Studies on Sugarcane Smut Disease caused by *Sporosorium scitamineum* under Sub- tropical India

ABSTRACT

Sugarcane (*Saccharum spp*.) is an important commercial crop, cultivated across the world in more than 90 countries including India. Diseases in sugarcane are mainly caused by fungi, bacteria, virus, and phytoplasma. Apart from the biotic factors nutritional imbalance is also responsible for various diseases. The major fungal diseases of sugarcane are red rot, smut, and wilt. The Smut of sugarcane is caused by the fungus *Ustilago scitamineum*. The first report of the disease incidence came from Natal, South Africa in 1877. Severe smut infection affects the sugar recovery as well as yield loss ranging from 10 to 70 per cent. The present study includes genotypes/varieties evaluation against smut disease (*Sporisorium scitamineum*) of sugarcane in sub-tropical region of India. The experiments were conducted in field condition during 2022-2023. Some total of 71 genotypes, maintained at ICAR-IISR, Lucknow. Three bud setts of each of the test genotypes were inoculated by dipping them in aqueous teliospores suspension (10^6/ml) for 30 minutes. Based on disease incidence of each genotype, these were categorized in five class intervals i.e., 0-1 (R), 1-10(MR), 10-20(MS), 20-30(S) & >30 % (HS) smut infection. Out of 71 genotypes tested, Forty five (45) genotype were rated as Resistant (R), Eight (8) genotypes were rated as Moderate Resistant (MR), Five (5) genotypes were rated as Moderate Susceptible (MS), and Ten (10) genotypes were rated as Susceptible (S) against smut disease of sugarcane. The genotypes rated resistant against smut of sugarcane can be exploited for development of smut resistant variety of sugarcane whereas rated susceptible genotypes can be exploited as susceptible check for screening against smut of sugarcane.

Key words: Smut, *Sporisorium scitamineum,* Sugarcane, evaluation and genotypes.

INTRODUCTION

Sugarcane smut disease caused by *Ustilago scitaminea*, is one of the most severe fungal diseases which causes reduction in cane thickness, intermodal length, and number of millable canes resulting in yield of the crop and affects sugarcane productivity and also leads to significant decrease in sucrose content witnessing reduced sugar recovery. The first report of the disease incidence came from Natal, South Africa in 1877 as reported by [1]. All the sugarcane producing countries have developed protocols for the protection and control of this smut disease [2, 3]. Smut disease of sugarcane causes loss in the yield of sugarcane in upcoming years, especially in dryland and perennial sugarcane [4, 5] and also known as "the death of sugarcane" [6]. The disease can be transmitted with the wind, and its teliospores can spread over a wide range and long distances [7]. A typical symptom of this disease is the development of a whip-like sorus from top of the infected stalks. The infected cane has a curled black whip that varies from a few to tens of centimeters [8]. The whip morphology differs from short to long, twisted, multiple whips etc. This whip-like structure consists of fungal sori which are covered by a thin layer of

the host tissue [9]. Once this thin layer is ruptured, the spores of the exposed sorus are spread by wind and rain [10], spread to other plants and result in a new disease if the environmental condition is favorable for the disease development.

Losses due to smut infection range from 30-40 % in plant crops and even up to 70 % in rations [11, 12]. In India, loss in yield due to smut infection is up to 50 % [13] and cane tonnage loss is recorded due to reduced number of millable canes. James, [14] reported that in a susceptible variety, the smut incidence increases tenfold from plant crop to first rations crop. This disease was responsible for the elimination of many high yielding varieties and major cause of varietal decline of varieties like Co 419, Co 1158, Co 740 and CoS 91269. In Japan, the use of varieties susceptible to smut disease, such as NCo310 and Ni9, caused high yield losses [15]. In China, an average smut infection rate is over 10%, and can reach over 50% in some fields, causing billions of economical loss every year [15]. Ratoon crops are more vulnerable to smut infection rather than plant cane. The yield loss of plant cane in China could reach up to 9%, while in ratoon crop it could up to 11% [17]. The smut disease was reported one of important diseases in Australia causes significant losses in susceptible sugarcane varieties and could lose their yield up to 60% [18].

