



Construction of full-length infectious clones of turnip mosaic virus isolates infecting *Perilla frutescens* and genetic analysis of recently emerged strains in Korea

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Received: 31 August 2021 / Accepted: 23 November 2021 / Published online: 8 March 2022
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Abstract

Perilla is an annual herb with a unique aroma and taste that has been cultivated in Korea for hundreds of years. It has been widely cultivated in many Asian and European countries as a food and medicinal crop. Recently, several viruses have been reported to cause diseases in perilla in Korea, including turnip mosaic virus (TuMV), which is known as a brassica pathogen due to its significant damage to brassica crops. In this study, we determined the complete genome sequences of two new TuMV isolates originating from perilla in Korea. Full-length infectious cDNA clones of these two isolates were constructed, and their infectivity was tested by agroinfiltration of *Nicotiana benthamiana* and sap inoculation of Chinese cabbage and radish plants. In addition, we analyzed the phylogenetic relationship of six new Korean TuMV isolates to members of the four major groups. We also used RDP4 software to conduct recombination analysis of recent isolates from Korea, which provided new insight into the evolutionary relationships of Korean isolates of TuMV.

Introduction

Perilla (*Perilla frutescens* Britton) is a member of the family Lamiaceae, which is composed of 236 genera and more than 7000 species. It is an annual herb with a unique aroma and taste that has been cultivated in Korea for hundreds of years [1–3]. Perilla is also cultivated in many other countries,

including China, Japan, and Vietnam. Furthermore, because of its increasing economic significance, some western countries, including European countries, the United States, and Russia, are now also growing perilla [4]. Perilla is a leafy vegetable that is a very common and popular in South Korea, and it is usually eaten as a kind of salted vegetable or consumed with barbecued meats [2]. Due to its importance as a food crop, it is grown widely in South Korea, including the provinces of Kangwan, Jeollabuk, Gyeongsangbuk, Kyonggi, Jeollanam, Chungchongbuk, Chungcheongnam, and Gyeongsangnam [3, 5].

Handling Editor: Ioannis E. Tzanetakis.

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In addition to its value as a food crop, perilla has been reported to have potential as a medicinal resource [6–9]. For example, a recently described substance, Pf-gp6, extracted from perilla has been reported to inhibit the replication of HIV-1 [7]. A leaf extract of perilla has been reported to inhibit SARS-CoV-2 by direct virus inactivation [6]. Thus, perilla is a very popular plant with high economic value.

In Korea, several pathogens have been found to infect perilla and cause disease. *Ramularia coleosporii* has been reported to induce leaf spots on perilla [10], and *Corynespora cassiicola* has been shown to cause stem blight [11]. Recently, other diseases caused by plant viruses have been reported in Korea. In the city of Yeongcheon, cucumber green mottle mosaic virus (CGMMV) has been found to cause mosaic and malformation on perilla leaves [12]. Turnip mosaic virus (TuMV) was first found to infect perilla plant in Korea in 2020, inducing mild mosaic and yellowing symptoms [13].

TuMV is known as a brassica pathogen due to its significant damage to brassica crops [14]. A recent report has indicated that TuMV spread from the west to the east across Eurasia beginning in approximately the 17th century CE [15]. In recent years, TuMV has been found to cause diseases in Brassicaceae plants in South Korea, such as Chinese cabbage (*Brassica rapa* var. *pekinensis*) and radish (*Raphanus sativus*) [16, 17].

Investigation of the molecular evolutionary history of TuMV is beneficial for studying its biological properties [18]. Variation in the genomes of viruses results from mutation, recombination, adaptation, and selection [18–21]. Recombination is one of the main forces that accelerate adaptation and variation, often resulting in the emergence of variants that are able to overcome resistance [21–25]. The development of software designed to detect recombination based on sequence comparisons has allowed an increasing number of recombination events in plant RNA viruses to be discovered [14, 18, 19].

In this study, the complete genomes of two new TuMV isolates were characterized, full-length infectious cDNA clones of these two isolates were constructed, and their infectivity was tested by agroinfiltration of *Nicotiana benthamiana* plants and sap inoculation of Chinese cabbage cv. CR Victory, radish cv. Iljin, and *P. frutescens*. We also investigated the relationship of six newly collected isolates from Korea by phylogenetic analysis. We found for the first time that recombination events have occurred in Korean TuMV strains, which helps us to better understand the evolutionary relationship among Korean isolates of TuMV.

Materials and methods

Sample collection and plant material

A *P. frutescens* plant sample with typical TuMV-like symptoms, including mosaic and chlorosis, was observed in the

city of Chuncheon, South Korea (sample collected by Professor Jin-Sung Hong, Kangwon National University). The *N. benthamiana*, Chinese cabbage, radish, and perilla plants used in this study were incubated in 25°C ± 2°C with 16 hours of light and 8 hours of dark. All of the soil used was sterilized before use.

RNA extraction, cDNA synthesis, and PCR detection

For virus detection, total RNA was extracted from plant tissues using TRIzol® Reagent (Life Technologies, Carlsbad, CA, USA), and the extracted samples were preserved at -70°C. cDNA was produced using a LeGene Express 1st Strand cDNA Synthesis System with an oligo dT primer. PCR was performed using TuMV CP forward primer 5'-TCT CAA TGG TTT AAT GGT CTG G-3' and the reverse primer 5'-AAC CCC TTA ACG CCA AGT AAG-3' [26].

Construction of full-length clones of TuMV

To obtain infectious clones, we performed full-length PCR using a cDNA template derived from infected perilla. The PCR mixture of 50 µl was composed of 2 µl of the template cDNA, 25 µl of 2x PCR buffer for KOD FX Neo, 10 pmol of a forward primer containing an *ApaI* site and T7 RNA polymerase promoter sequence (5'-GAG GGG CCC TAA TAC GAC TCA CTA TAG GAA AAA TAT AAA AAC TCA ACA CAA CAT ACA CAA AAC G), 0.4 mM dNTPs, 10 pmol of a reverse primer containing an *XmaI* site (5'-GAG CCC GGG TTT TTT TTT TTT TTT TTT TTT TTT TTT GTC CCT TGC ATC CTA TCA AAT G)[17], and 1 µl of Taq polymerase (KOD FX Neo, Toyobo, Osaka, Japan). A cDNA template from TuMV that has been successfully amplified before was used as a positive control. The conditions for full-length PCR were 94 °C for 2 min, followed by five cycles of 10 s at 98 °C, 30 s for annealing at 59 °C, and 6 min for extension at 68 °C, and then by 30 cycles of 10 s at 98 °C, 30 s at 65 °C, and 6 min at 68 °C, and finally incubation at 4 °C. Full-length PCR products were analyzed by 0.8% agarose gel electrophoresis with dye incorporated in the gel. The PCR product was digested using *ApaI* and *XmaI* and subsequently cloned into the binary vector pJY that had been digested using the same enzymes [27, 28]. The recombinant plasmids were then introduced into competent *E. coli* DH5a cells. The recombinants were screened by colony PCR and double enzyme digestion.

