New Disease Reports

First report of *Radish leaf curl virus* infecting okra in India

J. Kumar [1]*, A. Kumar [2], S.P. Singh [1], J.K. Roy [1], A. Lalit [3], D. Parmar [3], N.C. Sharma [3] and R. Tuli [1]

[1] *National Agri-Food Biotechnology Institute, Mohali -160071, Punjab, India*; [2] *School of Biochemistry, Devi Ahilya Vishwavidyalaya, Indore, India*; [3] *Department of Biochemistry and Genetics, Barkatullah University, Bhopal, India*

*E-mail: jitsingh27281@gmail.com

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Okra (*Abelmoschus esculentus*) is one of the important vegetable crops of India cultivated in 452 hectares and yielding 4803 tonnes (Anonymous, 2010). In Bihar State, India, leaf curl disease on okra was observed in the field causing crop losses of about 30% in 2009 and 35% in 2010-11. Characteristic symptoms of this disease were leaf curling and overall stunting of plants that bore no fruit (Fig. 1A). To test for a begomovirus-infection, total DNA was extracted from symptom-bearing leaves of six infected plants (2 plants ´ 3 fields). A PCR approach was used to amplify viral genomes (primers F1For/Rev and F2For/Rev; Kumar *et al*., 2011) or alpha- and betasatellites (primers 'nanofor'/'nanorev' and 01/04; Kumar *et al*., 2010). All samples yielded PCR-products for a begomovirus and associated satellites, which were cloned and sequenced. The sequence deposited in GenBank for the monopartite begomovirus (Accession No. HQ257375) showed 97% and 92% nucleotide identity to *Radish leaf curl virus* (RaLCV) depositions GU732203 and EF175733, respectively. The alphasatellite (Accession No. HQ728354) possessed 98% and 96% nucleotide identity to Cotton leaf curl Burewala alphasatellite (CLCuBwA) sequences HM004548 and FN658728, respectively. The betasatellite (Accession No. HQ257376) exhibited 96% and 94% nucleotide identity to Tomato leaf curl Bangladesh betasatellite (ToLCBDB) sequences GU732208 and EF190215, respectively. Phylogenetic analysis of the begomovirus genome revealed a close relationship with RaLCV,GU732203 and EF175733 but a distant relationship with other okra infecting begomoviruses in India, AF241479 and FJ176236 (Fig. 2A). Phylogenetic analysis of the alphasatellite showed close relationship with CLCuBwA (Fig. 2B) and the betasatellite with ToLCBDB (Fig. 2C).

Rolling circle amplification (RCA) was performed (TempliPhi amplification kit; GE Healthcare, USA) to construct infectious clones. RCA products were partially digested with *BamHI* to obtain monomer and head-to-tail tandem repeat dimers of full-length begomoviral DNA. Monomers and dimers were cloned into the pCAMBIA1301 vector. Infectious head-to-tail tandem repeat clones of both satellites were prepared analogously. Sequencing of 30 begomoviral monomer clones confirmed the presence of identical viral DNAs. Healthy whiteflies (~25) were used for virus and satellite transmission from field-collected infected plants to healthy tobacco and okra. Infectivity testing was performed by inoculation of tobacco and okra (10 plants each) with a mixture of begomovirus and alpha- and betasatellite infectious clones. Plants of both assays yielded typical symptoms of leaf curling and stunting identical to those observed previously in the field. Thus RaLCV and its associated satellites were confirmed as the causal agent of okra leaf curl disease. However, the functional role of both satellites for symptom development remains to be determined. This is the first report providing the evidence for RaLCVinfecting okra in India.

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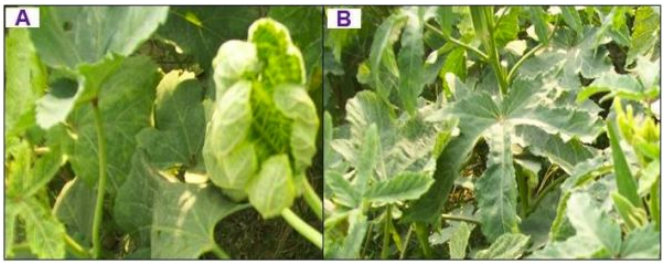

Figure 1

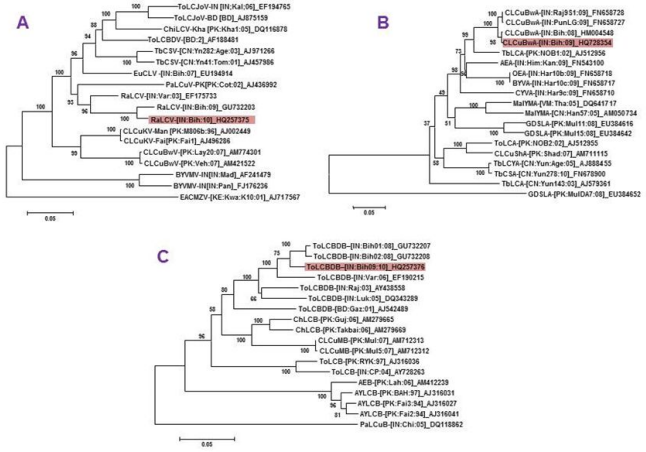

Figure 2

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