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.
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 coeleosporii has been reported to induce leaf spots on perilla [10], and Corynespora cassicola 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. Ilijin, 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 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 *Apa*I 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 *Xma*I site (5’-GAG CCC GGG TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT CCT TGC TGC ATC TCA 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 *Apa*I and *Xma*I 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* DH5α 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 rifamycin, and the agrobacterium cells collected...
Infectious clones of turnip mosaic virus isolates 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 study.

**Table 1** Sequencing primers designed and used in this study

| No. | Name   | Sequence (5' to 3')       |
|-----|--------|---------------------------|
| 1   | KPF_F1 | AGTGCCATTGCGAACAC         |
| 2   | KPF_F2 | CAAGATCTTCAGGCCGAG         |
| 3   | KPF_F3 | TGACGGTAGTGGAGTTGCT         |
| 4   | KPF_F4 | AACGGATACAGAAGGCT         |
| 5   | KPF_F5 | ATATCTTCAAGACAAACC         |
| 6   | KPF_F6 | ACCGAATAATGAGCTGC         |
| 7   | KPF_F7 | ATTCAGCGTATATGCT         |
| 8   | KPF_F8 | TTGGGAACTCCCTGACC         |
| 9   | R1     | AATCCACACATAAGCCTAA       |
| 10  | R2     | TTGGGGAGTTTCATCTCT       |
| 11  | R-cover| ACATCCAGATGAAAGCAG       |
| 12  | R3     | GTCGATCATCATCAGTCC         |
| 13  | R4     | TTCATAAAATCTCAAGCGGAT     |
| 14  | R5     | AGTTGCGATGTGATTTTTC       |
| 15  | R6     | TTGGTGCTGAATCCAGTGT       |
| 16  | R7     | CATGGAGTACACACACT       |
| 17  | KPF_R8 | TAAGCTTTTATTCCACTTTTGCC       |
| 18  | KPF_R9 | ATACTGCGTTGCTGTGAG       |

**Table 2** Details about the TuMV isolates analyzed in this study

| No. | Accession number | Isolate | Original host          |
|-----|------------------|---------|------------------------|
| 1   | AB701698         | BEL1    | *Nasturtium officinale* |
| 2   | AB093611         | BZ1     | *Brassica oleracea*    |
| 3   | D10927           | Q-Ca    | *Brassica napus*       |
| 4   | AF530055         | YC      | Zantedeschia sp.       |
| 5   | AB252106         | CHZJ26A | *Brassica campestris*  |
| 6   | AB093627         | HRD     | *Raphanus sativus*     |
| 7   | AB252103         | CH6     | *Raphanus sativus*     |
| 8   | AB252107         | CZE5    | *Brassica oleracea*    |
| 9   | AB701703         | DNK3    | *Brassica rapa*        |
| 10  | AB701708         | FRA2    | *Brassica napus*       |
| 11  | NC001616         | Potato virus Y | *Solanum sp.* |
| 12  | AB701697         | ASP     | *Allium sp.*           |
| 13  | AB701734         | TIGA    | *Tigridia sp.*         |
| 14  | AB701735         | TIGD    | *Tigridia sp.*         |
| 15  | AB701700         | DEU2    | *Raphanus sativus*     |
| 16  | AB701699         | DEU1    | Unknown                |
| 17  | AB252117         | GRC42   | *Wild Allium sp.*      |
| 18  | AB701696         | GK1     | *Matthiola incana*     |
| 19  | AB701719         | HUN1    | *Alliaria petiolata*   |
| 20  | AB440238         | IRNTra6 | *Rapistrum rugosum*    |
| 21  | AB440239         | IRN55S  | *Sisymbrium loeselii*  |
| 22  | AB093602         | IS1     | *Allium ampeloprasum*  |
| 23  | AB701720         | ITA1A   | *Brassica rapa*        |
| 24  | AB093598         | Al      | *Alliaria officinalis* |
| 25  | AB701721         | ITA2    | *Cheiranthus cheiri*   |
| 26  | AB701725         | ITA8    | *Abutilon sp.*         |
| 27  | AB093600         | ITA7    | *Raphanus sativus*     |
| 28  | AB093601         | Cal1    | *Calendula officinalis* |
| 29  | AB252125         | KWB779J | *Brassica rapa*        |
| 30  | MG200170         | KBJ5    | *Raphanus sativus*     |
| 31  | MG200169         | KBJ4    | *Raphanus sativus*     |
| 32  | MG200168         | KBJ3    | *Raphanus sativus*     |
| 33  | MG200167         | KBJ2    | *Raphanus sativus*     |
| 34  | MG200166         | KBJ1    | *Raphanus sativus*     |
| 35  | KX674727         | HIY1    | *Raphanus sativus*     |
| 36  | KX674728         | HIY2    | *Raphanus sativus*     |
| 37  | KX674729         | KIH1    | *Raphanus sativus*     |
| 38  | KX674730         | KIH2    | *Raphanus sativus*     |
| 39  | KX674731         | GJS1    | *Raphanus sativus*     |
| 40  | KX674732         | GJS2    | *Raphanus sativus*     |
| 41  | KX674733         | GJS3    | *Raphanus sativus*     |
| 42  | KX674734         | GJS4    | *Raphanus sativus*     |
| 43  | KY111268         | SW1     | *Raphanus sativus*     |
| 44  | KY111267         | SW2     | *Raphanus sativus*     |
| 45  | KX674726         | BE      | *Raphanus sativus*     |
| 46  | KY111274         | DJ1     | *Raphanus sativus*     |
| 47  | KY111273         | DJ2     | *Raphanus sativus*     |
| 48  | KY111272         | DJ3     | *Raphanus sativus*     |
| 49  | KY111271         | DJ4     | *Raphanus sativus*     |
| 50  | KY111270         | DJ5     | *Raphanus sativus*     |
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

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### Table 2 (continued)

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

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(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 Nla-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

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**Table 3** Results of RT-PCR detection assays

|         | N. benthamiana | Chinese cabbage cv. CR Victory | Radish cv. Iljin | P. frutescens cv. Leaf | P. frutescens 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
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 * | p-value |
|--------------|-------------|--------------|--------------|----------------------|---------------------|---------|
| 1            | BE          | KBC-8        | R65          | World-B × Basal-BR   | R G B M C S T       | 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 ARGONAUT1 (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.

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