The best control practice is the use of resistant varieties [10, 19]. Sakaigaichia et al., [15] identified first Japanese wild sugarcanes with high resistance to smut disease and contribute to the improvement of sugarcane breeding program in Japan. Currently, control of smut disease of sugarcane mainly relies on the breeding of resistant cultivars [20]. Disease-resistance breeding is the main way to control diseases in crop [21]. The most effective method of managing the smut disease of sugarcane is via resistant varieties [22]. Cultivation of sugarcane resistant varieties is the most feasible strategy to combat the harms of this devastating disease Rajput et al., [23]. Therefore, the use of resistant varieties is believed to be a promising control method for the sugarcane smut disease [24]. Screening of varieties for the resistance to disease infection is one of the important aspects for the development of resistant varieties. The present study focuses on the evaluation of genotypes/ varieties against smut disease of sugarcane and aimed to identify the resistant sugarcane varieties for smut disease caused by *S. scitamineum.*

MATERIALS AND METHODS

The experiment was conducted at ICAR-Indian Institute of Sugarcane Research, Lucknow, Uttar Pradesh. Sampling site is geographically located on Northern gangetic plain of India between 26.51° North and 80.57° East. Freshly collected smutted whips were air dried by keeping under shade and teliospores were collected in butter paper bags and were stored in desiccators under anhydrous calcium chloride.

Pathogenicity test

Pathogenicity test for smut was performed with smut susceptible variety CoLk 7701. The three budded setts of CoLk 7701 were pre-soaked in smut teliospore suspension (spore load @ 10^6 spores/ ml) for a period of 30 min and planted in 3 rows of 5m length with row to row spacing of 90 cm. The incidence of smut disease was recorded at fortnightly intervals with first record at the time of whip emergence (around 45 days). The total

number of smut infected clumps was also recorded and it was found that the variety CoLk 7701 was highly susceptible and hence a good choice for pathogenicity testing. [25].

Evaluation of genotypes/germplasm/varieties against smut of sugarcane

An experiment with 71 genotype, maintained at Indian Institute of Sugarcane Research, Lucknow, was conducted at different rating scale. Three budded setts of each of the test genotypes were inoculated by dipping them in aqueous teliospores suspension (10^6/ml) for 30 minutes by following the technique given by Srinivasan [26] along with the respective checks/standards for resistant and susceptible categories in separate plot of size 13.5 m^2 (three row of five meter each) with row to row spacing of 90 cm. At post-emergence stage, the crop was regularly observed for emergence of smut whips from the tillers. In the end of June, numbers of smut affected clumps per plot were observed. Thereafter, the final diseases incidence was recorded before harvest of the crop in the month of February. After harvest of each genotypes, the cane yield and average single cane weight were recorded. Based on disease incidence of each genotype, these were categorized in five class intervals i.e., 0-1,1-10, 10-20, 20-30 and >30 % smut incidence as suggested by Alexander [27]. (Table 1).

Table 1: Disease rating scale for smut disease of sugarcane

S. No.	% Infestation	Category
1.	0-1 %	Resistant(R)
2.	1-10 %	Moderately resistant(MR)
3.	10-20 %	Moderately susceptible(MS)
4.	20-30 %	Susceptible(S)
5.	Above 30%	Highly susceptible (HS)

Fig. 1: Sett treatment with smut teliospores suspension

Collection of smut whips and spores

Smutted whips and spores were collected from the ICAR-Indian Institute of Sugarcane Research Lucknow during the month of September, 2022 and studied for the variability amongst the *Sporisorium scitamineum* isolates. Fully developed smut whips were collected. Whips were spread out in a tray and maintained in a drying cabinet at room temperature for ten days. Spores were collected from whips by scraping with a plastic knife, and subsequent sieving through nylon net (1×1mm) to remove plant material. Spores were stored in airtight containers at 4°C for further experimentation. [25].

Fig. 2: Whip formation in smut infected sugarcane

Isolation of smut pathogen

Smut samples were collected from experimental farm of ICAR- Indian Institute of Sugarcane Research, Lucknow and scraped out from the infected portion in the laboratory under sterile condition. Collected spores were stored in sterilized airtight containers. Spores were placed in Petri plates containing PDA medium under sterilized condition and incubated for 5-6 days at 27°C [25].

