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RESEARCH ARTICLE

Status of leaf fleck caused by *Sugarcane bacilliform virus* incidence and severity in different sugarcane growing areas of Kerala and Tamil Nadu

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Abstract
Sugarcane bacilliform virus (SCBV), causing leaf fleck in sugarcane (*Saccharum* spp), is a major threat affecting exchange of germplasm globally. Surveys conducted in four major sugarcane growing districts of Kerala and two in Tamil Nadu during 2018-2019 revealed that the disease incidences ranged between 12 and 51% in Kerala and 28 and 56 % in Tamil Nadu. Symptoms associated with the disease include mild to severe mottling of the leaves, which were more prominent on leaf positions four to six. In Kerala, ratoon crop of the cv. Madhuri in Pathanamthitta district recorded the highest incidence (51%) and severity (36%) of leaf fleck. Least incidence (12%) and severity (10%) were recorded in Idukki district in the cv. Co 86032. Among the surveyed locations in Tamil Nadu, plant crop of cv. CoV 09356 in Villupuram district recorded the maximum percent of disease incidence (56%) and severity (50.41%) followed by Radhapuram with an incidence of 55% and severity of 43.75%. Least incidence and severity were recorded in plant crop of cv. Co 86032 at Kuchipalayam (28%). In comparison to Kerala, Tamil Nadu recorded higher incidences and severities of leaf fleck. PCR assays using primers specific to Badna viruses, confirmed the presence of the virus in collected samples. Detailed surveys conducted for leaf fleck for the first time under field conditions indicated severe occurrence of the disease in the states of Kerala and Tamil Nadu.

Keywords: Sugarcane; Leaf fleck; PCR assay; Disease symptoms

Introduction

Sugarcane is one of the major cash crops cultivated worldwide under varied agro climatic conditions. India is the second largest producer of sugarcane with an area of 4.7 million ha and production of 376.90 million tonnes (http://agricoop.gov.in/). One of the major factors affecting yield and productivity of sugarcane is stresses posed by various biotic and abiotic agents. Among the biotic constraints, various diseases of viral origin cause huge impact on sugarcane production and they are prevalent throughout India. Planting material (setts) is the chief source for disease spread in the country and to other countries through germplasm exchange (Viswanathan et al. 2018). The important viral diseases that cause significant damages to sugarcane production till date are yellow leaf, mosaic and leaf fleck in India (Viswanathan 2018). Among them, leaf fleck caused by *Sugarcane bacilliform virus* (SCBV) is one of the major threats, which hinders exchange of sugarcane germplasm globally. In India, the presence of the virus was suspected in germplasm clones maintained at Kannur, Kerala and was later confirmed through electron microscopy and enzyme linked immunosorbent assay (ELISA) technique (Viswanathan 1994; Viswanathan et al. 1996). Symptoms of SCBV include mild to

intense flecks in the lamina followed by chlorotic stripes or streaks that may result in reddening and premature drying of leaves (Viswanathan et al. 1999). Some of the affected clones were stunted and exhibited a canopy resembling bunchy top with few tillers and reduced internodal length in the germplasm collections of *Saccharum* spp. especially *S. officinarum, S. barberi, S. robustum* and in some of the hybrid clones (Viswanathan and Premachandran 1998).

SCBV belongs to the genus *Badnavirus,* family *Caulimoviridae,* is characterized by a circular, double stranded DNA genome of 7.8 kb and is transmitted naturally through its mealy bug vector, pink mealy bug *Saccharicoccus sacchari* (Lockhart et al. 1992; 1996). Transmission of the virus by vegetative cuttings, setts was also reported (Lockhart et al. 1996; Viswanathan *et al.,* 1996). Earlier studies conducted in India were confined to germplasm clones (Viswanathan et al.1996, 1999) and later Karuppaiah et al. (2013) reported and characterized five new SCBV species from India, four from *Saccharum* spp clones and a new SCBV species from the hybrid cv. BO 91. Recently, Viswanathan et al. (2019) reported virus diagnosis from *Saccharum* spp clones and cultivated varieties. The same study elaborated on the progressive symptoms of the disease in a set of *Saccharum* spp genotypes and hybrid clones. However, no studies were made under field conditions on the actual disease spread in the major sugarcane varieties and its impact to sugarcane cultivation. Hence, we conducted intensive surveys for the disease scenario in major sugarcane growing districts in Kerala and Tamil Nadu and presence of the virus was confirmed through PCR assays using primer specific to Badnaviruses.

