*, *Brambyvirus*, *Bymovirus*, *Celavirus*, *Ipomovirus*, *Macluravirus*, *Poacevirus*, *Potyvirus*, *Roymovirus*, *Rymovirus*, and *Tritimovirus* (Palani *et*

al., 2021). Members of the genus *Bymovirus* have a bipartite genome, whereas those from the remaining 11 genera have a monopartite positive-sense single-stranded RNA genome (Revers and Garcia, 2015).

The genomic RNA of potyvirids encodes an open reading frame (ORF) for a large polyprotein, which undergoes proteolytic cleavage by virus-encoded proteases (Adams *et al.*, 2005; Goh and Hahn, 2021; Palani *et al.*, 2021). The polyprotein of potyviruses (the genus *Potyvirus*) is processed into ten mature peptides (from N-terminus to C-terminus): protein 1 protease (P1), helper component-protease (HC-Pro), protein 3 (P3), 6-kilodalton (kDa) peptide 1 (6K1), cylindrical inclusion protein (CI), 6-kDa peptide 2 (6K2), viral protein genome-linked (VPg), nuclear inclusion a protease (NIa-Pro), nuclear inclusion b protein (NIb), and coat protein (CP) (Adams *et al.*, 2005; Goh and Hahn, 2021). P1 and HC-Pro proteases cleave themselves from the polyprotein, and NIa-Pro cleaves the remaining seven junctions. The NIb protein is an RNA-dependent RNA polymerase (RdRp)

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Abbreviations: BVMoV = Bellflower veinal mottle virus; CeLV = Celery latent virus; ORF = open reading frame; PIPO = pretty interesting *Potyviridae* ORF; RdRp = RNA-dependent RNA polymerase; SaPIV1 = Striga-associated poty-like virus 1; SaPIV2 = Striga-associated poty-like virus 2

that is responsible for viral genome replication (Shen *et al.*, 2020). Potyvirids from other genera have the same or similar genomic organization.

Eight mature peptides, P3, 6K1, CI, 6K2, VPg, NIa-Pro, NIb, and CP, are commonly produced from potyvirus polyproteins (Revers and Garcia, 2015). However, the P1 and HC-Pro segments among potyvirids are variable, probably resulting from diversifying evolution involving recombination and gene duplication (Valli *et al.*, 2007). For example, macluraviruses (the genus *Macluravirus*) lack the P1 coding region and HC-Pro is absent in cassava brown streak virus (Mbanzibwa *et al.*, 2009; Revers and Garcia, 2015; Elangovan *et al.*, 2019).

An additional short ORF termed “pretty interesting Potyviriidae ORF” (PIPO) is universally present within the P3 coding region of potyvirus genomes (Chung *et al.*, 2008). A polymerase slippage inserts an additional A nucleotide within the highly conserved GAAAAA (GA₆) motif. This insertion, termed “+1A insertion event,” results in the formation of a “trans-frame” fusion protein with the N-terminal half of P3 and PIPO; this protein is named the P3N-PIPO protein (Olsper *et al.*, 2015; Rodamilans *et al.*, 2015; White, 2015).

The majority of ICTV-approved Potyviriidae species (190 out of 235) belong to the genus *Potyvirus*. The other 11 genera each contain one to ten recognized species. The genus *Bevemovirus* has a single approved species, the *Bellflower veinal mottle virus* (BVMoV), which was isolated from a bellflower (*Campanula takesimana*) (Seo *et al.*, 2017). The BVMoV polyprotein is predicted to be cleaved to produce nine mature proteins, HC-Pro, P3, 6K1, CI, 6K2, VPg, NIa-Pro, NIb, and CP; this is the same as that of macluravirus polyproteins. Phylogenetic analysis using BVMoV and selected potyvirus polyprotein sequences suggested that the genus *Bevemovirus* is the sister taxon of the genus *Macluravirus* (Seo *et al.*, 2017).

Celavirus is another genus represented by only one species, the *Celery latent virus* (CeLV), which was first identified in celery (*Apium graveolens* var. *dulce*) and celeriac (*A. g.* var. *rapaceum*) without visible symptoms (Bos *et al.*, 1978; Rose *et al.*, 2019). The CeLV polyprotein has a low sequence similarity to other potyvirus polyproteins, suggesting that *Celavirus* is the most divergent genus of the family Potyviriidae (Rose *et al.*, 2019).

RNA-Seq data obtained from plant tissue samples often contain genomic RNA or mRNA fragments derived from latently infected RNA viruses (Bejerman *et al.*, 2020; Park and Hahn, 2021). Comprehensive analyses of diverse plant transcriptome data have yielded many novel RNA virus genome sequences (Park *et al.*, 2018; Goh *et al.*, 2021; Park *et al.*, 2021; Park and Hahn, 2021). In this study, we identified the genome sequences of two novel viruses belonging to the genera *Bevemovirus* and *Celavirus* (the

family Potyviriidae) from the transcriptome data of purple witchweed (*Striga hermonthica*) (Yoshida *et al.*, 2019). Purple witchweed is a hemiparasitic plant that infests grain crops, such as sorghum, maize, and sugar cane, and causes major grain losses (Spallek *et al.*, 2013).

Materials and Methods

The purple witchweed transcriptome data analyzed in this study are available in the Sequence Read Archive (SRA) of the National Center for Biotechnology Information (NCBI) (Yoshida *et al.*, 2019). The SRA Acc. Nos. are DRR183243, DRR183244, DRR183245, DRR183246, DRR183247, DRR183248, DRR183249, and DRR183250. RNA-Seq reads were trimmed using the sickle program (version 1.33; <https://github.com/najoshi/sickle>) with the parameter “-q 30 -l 55.” Filtered high-quality reads from all nine sequencing runs were pooled into a single dataset and assembled into contigs using the rnaviralSPAdes pipeline of the SPAdes assembler (version 3.15.1; <http://cab.spbu.ru/software/spades>) (Bushmanova *et al.*, 2019).

Known viral RdRp domain sequences were downloaded from the Pfam database (release 33.1; <https://pfam.xfam.org>). Pfam Acc. Nos. for the viral RdRp families are PF00602, PF00603, PF00604, PF00680, PF00946, PF00972, PF00978, PF00998, PF02123, PF03035, PF03431, PF04196, PF04197, PF05788, PF05919, PF06317, PF07925, PF08467, PF08716, PF08717, PF12426, and PF17501. The DIAMOND program (version 2.0.4; <http://www.diamondsearch.org/index.php>) was used to compare the purple witchweed contigs and known viral RdRp sequences.

