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Posted Date: 31 August 2023

doi: 10.20944/preprints202308.2064.v1

Keywords: Fusarium species, Sarocladium species, rice, pathogenic variability, genetic diversity, mycotoxins, sub-Saharan Africa

Article

Fusarium and *Sarocladium* Species Associated with Rice Sheath Rot Disease in Sub-Saharan Africa

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Abstract: *Sarocladium* and *Fusarium* species are commonly identified as causal agents of rice sheath rot disease worldwide. However, limited knowledge exists about their genetic, pathogenic, and toxigenic diversity in sub-Saharan African (SSA) countries, where an increasing incidence of this disease has been observed. This study aimed at identifying, characterizing, as well as assessing the genetic, pathogenic, and toxigenic diversity of the pathogens associated with rice sheath rot disease in Mali, Nigeria, and Rwanda. In this study seventy fungal isolates were obtained from rice plants displaying disease symptoms on research and farmer's fields in Mali, Nigeria, and Rwanda. Thus, an extensive comparative analysis was conducted to assess their genetic, pathogenic, and toxigenic diversity. The *Fusarium spp*. were characterized using the translation elongation factor (*EF-1α*) region, while a concatenation of Internal Transcribed Spacer (ITS) and Actin-encoding regions were used to resolve *Sarocladium* species. Phylogenetic analysis revealed four *Fusarium* species complexes. The dominant complex in Nigeria was the *Fusarium incarnatum-equiseti* species complex (FIESC), comprising *F. hainanense, F. sulawesiense, F. pernambucatum,* and *F. tanahbumbuense*, while *F. incarnatum* was found in Rwanda. The *Fusarium fujikuroi* species complex (FFSC) was predominant in Rwanda and Mali, with species such as *F. andiyazi, F. madaense,* and *F. casha* in Rwanda, and *F. annulatum* and *F. nygamai* in Mali. *F. marum* was found in Nigeria. Furthermore, *Fusarium oxysporum* species complex (FOSC) members, *F. callistephi* and *F. triseptatum,* were found in Rwanda and Mali, respectively. Two isolates of *F. acasiae-mearnsii*, belonging to the *Fusarium sambucinum* species complex (FSAMSC) were obtained in Rwanda. Isolates of *Sarocladium* which were previously classified in three phylogenetic groups were resolved into three species which are: *attenuatum, oryzae* and *sparsum*. *S. attenuatum* was dominant in Rwanda, while *S. oryzae* and *S. sparsum* were found in Nigeria. Also, the susceptibility of FARO44, a rice cultivar released by Africa Rice Centre (AfricaRice) was tested against isolates from the four *Fusarium* species complexes and the three *Sarocladium* species. All isolates evaluated could induce typical sheath rot symptoms albeit with varying disease development levels. In addition, liquid chromatography-tandem mass spectrometry (LC-MS/MS) was used to determine variation in the *in vitro* mycotoxins of the *Fusarium* species. Regional differences were observed in the *in vitro* mycotoxins profiling. Out of the forty-six isolates tested, nineteen were able to produce one to four mycotoxins. Notably, very high zearalenone (ZEN) production was specific to the two *F. hainanense* isolates from Ibadan-Nigeria, while *Fusarium nygamai* isolates from Mali produced high amounts of fumonisins. To the best of our knowledge, it seems this study is the first to elucidate genetic, pathogenic, and toxigenic diversity of *Fusarium* species associated with the rice sheath rot disease complex in selected countries in SSA.

Keywords: *Fusarium* species; *Sarocladium* species; rice**;** pathogenic variability; genetic diversity; mycotoxins; sub-Saharan Africa