Agrobacterium infiltration and sap inoculation

The recombinant plasmids were introduced by transformation into *Agrobacterium tumefaciens* GV2260. Colonies of each clone were grown on LB plates supplemented with kanamycin and rifampicin, and the agrobacterium cells collected

from fresh plates were diluted to an OD₆₀₀ of approximately 0.6 in infiltration buffer (10 mM MES, 10 mM MgCl₂, and 150 μM acetosyringone). *N. benthamiana* plants inoculated with the constructed clones were incubated in a growth chamber at 24-26 °C (16/8h, light/dark cycle) [17]. Leaves of the infiltrated *N. benthamiana* plants with symptoms were used to inoculate Chinese cabbage cv. CR Victory, radish cv. Iljin, perilla cv. Okdong, or perilla cv. Leaf as described previously [17].

Sequencing of TuMV infectious cDNA clones

After the infectivity of each full-length cDNA clone was assessed by agroinfiltration, the full-length clones that were shown to have infectivity were sequenced by Bioneer Corporation (Daejeon, South Korea). Sequencing was initiated from each terminus using vector-specific primers and continued using primers designed based on the sequences obtained (Table 1). The complete genome sequences were compared and assembled using DNAMAN software (Version 5.2, Lynnon BioSoft).

Phylogenetic analysis

The maximum-likelihood method was used to construct a phylogenetic tree with 1000 bootstrap replicates in MEGA software (version 7.0). The complete genome sequences of TuMV strains used in this study were obtained from NCBI (Table 2), including isolates previously reported in South Korea [16, 17, 29], two infectious clones constructed in this

Table 2 Details about the TuMV isolates analyzed in this study

No.	Accession number	Isolate	Original host
1	AB701698	BEL1	<i>Nasturtium officinale</i>
2	AB093611	BZ1	<i>Brassica oleracea</i>
3	D10927	Q-Ca	<i>Brassica napus</i>
4	AF530055	YC5	<i>Zantedeschia</i> sp.
5	AB252106	CHZJ26A	<i>Brassica campestris</i>
6	AB093627	HRD	<i>Raphanus sativus</i>
7	AB252103	CH6	<i>Raphanus sativus</i>
8	AB252107	CZE5	<i>Brassica oleracea</i>
9	AB701703	DNK3	<i>Brassica rapa</i>
10	AB701708	FRA2	<i>Brassica napus</i>
11	NC 001616	Potato virus Y	<i>Solanum</i> sp.
12	AB701697	ASP	<i>Allium</i> sp.
13	AB701734	TIGA	<i>Tigridia</i> sp.
14	AB701735	TIGD	<i>Tigridia</i> sp.
15	AB701700	DEU2	<i>Raphanus sativus</i>
16	AB701699	DEU1	Unknown
17	AB252117	GRC42	Wild <i>Allium</i> sp.
18	AB701696	GK1	<i>Matthiola incana</i>
19	AB701719	HUN1	<i>Alliaria petiolata</i>
20	AB440238	IRNTra6	<i>Rapistrum rugosum</i>
21	AB440239	IRNSS5	<i>Sisymbrium loeselii</i>
22	AB093602	IS1	<i>Allium ampeloprasum</i>
23	AB701720	ITA1A	<i>Brassica ruvo</i>
24	AB093598	AI	<i>Alliaria officinalis</i>
25	AB701721	ITA2	<i>Cheiranthus cheiri</i>
26	AB701725	ITA8	<i>Abutilon</i> sp.
27	AB093600	ITA7	<i>Raphanus sativus</i>
28	AB093601	Cal1	<i>Calendula officinalis</i>
29	AB252125	KWB779J	<i>Brassica rapa</i>
30	MG200170	KBJ5	<i>Raphanus sativus</i>
31	MG200169	KBJ4	<i>Raphanus sativus</i>
32	MG200168	KBJ3	<i>Raphanus sativus</i>
33	MG200167	KBJ2	<i>Raphanus sativus</i>
34	MG200166	KBJ1	<i>Raphanus sativus</i>
35	KX674727	HJY1	<i>Raphanus sativus</i>
36	KX674728	HJY2	<i>Raphanus sativus</i>
37	KX674729	KIH1	<i>Raphanus sativus</i>
38	KX674730	KIH2	<i>Raphanus sativus</i>
39	KX674731	GJS1	<i>Raphanus sativus</i>
40	KX674732	GJS2	<i>Raphanus sativus</i>
41	KX674733	GJS3	<i>Raphanus sativus</i>
42	KX674734	GJS4	<i>Raphanus sativus</i>
43	KY111268	SW1	<i>Raphanus sativus</i>
44	KY111267	SW2	<i>Raphanus sativus</i>
45	KX674726	BE	<i>Raphanus sativus</i>
46	KY111274	DJ1	<i>Raphanus sativus</i>
47	KY111273	DJ2	<i>Raphanus sativus</i>
48	KY111272	DJ3	<i>Raphanus sativus</i>
49	KY111271	DJ4	<i>Raphanus sativus</i>
50	KY111270	DJ5	<i>Raphanus sativus</i>

Table 1 Sequencing primers designed and used in this study

No.	Name	Sequence (5' to 3')
1	KPF_F1	AGTGCCATTGCGAACCAC
2	KPF_F2	CAAGATCTTCAAGGCGAAG
3	KPF_F3	TGACGGATAGTGAGTTGTCT
4	KPF_F4	AACGGATCACGAAGGCT
5	KPF_F5	ATATCCTCAAGACAAACACC
6	KFP_F6	ACCGAATAATGAGCTGC
7	KPF_F7	ATTCACAGCGTATATTGCT
8	KPF_F8	TCTTGAACCAACTCGACC
9	R1	AATCTCACCATGCGCTAA
10	R2	TTGGGGAGGTTCCATTCT
11	R-cover	ACATCCAGATGAAAGCAG
12	R3	GTTCGATCATCCATGCGT
13	R4	TTCATAAATTCCAAGCGGAT
14	R5	AGTTCGCGATCGATATTC
15	R6	TTGGTGCTAATCCAGTGTT
16	R7	CATGGAGGTCACACACCT
17	KPF_R8	TAACGTTTTATCCATTTGCC
18	KPF_R9	ATACTCGCTTGCTGTGAG

Table 2 (continued)

