SEQUENCE ANALYSIS OF CP AND HC-Pro GENES OF TURNIP MOSAIC VIRUS ISOLATES FROM CHINA

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Summary. – Fourteen isolates of Turnip mosaic virus (TuMV) were obtained from the leaves of diseased cruciferous plants in China. According host tests, the isolates were classified into B-host and BR-host group. The nucleotide sequences of the coat protein (CP) and helper component proteinase (HC-Pro) genes of the isolates were determined. The CP genes consisted of 864 nucleotides encoding a polypeptide of 288 amino acids. The HC-Pro genes comprised 1374 nucleotides encoding a polypeptide of 458 amino acids. The genes CP and HC-Pro of the 14 isolates shared nucleotide sequence identities ranging from 89.2 to 99.5% and 79.1 to 99.9%, respectively. Amino acid sequence identities of CP and HC-Pro proteins ranged from 95.1 to 100% and 94.8 to 99.8%, respectively. Phylogenetic tree based on the CP gene indicated that 13 of the 14 TuMV isolates belonged to the world-B group, while the remaining isolate ZJ1 belonged to the basal-BR group. The phylogenetic tree based on the HC-Pro gene was similar to that of CP gene with the exception of the isolate JX that clustered with the Asian-BR group. Our results were consistent with the previous results demonstrating that a majority of the isolates collected from *Brassica spp.* belonged to the world-B group.

Key words: Turnip mosaic virus; HC-Pro gene; CP gene; phylogenetic relationship

Introduction

TuMV is a member of the family *Potyviridae*, the genus *Potyvirus.* It has a very wide host range and is transmitted by aphids in a non-persistent manner. TuMV is distributed throughout the world including the temperate and tropical regions of Africa, Asia, Europe, Oceania, and North and South America (Provvidenti, 1996; Ohshima *et al*., 2002). It is regarded as the most important pathogen of cruciferous crop in China causing severe reductions in yield.

TuMV is a single-stranded positive-sense RNA virus. Its genome has a single ORF, which is translated into a large polyprotein hydrolyzed into ten proteins including CP and HC-Pro. Both CP and HC-Pro are involved in regulation of the viral RNA amplification, aphid transmission, and movement (Urcuqui-Inchima *et al*., 2001; Tomimura *et al*., 2004; Kasschau and Carrington, 2001; Plisson *et al*., 2003; Roudet-Tavert *et al*., 2002). In addition, HC-Pro is regarded as a pathogenicity determinant (Revers *et al*., 1999; Redondo *et al*., 2001) and a suppressor of gene silencing (Anandalakshmi *et al*., 1998; Kasschau and Carrington, 1998, 2001). Recent studies indicate that there is considerable variation and recombination within the TuMV genome (Ohshima *et al*., 2002; Tan *et al*., 2004; Tomimura *et al*., 2003, 2004). Host tests grouped TuMV isolates into the B-host type (infecting *Brassica spp*. but not *Raphanus sativus*) and the BR-host type (infecting both *Brassica spp.* and *R. sativus*).

In this paper, we report the molecular characterization of the CP and HC-Pro genes of 14 TuMV isolates from different hosts in China and their phylogenetic relationship.

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Abbreviations: CP = coat protein; HC-Pro = helper component proteinase; JYMV = Japanese yam mosaic virus; ScMV = Scallion mosaic virus; TuMV = Turnip mosaic virus

Materials and Methods

Virus source. Fourteen virus isolates were collected from the infected leaves of cruciferous crop with typical disease symptoms in six provinces of China (Table 1). The collected samples were tested using monoclonal antibodies against TuMV by ELISA (Yu *et al*., 2005). All samples were positive for TuMV. Host, geographical origin, date of isolation, and sequence database Acc. Nos. of TuMV isolates are shown in Table 1.

Host tests. Samples of diseased leaves were homogenized in 0.01 mol/l potassium phosphate buffer (pH 7.0), and then inoculated mechanically to young plants of *Brassica chinensis* L., *B. pekinensis*, *B. oleracea*, *B. juncea*, and *R. sativus.* The inoculated plants were kept in a greenhouse for at least 6 weeks. The asymptomatic plants were tested for the presence of TuMV antigens by ELISA.