 doi:10.20944/preprints202308.2064.v1

1. Introduction

Rice (*Oryza sativa*) holds significant economic importance in Africa, with Nigeria being one of the leading contributors to the continent's global rice production share (4.2%), accounting for 24% [1]. Despite this, Nigeria remains the second-largest importer of rice worldwide, trailing only behind China. In 2018 alone, Nigeria imported approximately 3 million metric tons of milled rice, struggling to meet its demand deficit for the past decade [2]. Mali, a landlocked country in West Africa, ranks fifth among African nations in terms of rice production. Its rice production is being managed through irrigated systems connected to the Niger River. In Rwanda, the demand for rice is estimated at 145,000 tons per year, while national supply accounts for about 40%, creating a 60% deficit that is met through imports [93]. While the rice cultivation area is expanding in Africa, the average yields (2.35 tons/ha in 2021) are low when compared to Asia (4.95 tons/ha in 2021) [1]. Rice production is constrained by various factors including biotic stresses such as pests and diseases. Africa has recorded a steady and substantial increase in the incidence of rice diseases such as *Rice Yellow Mottle Virus* (RYMV), Rice blast (*Pyricularia oryzae*), Bacterial Leaf Blight (*Xanthomonas oryzae pv oryzae*), Bacterial Leaf Streak (*Xanthomonas oryzae pv oryzicola*), and *Rice Stripe Necrosis Virus* (RSNV) [3–7].

Rice sheath rot is an emerging disease worldwide [8]. *Sarocladium oryzae*, [9], formerly *Acrocylindrium oryzae*, was the first organism to be associated with rice sheath rot symptoms in Taiwan in 1922 [10] *Sarocladium attenuatum* was originally described as a distinct species causing rice sheath rot, was then considered a synonym of *Sarocladium oryzae* [11], but has recently been re-established as a separate species causing rice sheath rot in Taiwan [12]. These authors also described a third species that causes sheath rot symptoms on rice called *S. sparsum*, which is closely related to *S. oryzae* and *S. attenuatum. Sarocladium* has been associated with rice sheath rot in thirty-eight countries [13].

Besides *Sarocladium* species, *Fusarium* spp. have been associated with the rice sheath rot complex. These mainly comprise isolates in the *F. fujikuroi* species complex (FFSC) including *F. proliferatum*, *F. verticillioides*, *F. incarnatum* and *F. fujikuroi* [14–19]. In addition, various bacterial species cause rice sheath rot symptoms. The most important one is *Pseudomonas fuscovaginae* which is known to cause sheath brown rot of rice at high altitudes. In Africa, this bacterium has been reported in Burundi [8] [20] and Madagascar.

In West Africa, rice sheath rot has been reported in Cote D'ivoire, Gambia, Niger, Nigeria, and Senegal [13]. However, no causative fungal strains were isolated and no detailed scientific information was provided except for Nigeria [21] where *S. attenuatum* was first reported in Nigeria as one of the causes of grain discoloration on rice [21]. In addition, an inhibitory effect of *S. oryzae* on seed germination was later observed [22]. Most information pertaining to the occurrence of *S. oryzae* in Africa relating to stored, marketed and field seeds especially with respect to mycotoxigenic potentials was enumerated by [23].

Rice sheath rot can cause high yield losses of 20-80% [24–26]. Furthermore, an extensive survey of rice fields across West Africa enabled the identification of sheath rot symptoms in Mali and Nigeria (AfricaRice disease database). Although yield losses due to the sheath rot disease has not been estimated in Mali and Nigeria, a field survey conducted in 2011 and 2013 revealed high incidence and severity of the disease in Rwanda [27].

Mycotoxin contamination of cereal products poses a serious concern for animal and human health. Several studies have reported *Fusarium* species as the major producers of mycotoxins contaminating cereals including rice [28–34]. In the African region, previous studies have reported several mycotoxins being synthesized by *Fusarium* species isolated from rice as a serious health threat to producers and consumer [35–40].

The comprehensive information regarding the incidence and distribution of sheath rot disease is the aspect notably lacking. Additionally, there has been a notable absence of research examining the genetic, pathogenic, and toxigenic variability of pathogens associated with this disease in East and West Africa. Acquiring this crucial information will offer valuable insights for disease control and enhance management strategies for breeding programs. Therefore, this study aimed at

 doi:10.20944/preprints202308.2064.v1

identifying, characterizing, as well as assessing the genetic, pathogenic, and toxigenic diversity of the pathogens associated with rice sheath rot disease in Mali, Nigeria, and Rwanda.

