International Journal of Science and Research (IJSR)
ISSN: 2319-7064
ResearchGate Impact Factor (2018): 0.28 | SJIF (2019): 7.583

Structure of *Sporisorium Scitamineum* Isolates, the Causative Agent of Sugarcane Smut in Côte D'ivoire

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Abstract: ***The aim of this work was to assess the macroscopic and microscopic characteristics and the variability between isolates of Sporisorium scitamineum present in Côte d'Ivoire. Smut whip samples were collected in 2017 and 2018 in Côte d'Ivoire from four agro-industrial complexes. The fungus was isolated out on PDA medium at the Laboratory. The morphological characters were described on the basis of the growth diameter, texture and colonies colors. The microscopic description focused on the spore's size from medium and teliospores from whips. An analysis of variance was applied to the measured parameters and the means comparison was performed according to the Newman-Keuls test (post-hoc ANOVA test) at the significance level of 5% using the STATISTICA 7.1 software. Macroscopic results indicated two groups of isolates. Group 1 contains 85% of the isolates and characterized by a white mycelial colony with a cottony appearance. The G2 group comprising 15% of the isolates and marked by a yeast-shaped mycelial colony. The diameter of the teliospores varied between 8.36 µm and 10.25 µm. The size of the spores varied between 9.14 and 11.65 µm. This study made it possible to highlight the variability between the isolates of Sporisorium scitamineum in Côte d'Ivoire.***

Keywords: Smut disease, Structures of isolates; *Sporisorium scitamineum*; Sugarcane; Côte d'Ivoire

1. Introduction

Sugarcane, *Saccharum officinarum* is a Poaceae, cultivated for the sugar contained in its stems **[1]**. It is cultivated in about 109 countries in the tropical and subtropical regions of the world **[2]**, on more than 26.9 million hectares of land. It contributes more than 60 per cent of global sugar production **[3]**. It plays a considerable role in the world economy, in terms of agri-food **[4]**. In recent years, sugarcane has been used for the production of ethanol, biofuels and energy **[5]**. In Côte d'Ivoire, it is mainly cultivated for sugar production, estimated at 214,000 tons per annum. It contributes about 0.9% of GDP and 3.3% of the agricultural sector. Unfortunately, plantations are attacked by several fungal pests, including *Sporisorium scitamineum* **[6]**, described as an agent of smut. This disease was reported in 1877 in Natal, South Africa **[7]**. It spread very rapidly in all sugarcane producing countries causing numerous yield losses ranging from 30 to 50% for susceptible varieties **[8]**; **[9]**. Control methods are mainly based on the use of resistant varieties. However, some authors have reported that the disease is difficult to control because the pathogen sometimes produces new physiological strains to overcome the host resistance mechanism **[9]**; **[10]**. To this end, several physiological strains have been reported in several countries including Hawaii, Taiwan, Brazil, and Pakistan **[10]**. These strains have been reported on the basis of morphological characters **[11]**; **[12]**. In the past in Côte d'Ivoire, knowledge on isolate strains has been limited to the study made by **[13]**. Therefore, as smut is and continues to be a threat to sugarcane cultivation, it seems necessary to initiate a study to assess the physiological and morphological variability of the pathogen in order to prevent the risk of an epidemic. This study carried out in the laboratory aims to evaluate the morphological and microscopic variability between isolates in order to develop against this pathogen, an effective control strategy at low cost without risk of contamination for the user, the environment and production.

