

## First report of *Tuberose mild mosaic potyvirus* from tuberose (*Polianthes tuberosa* L.) in India

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**Abstract.** A potyvirus was detected molecularly by reverse-transcription polymerase chain reaction (RT-PCR) and serologically by Ouchterlony's double diffusion test from a tuberose (*Polianthes tuberosa* L.) plant with mild mosaic disease. Potyvirus group-degenerate primers specific to the core region of coat protein gene were used in RT-PCR and the subsequent amplicon was sequenced. The sequence shared highest 98% and 99% identities at nucleotide and amino acid level, respectively and had a close phylogenetic relationship with the sequence of *Tuberose mild mosaic potyvirus* (TuMMV). This is the first report of TuMMV being present in India.

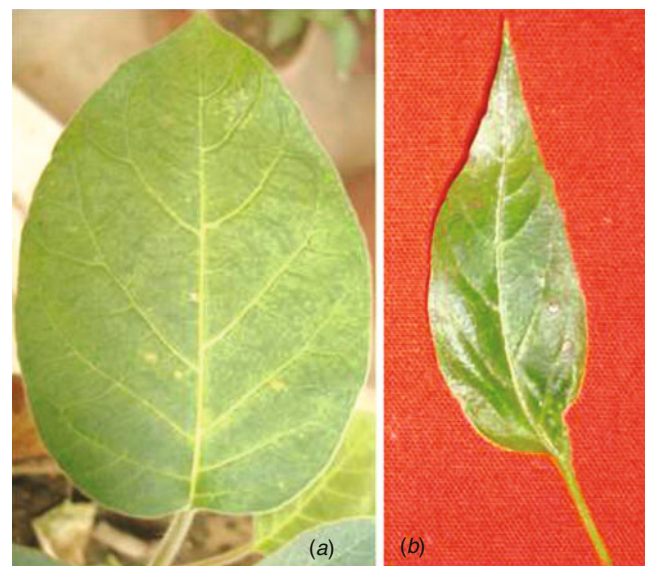
Tuberose (*Polianthes tuberosa* L.) of family Agavaceae is a bulbous ornamental plant, native of Mexico. It is grown commercially for cut flowers and occupies a prime position in the floriculture industry of India. Two cultivars of *P. tuberosa* namely, Rajat Rekha and Svarna Rekha, were bred and maintained at NBRI, Lucknow (Sharga 1999). During the survey in May–June 2007, a mild mosaic disease was observed on several commercial plants of cultivar Rajat Rekha at the botanic gardens of NBRI, Lucknow, India. The observed symptoms of the disease were green stripes and a mild mosaic on leaves (Fig. 1a, b) as compared to healthy one (Fig. 1c, d). Small sized floral spikes typically develop in affected plants as compared to healthy ones. Based on the reports of potyviruses on tuberose in India and abroad (Pearson and Horner 1986; Chen

and Chang 1998; Kulshrestha *et al.* 2005; Krishnareddy *et al.* 2007) and mild mosaic symptoms similar with those of potyvirus (Kulshrestha *et al.* 2005; Krishnareddy *et al.* 2007), the infection of a potyvirus was suspected.

Mechanical inoculations, using sap of naturally infected tuberose, induced necrotic local lesions on *Chenopodium amaranticolor*, *Datura metal* (Fig. 2a) and *Capsicum annum* (Fig. 2b) leaves, 10 days post-inoculation. However, neither local nor systemic symptoms developed on similarly inoculated plants of *Lycopersicon esculentum*, *Nicotiana glutinosa*, *N. rustica* and *N. tabacum* cv. White Burley even after 30 days post-inoculation.



**Fig. 1.** Leaf of infected tuberose showing green stripes and mild mosaic symptoms on (a) upper and (b) lower side, respectively as compared to healthy ones (c and d).

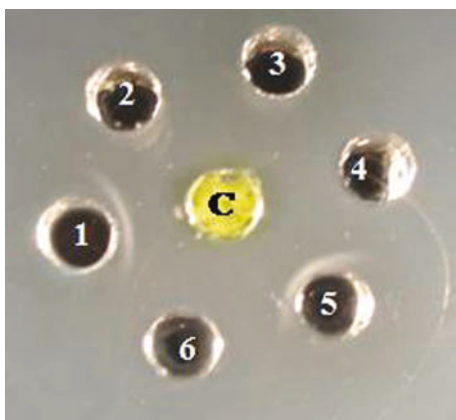


**Fig. 2.** Necrotic local lesions on (a) *Datura metal* and (b) *Capsicum annum* leaves after 10 days of inoculation of sap from naturally infected tuberose.

