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Complete genomic sequence of turnip mosaic virus infecting passionfruit in Fujian province of China

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Abstract

The complete genome sequence of turnip mosaic virus (TuMV), isolated from diseased passion fruit (*Passiflora edulia*) in Fujian Province, China (TuMV-FJ) was determined. The viral genome was found to be 9,833 nucleotides in length excluding the poly(A) tail and to encode a polyprotein of 3164 amino acids, which was predicted to be cleaved into ten functional proteins by viral proteases. Phylogenetic analysis indicated that TuMV-FJ had closest phylogenetic relationship to BJ-B01, an isolate from *Brassica oleracea* in the world-B phylogenetic group of TuMV. Sequence analysis showed that TuMV-FJ shared more than 94% nucleotide and amino acid identities with BJ-B01. These results suggest that TuMV-FJ is a novel isolate of TuMV. To our knowledge, this is the first report of the complete genome sequence of TuMV infecting *P. edulia*.

Full Text

Passion fruit (*Passiflora edulis*) is a fragrant fruit widely planted in tropical and subtropical regions around the world [13]. In China, passion fruit plants mainly in Fujian, Guangdong, Guangxi and Hainan Provinces. In recent years, passion fruit cultivation area in China is increasing, especially in Fujian Province, which has exceeded 4700 hm^2 [14]. However, the cultivation and production of passion fruit are severely affected by the infection of plant viruses. It has been documented that passion fruits are susceptible to infection of no less than 25 plant viruses in the world and potyviruses are the dominant types of plant pathogens [1]. To date, only four potyviruses have been found in passion fruit in China: East Asia Passiflora virus, telosma mosaic virus, passion fruit severe mottle virus and turnip mosaic virus (TuMV), a potyvirus that identified as new host of yellow passion fruit in China [2, 4, 15].

TuMV is a member of the genus *Potyvirus* in the family *Potyviridae* and has a single-stranded positive-sense RNA genome ~10 kb in size [8]. The viral genome has one major open reading frame (ORF), which is translated into a polyprotein and autocatalytically hydrolyzed into 10 mature multifunctional proteins. In addition, a small overlapping ORF called PIPO (for pretty interesting *Potyviridae* ORF) was found to be embedded in the P3 cistron, resulting in a P3-PIPO fusion protein [3].

TuMV has a wide host range and mainly damages dicotyledonous domestic brassica crops [7]. Like other potyviruses, the virus is transmitted by aphids in a non-persistent manner. Depending on the ability to infect different hosts, TuMV isolates can be phylogenetically divided into six groups: basal-Brassica (basal-B), basal-Brassica/Raphanus (basal-BR), Asian-Brassica/Raphanus (Asian-BR), world-Brassica (world-B), Iranian and Orchis [7, 11]. Though TuMV on passion fruit has recently been identified in Guangdong Province, China [2], the genome sequence of TuMV in passionfruit was, until now, not available. Here, we report the first complete genome sequences of a TuMV isolate infecting passionfruit, which is referred to as TuMV-FJ.

Leaves samples from passion fruit samples showing mosaic, crinkle and yellow spots symptoms (Fig. 1) were collected in 2018 from a commercial orchard in Fujian Province, China. Total RNA was extracted from the positive samples using TRIzol Reagent (Invitrogen, USA). RNA purification and reverse

transcription were performed according to the manufacturer's instruction. A library of small interfering RNA (siRNA) was constructed and sequenced using a HiSeq X Ten sequencer (Illumina, USA) by Sangon Biotech Co., Ltd. (Shanghai, China). A total of 4,392,365 clean reads were generated followed by contigs assembly. Among the obtained contigs, eight contigs were found to share highly similarity with reported genomic sequences of TuMV. In addition, contigs with homologies to telosma mosaic virus and cucumber mosaic virus were also identified. TuMV infection was confirmed by RT-PCR using specific primers (5 -TGTGTTTATVAYCAYCARGCAGGYGA-3´ and 5 -CGCTGAAGACCATATCGTGGCATG-3´). To determine the complete nucleotide sequence of TuMV-FJ, the gap regions of the virus genome between contigs were amplified and sequenced by RT-PCR using four virus-specific primer sets (Table S1) designed based on the contig sequences. Terminal sequences of the viral genome were determined by rapid amplification of cDNA ends (RACE) using commercial kits (Invitrogen, USA). The RT-PCR amplicons of the expected sizes were cloned and sequences in both directions by Sangon Biotech Co., Ltd. (Shanghai, China). The final sequences were assembled and analysed using the DNAMAN 9 program (Lynnon, Quebec, Canada). The complete genome sequence of TuMV-FJ has been deposited in GenBank under the accession number MK340758.