2. Material and methods

2.1 Plant Material

The research was carried out on four sugar complexes in Côte d'Ivoire in 2017 and 2018. The Integrated Agricultural Unit (IAU) of Zuénoula is located between 7°30 and 7°40 north latitude, and between 6°50 and 6°15 west longitude; The Integrated Agricultural Unit (IAU) of Borotou-Koro is located between 08°31 north latitude and 7°17 west longitude. The two sugar perimeters of Ferké1 and 2 are located in the north of Côte d'Ivoire between 9°20' and 9°60' north latitude and between 5°22' and 5°40' west longitude respectively. Surveys were carried out in the four production areas. Nursery plots and industrial plantations were visited. Samples of charcoal whips were collected from the sugarcane varieties encountered and stored in plastic bags. A collection of 556 whips of *Sporisorium scitamineum* was collected from different varieties and stored in the laboratory at room temperature. Also, in order to highlight the variability among isolates, a collection of nine (09) samples was collected specifically from the sugarcane varieties mainly grown in the different zones and coded **(Table I)**.

Table I: Isolates taken from different varieties in four production zones

Locations	Plots	Varieties	Isolate codes	Symptoms	Geographical coordinates
Zuénoula	PIV39D	R570	R570Z	Whip	07°36.575'N 006°11.852W
Zuénoula	A47	CO997	CO997Z	Whip	07°37.555'N 006°09.375'W
Zuénoula	A54	R570	R570BK	Whip	07°37.955'N 006°10.393'W
Ferké 2	P3-53	SP711406	R93/2140Y	Whip	09°20.336'N 005°20.491'W
Borotou-Koro	Piv16d	R93/2140	SP711KD	Whip not emerged	08°27.530'N 007°09.747W
Borotou-Koro	36C	R97/6177	R97/6177K	Whip	08°27.953'N 007°11.434'W
Borotou-Koro	E68	CO997	CO997E	Whip	08°29.928'N 007°15.982'W
Ferké 1	B3-12	SP701006	SP711M	Whip	09°30.813'N 005°16.616'W
Borotou-Koro	Piv7D	NCO376	NCO376B	Red Whip	08°30.731'N 007°16.851'W

2.2 Isolation and identification of strains

The whips were cut into 1 cm fragments using a sterilized scalpel at a rate of eight explants per sample. These pieces were disinfected with 70% alcohol for 1 minute, followed by soaking in 5% sodium hypochlorite for 03 min **[14]**. These fragments were thoroughly rinsed with sterile distilled water. Using sterile forceps, the explants were removed and inoculated onto PDA medium in Petri dishes and sealed with stretch film before being kept in the dark for 5 days at 25 ± 2°C **[14]**. After 7 days of incubation, mycelial strands of the fungus at the growing front were removed with a punch and transplanted on PDA medium into a Petri dish to obtain a pure colony **[15]** Strains were identified on the basis of their cultural traits described by **[16]** and by referring to the identification key of **[17]** charter. The strain isolation rate was calculated according to localities according to the following formula: $Tx\ (\%) = \frac{Ni*100}{Nt}$**Tx**

Isolation rate; **Ni**: the number of samples from which isolations were made; **Nt**: the total number of samples.

2.3 Morphological observation of isolates

The description of the crops was done according to the method described by **[14]**. based on the cultural aspects of the strains. These were the texture and colouring of thallus **[16].**

2.4 Microscopic observation of isolates

The microscopic description was carried out according to the method described by Rosalyn *et al.,* (2006) **[18]**. The size of spores from the cultures and the diameter of teliospores from the carbon whips were evaluated. A teliospore suspension was prepared in test tubes from the carbon whips. Using a computer-coupled camera, the diameters of 50 teliospores were measured and repeated 4 times for each isolate, i.e. 200 teliospores/strain **[18]**. The same methodology was adopted for the measurement of spores. After 15 days of culture, a spore suspension was prepared in the test tubes. From this solution, the sizes of 50 spores were taken at a rate of 4 replicates per suspension, 200 spores/strain **[18]**. Spore and teliospore shapes were also describe

2.5 Statistical analyse

An analysis of variance was applied to the measured parameters and the comparison of the means was performed according to the Newman-Keuls test (ANOVA post-hoc test) at the 5% significance level using the STATISTICA 7.1 Software. This was followed by a Principal Component Analysis (PCA) using the same software.