2. Materials and Methods

2.1. Collection of Samples

Samples were collected from rice research programs and farmer's fields in Mali, Nigeria, and Rwanda (Figure 1). Naturally infected whole rice plants with sheath rot symptoms having sheath browning, necrosis, grain emptiness and rotting as indicated in Figure 2 were collected. Samples from farmer's field were collected at the office of the rural development, Selingue village near Bamako, Mali and from two fields located at Ibadan, Oyo State and Katcha near Badeggi, Niger State in Nigeria during the 2017 rice growing season. Samples were randomly collected 25 m apart at each location. The samples collected were conserved in dry paper bags, while hands were disinfected with 70% alcohol after each sampling. Samples were later stored in dry bags in the refrigerator at 4°C in the laboratory. Isolates earlier collected from Rwanda in 2011 and 2013 were also included in this study. Agro-climatological details of the selected three countries with their various agroecologies are presented in Table 1. Isolation and identification of pathogens was carried out at the Phytopathology laboratory of Ghent University, Belgium.

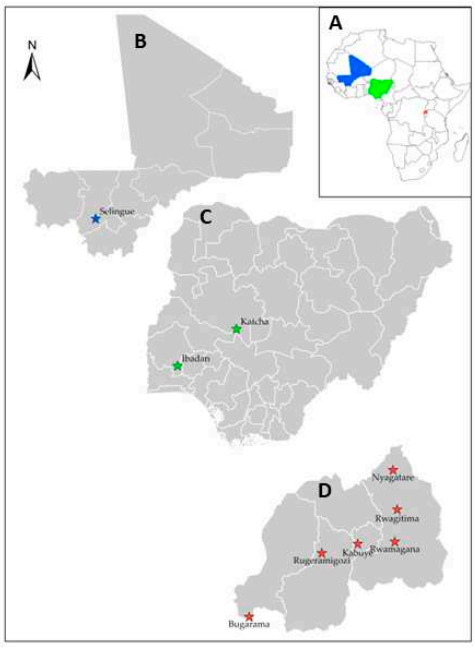

Figure 1. Locations in Mali, Nigeria, and Rwanda where rice samples were taken. A. Locations of Mali, Nigeria, and Rwanda in Africa; B, location of the area where samples were collected in Mali; C, locations in Nigeria; D, locations in Rwanda.

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Figure 2. Diseased rice plants showing typical sheath rot disease symptoms. A: Typical sheath browning characteristic of sheath rot disease on rice field at AfricaRice experimental field, Ibadan-Nigeria. B Greyish-brown lesions on the leaf flags enclosing the panicle observed during the screenhouse experiment. C: Emerged brownish panicles, chaffy and sterile grains showing typical sheath rot disease symptoms.

Table 1. Agro-ecological details of the sampling regions in Mali, Nigeria, and Rwanda

Location	Ecology	Annual precipitation (mm)	Temperature (°C)	Ecosystem	Elevation (m)
Nigeria					
Ibadan	Derived savannah	1300-1500	25-35	Irrigated lowland	225
Katcha	Southern Guinea Savannah	900-1000	28-40	Rainfed lowland	123
Mali					
Selingue	Sudan Guinea Savannah	≤600	35-50	Irrigated lowland	351
Rwanda					
Bugarama	Mosaic Vegetation and Forest (West)	1098	24	Irrigated marshland	900
Kabuye	Mosaic Vegetation and Forest (Central)	951	22	Irrigated marshland	1270
Nyagatare	Savannah (East)	783	20	Irrigated marshland	1470
Rwamagana	Savannah (East)	979	19	Irrigated marshland	1680
Rugeramigozi	Mosaic Vegetation and Forest (South)	1154	19	Irrigated marshland	1706

2.1.1. Isolation and Purification of Sheath Rot-Associated Isolates

Infected sheath and seed samples showing symptoms of sheath rot were surface - sterilized in 2% sodium hypochlorite for two minutes and then rinsed thrice in sterile distilled water. They were drained using sterile paper towels, and thereafter cut into small pieces of about 0.5 cm^2 and plated on 90 mm diameter Petri dishes containing Potato Dextrose Agar (PDA). The cultured Petri dishes were incubated at 28°C in darkness for 7-14 days. Cultures were further purified by plating on fresh PDA.

 doi:10.20944/preprints202308.2064.v1

2.1.2. Identification of Pathogens

Identification of fungal pathogens was done based on their typical structure and basic characters as described by Barnett and Hunter (1972). The incidence and diversity of fungi was observed and recorded. After 5 days, all plates were examined under a compound microscope for the presence of several fungal pathogens. Conidia of these fungi were mounted on glass slides in water and examined under a compound microscope for identification at genus level. For *Fusarium* species, pure cultures were plated and stored on PDA slants at room temperature and maintained at -80°C with 40% glycerol. A similar method of storage was used for *Sarocladium* species except for the use of 20% glycerol.

