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LETTER TO THE EDITOR

Characterization and full genome sequence analysis of a Chinese isolate of tomato chlorosis virus

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China, the world´s largest producer of tomato (*Solanum lycopersicum*), has been suffering from whitefly-transmitted viruses, such as tomato chlorosis virus (ToCV), in recent years (*1*). ToCV causes a severe yellowing disease on tomato plants and is emerging as a serious problem for field and greenhouse tomato production in many parts of the world, including Europe (*2*), America (*3, 4*), Asia (*1, 5, 6*), and Africa (*7, 8*). The infected tomato plants exhibit symptoms including interveinal chlorosis, leaf brittleness, and limited necrotic flecking or leaf bronzing (*9*). ToCV is a member of the genus *Crinivirus* in the family *Closteroviridae* and has a bipartite genome of single-stranded positive-sense RNA (*10*). The two RNA molecules are encapsidated separately in long and flexuous virions approximately 800 to 850 nm in length. More recently, the complete nucleotide sequences of ToCV isolates from the United States (*11*), Spain (*12, 13*), Greece (*14*), and Brazil (*15*) have been determined. RNA 1 contains four open reading frames (ORFs) and RNA 2 encodes up to nine ORFs, most of which are not well studied (*11–13*). Despite ToCV outbreaks in Japan and China (*1, 5, 6*), no analysis on the local isolates and their sequences was reported. Here, we firstly report and characterize the bipartite genome sequence of ToCV-BJ, an isolate of ToCV from Beijing, China.

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Abbreviations: ToCV = tomato chlorosis virus; UTR = untranslated region

Tomato plants showing interveinal chlorosis on lower leaves were collected from a plastic house in Haidian district, Beijing, and were screened using ToCV-specific primers (*6*). Positive tomato plants were moved to a greenhouse in the west campus of China Agricultural University. Virions were purified from 80 g of infected tomato leaves and viral RNA was extracted following the Klaassen's method (*16*). Most parts of the genome were amplified by reverse transcription polymerase chain reaction (RT-PCR) with specific primers designed according to the full sequence of ToCV Florida isolate (NC_007340.1 and NC_007341.1) or with those used previously (*17, 18*). The 5´- and 3´-terminal sequences of viral RNA were determined basically by following the 5´- and 3´- rapid-amplification of cDNA ends (RACE) protocols described previously (*19*). To obtain the two 3´-terminal sequences, RNA was polyadenylated for 10 min prior to the cDNA synthesis, using the *Escherichia coli* poly (A) polymerase (New England Biolabs, America) according to the manufacturer's instructions. All PCR products were gel-purified using TIANgel Midi Purification Kit (Tiangen, Beijing, China) and the resulting products were ligated to the vector pMD-18T (TaKaRa, Dalian, China). Positive clones were confirmed by colony PCR before sequencing. At least three clones from independent PCR reactions were sequenced (BGI, China) from both directions. For sequencing of the 5´- and 3´-terminus, at least four clones were determined to obtain a consensus sequence.

The complete sequence was assembled from overlapping RT-PCR clones (including 5´- and 3´- RACE) using the

program DNAMAN (version 6.0). The NCBI ORF Finder (http://www.ncbi.nlm.nih.gov/gorf/gorf.html) was used to identify ORFs and SMS (The Sequence Manipulation Suite, http://www.bio-soft.net/sms/index.html) was applied to translate ORFs to amino acid sequences. Sequence alignment was performed by DNAMAN (version 6.0). Phylogenetic trees were constructed by neighbor-joining (NJ) method in the MEGA (version 4.0) software with 1000 replicates bootstrapping (*20*).

The complete RNA 1 sequence of ToCV-BJ consisted of 8594 nt (GenBank Acc. No. KC887998) containing a 301 nt 5′-untranslated leader sequence, four ORFs and a 176 nt 3′-untranslated region (UTR), which was consistent with a Greek isolate (Gr-535) (*14*). The first two ORFs of RNA 1, ORF 1a (nt 302 to 6139) and ORF 1b (nt 6141 to 7655), encoded the conserved closterovirus proteins involved in the replication of viral RNA based on the homology with other viral replication factors (*11*). ORF 1a encoded a 221 kDa multifunctional protein containing protease, methyltransferase and helicase (Pro-MT-HEL) domains, which was common among members of the family *Closteroviridae* (*13, 21*). ORF 1b encoded a 59 kDa RNA-dependent RNA polymerase (RdRp) that was conserved among members of the genus *Crinivirus* (*11*). ORF 2 (nt 7662–8243) encoded a 22 kDa protein (p22), which was identified to be an effective silencing suppressor (*22*). ORF 3 (nt 8263–8418) encoded a 5 kDa protein (p5) possessing a central transmembrane domain, but its function remains unknown (*11*).

Similar to the RNA 2 of the isolate from Greece (Gr-535), the complete sequence of RNA 2 of ToCV-BJ was 8242 nt (GenBank Acc. No. KC887999), possessing 238 nt and 213 nt 5′- and 3′-UTRs, respectively, and encoding nine putative proteins, p4 (ORF 1, nt 239 to 340), Hsp70h (ORF 2, nt 733 to 2397), p8 (ORF 3, nt 2407 to 2610), p59 (ORF 4, nt 2562 to 4115), p9 (ORF 5, nt 4097 to 4333), CP (ORF 6, nt 4333 to 5106), CPm (ORF 7, nt 5112 to 7121), p27 (ORF 8, nt 7125 to 7823) and p7 (ORF 9, nt 7832 to 8029). Hsp70h, p59, CP and CPm may participate in viral movement, encapsidation, vector-mediated transmission and silencing suppression (*11, 12, 14, 22*). P4 and p7 were transmembrane proteins, but their functions remain unknown, and p7 was a novel protein that had no equivalent in genomes of other criniviruses (*12*). P9 might self-interact based on the homology with lettuce infectious yellows virus (*23*). P27 was unique to criniviruses and shared a homology with the p27-like proteins of other criniviruses (*11*).

