

Novel *Foveavirus* (the family *Betaflexiviridae*) species identified in ginseng (*Panax ginseng*)

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Summary. – Ginseng (*Panax ginseng*) is a valuable herb that is widely cultivated in Korea, China, and Japan because it contains a variety of pharmacologically active substances with a wide range of positive effects on human health. Identification and prevention of disease-causing viral pathogens of ginseng is important for improving the yield and quality of ginseng-derived bioactive molecules. In this study, the genome sequence of the virus Panax ginseng flexivirus 1 (PgFV1) was identified from a ginseng root transcriptome data set. Sequence comparison and phylogenetic analysis showed that PgFV1 is a novel plant RNA virus species of the genus *Foveavirus* (the family *Betaflexiviridae*). Foveaviruses have flexuous and filamentous virions with a single-stranded positive-sense mono-segmented RNA genome. Its infection causes diseases with mosaic and ringspot symptoms in the stems and leaves. The PgFV1 genome encodes for 5 open reading frames: a replicase polyprotein for viral genome replication, 3 triple gene block proteins for viral cell-to-cell movement, and coat protein. Phylogenetic trees inferred from replicase polyprotein or coat protein sequences showed that PgFV1 is most closely related to grapevine virus T. PgFV1 is the first foveavirus identified to be associated with ginseng. Given the potential pathogenic features of previously known foveaviruses and importance of ginseng in the health industry, the PgFV1 genome sequence may be highly useful for studying ginseng foveaviruses.

Keywords: ginseng; *Panax ginseng* flexivirus 1; *Foveavirus*; *Betaflexiviridae*

Introduction

Ginseng (*Panax ginseng*) is a slowly growing perennial plant belonging to the genus *Panax* of the family *Araliaceae*, which is widely cultivated in Korea, China, and Japan. Ginseng has long been used as a valuable herb in oriental medicine because it contains various pharmacologically active substances such as ginsenosides (dammarane-type tri-

terpenoid saponins) and gintonin (Briskin, 2000; Yun, 2001; Baeg and So, 2013). Ginseng is known to have a wide range of positive effects on human health including preventing some types of cancers, improving diabetes and vascular diseases, enhancing host immunity, and protecting against some virus infections (Vuksan *et al.*, 2010; Kim and Park, 2011; Im *et al.*, 2016). Several comprehensive transcriptomic analyses using next-generation sequencing technology have been performed to understand the underlying molecular genetic mechanisms of the beneficial traits of ginseng (Jayakodi *et al.*, 2015; Wang *et al.*, 2015; Zhen *et al.*, 2015; Jo *et al.*, 2017a; Xu *et al.*, 2017; Zhang *et al.*, 2017).

Identification of pathogens that cause disease in ginseng is important for improving the quality and yield of ginseng-derived substances. For example, fungal pathogens, such as *Cylindrocarpon destructans* and *Fusarium solani*, cause root rot of ginseng and lead to yield losses (Ohh, 1981; Jang *et al.*, 2010). Several viruses, such as a *Closterovirus* species (the

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Abbreviations: ASPV = apple stem pitting virus; CP = coat protein; GRSPaV = grapevine rupestris stem pitting-associated virus; GVT = grapevine virus T; ORF = open reading frame; PgFV1 = Panax ginseng flexivirus 1; PVM = potato virus M; REP = replicase polyprotein; RdRp = RNA-dependent RNA polymerase; sgRNA = subgenomic RNA; SRA = Sequence Read Archive; TGB = triple gene block

family *Closteroviridae*) and watermelon mosaic virus (the genus *Potyvirus*, the family *Potyviridae*), have been identified to infect or be associated with ginseng (Mishchenko *et al.*, 2009; Jung *et al.*, 2013; Park *et al.*, 2017).

Application of next-generation sequencing methods has been demonstrated to be a rapid, efficient, and inexpensive method for the detection and characterization of viral sequences (Barba *et al.*, 2014). When transcriptomic analyses of plant samples are performed, genome sequences of plant RNA viruses present in the sample may be isolated together with host RNA molecules. Thus, RNA virus genome sequences can be found in plant transcriptome data deposited in publicly available databases such as the Sequence Read Archive (SRA) or Transcriptome Shotgun Assembly databases of the National Center for Biotechnology Information (NCBI) (Liu *et al.*, 2012; Jo *et al.*, 2017b). In this study, a ginseng transcriptome dataset was analyzed to identify RNA viruses that potentially infect ginseng (Wang *et al.*, 2015). As a result, a genome sequence of a novel *Foveavirus* species was found and annotated.

Materials and Methods

RNA-seq assembly. A previously published ginseng transcriptome data set (SRA accession number SRP066368) was analyzed (Wang *et al.*, 2015). The data set contained approximately 26.5 gigabases of paired-end reads obtained from 18 RNA samples, which were prepared from various tissues of ginseng at different ages. High-quality sequences were collected by filtering raw RNA-seq reads using the sickle program (version 1.33; <https://github.com/najoshi/sickle>) with the option “-q 30 -l 55.” The SPAdes Genome Assembler (version 3.10.1; <http://spades.bioinf.spbau.ru>) with the “--rna” option was used for *de novo* assembly of RNA-seq reads (Bankevich *et al.*, 2012). Eighteen transcriptome data sets were separately assembled into contigs.

Detection of virus-derived contigs. All RNA sequence contigs were analyzed to detect potentially virus-derived contigs containing a viral RNA-dependent RNA polymerase (RdRp) motif. The reference sequences of viral RdRps were prepared from the Pfam database (release 31.0; <http://pfam.xfam.org>). Pfam accession numbers for viral RdRp motifs were PF00602, PF00603, PF00604, PF00680, PF00946, PF00972, PF00978, PF00998, PF02123, PF03431, PF04196, PF04197, PF05788, PF05919, PF07925, PF08467, PF08716, PF08717, and PF12426. A total of 394 non-redundant viral RdRp motif sequences were collected and converted into a custom-built BLAST-searchable database. BLASTX searches were performed against RdRp motif sequences using assembled contigs as queries with the parameter “-evaluate e-5.” Mapping of RNA-seq reads to a viral genome contig was carried out using BWA program (version 0.7.16a-r1181; <https://github.com/lh3/bwa>) with the “mem” method (Li and Durbin, 2009). Sequence variants were

analysed using SAMtools/BCFtools programs (version 1.6; <http://www.htslib.org>) (Li *et al.*, 2009).

Multiple sequence alignments. The NCBI BLAST (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>) searches were performed to identify and collect closely related viruses. Open reading frames (ORFs) were predicted based on BLASTX searches and ORF finder analysis (<https://www.ncbi.nlm.nih.gov/orffinder>). Functional domains of viral proteins were identified by using Pfam and TMHMM (version 2.0; <http://www.cbs.dtu.dk/services/TMHMM>) (Sonnhammer *et al.*, 1998). Pair-wise identities among protein sequences were calculated using the FASTA program (version 36.3.6; https://fasta.bioch.virginia.edu/fasta/www2/fasta_down.shtml).

Multiple alignments of protein sequences were generated using MUSCLE software (version 3.8.425; <https://www.drive5.com/muscle>) (Edgar, 2004). A phylogenetic relationship among viruses was inferred using the neighbor-joining method implemented in the ClustalW2 program (version 2.1; <http://www.clustal.org>) (Larkin *et al.*, 2007).

Results and Discussion

A 9002 nucleotide (nt) long contig isolated from a 12-year old ginseng root sample (SRA accession number SRR2952882) showed amino acid (aa) sequence similarity with the RdRp motif sequence of apple stem pitting virus (ASPV) (UniProt Acc. No., Q64962; Pfam Acc. No., PF00978). ASPV is the type species of the genus *Foveavirus* of the family *Betaflexiviridae* (Jelkmann, 1994), suggesting that the putative viral contig was derived from a foveavirus or related virus.

A BLASTX search against the non-redundant protein database using the NCBI BLAST confirmed that the putative viral contig was related to foveaviruses, including grapevine virus T (GVT) (Jo *et al.*, 2017b) and grapevine rupestris stem pitting-associated virus (GRSPaV) (Meng *et al.*, 1998). The contig was considered a novel ginseng RNA virus of the genus *Foveavirus* of the family *Betaflexiviridae* and named as Panax ginseng flexivirus 1 (PgFV1). The PgFV1 genome sequence with annotation information is available in the NCBI nucleotide database under Acc. No. MH036372.

The ginseng root RNA-seq reads (SRA Acc. No., SRR2952882) were mapped to the PgFV1 genome contig. A total of 22,513 reads were mapped to the PgFV1 genome. There were only two single-nucleotide polymorphism sites at nt positions 52 and 55, indicating that the PgFV1 genome contig was assembled from a highly homogeneous viral population. The two single-nucleotide polymorphism sites were in the 5'-untranslated region of the virus genome.