3. Results

3.1 Isolation rates by locality

Table II presents the isolation rates of *Sporisorium scitamineum* by locality. Statistical analysis indicated a significant effect of locality on the isolation rate of isolates ($P < 0.05$). The localities of Zuénoula and Ferké 2 had the highest isolation rates with 52.85% and 52.63% respectively. As for the Borotou-Koro zone, the isolation rate was the lowest with 37.63% or 102 isolates. The locality of Ferké1 showed an intermediate rate of isolation with 43.47% (10 isolates). On average, the isolation rate was estimated at 45.31% or 261 isolates obtained.

3.2 Macroscopic description of the culture on PDA medium of *Sporisorium scitamineum*

Macroscopic description of the isolates showed two groups according to texture, box coloration and growth topography. Group 1 (G1) represents about 85% of the collected isolates. Isolates are characterized by abundant mycelial texture with white coloration on the upper surface of the petri

Table II: Isolation rate of *Sporisorium scitamineum* in different localities

Locations	Total samples	Isolation rate (%)	Total isolates obtained
Borotou-Koro	271	37,63 %	102
Zuénoula	263	52,85 %	139
Ferké 1	23	43,47 %	10
Ferké 2	19	52,63 %	10
Total	576	45,31%	261

Means followed by the same letters in the same row are not significantly different at the 5% threshold according to the Newman-Keuls test dish and light yellow on the reverse side of the dish **(Table III)**. The second group (G2) comprises 15% of the collected isolates. This group is characterized by a sparse mycelial texture with a yellow coloration on both sides of the petri dish and is yeast-like. Both groups showed flat and radial growth **(Table III).**

3.3 Microscopic description of spores and teliospores of *Sporisorium scitamineum*

Microscopic description of the isolates revealed that spore length and teliospore diameter vary between isolates. All the spores obtained on culture medium are cylindrical in shape. Among the G1 group isolates, spore length ranged from 9.2 µm to 12.42 µm, i.e. an average of 10.61 µm. For group G2, spore length ranged from 9.68 µm to 12.07 µm, with an average of 10.43 µm (Table IV). Teliospore diameter ranged from 7.33 µm to 10.25 µm with an average of 8.85 µm. Teliospores are circular in shape with a thick wall, the diameters of which varied according to the isolates collected

Table III: Macroscopic characteristics of *Sporisorium scitamineum* isolates

		Macroscopic description			
Groups	Proportion of isolates	Texture	Colour		Topography
			Surface	Reverse	
Group 1	(85 %)	Abundant Aerial mycelium	White Light	yellow	Flat growth
Group 2	(15 %)	Short aerial mycelium	Light yellow	Light yellow	Flat growth

Two groups were formed; in group G2, the length of teliospores ranged from 7.35 to 10.30 µm with a mean of 8.80 µm **(Table IV).**

3.4 Studies of the morphological variability of strains collected from different sugarcane varieties

Analysis based on colony texture and colour revealed two types of *Sporisorium scitamineum* strain colonies. Among the isolates, more than 77.77% showed a very abundant mycelial texture of white on the upper surface and yellow on the reverse side of the culture dish and were formed by strains R580Z, R93/2140Y, R97/6177K, SP711KD, R570BK, CO997E and NCO376B (Figure 1). Whereas, strains coded by CO997Z and SP711M accounted for 22% of the isolates and showed a sparse mycelial texture with a yellow coloration on both sides similar to yeast **(Figure 1)**. Analysis of the mean colony growth of the strains indicated a significant difference ($P < 0.05$), as shown in Table V. Based on their radial growth diameter, the strains were classified into three groups. The first group has rapid growth and is characterized by a growth diameter between 7.70 cm and 7.25 cm; this group is composed of seven strains coded R580Z, CO997Z, R93/2140Y, R97/6177K, 570BK, CO997E and NCO376B. The second group has intermediate growth, with an average growth diameter of 7.0 cm composed by the coded strain SP711M. Finally, the third group called the slow growing group is marked by an average growth diameter of 6.40 cm and comprises the coded strain SP711KD **(Table V).**