Sequencing depth was examined by mapping high-quality RNA-Seq reads to the contig sequence using the bwa-mem2 program (version 2.0pre2; <https://github.com/bwa-mem2/bwa-mem2>). Conserved domains in the polyprotein sequences were predicted using the InterPro web server (version 84.0; <https://www.ebi.ac.uk/interpro>). The SignalP web server (version 5.0; <https://services.healthtech.dtu.dk/service.php?SignalP>) was used to predict the signal peptide.

Pairwise identities of the protein sequences were calculated using the needle program of the EMBOSS package (version 6.6.0.0; <http://emboss.open-bio.org>). Multiple sequence alignments were performed using the MAFFT program (version 7.475; <https://mafft.cbrc.jp/alignment/software>) (Nakamura *et al.*, 2018). Gap-rich segments in the aligned sequences were removed using the trimAl program (version 1.4.rev22; <http://trimal.cgenomics.org>) with the “-gappypout” option (Capella-Gutierrez *et al.*, 2009). A maximum-likelihood phylogenetic tree was constructed using the IQ-TREE program (version 2.1.2; <http://www.iqtree.org>) (Minh *et al.*, 2020). Bootstrap supports were calculated from 1,000 replicates using the UFBoot2 method implemented in the IQ-TREE program. Sequence logos were generated using the WebLogo 3 server (<http://weblogo.threeplusone.com>) (Crooks *et al.*, 2004).

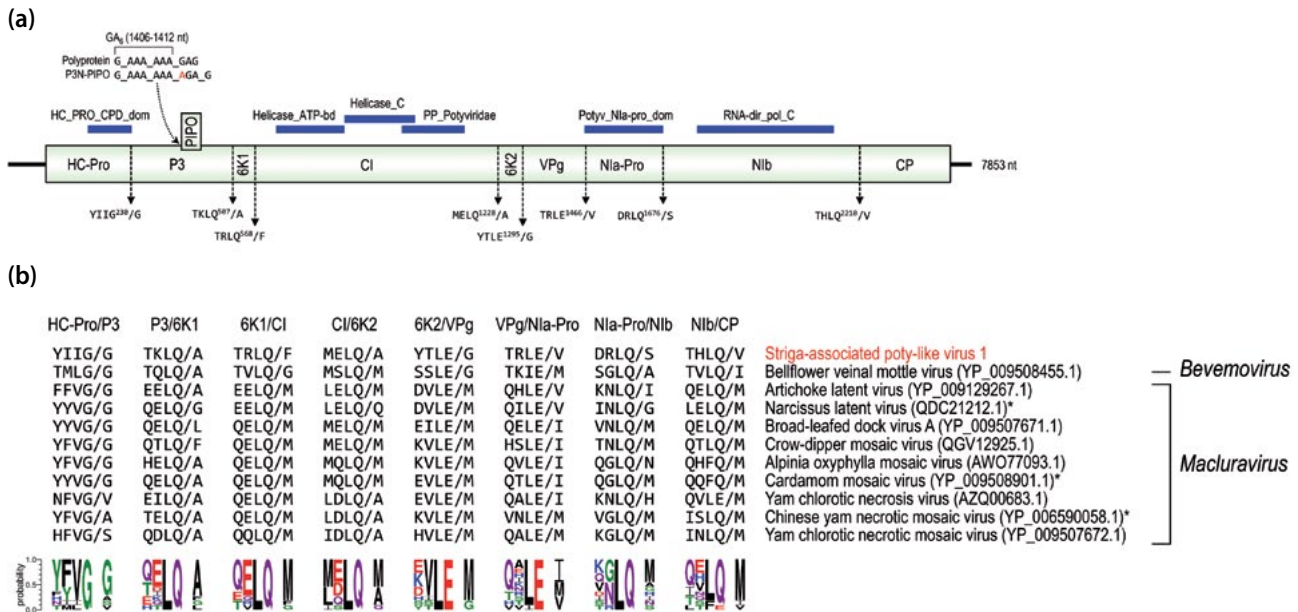


Fig. 1

Genome organization and cleavage sites of SaPIV1

(a) Schematic figure of the SaPIV1 genome is presented. ORFs for the large polyprotein and PIPO are depicted as boxes. Predicted InterPro domains are marked above the polyprotein ORF with the corresponding InterPro short name. The GA_n motif causing a polymerase slippage is shown above the genome (the +1A insertion is indicated in red and the underscores (‘_’) indicate the codon boundaries). Predicted cleavage sites are presented below the polyprotein ORF. (b) Five aa residues at the eight cleavage sites of the SaPIV1, BVMoV, and nine macluravirus polyprotein sequences are presented. The slash (‘/’) indicates the cleaved bond. Three macluraviruses with annotated cleavage site information are indicated by asterisks. Sequence logos showing the frequencies of residues at each position are displayed at the bottom.

Results and Discussion

We re-analyzed the transcriptome data obtained for the investigation of gene expression dynamics during haustorium development in purple witchweed (Yoshida et al., 2019). Transcriptome contigs assembled from high-quality RNA-Seq reads were compared with known viral RdRp sequences. Several contigs were found to contain a viral RdRp motif. Two different contigs showing significant sequence similarity to potyvirus RdRp motifs were chosen for further studies. To obtain a high-quality contig sequence, the sequencing depth of each contig was examined by mapping RNA-Seq reads to a contig, and bases supported by only one read were trimmed off at both ends. Two putative viral contigs that were 7853 and 10767 nucleotides (nt) in length were obtained.

BLASTX searches of the NCBI protein database using two contigs as query sequences confirmed that they encoded a large protein showing significant sequence similarity to known potyvirus polyproteins. Therefore, the 7853-nt and 10767-nt contigs were considered genome sequences of novel potyvirids and tentatively named Striga-associated poty-like virus 1 (SaPIV1) and Striga-associated poty-like virus 2 (SaPIV2), respectively. The

genome sequences were deposited in the NCBI database (Acc. Nos. MW699352 and MW699353, respectively).

Genome sequence of Striga-associated poty-like virus 1 (SaPIV1)

The SaPIV1 genome was 7853-nt long and predicted to encode a 2462-amino acid (aa) polyprotein (Fig. 1a and Table 1). The InterPro domain analysis indicated that the SaPIV1 polyprotein has conserved domains that are typically found in other potyvirus polyproteins, including the HC-Pro cysteine protease, helicase, NIa protease, and RdRp domains.