Viruses in the genus *Foveavirus* (the family *Betaflexiviridae*) have flexuous and filamentous virions with a single-stranded positive-sense mono-segmented RNA genome

Table 1. ORFs and functional domains of PgFV1

Protein	nt position	nt length	aa length	Domain	Pfam	aa position
Replicase polyprotein (REP)	57-6476	6420	2139	Viral methyltransferase	PF01660	43-354
				Carlavirus endopeptidase	PF05379	1163-1250
				Viral (superfamily 1) RNA helicase	PF01443	1334-1599
				RNA-dependent RNA polymerase	PF00978	1711-2130
Triple gene block protein 1 (TGB1)	6559-7224	666	221	Viral (superfamily 1) RNA helicase	PF01443	24-221
Triple gene block protein 2 (TGB2)	7228-7575	348	115	Plant viral movement protein	PF01307	4-107
				Transmembrane		12-29
				Transmembrane		71-93
Triple gene block protein 3 (TGB3)	7502-7738	237	78	7 kDa viral coat protein	PF02495	2-63
				Transmembrane		7-26
Coat protein (CP)	7755-8633	879	292	Viral coat protein	PF00286	110-247

of approximately 8.4–9.3 kb (Martelli and Jelkmann, 1998; Martelli *et al.*, 2007). The natural hosts of foveaviruses include dicotyledonous woody plants such as grapevines, apple trees, peach trees, or apricot trees (Jelkmann, 1994; Meng *et al.*, 1998; James *et al.*, 2007; Youssef *et al.*, 2011). In many cases, foveavirus infection causes diseases manifested as mottle, mosaic, and ringspot symptoms in the stems and leaves. Foveaviruses are transmitted via grafting and no biological vectors have been reported.

The PgFV1 genome sequence was predicted to contain 5 ORFs (Table 1 and Fig. 1, top), which are commonly shared with other foveaviruses (Jelkmann, 1994; Martelli and Jelkmann, 1998; Martelli *et al.*, 2007). ORF1, which is the longest ORF, encodes a 2139 aa long replicase polyprotein (REP). REP was predicted to contain 4 known domains: a viral methyltransferase, carlavirus endopeptidase, viral RNA helicase, and RdRp.

The next 3 ORFs (ORF2, ORF3, and ORF4) encode for triple gene block protein 1 (TGB1), triple gene block protein 2 (TGB2), and triple gene block protein 3 (TGB3), respectively, which constitute tripartite movement proteins involved in the cell-to-cell movement of viruses (Kalinina *et al.*, 2002; Rebelo *et al.*, 2008). The sizes of these proteins are 221, 115, and 78 aa, respectively. TGB1 contains a viral RNA helicase domain, which shows approximately 17.5% aa sequence identity with the viral RNA helicase domain of REP. The two RNA helicase domains belong to the same Pfam domain family PF01443. TGB2 has a plant viral movement protein domain and TGB3 has a 7 kDa viral coat protein domain. Transmembrane domain prediction using the TMHMM showed that the TGB2 and TGB3 proteins contain 2 and 1 transmembrane domains, respectively. The presence of transmembrane domains in these proteins agrees with a previous report showing that these two proteins are membrane-associated and are essential for viral mobilization (Rebelo *et al.*, 2008).

ORF5 encodes a 292 aa long coat protein (CP). CP contains a viral coat protein domain that is shared with coat proteins of other *Betaflexiviridae* viruses such as members of the genera *Potexvirus* and *Carlavirus* (Rupasov *et al.*, 1989; Querci *et al.*, 1993).

ORF1 (REP) of the family *Betaflexiviridae* is directly translated from genomic RNA. In contrast, the other ORFs are translated from subgenomic RNAs (sgRNAs), which were transcribed from genomic RNA (Martelli *et al.*, 2007). It is thought that foveaviruses generate two sgRNAs, one for the 3 TGB proteins and another for the CP, although their presence in the plant cells has not been confirmed (Fig. 1, arrows). When RNA-seq reads isolated from the ginseng root sample (SRR2952882) were analyzed, a total of 22,513 reads were found to be derived from PgFV1. Interestingly, the sequencing depth plot of the PgFV1 genome showed an elevated sequencing depth at the 3' region, which approximately coincides with putative sgRNA segments (Fig. 1, graph). The observed read depth elevation suggests the presence of sgRNAs in ginseng cells. However, it is also possible that the pattern resulted from biased cDNA synthesis because of a local RNA structure or the experimental procedure employed.

For phylogenetic analysis of PgFV1, a BLAST search against the NCBI protein database was performed using the PgFV1 genome sequence as a query. A total of 11 related viral genomes were collected, including GVT, GRSPaV, peach chlorotic mottle virus (PCMV), apricot latent virus (ApLV), ASPV, apple green crinkle associated virus (AGCaV), rubus canadensis virus 1 (RuCV1), Asian prunus virus 1 (APV1), Asian prunus virus 2 (APV2), Asian prunus virus 3 (APV3), and potato virus M (PVM). The first 10 viruses are members of the genus *Foveavirus*. PVM, the type species of the most closely related genus *Carlavirus*, was included as an outgroup (Rupasov *et al.*, 1989).

Protein sequence identities between PgFV1 proteins and their respective orthologs of related viruses were calculated

Table 2. Protein sequence comparison of the REP, TGB1, TGB2, TGB3, and CP of PgFV1 and related viruses

No.	Acronym	Full name	RefSeq	REP	TGB1	TGB2	TGB3	CP
1	GVT	grapevine virus T	NC_035203.1	1282/2147 (59.7%) ^a	144/221 (65.2%)	69/115 (60.0%)	33/78 (42.3%)	157/219 (71.7%)
2	GRSPaV	grapevine rupestris stem pitting-associated virus	NC_001948.1	1146/2170 (52.8%)	137/221 (62.0%)	53/115 (46.1%)	24/74 (32.4%)	125/245 (51.0%)
3	PCMNV	peach chlorotic mottle virus	NC_008992.1	980/2171 (45.1%)	94/221 (42.5%)	55/115 (47.8%)	19/62 (30.6%)	78/208 (37.5%)
4	ApLV	apricot latent virus	NC_014821.1	979/2208 (44.3%)	96/222 (43.2%)	60/114 (52.6%)	23/64 (35.9%)	74/191 (38.7%)
5	ASPV	apple stem pitting virus	NC_003462.2	965/2216 (43.5%)	93/222 (41.9%)	55/114 (48.2%)	28/64 (43.8%)	71/193 (36.8%)
6	AGCaV	apple green crinkle associated virus	NC_018714.1	956/2216 (43.1%)	91/222 (41.0%)	55/114 (48.2%)	23/68 (33.8%)	68/202 (33.7%)
7	RuCV1	rubus canadensis virus 1	NC_019025.1	887/2208 (40.2%)	82/231 (35.5%)	42/108 (38.9%)	24/67 (35.8%)	91/286 (31.8%)
8	APV2	Asian prunus virus 2	NC_028868.1	887/2201 (40.3%)	78/203 (38.4%)	47/106 (44.3%)	26/67 (38.8%)	65/193 (33.7%)
9	APV1	Asian prunus virus 1	NC_025388.1	873/2202 (39.6%)	77/204 (37.7%)	45/106 (42.5%)	25/66 (37.9%)	87/289 (30.1%)
10	APV3	Asian prunus virus 3	NC_028975.1	872/2201 (39.6%)	76/203 (37.4%)	46/106 (43.4%)	23/66 (34.8%)	67/193 (34.7%)
11	PVM	potato virus M	NC_001361.2	816/2158 (37.8%)	89/222 (40.1%)	36/106 (34.0%)	15/52 (28.8%)	84/288 (29.2%)