Materials and Methods

Survey

Surveys were conducted during 2018-2019 seasons to record the incidence of leaf fleck in major sugarcane growing areas in four districts of Kerala and two districts of Tamil Nadu. The list of plots to be surveyed was prepared in consultation with Agricultural department officials, scientists in research stations and officials in sugar factories. Sugarcane fields with minimum coverage of one acre were taken for recording observations. Minimum of hundred plants were observed at random in a zigzag manner across the fields. Healthy and diseased plants were counted and percent disease incidence was calculated. Disease incidence refers to the percentage of visibly diseased plants, in relation to the total number of plants assessed. The percentage disease severity (PDS) was recorded using the newly created 0-5 grade system to score disease severity (Table 1, Fig1a-f).

Percentage disease incidence and severity was calculated using the formula

$$\text{Per cent disease incidence} = \frac{\text{Number of diseased plants}}{\text{Total number of plants examined}} \times 100$$

$$\text{Percent disease severity} = \frac{\text{Sum of all individual disease ratings}}{\text{Total no. of plants observed x Maximum disease grade}} \times 100$$

Leaf samples were collected from each location, labelled and kept in polythene bags, brought to the laboratory for virus diagnosis.

DNA isolation

CTAB method was followed to isolate total DNA of selected samples. Leaf samples (each 100 mg), stored at −80 °C, were ground to a fine powder using liquid nitrogen in mortar and pestle. The powdered samples were transferred to 1.5 ml sterile microfuge tube and 1ml of CTAB buffer was added (Doyle and Doyle 1987). The samples

Table 1. Newly developed 0-5 scale to score leaf fleck severity in sugarcane

Grade	Description
0	Plants free from symptoms Plants with no leaf fleck symptom. Leaves appear dark green in colour and free from symptoms when observed farther and closely in the leaf positions -2 to 6.
1	Apparently free from symptoms but mild specks throughout the lamina Mottles are concentrated more on distal end. Matured leaves showed more intense mottling compared to younger leaves
2	Few widely distributed visible flecks in 2nd and 3rd leaves from top
3	Intense flecks with yellowish discoloration covering partial or complete leaf lamina in 4th to 6th the leaves. The symptoms are very distinct even when observed from few meters away
4	Almost all the leaves show severe fleck symptoms with reddish discoloration or yellow blotches. No apparent growth reduction. When viewed from a distance, symptoms are very clear. Symptom expression was more prominent on mature leaves. As the leaf matures mottles become yellow or reddish later turns necrotic.
5	All the leaves exhibit reddish discoloration of flecks covering entire leaf lamina accompanied by drying of matured leaves. The plants appear to be sick and stunted growth / degeneration.

were then incubated at 55 °C for 45 min and centrifuged at 12,000 rpm for 10 min at 4 °C. To the supernatant, equal volume of chloroform: isoamyl alcohol (24:1) was added. It was then mixed thoroughly and centrifuged at 12,000 rpm for 12 min at 4 °C. Three separate layers were formed out of which aqueous phase, which contained DNA was transferred to a new microfuge tube