No.	Accession number	Isolate	Original host
51	KY111269	DJ6	<i>Raphanus sativus</i>
52	KU140420	R007	<i>Raphanus sativus</i>
53	KU140421	R041	<i>Raphanus sativus</i>
54	KU140422	R65	<i>Raphanus sativus</i>
55	MZ570590	KPF-1	<i>Perilla frutescens</i>
56	MZ570591	KPF-2	<i>Perilla frutescens</i>
57	MW556024	KBC-1	<i>Brassica rapa</i>
58	MW556025	KBC-8	<i>Brassica rapa</i>
59	MW556026	KRS-3	<i>Raphanus sativus</i>
60	MW556027	KRS-8	<i>Raphanus sativus</i>
61	DQ648591	CAR37A	<i>Cochlearia armoracia</i>
62	AB701731	POL2	<i>Papaver somniferum</i>
63	AB701728	POL1	<i>Brassica napus oleifera</i>
64	AB701729	PRT1	<i>Brassica oleracea acephala</i>
65	AB093606	RUS1	<i>Armoracia rusticana</i>
66	AB362513	TUR9	<i>Raphanus sativus</i>
67	AB701717	GBR83	<i>Brassica oleracea</i>
68	AF169561	UK1	<i>Brassica napus</i>
69	KM094174	JPN 1	<i>Raphanus sativus</i>

study (KPF-1 and KPF-2), and four newly collected isolates, KRS-3, KRS-8, KBC-1, and KBC-8 (submitted to NCBI but not yet published).

Recombination analysis

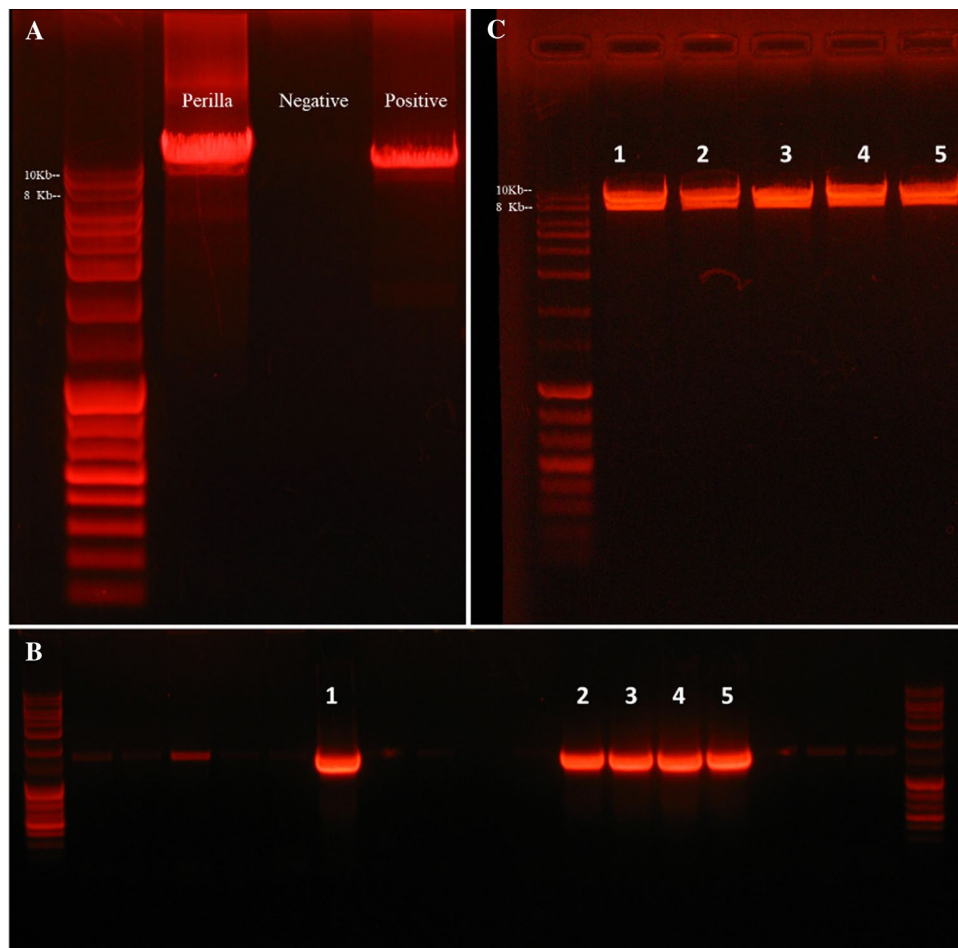
First, the complete genome sequences of all of the Korean TuMV isolates were aligned using Clustal X in MEGA 7.0. Recombination analysis was performed using the RDP4 software package, employing seven detection methods: RDP [30], GENECONV [31], Bootscan [32], Maxchi [33], Chimaera [34], SiScan [35], and 3SEQ [36]. Recombination events were noted if supported by at least four different methods ($p < 1.0 \times 10^{-6}$) [18].

Results

Construction of full-length cDNA clones

Full-length PCR products amplified from cDNA prepared from infected perilla were analyzed by gel electrophoresis

Fig. 1 (A) Results of PCR amplification of the full-length genome of TuMV from perilla, with negative and positive controls. cDNA from a TuMV isolate that had previously been amplified successfully served as a positive control. (B) Colony PCR results of screening for full-length TuMV clones. (C) Confirmation of the positive colonies by digestion with *Apa*I and *Xma*I.



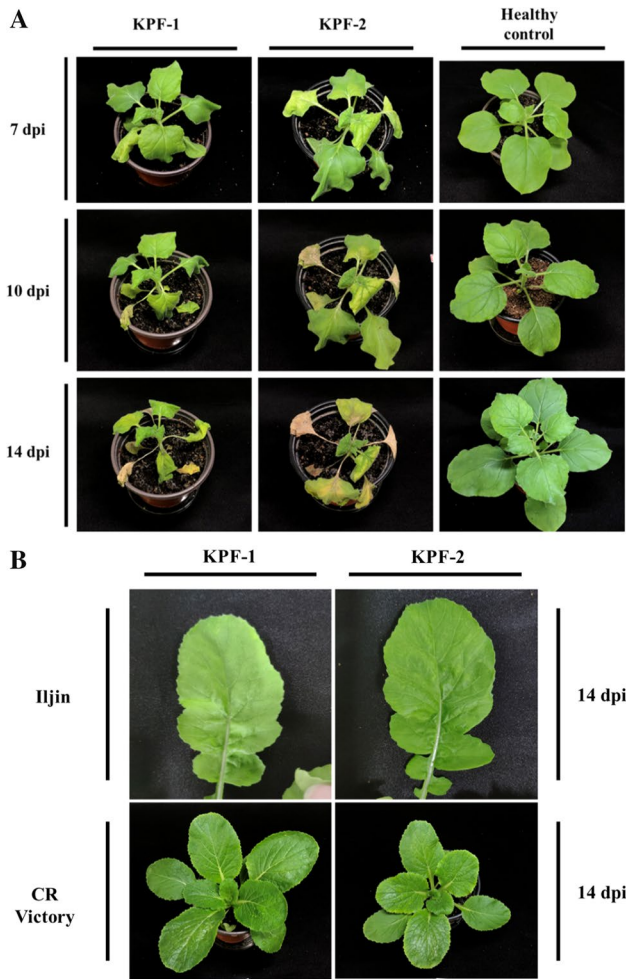


Fig. 2 (A) Symptoms induced by the TuMV isolates KPF-1 and KPF-2 on *Nicotiana benthamiana* at 7, 10, and 14 dpi. (B) Symptoms induced by the isolates KPF-1 and KPF-2 on radish cv. Iljin and Chinese cabbage cv. CR Victory at 14 dpi

