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Occurrence of natural infection of tomato by *Potato Spindle Tuber Viroid* (PSTVd) in India

Shilpa Natarajamurthy[1,2] · Sumashri Kepu Shankaranarayana Bhat[1] · Janardhana Gottravalli Ramanayaka[1]

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Abstract
A field survey was carried out in districts of southern Karnataka to assess the pospiviroid infections on tomato crop. The tomato plants showing disease symptoms such as severe leaf curling, epinasty, chlorosis, purpling and stunted growth that are characteristics of viroid infection were collected along with asymptomatic plants and the infection was analysed by RT-PCR. A sample collected from the Mandya district showed an amplicon size of ~ 361 bp which confirmed PSTVd infection, while no other Pospiviroids were detected. The amplified PCR products were cloned, sequenced and the resultant sequences were deposited in GenBank. The PSTVd sample inoculated tomato plants also showed typical disease symptoms of viroid infection after four weeks of post-inoculation and was re-confirmed through RT-PCR. This is the first report of the occurrence of natural infection of tomato by PSTVd in India.

Keywords Bioassay · Pospiviroids · *Potato spindle tuber viroid* · RT –PCR

Potato spindle tuber viroid (PSTVd) is a non-coding infectious RNA molecule known to cause serious diseases in potato and tomato crops. Tomato (*Solanum lycopersicum* L.; Family-Solanaceae) is one of the most important vegetable crops grown for its edible berries. In India, the tomato is being cultivated as rain-fed and irrigated crop. Throughout 2017–18, 789,000 hectares of land was under tomato cultivation with an annual production of 19,759 Metric tonnes (Anonymous 2018). In the recent past, the crop yield has been seriously affected by both biotic and abiotic factors. During 2017 to 2019, a field survey was carried out to detect the presence of PSTVd in 37 tomato crop-growing fields each measuring 0.5–1 acre covering an area of approximately 25–30 acres. The areas include Mysore, Mandya, Chamarajanagara, Hassan and Bangalore Rural districts of Karnataka state (India) (Suppl. Table 1). All the symptomatic tomato plants (83) showing severe leaf curling, epinasty, chlorosis, purpling and stunted growth that are characteristics of viroid infection were collected along with two asymptomatic plants from each field for further analysis (Fig. 1).

The total RNA was extracted from the collected samples using 2X CTAB buffer, followed by 4 M Lithium chloride precipitation to enrich low molecular weight RNAs (Adkar-Purushothama et al. 2011). A two-step reverse transcription-polymerase chain reaction (RT-PCR) was performed to confirm the presence of viroid using universal primer pair Pospi1-FW/ Pospi1-RE (Verhoeven et al. 2004) and the asymptomatic tomato plants served as a negative control. Among the 83 screened samples, a sample from Banaghatta (12°31′37.7"N 76°40′05.5"E), Mandya district, sampled during 2019, showed an expected amplicon size of ~ 200 bp, while others did not. The amplified PCR product was sequenced directly and analyzed through BLASTn, which revealed the association of PSTVd. Further, a full-length viroid genome (~ 361 bp) was amplified using a specific set of primers 3H1/ 2H1 for PSTVd (Shamloul et al. 1997), purified, cloned into pGEM®-T Easy vector (Promega, Madison, USA) and sequenced. The pathogenicity of the isolated viroid sample was tested on six 15-days old tomato seedlings (cv. Rutgers) at the two-leaf stage. Briefly, two leaves of each seedling were dusted with carborundum and 20 µl of

✉ Janardhana Gottravalli Ramanayaka
grjbelur@gmail.com

[1] Molecular Phytodiagnostic Laboratory, Department of Studies in Botany, University of Mysore, Manasagangotri, Mysuru, Karnataka 570 006, India

[2] Department of Studies in Microbiology, University of Mysore, Manasagangotri, Mysuru 570 006, Karnataka, India

