First Report of Cucurbit Chlorotic Yellows Virus Infecting Pumpkin in India

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Pumpkin (*Cucurbita moschata*), a member of the family Cucurbitaceae, is widely cultivated throughout the world including India. During August 2020 to January 2021, stunted pumpkin plants (cv. Pusa Vishwas) showing chlorotic patches, mosaic, and vein banding on leaves were observed in the experimental fields of the Indian Agricultural Research Institute (IARI), New Delhi, India. Leaf-dip electron microscopy of the symptomatic plants (12 out of 37 samples) revealed the association of long flexuous virus particles measuring 650 to 950 × 10 to 12 nm, which was suggestive of the presence of either crinivirus or potyvirus or both. Subsequently, a reverse transcription (RT)-PCR was performed on RNA extracted from the samples that had long flexuous virus particles using generic primers for criniviruses (i.e., CriniPol-F: GCYCCSAGRGTKAATGA and CriniPol-R: ACCTTGRGAYTTRTCAAA targeting partial RNA-dependent RNA polymerase coding region) (Martin et al. 2004) and specific primers for papaya ringspot virus (PRSV) targeting a part of 3' NIB and the full coat protein (CP) gene (Basavaraj et al. 2019), separately. All tested samples were positive for both crinivirus and PRSV as expected size amplicons were obtained, accounting for about 32% prevalence. As PRSV is a well-studied virus infecting cucurbits, further work was not carried on this virus, and only the RT-PCR amplicon indicative of crinivirus (~515 bp) was cloned into the pGEM-T Easy cloning vector (Promega, Madison, WI) and sequenced for further confirmation of the virus presence. The obtained sequence (GenBank accession no. MZ318672) shared up to 90%

nucleotide and 100% amino acid sequence identity with the corresponding genomic region of a cucurbit chlorotic yellows virus (CCYV) isolate from Greece (LT841297). To confirm the identity of the crinivirus species present in the same pumpkin sample, the CP gene (753 bp) was amplified and sequenced using CCYV CP gene-specific primers CP-F (5'-ATGGAGAAGA CYGACAATAAACAATAATGATGA-3') and CP-R (5'-TTATTTACTA CAACCTCCCGGTGCCAAC-3') (modified from Kheireddine et al. 2020). Sequence analysis using the BioEdit tool (version 2.0) revealed that the crinivirus present in pumpkin (KC577202) shared 95 to 100% nucleotide (and 98 to 100% amino acid) sequence identity with the corresponding gene sequences of CCYV isolates originating from cucurbitaceous hosts from diverse locations. The presence of CCYV was further validated by a whitefly transmission-based bioassay followed by RT-PCR confirmation. The bioassay was performed by the whitefly species *Bemisia tabaci* (biotype Asia II7) using an acquisition access period and inoculation access period of 24 h. Six whitefly individuals per plant were used for inoculating 10 pumpkin plants (cv. Pusa Vishwas) at the first true-leaf stage grown in pots containing Soilrite as the medium in insect-proof cages. All 10 plants inoculated using whiteflies exhibited chlorosis and stunting symptoms 12 to 15 days post-inoculation and were found positive for CCYV in an RT-PCR assay performed using CCYV CP gene-specific primers. Although CCYV had been reported worldwide (Tzanetakis et al. 2013), its occurrence had not been reported from India. Results of the present study confirm the infection of pumpkin plants by CCYV and constitute the first report of its presence in India. There is a further need to investigate the extent of its spread and impact of this virus on the production of cucurbitaceous crops in the country.

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