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A new strain of Papaya ring spot virus infecting ivy gourd (*Coccinia grandis* L.) in India

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Abstract

Ivy gourd (*Coccinia grandis* L.) plants showing virus like symptoms such as mosaic mottling and thickening of leaf lamina were collected from Tindivanam, Tamil Nadu. Through Reverse transcription polymerase chain reaction, infection of potyvirus was confirmed with the yield of desired amplicon (~1.0 kb) using universal primer pair. Sequence analysis of amplified product showed the infection of Papaya ring spot virus (PRSV). Further association of PRSV was confirmed with PRSV specific primer pair (GK PRSV F/R). The nucleotide sequence analysis showed identity of coat protein region shared only 84% with PRSV isolate from Thailand in BLAST analysis. In phylogenetic analysis, PRSV infecting ivy gourd formed a separate cluster from other PRSV isolates. This confirms the association of distinct strain of PRSV on Ivy gourd in India.

Keywords: Cucurbits virus; *Potyvirus*; RT-PCR; Vegetable virus; Papaya ring spot virus

Introduction

Coccinia grandis (L.) Voigt, commonly known as ivy gourd, belonging to the family Cucurbitaceae, is mainly grown for its edible fruits. It is widely cultivated in Southeast Asian countries for culinary purposes. Fruits are used in traditional medicine for the treatment of leprosy, fever, asthma, bronchitis and jaundice. Globally, cucurbitaceous crops are susceptible to many viral diseases including *Potyvirus*, *Begomovirus, Cucumovirus, Tobamovirus*, *Tospovirus*, *Polerovirus*, *Crinivirus*, *Tymovirus*, etc (Kumari et al. 2021; Nagendran et al. 2022; Bernal et al. 2000). Among these viruses, aphid borne potyviruses are causing major economic losses in cucurbit crops worldwide. It consists of ssRNA in messenger sense to a size of ~10 Kb encoding a single large polyprotein which is cleaved and processed proteolytically by the viruses encoded proteases to yield 8 proteins of various functions (Gonsalves et al. 2010). Virions are flexuous rods measuring 760-800 × 12 nm size. Among the potyviruses, Papaya ring spot virus (PRSV) is infecting specifically cucurbits and papaya there by causing serious threats for their cultivation. PRSV infection on cucurbits shows mosaic mottling on leaves; narrowing and shoe string of leaves; blistering and puckering of leaves; chlorotic spots on leaves and fruits; malformation of fruits, etc. In order to characterize the potyviruses, nucleic acid regions coding for nuclear inclusion bodies and coat protein are being widely used (Miglino et al. 2010; Naidu and Karthikeyan 2008; Saqib et al. 2000). In this study, we have characterized the novel strain of PRSV causing mosaic disease of ivy gourd. We have established the phylogenetic relationship with other PRSV strains.

Materials and Methods

Sample collection and RT-PCR assay: Ivy gourd sample showing mosaic mottling symptoms was collected from Tindivanam region of Tamil Nadu. Total RNA was extracted from a total of five symptomatic field samples along with an apparently healthy leaves using Tri Reagent (Sigma Aldrich, USA). Initially cDNA has been synthesized with the extracted total RNA using the RevertAid First Strand cDNA synthesis kit (Thermo Scientific, USA) according to the manufacturer's instructions. Then cDNA has been subjected to PCR assay using potyvirus degenerate primers (PNIbF1: 5'-GGBAAYAATAGTGGNCAACC-3' and PCPR1: 5'-GGGGAGGTGCCGTTC TCDATRCACCA-3') (Hsu et al., 2005). A fragment of ~1200 bp covering 3' end of the NIb gene and 5' end of the coat protein gene was amplified only from the symptomatic samples but not from the asymptomatic leaves. Amplified product from

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the representative sample was cloned in the pGEMT vector (Promega, USA) according to manufacturer's instruction and transformed in to the *E. coli* cells of DH5á strain. Two independent clones were sequenced in both orientations for the representative sample at Xcelris Labs, Pvt. Ltd, Ahmedabad, India. Further, RT-PCR was carried out with the PRSV specific primer pair (GK PRSV F – 5' GCAAT GATAGARTCATGGGG 3'; GK PRSV R – 5' AAGCGGTGGCGCAGCCACACT 3') (Nagendran et al. 2017) and the resultant product was cloned and sequenced.