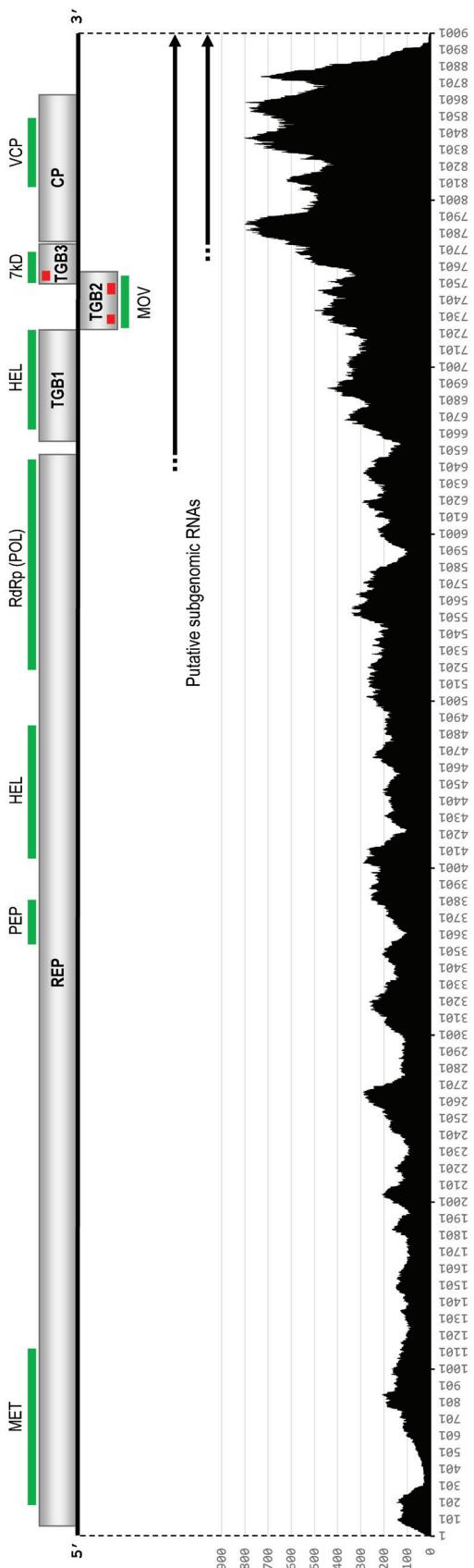
^aAmino acid sequence identities in a format of “identical residues/aligned length (% identity).”

Fig. 1

Schematic representation of PgFV1 genome organization

At the top, ORFs are depicted as gray boxes: replicate polyprotein (REP), triple gene block 1 (TGB1), triple gene block 2 (TGB2), triple gene block 3 (TGB3), and coat protein (CP). Domains identified by Pfam are marked by green lines: viral methyltransferase (MET), carlavirus endopeptidase (PEP), viral RNA helicase (HEL), RNA-dependent RNA polymerase (RdRp or POL), plant viral movement protein (MOV), 7 kDa viral coat protein (7kD), and viral coat protein (VCP). Predicted transmembrane domains of TGB2 and TGB3 are indicated by red lines. Coordinates and lengths of ORFs and domains are presented in Table 1. Arrows indicate putative subgenomic RNAs, which were presumed based on other foveavirus genomes. Graph represents sequencing depth of the PgFV1 genome contig. The X-axis represents genomic coordinates and Y-axis represents sequencing depth towards the 3'-end of the PgFV1 genome.

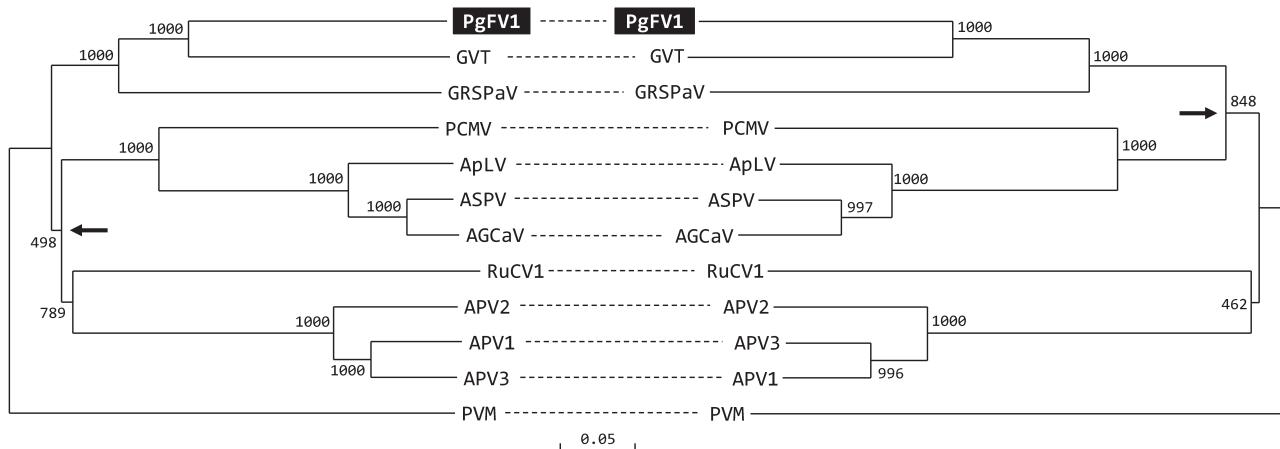


Fig. 2

Phylogenetic tree of PgFV1 and related viruses

Phylogenetic trees were inferred from multiple alignments of REP (left) and CP (right) sequences. Trees were rooted using sequences of PVM, the type species of the genus *Carlavirus*. The node showing a topology discrepancy between two trees is marked by an arrow in each tree. The bootstrap values obtained from 1000 replicates are shown at the nodes. Full names and accession numbers of viruses are presented in Table 2.

(Table 2). The highest aa sequence identity of the PgFV1 REP was found with the GVT REP (59.7% identity over 2147 aa overlap) and the second highest with the GRSPaV REP (52.8% identity over 2170 aa overlap). REP proteins of the remaining viruses showed lower identities (37–45%) with the PgFV1 REP.

The other PgFV1 proteins (TGB1, TGB2, TGB3, and CP) also showed the highest identities with respective proteins of GVT, indicating that GVT is the most closely related known virus to PgFV1. The sequence identity between the PgFV1 CP and GVT CP was 71.7% over 219 aa overlap. The CP or REP protein sequence identity threshold for assigning foveaviruses to different species is approximately 80% (Adams *et al.*, 2004), confirming that PgFV1 is a novel species.

Multiple sequence alignment of PgFV1 proteins with the respective orthologs of 10 other foveaviruses and 1 carlavirus revealed conserved regions mainly corresponding to known functional domains (Supplementary Figs. 1–5). Phylogenetic trees inferred from REP and CP sequences confirmed that PgFV1 is a member of the genus *Foveavirus* and formed a subclade with GVT and GRSPaV (Fig. 2). Phylogenetic tree topologies from two proteins were nearly the same except for a difference of the sister clade from the PgFV1/GVT/GRSPaV subclade. The tree inferred from REP sequences showed that PgFV1, GVT, and GRSPaV formed a sister clade of all other foveaviruses (Fig. 2 left). However, the tree from CP showed that the PgFV1/GVT/GRSPaV subclade was a sister clade of the PCMV/ApLV/ASPV/AGCaV subclade (Fig. 2 right). This discrepancy may be related to ancestral recombination events which have been reported for several foveaviruses (Komorowska *et al.*, 2011; Glasa *et al.*, 2017).

In conclusion, the genome sequence of PgFV1, a novel member of the genus *Foveavirus* of the family *Betaflexiviridae*, was identified from a ginseng root transcriptome data set. Sequence comparison and phylogenetic analysis indicated that PgFV1 is most closely related to GVT. PgFV1 is thought to be the first foveavirus found to be associated with ginseng although no disease symptoms have been confirmed. Given the important status of ginseng in the health industry and tendency of foveaviruses to cause diseases, further studies of PgFV1 and other foveaviruses potentially associated with ginseng are needed. The genome sequence of PgFV1 reported in this report may be useful for investigating ginseng foveaviruses.

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

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Supplementary information

Novel *Foveavirus* (family *Betaflexiviridae*) species identified in ginseng (*Panax ginseng*)

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Fig. S1. Multiple sequence alignment of replicase polyprotein sequences of PgFV1 and related viruses