Cloning of CP and HC-Pro genes. Viral RNA was extracted from TuMV-infected leaves using the RNeasy Plant Mini kit (Qiagen). First-strand cDNA was synthesized using AMV-reverse transcriptase (Promega) and amplified by RT-PCR using degenerate primers PA1 (5'-GCAGATGAAACGCTTGACGCAG-3') and PB1 (5'-TACAACTTCATAACCCCTKAACGC-3') for the CP gene, PA2 (5'-ATGAGCTCGCASSDGGMGCCAACTTYTGGAAAG-3') and PB2 (5'-TTGTCGACATGAGTGTCCTCCCATTCTGTTCC-3') for the HC-Pro gene together with *Pfu* DNA polymerase (Promega). The PCR products were cloned into pGEM-T Vector (Promega) and sequenced in both directions using an automated model 3730 DNA sequencing system (Perkin-Elmer Applied Biosystems).

Sequence and phylogenetic analysis. Sequence data were analyzed by the DNAStar (DNAStar Inc.) and a phylogenetic tree was generated using the neighbor-joining method in DNAMAN Version 5.22 software (Lynnon Biosoft). Vertical distances were arbitrary; horizontal distances were proportional to sequence distances. The Acc. Nos. and hosts of the 24 TuMV isolates reported elsewhere were: C1 (AF394601, host unknown), CH6 (AB252103, *R. sativus*), CHK16 (AB252104, *R. sativus*), CHL13 (AB252105, *R. sativus*), CHN1 (AB093626, *Brassica spp.*), CHN12 (AY090660, host unknown), CHZJ26A (AB252106, *B. campestris*), CZE1 (AB093608, *B. oleracea*), DMJ (AB093623, *R. sativus*), HRD (AB093627, *R. sativus*), HZ6 (AB252119, *Brassica spp.*), IS1 (AB093602, *Allium ampeloprasum*), KEN1 (AB093605, *B. oleracea*), KYD81J (AB093613, *R. sativus*), NZ290 (AB093612, *B. pekinensis*), PV0104 (AB093603, *Lactuca sativa*), PV376-Br (AB093604, *B. napus*), RC4 (AY134473, *Zantedeschia spp.*), RUS2 (AB093607, *B. napus*), SGD311J (AB093619, *R. sativus*), St48 (AB093596, *Limonium sinuatum*), Tu3 (AB105134, *B. oleracea*), TW (AF394602, host unknown), and YC5 (AF530055, *Zantedeschia spp.*). The homologous sequences of two isolates of Japanese yam mosaic virus (JYMVj1, AB016500; JYMVm, AB027007) and an isolate of Scallion mosaic virus (ScMV, AJ316084) were used as the outgroup.

The GenBank Acc. Nos. of the sequences reported in this paper are AJ576320, AJ831793-802, AJ831804-07 and AJ831809-21.

Results and Discussion

Host specificity

The three isolates (JX, YN2, and ZJ1) collected from *R. sativus* were able to infect both *Brassica spp.* and *R. sativus* plants inducing strong or mild mosaic symptoms, and consequently were classified as the BR-host type (Ohshima *et al*., 2002). Conversely, the other 11 isolates (AH, GX, JS, YN1, YN3, ZJ2, ZJ3, ZJ4, ZJ5, ZJ6, and ZJ7) obtained from diseased *Brassica spp.* plants were not able to induce symptoms in *R. sativus* plants and the presence of virus in the inoculated leaves was not detected by ELISA. Obtained results indicated that these isolates belonged to the B-host type.

Sequence comparisons

The CP and HC-Pro sequences of 14 TuMV isolates were determined and their Acc. Nos. were listed in Table 1. The

Table 1. Host, geographical origin, year of isolation, and sequence Acc. Nos. of TuMV isolates

Isolate	Acc. No. HC-Pro	Acc. No. CP	Host	Locality (city, province)	Year of isolation
AH	AJ831820	AJ831806	*B. chinensis* L.	Hefei, Anhui	2004
GX	AJ831799	AJ831814	*B. chinensis* L.	Nanning, Guangxi	2002
JS	AJ831805	AJ831819	*B. chinensis* L.	Yixing, Jiangsu	2002
JX	AJ831796	AJ831812	*R. sativus*	Nanchang, Jiangxi	2003
YN1	AJ831801	AJ831816	*B. pekinensis*	Yuanjiang, Yunnan	2004
YN2	AJ831802	AJ831817	*R. sativus*	Kunming, Yunnan	2003
YN3	AJ831804	AJ831818	*B. chinensis* L.	Kunming, Yunnan	2003
ZJ1	AJ831821	AJ831807	*R. sativus*	Hangzhou, Zhejiang	2004
ZJ2	AJ831793	AJ831809	*B. rapa*	Hangzhou, Zhejiang	2004
ZJ3	AJ831794	AJ831810	*B. narinosa*	Hangzhou, Zhejiang	2004
ZJ4	AJ831795	AJ831811	*B. juncea Var. tumida*	Hangzhou, Zhejiang	2004
ZJ5	AJ831797	AJ576320	*B. juncea Var. multiceps*	Ningbo, Zhejiang	2002
ZJ6	AJ831798	AJ831813	*B. juncea Var. multiceps*	Ningbo, Zhejiang	2003
ZJ7	AJ831800	AJ831815	*B. juncea Var. tumida*	Xiaoshan, Zhejiang	2003

