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Fungal Profile of Sugar Cane (*Saccharum Officinarum* L.) Pathogens Produced In Côte D'ivoire

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Sugar cane occupies an important place in the Ivorian economy. However, it is subject to fungal attacks during its production, leading to enormous economic losses for farmers and manufacturers. The aim of this work was establish profile of pathogenic fungal strains of sugar cane in Côte d'Ivoire. To do this, 250 sugarcane samples composed of leaves, stems and shoots of sugarcane presenting pathologies were collected, then phenotypic and molecular identifications of the pathogenic fungal strains were carried out by PCR followed by sequencing. After identification, a pathogenicity test was carried out to demonstrate the involvement of these fungal strains in sugar cane pathologies. Three (3) fungal species including *Fusarium verticillioides, Fusarium subglutinans* and *Schizophyllum commune* were identified. *Fusarium verticillioides* is believed to be the agent responsible for red movre disease and *Fusarium subglutinans* responsible for "Pokkah Boeng" disease. The fungus *Schizophyllum commune* is thought to be responsible for anthrax.

Keywords: *Fusarium subglutinans; Fusarium verticillioides;* Pathogen; PCR; Sugar cane*; Schizophyllum commune*.

Ivorian sugar production amounts to 330,000 t. The cultivation of sugar cane is subject to several constraints. The pathologies associated with this crop are caused by viruses, stem-boring insects, bacteria and fungi[1]. The monoculture practiced for several years in the sugar complexes leads to a decline in soil fertility. All of these factors contribute to a drop in production[2]. Biotic constraints have caused reductions in yield in the sugar industries of Côte d'Ivoire, notably those of Borotou-Koro and Zuénoula. Fight against these pathogens, pesticides, especially synthetic fungicides such as benzimidazoles, triazoles and strobilurins, are mainly used. However, the excessive use of these products in sugar cane plantations could encourage the appearance and proliferation of resistant strains, leading to an increase in diseases[3]. Faced with these harmful effects, it is essential to precisely identify and know the pathogenic fungal profile, in order to effectively fight against these phytopathogens responsible for serious pathologies. The fungal agents responsible for these diseases are often unknown. It is within this framework that this study falls, the general objective of which is to establish the profile of pathogenic fungal strains of sugar cane in Côte d'Ivoire.

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MATERIALS AND METHODS

Material

Organs (stems, leaves and shoots) of sugar cane presenting pathologies were collected in the village plantations of Borotou-koro (08°31 North latitude and 7°17 West longitude) and Zuénoula (7°30 and 7°40 North latitude, and between 6°50 and 6°15 West longitude) in Côte d'Ivoire.

Sampling

Samples of shoots, leaves and stems showing symptoms of pathology were collected. Thus, 5 samples consisting of 5 leaves, 5 stems and 5 shoots presenting pathologies were collected per plantation. Or 50 samples at a rate of 25 samples per production area. The collected samples were packaged in sterile stomacher bags, labeled, sealed then kept in a cooler containing cold accumulators where the temperature was from 4°C.

Phenotypic identification of pathogens

Isolation was carried out according to the method of Silué et al[4]. Thus, the sugarcane organ fragments were sterilized in ethanol (70%) during one minute, immersed in 3% bleach for 3 minutes, and rinsed with sterile distilled water three times. The fragments collected were inoculated by direct contact on medium Potato Dextrose Agar with Chloramphenicol. The culture were then incubated at 27±1°C for 24 to 72 hours. Macroscopic identification was carried out according to the method of Botton et al[5] by examining the culture. The cultural characteristics determined were the texture, the color of the thallus and the color of the underside of the crop. As for the microscopic identification, it was carried out according to the method described by Guiraud[6] taking a filament using sterile tweezers then placed in a drop of methylene blue, placed on object slide and covered with a coverslip then observed under a LEICA DM750 optical microscope with an X40 objective. The characteristics observed were the appearance of the mycelium (compartmentalized or not), the presence and shape of the spores (oval, spherical, round, etc.), the shape of the conidial heads and the size of the conidiospores (short or long).