To identify virus-derived sequences with close similarity to TuMV-FJ, a homology search was performed against the GenBank database. Multiple sequence alignment was performed using the MAFFT algorithm [6] implemented in PhyloSuite 1.21 [16] and ambiguously aligned regions were then trimmed using the program Gblock 0.91b [12]. Phylogenetic analysis was carried out by the maximum-likelihood method implemented in IQ-tree 1.6.8 [10] using the GTR+I+G4+F substitution model, which was determined by ModelFinder [5]. Support for the inferred tree was assessed by ultrafast bootstrapping with 10000 replicates. The presence of putative recombination events were identified using RDP 5.3 [9] implementing seven different algorithms, including RDP, GENECONV, Bootscan, MaxChi, Chimaera, Siscan and 3Seq.

RT-LAMP assay was developed quickly investigate the incidence of TuMV in commercial orchard in this study. Primer sets (Table S2) for the RT-LAMP assay were designed based on the published coat protein sequences of TuMV using the online software of Primer Explorer version 4 (Eiken Chemical Co. Ltd., Japan). The RT-LAMP reaction was conducted in a total volume of 25 μL containing 1× Isothermal Amplification Buffer (20 mM Tris-HCl, 10 mM $(NH4)_2SO_4$, 50 mM KCl, 2 mM $MgSO_4$, and 0.1% Tween® 20, PH 8.8), 1.6 μM of each FIP and BIP primers, 0.2μM of each F3 and B3 primers, 1.4 Mm each dNTP, 6 mM $MgCl_2$, 8 U of Bst 2.0 DNA polymerase (NEB, USA), 10.5 μL double-distilled water, together with 2 μL template cDNA solution. The RT-LAMP assay was carried out at 65°C for 60 min using a real-time turbidimeter instrument (LA-200, Teramecs, Japan). Visual detection of RT-LAMP products was performed by naked eyes with the addition of SYBR Green I (Solarbio, China).

The complete genome sequence of TuMV-FJ consists of 9833 nucloetides (nts) excluding the poly(A) tail. The 5´ and 3´ untranslated regions (UTRs) were found to be 129 and 209 nts in length, respectively, in length. Its genome contains an open reading frame (ORF, nucleotides 130-9264) encoding a polyprotein of 3164 amino acid residues. The polyprotein has nine putative cleavage sites and is cleaved into ten mature

proteins: P1 (nt 130–1215, 362 aa, 40.15kDa), HC-Pro (nt 1216-2589, 458 aa, 51.72 kDa), P3 (nt 2590-3654, 355 aa, 40.13 kDa) 6K1 (nt 3655-3810, 52 aa, 5.93 kDa), CI (nt 3811-5742, 644 aa, 71.76 kDa), 6K2 (nt 5743-5901, 53 aa, 5.98 kDa), VPg (nt 5902-6477, 192 aa, 21.95 kDa), NIa (nt 6478-7206, 243 aa, 27.32 kDa), NIb (nt 7207-8757, 517 aa, 59.65 kDa), and CP (nt 8758-9621, 288 aa, 33.06 kDa). A recently defined pipo gene was also identified in TuMV-FJ that includes the highly conserved frameshift motif G_1A_6 (nt 3076-3082) within the P3 cistron encoding a very short protein of 60-amino acids which expected to be a P3N-PIPO fusion product. Details of the genome organization of TuMV-FJ are presented in Table 1.

