Construction of Full-length Infectious cDNA Clones of Two Korean Isolates of Turnip Mosaic Virus Breaking Resistance in *Brassica Napus*

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Research Article

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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 which showed typical TuMV-like symptoms and was collected from Gimcheon city, South Korea in 2020. Complete genomes and infectious clones of each isolate were obtained. Phylogenetic analysis indicated that the strains isolated from canola belonged to the World-B group. Both infectious clones which were driven by 35S and T7 promoters induced systemic symptoms on *Nicotiana benthamiana* and *B. napus*. To our knowledge, this is the first report of TuMV infecting *B. napus* in South Korea.

Main Text

Brassica napus (canola or oilseed rape), belonging to the genus *Brassica*, in the family Brassicaceae, is extensively utilized as 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 brassica vegetables worldwide [2–4]. Symptoms caused by TuMV in *Brassica* crops depend on virus isolate, host plant and environmental conditions, mainly including systemic vein clearing, mosaic, necrosis, and plant stunting [3]. 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 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].

Turnip mosaic virus is a member of the genus *Potyvirus* in the family *Potyviridae*. Its genome is a single-stranded, positive-sense RNA molecular of about 9830 nucleotides (nt), covalently linked to a virus protein genome-linked (VPg) attached at the 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 P3 region as a result of polymerase slippage and resulting change of reading frame [12]. The world population of TuMV has probably been more thoroughly sampled and sequenced than that of other potyviruses [13]. 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 [14].

In recent years, an increasing number of studies have reported new TuMV isolates occurring in China, South Korea and Japan, which reflects the prevalence of TuMV in east Asia [15–17]. TuMV is transmitted by aphids in the non-persistent stylet-borne manner. At least 89 aphid species were reported to be able to transmit TuMV, including the well-known *Myzus persicae* and *Brevicoryne brassicae* [18]. Recently, incidences of insect-transmitting viruses have increased in China, South Korea, and Japan [19]. Climate

change, leading to the rise of 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 affiliation was clarified by phylogenetic analysis. Moreover, full-length infectious cDNA clones of each isolate were constructed to characterize their biological properties. To the best of our knowledge, this is the first report of TuMV infecting *B. napus* in South Korea.

A single canola sample showing obvious mosaic symptoms was collected from Gimcheon city, South Korea in 2020. In order to detect viruses, total RNAs were extracted from the sample using TRIzol® Reagent (Life Technologies, Carlsbad, CA, USA), stored at -70°C, and the cDNAs were subsequently produced with LeGene Express 1st Strand cDNA Synthesis System and an oligo dT primer (LeGene Biosciences, San Diego, CA, USA). Then detection was conducted by PCR using TuMV-CP primers (Supplementary Table 1), and gel electrophoresis result showed that the sample was positive for TuMV. In order to construct full-length cDNA clones, PCR products amplified by TuMV-specific 5' primer (with an *SalI* restriction site and T7 promoter) and 3' primer (with an *XmaI* restriction site and an oligo T(30) sequence) (Supplementary Table 1) were digested with *SalI* and *XmaI* and subsequently cloned into the binary vector pJY which was digested by the same enzymes [20, 21]. The infectivity of these full-length clones was evaluated by agroinfiltration on *Nicotiana benthamiana* [17]. *N. benthamiana* plants inoculated by each full-length clone were incubated 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 leaf curling was observed at 5 days post inoculation (dpi) on the top leaves. At 10 dpi, the inoculated plants showed leaf malformation and obvious growth stunting compared with healthy control. Both isolates Canola-12 and Canola-14 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 subsequent sap inoculation. Briefly, the plant leaves were ground into powder in liquid nitrogen, which was suspended in 1 x PBS buffer and mechanically inoculated to leaves of canola [17]. About 30 days later, mosaic symptoms appeared on the newly grown leaves of *B. napus* plants inoculated by isolate Canola-12 or Canola-14 (Fig. 2), which is consistent with the symptoms we observed on the original sample. All of the infections were confirmed by RT-PCR as above (data not shown) and the experiment was repeated twice.

Sequences of these two infectious clones were determined by sequencing with vector-specific primers and sequential TuMV-specific primers designed from the initially obtained sequences (Supplementary Table 1) and assembled in DNAMAN (Version 5.2.10). Each genome is composed of 9833 nt, excluding the poly(A) tail, and is predicted to encode a polyprotein of 3164 aa. There are only 3 nt 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 using the Maximum-likelihood method with 1000 bootstrap replicates in MEGA (version 7.0) and based on the complete genomic

sequences of TuMV isolates from NCBI, including 25 isolates previously identified in South Korea [16, 17, 21]. Unlike those strains which were characterized in South Korea previously and mostly belonged to the Basal-BR group, these two isolates collected from *B. napus* were grouped into the World-B clade. Isolates Canola-12 (MW556022) and Canola-14 (MW556023), sharing 99.97 % nucleotide identity, clustered together and fell into a subgroup that consists of two strains collected in China and one European strain UK1 infecting *B. napus* [22] with bootstrap support at 100% (Fig. 3). The Korean canola isolates were distinct from isolates 12.1 and 12.5 which have emerged in Australia recently as new isolates breaking TuMV resistance in *B. napus* [23], showing that TuMV has been constantly evolving to overcome host resistance.

Recently, researchers have shown that TuMV, probably originating from European wild orchids, likely spread from west to east across Eurasia from about the 17th century CE [13, 24, 25]. Previous studies about TuMV have revealed the prevalence of Basal-BR isolates of TuMV in South Korea [16–17], whereas the isolates reported here belonged to the World-B group and showed a close relationship with two Chinese isolates and one European isolate (Fig. 3). Besides, a genomic analysis of TuMV indicated that northeast China was a center for the spread of World-B3 isolates in Asian areas, while the United Kingdom played an important role in European spread [25], which could be a reasonable explanation for the emergence of the World-B isolates in South Korea. The high genetic variability, wide host range and mode of transmission of TuMV make the 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 [23, 26–28].

Declarations

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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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Figures

Figure 1

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.

Figure 2

Symptoms of original sample and sap inoculated *Brassica napus* plants. (A) Symptoms of the originally collected sample. (B, C) Symptoms induced by Canola-12 and Canola-14 on *B. napus* about one month after sap inoculation. (D) Healthy plant.

Figure 3

A phylogenetic tree was constructed by the Maximum-likelihood method with 1000 bootstrap replicates, based on the complete sequence of TuMV isolates from NCBI, including 25 isolates previously identified in South Korea [16, 17, 21]. The new canola isolates are indicated by arrows to the right of the isolate/accession number/country label.

Supplementary Files

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