Molecular identification of pathogens

DNA extraction

Fungi colonies were collected and bubbled in 1 ml of sterile water. The suspension thus obtained is centrifuged at 12,000 rpm for one minute. The pellet of this suspension was collected and suspended in 200 µl of Chelex 100 (Bio-Rad) previously prepared following to the manufacturer's instructions. The new suspension obtained was homogenized using a vortex for ten seconds. Heating in a water bath at 100°C for eight minutes was carried out followed by sudden cooling in ice. This thermal shock allows the lysis of cells and the release of DNA. A new homogenization step using a vortex for ten seconds was carried out at 12,000 rpm for two minutes. In the base are the proteins captured by the Chelex 100 and in the supernatant is the DNA. DNA has been preserved at -20°C for PCR amplification.

Polymerase Chain Reaction

Primers ITS1 (5'-TCCGTAGGTG AACCTGCGG-3') and ITS4 (5' TCCTCCGCTTATTGATATGC-3') were used. For the preparation of the reaction mixture, an Invitrogen kit was used. This kit consists of Taq DNA polymerase, polymerase buffer and $MgCl_2$[7]. dNTPs and primers were also used. Final volume of amplification reaction was 50 µl. PCR medium was composed of 26.35µL of MilliQ Water, 5µL of ITS1, 2.5 µL of ITS4, 0.25 µL of Taq polymerase (Promega) and 10 µL of template DNA. The amplification reaction was made in a thermal cycler (Techne® Prime Thermal Cycler Range, USA) according to the program described by Bessadat et al[8]. The first step is that of initial denaturation. It is done at 94°C for five minutes. The second stage contains 35 cycles, each of which is composed of a denaturation phase at 94°C for thirty seconds, annealing phase at 55°C for thirty seconds and elongation phase at 72°C. Finally, the third stage is that of the final elongation which is done at 72°C for ten minutes.

Profile visualisation

The PCR profile were revealed by electrophoresis on 1.5% agarose gel. For gel preparation, the agarose was suspended in 50 ml of 0.5X TBE. The suspension was heated and left to room temperature for five minutes then a drop of ethidium bromide was incorporated before pouring it into a gel holder. Once solidified, the gel was immersed in an electrophoresis tank containing 0.5X TBE. 10 µl of amplified fragments were mixed with 2 µl of loading buffer (50% TE, pH 8; 50% glycerol; bromophenol blue) before being loaded into the gel wells. Profile was visualized

using a transilluminator (Vilber Lourmat) under UV illumination after migration at 90 Volts for 90 minutes. The size of the DNA fragments was determined using the Reddy Run Super ladderlow 100 bp molecular size marker (Thermo Scientific) used as a reference.

Sequencing

Sequencing was carried out from the rDNA products amplified with an automated sequencer. The primers used for the amplification of the regions studied were also used as sequencing primers. All DNA sequences obtained during sequencing were combined in a database (GenBank), used for the reconstruction of taxonomic relationships between the species studied. The homology of related sequences was investigated using the bioinformatics search tool BLAST. Multiple sequence alignment was carried out using Clustral W software[9].

Pathogenicity of isolated fungi

Preparation of fungal inoculum

Ten milliliters (10 mL) of sterilized distilled water were added to 14-day-old mycelial cultures of pathogenic fungi. The surface of these cultures was scraped using a sterilized metal spatula. The resulting solution was homogenized in a test tube using a vortex. A few drops (200 µL) of this suspension were used to fill the wells of a Malassez cell in order to estimate the number of spores. This number was adjusted to 10^6 spores/ml by dilution or by increasing the spore concentration of the solution by returning the spore solution to another mycelial culture. One milliliter (1 mL) of a 1% glucose and agar solution is added to the spore suspension before inoculation. The role of this solution is to facilitate the adhesion and germination of spores on the leaves[10].

Infection of sugarcane seedlings with spore suspensions of pathogenic fungi

Using a syringe, the underside of the leaves of sugarcane seedlings was infected with 1 mL of inoculum of the pathogenic fungus. The greenhouse was regularly humidified (twice a day) by watering using a watering can in order to maintain the high humidity level (95 – 100%). The purpose of humidification was to facilitate the germination of spores of the fungal pathogen. The evolution of fungal disease symptoms was monitored regularly for three (3) months. Symptomatic organ samples were collected for the isolation of inoculated pathogenic fungi[10].

