SHORT COMMUNICATION

A NEW ISOLATE OF *CHILLI VEINAL MOTTLE VIRUS* THAT INFECTS TOBACCO IN CHINA

J. Yang[1*], J.H. Dong[2*], T.J. Zhang[2], R. Wang[1], Z.P. Luo[1], H.Y. Luo[3] and Z.K. Zhang[2]

[1]Zhengzhou Tobacco Research Institute, CNTC, Zhengzhou 450001, China
[2]Key Laboratory of Agricultural Biotechnology of Yunnan Province, Biotechnology and Genetic Germplasm Institute, Yunnan Academy of Agricultural Sciences, Kunming 650223, China
[3] Hongyun-Honghe Tobacco (Group) Limited Company, Yunnan Kunming 650202, China
**These authors have contributed equally to this work*

SUMMARY

An isolate of *Chilli veinal mottle virus* (ChiVMV) was characterized, which infects tobacco (*Nicotiana tabacum* L.) in the Chinese province of Yunnan inducing mottling and necrotic spots on the leaves. Nine solanaceous crops inoculated mechanically proved susceptible to this viral isolate (ChiVMV-To) and reacted with symptoms including systemic mottling and leaf distortion. ChiVMV-To has filamentous particles *ca.* 760 nm long and a genome composed of 9,724 nucleotides (nts), excluding the poly(A) tail, comprised in a single open reading frame encoding a polypeptide 3,089 amino acids (aa) in size. The 5'- and 3'-untranslated regions (UTRs) are 168 and 286 nts in size, respectively. The complete genome sequence of ChiVMV-To shares 78.5 to 79.1% nt and 83.8 to 86.7% aa identities with those of six other ChiVMV chilli isolates reported previously. Phylogenetic analysis based on the polyprotein sequences of other potyviruses suggests ChiVMV-To to be most closely related to *Wild tomato mosaic virus* (WTMV). This is the first complete genome sequence characterization of ChiVMV isolated from tobacco in China.

Key words: ChiVMV, sequencing, tobacco, polyprotein, potyvirus, phylogenetic tree.

Tobacco (*Nicotiana tabacum* L.) is the leading cash crop in the Chinese province of Yunnan and virus diseases are important constraints on its production. Since 2008, symptoms consisting of mosaic and mottling of the upper leaves and white lesions in the middle and lower leaves have been observed in major tobacco-growing areas of this province, with an incidence of up to 10-15%. Initial studies of this disease using RT-PCR amplification followed by sequencing of the 3'-terminal sequences of the genome of a virus associated with it, revealed the presence of *Chilli veinal mottle virus* (ChiVMV) (Ding *et al.*, 2011).

Corresponding author: Z.K.Zhang and H.Y. Luo
Fax: +86.871.5160084
E-mail: zhongkai99@sina.com; huayuanluo@126.com

ChiVMV, a member of the genus *Potyvirus*, family *Potyviridae* (King *et al.*, 2011), is an important pathogen of chilli crops in some Asian and African countries (Agranovsky *et al.*, 1993; Ravi *et al.*, 1997; Chiemsombat *et al.*, 1998; Nono-Womdim *et al.*, 2001; Tan *et al.*, 2003; Moury *et al.*, 2005; Wang *et al.*, 2006).

ChiVMV virions are flexuous filaments 765x13 nm in size (Siriwong *et al.*, 1995), containing a single-stranded RNA genome of *ca.* 9.7 kb with a 3'-terminal poly(A) tail, which encodes a polyprotein of *ca.* 350 kDa cleaved by virus-encoded proteinases into 10 functional proteins (Siriwong *et al.*, 1995; Anindya *et al.*, 2004). The virus is transmitted by *Aphis gossypii* (Glov.) to solanaceous crops in a non-persistent manner (Ong *et al.*, 1979; Green *et al.*, 1999; Shah *et al.*, 2008).

The complete sequences of six ChiVMV isolates from chilli (ChiVMV-Chi) are available in GenBank. Here, we report the complete nucleotide sequence of a ChiVMV isolate infecting tobacco (ChiVMV-To) in Yunnan, and its phylogenetic relationships with ChiVMV-Chi isolates and other potyviruses.

Samples from diseased tobacco leaves were collected in the surrounding of Mengzi city (Honghe prefecture, Yunnan). Sap extracts from symptomatic leaves negatively stained with 2% ammonium molybdate (pH 5.5) and examined with an electron microscope as previously described (Zhang and Li, 2001) contained potyvirus-like filamentous particles, *ca.* 760 nm long. All samples were then screened by DAS-ELISA using a potyvirus-specific commercial kit (Agdia, USA).