Sequence analysis: Sequences were assembled using CLUSTALW2 and phylogenetic trees were drawn using the MEGA 7.0 constructed with the Maximum-Likelihood algorithm, bootstrapped with 1000 replicates (Kumar et al. 2016). Sequence identity percentage at the nucleotide and amino acid levels were calculated using the BioEdit tool (Version 7.2) (Hall 1999).

Results and Discussion

Ivy gourd showing symptoms of mosaic mottling with brittleness of leaf lamina were collected from Tindivanam of Tamil Nadu (isolate named as TN TDV Coc2) along with healthy leaves. RT-PCR analysis has been carried out with the RNA extracted from the symptomatic samples along with the apparently healthy samples. A fragment of ~1000 bp covering 3' end of the NIb gene and 5' end of the coat protein gene was amplified only from the symptomatic samples but not from the asymptomatic leaves. Amplified product from the representative sample was cloned and sequenced. BLAST analysis of resultant sequence showed 77% nucleotide identity towards the *Papaya ringspot virus* (AY010722) from Thailand.

Further, specific primer pair (GK PRSV F/R) designed has amplified an amplicon of 1255 bp in infected ivy gourd sample covering 303 nt of NIb, 855 nt of CP and 97 nt of 3'UTR. The whole sequences shared a maximum nucleotide identity of 84 % with PRSV-W (AY010722) from Thailand in BLAST analysis. The sequences were submitted in the NCBI database (KM279931). Then nucleotide sequences and their putative translated products were compared with the corresponding regions of other GenBank sequences of PRSV using BioEdit (Table 1).

The 1284 nt sequence obtained, shares maximum nucleotide identity of 82.7 % with PRSV [W] (AY010722), included 332 nt of 3' NIb (110 aa), 855 nt of complete CP coding region (285 aa) and 97 nt of the 3' UTR region. The transduced amino acid similarity of core CP is 86.6% with PRSV P (AF063220). The putative coat protein contained the MVWCIENGTSP, AFDF and QMKAAAL motif s at 133-143, 216-219 and 236-242 aa respectively from the cleavage site Q/S of putative NIb and CP. These are conserved motif for the genus *Potyviridae* reported earlier by Miglino et al. (2010) and Shukla et al. (1994). The 3' UTR region shared a maximum identity of 89.6 % with PRSV (AB583217). Also, in the amino-acid sequence analysis, deletions of amino-acids *viz*., Serine (S) at 14th position and Glycine-Alanine-Serine (GAS) at 54 to 56th position and addition of one Histidine (H) at 278th position were

Table 1: Percentage identity of nucleotide (NT) and amino acid (AA) sequences of different regions encoded by PRSV (TN TDV Coc 2) with other selected PRSV isolates from different countries for the study

Accession No.	Virus	CP		NIb		3'UTR	Total NT	Total AA
		NT	AA	NT	AA			
DQ374152	*Papaya ringspot virus* [PRSV-W-C]	85.4	85.5	69.8	68.8	84.5	81.5	84.5
DQ374153	*Papaya ringspot virus* [PRSV-W-1]	84.5	84.2	69.8	69.7	83.5	80.8	84.1
AB583217	*Papaya ringspot virus* [JIK2]	86.3	86.5	69.8	66.9	89.6	82.4	84.5
AB583216	*Papaya ringspot virus* [JTK9]	86.3	86.8	71.0	70.6	86.5	82.5	85.3
JX448371	*Papaya ringspot virus* P [prT3-AX-M-Ph]	85.9	86.5	69.8	66.9	82.4	81.6	84.5
DQ340771	*Papaya ringspot virus* [Leaf-mottling]	85.9	86.5	69.8	66.9	82.4	81.6	84.5
KF791028	*Papaya ringspot virus* [PRSV-HNVb]	85.8	85.8	71.0	66.9	83.8	82.0	83.8
AB369277	*Papaya ringspot virus* [Korean watermelon]	85.5	86.9	70.4	66.0	85.5	81.8	85.1
JX448373	*Papaya ringspot virus* P i[pFT3-NP]	85.8	86.5	68.5	65.1	82.4	81.2	84.3
AY027810	*Papaya ringspot virus* W [CI]	85.3	85.3	69.8	66.9	86.5	81.5	84.4
DQ340769	*Papaya ringspot virus* [Leaf-deformation]	85.8	86.2	68.5	65.1	82.4	81.2	84.0
AY010722	*Papaya ringspot virus* [W]	86.4	86.2	71.3	70.6	87.6	82.7	85.1
KC345609	*Papaya ringspot virus* [E2]	86.2	86.5	71.0	70.6	85.5	82.4	85.8
AF063220	*Papaya ringspot virus* [P]	86.6	87.2	69.4	67.8	82.4	82.0	84.0
D00594	*Papaya ringspot virus* (strain W)	85.8	85.9	69.1	69.7	85.5	81.6	84.5
EF017707	*Papaya ringspot virus* P [DEL]	85.5	85.5	69.8	67.8	86.5	81.7	83.3
EU475877	*Papaya ringspot virus* W [India]	85.5	85.9	68.8	66.9	84.5	81.3	84.8
EF183499	*Papaya ringspot virus* P	86.5	86.2	69.4	69.7	85.5	82.2	84.0
AY162218	*Papaya ringspot virus* type P [Thailand]	85.5	85.5	71.6	68.8	84.5	82.0	83.0