2.2. Molecular Characterization of Isolates

2.2.1. DNA extraction, amplification, and sequencing

Fungal isolates were grown on potato dextrose broth (PDB) at 28 °C for seven days. Mycelia mats were harvested by filtration, dried by blotting using sterile paper towels, frozen in liquid nitrogen, and pulverized using a tissue lyser (MM400, Retsch GmbH, Haan, Germany).

Genomic DNA was extracted using the Wizard Genomic DNA Purification Kit (Promega). Quantification and purity were determined using Nanodrop 3000 (Thermo Scientific, Asheville, NC, USA) and diluted to a concentration of 20 ng µL-1.

Fusarium isolates were further identified by amplifying the Translation Elongation Factor (EF-1α), using primer pair TEF-1-F (5′-ATG GGT AAG GAA GAC AAG AC-3′) and TEF-2-R (5′-GGA AGT ACC AGT GAT CAT GTT-3′) [41]. PCR reactions were done in 25 µL of a solution consisting of 2 µL genomic DNA (100 ng µL-1), 5 µL PCR buffer (5x; Promega), 5 µL Q solution (Qiagen), 0.5 µL dNTPs (10 mM; Fermentas GmbH), 1.75 µL of each primer (10 µM), 0.15 µL Taq DNA polymerase (5 units µL−1; Fermentas GmbH) and 8.85 µL ultrapure sterile water. Amplification was performed with initial denaturation step at 94 °C for 1 min, followed by 35 cycles of denaturation at 94°C for 30 s, primer annealing at 53°C for 45 s and extension at 72 °C for 1 min. Cycling ended with a final extension step at 72 °C for 5 min [41]. The amplicons were separated by horizontal electrophoresis using 1.5 % agarose gels in TAE-buffer at 100 V for 25 min and visualized by ethidium bromide staining on a UV trans illuminator. Amplified products were purified with exosap and sequenced by LGC Genomics GmbH (Berlin, Germany) using Sanger sequencing.

For *Sarocladium* isolates, two genomic regions, the Internal Transcribed Spacer (ITS) and Actin, were amplified and sequenced. For the ITS region, primers ITS1 (5′-TCC GTA GGT GAA CCT GCG G-3′) and ITS4 (5′- TCC TCC GCT TAT TGA TAT GC -3′) were used (White et al., 1990). The actin region was amplified using ACT1 (5′- TGG GAC GAT ATG GAG AAG ATC TGG CA -3′) and ACT4 (5′- TCG TCG TAT TCT TGC TTG GAG ATC CAC AT-3′) [42].

For both primer pairs, PCR reactions were done in 25 µL of a solution consisting of 2 µL genomic DNA (100 ng µL-1), 5 µL PCR buffer (5x; Promega), 0.5 µL dNTPs (10 mM; Fermentas GmbH), 1.75 µL of each primer (10 µM), 0.15µL Taq DNA polymerase (5 units µL−1, Fermentas GmbH) and 13.85µL ultrapure sterile water. Amplification was performed using a Flexcycler PCR Thermal Cycler (Analytik Jena). For ITS amplification, the thermal profile consisted of an initial denaturation step at 94 °C for 10 min, followed by 35 cycles of denaturation at 94 °C for 1 min, primer annealing at 55 °C for 1 min and extension at 72 °C for 1 min. Cycling ended with a final extension step at 72 °C for 10 min [43]. ACT fragments were amplified using an initial denaturation step at 94°C for 1 min, followed by 39 cycles of denaturation at 94 °C for 45 sec, primer annealing at 59 °C for 30 sec and extension at 72 °C for 30 sec. Cycling ended with a final extension step at 72 °C for 8 min [42].

2.2.2. Phylogenetic Analysis

The nucleotide sequences generated by the forward and reverse primers were used to obtain consensus sequences after editing via BioEdit version 7.2.5. [44]. From each duplicate identical sequence, a representative sequence from each identical set of sequence was compared to other

sequences available at GenBank. Sequences were first aligned via muscle alignment in Mega V.11. [45] after which a maximum-likelihood tree was constructed based on the matrix of pairwise distances obtained using the General Time Reversible (GTR) Model. Reference sequences *of Fusarium* (Table 5) and *Sarocladium* (Table 6) species representing the three countries were used for phylogenetic analysis. For *Fusarium*, Phylogenetic tree was constructed based on maximum-likelihood inferred from partial *EF-1α* sequences of four *Fusarium* species complexes using IQ-Tree with GTR + G + I model and annotated using iTol software [46]. *Cylindrocarpon* sp. AC2011 strain CPC 13531 was used as an outgroup. However, for *Sarocladium* characterization, concatenated alignments of ITS and ACT region were performed after which a single phylogenetic tree was generated. To root the tree, *Sarocladium zeae* strain CBS 800.69 was used as an outgroup.