Table 3 Results of RT-PCR detection assays

	<i>N. benthamiana</i>	Chinese cabbage cv. CR Victory	Radish cv. Iljin	<i>P. frutescens</i> cv. Leaf	<i>P. frutescens</i> cv. Okdong
KPF-1	+	-	+	-	+
KPF-2	+	-	+	-	+
KPF-3	-	o	o	o	o
KPF-4	-	o	o	o	o
KPF-5	-	o	o	o	o

+, positive; -, negative; o, not tested

(Fig. 1A) and digested using *ApaI* and *XmaI*. Subsequently, the digested product was ligated with the pJY vector, which had been treated with the same enzymes. Colony PCR was

performed to identify colonies harboring recombinants (Fig. 1B), and ultimately, we obtained five positive colonies, namely KPF-1, KPF-2, KPF-3, KPF-4, and KPF-5, and the presence of the plasmid constructs was confirmed by double enzyme digestion (Fig. 1C).

Agroinfiltration and sap inoculation

At 4 days post-inoculation (dpi), we observed weak leaf curling symptoms on the top leaves of *N. benthamiana* plants inoculated with KPF-2, while no symptoms were observed in plants infiltrated with other isolates. At 6 dpi, plants inoculated with the construct KPF-1 also showed symptoms. Symptoms were further recorded at 7, 10 and 14 dpi (Fig. 2A). Finally, infectivity was only confirmed for the full-length cDNA clones KPF-1 and KPF-2. KPF-2 was found to cause symptoms more quickly and to induce obvious chlorosis, which was not induced by KPF-1. The infection was confirmed by RT-PCR as described above, and the RT-PCR result was consistent with symptom development (Table 3).

For sap inoculation, both isolates infected radish cv. Iljin systemically, causing mild mosaic symptoms, while neither isolate could infect Chinese cabbage cv. CR Victory systemically (Fig. 2B). In addition, sap inoculation of perilla plants showed that our isolates could infect perilla cv. Okdong but not cv. Leaf. Infection was confirmed by RT-PCR (Table 3).

Nucleotide and amino acid sequence analysis

The genomes of isolates KPF-1 and KPF-2 are both composed of 9832 nucleotides, excluding the poly(A) tail, and the genome is predicted to encode a polyprotein of 3164 amino acids. However, the complete genome size of Korean TuMV isolates that we obtained previously was 9833 nt [16, 17]. By contrast, the complete genome sequences of the new isolates reported here lack a base in the 3' untranslated region at nt 9753. The two isolates share 99.92% identity in their nucleotide sequence and 99.84% in their predicted amino acid sequence. A sequence alignment revealed that there are eight nucleotide differences between their genomes, located in P1 (nt 579), HC-Pro (nt 1279 and 2201), P3 (nt 3528 and 3653), CI (nt 4419), VPg (nt 6233), and NIa-Pro (nt 6597). An amino acid sequence alignment showed five differences, in P1 (R150S), HC-Pro (D384N and P691H), P3 (R1175Q), and VPg (E2035G) (Fig. 3).

Phylogenetic analysis

Two phylogenetic trees were constructed by the maximum-likelihood method with 1000 bootstrap replicates in MEGA 7.0. The first phylogenetic tree consisting of

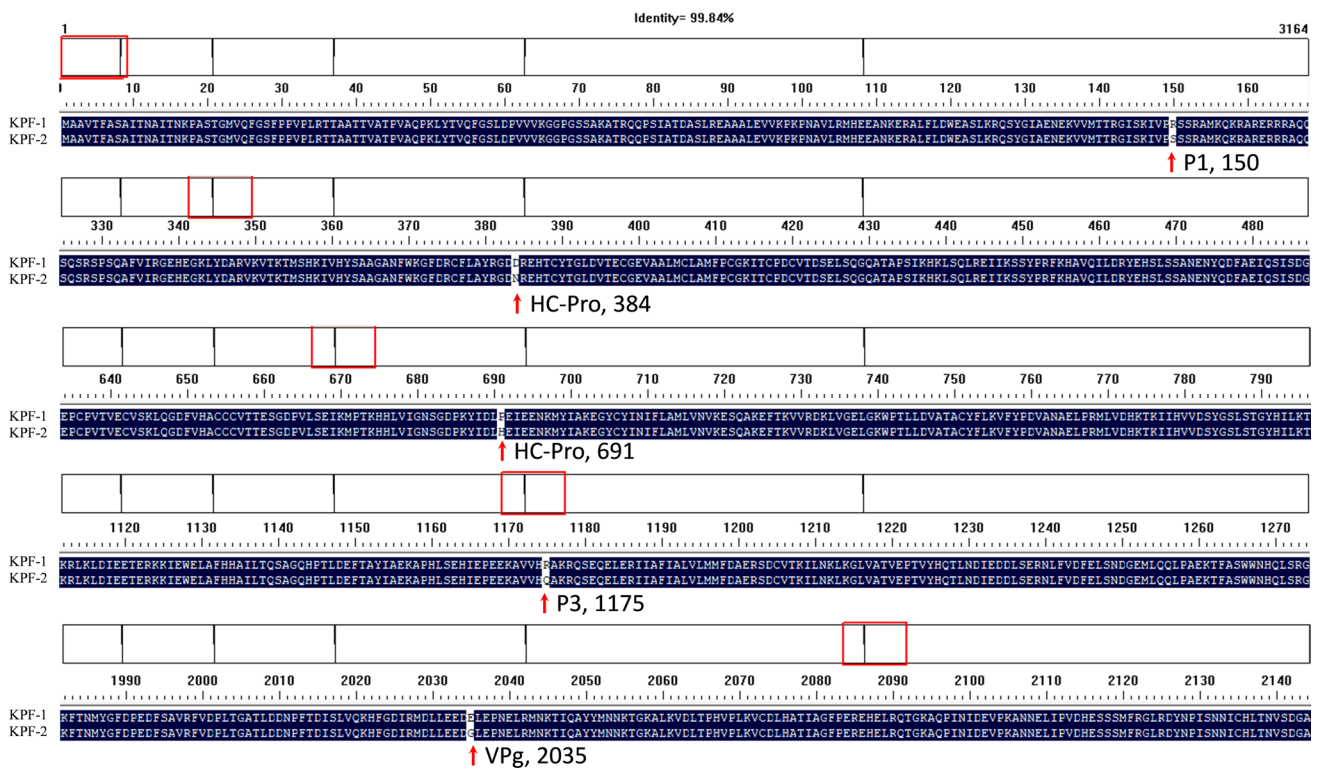


Fig. 3 The positions of amino acid differences in the polyproteins of TuMV isolates KPF-1 and KPF-2

