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# Yellow vein netting of Bimili jute (*Hibiscus cannabinus* L.) in India caused by a strain of *Tomato leaf curl New Delhi virus* containing DNA β

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**Abstract.** A strain of DNA β-containing *Tomato leaf curl New Delhi virus*, causing yellow vein net disease of Bimili jute (*Hibiscus cannabinus* L.) in India, was identified by dot blot hybridisation, PCR and sequence analysis.

Bimili jute (*Hibiscus cannabinus* L.), a member of the family Malvaceae, is one of the important fibre yielding plants. The plant has been under cultivation for many years and is widely distributed throughout India. The leaves and tender twigs of the plant are used for cattle fodder, the dried stem stalks as firewood and fibre for sacking and cordage ropes. Recently, a cultivar of *H. cannabinus* was introduced as an ornamental plant at the NBRI garden because of its beautiful blooms (Sharga 2004). A yellow vein net disease on a *H. cannabinus* cultivar with >20% incidence in the garden has been observed over four successive years (2003–06). Naturally infected plants showed yellow leaf vein netting symptoms accompanied by excessive yellowing and curling of leaves and stunting of the whole plant (Fig. 1). A population of whiteflies (*Bemisia tabaci*) was also observed in the growing area, therefore, an association of begomovirus with the disease was suspected. To determine the cause of this yellow vein netting symptom, total DNA was isolated from symptomatic and asymptomatic *H. cannabinus* leaf samples, dot blotted on nylon membranes and allowed to hybridise with the radiolabelled probes prepared from cloned DNA A of a well-characterised begomovirus isolate of *Indian tomato leaf curl virus* (Srivastava *et al*. 1995). The total DNA of infected samples hybridised well with the probe and developed strong signals but not with DNA of healthy plants, indicating the presence of a begomovirus.

To identify the begomovirus associated with the disease, PCR was performed using the total DNA of infected and healthy samples with a pair of primers specific to the coat protein (CP) gene of the genus *Begomovirus* (Singh 2005). The electrophoresis of PCR products on 1% agarose gel showed the expected size (~800 bp) amplicons in infected samples but no such amplicons were obtained in healthy samples. The amplicon was cloned and sequenced and the data obtained from three clones was submitted to GenBank (Accession number EF123060). BLAST search analysis of nucleotide sequence EF123060 revealed 96–98% sequence identity with various strains of *Tomato leaf curl New Delhi virus* (ToLCNDV) isolated from cotton (EF063145), *Luffa* (AY939926), tomato (U15016, AY691902), potato (DQ272541), bottle guard (DQ272540), *Solanum* (DQ116885) and chilli (DQ141676, DQ116880).

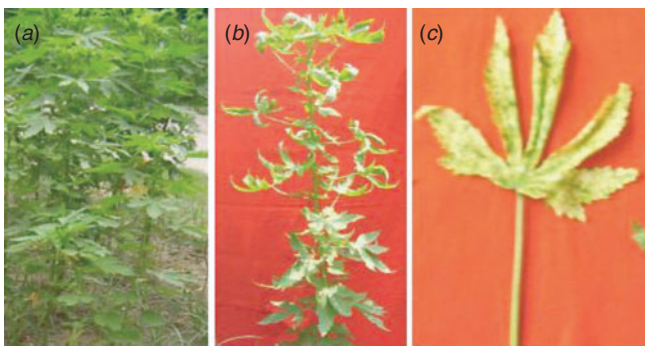


**Fig. 1.** (*a*) Healthy *Hibiscus cannabinus* plant, in comparison with a (*b*) naturally infected plant showing yellow vein net and leaf curl symptoms and (*c*) a close view of an infected leaf.

Pair-wise alignment and phylogenetic analyses of nucleotide (nt) and amino acid (aa) sequences of the CP gene of the virus isolate EF123060 was also completed to compare it with CP gene sequences of selected ToLCNDV isolates originating from diverse plant species, and other begomoviruses reported from malvacious host species. Pair-wise alignment of the virus isolate EF123060 revealed the 96–98% similarity at the nt level and 98% similarity at the aa level with various isolates of ToLCNDV (Table 1). The virus isolate did not reveal a close identity to *Cotton leaf curl Rajasthana virus* (NC_003199) used earlier for cross hybridisation (Chatterjee *et al*. 2005), nor with *Mesta yellow vein mosaic virus* (DQ298138) (Paul *et al*. 2006). Moreover, the phylogenetic analyses of both nt and aa sequences of the virus isolate showed a close relationship with strains of ToLCNDV (Fig. 2). Therefore, we identified the causal agent of yellow vein net symptoms of *H. cannabinus* as a strain of *Tomato leaf curl New Delhi virus.*


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**Table 1. Coat protein-based analysis of the virus isolate (EF123060) based on the Genomatix DiAlign programme**