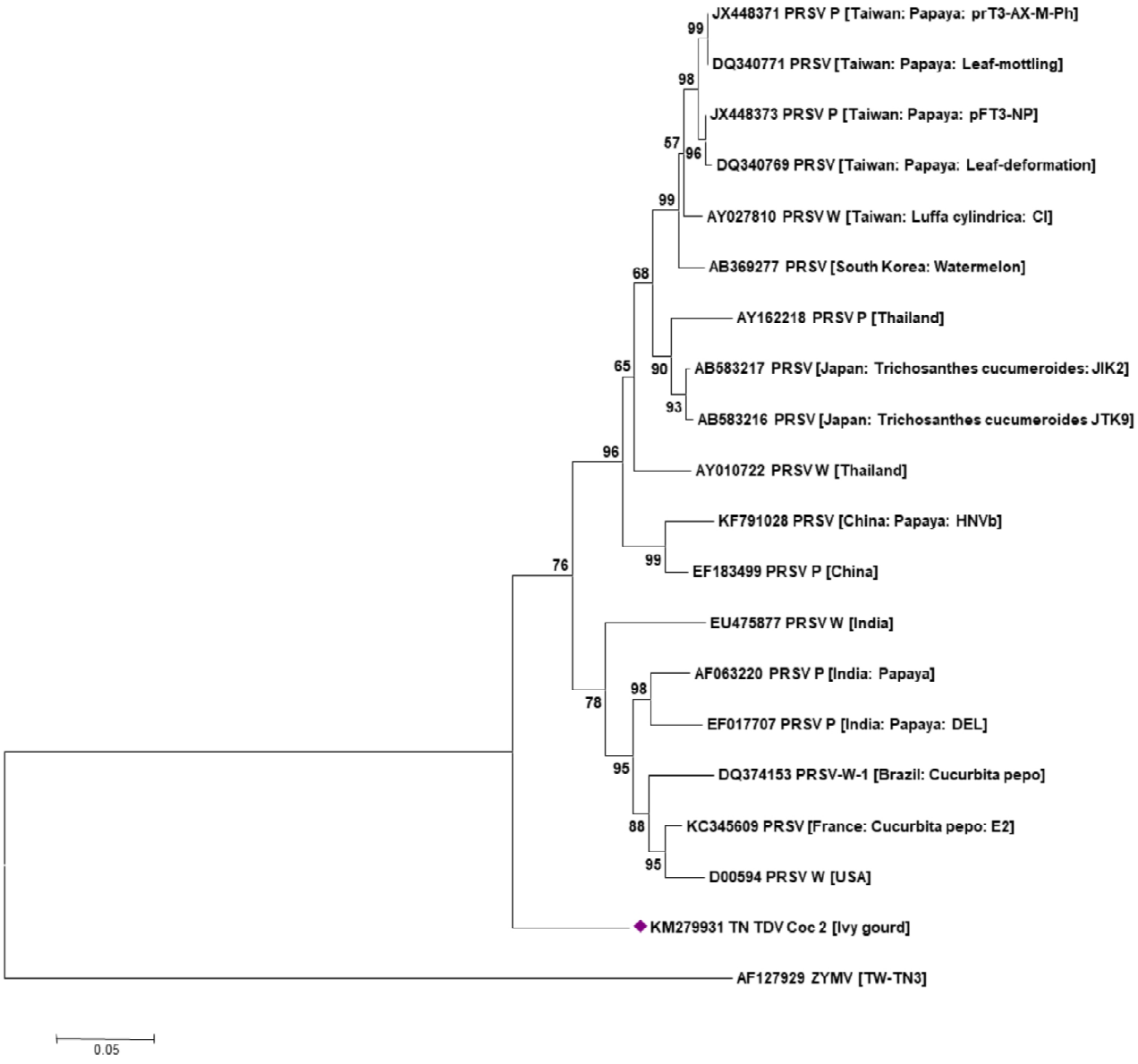

Figure 1: Phylogeny of complete nucleotide sequence of CP region of PRSV (TN TDV Coc2) with other PRSV isolates. The tree was generated using the Maximum-Likelihood method in MEGA 7.0. A bootstrap analysis with 1000 replicates was performed.

observed in PRSV isolate (TN TDV Coc2) infecting ivy gourd in Tamil Nadu in comparison with the PRSV [W] (AY010722). The phylogenetic relationships obtained from the coat protein region 855nt showed that this isolate is forming a separate cluster without grouping with other existing strains of PRSV (Figure 1). Hence this suggests that the study PRSV isolate might be a new strain of PRSV. Also, it has been proposed that amino acid sequence of coat protein region identity should range from 90-99% among the strains of the same virus (Hull 2002; Shukla et al. 1994; Ward et al. 1992). Further, Fauquet et al. (2005) and Adams et al. (2005) has proposed that threshold of minimum 80 % of CP gene nucleotide sequence identity is needed to define a new potyvirus species. This suggests that the virus infecting ivy gourd should be a distinct PRSV strain belongs to the genus *Potyvirus*.

According to Frankel et al. (1989), the identity of 3' UTR nucleotide sequence should share 83-99% among strains of the same potyvirus species. The 3' UTR region of 97 nt of this isolate (TN TDV Coc2) share 89.6% identity with the existing PRSV strians. Hence in the 3' UTR relationship also indicated that the virus isolates (TN TDV Coc2) associated with the mosaic mottling