The phylogenetic relationship between TuMV-FJ and the other TuMV isolates is illustrated in the phylogenetic tree (Fig. 1). The maximum-likelihood tree showed that TuMV isolates could be separated into six phylogenetic groups. TuMV-FJ was clustered into a monophyletic clade with high confidence (bootstrap value, BP =100%, Fig. 1), together with TuMV isolates in the world-B phylogenetic group with a considerable diversity of sampling locations and host origins. Within the clade, the clade, TuMV-FJ had closet phylogenetic relationship to BJ-B01, an isolate from *Brassica oleracea.*

TuMV-FJ shares 77%-98% nucleotide and 87%-99% amino acid sequence identities with TuMV isolates from six phylogenetic groups at the genome level. At the individual cistron level, TuMV-FJ shares the highest nucleotide (≥94%) and amino acid (≥100%) sequence identities with BJ-B01. Similarly, either the CP or PIPO of TuMV-FJ share highest nucleotide and amino acid sequence identities with BJ-B01 (Table 1). Comparing all TuMV isolates, recombination analyses did not identify recombination event in the TuMV-FJ genome. Taken together, these analyses suggest that TuMV-FJ is a novel isolate of TuMV from passionfruit. To our knowledge, this is the first report of the complete genome sequence of TuMV infecting *P. edulia*. In addition, the established RT-LAMP in this
study showed good specificity and high sensitivity *(*Fig. S2*). The* results from our field survey indicated that although TuMV incidence (1.3%) in passion fruit in the commercial orchard seems to be low, additional surveillance for it may be needed.

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Declarations

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Competing interests The authors declare no completing interests.

Author Contributions J. Shen and F. Gao contributed to the study conception and design. Material preparation, data collection and analysis were performed by X. Li, L. Xie, X. Chen and J. Chen. The first draft of the manuscript was written by J. Shen and F. Gao. All authors read and approved the final manuscript.

Data Availability Sequence data obtained in this study has been deposited in GenBank under the accession number MK340758.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

Tables

Table 1 Genome positions, protein sizes of TuMV-FJ and percentage of nucleotide and amino acid sequence identity to representative sequences from six TuMV phylogenetic groups

	Nucleotide Position	Protein Size		Nucleotide/amino acid sequence identity (%)					
		amino acid	kDa	BJ-B01 (World-B)	CCLB (Asian-BR)	TUR16 (basal-BR)	GRC42 (basal-B)	IRNTlm1 (Iranian)	OS (Orchis)
Polyprotein	130-9624	3164	357.48	98/99	84/93	81/90	80/90	80/90	77/87
5´-UTR	1-129	-	-	100/-	92/-	82/-	82/-	82/-	84/-
P1	130-1215	362	40.15	98/97	80/81	75 /74	76/74	76/73	81/82
HC-Pro	1216-2589	458	51.72	98/99	81/95	79/93	77/93	79/93	79/92
P3	2590-3654	355	40.13	99/100	79/84	77/80	77/81	77 /80	70/71
PIPO	3078-3260	60	7.11	100/100	92/90	88/80	91/83	88/82	84/75
6K1	3655-3810	52	5.93	98 /100	84/98	84/98	82/96	79/96	77/96
CI	3811-5742	644	71.76	98/100	86/98	82 /95	83/96	82/96	79/92
6K2	5743-5901	53	5.98	97/100	85/98	82/81	76/79	79/89	73/74
VPg	5902-6477	192	21.95	94/94	83 /93	80 /88	81/87	79/86	80/88
NIa	6478-7206	243	27.32	97/100	83/96	82/95	81/90	82/95	77/90
NIb	7207-8757	517	59.65	99/99	86/97	81/95	80/95	83/95	77/91
CP	8758-9621	288	33.06	99/99	90/95	87/94	89/94	87/91	82/90
3´-UTR	9625-9833	-	-	98/-	96/-	91/-	99/-	92/-	82/-

The accession numbers of BJ-B01, CCLB, TUR16, GRC42, IRNTlm1 and OS are KC119185, KR153038, AP017728, AB252117, AP017796 and AB701693, respectively.

