New Disease Reports

First report of *Cucurbit aphid-borne yellows virus* infecting bitter gourd (*Momordica charantia*) and teasel gourd (*Momordica subangulata* subsp. *renigera*) in India

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Bitter gourd is one of the most important cucurbit vegetable crops grown throughout India. For the last two years a new disorder of yellowing has brought serious economic losses in bitter gourd production. Symptoms include interveinal chlorosis, green vein banding, wrinkling and thickening of younger leaves while older leaves show chlorotic patches or complete yellowing and downward rolling of leaves (Fig. 1). In teasel gourd, a perennial dioecious climber with a tuberous root, symptoms of chlorotic lesions followed by yellowing and thickening of older leaves were observed (Fig. 2). Surveys of viral symptoms were done in 2016 and the incidence of symptoms in bitter gourd varied from 5 to 65% in Andhra Pradesh, Chhattisgarh and Karnataka states and in teasel gourd varied from 1.5 to 14.5% in Karnataka and Orissa states. The symptoms in bitter gourd resembled those caused by viruses of the genera *Crinivirus* (Tomassoli *et al*., 2003) and *Polerovirus* (Lecoq *et al*., 1992).

During the survey 21 symptom-bearing samples from bitter gourd and 15 from teasel gourd were collected, washed with RNase-free sterile distilled water and leaf dip preparations were examined under an electron microscope This examination showed the presence of isometric particles in five bitter gourd and three teasel gourd samples. To identify the causal virus, generic crinivirus (Wintermantel *et al*., 2010) and novel polerovirus primers (POLF 5′-CTCAARGCCTACCATGARTATAARATC-3′/ POLR 5′-CGTCTACCTATTTNGGRTTNTG-3′) were used for RT-PCR amplification. Total RNA was isolated from diseased and healthy leaf samples using TRI Reagent (Sigma-Aldrich, USA). RT-PCR resulted in amplification of a single DNA fragment of approximately 350 bp, from 18 of 21 samples of bitter gourd, and 12 of the 15 teasel gourd samples, but not from healthy control samples. None of the samples produced an amplicon using the crinivirus primers. Cloned and sequenced RT-PCR products were subjected to a Blast search, which showed 89.5-95.0% nucleotide identity with poleroviruses and >96% nucleotide identity with *Cucurbit-aphid borne yellows virus* (CABYV) from Thailand (GenBank Accession No. KF815679) and Spain (JF939814). To confirm the identity of the virus total RNA isolated from the different samples was tested by RT-PCR using specific primers for the CABYV coat protein gene (Choi *et al*., 2015). A DNA fragment of 600 bp was amplified from the polerovirus-positive samples but not from the healthy control (Fig. 3). Cloning, sequencing and analysis of the coat protein gene (bitter gourd: MF281974; teasel gourd: KY711340) revealed that it had 95-97% nucleotide and 94-96% amino acid identity with different isolates of CABYV deposited in Genbank (Fig. 4). Based on electron microscopy and nucleotide sequence identity, the virus was identified as CABYV.

CABYV seems to be widespread throughout the world (Xiang *et al*., 2008; Knierim *et al*., 2014) but to our knowledge this is the first report of the occurrence of CABYV on bitter gourd in India and first report of its occurrence in teasel gourd in the world. The high frequency with which CABYV was detected in the samples indicates that the virus is an important and emerging virus infecting cucurbits in India.

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Figure 1

Figure 2

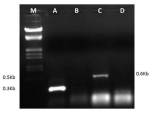

Figure 3

Figure 4

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