Fig. S1. Continued

PgFV1	386	KNFLNEMISLFPSCVGRILFSIIEEKCIADFIYDLKPKEIIVKTVAVK--RDFEDELHFLSMT-----DPNPDYDIAKID
GVT	386	KDCKNFLIGLFPGFISRMQIYIKDKCISDFIYDLEPENBYVDIVDVK--GDSDEFEWDFDTH-----DLLDSFDVSEGID
GRSPaV	387	KLVISFFCRMPNAARLSSSRECSLSDSFVYSLEPFNSVNLVDIT--PDSFEHLFLFSCL-----NELIEEDVEEVMD
PCMV	398	KLIISNEFIGLFPDSVARNEKFVQOQMSLDNFISEMEENESVOTSTIS--LSLDDIREVDEN-----FDIKPVIDLMLF
ApLV	388	RIVQGFFLKLFPNDIARNFKVVQQQLHLDNFIELTLEDIFSECVPTEETLT--LDKDDLEFVNLT-----FGETDFDIDESF
ASPV	388	RIVQGFFLKLFPNPISRNFKVVQQQLHLDNFIELTLEEFNSINTESIS--LNKDDLEFVNLT-----FGDTDFNVEDSF
AGCaV	388	RIVQGFFLKLFPNPISRNFKVVQQQLHLDNFIELTLEEFNSINTEDIS--LNKDDLEFVNLT-----FGDIDFNVEESEF
RuCV1	388	KFLHSLMSIFPSSWVRNENSEQETILDENISSLAFLRVRELK--K--LDSDDL-FSDIGLSHEEEFPSIPFDPBQLM
APV2	386	KLIIGALFASFLPDKMARFFDCMKSSSLDKFIHDLPEPSFSVPTEVIN--CNTMPAFATSLG-----ESMSPEVVIQIL
APV1	387	KLIIGSLFSSFLPDKMARFFDCMKACSLDKFIHDLPEPSFSVETKVIN--SRSNPMEAVTIG-----ETMSSEVVLKIL
APV3	386	KLIIGALFASFLPDKMARFFECMKACSLDKFIHDLPEPSFSVETKVIN--CRSQTFEAVTIG-----ETMGPEVVMQID
PVM	387	KEFMGNWLGKMPSVIARRSSVRAVCVNEKIRGLKPSFHLRLNEITWWNIENSYAWFF-----DTDAEVDVPEKID
consensus	401	...
PgFV1	458	DDWVFGVDKSA--DRIAQRYCINSQFWRISRLRIFYSKFEC-----SAYSICLDHFIFDCPGLNFEIVINFRSV
GVT	458	DMKVTIKNWNN--TRSPQSYFIGGREWESSSSKFYKFQAA--FERGD-YYLYLQDL-----SHDMVNEEVVRR
GRSPaV	459	DNSFGLGLDLQF--NRQRAPPFIGSSYWLNKSFVSEHKFSG--TINSQIMQVILSLIPFSDDPFRPSSTEVNIALS
PCMV	470	QKGWGLVKEVTLI--ERSREPYSFYKDSDYDAQFTSVPSTN--FEGFAAISYIIRS-----KPLLPCSWYFENVRDV
ApLV	460	DSTWGVKKDHVNITTVVHHAPYKSKHEAYEYQFHSILNDS--TAALTR-FAKIVVSLY-----SVSLVESYDCSKLCSL
ASPV	460	AEATGKTKDVKVNITTVVHSPYLVSKEEYDHQEHILSLSKS--ISALTR-TAKIVLSLY-----SVSLVESYDCSKLCSL
AGCaV	460	ASAAGTRKDVVDTTVVHSPYLVSKEEYDHQEHILSNTS--TTAATK-TAKIVLSLY-----DPCVVGAFSECRVSNL
RuCV1	463	PS-E SIGRSKKAIPDRVPSPYNNLIE-----HGAEP-VDTRDKSVLIO-YHCVFNAF-----SNSDGFYVSSRTFFSV
APV2	458	NRMEMG-----AFAGSCNLGKFKYSNEAYHCHHYLDGCKPEVFKR-IIRVAGSF-----CNEFGVYRKSSYVASV
APV1	459	AKLDMG-----APTSLEGTLGKFKYSSEAYHGHHYLDGCGPHVFR-LVRKIADS-----CNEFGIYRKSSYVSSV
APV3	458	ERMEELS-----APVRLENLTGKFKYSSEAYHGHHYLDGCGPHVFR-LVRKIADS-----CNEFGVYRKSSYVSSV
PVM	459	DSLEMGEGLAGLVAHITSRPYGTVPILADREWNALCDNSQKLLAHMRMFMGAWGAHMCVISREFLLKYVEARIKSS
consensus	481	...
PgFV1	529	--I KARKL----DPQLCYVINDD--FFDVFSRKLISR--KLRDRLNKVDARVDICWF--KLNRVNSKFI-SFNTGK
GVT	526	RDFLRRRG----TESLAYMISCNLDI--TALKKTVN--WTLEARKEN-LRVRVRPQWF--LLVGRINTKFI-CESSSS
GRSPaV	532	E KAALEAT----GQSCLKERFLVDDCAMREV--RSS-KVGLFKHIKALTHCNSCGIQWF--ILEORSNLKFLKDRASS
PCMV	542	AQGVLVKG----GLGKSVSISCFDTADLRAFLDFSSVKP---KLHQPPPPLIEWEVSVLWF--FKEENRINCHFLREYSE
ApLV	532	ASEVIVSA----NLRACTFVTDLWKTFRALIKEGNR--SKGMRKKYFELGIKWF--IFTNAANVKFLTPGRD
ASPV	532	ANVIIIAA----NLRACTFAVTDLWRFTEGILLKECKR--AQGKMRKR-YFELGIRWF--FVVDVSNOFLPPCRD
AGCaV	532	ANVIIIAA----NLRACTFAVTDLWTFTEGILLKECKK--AQGKMRKR-YFELGIRWF--FVVDVSNOFLPPCRD
RuCV1	530	ADGYARNSIFSNSKALRLAFLISMCSGRVLKDVKYLLNIRRKIFFVRSPSTSQAQIRTAQW-FIKRGNOKFLRDSA
APV2	525	ANVFORRS----NLNLDFIIGSDLNQKVVCIVRNFS---SKKSQLIWIYSTNSMAWERFS-LRANOKFLRSPPIFP
APV1	526	ATVLOQRT----NSNLSEIIFGTDLNPMIIGFVRYN-A--SRSSLLIWEHDRPESWEQFS-LRANOKFLRSPPIWP
APV3	525	ANVLOQRT----NSNLSEIIFGADLNPMIVGFVRYN-S--SKKASLLIWEADRPSWEQFS-LRANOKFLRSPPIFP
PVM	537	CIAKARRR----GQHKE-KLEAEWVLGLKSSDALRAMTYLCNARLEPM-SESGRFIT-TGGRNNLYGLTNYTEG
consensus	561	...
PgFV1	593	ENGIDRTSQSRYNAIT-----SDLTSRKDFNLKVYWGFD-----KLA SKD V
GVT	593	ISNISVESVNLNYRSIV-----KEVEFKFKLESLDVFW-----QTTSA
GRSPaV	603	FADIDCEVIVKQVQIVT-----SQAILPEALLSLTKVFRVD-----SDS GVS IP
PCMV	609	--D-YCR-----ADFRCSFKIFKSR-FTN-----EVNSNGSKL
ApLV	599	-GIVFVRSLFNDFRK-----NCQCNLFSFHGERMAIH-----QTIKGERIE
ASPV	599	--GIIARVSFQDFRK-----GCQRDNLSLHNCRMSDR-----QVL GPK Q
AGCaV	599	--GIIARSVLFQDFRK-----GCQRHNSFHNCRMAIR-----QVF KEK Q
RuCV1	609	ESNIETKVFTSWMKVDEVLLINSSSSLGLMRSTLRSCL-NIWEYNCSTTSYPAPSATESVEKV-----EEIEEESV
APV2	595	-ASVNCKDLSRKNI-----LDFEVFGFCACI-KUGSSGVSSVPPKPVLGGEFVP-----PGEEA EDDIE
APV1	596	-NDIGLVEFRMRKNA-----LEISNFDRNSNIEKLGFSQTAPTSKNTSSSEGASNHOREQ-RDQCDEATLQ
APV3	595	-DQGLSDFRJQKSIT-----AEVKEFDRKCL-KLGFSNLSRPYQVQDQLPPLKSVNEANCVSGDIEQGSNPE
PVM	608	-KRAVTGVQNLWSNV-----HEVSTKRHKGMIRUE-----KARVTEQPR
consensus	641	...
PgFV1	637	V\$INGDSELND-SPTHSLKSD-LVSSNFQNEEVLTTEQNGSVK-----GDCSIREDSGEDK-----SFEV
GVT	631	MEFGGPKEISEEPVVEEQ-APEEVIDVGSKQTSAVHAES-QATAPSXVDT-----SVS
GRSPaV	647	RIVSRNELEELAHPANSALEP---QSVDNCAGRQASVSSQ-QLADTHSLGSVKSSIETANK-----AFNI
PCMV	638	NHCTSPPIFGLESSSTLSTID--EPIPCTPKS-TPDALSDS-----NFTFIHSC-GNLR-----CAK
ApLV	638	SLLDEVELNSHKKFVEKQDVRT-VEE EKEQD-PHTFENHENSSP-----ATVCGNVPCKCACSTN-----CFVQ
ASPV	638	ALFDVSELSIIHVNEMENAPPA-GST-DAGIKPTSSPLEVPIE-----NARONLAPCKCDLN-----CFIQ
AGCaV	638	ALLSESELSVHHMVEIRTPEV-EPTVDEQHQLTA-PSEVPI-----TVCQNVVPCPKCMLN-----CFIQ
RuCV1	682	THENVPILKRDVSSFLKIAFNV-PTVQEEVVEVKD-VET-GTEE-----EFEAPALEKFGDAGI-----VFGR
APV2	658	VKSNTMAKMFDPSPQAVLGI-G-PAAGVHEIDPQDQTLSPKAADLEERNLIFGCVPEPSLMNFSFSSANQGDRLFANI
APV1	663	VNFQREIEFCMGSILPEANSFKSFTPC-EAVAETSYEVQEPEP-EANGLVLGCI-IPDASTPAFTFNSANQKDRLFSSV
APV3	663	VCFAMEMEFGRRSPSMSGTSEILPNAAELAVKE-SDSTAPER-KHEGLVFGCIPDSSSTGAFACFSANQHENTFAAV
PVM	647	SEFASCVLEPEVWRDVEAALDIELGEACACNARFQGVILSNQ
consensus	721	...