A sequence similarity search of all known viral proteins revealed that the SaPIV1 polyprotein was the most similar to the BVMoV polyprotein (NCBI Acc. No. YP_009508455.1), with 52.6% identity in the 2496-aa overlap. BVMoV is a species within the genus *Bevemovirus* (Seo et al., 2017). The next similar sequences were polyproteins of various macluraviruses (the genus *Macluravirus*) such as the narcissus latent virus (QDC21212.1), cardamom mosaic virus (YP_009508901.1), and Chinese yam necrotic mosaic virus (YP_006590058.1), with 32%–34% identity in an approximately 2700-aa overlap (Kondo and Fujita, 2012;

Table 1. ORFs and domains of SaPIV1 and SaPIV2 genome sequences

Virus	ORF	ORF position (nt)	Protein length (aa)	Domain position (aa)	InterPro domain ^a
SaPIV1	Polyprotein	309-7679	2462	113-230	HC_PRO_CPD_dom (IPR031159): Helper-component proteinase (HC-Pro) cysteine protease (CPD) domain-like methyltransferase (MT) domain
				625-810	Helicase_ATP-bd (IPR014001): Helicase superfamily 1/2, ATP-binding domain
				810-1000	Helicase_C (IPR001650): Helicase, C-terminal
				967-1136	PP_Potyviridae (IPR013648): Polyprotein, <i>Potyviridae</i>
				1463-1674	Potyv_NIa-pro_dom (IPR001730): Potyvirus NIa protease (NIa-pro) domain
				1769-2139	RNA-dir_pol_C (IPR001205): RNA-directed RNA polymerase, C-terminal domain
	PIPO	1412-1573	53		
SaPIV2	Polyprotein	278-10267	3329	1180-1342	Helicase_C (IPR001650): Helicase, C-terminal
				2067-2285	Potyv_NIa-pro_dom (IPR001730): Potyvirus NIa protease (NIa-pro) domain
				2381-2726	RNA-dir_pol_C (IPR001205) RNA-directed RNA polymerase, C-terminal domain
					PIPO

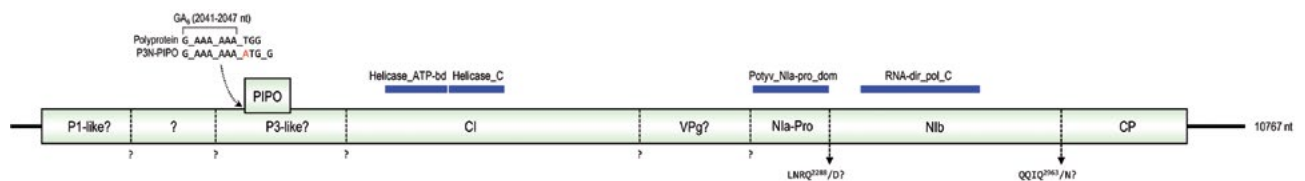
^aInterPro domain in the format of “short name (accession number): full name.”

Elangovan *et al.*, 2019; Wylie *et al.*, 2019). Thus, SaPIV1 is the most closely related to BVMoV and possibly, a novel member of the genus *Bevemovirus*.

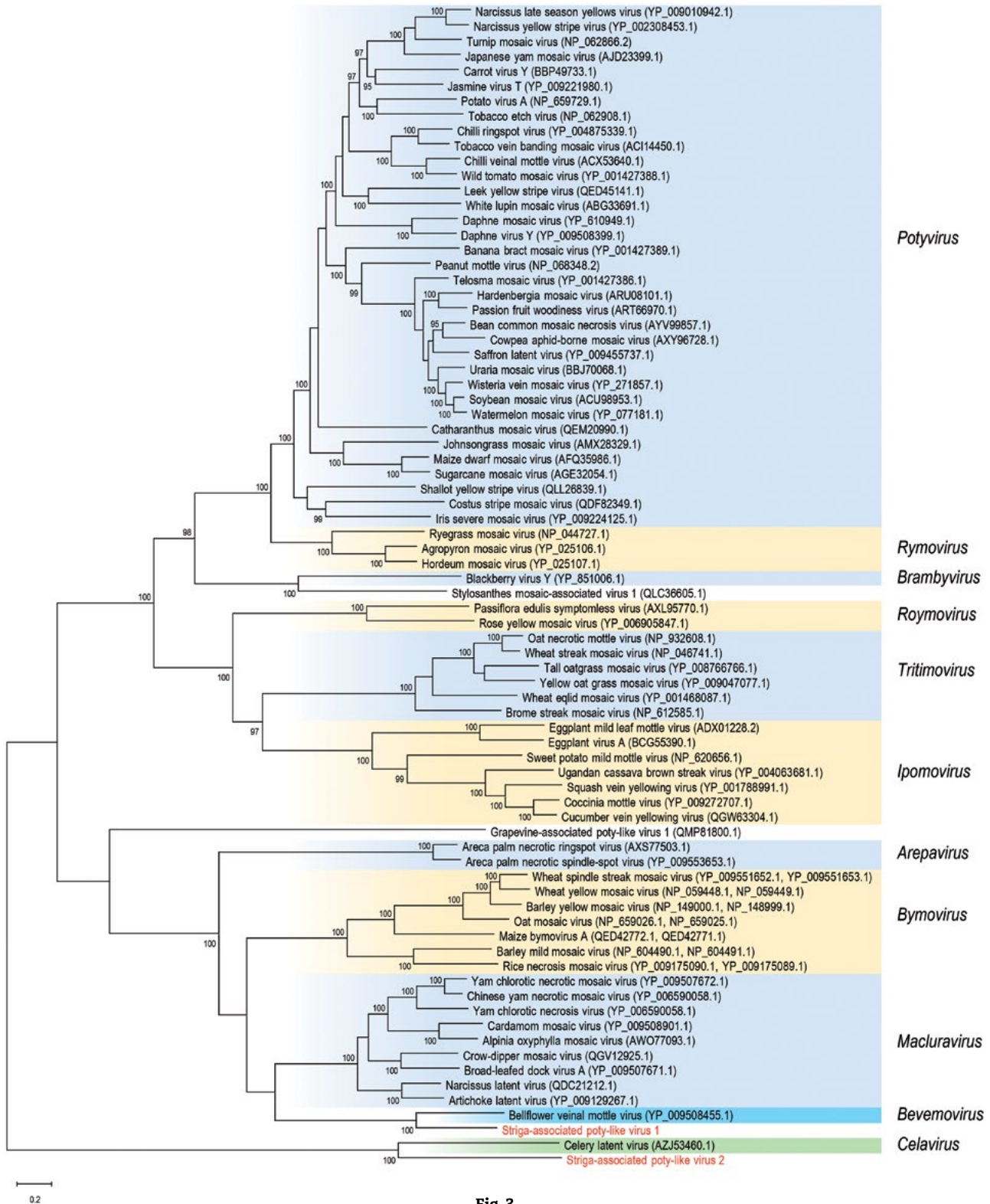
The polyprotein of potyvirids is post-translationally cleaved at conserved cleavage sites to produce mature peptides (Adams *et al.*, 2005; Revers and Garcia, 2015; Goh and Hahn, 2021; Palani *et al.*, 2021). BVMoV and macluravirus polyproteins are processed into nine mature peptides: HC-Pro, P3, 6K1, CI, 6K2, VPg, NIa-Pro, NIb, and CP (Revers and Garcia, 2015; Seo *et al.*, 2017). The HC-Pro/P3 cleavage site is self-cleaved by HC-Pro, and the remaining seven sites are processed by NIa-Pro. To predict the putative cleavage sites and mature peptides of the SaPIV1 polyprotein, the polyprotein sequences of SaPIV1, BVMoV, and nine macluraviruses were multiply aligned (Supplementary Fig. S1 and Fig. 1b).