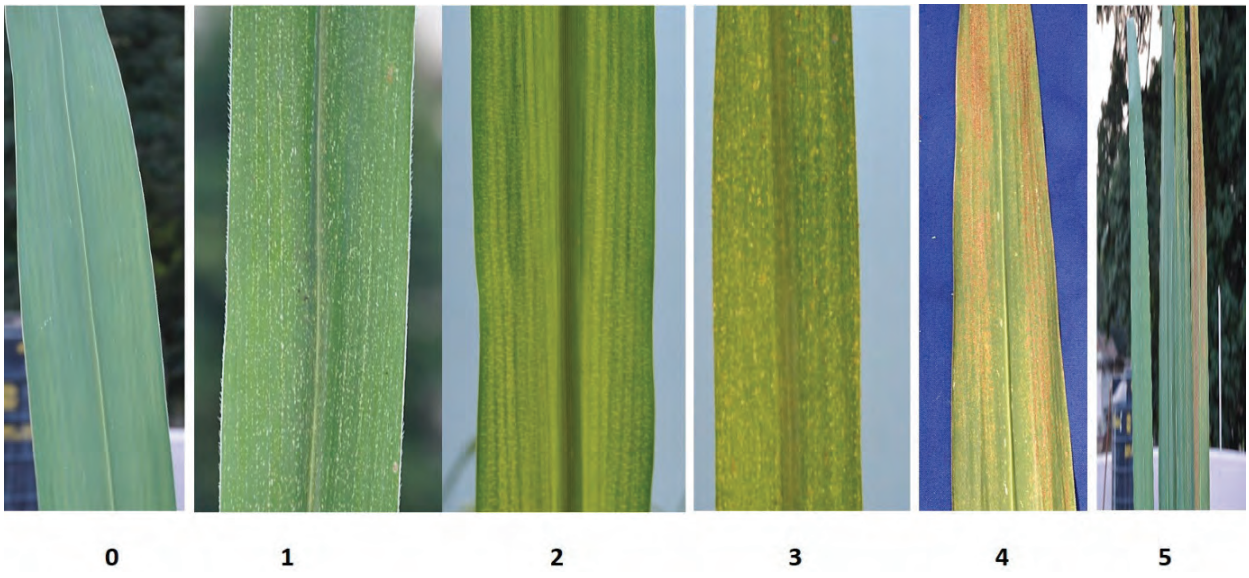

Figure 1. Progressive increase in disease severity in sugarcane leaves is given as per 0-5 grading system (Table 1). '0' depicts disease free condition and other leaves show increase in severity starting from isolated flecks in grade 1 to intense flecks in 2 and 3. The grades 4 and 5 show increased yellow/ reddish discolouration accompanied by drying of lamina tissue.

Table 2. Details of primers used in this study

Primer	Sequence (5'-3')	Length	Relative genome position	Product size(bp)
BadnaFP	ATGCCITTYGGIITIAARAAYGCICC	26	5725-6304	579
BadnaRP	CCAYTTRCAIACISCICCCCAICC	24		

with addition of 0.7 volume of isopropyl alcohol and mixed by gentle inversion. It was then incubated for 20 min at 4 °C. Afterwards, DNA was pelleted by centrifugation at 12000 rpm for 10 min. Then the pellet was washed using 75 % ethanol and centrifuged at 10,000 rpm for 5 min at 4 °C. The supernatant was discarded and the pellet was retained. Finally, the pellet was air dried and dissolved in 40µl of sterile distilled water.

The quantity and quality of DNA were determined through spectrophotometry at 280 nm wavelength in Nanodrop (2000C, Thermo Fisher Scientific, USA). Purity of samples analyzed was within the optimal range of 1.8-2.0 and their concentration ranged from 850 to 2100 ng. Further, the integrity of DNA was checked in 0.8 % agarose gel having ethidium bromide as stain (10 mg ml^{-1}) at 120 volts for about 45 minutes in 1X- Tris-Acetate–EDTA (TAE) buffer of pH 8. The gel images were documented in a gel documentation system (Syngene G: Box, UK).

