Construction of full-length infectious cDNA clones of two Korean isolates of turnip mosaic virus breaking resistance in *Brassica napus*

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**Abstract**

In this work, two new turnip mosaic virus (TuMV) strains (Canola-12 and Canola-14) overcoming resistance in canola (*Brassica napus*) were isolated from a *B. napus* sample that showed typical TuMV-like symptoms and was collected in the city of Gimcheon, South Korea, in 2020. The complete genome sequence was determined and an infectious clone was made for each isolate. Phylogenetic analysis indicated that the strains isolated from canola belonged to the World-B group. Both infectious clones, which used 35S and T7 promoters to drive expression, induced systemic symptoms in *Nicotiana benthamiana* and *B. napus*. To our knowledge, this is the first report of TuMV infecting *B. napus* in South Korea.

*Brassica napus* (canola or oilseed rape), belonging to the genus *Brassica* of the family Brassicaceae, is extensively used to produce vegetable oil, biodiesel, and livestock feed around the world [1]. Turnip mosaic virus (TuMV) is one of the three principal viruses that are very harmful to brassica crops, causing severe economic losses and threatening brassicaceous vegetables worldwide [2–4]. Symptoms caused by TuMV in *Brassica* crops include systemic vein clearing, mosaic, necrosis, and plant stunting [3], depending on the virus isolate, the host plant, and environmental conditions. The spread of TuMV has caused significant economic losses in many regions, especially in Europe, Asia, and North America [5–8]. Examples of the damage it has caused include a loss of 30% in *B. napus* yield in Canada [9], seed yield losses of up to 70% in *B. napus* in the UK [10], and a 50% decrease in *B. oleracea* var. *capitata* (cabbage) head production in Kenya [11]. In 2014, a nationwide survey conducted in radish fields in South Korea showed that 47 of 108 samples with virus-like symptoms were infected with TuMV and that the incidence of TuMV was higher than that of cucumber mosaic virus and radish mosaic virus [12]. In addition, TuMV infection was also found in some Chinese cabbage fields in South Korea in 2015 [13]. The epidemic of TuMV in South Korea may adversely affect the quality of some vegetables that are common hosts of TuMV.

Turnip mosaic virus is a member of the genus *Potyvirus* in the family *Potyviridae*. Its genome is a single-stranded, positive-sense RNA molecule of about 9830 nucleotides.

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(nt) that is covalently linked to a genome-linked viral protein (VPg) attached at its 5′ end [2]. The large open reading frame (ORF) is translated into a polyprotein, which is cleaved into 10 mature proteins. An additional coding region, PIPO, is embedded within the P3 region as a result of polymerase slippage and a resulting change of reading frame [14]. The world population of TuMV has probably been more thoroughly sampled and sequenced than that of other potyviruses [15, 16]. Four major groups have been identified based on their host type. Isolates from host type [(B)] occasionally infect Brassica plants (usually latently), but not Raphanus plants, and isolates from host type [B] infect most Brassica species systemically with mosaic symptoms but do not infect Raphanus plants. Isolates from host type [B(R)] cause systemic mosaics in most Brassica species and occasionally infect Raphanus plants latently, while isolates from host type [BR] are able to infect both Brassica and Raphanus plants [17].

In recent years, an increasing number of studies have led to the discovery of new TuMV isolates in China, South Korea, and Japan, which reflects the prevalence of TuMV in East Asia [18–20]. TuMV is transmitted by aphids in a non-persistent stylet-borne manner. At least 89 aphid species have been reported to be able to transmit TuMV, including the well-known Myzus persicae and Brevicoryne brassicae [2]. Recently, the incidence of insect-transmitted viruses has increased in China, South Korea, and Japan [21–23]. Climate change, leading to a rise in temperature, may be one of the reasons for the increase in viral diseases. A suitable environment facilitates the reproduction of insects, which also accelerates the transmission of some viral diseases.

In the present study, the complete genome sequences of two TuMV isolates originating from canola in South Korea were determined, and their genetic relationships were clarified by phylogenetic analysis. Moreover, a full-length infectious cDNA clone of each isolate was constructed to study the biological properties of these isolates. To the best of our knowledge, this is the first report of TuMV infecting B. napus in South Korea.

![Symptoms induced by full-length infectious clones of TuMV in Nicotiana benthamiana at 5 dpi and 14 dpi. Both Canola-12 and Canola-14 caused severe leaf curling and growth stunting symptoms.](image1)