Mechanical inoculation on tuberose also resulted in the similar mild mosaic symptoms after 30 days.

The Ouchterlony's double diffusion test (ODDT) was as described by (Ouchterlony 1958). Briefly, crude sap from leaf tissue of naturally infected tuberose was prepared by homogenising 1 g of leaf tissue in 10 mL of sodium phosphate buffer (0.1 M, pH 7.0) and used as the test antigen in the ODDT. The crude sap was reacted against antiserum of three *Potyviridae* species, *Cardamom mosaic virus*, *Bean yellow mosaic virus* and an unknown potyvirus from *Narcissus tazetta* (Aminuddin *et al.* 1999) and two species from unrelated virus families, *Cucumber mosaic virus* (*Bromoviridae*) and *Carnation mottle virus* (*Tombusviridae*). The antigen only reacted positively with antiserum to *Cardamom mosaic virus* and the unknown potyvirus from *N. tazetta* as observed by precipitin lines in the media (Fig. 3). Based on results of the ODDT the presence of a potyvirus was suspected.

For molecular identification of the putative potyvirus, total RNA was extracted from infected leaf samples following the method described by Pawlowski *et al.* (1994). RT-PCR was performed as described earlier (Raj *et al.* 2009) using the degenerate forward primer 'oligo1n' encoding the MVWCIE motif and reverse primer 'oligo2n' corresponding to the reverse complimentary of the nucleotide sequence encoding the QMKAAA motif in the core of the coat protein gene of potyviruses (Marie-Jeanne *et al.* 2000). Complementary DNA (cDNA) synthesis was carried out in a 20  $\mu$ L reaction mixture containing 1.0  $\mu$ g of RNA template, 1 $\times$  reverse transcriptase buffer, 1.0 mm each dNTPs, 25 p.m. reverse primer, 25 U RNA guard (MBI Fermentas, USA) and 200 U of MMuLV Reverse transcriptase Revert Aid H-minus (MBI Fermentas, USA). The reaction was performed at 42°C as per manufacturers' instruction. PCR was carried in a 50  $\mu$ L reaction mixture containing: 50 ng cDNA template, 1 $\times$  *Pfu* DNA polymerase buffer, 200  $\mu$ M dNTPs, 25 p.m. each of forward and reverse primers and 3U *Pfu* DNA Polymerase (MBI Fermentas, USA) in a Peltier thermal cycler PTC200 engine (MJ Research, USA). The PCR condition were an initial template denaturation at 94°C for 5 min then 29 cycles consisting of denaturation at 94°C for 45 s, primer

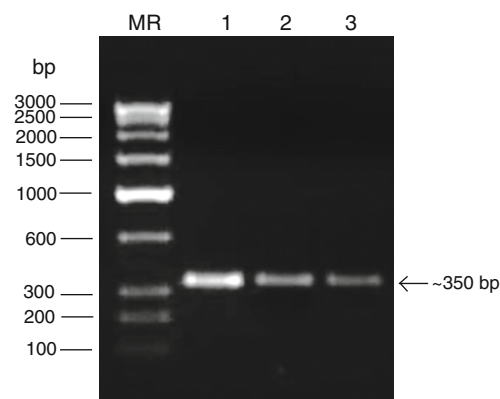


**Fig. 3.** Ouchterlony's double diffusion test showing positive reaction of tuberose isolate (central well) with *Cardamom mosaic virus* and *Narcissus tazetta* potyvirus (Aminuddin *et al.* 1999) (peripheral wells 2 & 5), but not with *Bean yellow mosaic virus*, *Cucumber mosaic virus* and *Carnation mottle virus*.

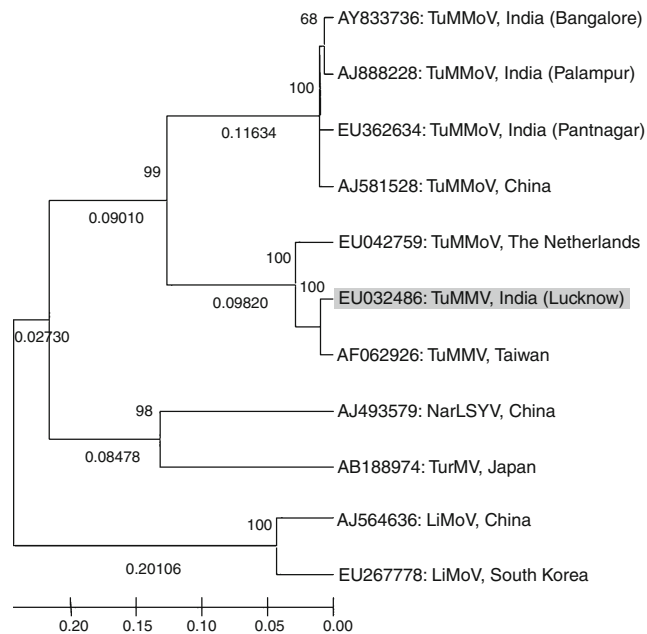
annealing at 50°C for 2 min and extension at 72°C for 50 s and a final extension at 72°C for 10 min. The amplified PCR products were electrophoresed with a known low range DNA ruler (Bangalore Genei Pvt. Ltd, India) using a 1.2% agarose gel to assess the size of amplicons. The electrophoresis of PCR products resulted in the expected size (*ca.* 350 bp) amplicons derived from leaf samples of diseased tuberose collected from three locations (Fig. 4), but not from two healthy plants.

