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The Detection of Plant Viruses in Korean Ginseng (*Panax ginseng*) through RNA Sequencing

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Korean ginseng (*Panax ginseng*) is a dicotyledonous, medicinal, perennial plant belonging to the genus *Panax* of the family Araliaceae. We investigated the occurrence and incidence of plant viruses in *Panax ginseng* in Korea. A total of 656 leaf samples were combined into one and total RNA was extracted from the polled sample, using RNA sequencing (RNA-Seq), a metatranscriptome analysis of the plant virome was conducted. The virus present in *Panax ginseng* was confirmed by reverse transcription polymerase chain reaction (RT-PCR) assay using virus-specific primers. In RNA-Seq data analysis, the multiplication protein of four viral contigs including *Aristotelia chilensis virus 1* (AcV1), *Turnip mosaic virus* (TuMV), *Watermelon mosaic virus* (WMV), and Tobamovirus multiplication protein were discovered. From our metatranscriptome analysis and RT-PCR assay, TuMV and WMV were detected, whereas the three viruses reported in China such as tomato yellow leaf curl China virus; panax notoginseng virus A; and panax virus Y were not found in this study. The distribution of domestic ginseng viruses seems different from that recorded in China. Overall, this is the first plant virome analysis of *Panax ginseng* in Korea.

Keywords **:** *Panax ginseng*, plant virome, RNA-Seq

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Korean ginseng, traditionally referred to as “Insam” (*Panax ginseng*) is dicotyledonous, medicinal, perennial plant belonging to the genus *Panax* of the family Araliaceae; there are six species in the genus *Panax* (*P. ginseng*, *P. japonicum* [Japanese ginseng], *P. notoginseng* [Chinese ginseng], *P. pseudoginseng* [Himalayan ginseng], *P. quinquefolius* [American ginseng], and *P. trifolius* [Dwarf ginseng]). In previous reports, six viruses were reported in the genus *Panax*: tomato yellow leaf curl china virus (TYLCCNV) (Li et al., 2014), panax notoginseng virus A (PnVA) (Guo et al., 2016), panax notoginseng virus B (reported only in the NCBI GenBank, MF614102), panax notoginseng virus Y (reported only in the NCBI GenBank no., FJ816101), panax virus Y (PanVY) (Yan et al., 2010), and watermelon mosaic virus (WMV) (Jung et al., 2013). TYLCCNV belongs to a member of the genus *Begomovirus* and was reported in China. TYLCCNV-infected *P. notoginseng* shows foliar yellowing, shrinking, and blistering symptoms. *Bemisia tabasi* is known as a potential insect vector of TYLCCNV. PnVA belongs to a member of the genus putative *Totivirus* and has been reported in China. PnVA-infected *P. notoginseng* shows mild mosaic symptom. PanVY, as a member of the genus *Potyvirus,* has also been reported in China. PanVY-infected *P. notoginseng* shows typical distortion and mosaic symptoms. The pathogenicity of panax notoginseng virus B and panax notoginseng virus Y is uncertain; only their nucleotide sequences have

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been reported in the GenBank database. In Korea, only the WMV has been reported in *P. ginseng*. The WMV is a member of the *Potyvirus*, which is the second largest genus of plant viruses based on the International Committee on Taxonomy of Viruses. The WMV strain Insam (WMV-Insam) infecting *P. ginseng* was first reported in Korea using large-scale oligonucleotide chip and reverse transcription polymerase chain reaction (RT-PCR) assay (Jung et al., 2013). Unlike other WMV isolates that cause economic damage to the Cucurbitaceae, including cucumber, pumpkin, and watermelon, it has been known that the WMV-Insam strain is not transmitted to the Cucurbitaceae plants by mechanical inoculation (Choi et al., 2014).