Fig. S1. Continued

PgFV1	696	EGFNIVKVAEPP-DIAQFASLLSFPPDQ-----KGRKACFIAVPEVSHYQVNGGFHENRGWPPFNSILKICGG-FGHYNS
GVT	689	DEIKIDIGNSS-ELKFHYGVLKSPDVI-----KGRKACFIAKSGIKSYAYNGGSHDSMGWPVKALDEILRLVDG-GIYNS
GRSPaV	711	EELRIMIRVLPEDFNWVAKNIGEKDR-----RGRGASFTSKPGISCHSYNCGSHTSLGWPKFNDQILSSTGG-RNLYNS
PCMv	696	RSDCTAIP-----LNLDPEPDK-----NNTRVAEVFSR-RGDGYSMTCFSHKSMGWPDELLDKELLDNQIALENMDH
ApLV	700	NAKDALH-----NNLTEHDFT-----GGARGRGAFFFSR-DLKGYSMTCFSHESRGWPVRLLDFLDDNIIPKHEYDQ
ASPV	699	PADVNSLH-----GNLVFLDFI-----GGSKGRGASEFSR-NLKGYSMTCFSHVSRGWPAFLDKELSDNKIPQKRYNQ
AGCaV	699	QADVSSLH-----GNLIELDFI-----GGARGRGAFFFSR-NLKGYSMTCFSHVSRGWPTBLKELDDDNKIPQKRYNQ
RuCV1	744	VKDMTLVGCLD-MV-----FENNLHDV-----HLKGRSAVETRCPCLRMHNGLQYLQPKWPBEEWLGENL---GKYN
APV2	737	NENITDVGVILP-P-----TELKIPLDAKVTQINGRDCYEFTRCSCIDMHNKIKYTANNWFSCLLQELSNEG---TMYNA
APV1	741	EENITTDIGILS-P-----MEVLVPSSAEATPLNGRQCYFFTRCGCIDMHNKIRYKPNKWFSCLLSILKDES---TMYNA
APV3	741	NENITDVGVILP-P-----TELVLVDAEATIVKGRCFFTRCECIDMHNKIRYKPNRWFSCLLALLKDEG---TMYNA
PVM	691	-----AGLNVROQ-----AGASVGLYTK-DRSNLK-GNSELLSNGWGRSHSVWMEINSV-SQKEDV
consensus	801	.
PgFV1	769	CIAQCLYDDGSDKMHRDNEKCYNENEKVLTVCIRGSCTENFCSTGKSRSRGDWVSIEMGPFEWEEMPRSFOAKMLHGVTN
GVT	762	CIAQCRYDGGSIGMSDNERCYAVGKVLTVNMLGSCQBYT-CRKQDRNLKERHINFIREGDYEMPRNFOENIHCYRE
GRSPaV	785	CIAQCIYEENSKLAIIHKDDESCYEIGHKVLTVNLIGSATTI-SKSRNLVGGNHCSLTIGPNEFFEMPRGMOCNIEHGVSN
PCMv	761	CLVCKYSQGAALCWHDSDNEDCQYCDLHQ-LTVNLICGEAKAVKCGAGSD-----QVDIRLPWDABIMPHGFOVSHKHCILD
ApLV	767	CLVQEYSTGHLISLHKDDEEIIDYDPNHO-LTVNVNSGDAIHCIECLGSGF-----EVKINGPQMLLMPFGFOKDHKHGCKN
ASPV	766	CLVQEYSTGHLISLHKDDEEIIDYDINHO-LTVNVNSGDAIHCIECLGSGF-----EIPLSGPQMLLMPFGFOKEHRHGCKS
AGCaV	766	CLVQEYSTGHLISLHKDDEEIIDYDINHO-LTVNVNSGDAIHCIECLGSGF-----EVPLSGPQMLLMPFGFOREHRHGCKS
RuCV1	813	CLVQREEVGAKICBHSDDEKHYSDDNDYTUVNLMGNAQLSIRPKGDKRK-ANEITRALVSDQS-IMPSGFQDK-BEISRS
APV2	808	CLVQVYRAGSGICBHSDNEKVYHRS-PKTVNLICGEADEILVAKAKRRDL-GTVARGHIFPGHY-TMDSNFQSY-QHSVQD
APV1	812	CLVQCIYEAGAGICBHSDNEKVYHRS-PKTIINFCEADEBVAKAKRRDL-GVNATCHMKTGQFT-TMDSNFQSY-QHSVQN
APV3	812	CLVQVYEAAGAGICBHSDNEKVYHRS-PKTIINFCEADEBVAKAKRRDF-GVSATCQKPGQFT-TMDSNFQSY-QHSVQN
PVM	746	AIRLSYSKETO-NVLLPSLDGIERGAGATVNVLRKCAIBIVRCARGWR-----IADAWMDHICIEVMANVA-CHECYM
consensus	881	.
PgFV1	849	CKGERISLTFR-----RHIVQDNETLQIP---NLNKNLAAFERVKALDVKW
GVT	842	TSDGRISLTFR-----RQLVPDSGDDEEE---CSPFAFGSIDRIAGR---KFW
GRSPaV	864	CIPGRVSLTFR-----RQLKEDDDLIIFINPQVPPIELNHEKLDR---SMW
PCMv	835	TSEGRVSLTFR-----KSKACLNGISRTLTVQGAQGPPIP-----TPSDASHSLAHDLQ
ApLV	841	PSKGRISLTFRLRSASNSQPVQEVVKV-DQDGAVKGASLEKLPSQQTQDESMGLGTGSSVFDEGFSADSSGSSPVQEFM
ASPV	840	PSKGRISLTFRLKEGDSQVPIQEVTICDHDSDRAALKALERSHQSGGRPAVELEGHEREKVNDSDDSAVQEFM
AGCaV	840	PSKGRISLTFRLKEGDSQVPIQEVTICDHDSDRAALKALERSHQSGGRPAVELEGHEREKVNDSDDSAVQEFM
RuCV1	892	MSEGCRVSLTFR-----
APV2	886	CSEGCRVSLTFR-----
APV1	890	CSEGCRVSLTFR-----
APV3	890	CSEGCRVSLTFR-----
PVM	819	RSGWTMDVVVF-----LKRATVSEQVTFESA-----
consensus	961	.
PgFV1	893	-----NLGKKMADLKTKRSCFISDIACFSCSNFDKSADLSKVSKALCAGCVAEADRI-ILFDQTAKPE-FRDALSR
GVT	884	-----PECSNYAAIMDGSGKGKRELDLCDFCSCPNTFHAKNIDGIRKVFVTSATGLAAGDRV-LVLN SKSSKAILEKPERL
GRSPaV	905	-----QMGLHGKKSISMNGTSFTSDICSCFSCNHFHKFKDILINNIRLALCAQCLGQCDRFFVATTGPGLSKVLEMPRSK
PCMv	883	-----VDDGSVVELIRGKGGKFGKGYQSDICCCNMSWATDEDEPILETRLSLFACFGFSNVDRV-LISDVNSITTSSLLEVE
ApLV	920	IQIDSSLLEYADKSISCLSSKDILNCDCICLONSPWLKNEELKFSEADRDLAFACSFNPTDRFSIAGKGVSGVRG-NRIISEL
ASPV	920	IQIDSSLLEYADKSISCLSSKDILNCDCICLONSPWLKNEELRFSEADRDLAFACQCLIQLIDF-LCLKVLRCAE-NRIISEL
AGCaV	919	IQIDSSILEYADKSISCLSKDVNLNCDCICLONSPWLKNEELKFSEADRDLAFACSFNPTDRFSIAGKIEGVVRG-NRIINEL
RuCV1	903	-----KVHDSDLQ-----
APV2	897	-----HHNNVTRGL-----
APV1	901	-----YHNNVAGL-----
APV3	901	-----YHNNVAGL-----
PVM	845	-----QEGLPEECK-----SDSGAPCV-----
consensus	1041	.
PgFV1	967	PVKFVLEGEQRVSDSEDLLILKSMQCGVV--RNFS-SKATCQLGFIAF-REKISSRGSIRVVIEECRFSGS-SEVILGC
GVT	958	RVTAVFSGCKIEFLDGLVPGIVSDGDLREV--KNFS-QNLKVELAVVIF-RGSTVSGSKVRLVREGECKFTICA-SEVILGC
GRSPaV	980	ILVLEGALSIETDYGPKVLSFEVFK---GDFHKKMEEGSIFVITYAPIRSTGRIRVHSSECSFSGSKDEVILLGC
PCMv	962	MGTWCISGFILVKSEGGEVKIGEMMAKF---MKMSIGWSKDFLSFFY-PRLGKGMQIRTNNECECELSDFTPEQIFGCC
ApLV	1000	PSHFPLRGSMSILDIDDKTVGNVKEGSFSGFRRWK-VSCSTDLLVV-AFLPKMTLGGEIRTHEDECELSDLTEKIHGC
ASPV	999	PTHVFPFLRGTMHIVDLDDESIRGDVKEGSFSGFRRWK-MSCSTDLLIM-AFLPKMTLGGEIRSHEDECELSDLTEKIHGC
AGCaV	999	PTHVFPFLRGTMHIVDLDKSIKGDVKEGSFSGFRRWK-TSCSTDLLIV-AFLPKMTLGGEIRSHEDECELSDLTEKIHGC
RuCV1	912	-----EIVHDSICLLDCFSENIFDV-----
APV2	906	-----PIKHTCGEFGDTEIFDV-----
APV1	910	-----PIKHTCGEFGDTEIFDV-----
APV3	910	-----PIKHTCGEFGDTEIFDV-----
PVM	862	-----GVNLIDGCV-----
consensus	1121	.