Three amplicons were obtained, sequenced and the 328 bp consensus sequence deposited in GenBank under accession number EU032486. National Center for Biotechnology Information Basic local alignment search tool (NCBI/BLAST/blastn suite) analysis of the deposited nucleotide sequence revealed that it was 98% identical with *Tuberose mild mosaic virus* (AF062926 from China, Chen *et al.* 2002), 94% with *Tuberose mild mottle virus* (EU042759 from The Netherlands) and only 80–79% with *Tuberose mild mottle virus* (EU362634, AJ581528, Lin *et al.* 2004; AJ888228, Kulshrestha *et al.* 2005; AY833736, Krishnareddy *et al.* 2007). The putative amino acid sequence (108 aa) of virus isolate was also analysed by BLAST which revealed 99% identities with the polyprotein of *Tuberose mild mosaic virus* (AAL71892), 97% with *Tuberose mild mottle virus* (ABU41879) and 88% with other isolates of *Tuberose mild mottle virus* (CAE46329, AAX24131 and CAI59781). Based on highest 99% identities, the potyvirus under study have been considered as a strain of *Tuberose mild mosaic virus*.

Phylogenetic analysis of nucleotide sequence of the core of the coat protein of the virus isolate with the most related potyviruses or potyvirus-like sequences was performed using molecular evolutionary genetics analysis (MEGA) neighbour joining version 4.0 tool (Tamura *et al.* 2007). The analyses revealed the virus isolate was most closely related to *Tuberose mild mosaic virus* (Chen *et al.* 2002) of Taiwan and has distant relationship with *Tuberose mild mottle virus* isolates of Indian origin (Fig. 5). The *Tuberose mild mottle virus* of The Netherlands (EU042759) which showed 94% sequence identity also shared a close relationship with the virus isolate under study, may be misidentified and has been considered as a strain of *Tuberose mild mosaic virus* based on its highest 92% identities



**Fig. 4.** RT-PCR using degenerated primers conserved in the core region of CP gene of a potyvirus, showing band of *ca.* 350 bp in leaf samples of infected tuberose (lanes 1–3). MR = low range DNA ruler (Genei Pvt. Ltd, Bangalore, India).



**Fig. 5.** Phylogenetic analysis (using MEGA v. 4.0 program) of virus isolate under study (EU132486, highlighted by grey) showing close relationships with *Tuberose mild mosaic* (TuMMV) and distant relationship with *Tuberose mild mottle potyvirus* (TuMMoV) isolates, and *Narcissus late session yellow virus* (NarLSYV), *Turnip mosaic virus* (TurMV) and *Lily mottle virus* (LiMoV) of various countries.

with *Tuberose mild mosaic virus* (AF062926, reported from Taiwan by Chen *et al.* 2002) and low identities 74% with strains of *Tuberose mild mottle virus* (AY833736, AJ581528, AJ888228). Furthermore, the sequence (EU042759) is available onto GenBank as an unpublished record so there are no other details provided to verify its identity. Based on highest 98% and 99% sequence identity at nucleotide and amino acid respectively of core region of coat protein, and close phylogenetic relationships, the virus under study was identified as a strain of *Tuberose mild mosaic virus*.

This is the first report on the occurrence of *Tuberose mild mosaic virus* on tuberose in India. *Tuberose mild mottle virus* (TuMMoV) from India (Kulshrestha *et al.* 2005; Krishnareddy *et al.* 2007), New Zealand (Pearson and Horner 1986) and *Tuberose mild mosaic virus* (TuMMV) from Taiwan have been reported earlier (Chen and Chang 1998; Chen *et al.* 2002) and China (Lin *et al.* 2004). Spread of these viruses in planting material is a concern for the Indian floriculture industry as propagation and commercial cultivation of tuberose is through

bulbs and/or tissue culture. Screening of this propagation material for viruses would be useful to establish and maintain a virus-free tuberose industry in India.

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