Table IV: Microscopic characteristics of spores and teliospores of *Sporisorium scitamineum*

	Teliospore diameter (µm)		
Groups	Minimum	Maximum	Medium
Group 1	7,33 ± 0,39a	10,25 ±1,30a	8,85 ±1,2a
Group 2	7,35 ± 0,42a	10,30 ±1,32a	8,80 ±1,24a
	Spore length (µm)		
Groups	Minimum	Maximum	Medium
Group 1	9,21 ±1,06a	12,42 ±3,64a	10,61 ±2,32a
Group 2	9,68 ±1,35a	12,07±1,52a	10,43 ±2,13a

The means followed by the same letters in the column are not significantly different at the 5% threshold according to the Newman-Keuls test.

Table V: Morphological characteristics and mycelial growth diameters of isolates

Isolate codes	Colony staining Surface / Reverse	Average Growth Diameters of colonies (cm) at 28°C
R580Z	White / Light yellow	7.40±0.14a
CO997Z	Yellow / Yellow	7.30±1.13a
R93/2140Y	White / Yellow	7.25±0.35a
R97/6177K	White / Yellow	7.25±0.35a
SP711KD	White / Yellow	6.40±0.14b
R570BK	White / Yellow	7.25±0.35a
CO997E	White / Light yellow	7.40± 0.14a
SP711M	Yellow / Yellow	7.0± 0.7ab
NCO376B	White / Yellow	7.70± 0.28 a

The means followed by the same letters in the column are not significantly different at the 5% threshold according to the Newman-Keuls test.

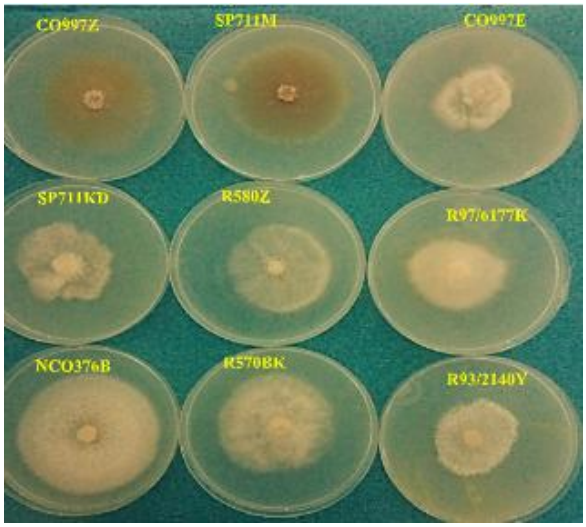

Figure 1: Morphological aspects of isolates after 5 days of culture

3.5 Studies of microscopic variability between *Sporisorium scitamineum* strains collected from different varieties

The analysis of variance shows a highly significant strain effect ($p \leq 0.01$) for the diameter of the teliospores **(Figure 2).** Two groups of isolates were observed based on teliospore size. Isolates R580Z and SP711KD had the largest teliospore diameters with values of 10.17 µm and 10.25 µm, respectively. Isolates coded as CO997Z, R570BK, R93/2140Y, R97/6177K, CO997E and SP711M had the smallest teliospore diameters ranging from 8.36 µm to 9.12 µm. Spore length analysis indicated a very significant

difference ($p \leq 0.01$) between isolates and allowed the isolates to be classified into two groups. Isolates coded CO997Z, R570BK, R93/2140Y, CO997E and SP711M obtained the highest spore sizes ranging from 10.41 µm to 11.65µm. Isolates coded as R580Z, R570BK, R97/6177K and NCO376B had the smallest spore sizes with values ranging from 9.14 to 10.17 µm.