The complete nucleotide sequences of RNA 1 and RNA 2 of ToCV-BJ showed 97.38%–99.42% and 97.56%–99.65% identities with those of ToCV isolates from the United States (*11*), Spain (*12, 13*), Greece (*14*), Brazil (*15*), and two latest isolates from China (SDSG from Shandong province with GenBank Acc. Nos. KC709509.1 and KC709510.1, and another from Nanjing city with GenBank Acc. No. KF018280.1), indicating that ToCV isolates worldwide were highly conserved. The RNA 1 and RNA 2 of ToCV-BJ had the highest nucleotide sequence identities with the isolate SDSG (KC709509.1 and KC709510.1) with 99.42% and 99.65%, respectively. The 5′- and 3′- UTRs of RNA 1 showed identities of 96.35%–99.34% and 98.30%–100.00% with other ToCV isolates, respectively. The 5′- and 3′- UTRs of RNA 2 shared

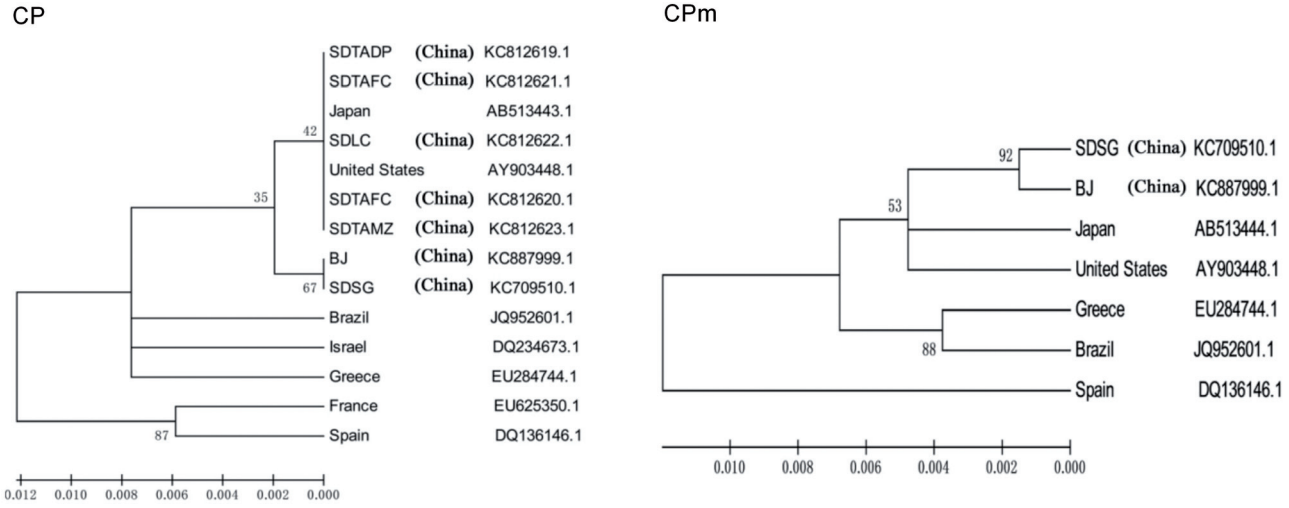

Fig. 1

Neighbor-joining tree generated based on ToCV CP and CPm amino acid sequences available in GenBank depicting the phylogenetic relationships

The bootstrapping replicates = 1000. The scale bar represents genetic distance (substitutions per nucleotide).

98.74%–100.00% and 97.25%–100.00% identities with other ToCV isolates, respectively. Sequences at the 5′- and 3′- ends of the two RNAs were highly conserved, and the first 6 nt and 10 of the first 12 nt of RNAs 1 and 2, respectively, were identical with the isolate ToCV-BJ, and with isolates from the United States (Florida) and Greece (Gr-535). The identity of 3′-UTRs between RNA 1 and 2 of ToCV-BJ was 74.18%.

Nucleotide and amino acid sequences of CP and CPm of ToCV-BJ were compared with other ToCV isolates. The identities of CP between ToCV-BJ and other ToCV isolates ranged from 97.29% to 99.87% at the nucleotide level and 97.28% to 100.00% at the amino acid level. The nucleotide and amino acid identities of CPm between ToCV-BJ and other ToCV isolates were 97.46%–99.35% at the nucleotide level and 97.46%–98.95% at the amino acid level, respectively. Sequence analysis and phylogenetic trees based on amino acid sequences of CP and CPm (Fig. 1) revealed that the isolate ToCV-BJ shared more homology with isolates from China, the United States and Japan, but less homology with European isolates from Greece, Spain, France and Israel.

In this study, we reported the complete genome sequence of a Chinese isolate, ToCV-BJ. To our knowledge, this is the first report of full sequence of ToCV from Asia. Though the potential impact of the virus in China is to be assessed, the data presented here provide a basis for further investigation on the genetic diversity and the epidemic spread of ToCV. As additional positive tomato samples have been found in Shandong province (a principle tomato production region in China), showing the virus may be widespread in east of China, further studies are in progress to determine the incidence and genetic relationships of ToCV from different regions of China.

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