OCCURRENCE OF TURNIP MOSAIC VIRUS IN PHALAENOPSIS sp. IN CHINA

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SUMMARY

A turnip mosaic virus (TuMV) isolate from asymptomatic Phalaenopsis sp. was detected by indirect ELISA. Its presence was confirmed by RT-PCR with a pair of degenerate primers whose design was based on reported coat protein gene sequences. Further analysis of the genomic sequence of this isolate designated as TuMV-ZH1 (GenBank accession No. KF246570) showed a high nucleotide sequence identity with isolate Lu2 (96.7%) and CHN 12 (96.3%) from China. Phylogenetic analysis indicated that TuMV-ZH1 belongs to the world-B lineage. To the best of our knowledge, the above may be the first indication that TuMV infects orchids in China. In addition, proteins P1 and P3 of TuMV-ZH1 are highly mutated as previously reported for other TuMV isolates.

Keywords: TuMV, coat protein, genome, phylogenetic analysis.

INTRODUCTION

Turnip mosaic virus (TuMV) is transmitted by at least 89 species of aphids in a non-persistent manner and is an important member of the genus Potyvirus (Walsh and Jenner, 2002). This virus infects more than 318 plant species of 156 genera in 43 families and it damages Cruciferae. TuMV is widespread in temperate and subtropical regions of five continents and occurs to most regions of China. Studies have shown that TuMV isolates can be grouped into two pathotypes designated as Brassica (B) and Brassica-Raphanus (BR), and further subdivided into four genogroups called basal-B, basal-BR, Asia-BR and world-B (Ohshima et al., 2002). Placing TuMV isolates into these groups has become the main classification method of this virus (Sanchez et al., 2007; Farzadfar et al., 2009; Wang et al., 2009).

TuMV has great genetic versatility in adapting to different environments and breaking host resistance. For example, TuMV with just a single-nucleotide mutation in the cylindrical inclusion gene (CI) can overcome the resistance gene TuRB01 (Walsh et al., 2002). A single mutation (+3394 T>C) in the viral P3 protein induces local necrotic lesions and overcomes extreme resistance in Brassica napus. Such a change results in systemic non-necrotic infection when another mutation (+5447 T>C) in CI protein is introduced (Jenner et al., 2002).

TuMV can infect 13 species of orchid plants, including Aceras anthropophorum, Anacamptis pyramidalis, Barlia longibracteata, Calanthe sp., Ophrys spheum, Ophrys tentrequinica, Orchis italica, Orchis morio, Orchis militaris, Orchis papilionacea, Orchis sinia, Cymbidium sp. and Pescatorea sp., in which it induces mosaic symptoms (Lesemann and Vetten, 1985; Inouye, 1992). A TuMV isolate, denoted TuMV-ZH1, was identified in Phalaenopsis sp. in a survey for orchid pathogens in China. This paper describes

Fig. 1. Electron micrograph of virus particles purified from infected Phalaenopsis sp. TuMV-ZH1 particles are filamen
tous and measure 15-18 \times 700-800 \text{nm}.
the identification process and the genomic sequence of TuMV-ZH1 to clarify its origin and genetic features.

MATERIALS AND METHODS

Collection of orchid plants. A total of 403 samples, distributed among 19 orchid genera (Cymbidium, Dendrobium, Cattleya, Paphiopedilum, Oncidium, Phalaenopsis, Vanda, Bulbophyllum, Coelogyne, Calanthe, Cephalanthera, Phaius, Malaxis, Miltonia, Neofinetia, Gastrochilus, Epidendrum, Rhynebytis, Dendrobium) were collected from several Chinese main orchid producing areas, such as Guangzhou, Zhuhai, Kunming and Xiamen.

I-ELISA detection of TuMV. The polyclonal antibodies to TuMV were purchased from AC Diagnostic Inc. (USA) and used at the working concentration of 1:2000. Alkaline phosphatase (AP)-labelled IgG was purchased from Beijing Biosynthesis Biotechnology Co. (China) and used at the working concentration of 1:10000. The collected samples were tested for TuMV by I-ELISA according to the method described by Hornbeck (1991).

Serological detection of other quarantine plant viruses (cymbidium ringspot virus, tobacco mosaic virus, tomato ringspot virus, odontoglossum ringspot virus, cymbidium mosaic virus, bean yellow mosaic virus, cucumber mosaic virus and orchid fleck dichorhavirus) was also performed.

Virion purification. TuMV particles were purified from asymptomatic or symptom-showing leaves (Thompson et al., 1988), were negatively stained with phosphotungstic acid (PTA) for 5 min and examined under a JEM2100 transmission electron microscope (JEOL, Japan).

RNA extraction and RT-PCR. Total plant RNAs were extracted from TuMV-infected leaves with the Total RNAExtractor (Sangon Biotech, China) following the instructions of the manufacturer. cDNA was synthesized with the M-MuLV First Strand cDNA Synthesis Kit (Sangon Biotech, China).