Fig. S1. Continued

PgFV1	1043	P	LACADYQIIDDENAEDVPADGNCFWHSGVHLMNCDCGMVKSGI-----G	HFRASDC--ESKAIDEQMEGVWAENDSW
GVT	1034	N	IAIDEYSIGNENLFEQVPADGNCFWHSGVHLMNCNGLDLKNGV-----	FNKFRSSGL--ESVKLEHQIEPNVFAENEV
GRSPaV	1056	Q	EACADYDIDDFNLSVPGDGNCFWHSGVELLSTDGLALKAGIRSFVES	RLVSPDL--SAPAISKQLEENAYAENEM
PCMV	1039	T	RILSRKFENPDEFEVBDVEGDGNCFWHSGVPLIGVDGIIILKCGIL	--RRCGCGNGI--THKEIIPQMSGDTWAEREA
ApLV	1080	S	IILSRKFEPDLVHSEVEDVADGNCFWHSGVPLIGVDGECLKRNIL	--HQAKIDCV--KCPRLSHOLENNVWAEREA
ASPV	1079	S	IILSRKFEPDLFHSSEDVEADGNCFWHSGVPLIGVDGEYLKRNIL	--HQAKIDGV--KCPRLSHOLEGNTWAEREA
AGCaV	1079	S	IILSRKFEPDLFHSSEDVEADGNCFWHSGVPLIGVDGEYLKRNIL	--HQAKIDGV--KCPRLSHOLEGNTWAEREA
RuCV1	932	K	SASQDLDFFKNEQLEPVEADGDCFWHSGVYLEGTNGIDMRKTC	--SAMYDLGV-DSNSGSLVIQOMIGFNWAEREA
APV2	924	I	RRRSFTYAAKNFHLEPVGPDGSCFWHSGALLGVDEELKRNIS	--AREIQNEILLSNLSDRQOMANKQYAERES
APV1	928	I	RRRSFTSYSSKNFHLEPVGPDGSCFWHSGALLGVDEELKRNIS	--AREILNEVLLSRNLISLSAQOMENKQYAERES
APV3	928	I	RRRNFSYSAKNFHLEPVGPDGSCFWHSGALLGVDEELKRNIS	--SREIQNEMLSNLSSERQOMEKGQYAERES
PVM	871		VGSEYPANGAEIKRVSGPBGDGCCWHSEAYLVGMHHMELKRIC	--TSHVFENAA--LNVELEQCKASGAEVTHAA

PgFV1	1116	A Y F C I M H K R O L I V T P E Y E V C W K F G L D D W - P L L G F I C I -- N G H Y O P C A P N G C M I R A V A E A L G A R D S D V L N F L C P E T
GVT	1107	A Y F C G M H K R R I I T L T P E Y D V E W N F G E K N W - P L L G F I C V -- Q O H F O P C A P N G C M I R A V A E A L G A R E R E V D V L N Y I C Q D A T
GRSPaV	1134	A I F C I R H H V R P I V T P E Y E V S W K F G E G E W - P L C G I L C K -- S N H F Q P C A P N G C M I T A I A S A L G A R E R E V D V L N Y I C R P S T
PCMV	1112	A B F C S E Y S I O H V I S I S E G V W I F K P A K V - V K S S T I L K C Q - D N H F M P C I P N G C V V R A I A S A L G A R E D V I A V L G K P E F
ApLV	1153	A Y F C S H Y G I K I N V I Y A R E E C T W I F K P H E V - L K A A T I L I C Q - D N H F K P C M P N G C V I R A I A S A L N A R E V D V L A V L G K P A F
ASPV	1152	A Y F C S H Y G I R I N V I Y T R E E C T W I F K P H E V - L K A A T I L I C Q - D N H F K P C M P N G C V I R A I A S A L N A R E V D V L A V L G K P A F
AGCaV	1152	A Y F C S H Y G I R I N V I Y T R E E C T W I F K P H E V - L K A A T I L V C Q - D N H F K P C M P N G C V I R A I A S A L S A R E V D V L A V L G K P A F
RuCV1	1006	A I F A R V M G V E V T V F Y I E E G V N W T F P L E I D S S K K C Y L V C K - G N H F E P C I L P K N G C V V R A V A A C G K E D V L S Y L G G E F
APV2	999	A A F C R I Q S I H V V I L P C G N F S Y E F L P S Q N - P E V T O L F K L T D E H F E P A L P N G C V V K S I A E T I N Q T E A K I I L S V I G R P T N
APV1	1003	A A F C R I Q S I H V V I L P D Q N F S Y E F L P M Q N - A E V T O L F K L S G E H F E P A L P N G C V V K S I A E T I N Q T E A K I I L S V I G R P N M
APV3	1003	A A F C R I Q S V H V V I L P D H N F S Y E F L P T L S - S E V T O L F K L S G E H F E P A L P N G C V V K S I A E T I L G Q T E A K I I L S V I G R P T N
PVM	944	I A T A L I R L R A E R V H N A G T G R V H R E A P K Q K - N M A L D I W E -- S E H Y E P O V L R N G C V I E S V A O L G T R N A D I I L A V V E E R C C
consensus	1281	----- * ----- * ----- * ----- * ----- *** *

PgFV1	1269	VKRSEVDILALINGWINYNSPSVORAEELIENCLLAGLTGVLC	DKRFFNNQKPDIESVKDQRKERSVLA	AIMGTFGCGKSLE
GVT	1260	VRKSEDEMIGVNGWIEYEPOVARAEKIEKCLLAGLTGV	CSERYDNOKAIDNDIAKDQRGKRNMA	IMGTFGSGKSLEVY
GRSPaV	1287	VRKSALDELMSNGKITYEPSEFRAEAKIOGCLLGGLTGV	SDEKFSDAKPILSGISTTDIKPRELLV	VLGTFGAGKSELV
PCMV	1265	VSKSALKLIMNGEISYSPSMDRAOTIANSIHAGTGVCA	TYNRSRRHIDGEANEI-AERKUCTI	GTFGCGKSLE
ApLV	1306	VSNSAVRFLAINGAEDYRPSIDRASLTLDSLELGATGVLC	QGIKETKQNKLSSMVPPEAVHDRLKLI	ILGTFGCGKSLE
ASPV	1305	VSNSAMRFLAINGAEDYRPSIDRASLTLDSFEIAGTVLQ	QGIKEAQDKLASKIPLEVHLVERKLI	ILGTFGCGKSLE
AGCaV	1305	VSNSAMRFLAINGAEDYRPSIDRASLTLDSFEIAGTVLQ	QGIKEAQDKLASKIPLEVHLVERKLI	ILGTFGCGKSLE
RuCV1	1162	VAKDQEIVISKCCIEVEYTPSFDRANTILS	SILLNGTGTGVLC	SRNLNNQVDLEGNEKLCKEKRNLMLI
APV2	1154	NGQGPDPDLEGKG-----	ARNIIESESLNGTGTGVLC	DRTVNLOKNI
APV1	1158	NGQGPEIFIRGIVSEVDYKPSWGRARNI	LEESILLNGTGT	LDTRKLNNDNSRNIGV
APV3	1158	NGQGPEIFIRGIVSEVDYKPSWGRARNI	LEESILLNGTGT	LDTRTINLQKNVTHDRKLNDDEVRSIG
PVM	1099	FAESAELLDLIENCGLKIDFEPWNWRAGCMIA	DSMYSQGATGVLGSALENKRNMRKFKRVNV--	SLSLHAIVGTFGSGKSLE
consensus	1441	.	*	*****