Positive samples were used for virus isolation. The virus was biologically purified by two passages of single local lesions in *Chenopodium amaranticolor*, then a single local lesion isolate was maintained in *Nicotiana glutinosa*. Leaves taken from diseased *N. glutinosa* were ground in 0.01 M phosphate buffer (pH 7.0) containing 0.1% sodium sulfite and 0.1% 2-mercaptoethanol (0.5 g leaf tissue/ml of buffer). The extracts were mechanically inoculated onto 10 different plant species or cultivars (Table 1), i.e. *N. tabacum* cvs Samsun, K326, and Yun85, *N. glutinosa*, *N. rustica*, *N. benthamiana*, *N. debneyi*, *Capsicum annuum*, *Datura stramonium*, and *C. amaranticolor*. Inoculated plants were grown in an in-

Table 1. Symptoms induced by ChiVMV-To on mechanically inoculated plants.

Host plants	Symptoms*	
	5 dpi	15 dpi
Nicotiana tabacum cv. Samsun	M	SM, LD
N. tabacum cv. K326	M	SM, LD
N. tabacum cv. Yun85	M	SM, LD
N. glutinosa	M	SM, LD
N. rustica	M	SM, NS
N. benthamiana	M, NS	SM, NS
N. debneyi	M	SM
Capsicum annuum L.	M	VB
Datura stramonium	M	C, M, "Rat-tailed leaf"
Chenopodium amaranticolor	NLL	—

*The symptoms first appeared on newly developed leaves at 5 dpi then gradually expanded to other leaves. M, mottling; SM, systemic mottling; LD, leaf distortion; NS, necrotic spot; VB, vein banding; C, chlorosis; NLL, necrotic local lesions (inoculated leaves only).

sect-proof greenhouse under a 25-30°C/18°C day/night temperature regime and monitored daily. Symptoms appeared on all plants five days post inoculation (dpi). Systemic mottling was observed on the leaves of inoculated solanaceous hosts, whereas *C. amaranticolor* reacted with local lesions on inoculated leaves. Severe mottling and "rat tail-like" deformations of the leaves appeared on *D. stramonium* 14 dpi, whereas *N. rustica* showed systemic mottling, necrotic spot and bud necrosis. A higher number of virus particles was observed in leaf extracts from *N. rustica* than in extracts from other plants tested (data not shown).

Total RNA was extracted from 100 mg of infected *N. glutinosa* leaves using the TRIzol reagent (Invitrogen, USA) following the manufacturer's instructions. cDNA was synthesized from total RNA extracts using the Moloney murine leukemia virus reverse transcriptase (Promega, USA), and was amplified by PCR under the following conditions: 94°C for 4 min, 30 cycles of 94°C for 40 sec, 46°C for 40 sec, 72°C for 2 min and a final extension at 72°C for 10 min. Initially, about 1,800 nts of the 3'-terminal sequence of the viral genome were obtained using the method described by Chen *et al.* (2001). Six additional primer pairs were designed on conserved regions of the complete genomic sequences of ChiVMV available in GenBank (Table 2). The resultant PCR products were purified using a PCR purification kit (TaKaRa, Japan), ligated into the pMD18-T vector (TaKaRa, Japan), which was then transformed into *Escherichia coli* DH5α. Positive clones were identified by colony PCR. Sequences were determined using an ABI prism 3770xl DNA Analyzer (Applied Biosystems,

Table 2. Primers used for the cloning of ChiVMV-To.

Primers	Sequence (5'- 3')	Genomic location	Tm
ChiV-F1	AATACAAACATACAGAAAACAAACG	V1-24	50°C
ChiV-R1	ACTCAACACGATTCTTGTGG	Vc1560-1579	
ChiV-F2	GGTGGAAGAGCAACAAGTG	V1495-1513	52°C
ChiV- R2	TTCCTGAGATTATCTCTTGCG	Vc3008-3028	
ChiV-F3	ATGGAAAAAATCTACAAGGAGG	V2893-2914	50°C
ChiV-R3	GGTCCACTTCATTRTAGCTTG	Vc4400-4420	
ChiV-F4	AGCATCGCAGATAACATTCTTG	V4368-4390	50°C
ChiV-R4	AGTCACTGGATCAACAAACCG	Vc5947-5967	
ChiV-F5	AAAGGCACATGGAATGGG	V5868-5885	50°C
ChiV-R5	CCACTTYTCACTTGCTTGCTC	Vc7012-7032	
ChiV-F6	CCAAATGAAATAGGATGGGG	V6907-6926	50°C
ChiV-R6	ATCACCGTTAGCAAAGAAAAC	Vc8050-8070	
Sprimer	GGXAAYAAYAGYGGXCAZCC; X=A, G, C or T; Y = T or C; Z =A or G	Degenerate primer for the 3'-terminal sequence (about 1800 nt) of potyviruses	
M4-T	GTTTTCCCAGTCACGACTTTTTTTTTTTTTTT		
M4	GTTTTCCCAGTCACGAC		