Based on the annotated cleavage sites of three macluraviruses (narcissus latent virus, cardamom mosaic virus,

and Chinese yam necrotic mosaic virus), eight cleavage sites were predicted in the SaPIV1 polyprotein (see Fig. 1a for their positions). The predicted HC-Pro/P3 cleavage site was YIIG/G, which is highly similar to the potyvirus HC-Pro recognition sequence YXVG/G, where ‘X’ indicates any aa and the slash (/) indicates the cleaved bond (Adams *et al.*, 2005; Goh and Hahn, 2021). Seven other predicted cleavage sites, which were processed by NIa-Pro, were TKLQ/A (P3/6K1), TRLQ/F (6K1/CI), MELQ/A (CI/6K2), YTLE/G (6K2/VPg), TRLE/V (VPg/NIa-Pro), DRLQ/S (NIa-Pro/NIb), and THLQ/V (NIb/CP). When the SaPIV1 NIa-Pro recognition sites were compared with those of BVMoV and macluraviruses, two consensus sequences were deduced: XXLQ/X for five sites (P3/6K1, 6K1/CI, CI/6K2, NIa-Pro/NIb, and NIb/CP) and XXLE/X for two sites (6K2/VPg, VPg/NIa-Pro) (see Fig. 1b). The same pattern was also observed in bymoviruses (the genus *Bymovirus*), which are

**Fig. 2**

Schematic figure of the putative genome organization of SaPIV2
Predicted genomic features of SaPIV2 are presented. See Fig. 1a for details.



more closely related to BVMoV and macluraviruses than to other potyvirids (Palani *et al.*, 2021).

Potyviruses have an additional overlapping ORF termed PIPO within the P3 segment (Revers and Garcia, 2015; Gibbs *et al.*, 2020). The trans-frame fusion protein P3N-PIPO is produced by a +1A insertion in the A-tract of the GA₆ motif by polymerase slippage (Olsper *et al.*, 2015; White, 2015). There was only one GA₆ motif in the SaPIV1 genome sequence within the P3 segment (nt 1406–1412) (Fig. 1a). To examine if the +1A insertion product was present in the purple witchweed transcriptome data, RNA-Seq reads were mapped to the SaPIV1 genome sequence. There were 18 reads spanning the GA₆ motif. Among them, 17 (94.44%) were the wild-type and 1 (5.56%) was the +1A insertion product, suggesting that this position may be a polymerase slippage site.

The SaPIV1 small polyprotein sequence containing the 54-aa PIPO segment was deduced by inserting an A nucleotide within the A-tract of the GA₆ motif. When the predicted SaPIV1 and BVMoV small polyprotein sequences were compared, the P3N and PIPO boundaries of both viruses exactly matched (Supplementary Fig. S2). The PIPO segments of the two viruses exhibited high sequence similarity, further indicating that the predicted GA₆ motif was a genuine polymerase slippage site for the production of the trans-frame fusion protein P3N-PIPO.

Genome sequence of Striga-associated poty-like virus 2 (SaPIV2)

The SaPIV2 genome was 10767-nt long and predicted to encode a 3329-aa polyprotein (Fig. 2 and Table 1). The SaPIV2 polyprotein had InterPro domains that are shared by potyvirid polyproteins, including helicase, NIa protease, and RdRp domains. No HC-Pro cysteine protease domains were detected. Sequence similarity search revealed that the CeLV polyprotein (3640-aa, NCBI Acc. No. AZJ53460.1) was the most similar to the SaPIV2 polyprotein, with 44.9% identity in a 3917-aa overlap, whereas the other potyvirid polyproteins showed lower (15%–20%) sequence identities. Thus, SaPIV2 is the most closely related to CeLV among the currently known viruses.

CeLV is the sole species in the genus *Celavirus* (Rose *et al.*, 2019). Because CeLV is distantly related to other potyvirids, annotation of proteolytic cleavage sites and mature peptides of its polyprotein is highly limited. The CeLV polyprotein was assumed to be cleaved into at least seven or eight mature peptides: one or two P1-like proteases, a P3-like peptide, CI, VPg, NIa-Pro, NIb, and CP (Rose *et al.*, 2019). However, only two cleavage sites (NIa-Pro/NIb and NIb/CP) have been previously predicted. Therefore, the annotation of the SaPIV2 polyprotein is difficult.

The SaPIV2 and CeLV polyprotein sequences shared a strong sequence similarity with their N-terminal regions (Supplementary Fig. S3). The SaPIV2 polyprotein was approximately 300-aa shorter than the CeLV polyprotein at the N-terminus. There was an in-frame stop codon upstream of the SaPIV2 polyprotein ORF, indicating that the SaPIV2 polyprotein was full-length. The CeLV polyprotein was predicted to contain a signal peptide at its N-terminus (Rose *et al.*, 2019). However, no signal peptide was predicted for the SaPIV2 polyprotein. Despite the difference in their N-terminal regions, it was assumed that the SaPIV2 polyprotein may be processed into seven or eight mature peptides similar to those of CeLV. The SaPIV2 and CeLV polyproteins lacked the HC-Pro segment. Among the six or seven potential cleavage sites, two were predicted in the SaPIV2 polyprotein: LNRQ/D (NIa-Pro/NIb) and QQIQ/N (NIb/CP) (see Fig. 2 and Supplementary Fig. S3).

