First Report of *Tobacco streak virus* Infecting Kenaf (*Hibiscus cannabinus*) in India

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Abstract The natural occurrence of *Tobacco streak virus* (TSV) in *Hibiscus cannabinus* was detected by enzyme-linked immunosorbent assay using an antiserum raised against TSV and reverse transcription polymerase chain reaction (PCR) using primers specific for the coat protein gene of the virus. Sequence analysis of the PCR products showed 99.6 and 99.5% of maximum identity at nucleotide and amino acid levels, respectively with TSV onion isolate from Kurnool (HM131490). This is the first report of the natural occurrence of TSV on kenaf in India.

Keywords DAC-ELISA · Host range · *Ilarvirus* · Kenaf · RT-PCR

Kenaf (*Hibiscus cannabinus* L.) a member of *Malvaceae*; is the second most important bast fibre crop after jute. Andhra Pradesh, West Bengal, Uttar Pradesh and Orissa are the major states cultivating kenaf in India. It is potentially a valuable industrial crop due to its fibre content, medicinal value and effective use in the paper industry [2]. Calyces and leaves of this plant are used as an ingredient in preparation of pickles and various food dishes in India. The leaves and tender twigs of the plants are also used as cattle

fodder and the dried stem stalks as firewood. *Tobacco streak virus* (TSV) a member of the genus *Ilarvirus*, family *Bromoviridae* consists of non-enveloped, isometric particles, ~25–35 nm in diameter. It has a wide host range, infecting more than 200 plant species belonging to 30 dicotyledonous and monocotyledonous plant families and its occurrence has been reported from more than 26 countries worldwide [4]. The virus is transmitted by thrips vector.

During June 2011 mosaic, necrotic spotting on leaves and necrosis of bud of kenaf was observed (Fig. 1) in fields of Chittoor district of Andhra Pradesh, India. Based on symptomatology TSV infection was suspected. The presence of the virus in symptomatic leaves was further confirmed by direct antigen coating (DAC)-ELISA using TSV polyclonal antibodies. RT-PCR tests of leaf tissue from kenaf plants using primers specific for the nucleocapsid gene of TSV [1] resulted in an amplicon of the expected size (~600 bp). The amplicon was cloned into pTZ57R/T vector (Fermentas, USA), deposited in GenBank (Accession No. JN254782). Sequence analysis (BioEdit v. 7.05) and comparison with 15 other TSV isolates (GenBank Accession No. JN254782, HM131490, GQ401240, HM131487, AF515823, AY590139, AY940155, DQ058079, EF159702, AY940151, AY501483, DQ864456, DQ864459, AF515825, DQ225172 and FJ447358) showed 99.6 and 99.5% of maximum identity at nucleotide and amino acid levels, respectively with TSV onion isolate from Kurnool (HM131490). Phylogenetic tree at nucleotide level was constructed using MEGA version 4.0 and it further showed clustering of kenaf (JN254782) with groundnut (FJ447357), green gram (HM131487), urd bean (DQ225172) and groundnut (GQ401240) (Fig. 2). Out of seven kenaf fields surveyed three fields showed 10–15% and remaining four fields showed 5–10% of disease incidence. The infected crop has now been removed to eradicate the infection. Based on

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Fig. 1 Symptoms associated with natural occurrence of *Tobacco streak virus* on kenaf: mosaic, necrotic spot of leaves and necrosis of the bud

Fig. 2 Phylogenetic tree using MEGA 4.0 obtained from Clustal W alignments of the coat protein gene of kenaf (JN254782) with the other TSV infected isolates

symptomatology, natural occurrence of TSV on kenaf was reported in Brazil during 1961 [3]. Molecular/Serological evidence confirming TSV infection on kenaf has not been

reported till date. To the best of our knowledge this is the first report of the natural occurrence of TSV on kenaf in India.

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