Due to the detection limit of conventional diagnostic

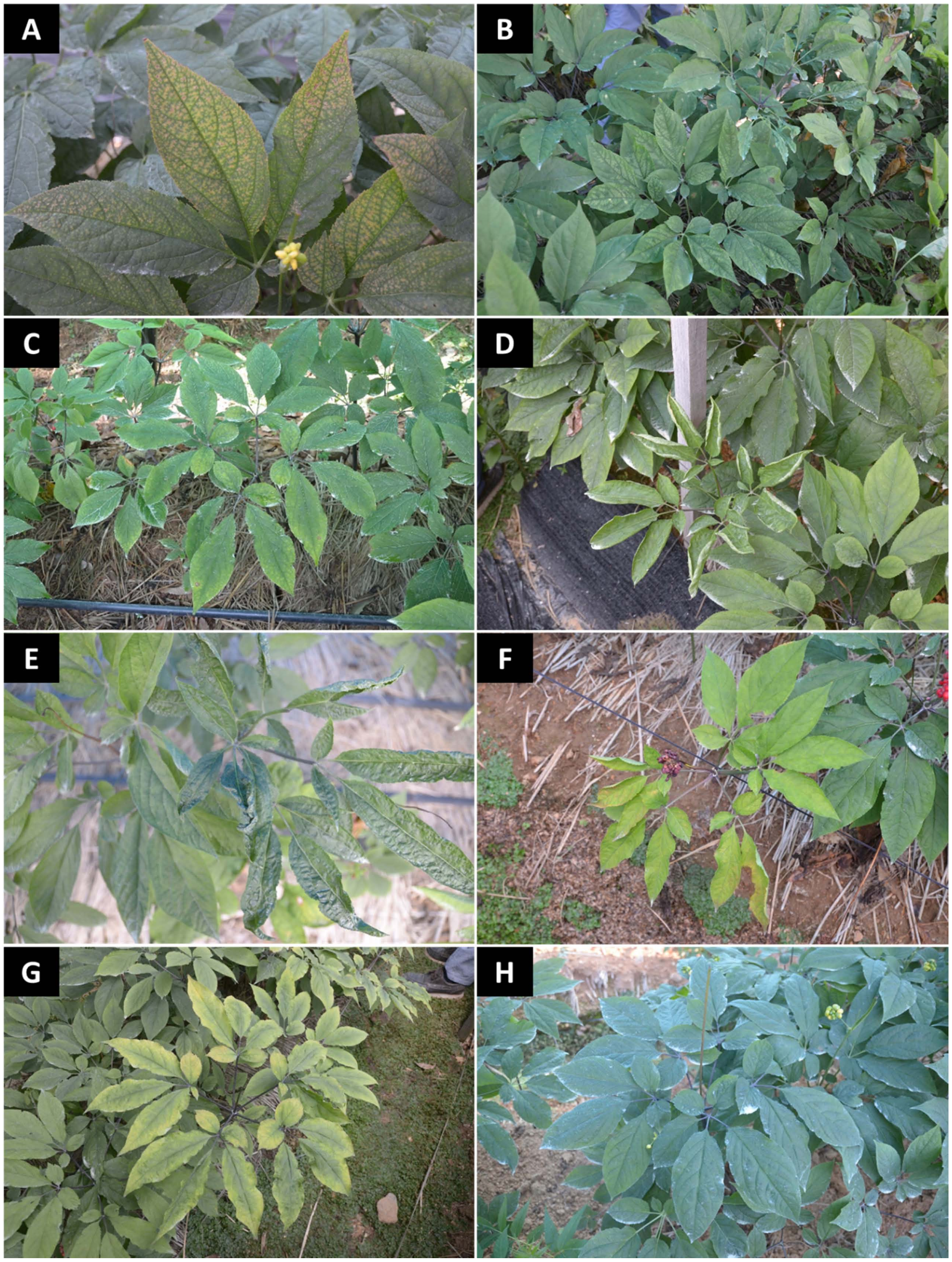

Fig. 1. Various symptom of *Panax ginseng*. (A) Chlorosis. (B) Vein banding. (C) Mosaic. (D) Leaf curl. (E) Malformation. (F) Yellowing. (G) Edge yellowing. (F) Symptomless.