A putative GA₆ polymerase slippage motif for the P3N-PIPO fusion was found within the SaPIV2 P3-like segment (nt 2041–2047). A total of 191 purple witchweed RNA-Seq reads mapped to the putative GA₆ motif: 175 (91.62%) were the wild-type and 16 (8.38%) were the +1A insertion products, implying that the position was a polymerase slippage site. The predicted SaPIV2 PIPO contained 130 aa whereas the CeLV PIPO contained 189 aa. Sequence alignment of the SaPIV2 and CeLV small polyproteins with the P3N-PIPO fusion revealed that the PIPO segments of the two viruses shared many conserved residues, although their start positions were different (Supplementary Fig. S4).

Phylogenetic positions of SaPIV1 and SaPIV2 among potyvirids

To investigate the phylogenetic positions of SaPIV1 and SaPIV2 among potyvirids, polyprotein sequences of 76 selected potyvirids were collected from 12 genera of the family *Potyviridae*. In the case of the genus *Bymovirus*, which has a bipartite genome, two polyprotein sequences were concatenated. Multiple sequence alignment was generated using the MAFFT program, followed by filtering out the gap-rich segments using the trimAl program. A maximum-likelihood tree was constructed using the IQ-TREE program (Fig. 3).

SaPIV1 and BVMoV, the founding and only member of the genus *Bevemovirus*, clearly formed a strong clade with 100% bootstrap support. SaPIV2 and CeLV, the type species of the genus *Celavirus*, also formed a distinct clade with 100% bootstrap support. Phylogenetic relationships and similarities of genome organizations strongly suggested that SaPIV1 and SaPIV2 may be novel members of the genera *Bevemovirus* and *Celavirus*, respectively.

Conclusion

In conclusion, the genome sequences of SaPIV1 and SaPIV2, novel members of the family *Potyviridae*, were identified from the transcriptome data of purple witchweed. Sequence comparisons and phylogenetic analyses suggested that SaPIV1 and SaPIV2 may be novel species from the genera *Bevemovirus* and *Celavirus*, respectively. The genome sequences of SaPIV1 and SaPIV2 may be useful for studying the evolution of potyvirus genome organizations.

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Supplementary information is available in the online version of the paper.

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Two novel poty-like viruses identified from the transcriptome data of purple witchweed (*Striga hermonthica*)

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Fig. S1. Alignment of the polyprotein sequences of SaPIV1 and related viruses Putative cleavage sites are marked by vertical bars (|) and highlighted in yellow.

Table with 3 columns: Virus Name, Sequence Alignment, and Line Number. The table lists various viruses such as Striga-associated poty-like virus 1, Bellflower veinal mottle virus, and others, with their corresponding amino acid sequences and line numbers. Vertical bars and yellow highlighting indicate putative cleavage sites. A red label 'HC-Pro|P3' is present at the top right of the alignment area.

Striga-associated poty-like virus 1	VAAATYEPKLSFKTKLGPTRRVLMDIPDNINQCHQIAVCLREHLKGMNLNVEARVVRVQESKLLCMF--PASKRLLSDQEKRIAVM	1183
Bellflower veinal mottle virus (YP_009508455.1)	TECCAKYEPYRAFQITIKTASMKKVVLLRDPENINICHSVASALLKQNDVLSLLNMQTCACRDKSLLSCF--RQTRRFSMDHQNRIEAT	1187
Artichoke latent virus (YP_009129267.1)	AEAAASYKPSMLTRWAKPAKEVSNVILHVNQKNCVCEIGVVRSLIATSKLSIQSKHNNQLHRESPLACLFTKRTTLEESLKGQIKLG	1243
Narcissus latent virus (QDC21212.1)	AEAVSSYKPSMITQWAKPAKEVANVILHVNQNVHEAINVVRSLIVTQAQILSKRHAQNLHRESPLACFFAKSTTMEARLGEQIKLG	1242
Broad-leafed dock virus A (YP_009507671.1)	AKAAQAYRSTVKSRRGKPKVEAAANVIMHANQSIHDAIRVAQSLRNEAQRQIQEKERVQNLHRESPLACFSKDTVRGLDXXIGEQIQMA	1233
Crow-dipper mosaic virus (QGV12925.1)	TKALAAQYQTVKERNGKPIKEAMNVMIMHVNQENIYETLAVARVMRSEAVKSMQQRQNTIKLHRNSPLSCLFGKKTIIQLEEKLGQVRLA	1233
Alpinia oxyphylla mosaic virus (AW077093.1)	VKAVANYKNMPLTVFGQPKPRNIGLIMRVDETNVFTMRMARLLKSDYEQIILNKKAALQAQKESPMAYFLSTRVVDVSLAKLSQSIQA	1242
Cardamom mosaic virus (YP_009508901.1)	AKAVAHYKPNMVTVFGSKPAKGVSLVMKVDENVTDFLTVARLLKRDYENQISSKTAINTQRESISYLLSTRIVDDLTAKLTQQIQRA	1242
Yam chlorotic necrosis virus (AZQ00683.1)	LRCADLYKPSVLTWRGPRVQSTNVLMHVNQNVHETIRVATLHRDYSQQIHMKQHAQQLHQNSPFAYFKSTKVTVDLSTNIGKQIATA	1245
Chinese yam necrotic mosaic virus (YP_006590058.1)	ANCVKEYRPNVLTWRGPRVQSTNVLMHVNQNIHATIRIANLRYDYOQQIQKKAQQLHDKSDYFAYFSSKVVDELASINIGKQVAMA	1244
Yam chlorotic necrotic mosaic virus (YP_009507672.1)	TNCVEKYRPSVLTWRGPRVQSTNVLMHVNQNIHATIRIVNLRFDYOQQIILQKKAQQLHDKSDYFAYFSTKVVNLENNIGKQIAMA	1244