isolates collected from a variety of hosts in multiple countries formed four branches, which is consistent with previous studies [18, 19] (Fig. 4). This tree also included isolates UK1, YC5, and JPN 1, which have been used in previous studies to construct infectious cDNA clones [37–39]. The newly collected perilla isolates (KPF-1 and KPF-2) and radish isolates (KRS-3 and KRS-8) grouped together within the Basal-BR group. The Chinese cabbage isolates (KBC-1 and KBC-8) were predicted to belong to the World-B group. However, most of the isolates collected from Korea belong to the Basal-BR group [16, 17]. The second phylogenetic tree only included Korean TuMV isolates with PVY as an outgroup (Fig. 5). Interestingly, the four strains previously identified as Basal-BR isolates clustered with KBC-1 and KBC-8, which belonged to the World-B group in the first tree, forming a separate branch from the other Basal-BR isolates.

Recombination analysis

Due to the close relationship between the four Basal-BR isolates and the two World-B isolates in the second phylogenetic tree, we conducted recombination analysis using RDP 4 software, which is commonly used to study recombination events that have occurred in virus populations.

As expected, the isolate BE and three very similar isolates (HJY1, HJY2, and R007, sharing 99.93% nt sequence identity) were identified as recombinants by all seven detection methods (Fig. 6). Three putative recombination events were detected. The information for each recombination event is shown in Table 4, with HJY-1 representing the three closely related isolates HJY1, HJY2, and R007.

Discussion

P. frutescens, a crop with high economic value, has been studied in recent years. With climate change resulting in rising temperatures, diseases caused by viruses are being reported continually. Increasing attention is being paid to diseases caused by viruses in perilla plants. In this study, we collected two TuMV isolates from a perilla sample collected in Kangwon, South Korea. The complete genome sequence of each isolate was determined and found to consist of 9832 nucleotides. Interestingly, they lack a nucleotide in the 3' UTR region when compared to all TuMV strains we have previously collected in South Korea [16, 17, 27].

In addition, we successfully constructed full-length infectious clones of these two isolates, namely KPF-1 and KPF-2. Their infectivity was evaluated by agroinfiltration of *N. benthamiana*. Both isolates are able to infect

Fig. 4 A phylogenetic tree constructed by the maximum-likelihood method with 1000 bootstrap replicates, using MEGA 7.0, based on the complete genome nucleotide sequence of TuMV isolates from around the world. The genome sequence of potato virus Y was used to root the tree. Labels show the isolate name or abbreviation/GenBank accession number/original host (Table 2). The labels of the new Korean isolates from perilla (KPF-1, KPF-2), radish (KRS-3, KRS-8), and Chinese cabbage (KBC-1, KBC-8) are shown in bold font on pink bars.

