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SHORT COMMUNICATION

Detection and characterization of an isolate of *Tomato mottle mosaic virus* infecting tomato in China

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Abstract

Tomato (*Solanum lycopersicum*) plants exhibiting severe leaf distortion, mottle and systemic crinkling symptoms were identified in Hainan Province in China in 2016. To survey and control the disease, it is necessary to identify and characterize the pathogen causing the disease. Dot enzyme-linked immunosorbent assay showed that the crude saps of the infected tomato samples reacted positively with the monoclonal antibody against *Tobacco mosaic virus* which indicated that one or more tobamoviruses are likely associated with the disease. RT-PCR and DNA sequence analysis results further elucidated that *Tomato mottle mosaic virus* (ToMMV) in *Tobamovirus* was the pathogen causing the mottle disease in tomato. We amplified and sequenced the full-length sequence of the genome which showed the highest nucleotide identity with ToMMV YYMLJ and ToMMV TiLhaLJ isolates. The putative virus isolate was named ToMMV Hainan. Biological indexing studies showed that ToMMV Hainan can infect *Nicotiana benthamiana*, *Capsicum annuum* and *Solanum lycopersicum* showing serious symptoms. This was the first identification and characterization of ToMMV infecting tomato in Hainan of China.

Keywords: *Tomato mottle mosaic virus*, tomato, *Tobamovirus*, dot-ELISA, biological characterization

1. Introduction

Tomato (*Solanum lycopersicum* L.) is one of the most economically important vegetable crops throughout the world. The annual tomato production showed an increasing tendency year by year, while the incidence and severity of diseases have limited the yield and quality of tomato causing serious losses. Viral diseases are the major constraints in tomato production among the known pathogen-induced diseases (Hanssen *et al*. 2010).

There are at least 136 characterized viruses that have been reported infecting tomato in the world which are much greater than the other vegetables (Brunt *et al*. 1996; Xu *et al*. 2017). Tobamoviruses are a kind of economically important viruses in the family *Virgaviridae*, which contains 37 members according to the taxonomy by the International Committee on Taxonomy of Viruses (https://talk.ictvonline.org/taxonomy/). The tobamoviruses were divided into different subgroups according to their genomic structure, host range, the amino acid composition of the coat proteins (CPs), etc. The phylogenetic relationship of the tobamoviruses conducted by Li *et al*. (2017) revealed that in the Solanaceae-infecting group, *Tobacco mosaic*

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virus (TMV), *Tomato mosaic virus* (ToMV), *Tomato mottle mosaic virus* (ToMMV) and *Tomato brown rugose fruit virus* (ToBRFV) are the four reported tomato-infecting tobamoviruses and are classified into one clade based on the complete genome and amino acid sequences. TMV and ToMV are the most epidemic viruses among the world, ToBRFV is a new species that has been characterized in Jordan (Salem *et al*. 2016) and Israel (Luria *et al*. 2017), while ToMMV was also a recently characterized species and showed fast spread in the world. ToMMV was first described in 2013 infecting tomato in Mexico (Li *et al*. 2013) and subsequently was detected on tomato in different parts of the world including the USA (Webster *et al*. 2014; Fillmer *et al*. 2015; Padmanabhan *et al*. 2015; Sui *et al*. 2017), Israel (Turina *et al*. 2016), Brazil (KT222999) and Spain (Ambrós *et al*. 2017). Additionally, Pirovano *et al*. (2014) showed its presence in *Cicer arietinum* L. in Italy and Li *et al*. (2014, 2017) established two isolates infecting pepper from Yunnan Province and Tibet Autonomous Region in China.