Striga-associated poty-like virus 1	KRNIQEVDKHIGKLTMYDTFVRCNVPSEFDMAEFQKVVHMELEQ ARGNNNETELKKVLDMKHSDLKVRLVHSHSGKIALGMVAMSG	1272
Bellflower veinal mottle virus (YP_009508455.1)	KRNVSKIRDHLAKLERYDFTVRDKEINGEFSHADFGSVDHMSLQ MRGEANNNEVRKLLQLEDNAQVNVSDLVTHNGDKIAIGL LATTG	1276
Artichoke latent virus (YP_009129267.1)	ERNLVLKTLKLNGLLEFMNLDEMKEEPEELSSGDMEEIGRVLLEQ MQTTTCNEHDHATVHLLEELPSTTFREAIIVGRKRACALVLMCI	1332
Narcissus latent virus (QDC21212.1)	ERNLSKLLKFAFANLEFANINVMQTELELITSSDMEEIGKVLLEQ QETTCEGHVQKVLHLEDMPTVSFREAIVVGRKRVAVALLMCI	1331
Broad-leafed dock virus A (YP_009507671.1)	RRNIEGLDKFISTLEIFAASQVGGIE-MEITSEDLSDIGRGMELQ MDTTCSENEHQVLEELPSTVFKDAIILGRQRVATAMMILCV	1321
Crow-dipper mosaic virus (QGV12925.1)	QRNLTKIDKFIGSLEMFATVNEHTDEGEFVTEPEEMDMNIGMELQ MNTTTCNEELQKVLLEELPSTVFRDAIIVGREKVMVATMILCV	1322
Alpinia oxyphylla mosaic virus (AW077093.1)	ERNIVKLTFTITNLEIFMNTANSNVE-QDMQTDLLEIIGRSMLQ MDGNLTRHALTNLKLIEDIPNVSFREAIIIGNRKSLIALSFLVC	1330
Cardamom mosaic virus (YP_009508901.1)	ERNIRKLDGFIITNLEVYANAPLNPEP-AVMGHGYYEIGRCMQLEQ MEGKLTRESMIESLQLEDIPNASLKDAIILGNRRSILALSFLAC	1330
Yam chlorotic necrosis virus (AZQ00683.1)	QRNIQKLDKFIISNLEIFVTMNEVDGD-TELTQDMHEIGQSLDLQ ADGKFSRDLTCDTLNLELSPSTTFRDAVIVGRKKAIVWIMILCC	1332
Chinese yam necrotic mosaic virus (YP_006590058.1)	QRNVSKLDKFIISRLMFATMNEMAGD-VEVTQQEMHEIGQCLDLQ AEGFTSKDNMNVLSLETLPQTTFRDAIIVGRKKAIVWIMILCC	1333
Yam chlorotic necrotic mosaic virus (YP_009507672.1)	QRNVAKLDKFIISRLMFATMNEMADD-VEVTQQEMHEIGQCLDLQ AEGSFNKENVNVILNLERLPQTTFRDAIIVGRKKAIVWIMILCC	1332

Striga-associated poty-like virus 1	AYLLSSLLHAL-----RSKEPDVAVYTL GKGGKPY-NRSKRSEAVLNQAFDM-----FEELKQRKEKRVHVKHERPS	1337
Bellflower veinal mottle virus (YP_009508455.1)	AYILGQIYAML-----RTTETITASSLE GKGGKPIRNRDRSEARAVNESFNPIE-RMRAESETQTVKKRRDKGK---QQSD	1348
Artichoke latent virus (YP_009129267.1)	AAFGLGLAWYLLWDDDTGLDNKWNKKNKAVLKDVL MKGKSF-NRDKRSA--TAYEKLLKDSYEGEDNYLDFRTRKGRRA---DADKT	1416
Narcissus latent virus (QDC21212.1)	AAFGLGLAWYLLWDDDTGLDNKWNKKNKRAVLKDVLE MKGKAF-NRDKRNP--AAEYKLLHDFAGDSLDELRFQFKMGKXK---GKQPQ	1415
Broad-leafed dock virus A (YP_009507671.1)	AAFSGLAWYMWSDDEGLDNDYKKNKQVYVEILE MKGKSF-NRDKRMP--AMQEHKMDADDYIENNPDIKFKSKRETKHR-SDGGA	1406
Crow-dipper mosaic virus (QGV12925.1)	AAFSGLAWYLLWDDDTGLDNEYNKQHEVESYTKVLE MKGKAF-NRDKRSE--ASLLAYEKADYVGENLDQVQMFKSKRGKSKMMDHSDK	1408
Alpinia oxyphylla mosaic virus (AW077093.1)	SAFAGLAWYLLWDDDEGLDNKQVAVVHNVKLE MKGKGL-NRDKRNV--AMQETDYEAESTRDEDFDRVRSRRQRAK---ETDIA	1414
Cardamom mosaic virus (YP_009508901.1)	GAFAGLAWYLLWDDDEGLDNKQVAVVHNVKLE MKGKHL-NRDKRNV--ALQDTFDEAYTARGDDEFERVRSRRKGAQ---EKDIA	1414
Yam chlorotic necrosis virus (AZQ00683.1)	AAFGLGLVWLLWDDDTGLDNEENKQRKAVCDVLE MKGKSF-NRDRRNP--MMQDTIDMADFVYMRDNEFVKLKKKNMNRADSDHKG	1419
Chinese yam necrotic mosaic virus (YP_006590058.1)	AAFGLGLAWYLLWDDDEGLDNDENKDRNEVHNVKLE MKGKSF-NRDRRNP--MMQDFHDAADFVYMRDNEFVSLRSRKRVS-VDVDS	1417
Yam chlorotic necrotic mosaic virus (YP_009507672.1)	AAFAGLAWYLLWDDDEGLNNTENKRRERDICHNVLE MKGKAF-NRDRRNP--AMQDFHDAADFVYMRDNEFQTLRSRRTSSGPNDAIS	1418

Striga-associated poty-like virus 1	VSNLLKKTNP-FVNFYDIADDSAVTEAVFKMLDGSVHKTTPPIADIPNLGRGRIIAEREMDDSPVLSWADEAERVDQLICEVTLKDG	1426
Bellflower veinal mottle virus (YP_009508455.1)	AVSRLTKTNP-FVNFYDIA-DADDETAIFSTLGGKVIHETATPVSDVKRL-DLSTNVDGDEGEPIN-LSDE-----DDLMCVYTLKDG	1428
Artichoke latent virus (YP_009129267.1)	PFENLMTKAAP-FITMYDITSDENVVAVFMDHKNQAFYETADPLKNMGEVKKHLEQMQLKQPV-FK-WGDGAN-----DDVFCOVITRDRG	1499
Narcissus latent virus (QDC21212.1)	VFNELMTKGGP-FVTMYDVTSDENVVAVFLDNTKQAFYETGNPLANMKVNRSHLEDHLLHNSKT-GL-WEISDD-----DEIFCDVTKKDG	1498
Broad-leafed dock virus A (YP_009507671.1)	PLDRFMSKPMVFNKYDIASDEKAKAIFSDENNHYFVETANPLKHMKEVEKHLNAHAKATSNL-LA-WGDGAN-----DLIYCVTKNDG	1490
Crow-dipper mosaic virus (QGV12925.1)	PLNKLFLNAEPP-FISLYDITDDNIVASVAFQDTRNQAIFYETANPLAHMKEVEEHLQKHEKGEI-IF-WADAAD-----DVTVCVITKNDG	1491
Alpinia oxyphylla mosaic virus (AW077093.1)	PVMRHMRSKPP-FITLYDLTDSDIHAFVSDHNSQAFYETANPLANLDKVKQLEHRRQGEKI-IF-WSDMTD-----DTIFMTITKDG	1497
Cardamom mosaic virus (YP_009508901.1)	PVMRHMRSKPP-FITLYDYLTDDETHAVFDHNSQAFYETANPLANLKDVEHLQSHKDDGKV-VF-WSDYAD-----DTIFMTITKDRG	1497
Yam chlorotic necrosis virus (AZQ00683.1)	LVQRMMSQSKP-FITLYDINNADIVAVFMDHKNQAFYETANPLKNMEVQRQLDHDHAKKEGGQTF-WGDGAD-----YTLFCNITKDRG	1503
Chinese yam necrotic mosaic virus (YP_006590058.1)	PVLRHMRSKPP-FITLYDINVDSEVAVAFQDHNGQAFYETANPLKNMMLVREHLNKHAKKAGDQTFI-WSDSESD-----FDIFCKITKTDG	1501
Yam chlorotic necrotic mosaic virus (YP_009507672.1)	PAMRYAMKSRP-FITLYDINVDSEVASEAFQDHNGQAFYETANPLKNMMLVRKHLLEHKQKNGTQLF-WSDSESD-----FDIFCKITKTDG	1502

