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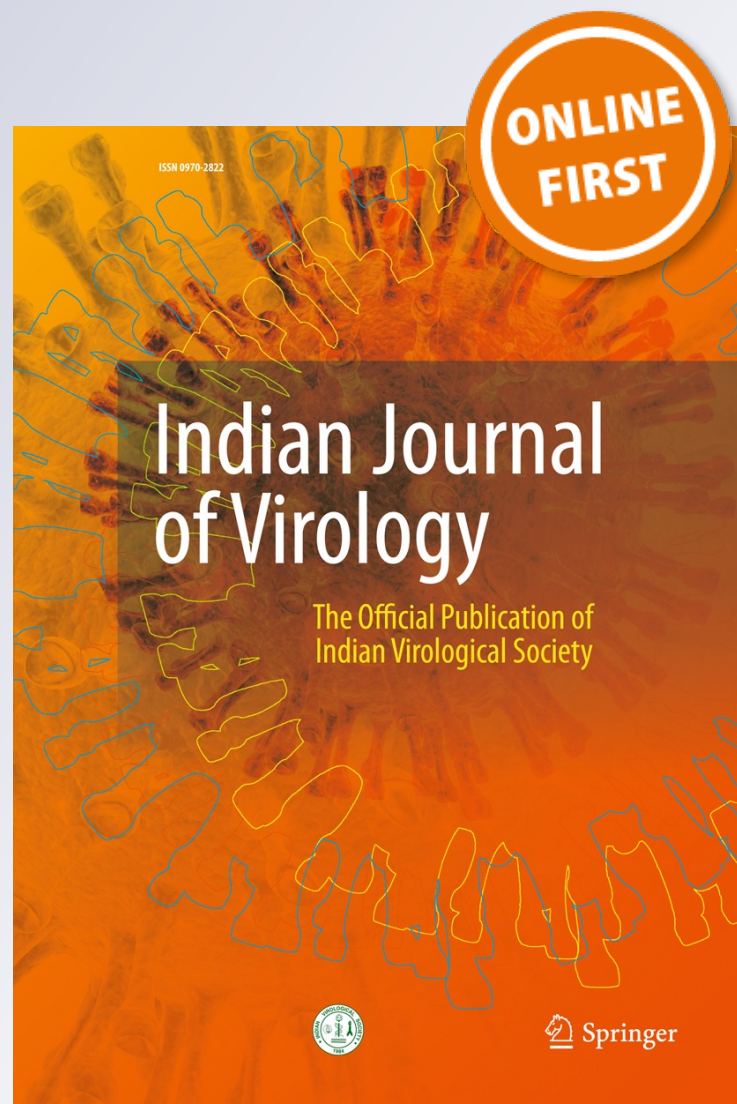
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Natural Occurrence of *Banana bunchy top virus* in *Ensete superbum* in India

R. Selvarajan · V. Balasubramanian

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The genus *Ensete* belongs to the family *Musaceae* of the order *Zingiberales* is grown throughout Africa and Southern Asia and contains as many as nine species. *Ensete superbum* (Roxb.) Cheesman, ($2n = 18$) commonly known as Ensete, is a large herb grown in India. The plant is well-known in the Western Ghats [2], Anaimalai hills, Sirumalai hills, Lower Pulney hills and other parts of the peninsular India. *Banana bunchy top virus* (BBTV; Genus *Babuvirus*; Family *Nanoviridae*) is a phytopathogenic virus associated with bunchy top disease of banana has become a major threat to banana and plantain cultivation in India and in several tropical regions of the world and cause significant yield losses [1]. The virus is phloem-limited and transmitted by the banana black aphid, *Pentalonia nigronervosa* (Coquerel) in a persistent circulative and non-replicative manner. The host range of BBTV is confined to *Musa* spp. In this paper we report natural occurrence of BBTV in *Ensete* in India. Samples of *Ensete superbum* plants exhibiting symptoms of intermittent dark green dots, dash, streaks of variable length like 'Morse code' pattern on leaf sheath, midrib, leaf veins and petioles and bunching of shorter and narrow leaves giving the appearance of bunchy top disease of banana were collected during December, 2011 from a garden in Coimbatore, TN (Fig. 1a, b). These *Ensete* plants also infested with banana black aphid. Aphids collected from symptomatic *Ensete* plants and healthy plants were transferred to healthy tissue culture raised Grand Nain banana plantlets for 48 h inoculation access. The inoculated plants were maintained in an

insect-proof growth chamber maintained at 25 ± 0.5 °C and 12 h light/dark photoperiod.