Fig. 1 Asymptomatic (**A**) and PSTVd infected (**B**) tomato plant showing stunted growth, leaf curling, epinasty and purpling on leaves

viroid RNA prepared in 0.5 M phosphate buffer (pH-7.0) was gently rubbed onto the upper surface of the leaves and rinsed gently with distilled water. The inoculated seedlings were maintained in a greenhouse (25 ± 2 °C) along with three mock-inoculated (control) plants (Sano et al. 2004). The experiment was repeated twice. Four weeks after post-inoculation, typical symptoms of PSTVd such as leaf curling, chlorosis and stunted growth were observed only in sap inoculated seedlings. Further, PSTVd infection in the symptomatic plants were re-confimred by RT-PCR

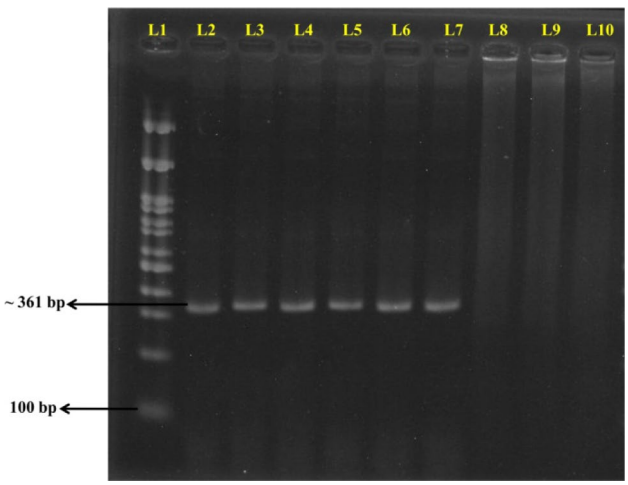

Fig. 2 A 2% Agarose gel showing amplification for the PSTVd viroids in bioassay. **L1**-100 bp ladder, **L2-L7** represent amplification of six challenge inoculated plants showing expected amplicon size (~ 361 bp) for PSTVd and **L8-L10** represent mock inoculated control plants and the presence of PSTVd was confirmed by RT-PCR

(Fig. 2) and sequenced. The two representative full-length sequences i.e. one from cloned product and other from bioassayed plant were deposited in NCBI GenBank with Accession No. MW114500 and MW114501, respectively. The PSTVd infection in tomato have been reported from many countries, including Australia (Hailstones et al. 2003), Belgium (Verhoeven et al. 2007), Ghana and Mali (Batuman et al. 2019), Italy (Navarro et al. 2009), New Zealand (Elliott et al. 2001), Turkey (Bostan et al. 2010), UK (Mumford et al. 2004) and the USA (Ling and Sfetu 2010). The nucleotide sequence analysis showed 97.10% similarity with PSTVd infecting potato in India (Accession No. MH758760), 96.63% similarity with PSTVd infecting potato in Russia (Accession No. JQ889847), 96.36% identity with China (Accession No. KR611357) along with 95.79% similarity with Indian isolate (Accession No. HQ639700). In India PSTVd is under strict quarantine and phytosanitary regulations of the Ministry of Agriculture and Farmers Welfare, Govt. of India, as it has been found to infect potato plants. Eventhough the inoculums exact source is unknown in the present study, it could have been infected from the other solanaceous crops including tomato seeds, as reported by earlier studies (Verhoeven et al. 2010). PSTVd infection has also been detected from the seeds of uncultivated Solanum species such as *Solanum anguivi*, *S. coagulans* and *S. dasyphyllum* collected from Ghana, Kenya and Uganda (Skelton et al. 2019). PSTVd infection could also be possible from the germplasm of the potato as reported by Roy et al. (2017) in India. Hence further investigation is needed to identify the source of viroid infection as it may threaten tomato and

other agriculturally important crops in India. To the best of our knowledge, this is the first report of natural infection of PSTVd in tomato crop in India.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s13314-021-00432-0.

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