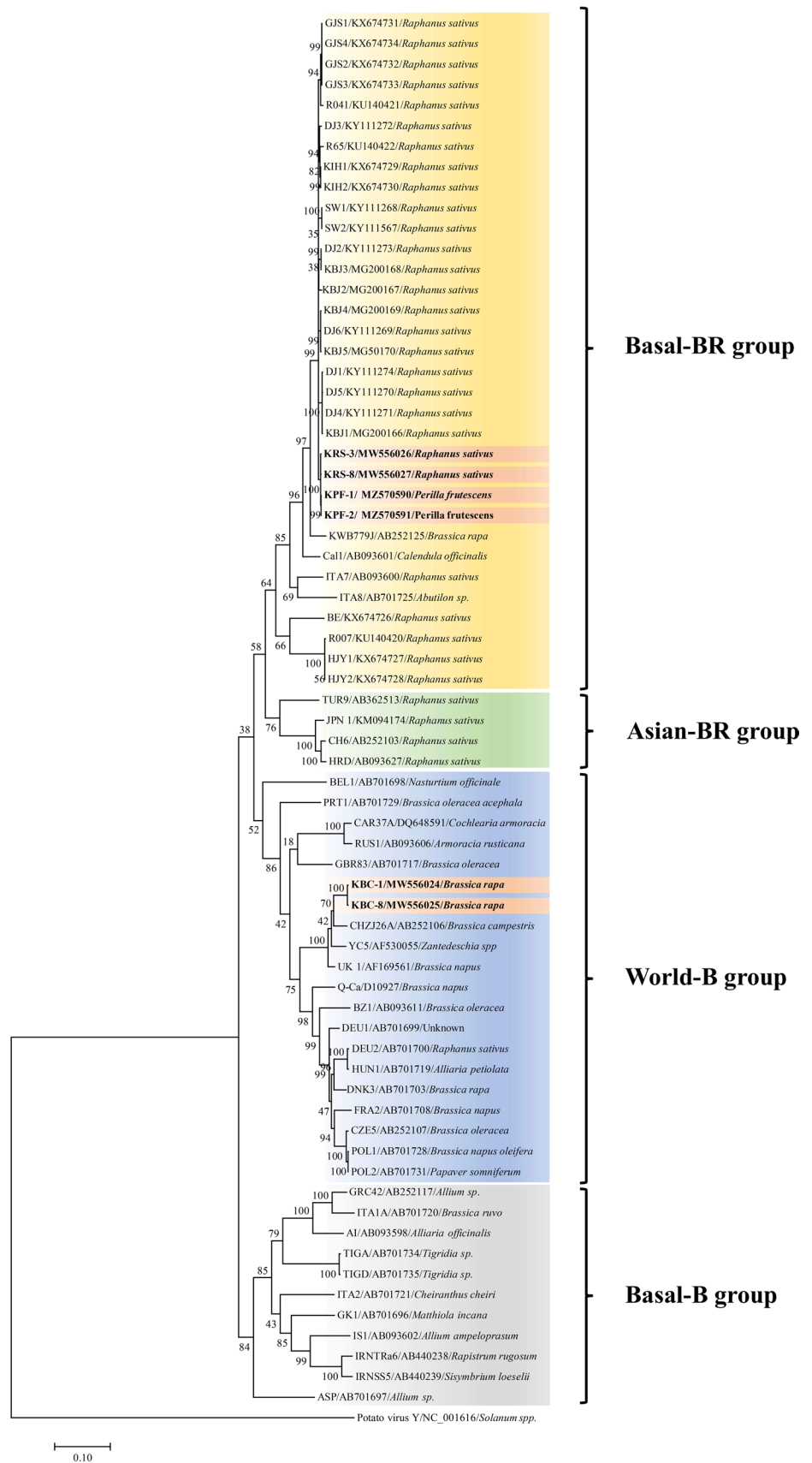


Fig. 5 A phylogenetic tree constructed by the maximum-likelihood method with 1000 bootstrap replicates, using MEGA 7.0, based on the complete genome nucleotide sequence of Korean TuMV isolates. The genome sequence of potato virus Y was used to root the tree. Labels show the isolate name or abbreviation/GenBank accession number. For further details including the host plant, see Table 2. The labels of the new Korean isolates from perilla (KPF-1, KPF-2), radish (KRS-3, KRS-8), and Chinese cabbage (KBC-1, KBC-8) are shown in bold font.

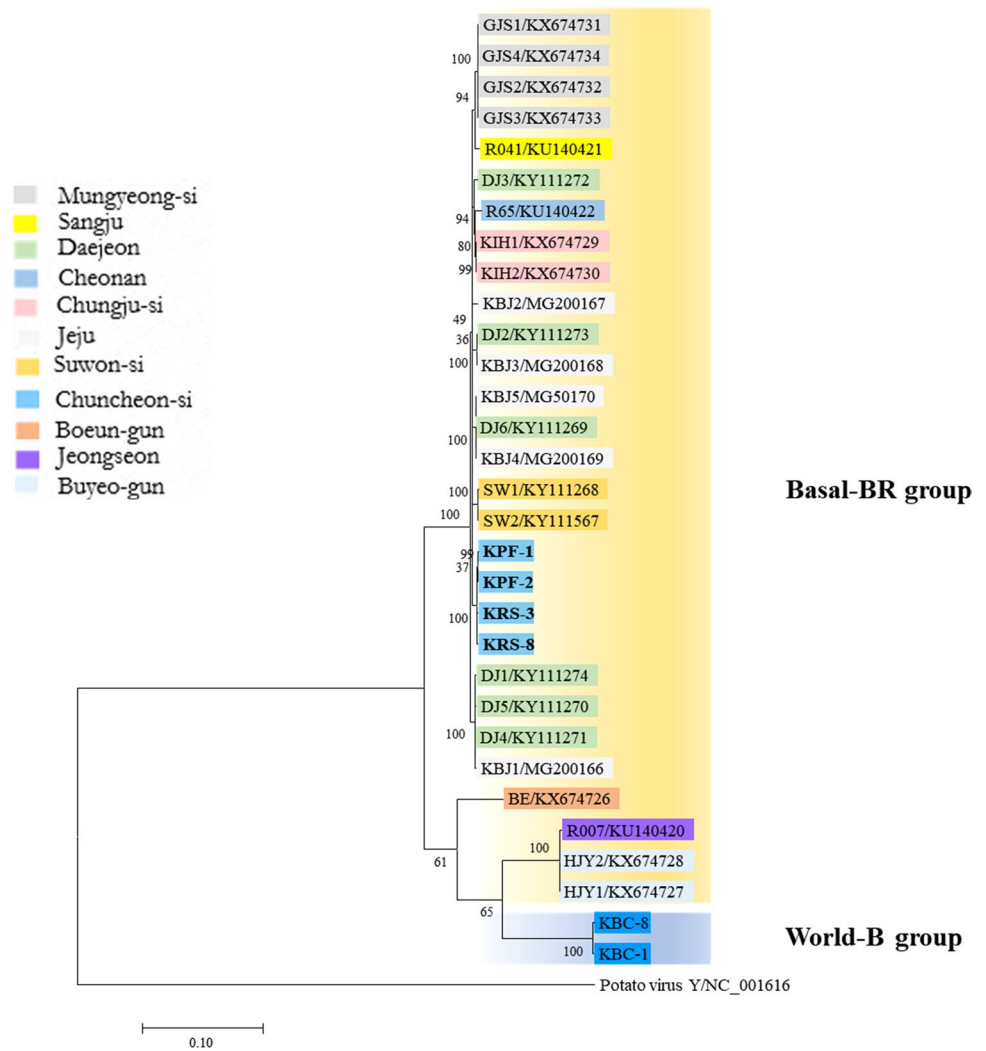


Fig. 6 Recombination events identified using RDP4, supported by all seven detection methods (see Table 4). Recombination events 1 and 3 were detected in isolate BE, and event 2 was detected in isolates HJY1, HJY2, and R007.

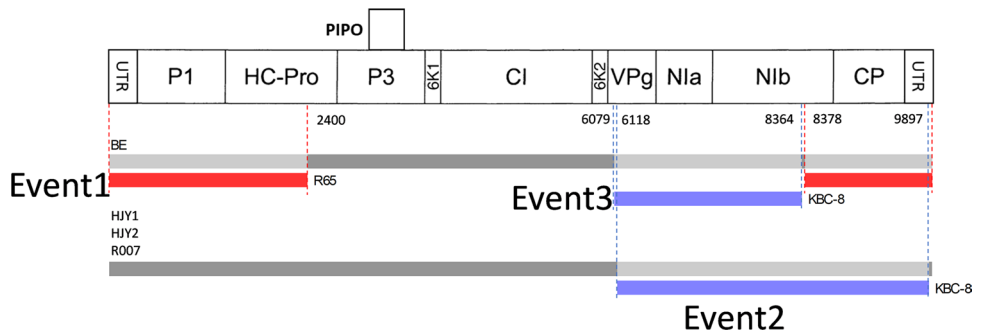


Table 4 Recombination events detected in Korea TuMV isolates

Event number	Recombinant	Major parent	Minor parent	Type of 'recombinant'	Detection methods *							
					R	G	B	M	C	S	T	p-value
1	BE	KBC-8	R65	World-B × Basal-BR	+	+	+	+	+	+	+	2.414×10^{-130}
2	HJY1	GJS1	KBC-8	Basal-BR × World-B	+	+	+	+	+	+	+	6.946×10^{-103}
3	BE	R65	KBC-8	Basal-BR × World-B	+	+	+	+	+	+	+	7.22×10^{-64}

* R = RDP; G = GENECONV; B = Bootscan; M = Maxchi; C = Chimaera; S = Siscan; T = 3Seq

N. benthamiana systemically, but isolate KPF-2 induced symptoms more quickly, usually two days earlier than isolate KPF-1. Amino acid sequence alignment suggested that there are five amino acid differences located in the P1, HC-Pro (2), P3, and VPg proteins. One conserved motif in HC-Pro region has been studied and shown to recruit and employ host ARGONAUTE1 (AGO 1) in the formation of stable virions, and this may be involved in achieving efficient systemic infection [40]. The P1 region is the most variable part of the genome [41], and the P1 protein has been shown to affect RNA silencing suppression activity indirectly through inhibition of its proteolytic activity, leading to an accumulation of the intermediate P1-HCPro [42]. The P3 region has also been reported to be an important symptom determinant, affecting the host range and cell-to-cell movement [43, 44]. In this study, we determined the complete genome sequences of two new TuMV isolates and constructed full-length infectious clones to investigate their corresponding symptom phenotypes on *N. benthamiana*, which will be useful tools in future studies.