Degenerate primers were designed based on the reported coat protein (CP) gene (864 bp) of TuMV (CP-F 5′-GCAGGYGARACRCTYGAYG-3′ and CP-R 5′-TAACCCCTTAACCGCAAGTA-3′). PCR was performed with 2.5 μl 10× PCR buffer, 1.0 μl dNTPs (10 mM each), 2.0 μl cDNA, 1.0 μl (200 nM) of each primer, 0.5 μl of FastPfu Fly DNA polymerase (TransGen Biotech, China) and 17 μl of double distilled water. Thermocycling conditions were 5 min at 94°C, 25 cycles of 30 s at 94°C, 20 s at 55°C, 60 s at 72°C, and a final extension at 72°C for 5 min. PCR
products were analyzed by electrophoresis in 1% (w/v) agarose gels and stained by GoldView.

The degenerate primers used for genome amplification were designed by Oligo7 using conserved regions of the genomic sequence of TuMV (Table 1). The 5' and 3' terminal sequences were determined according to the sequence most related to the TuMV-ZH1 isolate. The schematic genome amplification strategy is shown in Fig. 2.

Cloning and sequencing. The expected fragments were excised from the gel, cleaned by SanPrep Column DNA Gel Extraction Kit (Sangon Biotech, China) and ligated to pUCm-T vector. After the transformation of Escherichia coli DH5α, plasmid insertion was confirmed by PCR and restriction enzyme digestion with PstI and BamHI. Recombinant clones were sequenced at Sangon Biotech (China).

Sequence and phylogenetic analysis. The TuMV CP sequence was matched against the international gene sequence databases by BLAST. The sequences were assembled with DNAstar (DNASTAR, USA) and DNAman (Lynnon Co., Canada) for further analyses including genetic variability. The genome sequence was aligned with those of other TuMV isolates retrieved from GenBank (Table 2) with Clustal W in DNAStar. Phylogenetic analyses were performed using neighbor-joining (NJ) method in MEGA5 (Tamura et al., 2011). The statistical significance of branches was obtained by applying bootstrap analysis with 1000 replicates. The genome sequence of Japanese yam mosaic virus (JYMV) (GenBank accession No. NC_000947) was used as outgroup (Ohshima et al., 2002).

RESULTS AND DISCUSSION

A TuMV isolate (ZH1) from a symptomless Phalaenopsis sp. plant was characterized in this study. The presence of TuMV was detected by ELISA in a symptomless. Phalaenopsis sp. sample. Of the 403 orchid samples tested, Phalaenopsis was the only one that reacted for TuMV by RT-PCR. As shown by electron micrographs, purified virions from the infected Phalaenopsis were filamentous, 15-18×700-800 nm in size (Fig. 1). Other viruses detected by ELISA in the orchid samples tested were odontoglossum ringspot virus (25%) and cymbidium mosaic virus (14%).

Sequence comparison revealed that the expected 864 bp CP fragment had a 99% identity at the nucleotide level with the homologous sequence of isolate BJ-B03 (KC119187). The complete genomic sequence of TuMV-ZH1 obtained from assembled fragments (Fig. 2) was deposited in GenBank under accession No. KF246570. Its genome was 9,833 nt in length excluding the poly(A) tail. Compared with other isolates, TuMV-ZH1 shared 96.7% and 98.2% sequence identity with isolate Lu2 at the nucleotide and amino acid levels, respectively; 96.3% and 98.0% with isolate CHN 12, and 95.8% and 97.5% with isolate UK1. The nucleotide sequence identity of TuMV-ZH1 with other isolates from world-B group was more than 95%, but less than 87% with other typical isolates from the remaining three groups. Phylogenetic trees showed that TuMV isolates ZH1, CHN 12, Lu2, UK1 and C42J grouped together (Fig. 3). A comparative amino acid sequence analysis of TuMV-ZH1 and isolates CHN 12 and Lu2 showed two highly variable regions (13-266 and 925-1171 aa) with 34 mutation sites accounting for 58.6% of total mutations (58 sites) between isolates ZH1 and CHN 12, and 40 mutation sites accounting for 63.5% of total mutations (63 sites) between isolates ZH1 and Lu2 (Fig. 4). The two highly variable regions are part of protein P1 (1-362 aa) and P3 (820-1175 aa).

TuMV-ZH1 was closely genetically related to other isolates from China, suggesting it may have originated in this country. Proteins P1 and P3 are the most variable among TuMV-ZH1-encoded proteins. TuMV P3 is an important factor determining host range and symptomatology (Jenner et al., 2003; Suehiro et al., 2004). Also, protein P1 of potyviruses modulates replication and host defense (Pasin et al., 2014), and for Plum pox virus (PPV), a species in the genus Potyvirus, variations in protein P1 are associated with host-dependent pathogenicity (Maligoka et al., 2012). Based on these findings, it is predicted that proteins
P1 and P3 of TuMV-ZH1 could be involved in sustaining TuMV infection in a symptomless host. More work is needed to identify the amino acid residues involved in the interaction of TuMV-ZH1 and *Phalaenopsis* sp.

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