Morphological characterization of *Sporisorium scitamineum*

Morphological studies of the isolates of smut pathogen was conducted to find out the size and shape of the spore. The sugarcane smut pathogen *Sporisorium scitamineum* was characterized through lacto phenol cotton blue wet mount and examined under compound microscope at 40x and 100x. The fungal morphological characters like teliospores shape, teliospores size (length and width) were also studied [28].

Radial growth rate of host pathogen CoLk 7701 on PDA media at different temperatures

Smut isolate of CoLk 7701 was cultured on PDA agar plates to study the effect of different temperature conditions on the radial growth. Isolate of host pathogen CoLk 7701 was used for the study. 5 mm disc of 7 days old culture of smut pathogen was placed in the middle of the Petri plate containing PDA media and incubated at three different temperatures 25ºC, 30ºC and 35ºC. The colony diameter of the fungus was recorded after 24 h, 48 h, 72h, 96h, 120h, 144h and 168h of time interval as represented in the table 4.

RESULT AND DISCUSSION

Evaluation of different genotypes/ varieties

The study on genotypes/varieties evaluation against smut disease (*Sporisorium scitamineum*) of sugarcane in sub-tropical region in India had been conducted under randomized block design in field condition during 2022-2023 by following seed sett inoculation techniques for pathogen. The symptoms were easily recognized by the formation of a whip-like structure at the top of the sugarcane stalk. After the first symptom emerged, the number of the infected stalk was then recorded. Sum total of 71 genotypes were subjected for field experiments. The result revealed from the table 2 that out of 71genotypes tested against smut of sugarcane, 45 genotype *viz.*, LG 19006, LG 19100, LG 19043, LG 19171, LG 19005, LG 19063, LG 19109, LG 19123, LG 19025, LG 19039, LG 19036, LG 19066, LG 19087, LG 19142, LG 19037, CoLk 19201, CoLk 19202, CoLk 19203, Co 19016, CoPb 19211, CoPb 19212, CoPant 19221, CoS 19231, CoH 19261, CoS 17231, Co 19017, Co 19018, CoPb19182, CoPb 19213, CoPb 19214, CoPant 19222, CoS 19232, CoS 19233, CoS 19235, CoH 19262, CoPb 18213, CoS 18214, CoS 18231, CoS 18232, CoLk 17204, CoPant 17215, CoS 17234, CoS 17236, CoH17261 and CoH 17262 were rated as Resistant (R) genotypes against the smut of sugarcane disease. Eight (8) genotypes viz., LG 19101, LG 19003, LG 19015, CoPb 19181, CoPb 18181,CoLk 19204, CoPb 17215 and CoPb 17235 were rated as Moderate Resistant (MR) against the smut of sugarcane disease. Five (5) genotypes viz. LG 19165, LG 19033, CoLk 18202, CoS 19234 and CoS 18233 were rated as Moderate Susceptible (MS) against the smut of sugarcane disease. Ten (10) genotypes viz., LG 19107, LG 19103, LG 19158, LG 19096, LG 19049, LG 19104, LG 19097, CoS 17232, Co 18202 and Co 17018 were rated as Susceptible (S) against smut of sugarcane disease. The genotypes rated resistant against smut of sugarcane can be exploited for development of smut resistant variety of sugarcane whereas rated highly susceptible genotypes can be exploited as susceptible check for screening against smut of sugarcane.

Whip smut has the potential to cause substantial losses in susceptible sugarcane cultivars, therefore varieties under cultivation should be replaced with resistant sugarcane cultivars [23]. A study conducted by Sumedha Thushari [29] in Sri Lanka revealed that out of 455 entries artificially infested with *Sporisorium scitamineum*, 124 were found free from smut infection, including 86 hybrids, 16 of *Saccharum spontaneum* and 16 cultivars of Erianthus arundinaceus. Sakaigaichi et al., [15] studied to identify Japanese wild sugarcane accessions with high resistance to smut disease. Thirty wild sugarcane varieties and three sugarcane cultivars were tested. The results obtained from the inoculation tests aided in identifying wild sugarcanes i.e., JW90, Iriomote8, and Iriomote15 with high resistance to smut disease. The highly resistant wild sugarcane accession had a much better impact on progeny distribution of smut resistance as compared to the susceptible accession. The study conducted by Hidayah et al., [24] revealed that out of 41 mutants, 11 of them appeared highly resistant when buds were exposed to smut pathogen *Sporisorium scitamineum* before planting.