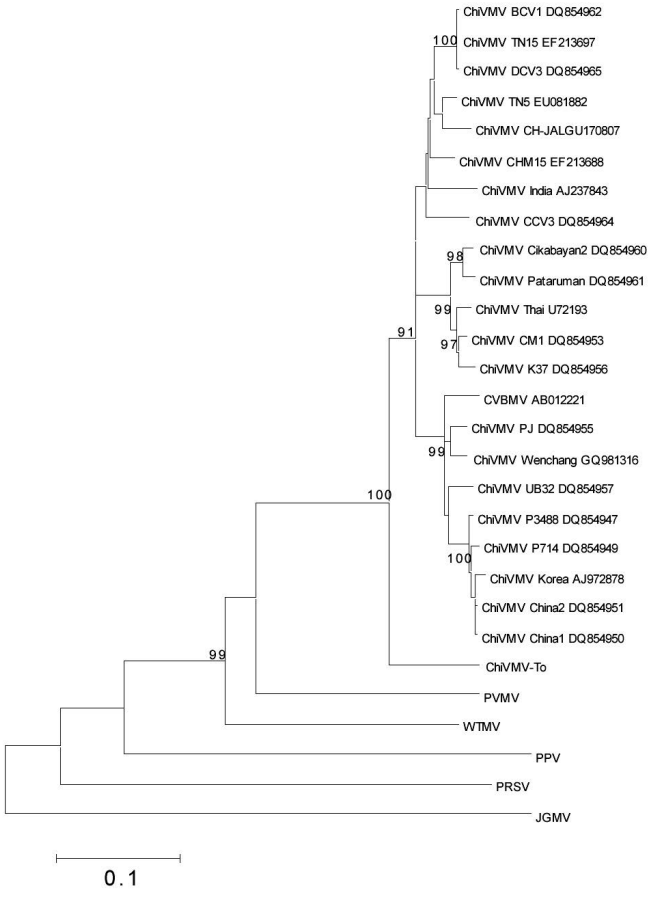

Fig. 1. Phylogenetic tree based on the polyprotein amino acid sequences of ChiVMV isolates and other potyviruses. Sequences of potyviruses for comparisons were obtained from GenBank Database: *Chilli veinal mottle virus* (ChiVMV): Wenchang isolate GQ981316 from China; two isolates from South Korea (AJ972878, AM909717); CH-JAL isolate GU170807, Ch-War isolate GU170808 and PVBV isolate AJ237843 from India; *Bean common mosaic virus* (BCMV), U34972; *Bean yellow mosaic virus* (BYMV), U47033; *Chilli ringspot virus* (CRSV), NC_016044; *Johnsongrass mosaic virus* (JGMV), NC_003606; Onion yellow dwarf virus (OYDF), AJ510223; *Papaya ringspot virus* (PRSV), NC_001785; *Pepper mottle virus* (PepMoV), AF501591; *Pepper veinal mottle virus* (PVMV), NC_011918; *Plum pox virus* (PPV) D13751; *Potato virus Y* (PVY) NC_001616; *Ryegrass mosaic viru*s (RGMV), Y09854; *Sugarcane mosaic virus* (SCMV), NC_003398; *Tobacco etch virus* (TEV), M15239; *Tobacco vein banding mosaic virus* (TVBMV), NC_009994; *Turnip mosaic virus* (TuMV), AF169561; *Wild tomato mosaic virus* (WTMV), DQ851495; *Zucchini yellow mosaic virus* (ZYMV), L31350.