Fig. S1. Continued

PgFV1	1583	RWITALSRFRMNSFINLLGMPPLSGAHFHAGKPLYHFLTESSGQSVIINDMLPGSPSFFSGEKISVGKDEGVKENKVAGD
GVT	1574	RWITALSRFRMNHIFVNLLGIPLBRANCHFAGKPLYHFMSKKSGEAIINDMLPGCPFKNGFISIGKDEGIVEKTKLAGD
GRSPaV	1602	RWITALSRFSHEIDLVNITGLRVSFISHFAGKPLYHFLLTAKSGENVIRDDILPGEPNFFSGFNVSIGKNEGVREKEKLCGD
PCMV	1577	RWITALSRFSHEITFTINGLSIEWNSVNLSEFHGKGALNKESKRASHDDVVDDILPGKPEFIEGFQVNIGRDEEVREPKLSDG
ApLV	1620	RWITALSRFSHDIEFTINGMGVTWDNATHFVVGKPLKEFTLTKRACNNDDIDLPGRPRELIEGFQSQVGADEVGVREAKLVGD
ASPV	1619	RWITALSRFSHDIEFTINGMGVTWDNATHFVVGKPLKEFTLTKRACNNDDIDLPGRPRELIEGFQSQVGADEVGVREAKLVGD
AGCaV	1619	RWITALSRFSHDIEFTINGMGVTWDNATHFVVGKPLKEFTLTKRACNNDDIDLPGRPRELIEGFQSQVGADEVGVREAKLVGD
RuCV1	1481	RWITALPRFRKLVEVSNTNLSYDIAQKAFGEPLMKELTEKASTDDLLEILPGKPNFVKHGFCF-VGKSEGEKEVKLQGD
APV2	1446	RWITALSRFRNIIIFVNLLVGNNDIDVCQVFHDRTLFKFLNKTATIGDIKQLPGNPPELTNDFCDKVVGKSEGVMDKLAGD
APV1	1463	RWITALSRFRNIIIFVNLLVGNCLDEDACQVFHDRTLDFLFTKRTATIANIDOLPGLPELTNDFCDKVGRSEGVMEAKLSDG
APV3	1463	RWITALSRFRNIIIFVNLLVGNCAIDVCQVFHGRTLDFLFTKRTATIANIDOLPGLPELTNDFCDKVGRSEGVMEAKLSDG
PVM	1406	RWITALSRFRNCFEVNCSCGMDDYQQLAGRYKGRVRSKFLCKTAIPDDNSMLPGQALFKSEPRLTGKDEGVREKKIAGD
consensus	1761	*** *** *** * *** * *** * * * * *

PgFV1	1663	PWLKTMIFLGQGEDMEVEHMEKEMSS	WFKTHLPISSENCRARWVGKIALKEAREFRIGMEVSSEQFKDDHHDFRC-ER
GVT	1654	PWLKTMIFLGQNEDIEIEEMEEEFKNEW	WFKTHLPITSMECSRARWVGKIALRESREVRCCMNITEOFKDDHHDLKG-VL
GRSPaV	1682	PWLKVMLFLGQDEDCEVEEMESECNSN	WFKTHLPISNLESTRARWVGKIALKEYREVRCCMELQQFFDEHRGGTC-EQ
PCMV	1657	PWLKTAIFLGQDPIEEEEMAEEIIQE	WFKTHLPIFELEAVR AVR WVKIIIAKEAREFRVGHEVTEQFIDEHSKNPC-KQ
ApLV	1700	PWLKTKIIFLGQSFDELLDFFEVQAEE	WFKTHLPIMCLEAVRAQWVSRLIAKEADREFRIGDITTEQFTDHSKNRG-LE
ASPV	1699	PWLKTKIIFLGQNEDIEIIADEVEAAE	WFKTHLPIMCLEAVRAQWVKHIIISRDREFRIGDITTEQFTDHSKNRG-QE
AGCaV	1699	PWLKTKIIFLGQNEDIEIIEVEVAE	WFKTHLPIMCLEAVRAQWVDRIIISREVREFRIGDITTEQFTDHSKNRG-QE
RuCV1	1560	PWLKEKIFLGQSEDIOEEIQIYEEIKN	WFKTHLPISSEQESCRIOQNRLISREAREKRMGESEISTOFAEHHPGCG-SA
APV2	1526	PWLKTEIDLQDDEQEMEELAEEVKNEE	WFKTHLPIFELESIRANWVKINNREYREVRCCGTEVTSQFPEDHPNKYK-VT
APV1	1543	PWLKTEIDLQDDEQEMEELAEEVKNEE	WFKTHLPIFELESIRASWVHRINNREYREVRCCGSETTQFPDDHPSGAK-IT
APV3	1543	PWLKTEIDLQDDEQEMEELVEEVVKNEE	WFKTHLPIFELESIRAGWVHRINNREYREVRCCGSETTAQFPDDHPNRSK-IT
PVM	1486	PWLKTMINLYQAPAEVIAEEPEVMMQG	WFKTHLPDELESVRAQWVKIIIAKEAREFRIGDMSSEQFTDHTKQLGAKQ
consensus	1841	***** * * * * *	***** * * * * *

Fig. S1. Continued

PgFV1	2058	I FEH G IFKRPQLVLERI N I A R E M N N L D C I D N Y A I E V S Y C Y N L G E L A G A A M D E E M E A H Y N C V R F I L V R Y R H K M C D I G K I
GVT	2049	I F K N G I F K R P Q L V L E R I N I A R E M N N L D C I D N Y A I E V S Y A Y S L G E L A V E V M S D E E L A H Y N C I R F I V K Y K N R M K S S I K E L
GRSPaV	2077	I F K E G I F K K P Q L V E R I C I A R E M G N L I N C I D N Y A I E V S Y A Y R L G E L A I E M T F E E V E A H Y N C V R F I L V R N K H K M C S I S G L
PCMV	2052	L C P E G V F K K P D L V L E R I L Q I A V E T N N L Q N C I D N Y A I E V S Y A Y S M G E S I S K Y L S E E M D A H Y N C V R F I V K H S H L L K C S V S D L
ApIV	2095	I C E H G V F K K P D L V L E R I L Q I A R E T R N L I N C I D N Y A I E V S C A Y Q M G E N I N L Y L S P H E M D A H Y N C V R F I I L H N H L L K S N I R D L
ASPV	2094	I C E H G V F K K P D L V L E R I L Q I A R E T R N L I N C I D N Y A I E V S C A Y K M G E N I N L Y L I P Q E V D A H Y N C V R F I V Q H N H L L K S N I R D L
AGCaV	2094	I C E H G V F K K P D L V L E R I L Q I A R E T R N L I N C I D N Y A I E V S C A Y K M G E N I N L Y L S P Q E V D A H Y N C V R F I V Q H N H L L K S N I R D L
RuCV1	1956	I S E F G L F K R P Q L V L E R I C I A V E R G N I A E C I D S Y A I E V S Y C Y V K U G E S I S A G K M S V E L D S H Y C V C R F I V K H O N L K S N I R D L
APV2	1921	I C L D G I F K R P Q L V L E R I L C V A R E K D N L I N C I D S Y A I E V C Y A F A L G E K V L O Y M D E A L O N H Y N C V R F I V K H S H L L K S S V R D L
APV1	1938	I C M D G I F K R P Q L V L E R I L C V A R E K D N L I N C I D S Y A I E V C Y A F A L G E K V L O Y M D E A L O N H Y N C V R F I I K H S H L L K S S V R D L
APV3	1938	I C L D G I F K R P Q L V L E R I L C V A R E K D N L I N C I D S Y A I E V C Y A F A L G E K V L O Y M D E A L O N H Y S C V R F I I K H S H M L K S S V R D L
PVM	1885	I C P D G I Y K K P Q L V L E R I C I A K E M N N L I S C I D N Y A I E V S A Y A Y K L G E K A V N R M D E E V A A F Y N C V R F I I L V R N K H L I R S D V Q V