techniques (PCR and serological assay), it has not yet been clear what kind of virus infects *P. ginseng* and little is known about plant virus in *P. ginseng* in Korea. Recently, metatranscriptomic analysis with RNA sequencing (RNA-Seq) and RT-PCR have been used in diagnostic tools for detecting novel or unknown viruses and the confirmation of the presence of viruses (Min et al., 2017, Oh et al., 2018, Park et al., 2019, Yang et al., 2019). This study reports the first metatranscriptomic analysis of the *P. ginseng* virome using RNA-Seq and RT-PCR assay for identifying the occurrence and incidence of *P. ginseng* viruses in Korea.

Plant materials and growth conditions. To investigate the incidence and occurrence pattern of viruses in Korean ginseng plant, a nationwide virus survey of *P. ginseng* was conducted from July to August 2018. A total of 656 leaf samples of symptomatic plants (showing virus disease-like symptoms such as chlorosis, vein banding, mosaic, leaf curl, malformation, yellowing, and edge yellowing) and asymptomatic plants were collected from 18 regions of the country (Yeoncheon, Yangpyeong, Yeoju, Anseong, Hwaseong, Icheon, Cheorwon, Hongcheon, Hoengseong, Seosan, Yesan, Sejong, Goesan, Chungju, Gochang, Namwon, Yeonggwang, and Gangjin) (Figs. 1 and 2); the samples then stored at –80°C for analyses.

Total RNA extraction and RNA library construction. To construct the RNA library of *P. ginseng* viruses, all collected samples were combined into one sample and total RNA was extracted from the polled sample using a Quant-IT RiboGreen (Invitrogen, Carlsbad, CA, USA). To assess the integrity of the total RNA, samples were subjected to the TapeStation RNA screentape (Agilent Technologies, Santa Clara, CA, USA). Only high-quality RNA preparations with RNA integrity number greater than 7.0 were used for RNA library construction. Total RNA quality and quantity were verified using a NanoDrop 1000 spectrometer (Thermo Scientific, Welmington, DE, USA) and Bioanalyzer 2100 (Agilent Technologies). A library was independently prepared with 1 µg of total RNA using TruSeq Stranded Total RNA Sample Prep Kit (Illumina, San Diego, CA, USA). The rRNA in total RNA was depleted by TruSeq Stranded Total RNA with Ribo-Zero Plant kit (Illumina). Following the depletion of the rRNA, the remaining purified, fragmented, and primed RNA was used for cDNA synthesis. The cleaved RNA fragments were copied into the first-strand cDNA using reverse transcriptase and random hexamer (5'-NNNNNN-3').