PCR amplification

The PCR reaction was performed using amplifying degenerative primers amplifying 579 bp, targeting RT/RNase H region of the viral genome (Table 2). The PCR reaction was performed in a total volume of 25 µl containing 2 µl DNA, 2.5 µl of 10X PCR buffer, containing 15 mM $MgCl_2$, 2µl of 2.5mM dNTP mix, 20 pmol of each primer pair, 1.25 units of *Taq*DNA polymerase (Takara Bio, USA) and sterile milliQ water to a final volume. The PCR reaction was performed with initial denaturation at 94 °C for 5 min, 32 cycles of 94 °C for 1 min, 55 °C for 45 seconds for annealing, 72 °C for 1 min for primer extension and a final extension of 72 °C for 10 min in a thermocycler (Mastercycler ProS, Eppendorf, Germany). The PCR products were separated by electrophoresis in 1.2% agarose gel having ethidium bromide as stain (10 mg ml^{-1}) at 120 volts for about 45 minutes in 1X- Tris-Acetate–EDTA (TAE) buffer of pH 8. The gel images were documented in a gel documentation system (SyngeneG: Box, UK).

Results and Discussion

Disease scenario

Detailed surveys were conducted in 35 locations to assess leaf fleck incidences and also to collect diseased samples from different sugarcane growing areas of Kerala and Tamil Nadu states to assess the virus diversity. During the course of surveys, symptoms on infected sugarcane plants, stage of crop and association of vectors percentage of leaf fleck incidence and symptom severity were recorded (Table 3). In Kerala, maximum incidence (51%) and severity (36%) of leaf fleck were recorded in ratoon crop of the cv. Madhuri in Pathanamthitta district. Plant crop of the cv. Co 86032 in Idukki district recorded least incidence (12%) and severity (10%). Among the surveyed locations in Tamil Nadu, ratoon crop of the cv. CoV 09356 in Moongilthuraipattu area of Villupuram district recorded the maximum percent of disease incidence (56%) and severity (50.41%). Plant crop of the cv. Co 86032 at Kuchipalayam recorded the least incidence and severity (28%) of leaf fleck (Table 4a, b).

Table 3. Leaf fleck incidence and severity in sugarcane varieties in surveyed areas of Kerala and Tamil Nadu

Districts (State)	No of fields surveyed	Locations	Crop growth stage	Variety	Leaf fleck incidence (Average)%	Leaf fleck severity (Average)%
Idukki (Kerala)	5	Keezhanthoor Kanthalloor Marayoor Nachivayal Podathivayal	6-10	Co 86032	30	22
Palakkad (Kerala)	4	Chittur Valiyavallampathy Elapully Pudussery	6-10	Co 86032	28	23
Pathanamthitta (Kerala)	4	Kallunkal Venpala Nedumpuram Kuttor	6-10	Madhuri	36	26
Alapuzha (Kerala)	2	Thiruvanmandoor Eramallikkara	6-10	Madhuri	28	23
Villupuram (Tamil Nadu)	10	Fields under Rajshree sugars	6-10	Co 86032, PI 1110, CoV 09356 , Co 06031	41	38
Cuddalore (Tamil Nadu)	10	Nellikuppam area under M/s EID Parry	6-10	Co 86032, PI 1110, CoV 09356, Co 06031	38	36

Leaf fleck symptoms were initiated as mild or intense flecks on entire lamina or prominently on distal end of leaf lamina (Fig.2a). Such symptoms were more distinct on the older leaves (leaf position 4-6). The cvs. Co 86032 and Madhuri were the major varieties under cultivation in Kerala and they exhibited symptoms of mild to intense flecks (Fig.2b). Symptom expression was more in ratoon crops where the canopy turns yellow due to intense flecks, which resulted in premature drying of the leaves (Fig.2c). Prominent varieties under cultivation in Tamil Nadu include Co 86032, PI 1110, Co 0212, CoV 09356, Co 06030 and Co 06022. In the cv. Co 0212, symptom initiated as mild to severe flecks, which were more distinct on distal portion of the leaf. Later this resulted in mottling of entire leaf lamina. Plants have a pale yellow canopy even at early stages of growth. Marginal reddening combined with necrosis results in complete drying of leaves. However, in cv. CoV 09356, chlorotic flecks turned to yellow, later to red and finally entire leaf dried off. Mild to

Table 4a. Leaf fleck incidences and severity during disease surveys in different locations of Kerala state