PgFV1	2138	FT-----
GVT	2129	F-----
GRSPaV	2157	FEAID-----
PCMV	2132	FRS-----
ApLV	2175	FRGEPTIVTLL
ASPV	2174	FKGESLPASS
AGCaV	2174	FRGEAAVGIK
RuCV1	2036	FAS-----
APV2	2001	FLQNM-----
APV1	2018	FLSGM-----
APV3	2018	FLGSM-----
PVM	1965	FEVL-----
consensus	2321	*

Fig. S2. Multiple sequence alignment of TGB1 protein sequences of PgFV1 and related viruses

PgFV1	1	MNNLLAALDLNFECSVSDKLSPVVIHCVPGGGKTSILRDLIKIDSNFVAFTAGEPDIPNIECKYIKRYSK-D--CAVKG
GVT	1	MNNLVSALELGFVRISEEARYPVIVHSVPGSGKTSILRSLIKLDGDFAEFTAGVPDTPNLECCYIIRSHFE-G--CASNK
GRSPaV	1	MNNLVKAISAEFVGVFVSVLKEPVIVHSVPGSGKSSLIRELISEDENETARTAGVPDSPNLTCRYIKPYSP-G--CAVPG
PCMV	1	METTVVSSLFEGFERTSVPIEDKLIVHVAPVPGSGKTTLIREALNRNLGIEAFSEGEFDPLNLUWCRYIKKAIS-G--QKGTG
ApLV	1	METVVLSSLNEFGFERTVEPLSDPVVHVAPVPGSGKTTLQIALAIRHNHIEAVTGVPEKANHGTYIKKARQ-G--QRGRG
ASPV	1	METVVLSSLNEFGFERTVEPLSDPVVHVAPVPGSGKTTLQIALIRNNHIEAVTGVPEKANHGTYIKKARQ-G--QRGRG
AGCaV	1	METVVLSSLNEFGFERTVEPLSDPVVHVAPVPGSGKTTLQIALIRNNHIEAVTGVPEKANHGTYIKKARQ-G--QRGRG
RuCV1	1	MEDLLHLLIEISSFERTSVPLSSVVVHVCAVAGAGKTSILRKWLGRNPNGEVTCGVPDKENITCRRRIKAWGG-NLESKSDD
APV2	1	MFVYDKLIDAGITRRLPISFPVIIHVCAVAGAGKSTLIREIEVDHKFEAFTYGVVPDPVNLSGIRIRGAELA-NARPE
APV1	1	MFVYDKLIEAGITRRLSISFPVIIHVCAVAGAGKSTLIREIEADNRFEAFTYGVVPDPVNLSGIRIKGAADIG-RARAD
APV3	1	MFVYDKLIEAGITRRLSISFPVIIHVCAVAGAGKSTLIREIEADNRFEAFTYGVVPDPVNLSGIRIKGAADIG-RARAD
PVM	1	MIVIVDILLYKRFERLSNKLVCPIVHVCPVPGAGKSSLIRELLELDSRGCAATAGVEDQPRLSGNWIRKWS-G-QQPEG
consensus	1	*. * .. * ... * .. * ***. * .. * .. * .. * .. * .. *
PgFV1	78	KLNILDEYLTAD---DWTFGSVLFSDPQNVK-APPLEAHFVGSRSSRGKETCKYLRNRCDEINSTS-----EDTVVIGS
GVT	78	KLSILDEYLTVD---NWEFGFDALFSDEYONDK-SPLVASVSKKTFRFCSTCAYLADYGDESEI-----EDIVVRGS
GRSPaV	78	KVNLILDEYLSVQ---DFSGFDVLFSDPQNIS-IPKEAHFLKSCTCRFCNTCKYLSSFGKSSDG-----LDKRVLVGS
PCMV	78	SFCILDEYLSG---FGTGFDCDFSDHQNSG-DCAPAHFVGRRSSORFCRNTAGLLQLSGLSNSAK-----DDELIIFEN
ApLV	78	NFSILDEYLSG---YSTGENCLFSDEYQNHG-DCLRAHFIGRCSHRCRNTVQVLRLNLSNASSK-----DIVEKKN
ASPV	78	NYSILDEYLSG---YSTGFNCNCLFSDEYQNHG-DCLRAHFIGRCSHRCRNTVQILRLDLSNASSK-----DIVEKKN
AGCaV	78	NFSILDEYLSG---YSTGFNCNCLFSDEYQNHG-DCLRAHFIGRCSHRCRNTVQILRLDLSNASSK-----DIVERKN
RuCV1	80	QVVVLDLEYCELGKHFDDYSKFGAIFCDNLQFPEDSCLEAHYICLNSHRLSRNTISFLNKEGNHSLKEVSKEEDVVF GG
APV2	79	SLKIIDDEYLGQD---LPEGTTFCADENOFPY-TCPDAHFTCYQTFRGDOTCALLGKIDCAAESYK-----QDQIIIFDK
APV1	79	SEKIIDDEYLGQH---RPEGTAVCFADENOFPY-SSPAHETCYQSFRGDOTCAFLGKIDCAAESYK-----SDQIIIFET
APV3	79	SIKIVDDEYLGQV---LPDGTAFCFADENOFPY-TCPDAHFTSYQTFRGDOTCSFLGKIDCAAESYK-----SDQIIIFET
PVM	77	KFVVLDEYTLLT---EVPPVFALECGDPIQSNTSAVQRADFVCVSSRRGSATCGLLREIGINRSEK-----ADLVQVSD
consensus	81	..***. * . * * * * * *
PgFV1	149	PHEV-KTEGQVICFGKAIDLAVSHSASEKLPCEVRGSTFEVVTVLK-SEEPNSENLHLYFALTRHKHKLILLE-----
GVT	149	PHEL-KVEGQVICFGKAAVLALSHEAEFKLPCEVRGSTFDVVTLK-SEEPSSVNRHLYFVCTRHRKKLILLE-----
GRSPaV	149	PETL-DVEGVVICFGKEAVDIAVAINSEKLPCEVRGSTFVVTLK-SRDPTPEDRHWFYIATRREKLIINQ-----
PCMV	149	VERA-ELEGAVICVEKVNVEDFLRWNHCEYRLLPCQVRGSTFEVVTFH-ELPLDQLVGPDPYIATLTRHSKKIQIIIN-----
ApLV	149	IIRLVEPEGAIICDEKGVEDFLKWESEVYKEPCQVRGATFDIVTFH-EKPLEELVGPDPWYIATLTRHKLVLVSN-----
ASPV	149	IQLIEPEGVIIICDEKGVEDFLKWESEVYKEPCQVRGATFDIVTFH-EKPLEELVGPDPFLVALTRHRSKLVLVSN-----
AGCaV	149	IQLVDPEGVIIICLEESVEDFLKWESEVYKEPCQVRGATFDIVTFH-EKPLEELVSPDLFVALTRHKTKLVLVTN-----
RuCV1	160	IYSE-ELEGQIIISLDREAEKLLKNEHSVAKRTFEVRCLFEVRCLVS-SRELVEIYPHVKYIATRHLRSVIMLSPDASH-----
APV2	150	IAG-DIEGOIIVYEKEIFDLDREGADYKKYCQIRGSTFDIVTFITASDTEFEPEDRYKVYIICLTRHRSILRILISPEGKF-----
APV1	150	VIEG-SIEDQIIVYEKEIFDLDREGADYKKDCQIRGSTFDIVTFITSSSEFEPEDRYKVYIICLTRHRSVLRILISPEGMF-----
APV3	150	IEG-SVEGOIIVYEKEIFDLDREGADYKRDCQIRGSTFDIVTFITASESFEPEDRYKVYIICLTRHRSVLRILISPEGMF-----
PVM	149	IYTK-DPLGKVVSEEVGCLIRSHGVAALSLQEITCOTFEVTFVTF-SENSPVLNRRAAYQCMTRHRTALHICPDATY-----
consensus	161 * * * * ***. . . .
PgFV1		-----
GVT		-----
GRSPaV		-----
PCMV		-----
ApLV		-----
ASPV		-----
AGCaV		-----
RuCV1	238	TS-----
APV2	229	LREDAKFDTTT
APV1	229	LRDNNAKFDATS
APV3	229	LRDNNAKFDATS
PVM	227	TAA-----
consensus	241	

Fig. S3. Multiple sequence alignment of TGB2 protein sequences of PgFV1 and related viruses

Fig. S4. Multiple sequence alignment of TGB3 protein sequences of PgFV1 and related viruses

PgFV1	73	IIIERC-
GVT	73	-LEGNCS
GRSPaV	75	EIDYHC-
PCMV		-----
ApLV		-----
ASPV		-----
AGCaV		-----
RuCV1		-----
APV2		-----
APV1		-----
APV3		-----
PVM		-----
consensus	81	

Fig. S5. Multiple sequence alignment of coat proteins of PgFV1 and related viruses

Fig. S5. Continued