The genome of ToMMV contains four open reading frames expressing four proteins like the other tobamoviruses in the family *Virgaviridae*, ~126 kDa protein and ~180 kDa read-through protein involved in virus replication, ~30 kDa movement protein and ~18 kDa coat protein. The sequence characteristic of ToMMV genome like the other tobamoviruses represents that the genome contains a Ω fragment in the 5′ untranslated region (UTR) which had no G residues except the most-proximal m^7G cap (Richards *et al*. 1977; Zhang *et al*. 2008), a conserved region TCCCTCCACTTAAATCGAAGGGTT located in the 3′ UTR with the CCCA ending sequence (Chng *et al*. 1996) and the '4404-50 motif' reported as the tobamoviruses-specific nucleotide motif which indicated that the 29 sites of the 47 nucleotides (nt) are invariant in all tobamoviruses sequences (Gibbs *et al*. 2004). Li *et al*. (2017) showed that the remaining 18 variable sites are also conserved among the previously reported ToMMV isolates and 9 of the 18 variable sites can distinguish ToMMV from ToMV. The ToMMV-specific sequence is as follows: GGTGATGTTACAACTTTCATAGGAAATACTGTTATTATA GCCGCGTG.

In a survey of viral diseases in 2016, a devastating disease infecting tomato crops was observed in the greenhouses and natural fields in Hainan Province in China. The tomato plants showing virus-like symptoms were collected for virus detection. The pathogen has been identified as ToMMV and characterized the full sequence of ToMMV Hainan isolate-infecting tomato.

2. Materials and methods

2.1. Plant growth conditions and inoculations

Tobacco, pepper and tomato plants were grown in a growth room (28°C day and 24°C night, 16 h/8 h photoperiod). Virus-infected leaf tissues were homogenized in 0.01 mol L^{-1} phosphate buffer (PBS, 0.01 mol L^{-1} KH_2PO_4:0.01 mol L^{-1} Na_2HPO_4=49:51 (v/v), pH=7.0) at 1:10 ratio (w/v). The crude extracts were rub-inoculated to the newly grown leaves of 4-week-old tobacco, pepper and tomato plants, respectively. The virus-inoculated plants were grown in the same conditions.

2.2. Dot enzyme-linked immunosorbent assay (dot-ELISA)

The dot-ELISA procedures were conducted as described previously with some modification (Li *et al*. 2015). Briefly, the 3 μL plant sample supernatants in 0.01 mol L^{-1} PBS (pH 7.4) were spotted onto the nitrocellulose membranes (Amersham Biosciences, Piscataway, NJ, USA). The TMV monoclonal antibody solution was diluted by 1:5 000-fold with blocking buffer (5% skimmed milk in PBS with 0.5% Tween-20). The membranes were developed with the substrate nitro-blue tetrazolium (NBT, 0.083 mg mL^{-1}) and 5-bromo-4-chloro-3-indolyl phosphate salt (BCIP, 0.05 mg mL^{-1}).

2.3. RNA extraction, RT-PCR, full-length genome amplification and sequencing

The total RNA was extracted from tomato leaves with TRIzol reagent (Invitrogen, Carlsbad, CA, USA) and treated with DNaseI. A total of 1 μg of total RNA, random primer, and M-MLV reverse transcriptase were used for synthesis of first-strand cDNA using Prime-Script™ RT Reagent Kit (TaKaRa Bio. Inc., Dalian, China). RT-PCR was performed according to the manufacturer's protocols. The primer pairs used in the RT-PCR experiments were listed in Appendix A. The RT-PCR products were purified with E.Z.N.A. Gel Extraction Kit (Omega, Norcross, GA, USA) and cloned into pGEM-T vector (Promega, Madison, WI, USA), then transfected into DH5α competent cells. The recombinant clones were sequenced with universal primer pairs M13F/M13R *via* Sanger sequencing using ABI 3730XL DNA Analyzers (Applied Biosystems, Foster City, CA, USA). At least three independent clones were sequenced.

The 5′- and 3′-terminal sequence of the ToMMV were obtained through 5′- and 3′-rapid amplication of cDNA ends (RACE) using SMARTer RACE cDNA Amplification Kit (Clontech, Mountain View, CA, USA) according to the