2.3. Pathogenicity Assay

Location of the isolates, and genetic groupings were used to select a subset of twenty-nine isolates for pathogenicity studies on rice plants. For pathogenicity tests with *Fusarium* species, representative isolates from the four Fusarium species complexes recorded in all the locations (FIESC, FFSC, FOSC, FSAMSC) were used for rice inoculation. To evaluate the pathogenicity of *Sarocladium* species, thirteen isolates comprising *S. attenuatum* (3), *S. oryzae* (6), and *S. sparsum* (4) were used. Indica rice cultivar (FARO 44) released by Africa Rice Center was used for the assay.

Inoculum was prepared according to the standard grain inoculum technique [47]. Briefly, rice grains were soaked in water for 60 min, excess water was removed, and the grains were autoclaved twice on two different days. For 4 g of rice grains, 1 plug (diameter = 5 mm) from the edge of a 14-day-old fungal colony was added together with 1 ml of sterile distilled water. Every two days, the grain inoculum was shaken to prevent the formation of clumps. After 10 days of incubation at 28°C, the inoculum was fully colonized.

The rice seeds were dehulled and surface sterilized in 2% sodium hypochlorite solution for 25 min, rinsed five times in sterile distilled water, and placed in Petri dishes containing sterile moistened filter papers (Whatman, grade 3). Following seedling emergence, six seedlings were transplanted into perforated plastic trays (22 x 15 x 6 cm) containing potting soil (Structural; Snebbout, Kaprijke, Belgium). Plants were watered daily, fertilized weekly with 0.2% iron sulphate and 0.1% ammonium sulphate, and maintained in a growth chamber (28°C, 60% relative humidity). Six-week-old plants were used for the inoculation.

One fully colonized fungal grain was introduced in the junction point between the sheath of the second youngest plant leaf and the stem. Inoculation points were covered with moist cotton wool and wrapped with parafilm to maintain humidity. High humidity was maintained for 24 h post inoculation by incubating in a controlled room at 28°C day and night, 16/12 light regime, and 85 % relative humidity. Subsequently, incubation was maintained at 65 % relative humidity, temperature, and light regime as above for 2-10 days. The disease development was evaluated eight days after inoculation by measuring the lesion length on the flag leaf sheath. Each treatment was replicated thrice while the experiment was conducted once.

2.4. Statistical Analysis

Lesion length was used as a measure of isolate virulence on rice plants. Since the conditions of normality were not met, a non-parametric analysis was carried out. Lesion length of the infected sheath were quantified using Kruskal-Wally's Rank Sum test followed by a post hoc Mann-Whitney test. Statistical significance was defined as P= 0.05.

2.5. Mycotoxin analysis

2.5.1. Culture Preparation

Pure cultures of seventy-seven identified isolates (*Fusarium* - 46, *Sarocladium* – 31) were sub-cultured on Petri dishes with PDA. The medium was poured into 90 mm Petri dishes. Two mm of

 doi:10.20944/preprints202308.2064.v1

clean and pure cultured isolates were sub-cultured on fresh PDA plates and incubated at 25 °C for 3 weeks. Each isolate was grown in triplicates.

2.5.2. Reagents and Standards

Ethyl acetate and dichloromethane (DCM) were purchased from (Thermofisher scientific, Merelbeke, Belgium). Analytical grade formic acid (100%) and ammonium acetate were from (Merck, Darmstadt, Germany). Purified water was from the Arium pro VF system (Millipore, Belgium). LC-MS grade acetic acid and methanol were from (Biosolve, Valkenswaard, the Netherlands).