***De novo* transcriptome assembly.** One hundred bp paired-end (2 × 100 bp) RNA-Seq based on next-generation se-

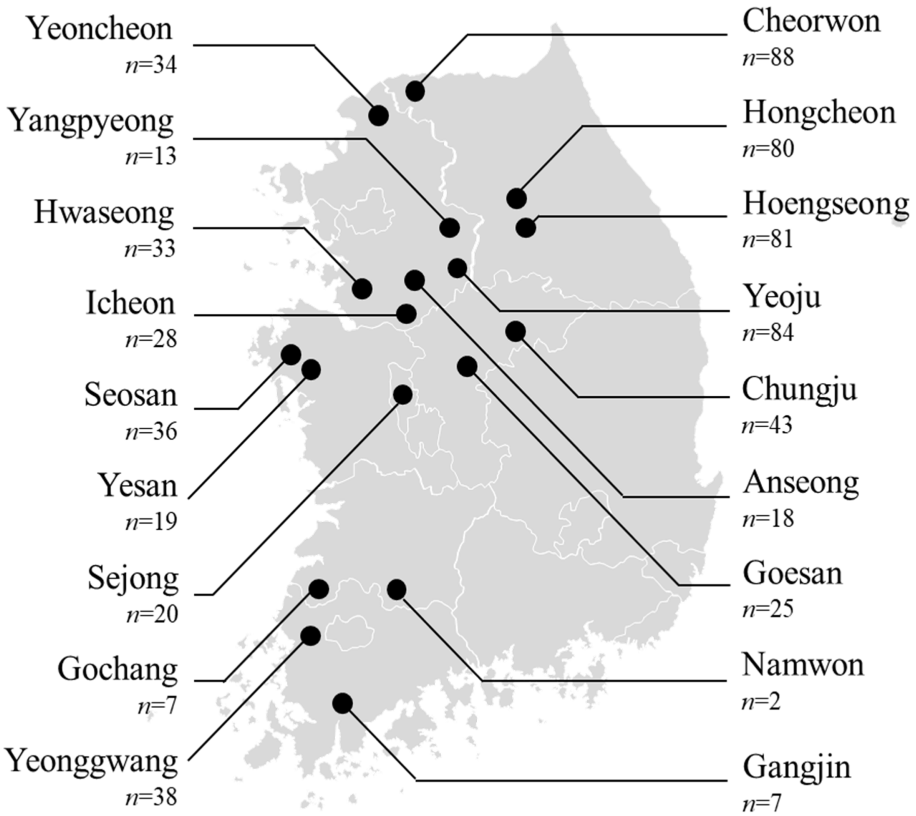

Fig. 2. The 18 locations of *Panax ginseng* cultivation areas and the number of samples collected from July to August 2018. *n*, number of collected samples.

quencing (NGS) was performed using Illumina Hiseq 4000 sequencer by Macrogen Inc. (Seoul, Korea). The quality of the sequence was verified using FastQC v0.11.7. Before the commencement of the analysis, raw reads, adapter sequences, and low-quality read sequences were filtered using Trimmomatic 0.38. Trimmed reads were assembled using a Trinity program with default parameters. The Trinity program was used for *de novo* transcriptome assembly, and was then assembled into transcript contigs. These contigs were further processed for read alignment and abundance estimation using Bowtie 1.1.2 and RSEM v1.2.29. The expression level of each contig was calculated using the fragments per kilobase of exon per million mapped fragments method, this was able to remove sequencing discrepancies in the calculation of gene expression and gene lengths. For annotation, the consensus sequences were searched against the GenBank non-redundant database (downloaded on May 3, 2018) and gene ontology database (released on March 19, 2018) using BLASTx (v2.2.25).

Confirmation of RNA-Seq result using RT-PCR assay. To confirm the RNA-Seq result, the total RNA of the pooled leaf sample was extracted using the easy-spin Total RNA Extraction Kit (iNtRON, Seongnam, Korea) and following the manufacturer's instruction RT-PCR assay using SR-8000 (GeNetBio, Daejeon, Korea) was conducted. The viral gene-specific primer, based on GenBank reference sequences and nucleotide sequences of the viral contigs from RNA-Seq, was designed for RT-PCR assay (Table 1). To verify the presence of reported viruses in *P. notoginseng* (TYLCCNV, PnVA, and PanVY) RT-PCR assay was performed using gene-specific primers (Table 1).

Metatranscriptomic analysis of plant virome from *P. ginseng*. Thirty Gbp of raw data were generated from RNA-Seq. Following *de novo* transcriptome assembly, the sequences of 57,610 contigs were compared by BLASTn with nucleotide (nt) sequences in the NCBI database and 23 viral contigs were identified as follows: 2 contigs of *Aristotelia chilensis virus 1* (AcV1, genus *Petuvirus*), 1 contig of *Turnip mosaic virus* (TuMV, genus *Potyvirus*), 17 contigs of WMV (genus *Potyvirus*), and 3 contigs of Tobamovirus multiplication protein (Table 2, Fig. 3, Supplementary Tables 1-3). Most WMV contigs identified were similar to other reported WMV-Insam strains with a nucleotide identity of 99-100%. Among the identified viral contigs, small contigs that corresponds to the WMV with a length less than 1,000 nt were also found, but mostly covered complete genome of WMV-Insam strains (Fig. 3C). Thus, it appears to be the same WMV isolate previously