![Symptoms on the original sample and sap-inoculated Brassica napus plants. (A) Symptoms on the original sample. (B, C) Symptoms induced by Canola-12 and Canola-14 on B. napus about one month after sap inoculation. (D) A healthy plant.](image2)
A single canola sample showing obvious mosaic symptoms was collected in the city of Gimcheon, South Korea, in 2020. In order to detect viruses, total RNA was extracted from the sample using TRIzol® Reagent (Life Technologies, Carlsbad, CA, USA) and stored at -70 °C, and cDNA was subsequently produced using a LeGene Express 1st Strand cDNA Synthesis System and an oligo dT primer (LeGene Biosciences, San Diego, CA, USA). Virus detection was performed by PCR, using TuMV-CP primers (Supplementary Table S1), and gel electrophoresis results showed that the sample was positive for TuMV. In order to construct full-length cDNA clones, PCR products amplified using a TuMV-specific 5’ primer (with a SalI restriction site and a T7 promoter) and a 3’ primer (with an XmaI restriction site and an oligo T(30) sequence) (Supplementary Table S1) were digested with SalI and XmaI and subsequently cloned into the binary vector pJY, which had been digested with the same enzymes [24, 25]. The infectivity of these full-length clones was evaluated by agroinfiltration of Nicotiana benthamiana plants [20], followed by incubation in a growth chamber at 22-25 °C (16/8h, light/dark cycle).

Two infectious clones, named Canola-12 and Canola-14, were obtained. Slight curling of the top leaves was observed at 5 days post-inoculation (dpi). At 10 dpi, the inoculated plants showed leaf malformation and obvious growth stunting when compared to healthy control plants. Both isolates were able to infect N. benthamiana and induced similar symptoms (Fig. 1). In addition, the leaves of plants inoculated by each infectious clone were collected to prepare an inoculum for subsequent sap inoculation. Briefly, the leaves were ground into powder in liquid nitrogen, suspended in 1x PBS buffer, and mechanically inoculated onto leaves of canola [20]. About 30 days later, mosaic symptoms appeared on the newly developed leaves of B. napus plants inoculated with isolate Canola-12 or Canola-14 (Fig. 2), which is consistent with the symptoms we observed in the original sample. All of the infections were confirmed by RT-PCR as above (data not shown), and the experiment was repeated twice.

The sequences of these two infectious clones were determined using vector-specific primers and sequential TuMV-specific primers designed from the initially obtained sequences (Supplementary Table S1) and assembled using DNAMAN (Version 5.2.10). Each genome was found to be composed of 9833 nt, excluding the poly(A) tail, and is predicted to encode a polyprotein of 3164 aa. There are only three nucleotide differences between their genomes (nt 526, 1508, and 4067), resulting in a single amino acid difference at aa 132 in P1 (E in Canola-12; G in Canola-14). A phylogenetic tree was constructed by the maximum-likelihood method with 1000 bootstrap replicates in MEGA (version 7.0), based on the polyprotein-encoding sequences of TuMV isolates from NCBI, including 25 isolates previously identified in South Korea [19, 20, 25], with Japanese yam mosaic virus (JYMV) used as the outgroup. Unlike strains that were characterized previously in South Korea, which mostly belonged to the Basal-BR group, these two isolates collected from B. napus were grouped in the World-B clade. The isolates Canola-12 (MW556022) and Canola-14 (MW556023), shared 99.97% nucleotide sequence identity and clustered together in a subgroup that also included two strains collected in China, and one strain from the UK (UK1) infecting B. napus [26], with 100% bootstrap support (Fig. 3). Isolates UK1 and JPN 1 have been used to construct infectious clones, and UK1, which is able to infect B. napus, showed a close relationship to our isolates in the World-B group, while JPN 1, a radish-infecting isolate, fell into a relatively distant branch within the Asian-BR group [27], while another Japanese isolate, KWB779J, fell into the Basal-BR group (Fig. 3). The Korean canola isolates were distinct from isolates 12.1 and 12.5, which recently emerged in Australia as new isolates breaking TuMV resistance in B. napus [28], showing that TuMV has been constantly evolving to overcome host resistance.

Recently, researchers have shown that TuMV, which probably originated in European wild orchids, likely spread from west to east across Eurasia around the 17th century CE [15, 16, 29]. Previous studies on TuMV have revealed the prevalence of Basal-BR isolates of TuMV in South Korea [19, 20], whereas the isolates reported here belonged to the World-B group and showed a close relationship to two Chinese isolates and one European isolate (Fig. 3). A genomic analysis of TuMV has indicated that the United Kingdom played an important role in the spread of the virus in Europe, while northeast China was a center for the spread of World-B3 subgroup isolates in Asia [16], which could be an explanation for the emergence of World-B isolates in South Korea. The high genetic variability, wide host range and mode of transmission of TuMV make this virus hard to control by traditional methods such as chemicals. Therefore, breeding of resistant host cultivars is a more effective and environmentally friendly strategy [4]. Additionally, identification of more resistance genes is also necessary, although resistance-breaking isolates sometimes may appear [28, 30–32].
Basal-BR group

Asian-BR group

World-B group

Basal-B group

Iranian group

Orchis group
Korean isolates of turnip mosaic virus

**Fig. 3** Phylogenetic tree constructed by the maximum-likelihood method with 1000 bootstrap replicates, based on the polyprotein-encoding sequences of TuMV isolates from the NCBI database, including 25 isolates identified previously in South Korea [19, 20, 25]. The new canola isolates are indicated by arrows to the right of the isolate/accession number/country label.

**Supplementary Information** The online version contains supplementary material available at https://doi.org/10.1007/s00705-022-05381-2.

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**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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