District	Location	Field	Stage of crop (Month)	Variety	Disease incidence %	Mean incidence %	Disease severity %	Mean severity %
Idukki	Keezhanthoor	F1	6	Co 86032	18	30.8	10.0	22.77
	Keezhanthoor	F2	6	Co 86032	12		10.0	
	Keezhanthoor	F3	8	Co 86032	30		23.33	
	Keezhanthoor	F4	6	Co 86032	21		30.83	
	Keezhanthoor	F5	10	Co 86032	35		16.0	
	Kanthalloor	F6	8	Co 86032	41		35.0	
	Kanthalloor	F7	6	Co 86032	42		30.83	
	Kanthalloor	F8	6	Co 86032	37		30.83	
	Kanthalloor	F9	8	Co 86032	28		13.0	
	Kanthalloor	F10	9	Co 86032	36		23.33	
	Marayoor	F11	10	Co 86032	42		35.0	
	Marayoor	F12	8	Co 86032	40		30.0	
	Marayoor	F13	6	Co 86032	27		23.33	
	Marayoor	F14	7	Co 86032	36		15.0	
	Marayoor	F15	6	Co 86032	32		35.0	
	Nachivayal	F16	6	Co 86032	31		10.0	
	Nachivayal	F17	8	Co 86032	30		12.5	
	Podathivayal	F18	9	Co 86032	39		35.0	
	Podathivayal	F19	9	Co 86032	19		16.0	
	Podathivayal	F20	9	Co 86032	20		10.0	
Palakkad	Chittur	F21	10	Co 86032	38	28	30.83	22.80
	Chittur	F22	6	Co 86032	40		35.0	
	Chittur	F23	6	Co 86032	23		13.0	
	Chittur	F24	8	Co 86032	37		35.0	
	Elapully	F25	10	Co 86032	18		30.83	
	Elapully	F26	10	Co 86032	22		15.0	
	Elapully	F27	10	Co 86032	34		35.0	
	Elapully	F28	10	Co 86032	27		23.33	
	Pudussery	F28	9	Co 86032	17		13.0	
	Pudussery	F29	9	Co 86032	19		10.0	
	Valiyavallampaty	F30	10	Co 86032	24		12.5	
	Valiyavallampaty	F31	10	Co 86032	30		22.5	
	Valiyavallampaty	F32	9	Co 86032	25		20.83	
	Valiyavallampaty	F33	10	Co 86032	31		10.0	
	Valiyavallampaty	F34	10	Co 86032	26		23.33	
	Kanjikode	F35	9	Co 86032	32		30.83	
	Kanjikode	F36	9	Co 86032	35		26.25	
	Kanjikode	F37	9	Co 86032	15		15.0	
	Kozhinjampara	F38	10	Co 86032	20		13.0	
	Kozhinjampara	F39	10	Co 86032	14		10.0	
	Kozhinjampara	F40	9	Co 86032	33		30.83	

Table 4a (Contd.)

District	Location	Field	Stage of crop (Month)	Variety	Disease incidence %	Mean incidence %	Disease severity %	Mean severity %
	Kallunkal	F41	9 (Ratoon)	Madhuri	51		36.25	
	Kallunkal	F42	9 (Ratoon)	Madhuri	48		30	
	Venpala	F43	9	Madhuri	32		23.33	
Pathanamthitta	Venpala	F44	8	Madhuri	27	36	18.75	25.66
	Nedumpuram	F45	9	Madhuri	21		22.5	
	Nedumpuram	F46	10	Madhuri	36		30.0	
	Pandalam	F47	9	Madhuri	25		30.0	
	Pandalam	F48	9	Madhuri	43		12.5	
	Thiruvalla	F49	8	Madhuri	38		27.08	
	Kuttor	F50	9	Madhuri	39		26.25	
	Thiruvanmandoor	F51	7	Madhuri	36		30.0	
	Thiruvanmandoor	F52	8	Madhuri	31		30.0	
	Thiruvanmandoor	F53	8	Madhuri	27		26.25	
Alapuzha	Eramallikkara	F54	9	Madhuri	21	28.33	15.0	22.29
	Eramallikkara	F55	8	Madhuri	33		10.0	
	Eramallikkara	F56	10	Madhuri	22		22.5	
Kannur	Talap	F 57	9	Co 86032	18	18	12.0	12