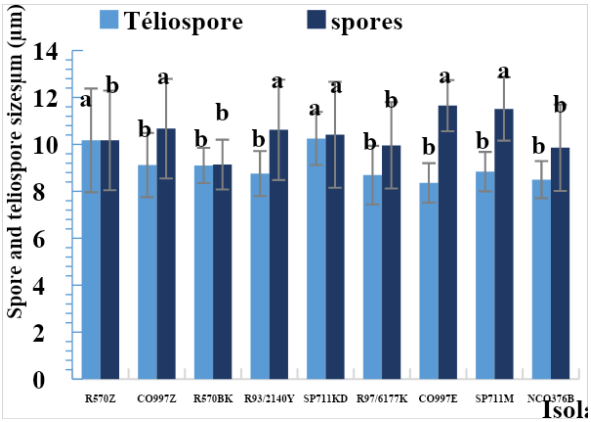

Figure 2: Average size of different strains *Sporisorium scitamineum*

3.6 Correlation between isolates

Principal Component Analysis (PCA) showed that factor 1 (axis 1) contributes 55.85% of the total variance and factor 2 (axis 2) contributes 34.64% of this variability **(Figure 3).** Teliospore diameter has a negative correlation with both factors F1 and F2 with coordinates -0.9 and -0.1. Spore size is positively correlated with the two factors F1 and F2 with 0.31 and 0.82 as correlation coefficients, respectively. As for growth diameters and phytosanitary parameters (disease incidence), they showed a positive correlation with the F1 factor and a negative correlation with the F2 factor with (0.62 and-0.64) and (0.75 and -0.32) respectively. The dispersal pattern of PCR individuals thus revealed four groups of strains **(Figure 3)**:

The first group consists of strains R580Z and R93/2140Y which showed a negative correlation with the two factors (F1 and F2). This group is characterized by large-diameter teliospores,

The second group consists of the strains coded Co997E and SP711M. They have recorded a positive correlation with both factors and are distinguished by large spore sizes, strains Co997Z and SP711KD form the third group with a negative correlation with the F1 factor and a positive correlation with the F2 factor. They are characterized by rapid mycelial growth with a high incidence of smut, finally, strains R570BK and NCo376B form the fourth group, characterized by a positive correlation with factor F1 and a negative correlation with F2. These strains are characterized by large spore sizes, with strong mycelial growth and a high rate of infection. It is also characterized by small diameter teliospores.

4. Discussion

The structure of isolates of *Sporisorium scitamineum*, an agent of smut, was studied. The localities of Zuénoula and Ferké 2 obtained the highest rates of isolation of fungal strains. Also, the morphological study, based on the appearance and coloration of the colonies, showed a significant difference between the colony growth diameters of the *Sporisorium scitamineum* strains. This study identified two types of *Sporisorium scitamineum* strains with white mycelial colonies and yellow yeast-like colonies. These results are in agreement with those **[14]** who found a difference between nine strains of *Sporisorium scitamineum* in Kenya with white and yellow yeast-like mycelial colonies. In addition, our results corroborate those of **[19].** who reported the existence of six all-white and yellow strains at the front and back of petri dishes. However, the studies of **[20]** contradict our results. These authors reported the existence of three types of *Sporisorium scitamineum* colonie. Teliospore analysis revealed strains with large teliospores with diameters ranging from 10.17 µm to 10.25 µm, and strains with small teliospores with diameters ranging from 8.36 µm to 9.12 µm. The spores obtained showed two forms. Long spores from 10.41 to 11.65 µm long and short spores from 9.14 to 10.17 µm long were observed. These results are similar to those obtained by **[18]** in the Philippines. However, the observed spore sizes do not agree with those obtained by **[19]** in Kenya. This author obtained smaller spore and teliospore sizes between 6.46 and 7.22 µm

