New Disease Reports

First report of *Ageratum enation virus* and Ageratum leaf curl betasatellite infecting *Calendula officinalis* in India

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Calendula officinalis (Asteraceae), commonly known as pot marigold, is grown as an annual ornamental plant in tropical countries including India. In January 2014, yellow vein net disease was observed on a number of *C. officinalis* plants (Fig. 1A) growing in a garden at Lucknow, India. The severely infected *C. officinalis* plants exhibited yellow vein net symptoms on leaves and leaf deformation (Fig. 1B) as compared to healthy plants (Fig. 1C). The severely infected plants remained stunted and bore smaller sized and fewer flowers. Based on the typical yellow net symptoms described earlier on *C. officinalis* plants caused by a begomovirus (Khan *et al*., 2005), begomovirus infection was suspected.

Total DNA isolated from fifteen infected samples and one healthy leaf sample was subjected to PCR amplification with begomovirus degenerate (Rojas *et al*., 1993) and betasatellite specific primers (Briddon *et al*., 2002). Following PCR amplification, products of the anticipated sizes of respectively ~1.2 and ~1.3 kb were successfully generated from all fifteen infected samples with no amplicon being generated from a healthy plant. These results suggest the association of a begomovirus and a betasatellite with the yellow net disease of *C. officinalis*. For molecular identification of the begomovirus, full length DNA genome was amplified from three independent DNA preparations of representative samples by the rolling circle amplification method using the RCA kit (GE healthcare, Buckinghamshire, UK) followed by restriction enzyme analysis. The resulting ~2.7 kb amplicons from three isolates (NBRI 1-3) were digested with *Bam*HI cloned, sequenced and the sequences were deposited in GenBank with Accession Nos. KM066975 (NBRI-1), KM262822 (NBRI-2) and KM262823 (NBRI-3). These three NBRI begomovirus isolates shared 99% sequence identity together and similar identities (98-99%) with various strains of *Ageratum enation virus* (AEV; JX436472, FN543099, FN794198, FN543099, JF682242 and JF728864). These isolates also shared a close phylogenetic relationship with well recognised strains of AEV (FN543099, HM149260 and AJ437618) as defined by the ICTV *Geminiviridae* study group (Brown *et al*., 2015) (Fig. 2). Based on these findings we propose that the begomovirus isolated from *C. officinalis* is an isolate of AEV. The three ~1.3 kb amplicons obtained by PCR using betasatellite specific primers from the same samples were also cloned and sequenced (KR922821, KR922822 and KR922823). Sequence analysis of associated betasatellites showed 99% sequence identity together and 92-96% identity and close phylogenetic relationships with Ageratum leaf curl betasatellite (ALCuB) (Fig. 3), and is nominated ALCuB.

To determine the infectivity of these new AEV and ALCuB isolates, infectious clones of both were generated using the pCAMBIA1300 vector backbone and agroinoculated in ten seedlings of *C. officinalis*. Combined agroinoculation of *C. officinalis* with AEV and ALCuB induced yellow vein net symptoms at 35 days post inoculation (Fig. 1D), which were similar to those observed in *C. officinalis* plants in the field, suggesting fulfilment of Koch's postulates. Reports of natural occurrence of *Cucumber mosaic virus* (Naqvi & Samed, 1985), an unidentified begomovirus (Khan *et al*., 2005) from India and *Bidens mottle virus* from Taiwan (Huang & Jan, 2011) on *C. officinalis* are known. However, this is the first report of AEV and ALCuB infecting *C. officinalis* in India.

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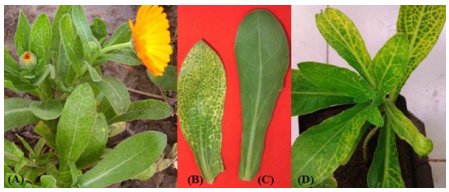

Figure 1

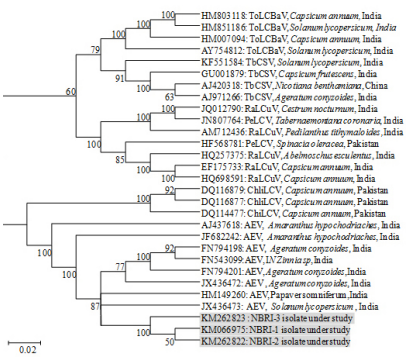

Figure 2

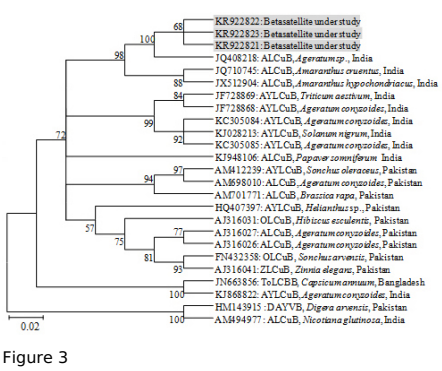

Figure 3

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