Table 4b. Leaf fleck incidences and severity during disease surveys in different locations of Tamil Nadu state

District	Location	Field	Stage of crop (Month)	Variety	Disease incidence %	Mean incidence %	Disease severity %	Mean severity %
	Radhapuram	F1	9	CoV 09356	55		43.75	
	Radhapuram	F2	8	CoV 09356	35		36.25	
	Radhapuram	F3	9	Co 06022	32		33.75	
	Radhapuram	F4	9	Co 86032	45		40.0	
	Radhapuram	F5	6 Ratoon	Co 86032	45		36.25	
Villupuram	Moongilpattu	F6	10	CoV 09356	56		30.0	
	Moongilpattu	F7	8	CoV 09356	49		50.41	
	Moongilpattu	F8	9	CoV 09356	49		36.25	
	Moongilpattu	F9	9	Co 86032	38	44.93	40.0	36.65
	Moongilpattu	F10	8	Co 86032	34		37.08	
	Kuchipalayam	F11	8	Co 86032	32		36.25	
	Kuchipalayam	F12	9	PI 1110	34		28.0	
	Kuchipalayam	F13	9	Co 86032	30		30.0	
	Kuchipalayam	F14	8	Co 86032	28		38.33	
	Maduraipakkam	F15	9	Co 86032	35		36.25	
	Maduraipakkam	F16	9	PI 1110	32		33.75	

Table 4b (Contd.)

District	Location	Field	Stage of crop (Month)	Variety	Disease incidence %	Mean incidence %	Disease severity %	Mean severity %
Cuddalore	Nellikuppam	F17	8	Co 0212	38	37.0	30.0	30.83
	Nellikuppam	F18	9	Co 0212	35		33.33	
	Nellikuppam	F19	9	CoV 09356	45		30.0	
	Nellikuppam	F20	9	Co 06030	30		30.0	

severe flecks were recorded in the affected plots. In ratoon crop of sugarcane, the disease incidence and severity were more combined with general yellowing, reddening and premature drying of leaves.

Figure 2a. Field view of leaf fleck in the cv. Madhuri in Kerala

PCR assay

The symptomatic leaf samples collected from different varieties in the surveyed locations were subjected for PCR assays. In Kerala, samples were collected from the cvs. Co 86032 and Madhuri whereas in Tamil Nadu from the cvs. Co 86032, PI 1110, Co 0212, CoV 09356, Co 06030 and Co 06022. Of the 100 symptomatic leaves collected from Kerala, 59 gave positive results whereas 26 of the 40 symptomatic samples from Tamil Nadu were found positive (Table 5). Amplification of 579bp amplicon confirmed positive amplification

Figure 2b. The cv. Madhuri with intense flecks

Figure 2c. Leaf fleck affected sugarcane cv. Co 0212 in Tamil Nadu shows severe yellow to reddish discolouration of foliage. Reddening of the distal portion of the leaf lamina is seen due to disease severity.

of SCBV and confirmed SCBV infection in the popular varieties under cultivation (Fig. 3).

During 1992, the disease was first suspected in the country in sugarcane germplasm clones and subsequently association of SCBV with the disease was confirmed through electron microscopic studies (Viswanathan 1994; Viswanathan et al. 1996). There were worldwide reports on the occurrence of the disease during the last decades that of the previous country suggested uniform distribution of the disease across the countries (Autrey 1985; Rodriguez-Lema et al. 1985; Lockhart and Autrey 1988; Comstock and Lockhart 1990; Teakle and Egan 1994; Braithwaite et al. 1995; Bailey 1996). However, none of the reports from other countries gave a clear description of leaf fleck symptoms. These studies described mild mottle or fleck symptoms and confirmed the virus through electron microscopic observations. Subsequent studies of Viswanathan and Premachandran