Striga-associated poty-like virus 1	RRVHDMTKHRSRMMTRTGGLAGYENKDFGQFRVLE-----STRLE VASSVPFSKINIALGRMVPYIADGSRVRLNAILYGDFFIICP	1507
Bellflower veinal mottle virus (YP_009508455.1)	RDVMVLRDRHRSRMTTRTGGLAGYENKDFGQFRVLE-----TTKIE MKGPFPFTNSTKCVNVMVGLYANRPMVNCVLYGDFIVCP	1509
Artichoke latent virus (YP_009129267.1)	TTVRRLTPHSHQRLTSTHGQYGAQVDFGTGDAEVLKMP HLE VDRTRADNMNVLNMGIGLVSITLQAG-SNANCLYLKDWVIMP	1587
Narcissus latent virus (QDC21212.1)	MVLRILRTPHSHMLRTSTSGRQYSVHEDGFRQTDGAEVLKQPD ILE VDRTRLSNHNVSVEAGAMIGVITLQAG-SNANCLYLKDWIIMP	1586
Broad-leafed dock virus A (YP_009507671.1)	SVYRVRLTPHNPRLTKHHGVQGFENKFGIFRQSGQTEVLQRP QELE IATHLQTSQVNLDAVMIGTIVTVDDG-LICICLYKDFLVMP	1578
Crow-dipper mosaic virus (QGV12925.1)	TVMRVKLTPHNPRLTNTFGTMYGHNKAGQYRGTGTEFLKHP HSL IATHLQTNQNLNLDVSGMIGIAEAGGG-QINCLYKDFIIMP	1579
Alpinia oxyphylla mosaic virus (AW077093.1)	SQRQVRLTPHSHQRLTSTHGQYGAQVDFGTGDAEVLKMP HLE IGTQLTNNQANLNDLIMIGHVNIETG-RNANCLYLKDFIIMP	1585
Cardamom mosaic virus (YP_009508901.1)	TVQKVRLLTPHSHERTRHGGQGGFKEHEGQYRGTGVEVLKQ PQTELE IDTRLSNNTNLEIMNIGHIIGEG-RLHCLLYKDFIIMP	1585
Yam chlorotic necrosis virus (AZQ00683.1)	SVLRVKLTPHIPSKTSTGGAGQFQNKADCYRGTGTEVFLVQ TQALE IDTRLPVNNINLDVANMIGKVRMANG-TLHCLYKDFIIMP	1591
Chinese yam necrotic mosaic virus (YP_006590058.1)	TIMKVKLTPHPLMARR-GTQGFVEKEDCYRGTGQAEVLQHP GNVLE MATRLPENKLNLDVADMIGKVSMSSEG-TIHCILYKDFIIMP	1588
Yam chlorotic necrotic mosaic virus (YP_009507672.1)	TVMKVKLTPHPTKRSKH-GTQGFNSMEDCYRGTGQAEVLHHP TQALE MATRLPDNKNLQIADVMGRVTMSEG-TIHCILYKDFIIMP	1589

Striga-associated poty-like virus 1	AHTQLVSLPITFRFHATCTVDHV-EAMKFEKCDLILIKRPNISAPVVKVSKATTITEPTLIQIIYRDLGSGFSCHVASDADLAVPFDNFRG	1596
Bellflower veinal mottle virus (YP_009508455.1)	AHILQVSLPITFRFHATCTCF-VVEYMAFTQCDLILIKRPREIAPVAVIAKCGVLTPEPSYQLAYRNGVLSASDICTPFDNFRG	1598
Artichoke latent virus (YP_009129267.1)	AHVMCKLPITLTFKHYTVTLSTLPECYSFVGFDLIAIKRPSLAPVRCASLTESAKSGMTVQMLKYPVINKLMTVTDVAVYKTEHRW	1677
Narcissus latent virus (QDC21212.1)	AHIMMRLLPITLTFKHYTCTLSEPECYSFVGFYDYLIRRRPRLAPVRCYATLDFAKDGMTIQMHKPKVNVKLVMTAAEAYRTEHRW	1676
Broad-leafed dock virus A (YP_009507671.1)	AHVMCKLPITLTFKHYTCTVTVVTEPEMYSFVGFYDYLIRRRPSELAPKICQAHCGTADHGDVQMLKYPVINKLMTVTDVAVYKTEHRW	1668
Crow-dipper mosaic virus (QGV12925.1)	AHVMCKLPITLTFKHAHVIVSELQYVSPFGFDLIVKRRPTLPPVKCVAYCGIAHENMVMQMLKYLKSHKLVTTITAPITHQDREHRW	1669
Alpinia oxyphylla mosaic virus (AW077093.1)	AHVMKQELPITLTFKHYTVKVEELVEYVAFEGFDLILKMRPHQLAPVRCVAVSQAQEAIVQVHVKHFTLVKPVITIAATHQTEHRW	1675
Cardamom mosaic virus (YP_009508901.1)	AHVMKQELPITLTFKHYTVKVEELVEYVAFEGFDLILKMRPQLAPKICASLATAQEGMIVQMLKHKFLTKNPITVITAPITHSELHRW	1675
Yam chlorotic necrosis virus (AZQ00683.1)	AHAMSKLPITLDFLKHKTITLSEFPEAYCFPGFDLILKRPSTLAPVACRATVAQASDGMIVQVHVKHFTLVKPVITVAPITHQDREHRW	1681
Chinese yam necrotic mosaic virus (YP_006590058.1)	AHAMIKQLPITLDFLKHKTITLSEFPEAYCFPGFDLILKRPSTLAPVACRATVAQASDGMIVQVHVKHFTLVKPVITVAPITHQDREHRW	1678
Yam chlorotic necrotic mosaic virus (YP_009507672.1)	AHAMITKLPMEITFKHYTKIGTLPAYCFPGFDLILKRPSTLAPTRCHATLATATDGMIVQMLHKKSVSDKTLTITAPITHQDREHRW	1679