RESULTS

Microscopic pathogenic fungi of sugar cane

Sugar cane samples are attacked by molds presenting colonies of various appearances, textures and colors. Thus, 129 fungal isolates were isolated taking into account the resemblance of thalli and spores. Phenotypic identification according to the macroscopic characteristics of the colonies and on the basis of the microscopic characteristics of the mycelium and conidia or spores made it possible to highlight 2 genera, namely *Fusarium* and *Schizophyllum* (Table 1).

Molecular characteristics of sugarcane pathogenic fungal isolates

Sequence analysis made it possible to identify 3 species with similarity rates of 99 % (Table 2). Thus, we obtained *Schizophyllum*

Table 1. Phenotypic characteristics of isolated genera

Genera	Characteristics
Schizophyllum	Rapid growth, a cottony appearance, abundant mycelial texture of white color on the upper side and yellow on the back of the culture box. Septate mycelium and spherical unicellular formations or teliospores.
Fusarium 1	A cottony appearance, a white mycelial texture on the surface and on the back of the box with a flaky appearance. Mycelium is septate with the presence of lunar crescent-shaped conidia.
Fusarium 2	White color on the surface and produces abundant false aerial buds and the back of the box has a flaky appearance. Mycelium is septate with the presence of conidia.
Fusarium 3	Flaky appearance. The mycelium is white in color with the presence of orange-colored spores. During growth, the orange color diffuses across the entire back of the box. The hyphae are septate and branched with the presence of ellipsoidal hyaline conidia

Table 2. Fungi strains identified from sugar cane samples

Groups	Nc	%H	Corresponding species	Sequences
I	606	99.49%	*Schizophyllum commune*	GGAAGGATCATTAACGAATCAAACAAGTTCAT CTTGTTCTGATCCTGTGCACCTTATGTAGTCCC AAAGCCTTCACGGGCGGCGGTTGACTACGTCT ACCTCACACCTTAAAGTATGTTAACGAATGTRA TCATGGTCTTGACAGACCCTAAAAAGTTAATA CAACTTTCGACAACGGATCTCTTGGCTCTCGCA TCGATGAAGAACGCAGCGAAATGCGATAAGTA ATGTGAATTGCAGAATTCAGTGAATCATCGAA TCTTTGAACGCACCTTGCGCCCTTTGGTATTCC GAGGGGCATGCCTGTTTGAGTGTCATTAAATA CCATCAACCCTCTTTTGACTTCGGTCTCGAGAG TGGCTTGGAAGTGGAGGTCTGCTGGAGCCTAA CGGAGCCAGCTCCTCTTAAATGTATTAGCGGAT TTCCCTTGCGGGATCGCGTCTCCGATGTGATAA TTTCTACGTCGTTGACCATCTCGGGGCTGACCT AGTCAGTTTCAATAGGAGTCTGCTTCCAACCGT CTCTTGACCGAGACTAGCGACTTGTGCGCTAAC TTTTGACTTGACCTCAAATCAGGTAGGACTACC CGCTGAACTTAAGCATATC
II	513	100 %	*Fusarium verticillioides*	GGAGGGATCATTACCGAGTTTACAACTCCCAA ACCCCTGTGAACATACCAATTGTTGCCTCGGCG GATCAGCCCGCTCCCGGTAAAACGGGACGGCC CGCCAGAGGACCCCCAAACTCTGTTTCTATATG TAACTTCTGAGTAAAACCATAAATAAATCAAA ACTTTCAACAACGGATCTCTTGGTTCTGGCATC GATGAAGAACGCAGCAAAATGCGATAAGTAAT GTGAATTGCAGAATTCAGTGAATCATCGAATC TTTGAACGCACATTGCGCCCGCCAGTATTCTGG CGGGCATGCCTGTTCGAGCGTCATTTCAACCCT CAAGCCCAGCTTGGTGTTGGGACTCGCGAGTC AAATCGCGTTCCCCAAATTGATTGGCGGTCAC GTCGAGCTTCCATAGCGTAGTAGTAAAACCCT CGTTACTGGTAATCGTCGCGGCCACGCCGTTAA ACCCCAACTTCTGAATGTTGACCTCGGATCAGG TAGGAATACCCGCTGAACTTAAGCAT
III	519	100 %	*Fusarium subglutinans*	TGCGGAGGGATCATTACCGAGTTTACAACTCC CAAACCCCTGTGAACATACCAATTGTTGCCTCG GCGGATCAGCCCGCTCCCGGTAAAACGGGACG GCCCGCCAGAGGACCCCCAAACTCTGTTTCTAT ATGTAACTTCTGAGTAAAACCATAAATAAATC AAAACTTTCAACAACGGATCTCTTGGTTCTGGC ATCGATGAAGAACGCAGCAAAATGCGATAAGT AATGTGAATTGCAGAATTCAGTGAATCATCGA ATCTTTGAACGCACATTGCGCCCGCCAGTATTC TGGCGGGCATGCCTGTTCGAGCGTCATTTCAAC CCTCAAGCCCAGCTTGGTGTTGGGACTCGCGA GTCAAATCGCGTTCCCCAAATTGATTGGCGGTC ACGTCGAGCTTCCATAGCGTAGTAGTAAAACC CTCGTTACTGGTAATCGTCGCGGCCACGCCGTT AAACCCCAACTTCTGAATGTTGACCTCGGATCA GGTAGGAATACCCGCTGAACTTAAGCATATC TGGCGGTCACGTCGAGCTTCCATAGCGTAGTA GTAAAACCCTCGTTACTGGTAATCGTCGCGGC CACGCCG

