Table: 1. Estimation of yield losses in sugarcane due Yellow Leaf Disease (YLD) at different degrees of infection during 2014-15 & 2015-16

Treatment (Sett infection with YLD)	NMC/ Ha	Cane Yield/ Ha	Sucrose %	CCS %	Sugar yield (t/Ha)
Complete Healthy setts	130	135.0	19.6	13.59	18.35
75% healthy setts	124	128.0	19.1	13.52	17.32
50% healthy setts	109	120.0	18.4	12.77	15.32
25% healthy setts	102	112.0	18.1	12.61	14.12
Completely Diseased setts	92	98.5	17.3	11.90	11.73
CD at 5%	4.76	5.32	1.07	1.27	9.35
C.V.%	9.61	15.01	2.86	2.08	10.32

NMC is Number of Millable Canes; CCS is Commercial Cane Sugar

Determining the prevalence of YLD, its distribution in Andhra Pradesh and the quantum of yield losses due to YLD are pre-requisites to comprehensively understand the disease pattern along the transect of cane growing areas of Andhra Pradesh. In this context, our studies assume significance. Our results indicated the increasing pattern of YLD over years in Andhra Pradesh, and the hot spot areas indicate the disease at alarming levels. Further, the quantum of yield losses and the quality parameters that deteriorate due to sett infection with YLD is a concern for researchers as well as cane growers. Therefore, it is necessitated to devise comprehensive control measures for YLD duly involving host plant resistance, vector management and sustainable agronomic practices. Our future studies are directed in understanding the vector-transmission, developing meristem derived tissue culture plants that ensure virus free seed material, along with biological control agents such as plant growth-promoting rhizobacteria (PGPR) that offer induced systemic resistance to canes through a holistic approach.

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IV-P5

LEAF FLECK CAUSED BY *SUGARCANE BACILLIFORM VIRUS*: AN EMERGING DISEASE IN SUGARCANE

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Sugarcane is an important cash crop contributing up to 72% sugar production in the world. Like any other crops it is also prone to many biotic stresses like pest and disease problems. Among the diseases, those caused by viruses are serious threats to sugarcane cultivation by causing varietal degeneration and pose challenges in germplasm maintenance (Viswanathan, 2016). Leaf fleck caused

by *Sugarcane bacilliform virus* (SCBV) is one of the important diseases reported in many sugarcane growing countries. It was first reported during 1985 from Cuba. Subsequently, this disease has been reported throughout the world including USA, Morocco, Australia, India, South Africa, Malawi, Guadeloupe and China. In most of the countries virus infection is confined to germplasm collection and its presence in the cane fields is not yet reported. However, in India the disease prevails in most of the sugarcane growing regions. Although its impact on cane growth is not assessed, its presence is documented (Viswanathan, 2012). SCBV is a plant *Pararetrovirus* belonging to *Badnavirus* with non-enveloped bacilliform particles of size 30 x 130 to 150 nm, with circular dsDNA genome of 7.5 to 8.0 kb (Lockhart, 1990) and it replicates via reverse transcription. Mealybug vectors like pink mealybug, *Saccharicoccus sacchari* and grey mealy bug, *Dysmicoccus boninsis* are the reported vectors of the virus and these transmit the virus in a semipersistent manner (Lockhart *et al*., 1992). Ninth Report of ICTV recognized two species of SCBV, *Sugarcane bacilliform IM virus* (SCBIMV) and *Sugarcane bacilliform MO virus* (SCBMOV). Muller *et al.* (2011) identified two more species viz. Sugarcane bacilliform Guadeloupe A virus (SCBGAV) and Sugarcane bacilliform Guadeloupe D virus (SCBGAV). Recently, Karuppaiah *et al.* (2013) reported three SCBV species from sugarcane germplasm collection and cultivated varieties in India and Sheng-Ren *et al.* (2015) reported two new SCBV isolates (SCBV-CHN1 and SCBV-CHN2) from China.

Earlier it was reported that the symptoms of SCBV infection were unreliable and infected clones might remain symptomless (Comstock and Lockhart, 1990). However, symptoms including varying degrees of chlorotic stripes, intense mottling and freckles on the foliage coupled with stunted growth were reported by Viswanathan and Premachandran (1998).They related symptoms expressed to virus concentration through ELISA or immunosorbent electron microscopy (ISEM). But in some instances, the virus particles were detected in symptomless plants. The symptoms described from sugarcane germplasm comprising different species of *Saccharum*, foreign hybrids and allied genera and Indian hybrids have indicated enormous variations. In further studies, Viswanathan (2012) described virus associated symptoms from cultivated varieties under field conditions. Overall, it was observed that the virus caused varying symptoms ranging from flecks to severe mottling, foliage discolouration and drying.

Resembling its type member, *Commelina yellow mottle virus* (ComYMV), the genome of SCBV also contains three ORFs. The exact functions of ORF1 and ORF2 have not been established and ORF3 encoded a large polyprotein containing domains associated with movement, coat protein, an aspartic protease, reverse transcriptase (RT) and ribonuclease H (RNase H) functions. A genome of 7568 bp with the three standard ORFs and intergenic region was identified through complete genome sequencing of the Morocco isolate (Bouhida *et al.,* 1993). SCBV isolates from various parts of the world exhibit remarkable genetic diversity signifying that the viral populations are complex and variable, even within a group. Muller *et al.* (2011) identified high molecular variability in the SCBV genome through phylogenetic analysis of 35 partial sequences along with the two known genome sequences.

