Diseases Caused by Viruses

First Report of Papaya ring spot virus (PRSV) Infecting Jute (*Corchorus olitorius*) in India

C. Biswas, P. Dey, V. Ramesh Babu,[†] N. M. Alam, and Gouranga Kar

ICAR-Central Research Institute for Jute and Allied Fibres, Barrackpore, Kolkata, West Bengal, India

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Jute is the most important bast fiber crop in the world, and is mainly cultivated in India, Nepal, Bangladesh, China, Indonesia, and South American countries. The fiber is utilized for making items such as apparel, ropes, bags, and carpets (Biswas et al. 2014). This bio-fiber is gaining importance due to growing environmental consciousness worldwide. In June 2019, we noticed jute plants (less than 2%) showing virus-like symptoms viz., downward curling, puckering, and angular brownish to yellowish spots in a farmer's field at Amdanga Block, North 24 Paraganas, West Bengal, India. To identify the virus, five symptomatic leaves from five different plants were used for high throughput sequencing (HTS). We extracted total RNA from each leaf, which was subjected to construction of cDNA libraries. Sequencing was done on an Illumina HiSeq 4000 (CytoScan, Thermo Fisher Scientific, Waltham, MA). Approximately 46 million 105-nt paired end reads were generated. Raw reads were trimmed and filtered to perform de novo assembly as described previously by Grabherr et al. (2011). The obtained contig was 10,326 nt long and showed highest identity in GenBank BLASTn with papaya ring spot virus (PRSV), with the contig covering 99.6% of the viral genome. The obtained contig shared 99.33% sequence similarity with PRSV strain P (accession no. MT470188). The selected leaf samples were also tested by double-antibody sandwich (DAS)-enzyme linked immunosorbent assay (ELISA) for PRSV along with some common viruses such as Potato leaf roll

virus (PLRV), Watermelon mosaic virus, Cowpea mosaic virus, and Cucumber mosaic virus with the help of commercial diagnostic kits (Agdia, Elkhart, IN). However, only the test with PRSV gave a positive reaction for the symptomatic samples. The major symptoms of PRSV on papaya are severe mosaic, chlorosis, and reduced lamina with curling and puckering (Gonsalves et al. 2010). To confirm PRSV infection, five symptomatic leaf samples (used for HTS) were collected and whole RNA was extracted from the samples using the RNeasy plant minikit (Qiagen, Germantown, MD). Reverse transcription (RT)-PCR was conducted by using isolated RNA. One pair of PRSV-specific primer (PSRV1F: 5'-TTAAATCTGATTCGTC-3' PRSV 1R: 5'-GAAATTCACGCAAAGTCGA-3') was developed by using primer BLAST software and used in RT-PCR assays. Amplified fragments were cloned and sequenced and all fragments shared 98% sequence identity with PRSV. One of the amplicons was deposited in GenBank (accession no. MN615832). Crude sap was prepared by homogenizing PRSV-infected jute leaf tissues in 0.1 M sodium phosphate buffer and 2% carborundum dust was added as abrasive (Holkar et al. 2018). The sap was then gently rubbed on to the healthy papaya leaves for inoculation. Typical PRSV-like symptoms appeared in inoculated leaves 10 days postinoculation, which confirmed the presence of PRSV-P. PRSV was detected by RT-PCR as well as DAS-ELISA from all inoculated infected papaya leaf tissues, but could not be detected from uninoculated healthy papaya tissues. To the best of our knowledge, this is the first report of PRSV-P infecting jute in India.

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[†]Indicates the corresponding author.
V. Ramesh Babu; veegalaramesh@gmail.com