Table 1. List of primer set used for RT-PCR assay[a]

Name[b]	Primer set	Sequences (5' to 3')	Locations[c]	Expected size (bp)
Tobamo	Tob-Uni 2	GTY GTT GAT GAG TTC RTG GA	5,482-5,501	805
	Tob-Uni 1	ATT TAA GTG GAS GGA AAA VCA CT	6,257-6,279	
TuMV	TuM-N60	ACA TTG AAA AGC GTA ACC A	9,329-9,347	461
	TuM-C10	TCA CCA CAT GCG CTA ACA CCA A	9,768-9,789	
WMV	WMV-Pu-F24	ATC AGA CAA CAT AAA CGC AAA CAA	24-47	572
	PGV-3R058	GCA CAG CAG TTC CAC AGA CAT TAC	557-580	
	WMV-Pu-F33	CAT AAA CGC AAA CAA ACT CTC AAG	33-56	585
	PGV-3R060	ATA TTC TTC GCC ACT TTT GTC TGC	579-602	
	WMV2-N40	GTT TAA CAC TCG AGC AA	9,207-9,223	613
	WMV2-C20	CTT ATA ACG ACC CGA AAT GCT A	9,798-9,819	
TYLCCNV	TYLCCNVFa	TGR TAG GWA CYT GAG TAG AGT GG	1,557-1,579	970
	TYLCCNVRa	TCR TCC ATC CAT ATC TTC CCA A	638-659	
PnVA	PnVA-F	GTG GTA CAC TTT TGC TGG CG	1,718-1,737	469
	PnVA-R	GAC AGG TCC ACC CCA TTC AG	2,167-2,186	
PanVY	CPJCF	GAC AAC AAC AAT GGA TGC	8,549-8,566	760
	CPJCR	ACT AAC ACT GCC ATC AAG	9,288-9,305	

[a] All reverse transcription polymerase chain reaction (RT-PCR) melting temperature was 55°C.

[b] Tobamo, *Tobamovirus degenerated primer* (Letschert et al., 2002); TuMV, *Turnip mosaic virus* (R.O.K. Patent No. 1006250190000) (Lee et al., 2006); WMV, *Watermelon mosaic virus* (Jung et al., 2013); TYLCCNV, *Tomato yellow leaf curl China virus* (Li et al., 2014); PnVA, *Panax notoginseng virus A*; PanVY, *Panax virus Y* (Yan et al., 2010).

[c] Tobamo (accession no. MT107885), TuMV (MG200166), WMV (KP100058), TYLCCNV (KU934106), PnVA (KT388111) and PanVY (GQ916624).