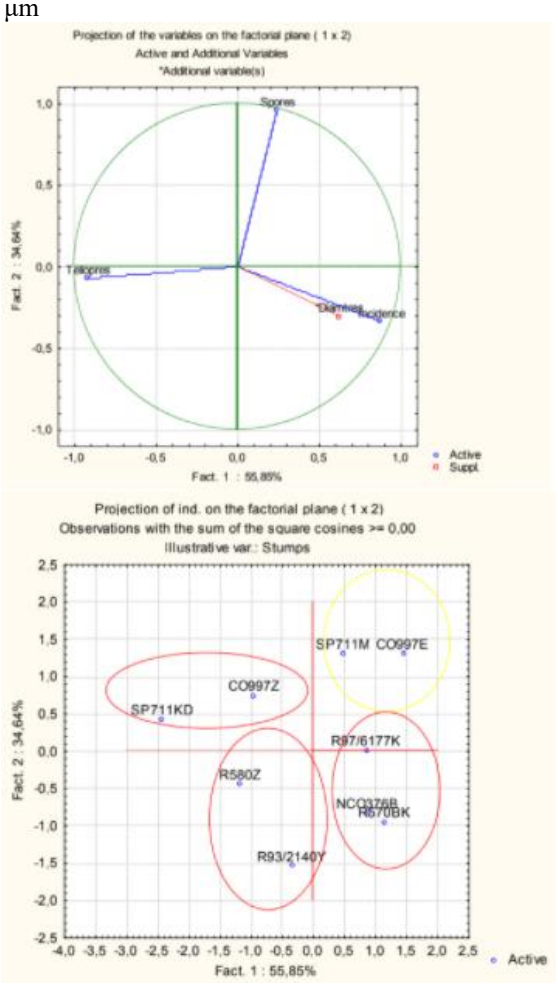

Figure 3: Circle of correlation and strain dispersion Groups of *Sporisorium scitamineum* obtained in Côte d'Ivoire

This physiological difference between strains can be correlated with changes in the genome of *Sporisorium scitamineum* (**[20]**, 1983; **[10]**. To this end, several countries have reported the presence of physiological strains of *Sporisorium scitamineum* (**[10]**. As for the work of **[9]**, they demonstrated that this variability of the pathogen may be due to the appearance of new physiological races over time. Moreover, it is also due to the influence of the climatic and environmental conditions of the area. This is in line with the work of **[21]** and **[22]** who demonstrated the effect of the environment in the variation of *Sporisorium scitamineum* strains. This shows that there may be variation in spore size that could be related to the strain or due to the strain's adaptation to the environment. To this effect, our results confirmed a variation between strains depending on sugarcane varieties and production areas. Two types of colonies were thus identified in Côte d'Ivoire. **[23]** demonstrated with similar results that the pathogen sometimes produces new physiological strains to overcome the host resistance mechanism. However, the work of **[10]** and **[24]** demonstrated using AFLP markers that there is a very low level of overall genetic variability except for the few isolates from South-East Asia. Thus, a further confirmatory study should be carried out using molecular diagnostic techniques. Our results showed that there could be variation between strains of *Sporisorium scitamineum* in Côte d'Ivoire. This could be a risk in the event of a serious epidemic, and methods for sustainable control of this pathogen should be considered.

5. Conclusion

This study allowed us to demonstrate that there is structural and physiological variability between isolates of *Sporisorium scitamineum* in Côte d'Ivoire. Based on morphological and microscopic description, differences were observed between isolates according to sugarcane varieties and production areas. This could be a risk in case of a severe epidemic. However, a further confirmatory study should be carried out using molecular diagnostic techniques and consideration should be given to establishing sustainable control methods for this pathogen.

6. Acknowledgement

We would like to thank the African Centre of Excellence on Climate Change, Biodiversity and Sustainable Agriculture (ECA-CCBAD) for funding this research. We are also grateful to the sugar companies, SUCAF-CI and SURCRIVOIRE, for having authorized the prospecting in their sugarcane plantations.

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