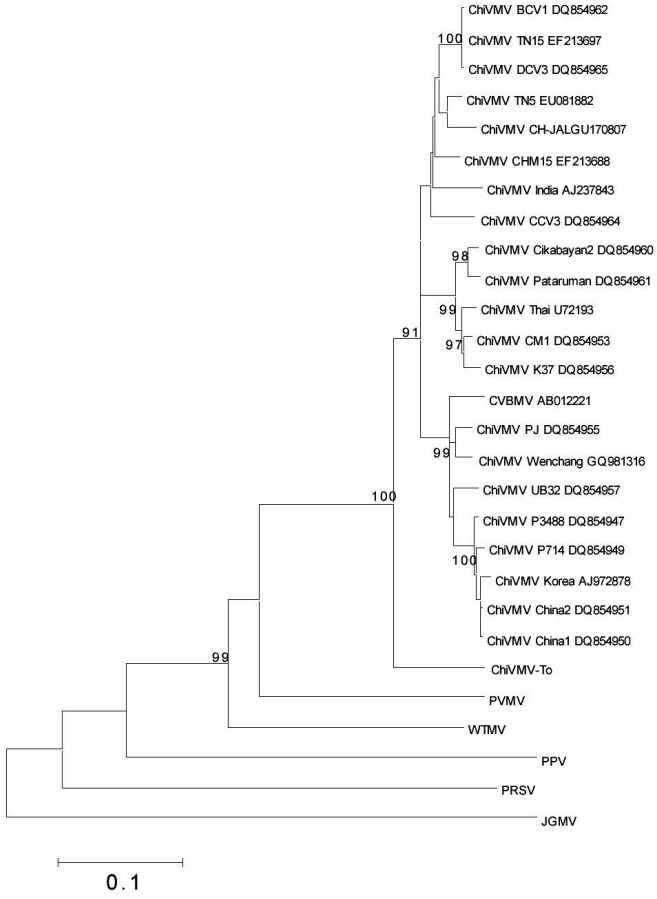

Fig. 2. Phylogenetic tree based on the CP gene and 3'-UTR nucleotide sequences of ChiVMV isolates and other potyviruses. Sequences of potyviruses for comparisons were obtained from the GenBank Database: *Johnsongrass mosaic virus* (JGMV), NC_003606; *Papaya ringspot virus* (PRSV), NC_001785; Pepper veinal mottle virus (PVMV), NC_011918; *Plum pox virus* (PPV), D13751; *Wild tomato mosaic virus* (WTMV), DQ851495.

USA). Sequence analysis and assembly were done using the DNAMAN Sequence Analysis Software (Lynnon Biosoft, Canada). Phylogenetic trees were constructed by the neighbor-joining method provided by the MEGA program version 5.0 and bootstrap was tested in 1000 replications (Tamura *et al.*, 2011).

The complete genome sequence of ChiVMV-To comprises 9,724 nts, excluding the poly (A) tail (GenBank accession No. JX088636) and contains 32.0% adenine, 26.5% uracil, 18.9% cytosine and 22.6% guanine. The 5'- and 3'-untranslated regions (UTRs) are 168 nts and 286 nts in size, respectively. The single open reading frame (ORF) was predicted to code for a polyprotein of 3,089 aa expected to be proteolytically processed to yield 10 mature proteins (i.e., P1, HC-Pro, P3, 6K1, CI, 6K2,VPg, NIa, NIb and CP) using consensus cleavage sites similar to those of other ChiVMV isolates (Adams *et al.*, 2005; Anindya *et al.*, 2004).

Comparisons of the complete genome sequences showed that ChiVMV-To exhibits nucleotide and amino acid sequence identities of 78.5-79.1% and 83.8-86.7%, respectively, with six other ChiVMV isolates (two from Korea, three from India and one from China) whose complete genome sequences were retrieved from GenBank, the closest being the Wenchang chilli isolate from the Hainan province of China (GQ981316). At the

amino acid sequence level identities are: PI (57.3-61.7%), HC-Pro (89.9-91.9%), P3 (75.0-76.5%), 6K1 (88.9-98.1%), CI (84.4-92.1%), 6K2 (78.4-88.2%), VPg (82.6-92.1%), NIa (88.4-91.3%), NIb (86.7-91.9%), and CP (89.5-91.3%). The 5'-UTR shared the highest identity (67.4%) with that of ChiVMV Ch-jal and Ch-war isolates from India, whereas the 3'-UTR shared the highest identity (90.7%) with that of the Wenchang isolate. Comparisons of the CP gene sequence of ChiVMV-To with those of ChiVMV-Chi from GenBank showed ChiVMV-To has the highest identities (87.3 and 91.6%) with the Thailand isolate CM1 (accession No. DQ854953) at the nucleotide and amino acid level, respectively.

Phylogenetic analysis based on the complete polyprotein sequences of six ChiVMV-Chi isolates and 20 representative potyviruses revealed that ChiVMV-To is most closely related to isolates of *Wild tomato mosaic virus* and closely related to *Pepper veinal mottle virus* (Fig. 1). Based on a phylogenetic analysis of the CP gene and 3'-UTR nucleotide sequences, all ChiVMV-Chi isolates could be classified into three groups (Tsai *et al.*, 2008). However, the phylogenetic tree based on the CP gene and 3'-UTR nucleotide sequences suggested that all ChiVMV isolates can be divided into two distinct groups, one of which contains the tobacco isolate and the other the chilli isolates (Fig. 2). The molecular composition of ChiVMV-To shows that it is a distinct strain of ChiVMV. Because chilli, an important crop host of ChiVMV, is one of the main vegetables grown in Yunnan, further studies on the variability and distribution of ChiVMV-To on chilli plants in Yunnan are needed.

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