Certified mycotoxin standard solutions, more specifically aflatoxin mix (AFB1, AFB2, AFG1 and AFG2), deoxynivalenol (DON), fumonisin mix (FB1 and FB2), nivalenol (NIV), neosolaniol (NEO), OTA, T2, HT2, 3-acetyldeoxynivalenol (3-ADON), diacetoxyscirpenol (DAS), 15-acetyldeoxynivalenol (15-ADON), fusarenon-X (F-X), sterigmatocystin (STC), zearalenone (ZEN) and deepoxy-deoxynivalenol (DOM) were purchased from Biopure (RomerLabs, Getzersdorf, Austria). Fumonisin B3 (FB3) and enniatin B (ENN B) were obtained from Fermentek (Jerusalem, Israel). Alternariol (AOH), alternariol monomethylether (AME), were purchased from Sigma-Aldrich (Bornem, Belgium) and roquefortine (ROQ-C) from Alexis Biochemicals (Enzo Life Sciences BVBA, Zandhoven, Belgium).

Working solutions were prepared by diluting the stock solutions in methanol and stored at -20°C. A standard mixture consisting of the above mycotoxins (without DOM) in a concentration range between 0.5 ng/µl and 40 ng/µl was prepared as well and stored at -20°C.

Mobile phase A (94% water, 5% methanol, 1% acetic acid and 5mM ammonium acetate) and mobile phase B (97% methanol, 2% water, 1% acetic acid and 5mM ammonium acetate) were prepared.

2.5.3. Sample Preparation and Extraction

The extraction process started with the preparation of the quality control samples. Briefly, three plugs each of blank agar (uninoculated) were removed and placed into each of the three 50 ml Falcon tubes (spike 1, spike 2, and blank) and were macerated into pieces using a sterile scalpel blade. Then 50 µL DOM internal standard (50 ng/µl) was added into each tube, after which 25 µL and 100 µL of the standard mixture were added to spike 1 and spike 2, respectively. The mixtures (spikes and blank) were left in the dark for 15 minutes.

Following the control sample preparation, *Fusarium* mycotoxins were extracted from pure cultures of different isolates by using a sterile 9 mm cork borer and scalpel to take three plugs (2 sides + centre). The plugs were transferred into 50 ml Falcon tubes and macerated into pieces using a sterile scalpel blade. Then 50 µL DOM internal standard (50 ng/µl) was added into each tube and left in the dark for 15 minutes. The samples together with the quality control samples were extracted by adding ethyl acetate + 1% formic acid. The content was agitated gently on a vertical shaker for 20 min and centrifuged at 3000 g for 15 min. Then a folded filter paper (VWR International, Zaventem, Belgium) moistened with ethyl acetate + 1% formic was placed on a new extraction tube to collect the upper layer of the filtrate. Thereafter, 5 ml of dichloromethane (DCM) was added to each of the samples. The mixtures were agitated on a vertical shaker for 20 min and centrifuged at 3000 g for 15 min. Following centrifugation, the bottom layer (DCM phase) was collected in the same Falcon tube with the same filter paper. The filtrates were evaporated to dryness at 40 °C under a gentle nitrogen stream. The dissolved residue was reconstituted in 200 µl injection solvent (60% mobile phase A and 40% mobile phase B), well vortexed, and ultra centrifuged for 5 min at 10000 rpm. Finally, 100 µl of the filtrates were transferred into HPLC vials for LC-MS/MS analysis.

2.5.4. Multi-metabolite analysis (LC-MS/MS)

The samples were analysed using a Quattro Premier XE triple quadrupole mass spectrometer coupled with a Waters Acquity UPLC system (Waters, Milford, MA, USA).

Liquid chromatography conditions and MS parameters were followed as described by [48].The analytical column used was a symmetry C18, 5 µm, 2.1 x 150 mm with a guard column of the same material (3.5 µm, 10 mm x 2.1 mm) (Waters, Zellik, Belgium) kept at room temperature. The injection

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volume was 10 µl. Capillary voltage was set at 3.2 kV with a source block temperature and desolvation temperature of 120 and 400 °C respectively. Data processing was done using the Masslynx and Quanlynx software.

3. Results

3.1. Sampling and Isolation

Information on *Fusarium* isolates obtained from diseased rice plants in Nigeria, Mali and Rwanda is presented in Table 2. Of the 46 *Fusarium* isolates evaluated in this study, nine isolates were obtained from seeds, while 37 isolates originated from the rice sheath. The highest number was obtained from Rwanda (24 isolates), followed by Nigeria (15 isolates), while Mali (seven isolates) had the least.