Table 2: Evaluation of different genotypes/ varieties against smut disease of sugarcane under field condition.

S. No.	Genotypes tested	Total no. of clumps observed	No. of Infected clumps				Maximum score on out of four	% infection
			2nd May	2nd Jun.	2nd Nov.	2th Dec.		
1.	LG 19104	18	0	5	4	5	5	5.55
2.	LG 19096	16	1	5	5	3	5	6.25
3.	LG 19107	24	2	6	5	4	6	4.17
4.	LG 19039	14	0	0	0	0	0	0.0
5.	LG 19105	16	1	5	4	3	5	6.25
6.	LG 19097	7	0	4	4	4	4	14.28
7.	LG 19015	18	0	1	2	2	2	5.55
8.	LG 19025	6	0	0	0	0	0	0.0
9.	LG 19006	2	0	0	0	0	0	0.0
10.	LG 19100	3	0	0	0	0	0	0.0
11.	LG 19171	7	0	0	0	0	0	0.0
12.	LG 19049	3	0	3	3	2	3	33.33
13.	LG 19043	13	0	0	0	0	0	0.0
14.	LG 19136	13	0	5	4	4	5	7.69
15.	LG 19109	10	0	0	0	0	0	0.0
16.	LG 19123	9	0	0	0	0	0	0.0
17.	LG 19005	9	0	0	0	0	0	0.0
18.	LG 19017	6	1	5	5	3	5	16.67
19.	LG 19003	23	0	1	1	1	1	4.34
20.	LG 19101	24	1	1	1	1	1	4.16
21.	LG 19063	23	0	0	0	0	0	0.0
22.	LG 19103	12	3	5	4	4	5	8.33
23.	LG 19087	24	0	0	0	0	0	0.0
24.	LG 19165	8	1	0	1	2	2	12.5
25.	LG 19066	18	0	0	0	0	0	0.0
26.	LG 19142	7	0	0	0	0	0	0.0
27.	LG 19037	12	0	0	0	0	0	0.0
28.	LG 19158	2	1	2	2	1	2	50.0
29.	LG 19036	4	0	0	0	0	0	0.0
30.	LG 19033	16	1	3	2	3	3	6.25
31.	Co 17018	17	1	0	0	0	1	5.88
32.	CoLk 17204	22	2	3	2	3	3	4.54
33.	CoPb 17215	14	0	2	1	1	2	7.14
34.	CoPant 17233	24	0	0	0	0	0	0.0