and thickening of leaf lamina of ivy gourd is the species of PRSV. Recently Kumari et al. (2021) has also been documented the infection of PRSV on ivy gourd from Uttar Pradesh state.

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कुन्दरू (*काक्सिनिया ग्रान्डीस* एल.) के पौध में दिखने वाले विषाणु के लक्षण जैसे–मोजैक माटलिंग तथा पत्ती की पटल को टिन्डीवानम (तमिलनाडु) से एकत्रित किया गया। रिवर्स ट्रान्सक्रिप्टन पालीमरेज चेन रिएक्सन से पोटी वायरस के संक्रमण का स्पष्टीकरण हुआ जो यूनिवर्सल प्राईमर जोड़ी के प्रयोग से उपजे वांछित एप्तलीकान (1.0 के.बी.) को स्पष्ट किया। प्रवर्धित उत्पादन के सिक्वेन्स विश्लेषण से पपाया रिंग स्पाट वायरस (पी.आर.एस.वी.) के संक्रमण की पुष्टि हुई। पी.आर.एस.बी. के सम्बन्ध की पुष्टि पी.आर.एस.वी. विशिष्ट प्राईमर जोड़ी (जी.के.पी.आर.एस.वी.एस.आर.) से हुई। न्यूक्लियोटाइड सिक्वेन्स विश्लेषण ने कोट प्रोटीन क्षेत्र की एकात्मकता को स्पष्ट किया जिनमें 84 प्रतिशत का योगदान बी.एल.ए.एस.टी. विश्लेषण से थाइलैण्ड के पी.आर.एस.वी. विलग से प्राप्त हुआ। बहुविध विश्लेषण से कुन्दरू के पौध में लगने वाले पी.आर.एस.बी. संक्रमण एक अलग झुण्ड पाया गया जो पी.आर.एस.वी. विलग से बिल्कुल अलग था। भारतवर्ष में कुन्दरू में लगने वाले पी.आर.एस.वी. के पृथक स्ट्रेन के सम्बन्ध की पुष्टि करता है।

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