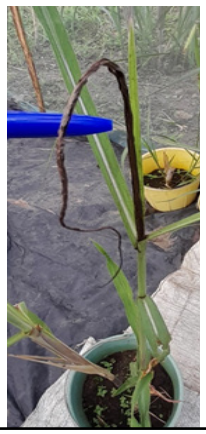
Nc: number of nucleotides compared. %H: percentage of sequence homology

commune (I), *Fusarium verticillioides* (II) and *Fusarium subglutinans* (III).

Pathogenicity of fungal isolates

Pathogenicity test is presented in Table 3. All species were found to be pathogenic

Table 3. Pathogenicity test

Strains of fungi	Control	Appearance of sugar cane after inoculation	Symptoms observed
Fusarium verticillioides			On the 11th day, Appearance on the leaves of small oval or elongated red lesions which develop on the midrib, sometimes along its entire length.
Fusarium subglutinans			At the 5th week, observation of a distortion of the stem with external particles and internal cut resembling lesions, At the 9th week, stem rot is noted.
Schizophyllum commune			At the 10th week, we noted the appearance of a whip at the top of the infested sugarcane stems, releasing brown-black spores. The infested canes had a grassy (thin leaves) and stunted (thin stem) appearance.

to sugarcane. Thus, *Fusarium verticillioides* presented the symptoms of red movre disease characterized by reddish spots and *Fusarium subglutinans* induced the characteristic symptoms of Pokkah Boeng disease. *Schizophyllum commune* caused black rots characteristic of anthrax. (Table 3).