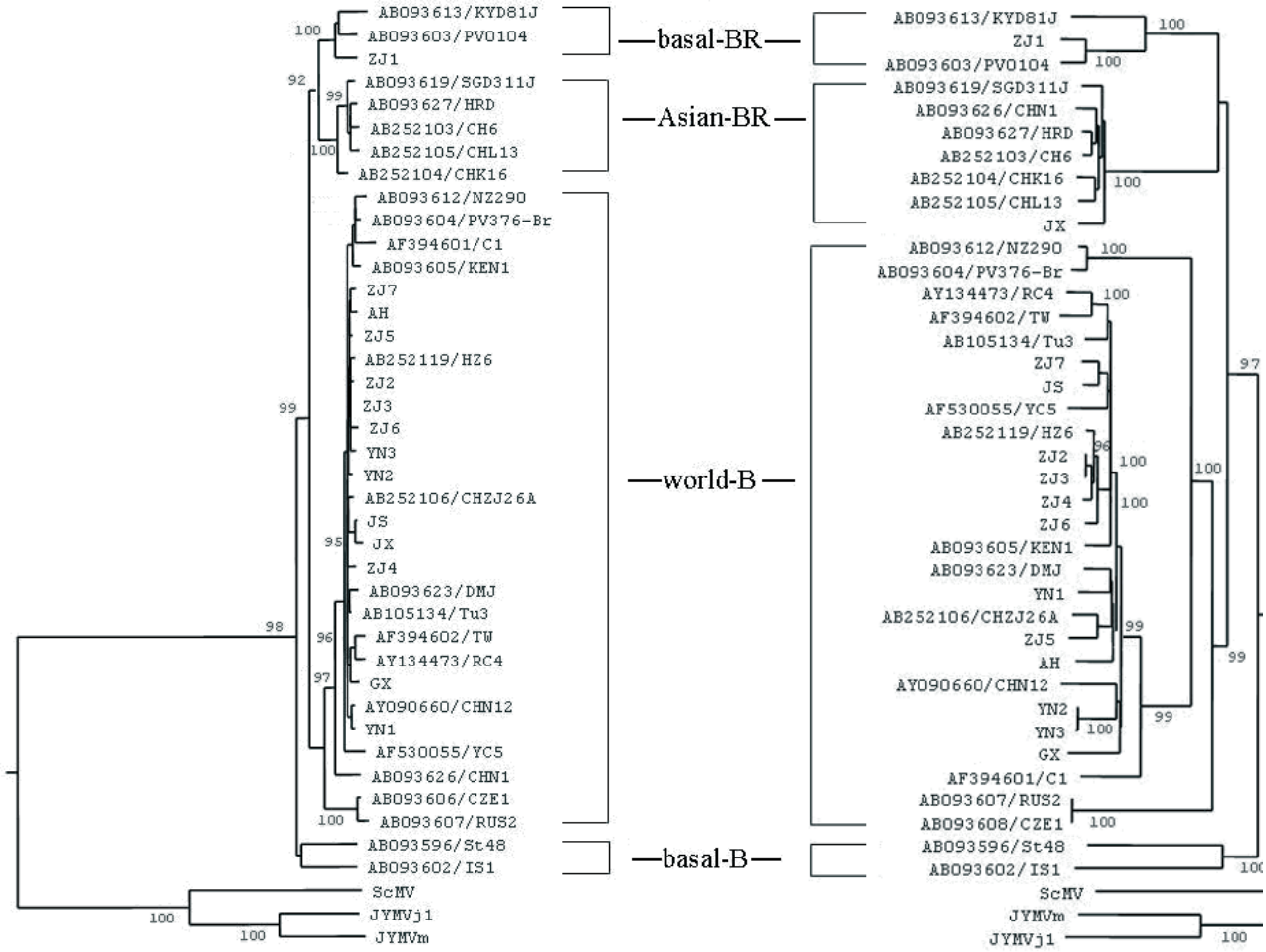

Fig. 1

Phylogenetic trees based on the CP (left) and HC-Pro (right) nucleotide sequences of 38 TuMV isolates from China and other countries

Numbers at nodes indicate percentage bootstrap scores. According to the hosts test, the isolates are grouped into four groups: basal-BR, Asian-BR, world-B, and basal-B.

