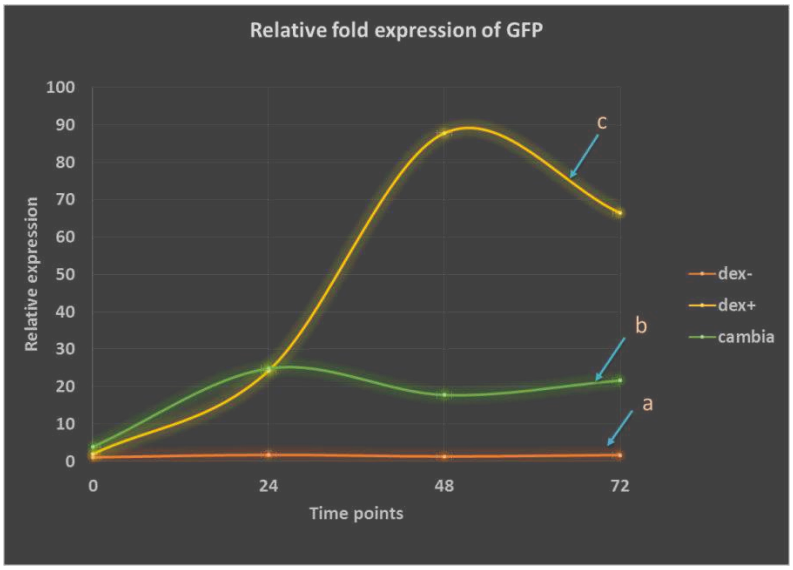

Figure 2: Transcription profiling of GFP in *N.tabacum* leavesinfiltrated with (a & c) pC1302DEX and (b) pCAMBIA1302. Induced [DEX+(5µM)] and pCAMBIA1302-infiltrated samples were compared with the mock-treated (un induced) samples. *Nt*L23 is used as the internal control.

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IV-SO27

EMERGENCE OF NOVEL *SUGARCANE BACILLIFORM VIRUS* GENOTYPES WITH EVIDENCES OF RECOMBINATION

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Sugarcane bacilliform virus (SCBV), belong to the genus *Badnavirus*, family *Caulimoviridae,* are a group of DNA viruses causing leaf fleck disease in sugarcane. Sugarcane is not only a potent sugar producing crop but also a source of renewable

bioenergy cultivated in various tropical and subtropical areas around the globe. The virus is primarily transmitted through seed canes and secondarily transmission takesplace through *Saccharicoccus sacchari* (pink mealy bug) in field conditions. The virus was first observed on a commercial cv B34104 in 1985 from Cuba (Rodriquez-Lema*et al.*1985). Later it was reported from more than 20 sugarcane cultivating countries in the world including Australia, Brazil, France, India and China (Karuppaiah *et al.*2013). It causes chlorotic leaf flecks, enlarged yellowish/reddish fleck and interveinal chlorotic streaks/stripes in sugarcane. The symptoms start to appear prominently from tillering phase of the crop and its severity aggravated with aging (Viswanathan *et al.* 2019).The disease was reported to decrease juice, sucrose content, purity and stalk weight in sugarcane from China (Li *et al.* 2010). Badnaviruses known for its variation within the species and this makes it difficult to characterize them through molecular diagnostics. Hence exploiting the genotypic variants among SCBV is an important step towards viral diagnosis. At present, 20 genotypes have been reported based on their nucleotide similarity viz SCBV A to T. More than 20% differences in the RT/RNase H region of ORF 3 functional polyprotein is considered as the species demarcation criterion among badnaviruses. Hence we made efforts is to find out the prevalence and characterize of SCBV in India.

To explore the genetic diversity, samples from 125 germplasm and 233 cultivated varieties were collected during the 2019-20 from different places of Tamil Nadu, Kerala, Karnataka, Maharashtra, Andaman and Nicobar islands. Germplasm clones comprised of *Saccharum officinarum, S. spontaneum, S. barberi, S. sinense*, *Erianthus arundinaceus*; cultivars included *Saccharum* hybrid varieties, *Saccharum* inter specific hybrid varieties and exotic clones. The leaf samples were ground in liquid nitrogen and DNA was isolated using CTAB buffer as reported earlier (Karuppaiah *et al.* 2013). The DNA samples were quantitatively checked in Nanodrop spectrophotometer and qualitatively by agarose gel electrophoresis (0.8%). A new set of degenerate primer targeting the conserved motifs of ORF3 polyprotein-RT/ RNase H region were designed which were capable of amplifying ~794base pair (bp) fragment. The amplicons were purified and sequenced through Sanger di-deoxy sequencing. Contiguous sequences were obtained from forward and reverse sequences using Cap contig assembly programme of Bioedit 7.0.5.3. The resulted sequence was used to query the National Centre for Biotechnology Information (NCBI) database (www.ncbi.nlm.nih.gov) with the BLASTn search functions, to match it with viral sequences. Phylogenetic relationship was performed in MEGA X using the maximum likelihood method with Tamura-Nei model (1993). An evidence of recombination was investigated using RDP4 software package (Martin*et al.* 2010).

Phylogenetic analysis of the sequences revealed formation ofthree major groups, in which most of the isolates clustered in 3rd monophyletic group. Apart from the known 20 genotypes, 4 novel genotypes were discovered from the study such as SCBV-U, SCBV-V, SCBV-W and SCBV X. About 56 SCBV isolates from the study were grouped

into a separate major cluster forming a new genotype SCBV-U. Another 16 isolates formed a new branch SCBV-V. The isolate CBJ46 alone formed a distinct branch (SCBV-W) outside the 3rd monophyletic tree, because of the sequence dissimilarities compared with other isolates from the study. This variant can be even considered as a new species, but it necessitates complete analysis of the genome. *S. officinarum* clones (Bangadya, Saipan G, Baragua); *S. spontaneum* 81-095; ISH 1 and Cym 08-666 formed a diverse cluster forming a novel genotype SCBV X. From published genotypes, the following genotypes were reported for first time from India viz., SCBV-G (France and China), SCBV-Q (China), SCBV-R (China) and SCBV-S (China). RDP4 recombination analysis suggested 19 recombinants from the study; MS 901, CB 96007, *S. officinarum* Khajuria and CBSe 95436 were major potent recombinants where default P- value used was 0.05.

In conclusion, SCBV-U, a novel genotype from the present study can be considered as the commonly occurring genotype in India especially in case of isolates from *Saccharum* hybrids. Predomination of one genotype was observed in the field conditions during the year 2019 and 2020. Analyzing large number of germplasm clones may reveal all possible genotypes of SCBV; thus ease the process of virus indexing and also lend a hand in molecular diagnostics. Phylogenetic analysis, recombination patterns and the knowledge about hot spots found in the RT/RNase H region of SCBV species can help to find the evolution within the species happened over the years. Present study provides a major portrayal of the prevalence, distribution and genetic diversity of the SCBV species complex in India. With the continued addition of new SCBV genotypes/strains, it is important that the distinct phylogenetic groups to be realigned to the level of species or strains in the genus *Badnavirus*.

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