ToLCNDV, *Tomato leaf curl New Delhi virus*; ToLCV, *Tomato leaf curl virus*; CLCuRV, *Cotton leaf curl Rajasthan virus*; BYVMV, *Bhindi yellow vein mosaic virus*; OYVMV, *Okra yellow vein mosaic virus*; MYVMV, *Mesta yellow vein mosaic virus*

| Accession number | Abbreviated virus name | Host plant | Location | % Nucleotide identity[A] | % Amino acid identity[A] |
|---|---|---|---|---|---|
| AY691902 | ToLCNDV | Tomato | Varanasi, India | 98 (0.985) | 98 (0.985) |
| EF063145 | ToLCNDV | Cotton | Hissar, India | 96 (0.959) | 98 (0.979) |
| U15016 | ToLCNDV | Tomato | New Delhi, India | 96 (0.911) | 98 (0.989) |
| DQ141676 | ToLCNDV | Chilli | Kanpur, India | 95 (0.918) | 98 (0.978) |
| DQ272541 | ToLCNDV | Potato | New Delhi, India | 95 (0.900) | 98 (0.975) |
| EF057795 | CLCuRV | Cotton | New Delhi, India | 75 (0.512) | 92 (0.609) |
| NC_003199 | CLCuRV | Cotton | Rajasthan, India | 77 (0.756) | 90 (0.899) |
| NC_003418 | BYVMV | Bhindi | Madurai, India | 76 (0.759) | 90 (0.902) |
| AJ002457 | OYVMV | Okra | Pakistan | 75 (0.750) | 90 (0.891) |
| DQ298138 | MYVMV | Mesta | Basirhat, India | 72 (0.646) | 78 (0.762) |
| AJ810357 | ToLCV | Malvestrum | Panipat, India | 72 (0.646) | 78 (0.761) |

[A]The % identity denotes number of identical sequences (in % of shorter sequence) and value given in brackets is the similarity (relative to maximum similarity).

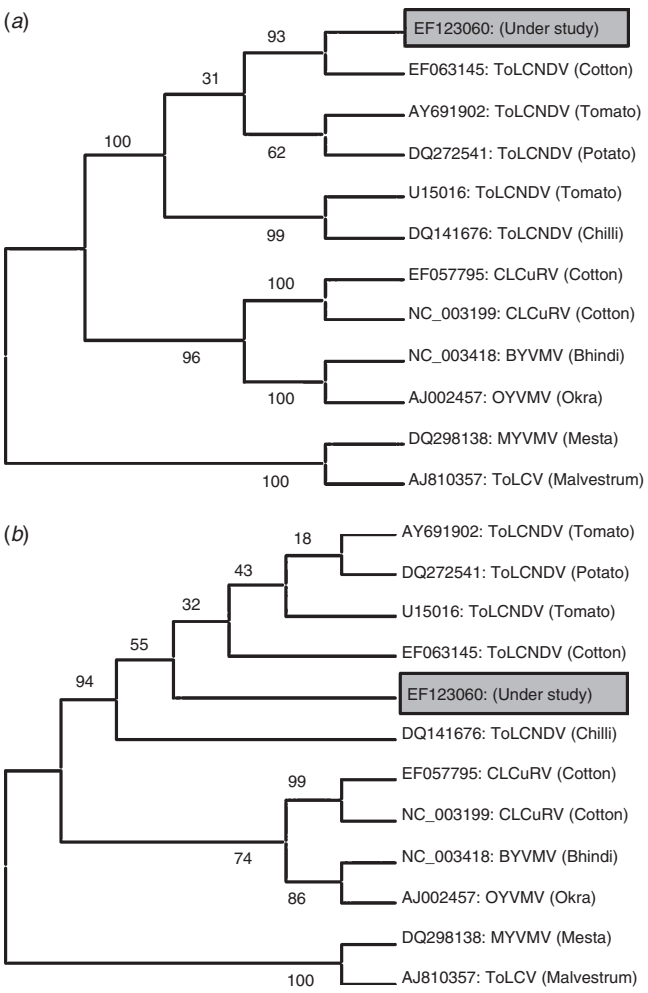


**Fig. 2.** Phylogenetic relationship of the virus isolate (EF123060) with other begomovirus strains reported from various host species at the (*a*) nucleotide and (*b*) amino acid level.

Recently, DNA β has been detected as being associated with ToLCNDV strains (Usharani *et al*. 2004; Reddy *et al*. 2005; Tahir and Haider 2005). A set of primers specific to the DNA β (Jose and Usha 2003) was therefore employed to test for its presence. Amplicons of the expected size, ~1350 bp, were successfully amplified from all of the infected samples. Furthermore, the amplicons cross-hybridised with the DNA β probe (DQ343289) under high stringency wash conditions. These findings confirm the association of the DNA β molecule with the ToLCNDV strain causing yellow vein net disease in *H. cannabinus*.

A literature survey revealed that *Hibiscus chlorotic ringspot carmovirus*, *Hibiscus yellow mosaic tobamovirus* and *Hibiscus latent ringspot nepovirus* have been reported on *Hibiscus* spp. (Brunt *et al*. 1996). Occurrence of a DNA β-containing begomovirus on *H. cannabinus* and *H. sabdariffa* has been recorded earlier (Chatterjee *et al*. 2005; Paul *et al*. 2006) based on PCR amplification and hybridisation with a *Cotton leaf curl Rajasthan virus* probe but without sequence analysis. Hence, molecular identification of a strain of *Tomato leaf curl New Delhi virus* containing DNA β as the cause of yellow vein net disease of *H. cannabinus* is a new report from India.

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