CP genes of all isolates consisted of 864 nucleotides encoding a 288 amino acid polypeptide, while the HC-Pro genes of all isolates consisted of 1374 nucleotides encoding a 458 amino acid polypeptide.

Sequence comparisons revealed that the CP genes of 14 isolates shared 89.2–99.5% nucleotide sequence identity and 95.1–100% amino acid sequence identity, respectively. Although the original host of isolates JX and YN2 was different from the 11 isolates (AH, GX, JS, YN1, YN3, ZJ2, ZJ3, ZJ4, ZJ5, ZJ6 and ZJ7) collected from *Brassica spp.* plants, the CP gene sequences of these two isolates were closely related to the mentioned 11 isolates with 96.8–99.5% nucleotide and 98.3–100% amino acid sequence identities, respectively. The CP gene of the ZJ1 isolate originating from *R. sativus* was distantly related to the JX, YN2, and other 11 isolates with nucleotide sequence identities of 89.2–90.5% and amino acid sequence identities of 95.1–96.5%, respectively.

The HC-Pro genes of the 14 isolates shared sequence identities ranging from 79.1–99.9% at the nucleotide level and 94.8–99.8% at the amino acid level. The HC-Pro gene sequences of the 11 isolates collected from *Brassica spp.* and the YN2 isolate collected from *R. sativus* were closely related, sharing nucleotide sequence identities ranging from 93.9–99.9% and amino acid sequence identities of 98.5–99.8%. The HC-Pro genes of isolates JX and ZJ1 collected from *R. sativus* had nucleotide identity 82.8% and amino acid identity 97.8%. They shared 79.1–81.6% nucleotide and 94.8–96.7% amino acid sequence identities with the other 12 isolates. HC-Pro gene was more variable than the CP gene among the examined isolates.

Comparison of genomic sequences showed that 14 isolates of TuMV fell into four well-defined close-knit groups: basal-

B, basal-BR, Asian-BR, and world-B (Ohshima *et al*., 2002; Tan *et al*., 2004; Tomimura *et al*., 2003). Thus, the 14 TuMV isolates were compared with representative isolates of these four groups. Sequence comparison showed the CP and HC-Pro genes of 12 isolates (except ZJ1 and JX) had higher nucleotide sequence identities with viruses of the world-B group than with those of the other three groups. For the isolate ZJ1, both the CP and HC-Pro genes had the highest nucleotide sequence identity with viruses of the basal-BR group. The CP gene of the isolate JX had the highest nucleotide sequence identity with viruses of the world-B group, while its HC-Pro gene was most closely related to the viruses of the Asian-BR group.

Phylogenetic relationships

The phylogenetic trees were constructed according to the nucleotide sequences of the CP and HC-Pro genes of the 14 TuMV isolates and 24 additional TuMV isolates reported elsewhere. All 38 TuMV isolates were classified into four groups in the phylogenetic trees based on CP and HC-Pro nucleotide sequences (Fig. 1). The CP gene of the isolate ZJ1 and two other TuMV isolates (KYD81J and PV0104) known as the basal-BR group (Ohshima *et al*., 2002) cluster together as a branch, while the other 13 isolates (AH, GX, JS, JX, YN1, YN2, YN3, ZJ2, ZJ3, ZJ4, ZJ5, ZJ6 and ZJ7) cluster together with the 15 isolates belonging to the world-B group. The five isolates of Asian-BR group and the two isolates of basal-B group also cluster together. A phylogenetic tree based on HC-Pro sequences is similar to that based on CP sequences with the exception of isolate JX. The isolate ZJ1 belonged to the basal-BR group in both CP and HC-Pro gene phylogenetic trees. This is the first report of a TuMV isolate from China belonging to basal-BR group. The isolate JX was included in the world-B group in the CP gene phylogenetic tree, while it was placed to the Asian-BR group in the HC-Pro gene phylogenetic tree. Among the TuMV virus population, a genetic recombination has been shown to be widespread (Ohshima *et al*., 2002), so it is not surprising that the isolate JX had parents from two different lineages (world-B and Asian-BR).

In China, a majority of the isolates collected from *R. sativus* belonged to the Asian-BR group, while most isolates collected from *Brassica spp.* belonged to the world-B group (Ohshima *et al*., 2002; Tan *et al*., 2004; Tomimura *et al*., 2003, 2004). Our results were consistent with the reported findings that a majority of the isolates collected from *Brassica spp.* belonged to the world-B group.

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