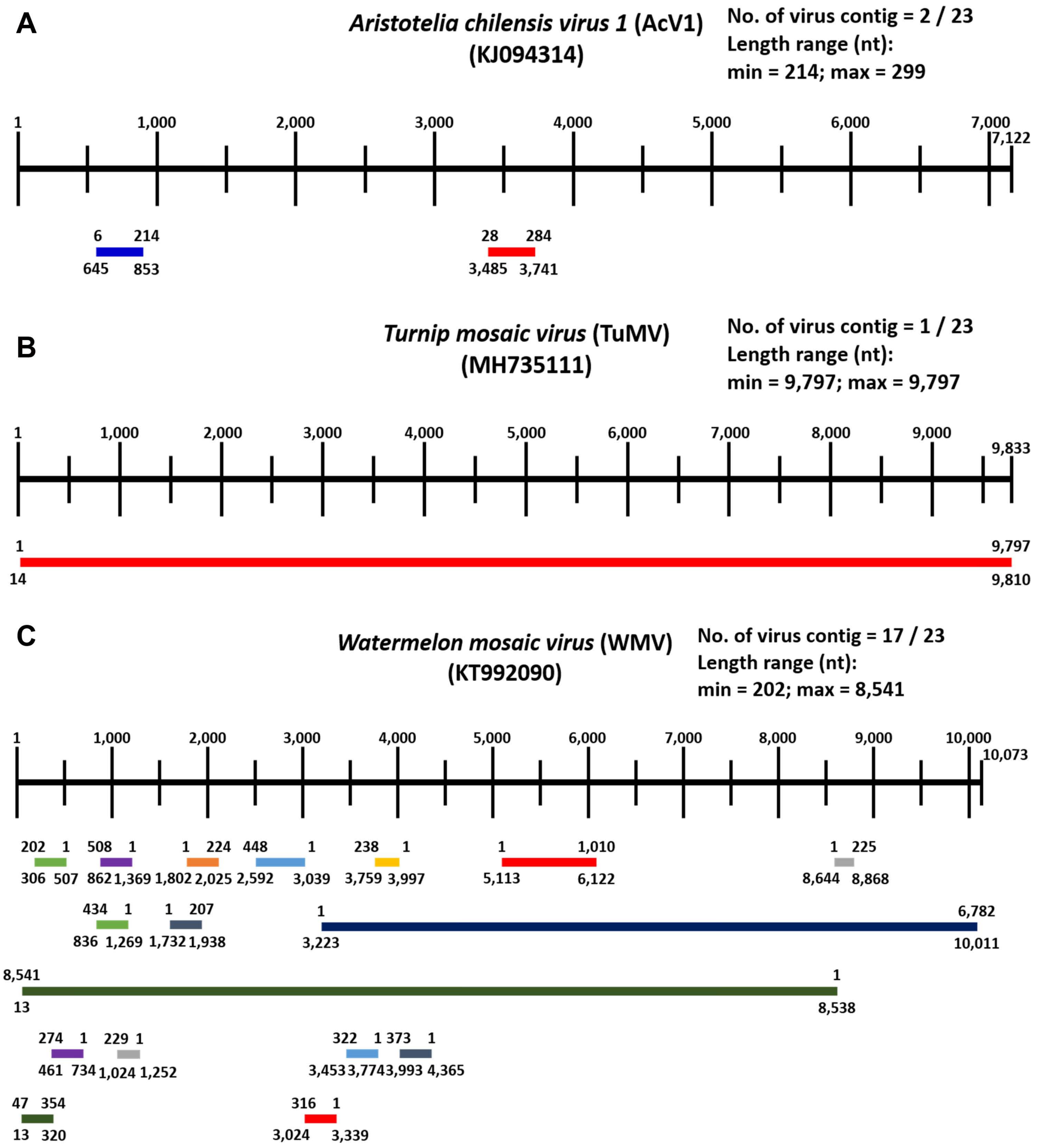

Fig. 3. Schematic overview of the alignment of contigs with NCBI reference sequences using BLASTn. (A) *Aristotelia chilensis virus 1* (family Caulimoviridae, dsDNA). (B) *Turnip mosaic virus* (genus *Potyvirus*, ssRNA). (C) *Watermelon mosaic virus* (genus *Potyvirus*, ssRNA). Colored bar indicates contig from RNA-Seq; upper number indicates the sequence region or location of contig matching NCBI reference sequence; and lower number indicates the region or location within NCBI reference sequence matching contig sequence.

reported in the Korean ginseng plant.

RT-PCR assay for RNA-Seq data verification. In RNA-Seq analysis large contigs (>8,000 nt) corresponding to WMV and TuMV, were mainly identified (Fig. 3). To verify the RNA-Seq data in individual samples, RT-PCR assay was conducted using gene-specific primers to detect TuMV (R.O.K. Patent No. 1006250190000) (Lee et al., 2006) and WMV (Jung et al., 2013). In RT-PCR assays for all collected samples (656 leaf samples), 3 (0.46%) samples were TuMV-positive (two samples of Yeoju and one sample of Hoengseong) and 139 (21.2%) samples were WMV-positive (Table 3). Interestingly, the infection rate of WMV showed noticeable regional differences, and

Table 2. The annotated viral contigs from metatranscriptome analysis of *Panax ginseng* virome