DISCUSSIONS

Identification of pathogenic fungal strains was carried out based on phenotypic and molecular characteristics. During this work, several species of *Fusarium* were identified. Their presence explains their involvement in the "Pokkah Boeng" disease. Indeed, the work of Hilton et al[11] and those of Lin et al[12] showed that several *Fusarium* species have been reported as causative agents of "Pokkah Boeng", including *F. sacchari, F. fujikuroi, F. verticillioides, F. andiyazi, F. proliferatum* and *F. moniliforme*. The identification of *Fusarium verticillioides* on canes in the locality of Zuénoula would implicate it in the transmission of red snot. The samples collected in this locality showed red spots. Thus, Suresh and Nelson[13] showed that the causative agent of red snot was *Fusarium verticillioides*. These results agree more or less with those of Kouadia et al[14] who highlighted a diversity of phytopathogenic fungi belonging to the genera *Colletotrichum, Fusarium, Aspergillus, Phoma, Penicillium, Curvularia, Botryodiplodia and Rhizoctonia* associated with spoilage of banana, avocado and mango fruits. Furthermore, the genera *Fusarium*, *Aspergillus, Botryodiplodia* and *Phoma* have been reported on avocado and mango fruits[15]. These fungi are known to cause the deterioration of many products such as fruits, seeds, vegetables and tubers both in the field and during storage[16]. The fungal diversity recorded could be explained by the fact that sugar cane is a food with high nutritional value, mainly consisting of carbohydrates, water, vitamins and minerals. Also, the pH of sugar cane also being lower than 4.6, would more favor the development of pathogenic fungi[17]. Naturally, sugar cane resists microbial attacks, the extent of which will depend on the stage of maturity. This is because sugar cane contains high concentrations of natural antimicrobial compounds. These compounds consist of mixtures of 5-substituted resorcinols such as resorcinol-5-(12-heptadecadienyl) and resorcinol-5-(pentadecyl) Prusky et al[18] accumulate in the stem. Faced with such a chemically unfavorable environment, the fungus suspends its development until the concentration of antimicrobial compounds decreases in the stem. The results of pathogenicity tests carried out on the canes showed that all of these isolated fungi were pathogens of sugarcane. *Schizophyllum commune* was the species that caused the most serious pathologies of sugar cane in the two localities after the Mosaic. This could be explained by the fact that this species is involved in anthrax. These results are in agreement with those of Yao et al[19] who showed that the pathology spread very quickly in all sugar cane producing countries causing numerous yield losses ranging from 30 to 50% for susceptible varieties.

CONCLUSION

This present study contributed to the identification of pathogenic fungal germs of sugar cane in Côte d'Ivoire. From this study, it should be noted that the fungal species, namely *Fusarium verticillioides, Fusarium subglutinans* and *Schizophyllum commune*, were identified as pathogens of sugar cane in Côte d'Ivoire. These fungi are believed to be involved in pathologies such as red snot, "pokkah boeng" and smut, which are commonly encountered in sugar cane plantations.

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Ethics Statement

This research did not involve human participants, animal subjects, or any material that requires ethical approval.

Informed Consent Statement

This study did not involve human participants, and therefore, informed consent was not required.

Clinical Trial Registration

This research does not involve any clinical trials**.**

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Not Applicable**.**

Author Contributions

Djédji Okoue, Armist Amoa Tehua and Ibrahima Kebe : Performed the majority of the experiments and data analysis; Mireille Waze Alloue-Boraud : Designed the experiments and analysed the data; Fulgence Yao Koffi : Wrote the manuscript; All authors read and approved the final version of the manuscript.