Information on *Sarocladium* isolates obtained from diseased rice plants in Nigeria, Mali and Rwanda is given in Table 3. Out of the 24 Sarocladium isolates obtained, four were from seeds and 20 from the rice sheath. The highest number of isolates were obtained from Nigeria (nine from Katcha, seven from Ibadan), six isolates were from Rwanda, and only two from Mali (Table 3).

Altogether, seventy isolates comprising *Sarocladium* species (24) and *Fusarium* species (46) were obtained. Four ITS characterized CBS *Sarocladium* species were added, and we sequenced their actin region in this study (Table 2).

Table 2. *Fusarium* isolates obtained from rice plants showing sheath rot disease symptoms in Nigeria, Mali and Rwanda.

Origin	Strain code	Species	Species complex	Host part	Year of isolation	Genbank EF-1α
Nigeria						
Ibadan	IBNGF0001	*F. sulawesiense*	FIESC 16	Seed	2017	MN539083
Ibadan	IBNGF0002	*F. pernambucanum*	FIESC 17	Sheath	2017	MN539084
Ibadan	IBNGF0003	*F. hainanense*	FIESC 26	Seed	2017	MN539085
Ibadan	IBNGF0004	*F. sulawesiense*	FIESC 16	Sheath	2017	MN539086
Ibadan	IBNGF0005	*F. hainanense*	FIESC 26	Sheath	2017	MN539087
Ibadan	IBNGF0006A	*F. sulawesiense*	FIESC 16	Sheath	2017	MN539088
Ibadan	IBNGF0006B	*F. sulawesiense*	FIESC 16	Sheath	2017	MN539089
Ibadan	IBNGF0007A	*F. sulawesiense*	FIESC 16	Sheath	2017	MN539090
Ibadan	IBNGF0012	*F. marum*	FFSC	Sheath	2017	MN539096
Ibadan	IBNGF0013	*F. sulawesiense*	FIESC 16	Sheath	2017	MN539091
Ibadan	IBNGF0016	*F. marum*	FFSC	Sheath	2017	MN539097
Ibadan	IBNGF0019	*F. sulawesiense*	FIESC 16	Sheath	2017	MN539092
Katcha	BDNGF0001	*F. tanahbumbuense*	FIESC 24	Sheath	2017	MN539091
Katcha	BDNGF0002	*F. sulawesiense*	FIESC 16	Seed	2017	MN539094
Katcha	BDNGF0003	*F. tanahbumbuense*	FIESC 24	Seed	2017	MN539095
Mali						
Selingue	SEMAF0004	*F. nygamai*	FFSC	Seed	2017	MN539098
Selingue	SEMAF0010	*F. nygamai*	FFSC	Seed	2017	MN539099
Selingue	SEMAF0012A	*F. nygamai*	FFSC	Seed	2017	MN539100
Selingue	SEMAF0012B	*F. nygamai*	FFSC	Sheath	2017	MN539101
Selingue	SEMAF17-225A	*F. annulatum*	FFSC	Sheath	2017	MN539103
Selingue	SEMAF17-225B	*F. nygamai*	FFSC	Seed	2017	MN539102