Query ID	Length	BLAST description	Query cover	Identities (%)	Accession no.
c122350_g1_i1	299	Aristotelia chilensis virus 1 isolate AcPLV-1 movement protein and reverse transcriptase genes, complete cds	85	73.93	KJ094314
c85745_g1_i1	214	Aristotelia chilensis virus 1 isolate AcPLV-1 movement protein and reverse transcriptase genes, complete cds	46	76.77	KJ094314
c101531_g1_i1	324	PREDICTED: Camellia sinensis tobamovirus multiplication protein 2A-like (LOC114279495), transcript variant X5, mRNA	100	84.88	XM_028221749
c103606_g1_i2	308	PREDICTED: Daucus carota subsp. sativus tobamovirus multiplication protein 1 (LOC108204642), transcript variant X2, mRNA	100	92.21	XM_017375367
c119698_g1_i1	250	PREDICTED: Vitis vinifera tobamovirus multiplication protein 1 (LOC104878681), mRNA	32	88.89	XM_010649283
c53614_g1_i1	9,797	Turnip mosaic virus isolate CN3, complete genome	100	99.37	MH735111
c116721_g3_i1	1,011	Watermelon mosaic virus isolate Buan2-1_2012, complete genome	99	99.6	KU240094
c110549_g1_i1	448	Watermelon mosaic virus isolate C06-666, complete genome	100	95.31	EU660588
c116728_g1_i1	6,782	Watermelon mosaic virus isolate Eumseong2-10_2014, complete genome	100	99.59	KT992074
c113261_g1_i1	202	Watermelon mosaic virus isolate Naju3-1_2012, complete genome	100	96.04	KU240100
c116735_g1_i1	8,541	Watermelon mosaic virus isolate Sangju3_2013, complete genome	100	95.96	KT992079
c41181_g1_i1	509	Watermelon mosaic virus isolate Sangju6_2012, complete genome	100	98.62	KU240105
c107961_g1_i1	207	Watermelon mosaic virus isolate WMV-WS, complete genome	99	97.09	KX664483
c108825_g1_i1	224	Watermelon mosaic virus isolate WMV-WS, complete genome	99	95.52	KX664483
c113261_g2_i1	354	Watermelon mosaic virus isolate Yeongam2_2013, complete genome	100	98.7	KT992081
c104119_g1_i1	229	Watermelon mosaic virus isolate Yeongju2-3_2013, complete genome	100	100	KT992085
c109339_g1_i1	240	Watermelon mosaic virus isolate Yeongju2-3_2013, complete genome	100	100	KT992085
c112142_g1_i1	373	Watermelon mosaic virus isolate Yeongju2-3_2013, complete genome	100	99.73	KT992085
c98826_g1_i1	434	Watermelon mosaic virus isolate Yeongju6-1_2013, complete genome	100	99.54	KT992086
c112889_g1_i2	297	Watermelon mosaic virus isolate Yeongyang8-1_2013, complete genome	100	99.64	KT992093
c116688_g4_i1	225	Watermelon mosaic virus Sa genomic RNA, complete genome	100	99.56	LC412927
c112898_g2_i1	322	Watermelon mosaic virus strain WMV-Fr, complete genome	100	95.34	AY437609
c93233_g1_i1	316	Watermelon mosaic virus strain WMV-Fr, complete genome	100	95.25	AY437609

this is probably related to the regional distribution of plant viral insect vectors such as aphids (Table 3). *Tobamovirus* was not detected using RT-PCR assay for the single pooled sample with a degenerated primer set for detecting Tobamovirus (Letschert et al., 2002). Therefore, the existence of the *Tobamovirus* is uncertain; therefore, the most commonly found virus of *P. ginseng* is the WMV. TuMV, on the other hand, is considered the minor virus of *P. ginseng*, given its low infection rate (0.61%). In RT-PCR assay using a polled samples, AcV1 was detected by RT-PCR assay using sequence-specific primers, designed based on the AcV1-like contigs from our RNA-Seq data. However, it is unclear whether AcV1 infects *P. ginseng* because only two small contigs (214 nt and 299 nt), corresponding to the partial regions of the genome of AcV1, were found in the RNA-Seq data and the pathogenicity of AcV1 was not yet been determined (Villareses et al., 2015). Viruses in the genus *Panax* that were reported in China, such as TY-