Recently, many studies of TuMV have focused on common recombinants of TuMV and the evolutionary history of the virus [18]. Some phylogenetic studies have emphasized the need to use non-recombinant sequences to reduce the ‘noise’ caused by recombinants in evolutionary analysis [14].

In this study, we analyzed the phylogenetic relationships of six new Korean TuMV isolates and determined their position with respect to the four major groups. Considering the close relationship among several Basal-BR isolates and two World-B isolates, we conducted a recombination analysis of Korean TuMV isolates using RDP4, which identified putative recombination events in isolates BE and HJY1 (and also HJY2 and R007) by all seven detection methods (Fig. 6). Recombinant events 2 and 3 could account for the close relationship among the Basal-BR isolates BE and HJY1 (plus HJY2 and R007) and the World-B isolate KBC-8 (KBC-1). Similar evidence of recombination of TuMV genomes has been reported previously, such as a recombination event between members of the Basal-B group and the World-B group identified in Australian TuMV isolates [14]. To our knowledge, there have been few studies involving recombination analysis of Korean TuMV isolates [15], and our analysis provides new insights into the evolutionary relationships among Korean isolates of TuMV.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s00705-021-05356-9>.

Acknowledgements This work was supported by a grant from the New Breeding Technologies Development Program (project no. PJ01477603), Rural Development Administration, Republic of Korea.

Funding This article was funded by Rural Development Administration (Grant no. PJ01477603).

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with human participants performed by any of the authors.

References

1. Lee BH, Lee ST, Kim YS (1998) Reference review for the scientific researches on perilla. RDA J Ind Crop Sci 40:80–112
2. Seo WH, Baek HH (2009) Characteristic aroma-active compounds of Korean perilla (*Perilla frutescens* Britton) leaf. J Agric Food Chem 57:11537–11542
3. Dhyani A, Chopra R, Garg M (2019) A review on nutritional value, functional properties and pharmacological application of Perilla (*Perilla frutescens* L.). Biomed Pharmacol J 12:649–660
4. Nitta M, Lee JK, Ohnishi O (2003) Asian Perilla crops and their weedy forms: Their cultivation, utilization and genetic relationships. Econ Bot 57:245–253
5. Nitta M, Lee JK, Kang CW, Katsuta M, Yasumoto S, Liu DJ, Nagamine T, Ohnishi O (2005) The distribution of *Perilla* species. Genet Resour Crop Ev 52:797–804
6. Tang W-F, Tsai H-P, Chang Y-H, Chang T-Y, Hsieh C-F, Lin C-Y, Lin G-H, Chen Y-L, Jheng J-R, Liu P-C, Yang C-M, Chin Y-F, Chen CC, Kau J-H, Hung Y-J, Hsieh P-S, Horng J-T (2021) Perilla (*Perilla frutescens*) leaf extract inhibits SARS-CoV-2 via direct virus inactivation. Biomed J 44:293–303
7. Kawahata T, Otake T, Mori H, Kojima Y, Oishi I, Okamoto Y, Sano K (2002) A novel substance purified from *Perilla frutescens* Britton inhibits an early stage of HIV-1 replication without blocking viral adsorption. Antivir Chem Chemother 13:283–288
8. Yamasaki K, Nakano M, Kawahata T, Mori H, Otake T, Ueba N, Oishi I, Inami R, Yamane M, Nakamura M, Murata H, Nakanishi T (1998) Anti-HIV-1 activity of herbs in Labiatae. Biol Pharm Bull 21:829–833
9. Asada M, Fukumori Y, Inoue M, Nakagomi K, Sugie M, Fujita Y, Tomizuka N, Yamazaki Y, Oka S (1999) Glycoprotein derived from the hot water extract of mint plant, *Perilla frutescens* Britton. J Agric Food Chem 47:468–472
10. Aktaruzzaman M, Afroz T, Choi H-W, Kim BS (2021) First report of *Ramularia coleosporii* causing leaf spot on *Perilla frutescens* in Korea. Plant Dis 105:2727
11. Lee HB, Kim CJ, Mun HY (2009) First report of stem blight on perilla (*Perilla frutescens*) caused by *Corynespora cassiicola* in Korea. Plant Dis 93:550–550
12. Cho I-S, Yoon J-Y, Chung B-N, Lim H-S (2020) First report of cucumber green mottle mosaic virus infecting *Perilla frutescens* in Korea and the world. J Plant Pathol 103:347–347
13. Park SH, Park JS, Park TS, Hong JS (2020) First report of turnip mosaic virus infecting *Perilla frutescens* in Korea. J Plant Pathol 102:969–969
14. Nyalugwe EP, Jones RAC, Barbetti MJ, Kehoe MA (2015) Biological and molecular variation amongst Australian Turnip mosaic virus isolates. Plant Pathol 64:1215–1223