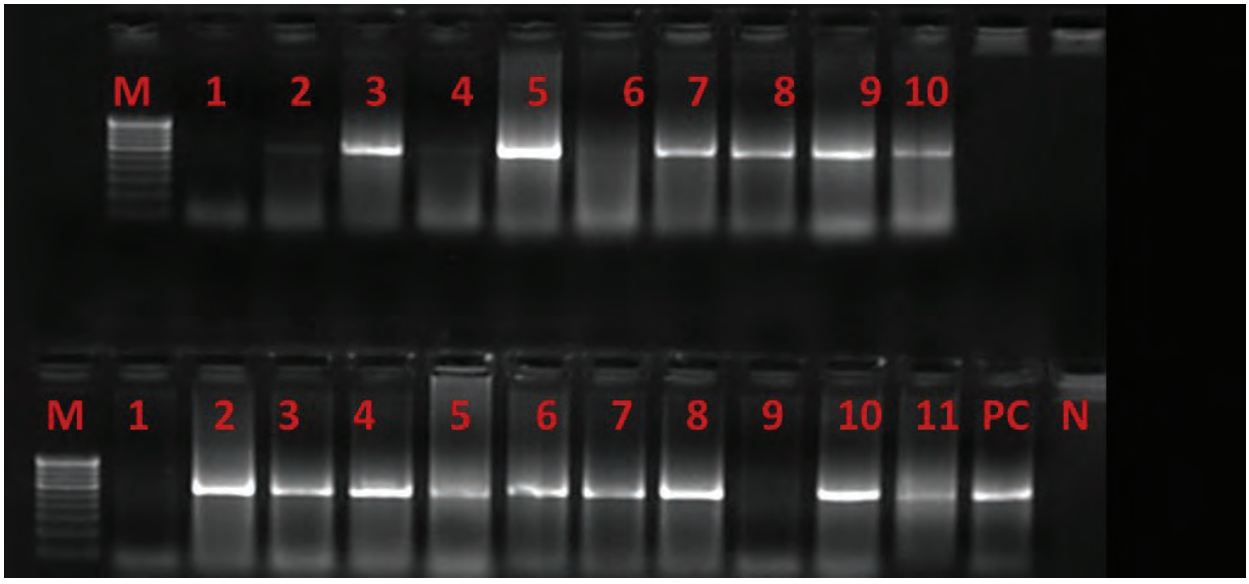

Figure 3. PCR amplification of SCBV targeting Rnase H/RT region (579 bp) of viral genome Upper row M: Marker; Lane 1-5:cv. Co 86032,6-10 cv. Madhuri Lower row M: Marker; Lane 1-3 Co 0212; 4-8, CoV 09356, cv. Co 86032,9-11 P1110 PC – positive; N -negative

Table 5. PCR assay for *Sugarcane bacilliform virus* in the field samples collected from Kerala and Tamil Nadu

SL No.	Variety	Location	Association of vector (Mealy bug)		Symptoms	Total no of samples	PCR +ve
1.	Co 86032	Keezhanthoor	Present	Adults per node =2	Mild mottles, intense flecks	70	47
		Kanthalloor	Present	Crawlers/ nymphs= 17-31			
		Marayoor	Present				
		Nachivayal	Present				
		Podathivayal	Present				
2.	Co 86032	Chittur	Present	Adults per node =4 Crawlers / nymphs = 19-34	Mild mottles, intense flecks yellowing	10	3
		Valiyavallampathy	Present				
		Elapully	Present				
		Pudussery	Present				
3.	Madhuri	Kallunkal	Present	Adults per node =3 Crawlers / nymphs= 22-31	Mild mottles, intense flecks severe yellowing	10	6
		Venpala	Present				
		Nedumpuram	Present				
		Kuttor	Present				
4.	Madhuri	Thiruvanmandoor	Present	Adult per node =1 Crawlers / nymphs = 28-36	Mild mottles, intense flecks severe yellowing	10	3
		Eramallikkara	Present				
5.	Co 86032, PI 1110, CoV 09356, Co 06031	Fields under Rajshree sugars, Villupuram Dt	Present	Adults per node =4 Crawlers / nymphs = 20-37	Mild mottles, intense flecks severe yellowing	28	19
6.	Co 86032, PI 1110, CoV 09356, Co 06031	Nellikuppam area under M/s EID Parry, Cuddalore Dt	Present	Adults per node =3 Crawlers / nymphs = 30-41	Mild mottles, intense flecks severe yellowing	12	7

