Disease Notes

Diseases Caused by Viruses

First Report of Natural Infection by Capsicum Chlorosis Virus on Amaryllis (*Hippeastrum hybridum*) Plants from India

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Capsicum chlorosis virus (CaCV) is a member of the genus *Orthotospovirus*, family *Tospoviridae* (Abudurexiti et al. 2019). In India, it was first identified on tomato (Kunkalikar et al. 2007) and thereafter on several other hosts belonging to different families (Basavaraj et al. 2017). Amaryllis or red trumpet lily (*Hippeastrum hybridum* Hort.; family Amaryllidaceae) is an ornamental plant being grown in the high ranges of the Western Ghats of India. In 2017 to 2018, chlorotic and necrotic ringspots rimmed by dark red margins, necrotic streaks, and sunken patches resembling the symptoms of tospovirus infection were observed on the leaves of amaryllis plants grown at the Indian Agricultural Research Institute, New Delhi, with an incidence of up to 36% (n = 18) out of 50 plants observed. In transmission electron microscopy, the symptomatic leaves revealed the presence of quasi-spherical virus-like particles measuring 80 to 120 nm in diameter. Direct antigen-coating enzyme-linked immunosorbent assay (ELISA) was performed using sap extracted from selected symptomatic (n = 11) and asymptomatic (n = 2) plants using the in-house raised polyclonal antibodies (pAb) to groundnut bud necrosis virus (GBNV) (Jain et al. 2005) and commercially available pAbs (Agdia, Elkhart, IN) to impatiens necrotic spot virus, iris yellow spot virus, and tomato spotted wilt virus. pAbs to the N protein of GBNV and watermelon silver mottle virus are known to react with GBNV, watermelon bud necrosis virus, and CaCV (Mandal et al. 2012). Thus, four symptomatic samples showed mild positive reactions with the pAbs to GBNV (with A_{405} 0.86 to 1.12), suggesting the association of a tospovirus antigenically related to the serogroup IV. Bioassay was done with the sap from ELISA-positive plants by mechanical transmission to amaryllis and cowpea (*Vigna unguiculata* cv. Pusa Komal) seedlings. Cowpea plants exhibited concentric chlorotic lesions followed by necrotic lesions at 5 to 7 days postinoculation, whereas the amaryllis plants exhibited symptoms as described above. To confirm the association of tospovirus species, total RNA was isolated from symptomatic leaves of amaryllis and cowpea plants using a PureLink RNA Mini Kit (Invitrogen, Carlsbad, CA). The isolated RNA (400 ng per 25-µl reaction) was subjected to cDNA synthesis using an IMPROM-II Reverse Transcription system (Promega, Madison, WI). The cDNA (2.0 µl per 25-µl reaction) was further subjected to RT-PCR with the generic primers of tospovirus (F, 5′-CCTTTAACAGTDGAAACAT-3′; R, 5′-CATDGCRCAAGARTGRTARACAGA-3′) (Chu et al. 2001) corresponding to a part of the L-RNA of tospoviruses (~800 bp) using GoTaq Flexi DNA polymerase (Promega). Amplicons of the expected size of ~880 bp were obtained from all four samples, cloned into the pGEM-T Easy Vector (Promega), and sequenced. The sequence (MT006242; 816 bp) showed up to 98 and 100% identities at nucleotide (nt) and deduced amino acid (aa) with the L-RNA sequence (KX108865) of the CaCV isolate of groundnut from Thailand. For further confirmation, the RT-PCR was performed using specific primers derived from the complete nucleocapsid (N) gene (828 bp) of CaCV (CaCV-BYB-F, 5′-ATGTCTAMCGTYAGG CAAC-3′; CaCV-BYB-R, 5′-TYACACYTCWATAGAWGTACTAG-3′), which resulted in the amplicons of ~800 bp from the ELISA-positive samples (n = 4). The gel-purified amplicons were cloned and sequenced. The sequence (MT018563; 828 bp) showed 97% (nt) and 99% (aa) identities with the N gene sequence of CaCV isolate (KX757228) from Iran. These results confirmed infection by CaCV on amaryllis plants. Infection of amaryllis plants by CaCV was previously reported in Taiwan (Chen et al. 2009). To the best of our knowledge, this is the first report of CaCV infection on amaryllis or red trumpet lily in India. However, CaCV is known to infect other hosts that belong to the families Amaranthaceae, Apocynaceae, Chenopodiaceae, Cucurbitaceae, Fabaceae, and Solanaceae in India (Basavaraj et al. 2017). This finding will help in understanding the expanded host range of CaCV.

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