Karuppaiah *et al*. (2013) reported complete genome sequencing of five SCBV isolates from India with a genome size ranging from 7568 to 7687 for the first time. The five Indian isolates shared 69–85% identities among themselves and 70–82% identity with SCBIMV and SCBMOV, indicating that the Indian SCBV isolates are distinct from other SCBV isolates. Phylogenetic analysis based on

the partial RT/RNase H sequence recognized three new species from India, which were named as Sugarcane bacilliform BR virus (SCBBRV), Sugarcane bacilliform BO virus (SCBBOV) and Sugarcane bacilliform BB virus (SCBBBV). Rao *et al*. (2014) found sequence variability up to 27% in the RT and RNase H (RT/RNase H) genetic region in 8 SCBV isolates from five states in India. Studies of Xiao-Bin *et al*. (2016) revealed significant haplotype diversity within individual SCBV isolates from China. Recombination analyses revealed weak signs of recombination among some of the SCBV sequences. In phylogenetic analysis it was revealed that there is segregation of global SCBV isolates into three major monophyletic clades encompassing 18 subgroups, including five previously undescribed subgroups named as SCBV-N to -R. Low levels of genetic exchange was observed between SCBV populations.

Many clones, particularly commercial hybrids, do not develop symptoms hence diagnosis of SCBV by phenotypic symptoms is unreliable. Reliable antigen-based (serology) and genome-based (PCR) methods for indexing SCBV were reported. Due to high degree of serological and genomic heterogeneity that exists among isolates of the virus both these approaches have their own restrictions and this may be explained by the mechanism of replication of *Badnavirus* that were prone to generation of genomic variants. Use of DAS-ELISA, DAC-ELISA and IEM were used for diagnosis of SCBV by various researchers (Autrey *et al*., 1991; Viswanathan, 1994; Balamuralikrishnan and Viswanathan, 2005). Real-time PCR and loop-mediated isothermal based assays were also reported for the detection of *Badnavirus* (Johnson *et al.,* 2014). As PCR-based detection methods could give false positive results due to the presence of endogenous *Badnavirus*, there is an urgent need for developing reliable method to detect and distinguish episomal and endogenous *Badnaviruses* in plants. Immunocapture-PCR or multiplex immuno-capture polymerase chain reaction (IC-PCR) was used for the detection of episomal virus DNA (Muller *et al.*, 2011). Rolling circle amplification (RCA) is a new promising method for the detection and amplification of the circular DNA genome of plant *Pararetroviruses* (Wambulwa *et al*., 2012). Bacteriophage Φ29 DNA polymerase is used to amplify circular DNA molecules in a sequence-independent manner in RCA. As episomal *Badnavirus* genomes are circular, RCA could discriminate between ds circular viral genome and endogenous viral sequences and thus overcome false positives. The RCA combined with RFLP was effectively used in the detection of *Banana streak badnavirus (BSV)*. Amalgamation of various methods like ISEM, IC-PCR, RCA, virus purification, Southern and *In situ* hybridizations, and complete sequencing of the virus genome might be required for detailed characterization of the virus. Currently no information is available on integration of the virus genome with sugarcane unlike BSV and further study in this direction is needed.

SCBV is a double stranded DNA virus belonging to *Caulimoviridae* which replicates via reverse transcription. Earlier, the disease was reported as the one confined to sugarcane germplasm. However, its severe occurrence under field conditions threatens sugarcane cultivation in India (Viswanathan, unpublished). This warrants detailed investigation on the disease including characterization of the virus. At present two SCBV species are confirmed by ICTV (King *et al*., 2011). Information on virus prevalence and its genetic diversity are crucial to manage this viral disease. Hence extensive studies are to be undertaken in sugarcane growing areas of the country to identify diverse strains using reliable diagnostics. Except for ORF III, the functions of the other ORFs are still not known. Conflicting views have been reported on endogenous nature of SCBV genome in sugarcane. Methods such as RCA and PCR have to be validated, for identifying virus-free plants used for

propagation and also for international germplasm exchange. Resistant sources need to be identified in germplasm and should be included in conventional breeding program to develop virus resistant progenies. Genome editing tools offer ways and means to delete the unwanted integrated viral sequences in sugarcane.

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IV-P6

MOLECULAR CHARACTERIZATION OF PHYTOPLASMA ASSOCIATED WITH SUGARCANE GRASSY SHOOT DISEASE

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Sugarcane (*Saccharum* spp. hybrid) is an important sugar and energy crop cultivated in more than 110 countries contributing more than 80% of world sugar requirement. India is the second largest producer of sugarcane after Brazil and it is a highly industry centric crop with more than 500 sugar factories. Since sugarcane is a vegetatively propagated crop planted through setts it is being affected by several disease causing pathogens viz. fungi, bacteria, phytoplasma and viruses. Among the sugarcane diseases 'Sugarcane Grassy Shoot' (SCGS) caused by sugarcane grassy shoot phytoplasma