Striga-associated poty-like virus 1	SYSVSTKDGMCAGIIVDTKGMITGIHVSANDLIKRNFMPEPSTSMVDVLQKAPFVRDE-----VMYFDSIASGAPHE DR Q S-	1677
Bellflower veinal mottle virus (YP_009508455.1)	TYTISTKKGCCGAIVIELTKGKFGVGHVANSVIRRNFMPEPSTSMVDVLQKQKEDFZPSR-----DWTFTTECCYMPG E SGLQ A-	1680
Artichoke latent virus (YP_009129267.1)	EHOIPTIAGMCGAPMLDVSSGKIVGHLGDKTARKNCTFEFPPDMLKLVNSNDPKVRLNFRERVKTEFVPEMHGHNSARI KNLQ I-	1765
Narcissus latent virus (QDC21212.1)	EHOIPTIAGMCGSPVFDVASKIVGHIILGIMKRNFTFAEFGDMKLVNSNDPKIKDLYKRNLEWFFAEVHGHDSRRL INLQ I-	1764
Broad-leafed dock virus A (YP_009507671.1)	AHQIPTIAGMCGAPMLDVVTGKIVGHIHVMGDGKLVNHFVFAEFPSEAIITMGNTDRKLVHAYFIRKNLVNHFVPEMHGYSRRL INLQ I-	1756
Crow-dipper mosaic virus (QGV12925.1)	AHQIPTIAGMCGAPMLDVATGKIVGHIHVMGDGKLVNHFVFAEFPSEAIITMGNTDRKLVHAYFIRKNLVNHFVPEMHGYSRRL INLQ I-	1758
Alpinia oxyphylla mosaic virus (AW077093.1)	AHQIPTIAGMCGAPMLDVGTGKIVGHIHVMGDGKLVNHFVFAEFPSEAIITMGNTDRKLVHAYFIRKNLVNHFVPEMHGYSRRL INLQ I-	1763
Cardamom mosaic virus (YP_009508901.1)	AHQIPTVAGMCGAPVDTGKIVGHIHVMGDSFKKHNVFETPFDALIELNTNDKIHQRYISQKIKWTFIPETHGYDPRKI QGLQ I-	1763
Yam chlorotic necrosis virus (AZQ00683.1)	AHQIPTVAGMCGAPVLEVATGKIVGHIHVMGDTFMKNHNVETFNNDIMTLMNTNDKIIISTFQRSKLSWTFMPEVHGYDPPK KNLQ I-	1769
Chinese yam necrotic mosaic virus (YP_006590058.1)	AHQIPTVAGMCGAPVIDVASKIVGHIHVMGDSFKKHNVFETPFDALIELNTNDKIHQRYISQKIKWTFIPETHGYDPPK KNLQ I-	1766
Yam chlorotic necrotic mosaic virus (YP_009507672.1)	AHQIPTVAGMCGAPVIDVASKIVGHIHVMGDSFKKHNVFETPFDALIELNTNDKIHQRYISQKIKWTFIPETHGYDPPK KNLQ I-	1767

Fig. S2. Alignment of the “small” polyprotein sequences of SaPIV1 and BVMoV
The PIPO segment is highlighted in cyan.

Striga-associated poty-like virus 1	MDYLALTRIVANRQIDMPRVLELAIKEVYGTYNRELNSAIDFK---GEQLETTTPATTDPLDVFIKNELKEINDAAGDCGKIACVRPRTL	87
Bellflower veinal mottle virus (NC_039002)	MDPLFLKRFNMDGGKLIQICAPISDALKAAAYNNHGVDIKSSYAVTAPCGTVISVENSTNEVLKDFVEAGMKFNDDVAGECPKGCQNEASKG	90
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Striga-associated poty-like virus 1	PEGFYATMS--YAMGGLKGVIPGPVSKRRRCFRDGYCYMNIFFVAIAPHVYDEDALVYAKFLNDCPLVLGWSPTMNSLAKAMIWIGQKIPNL	175
Bellflower veinal mottle virus (NC_039002)	RSSWFLNCSPTLAVGGRSATFIMPSPRRVFSFGYCYLNLAVAMSPYIFDEDAIKFAEFLHDLPIVLGKWPAMVKVARSFAWLLQHMPYL	180
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Striga-associated poty-like virus 1	YNKAVPAVSISHTAEACHVVDQRGPVKGWHILDAPTFRDVIILFSLKSDLSEYIIGG-DPVTAAVMHLSKICIRHANNRQPWVTFNKMVLE	264
Bellflower veinal mottle virus (NC_039002)	CDRHIPHISINHNLNAAHVSDQRGPMIGCHILNAYTLKDFVLTGMDTLNANTMLGGMDALTSAYQSLDKAIMQANKSFQPWLTFRMICA	270
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Striga-associated poty-like virus 1	DSALAATLVLPATLILKLESWFEDREERLIVATIESVGDALTRIIIVIKRAIEGVKIHFLDNDIETATAALHQRVLEYIRNADDGGA--	352
Bellflower veinal mottle virus (NC_039002)	DSMLAATLIMSPATLQKLESWFEDDEGFRRVVATLETTVSDALLRMVVIKRAIHGVMHFRTDNVERSTNELHRAIIAYIDSQDDQSAKD	360
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Striga-associated poty-like virus 1	LNAATRFSNLVTEKKRAVLLFRKTDLSLTFRFSLATRARFCYIYRRKLLYMSYSHVAKHFTISRKTRS-----	421
Bellflower veinal mottle virus (NC_039002)	LGDAARRFETMALEKKDAIRVLRKFN SPLFGLSLASRARFQLYIMWRKFFVFAAIKARKLYTCNSAPRAEGTAARITHRGQIHMGHLLV	450
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Striga-associated poty-like virus 1	-----	421
Bellflower veinal mottle virus (NC_039002)	RASSKPHVAGVVFN	464