REFERENCES

1. Péné B.C, Souleymane N, Chantal N. K. Sprinkler irrigation and soil tillage practices in sugarcane plantations as influenced by soil texture and water storage in northern Ivory Coast. *J. Appl. Biosci*. 2012;54:3916-3924.
2. Kouamé D. K, Péné C. B, Zouzou M. Évaluation de la résistance variétale de la canne à sucre au foreur de tiges tropical africain (*Eldana saccharina* Walker) en Côte d'Ivoire. *J. Appl. Biosci*. 2010;26:1614-1622.
3. Cawoy H, Bettiol W, Fickers P, Ongena M. Bacillus-Based Biological Control of Plant Diseases, Pesticides in the Modern World - Pesticides Use and Management, Dr. Margarita Stoytcheva (Ed.), ISBN: 978-953-307-459-7, InTech, 2011. Available from: http://www.intechopen.com/books/pesticides-in-the-modern-world-pesticides-use-and-management/bacillus-based-biological-control-of-plant-diseases
4. Silué N, Soro K, Koné T, Abo K, Koné M, Koné D. Parasitical fungi in cashew (*Anacardium occidentale* L.) orchard of Cote d'Ivoire. *Plant Pathol. J*. 2017;16:82-88. DOI: 10.3923/ppj.2017.82.88
5. Botton B., Breton A., Fevre M., Gauthier S., Guy P. H, Larpent J. P, Reymond P, Sarglier J. J, Vayssier Y, Veau P. (ed) : Usefull moist and nusible: industrial importance , 2nd edn Paris. Milan. Barcelone : Masson. « Collection Biotechnologies (Paris) ».1990;52 p.
6. Guiraud J. P. (ed) : Microbiologie alimentaire, Paris : Dunod, (1998),652p.
7. Maymonna S.N. Recherche de diversité génétique au sein d'une population *Stemphylium solani* Weber au Sénégal. *Rapport de stage*. 1998 : 66 p.
8. Bessadat N., Simoneau P., Benichou S., Setti B., Kihal M, Henni D. E. Morphological, physiological and pathogenic variability of small-spore *Alternaria* sp. causing leaf blight of Solanaceous plants in Algeria. *Afr. J. Microbiol. Res.*2014 ;8(37):3422-3434. https://doi.org/10.5897/AJMR2014.6802
9. Hall T.A. BioEdit: A User-Friendly Biological Sequence Alignment Editor and Analysis Program for Windows 95/98/NT. *Nucleic Acids Symp. Ser*.1999;41:95-98.
10. Silué N, Abo K, Johnson F, Camara B, Koné M, Koné D. Evaluation *in vitro et in vivo* de trois fongicides de synthèse et d'un fongicide biologique sur la croissance et la sévérité de *collectotrichum gloesporioides* et *pestalotia heterocornis*, champignons responsables de maladies foliaires de l'anacardier (*Anacardium occidentale* L.) en Cote d'Ivoire. *Agron. Afr*. 2018;30:107-122.
11. Hilton A, Zhange H, Yu W, Shim B. W. Identification and Characterization of Pathogenic and Endophytic Fungal Species Associated with Pokkah Boeng Disease of Sugarcane. *Plant Pathol. J.* 2017;33(3): 238-248. DOI: 10.5423/PPJ.OA.02.2017.0029.
12. Lin Z, Xu S, Que Y, Wang J, Comstock J. C, Wei J, Zhang M. Species-specific detection and identification of Fusarium species complex, the causal agent of sugarcane pokkah boeng in China. *PloS One*. 2014;9(8):104195. DOI: 10.1371/journal.pone.0104195.
13. Suresh N, Nelson R. Diversity of Arbuscular Mycorrhizal fungi (AMF) in the rhizosphere of sugarcane. *Eur. J. Exp. Biol.*2016;5(3):13-19.
14. Kouadia A.M-J, Abo K , Kouadio K.T. Evolution des infections naturelles sur les mangues, les avocats et les bananes en Côte d'Ivoire et principaux champignons reponables. *J. Appl. Biosci*.2019;134:13710-13721. DOI:10.4314/jab.v134i1.8.
15. Djeugap F.J, Tsompbeng G.N, Keuete K.E, Yaouba A, Serferbe S. Isolation and Identification of Fungi Associated with Avocado fruits from Local markets of the West Region of Cameroon. *Inter J Agri Biosc*. 2015;4(2):64-68.
16. Dongmo G.Z, Djeugap F.J, Fenohi N, Kenfack N.D, Takuete R, Teguefouet P. Contribution à l'identification des champignons de post-récolte associés aux amandes de *Ricinodendron heudelotii* et *Garcinia kola* collectées dans les Hauts Plateaux de l'Ouest Cameroun. *Int. J. Biol.*

Chem. Sci. 2017 ;11(4) :1840-1850.DOI : http://dx.doi.org/10.4314/ijbcs.v11i4.33

17. Cissé M. .Immobilisation d'un système lactoperoxyde dans un enrobage de chitosane dans le but de prolonger la conservation des mangues. *Thèse de doctorat. Université Montpellier Supagro, France.* 2012:159p. Cirad - Agritrop (https://agritrop.cirad.fr/566848/).
18. Prusky D, Kobiler I, Miyara I, Alkan N. Fruit diseases" *The Mango: Botany, Production and Uses*. 2009:210-230. https://doi.org/10.1079/9781845934897.0210.
19. Yao K. J-E, Kouamé K. D, Kouamé K. G, Kassi K. F. J-M, N'guessan A. C, Eponon E. C. G, Koné D. Structure of *Sporisorium scitamineum* isolates, the causative agent of sugarcane smut in Côte D'ivoire. *IJSR.* 2020; 9(3):1376-1381. DOI: 10.21275/SR20307072052.