+ve: positive to SCBV in PCR

(1998) made a detailed description on the disease symptoms of the disease in different clones of germplasm. Most prominent symptom of the disease was stripe symptoms in the interveinal region in leaf lamina in certain clones of *S. officinarum* and foreign hybrids. Many other clones expressed symptoms of mild freckling, mild mosaic, intense mottle etc. No further studies were made on disease symptomatology under field conditions with disease incidence and severity except the studies of Viswanathan et al. (2019). Hence the present study was conducted to assess the disease situation under field conditions in Tamil Nadu and Kerala.

Surveys revealed that the leaf fleck, which was earlier confined to germplasm clones has spread

to various sugarcane growing areas of Kerala and Tamil Nadu. When compared to Kerala, Tamil Nadu recorded highest incidence and severity of the leaf fleck. Higher incidence of disease in Tamil Nadu may be due to extended area under the cultivation, practice of ratooning, prevailing weather conditions favoring vector population and management practices. Studies conducted by Jayanthi et al. (2016) revealed how prevailing climatic and crop management practices influence the vector population, both positively and negatively. Symptoms of leaf fleck initiated as mild or intense flecks on entire lamina, which were more prominent on distal end of leaf lamina. These symptoms were more distinct on the older leaves. In some cvs. like Co 0212 and CoV 09356, the symptoms are more intense. In the cv. Co 0212, a pale yellow canopy was witnessed even at early stages of plant growth. Marginal reddening followed by necrosis was also observed in severe cases. While in cv. CoV 09356, colour of mottles changed to red followed by marginal necrosis and subsequent drying. These symptoms were different from those reported earlier (Viswanathan and Premachandran 1998) and similar to the recent report in hybrid varieties (Viswanathan et al. 2019). Symptom severity was more in ratoon crops where the canopy turns yellow due to intense flecks which resulted in premature drying of the leaves. Earlier, Rao et al. (2014) reported yellow to chlorotic freckling and mild to severe chlorosis with a striate mosaic pattern or yellow chlorotic streaks with freckling in different varieties in eight states in the country. However, they have not assessed severity of the disease nor incidence under field conditions. The disease incidence and severity varied with stage of the crop, variety, prevailing agro-climatic conditions and crop management practices. Disease progression was more intense in summer months. This may be attributed to abundant vector population and other abiotic stresses which may contribute positively towards the symptom development. Universal primer specific to BADNA viruses was used to amplify the targeted region of viral genome. The PCR assays using this primer confirmed the presence of virus. Absence of amplification in some symptomatic samples collected may be due to very low virus titre or probable genomic variation in the viral genome. The amplified PCR products were further sequenced, their identities were compared with the GenBank sequences using BLASTn and confirmed as isolates of SCBV.

The study represents a comprehensive report, to date, of the status of SCBV in major sugarcane growing areas of Kerala and Tamil Nadu. Results from this study provide evidence for the occurrence of SCBV infection in sugarcane fields of Kerala and Tamil Nadu and points to an expanded geographical distribution of SCBV mainly through infected setts/planting material. Several studies have reported oligonucleotide primers for specific detection of SCBV in epidemiological and phytosanitary programs. Although we could detect the virus in most of the symptomatic plants, some of the samples from the symptomatic plants were negative in PCR assay. Hence, we have to establish complete genomic variation in the field population of SCBV to develop assays with great accuracy and reliability. SCBV leaf fleck a minor disease, is now becoming a major issue under field conditions as a result of exchange of germplasm and transport of planting material across the states within India without any domestic quarantine. We have established a grading system to assess SCBV severity for the first time and this will be further helpful to identify the severely affected clones or varieties. Such diseases can be combated through adopting “clean” planting materials for propagation. Also, further focus is needed to eliminate the virus from the host through newer techniques like CRISPR-CAS.

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