Table 3. Occurrence, distribution, and infection rate (%) of WMV in *Panax ginseng* collected from the cultivation areas located at the 6 provinces

Cultivation area		No. of collected samples	No. of detected samples	Infection rate (%)
Gyeonggi-do	Yeoju	84	2	2.4
	Yeoncheon	34	3	8.8
	Yangpyeong	13	0	0
	Icheon	28	17	60.7
	Hwaseong	33	0	0
	Anseong	18	11	61.1
Gangwon-do	Hoengseong	81	0	0
	Hongcheon	80	1	1.3
	Cheorwon	88	0	0
Chungcheongbuk-do	Chungju	43	19	44.2
	Goesan	25	5	20
Chungcheongnam-do	Yesan	19	7	36.8
	Seosan	36	11	30.5
	Sejong	20	10	50
Jeollabuk-do	Gochang	7	6	85.7
	Namwon	2	2	100
Jeollanam-do	Yeonggwang	38	38	100
	Gangjin	7	7	100
Total	18 areas	656	139	21.2

WMV, *Watermelon mosaic virus*.

LCCNV, PnVA, and PanVY were not found in the RNA-Seq data from *P. ginseng*. TYLCCNV, PnVA, and PanVY were also not detected by RT-PCR assay. From these results, the distribution of domestic ginseng virus species seems different from those recorded in China; only two viruses (WMV and TuMV) were detected in domestically grown *P. ginseng* by RNA-Seq metatranscriptome analysis and RT-PCR assay (Table 4).

This is the first metatranscriptome analysis of plant virome from *P. ginseng* using NGS-based RNA-Seq and RT-PCR based analyses for virus identification and detection and its distribution, which will provide important basic data

Table 4. Summary of metatranscriptome analysis and RT-PCR diagnosis for viruses in *Panax ginseng*

Name[a]	Metatranscriptome analysis	RT-PCR assay	Description
AcV1	○	○	Only 2 small contigs corresponding to AcV1 were found in metatranscriptome analysis. It is uncertain whether the virus is AcV1 or novel (unreported) virus.
Tobamo	○	×	The presence of *Tobamovirus* or *Tobamo-like-virus* has not been confirmed in RT-PCR assay.
TuMV	○	○	Of the 656 samples tested, 3 (0.46%) samples were TuMV-positive in RT-PCR assay.
WMV	○	○	Of the 656 samples tested, 139 (21.2%) samples were WMV-positive in RT-PCR assay. WMV of *P. ginseng* has been reported in Korea (Jung et al., 2013).
TYLCCNV	×	×	Virus of *P. notoginseng*, reported in China (Li et al., 2014).
PnVA	×	×	Virus of *P. notoginseng*, reported in China (Guo et al., 2016).
PanVY	×	×	Virus of *P. notoginseng*, reported in China (Yan et al., 2010).

RT-PCR, reverse transcription polymerase chain reaction.

[a] AcV1, *Aristotelia chiliensis virus 1*; Tobamo, *Tobamovirus multiplication protein*; TuMV, *Turnip mosaic virus*; WMV, *Watermelon mosaic virus*; TYLCCNV, *Tomato yellow leaf curl china virus*; PnVA: *Panax notoginseng virus A*; PanVY: *Panax virus Y.*

for the development of virus-free plants. Further research and continuous field monitoring are therefore needed to clarify potential pathogenicity and economic impact of viruses on *P. ginseng*.

Conflicts of Interest

No potential conflict of interest relevant to this article was reported.

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Electronic Supplementary Material

Supplementary materials are available at The Plant Pathology Journal website (http://www.ppjonline.org/).

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