Figures

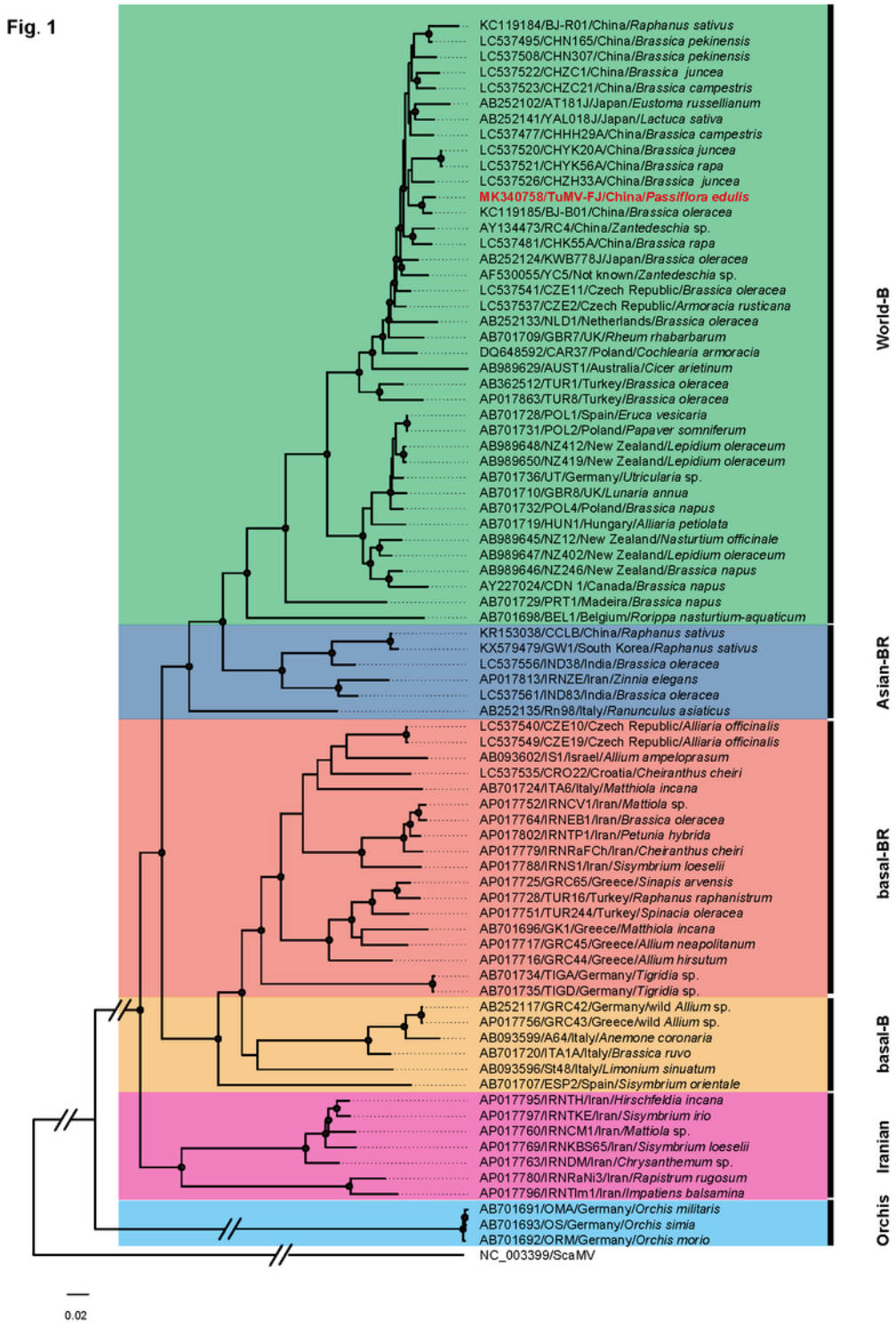

Figure 1

Maximum-likelihood phylogenetic tree based on the codon-aligned nucleotide sequences of polyproteins from TuMV isolates. Reference isolates were downloaded from GenBank, and a scallion mosaic virus isolate (accession no. NC_003399) was used as an outgroup. Black circles indicate strong node support (bootstrap support ≥ 95%). Isolate TuMV-FJ from this study is shown in bold red font.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Fig.S1lited.pdf
- Fig.S2.pdf
- TableS1.docx
- TableS2.docx
- TuMVFJ.txt