15. Kawakubo S, Gao F, Li S, Tan Z, Huang Y-K, Adkar-Purushothama CR, Gurikar C, Maneechoat P, Chiemsoat P, and San Aye S (2021) Genomic analysis of the brassica pathogen turnip mosaic potyvirus reveals its spread along the former trade routes of the Silk Road. *Proc Natl Acad Sci USA* 118:e2021221118
16. Hu W-X, Kim B-J, Kwak Y, Seo E-Y, Kim J-K, Han J-Y, Kim I-H, Lim YP, Cho I-S, Domier LL (2019) Five newly collected turnip mosaic virus (TuMV) isolates from Jeju Island, Korea are closely related to previously reported Korean TuMV isolates but show distinctive symptom development. *Plant Pathol J* 35:381–387
17. Gong J, Ju HK, Kim IH, Seo EY, Cho IS, Hu WX, Han JY, Kim JK, Choi SR, Lim YP, Hammond J, Lim HS (2019) Sequence variations among 17 new radish isolates of *Turnip mosaic virus* showing differential pathogenicity and infectivity in *Nicotiana benthamiana*, *Brassica rapa*, and *Raphanus sativus*. *Phytopathology* 109:904–912
18. Ohshima K, Tomitaka Y, Wood JT, Minematsu Y, Kajiyama H, Tomimura K, Gibbs AJ (2007) Patterns of recombination in turnip mosaic virus genomic sequences indicate hotspots of recombination. *J Gen Virol* 88:298–315
19. Ohshima K, Yamaguchi Y, Hirota R, Hamamoto T, Tomimura K, Tan Z, Sano T, Azuhata F, Walsh JA, Fletcher J (2002) Molecular evolution of Turnip mosaic virus: evidence of host adaptation, genetic recombination and geographical spread. *J Gen Virol* 83:1511–1521
20. Zhu FX, Sun Y, Wang Y, Pan HY, Wang FT, Zhang XH, Zhang YH, Liu JL (2016) Molecular characterization of the complete genome of three Basal-BR isolates of *Turnip mosaic virus* infecting *Raphanus sativus* in China. *Int J Mol Sci* 17:888
21. Nagy PD (2008) Recombination in Plant RNA Viruses. In: Roossinck MJ (ed) *Plant Virus Evolution*. Springer, Berlin, Heidelberg, pp 133–156
22. Wylie SJ, Jones RAC (2009) Role of recombination in the evolution of host specialization within *Bean yellow mosaic virus*. *Phytopathology* 99:512–518
23. Harrison BD (2002) Virus variation in relation to resistance-breaking in plants. *Euphytica* 124:181–192
24. Miras M, Sempere RN, Kraft JJ, Miller WA, Aranda MA, Truniger V (2014) Interfamilial recombination between viruses led to acquisition of a novel translation-enhancing RNA element that allows resistance breaking. *New Phytol* 202:233–246
25. Gibbs AJ, Hajizadeh M, Ohshima K, Jones RAC (2020) The potyviruses: An evolutionary synthesis is emerging. *Viruses* 12:132
26. Hu WX, Seo EY, Cho IS, Kim JK, Ju HK, Kim IH, Choi GW, Kim B, Ahn CH, Domier LL, Oh SK, Hammond J, Lim HS (2019) Amino acid differences in the N-terminal half of the polyprotein of Chinese turnip mosaic virus isolates affect symptom expression in *Nicotiana benthamiana* and radish. *Arch Virol* 164:1683–1689
27. Han J-Y, Chung J, Kim J, Seo E-Y, Kilcrease JP, Bauchan GR, Lim S, Hammond J, Lim H-S (2016) Comparison of helper component-protease RNA silencing suppression activity, subcellular localization, and aggregation of three Korean isolates of Turnip mosaic virus. *Virus Genes* 52:592–596
28. Park C-H, Ju H-K, Han J-Y, Park J-S, Kim I-H, Seo E-Y, Kim J-K, Hammond J, Lim H-S (2017) Complete nucleotide sequences and construction of full-length infectious cDNA clones of cucumber green mottle mosaic virus (CGMMV) in a versatile newly developed binary vector including both 35S and T7 promoters. *Virus Genes* 53:286–299
29. Kim I-H, Ju H-K, Gong J, Han J-Y, Seo E-Y, Cho S-W, Hu W-X, Choi SR, Lim YP, Domier LL (2019) A turnip mosaic virus determinant of systemic necrosis in *Nicotiana benthamiana* and a novel resistance-breaking determinant in Chinese cabbage identified from chimeric infectious clones. *Phytopathology* 109:1638–1647
30. Martin DP, Murrell B, Golden M, Khoosal A, and Muhire B (2015) RDP4: Detection and analysis of recombination patterns in virus genomes. *Virus Evol* 1:vev003
31. Sawyer S (1999) GENECONV: a computer package for the statistical detection of gene conversion. <http://www.math.wustl.edu/~sawyer>
32. Salminen MO, Carr JK, Burke DS, McCutchan FE (1995) Identification of breakpoints in intergenotypic recombinants of HIV type 1 by bootscanning. *AIDS Res Hum Retroviruses* 11:1423–1425
33. Smith JM (1992) Analyzing the mosaic structure of genes. *J Mol Evol* 34:126–129
34. Posada D, Crandall KA (2001) Evaluation of methods for detecting recombination from DNA sequences: computer simulations. *Proc Natl Acad Sci U S A* 98:13757–13762
35. Gibbs MJ, Armstrong JS, Gibbs AJ (2000) Sister-scanning: a Monte Carlo procedure for assessing signals in recombinant sequences. *Bioinformatics* 16:573–582
36. Boni MF, Posada D, Feldman MW (2007) An exact nonparametric method for inferring mosaic structure in sequence triplets. *Genetics* 176:1035–1047
37. Pasin F, Tseng XA, Bedoya LC, Heydarnejad J, Deng TC, García JA, Chen YR (2018) Streamlined generation of plant virus infectious clones using the pLX mini binary vectors. *J Virol Methods* 262:48–55
38. Lin YY, Fang MM, Lin PC, Chiu MT, Liu LY, Lin CP, Lin SS (2013) Improving initial infectivity of the *Turnip mosaic virus* (TuMV) infectious clone by a mini binary vector via agro-infiltration. *Bot Stud* 54:22
39. López-González S, Aragonés V, Daròs JA, Sánchez F, Ponz F (2017) An infectious cDNA clone of a radish-infecting *Turnip mosaic virus strain*. *Eur J Plant Pathol* 148:207–211
40. Pollari M, De S, Wang A, and Mäkinen K (2020) The potyviral silencing suppressor HC-Pro recruits and employs host ARGONAUTE1 in pro-viral functions. *PLoS Pathog* 16:e1008965
41. Adams MJ, Antoniw JF, Fauquet CM (2005) Molecular criteria for genus and species discrimination within the family Potyviridae. *Arch Virol* 150:459–479
42. Pasin F, Simon-Mateo C, and Garcia JA (2014) The hypervariable amino-terminus of P1 protease modulates potyviral replication and host defense responses. *PLoS Pathog* 10:e1003985.
43. Chai M, Wu X, Liu J, Fang Y, Luan Y, Cui X, Zhou X, Wang A, Cheng X (2020) P3N-PIPO interacts with P3 via the shared N-terminal domain to recruit viral replication vesicles for cell-to-cell movement. *J Virol* 94:e01898-e1919
44. Suehiro N, Natsuaki T, Watanabe T, Okuda S (2004) An important determinant of the ability of *Turnip mosaic virus* to infect *Brassica spp.* and/or *Raphanus sativus* is in its P3 protein. *J Gen Virol* 85:2087–2098

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