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First Report of *Banana mild mosaic virus* Infecting Banana in India

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ABSTRACT

Banana is one of the important fruit crops grown in the tropical and subtropical regions in developing countries. Viruses are important constraints to the movement and propagation of plant germplasm, especially for vegetatively propagated crops such as banana and plantain. Four major viruses, *Banana bunchy top virus* (BBTV), *Banana streak viruses*(BSVs), *Banana bract mosaic virus* (BBrMV), and *Cucumber mosaic virus* (CMV) are known to affect the production in banana and plantain. Viruses of minor importance such as *Abaca mosaic virus*, *Abaca bunchy top virus*, *Banana mild mosaic virus*, and *Banana virus X* are also reported to occur in banana. We have tested 347 leaf samples showing mild chlorotic streak symptoms during 2013 to 2015 collected from Tamil Nadu, Kerala, Goa, and the research fields of ICAR-National Research Centre for Banana, Tiruchirapalli, India, and disease incidence was estimated to be 0 to 33%. Based on the nature of the symptoms observed (Gambley and Thomas 2001), infection by *Banana mild mosaic virus* (BanMMV, family *Flexiviridae*) was suspected. Electron microscopy of symptomatic tissue extracts revealed the presence of filamentous virus like particles approximately 800 nm long. Negative results were obtained using potyvirus group degenerate PCR primers and potyviruses specific antibodies. RT-PCR using total RNA was isolated from leaf tissue by the RNeasy Plant Mini Kit (Qiagen). Primer BanCP1 (5′-GGATCCCGGGTTTTTTTTTTTTTTT-3′), which is an oligo (dT) with additional cloning sites at its 5′ end, was used for the reverse transcription step (Teycheney et al. 2005). Virus specific primers, BanMMVCPFP: 5′-ATGGCAACDGGGGAAAAGAAGG-3′ and BanMMVCPRP: 5′-TTAATTATTCAATTTGAGGCTCA-3′ were used for PCR amplification. The coat protein (CP) gene of the BanMMV isolate consisted of 717 nucleotides. The amplicon was cloned in pTZ57R/T vector (Fermentas, USA), sequenced, and deposited in GenBank (Accession Nos. KT780866 and KU378053). The CP gene of the Indian BanMMV isolates were compared with the CP of the published isolates. Sequence of the Indian isolates shared 74.4 to 91.7% nucleotides and 87.3 to 95.7% deduced amino acid similarities with other BanMMV isolates. To further confirm the identification, nucleic acid spot hybridization (NASH) was done using digoxigenin-labeled DNA probe for BanMMV and partial cp gene fragment of 250 bp was generated using PCR-DIG probe synthesis kit as per manufacturer's instructions. In NASH, RNA extract was spotted and tested from 26 leaf samples exhibiting mild chlorotic streaks. Symptoms which were positive in PCR gave strong signals of hybridization, confirming the presence of BanMMV. To the best of our knowledge, this is the first molecular evidence for the occurrence of BanMMV in banana in India and the genetic variability appears to be high between the Indian isolates and others. This report is the primary step to initiate research on the impact of the virus in banana production and germplasm exchange.

References:

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