35.	CoS 17234	14	0	0	0	0	0	0.0
36.	CoS 17235	26	1	1	2	2	2	3.84
37.	CoH 17261	9	0	0	0	0	0	0.0
38.	CoH 17262	7	0	0	0	0	0	0.0
39.	CoS 17236	8	0	0	0	0	0	0.0
40.	CoPb 18181	40	2	1	2	2	2	2.5
41.	CoS 17232	10	2	1	1	0	2	10.0
42.	CoLk 18202	18	2	3	4	4	4	5.55
43.	CoS 17231	14	0	0	0	0	0	0.0
44.	Co 18022	10	0	0	0	0	0	0.0
45.	Co 18234	35	0	0	0	0	0	0.0
46.	CoS 18231	11	0	1	0	1	1	9.09
47.	CoS 18232	31	0	0	0	0	0	0.0
48.	CoS 18233	10	1	1	1	1	1	10.0
49.	CoPb 18213	20	0	0	0	0	0	0.0
50.	Co 19016	23	0	0	0	0	0	0.0
51.	CoPb 19181	30	0	1	1	1	1	3.33
52.	CoLk 19201	9	0	0	0	0	0	0.0
53.	CoLk 19202	4	0	0	0	0	0	0.0
54.	CoLk 19203	8	0	0	0	0	0	0.0
55.	CoPb 19211	12	0	0	0	0	0	0.0
56.	CoPb 19212	1	0	0	0	0	0	0.0
57.	CoPant 19221	11	0	0	0	0	0	0.0
58.	CoS 19231	12	0	0	0	0	0	0.0
59.	CoH 19261	12	0	0	0	0	0	0.0
60.	Co 19017	13	0	0	0	0	0	0.0
61.	Co 19018	17	0	0	0	0	0	0.0
62.	CoPb 19182	13	0	0	0	0	0	0.0
63.	CoLk 19204	15	0	1	1	1	1	6.67
64.	CoPb 19213	31	0	0	0	0	0	0.0
65.	CoPb 19214	37	0	0	0	0	0	0.0
66.	CoPant 19222	19	0	0	0	0	0	0.0
67.	CoS 19232	14	0	0	0	0	0	0.0
68.	CoS 19233	15	0	0	0	0	0	0.0
69.	CoS 19234	17	0	2	2	1	2	5.88
70.	CoS 19235	14	0	0	0	0	0	0.0
71.	CoH 19262	17	0	0	0	0	0	0.0
*	CoLk 7701	18	0	0	0	1	1	34.00

*Standard check for smut of sugarcane

Morphological characterization of *Sporisorium scitamineum*

Sporisorium scitamineum spore width measurement studies revealed that Spherical shape of *Sporisorium scitamineum* was recorded with the isolates of the sugarcane host variety CoLk 7701 spore length of 1.52 µm and spore width of 0.44 µm was recorded (Table 3). Similar study was conducted by Singh et al., [25] for 10 test isolates of *Sporisorium scitamineum* and found great variation in spore length and width of the different test isolates. Maximum spore length of 1.58 µm and spore width of 0.45 µm was recorded and spherical shape of *Sporisorium scitamineum* was recorded in all the isolates. The existence of physiological specialization has been demonstrated by Alexander and Padmanaban, [30] and Amire et al., [31]. Classification of races of *U. scitaminea* was based on differences in spore morphology, germination characteristics or pathogenic nature [32]. The pathogen develops systemically throughout the stalk, but teliospores are formed only in peripheral tissues of the whip-like structure. The fungus is capable of mutating and hybridizing in nature in order to produce new virulent pathogenic races [33].

Table 3: Morphological characterization of smut teliospores of CoLk 7701

S.No.	Isolate	Shape	Spore length (µm)	Spore width (µm)
1.	*Sporisorium scitamineum*	Spherical	1.52	0.44

Radial growth rate of host pathogen CoLk 7701 on PDA media at different temperatures

Optimization of physiological condition with special reference to temperature and variability of isolate of CoLk 7701 was studied under completely randomized block design with three replications. The results of experiment conducted at 25°C, 30°C and 35°C temperature (Table 4) revealed that isolates CoLk 7701 recorded fastest growth rate attaining 1.1 cm maximum radial growth at 30°C followed minimum of 0.6 cm at 30°C at 24 h duration. Whereas at full plate growth was recorded for temp 30°C at 120h. A similar kind of study was conducted by Singh et al., [25] and found that out of 10 isolates A-4 recorded the fastest growing rate followed by the slowest growing isolates A-5 at 25ºC temperature.

Table: 4 Colony growth rate of host pathogen CoLk 7701 on PDA media at different temperatures

Temp.	Radial mycelial growth rate of colony in cm					
	24 h	48 h	72 h	96 h	120 h	144 h
25°C	1.0	1.8	2.7	3.5	4.1	4.5(F)
30°C	1.1	2.2	3.0	4.0	4.5(F)	-
35°C	0.6	1.3	2.0	3.1	3.9	4.5 (F)

CONCLUSION

The genotypes/varieties rated resistant against smut disease of sugarcane can be exploited for development of smut resistant variety of sugarcane whereas genotypes/varieties rated

susceptible genotypes can be exploited as susceptible check for screening against smut disease of sugarcane. And the development of resistant varieties is the eco-friendly way to control the smut disease of sugarcane.

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