First Report of Association of a Begomovirus with the Leaf Curl Disease of a Common Weed, *Datura Innoxia*

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Begomovirus is one of the largest genus of the family Geminiviridae and the vector white fly (*Bemisia tabaci*) is prevalent in the tropical and subtropical regions of the world [4]. Weeds may infect crops and those weeds may serve as a reservoir for crop-infecting geminiviruses [1, 5]. In the present report we identified a begomovirus infecting *Datura innoxia*, a common hallucinogenic alkaloid containing toxic weed found in India annually.

D. innoxia showing typical begomovirus symptoms i.e., leaf curling, decrease in leaf size, and growth stunting as well as healthy leaves were collected from the fields of Rajasthan, India (Fig. 1). The DNA was extracted from infected leaves using CTAB method and PCR was carried out by using universal coat protein primer AC 1048 and AV 494 [2]. A typical PCR contained about 2.5 µl DNA template, 2.5 µl Taq 10× buffer (10 mM Tris–HCl, pH 8.8, 50 mM KCl and 1 % [v/v]), 1.5 µl MgCl₂, 2 µl of each dNTPs, 0.5 µl of Taq DNA Polymerase, and 1 µl of each primer. The PCR thermal profile were pre-PCR denaturation at 94 °C for 60 s followed by 35 cycles of denaturing at 94 °C for 60 s, annealing at 55 °C for 2 min and extension at 72 °C for 3 min, and a final extension at 72 °C for 5 min. The PCR product was partially sequenced (JN000702) and 550 nucleotides sequence was used to construct a phylogenetic tree using the neighbor-joining method in DNASIS Pro ver. 2.6. In order to confirm the geminivirus infectivity, Southern blotting was performed

according to the method of Kon et al. [3]. Cloned DNA-A (2.7 Kb) of Chilli leaf curl virus was used to develop the radio-labeled probes.

Sequence analysis of the virus under study showed 82–83 % nucleotide sequence identity with the corresponding region of Chilli leaf curl virus (FM210476), Tomato leaf curl virus (AJ810360), and Croton yellow vein virus (FN543112). In neighbor-joining tree, the coat protein gene of the virus in datura leaf curl formed a separate monophyletic cluster with a low bootstrap value (Fig. 2). To complement the information the amplified product was used for the Southern hybridization. The strong signal showed that the virus in *D. innoxia* had some homology with Chilli leaf curl virus. The result suggested that the virus in *D. innoxia* samples belonged to the genus begomovirus. To the best of our knowledge, this is the first report of begomovirus associated with leaf curl of *D. innoxia*.

Fig. 1 *Datura innoxia* showing leaf curl symptoms in Lakshmangarh, Rajasthan in 2011

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Fig. 2 Neighbor-Joining tree based on the partial sequence of coat protein gene (JN000702), of the virus isolate obtained from leaf curl disease of *Datura innoxia* (DaLC) and other begomovirus sequences available in GenBank. Cotton leaf curl Allahabad virus (CLCuAV) [India:Karnal:OY81B:2005] (GU112004), Bhendi yellow vein mosaic virus (BYVMV) [India:Bhemarayanagudi:OY45:2005] (GU112069), Bhendi yellow vein mosaic virus (BYVMV) [India:Karnal:OY79A:2005] (GU112076), Tomato leaf curl Sri Lanka virus

segment A (ToLCSLV) (AF274349), Chilli leaf curl virus partial cp gene (ChiLCuV) (FM210476), Tomato leaf curl virus AV1 gene (TLCV) (AJ810360), Cotton leaf curl Bangalore virus segment A (CLCuBV) (AY705380), Croton yellow vein virus (CYVV) (FN543112), Euphorbia leaf curl virus (EuLCV) (EU194914), Papaya leaf curl virus (PaLCuV) (Y15934) and Tomato leaf curl Patna virus (ToLCPaV) (EU862323). The tree was constructed using MEGA 4.0 software. Percent bootstrap values are indicated in the *node*

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