The presence of the virus in symptomatic leaves of *Ensete* was confirmed by direct antigen coating (DAC) ELISA using BBTV polyclonal antiserum and the healthy leaf of *Ensete* was negative. Total DNA from the symptomatic *Ensete* plants and aphids were extracted by the modified CTAB method [3]. PCR was performed using primer specific for replicase gene (Rep) (RSREFPF: 5'-TTGGATCCATGGCGCGATATGTGGTATGC-3' and RSREPRP: 5'-TTGGATCCATGGCGCGATATGTGGTATGC-3') and coat protein (CP) gene (RSCPFP: 5'-ATG GCTAGGTATCCGAAGAAATCC-3' and RSCP RP: 5'-TCAAACATGATATGTAATTCTGTTC-3') of BBTV [4] which resulted with expected amplicon of sizes 861 and 513 bp, respectively in both the symptomatic plant sample and aphid sample. The purified PCR product was cloned and sequenced. The CP and Rep gene sequences of isolate obtained in this study were deposited in NCBI GenBank (JX171699; JX197072). 16 out of 20 inoculated banana plantlets expressed typical BBTD symptoms such as choked plant top with a rosette of narrow, short leaves, short dark green dots and dashes on midrib and the leaf veins 25 days after inoculation, whereas aphids from healthy *Ensete* plants did not produce any symptom on inoculated banana. The CP gene sequence of *Ensete* bunchy top isolate shared 95–99 % sequence identity with the BBTV isolates of South Pacific group and 90–94 % sequence identity with the isolates of Asian group. The Rep gene sequence of *Ensete* bunchy top isolate showed 97–99 % sequence identity with the South Pacific group isolates and 92 % sequence similarity with the Asian group isolates. In phylogenetic analysis based on the amino acid sequence of the CP gene, the *Ensete* bunchy top isolate grouped with BBTV isolates of Shevaroy and Kolli hills

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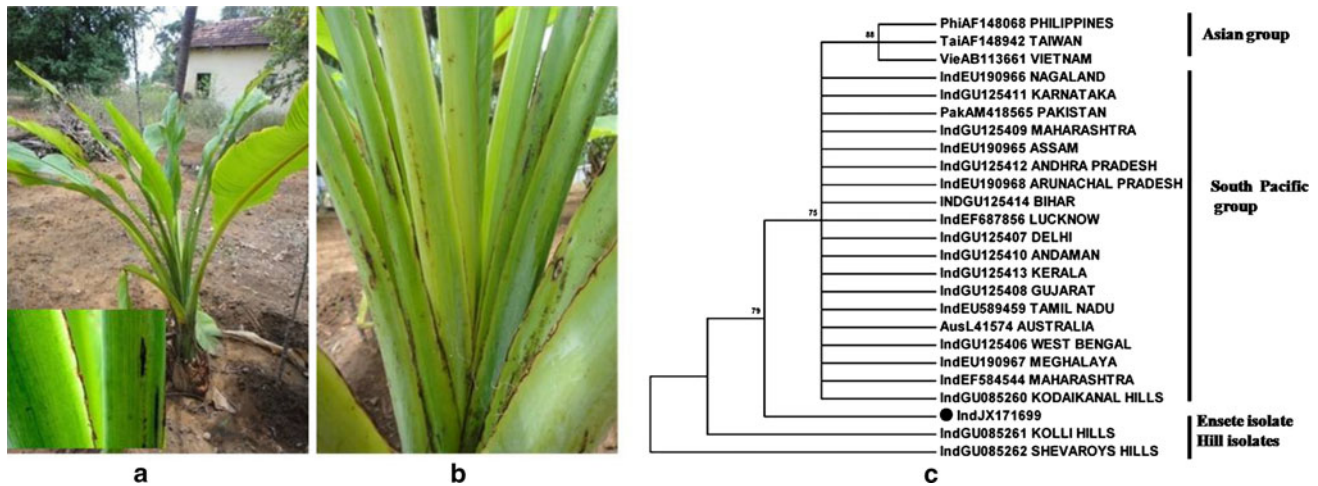


Fig. 1 **a** Bunchy top symptoms associated with *Ensete superbum*; the leaves are *short and narrow*; Insert: *Dark green* Morse code like streaks on midribs. **b** Closely packed leaf petioles bunching at the top. **c** Neighbor-joining tree based on the deduced amino acid sequences of the Ensete isolate of BBTV (JX171699) coat protein with other BBTV isolates. Bootstrap analysis was applied using 1,000 replicates.

The *scale bar* represents a genetic distance of 0.005. The phylogenetic tree was generated using MEGA4.0 and the neighbor joining algorithm. The distances computed using the Poisson correction method was derived from a multiple alignment using ClustalW. (Color figure online)

(TN) and formed a separate cluster (Fig. 1c) whereas remaining Indian BBTV isolates formed the core of South Pacific group. Wanitchakorn et al. [5] reported high degree of divergence in the sequence of CP gene of Asian group. Phylogenetic analysis based on amino acid sequence of the Rep gene of Ensete bunchy top isolate showed that this isolate grouped with isolates of Tamil Nadu, Maharashtra and Andaman (data not shown).

Both Ensete and wild valuable diploid bananas are conserved naturally. Infected plants of Ensete need to be identified and eradicated so as to protect the wild bananas in Western Ghats and other hill regions. An interesting finding of this study is that the Ensete bunchy top isolate is distinct based on amino acid sequence of CP gene and grouped with the BBTV-hill isolates of Tamil Nadu. Selvarajan et al. [4] distinguished the hill isolates of BBTV of TN with the isolates occurring in other hilly regions of India. Possibly this Ensete isolate would have originated from hill regions of Tamil Nadu. To the best of our knowledge, this is the first report of natural occurrence of BBTV infecting *Ensete superbum* in India.

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