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Selingue	SEMAF0043	*F. triseptatum*	FOSC	Sheath	2017	MN539104
Rwanda						
Kabuye	RFKB4	*F. callistephi*	FOSC	Seed	2013	KX424544
Kabuye	RFKB6	*F. madaense*	FFSC	Sheath	2013	KX424545
Nyagatare	RFNG10	*F. andiyazi*	FFSC	Sheath	2011	KX424546
Nyagatare	RFNG13	*F. andiyazi*	FFSC	Sheath	2011	KX424552
Nyagatare	RFNG16	*F. andiyazi*	FFSC	Sheath	2011	KX424553
Nyagatare	RFNG20	*F. andiyazi*	FFSC	Sheath	2011	KX424554
Nyagatare	RFNG32	*F. andiyazi*	FFSC	Sheath	2011	KX424555
Nyagatare	RFNG54	*F. callistephi*	FOSC	Sheath	2011	OQ909428
Nyagatare	RFNG57	*F. madaense*	FFSC	Sheath	2011	KX424556
Nyagatare	RFNG59	*F. callistephi*	FOSC	Sheath	2011	KX424557
Nyagatare	RFNG60	*F. callistephi*	FOSC	Sheath	2011	OQ909429
Nyagatare	RFNG61	*F. incarnatum*	FIESC 38	Sheath	2011	OQ909431
Nyagatare	RFNG72	*F. andiyazi*	FFSC	Sheath	2011	OQ909425
Nyagatare	RFNG96	*F. callistephi*	FOSC	Sheath	2011	OQ909430
Nyagatare	RFNG110	*F. madaense*	FFSC	Sheath	2011	OQ909426
Nyagatare	RFNG113	*F. madaense*	FFSC	Sheath	2011	KX424548
Nyagatare	RFNG114	*F. madaense*	FFSC	Sheath	2011	KX424549
Nyagatare	RFNG115	*F. andiyazi*	FFSC	Sheath	2011	KX424550
Nyagatare	RFNG127	*F. acasiae mearnsii*	FSAMSC	Sheath	2013	KX424551
Rwamagana	RFRM13	*F. incarnatum*	FIESC 38	Sheath	2013	OQ867255
Rwamagana	RFRM17	*F. incarnatum*	FIESC 38	Sheath	2013	OQ909427
Rwamagana	RFRM18	*F. madaense*	FFSC	Sheath	2013	KX424559
Rwamagana	RFRM19	*F. acasiae-mearnsii*	FSAMSC	Sheath	2013	KX424560
Rwamagana	RFRM35	*F. casha*	FFSC	Sheath	2013	KX424561

Table 3. *Sarocladium* isolates obtained from rice plants showing sheath rot disease symptoms in Nigeria, Mali and Rwanda.

	Strain code	Species	Host/Part	Year of Isolation	Genbank ITS	Genbank ACTIN
Nigeria						
Ibadan	IBNG0001	*S. sparsum*	Sheath	2017	MN389594	MN783308
Ibadan	IBNG0002	*S. sparsum*	Sheath	2017	MN389595	MN783309
Ibadan	IBNG0008	*S. sparsum*	Sheath	2017	MN389596	MN783310
Ibadan	IBNG0009	*S. sparsum*	Seed	2017	MN389597	MN783311
Ibadan	IBNG0011	*S. oryzae*	Sheath	2017	MN389589	MN783312
Ibadan	IBNG0012	*S. oryzae*	Sheath	2017	MN389590	MN783313
Ibadan	IBNG0013	*S. oryzae*	Sheath	2017	MN389591	MN783314
Katcha	BDNG0004	*S. oryzae*	Seed	2017	MN389581	MN783299
Katcha	BDNG0005	*S. oryzae*	Sheath	2017	MN389582	MN783300
Katcha	BDNG0007	*S. oryzae*	Seed	2017	MN389583	MN783301
Katcha	BDNG0009	*S. oryzae*	Sheath	2017	MN389584	MN783302
Katcha	BDNG0012	*S. oryzae*	Sheath	2017	MN389585	MN783303

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Katcha	BDNG0014	*S. oryzae*	Sheath	2017	MN389586	MN783304
Katcha	BDNG0022	*S. oryzae*	Sheath	2017	MN389587	MN783305
Katcha	BDNG0023	*S. oryzae*	Sheath	2017	MN389588	MN783306
Katcha	BDNG0025	*S. sparsum*	Seed	2017	MN389593	MN783307
Mali						
Selingue	SEMA0013A	*S. oryzae*	Sheath	2017	MN641009	MN783315
Selingue	SEMA0029	*S. attenuatum*	Sheath	2017	MN641010	MN783316
Rwanda						
Bugarama	RFBG3	*S. attenuatum*	Sheath	2011	KX424828	OP374130
Nyagatare	RFNG30	*S. attenuatum*	Sheath	2011	KX424536	OP374131
Nyagatare	RFNG33	*S. attenuatum*	Sheath	2011	KX424537	OP374132
Nyagatare	RFNG41	*S. attenuatum*	Sheath	2011	KX424538	OP374133
Nyagatare	RFNG122	*S. attenuatum*	Sheath	2011	KX424531	OP374134
Rugeramigozi	RFRG2	*S. oryzae*	Sheath	2013	KX424542	OP374135
CBS isolates						
Mexico	CBS 101.61	*S. attenuatum*	NA	1959	MN389592	MN783317
Kenya	CBS 361.75	*S. oryzae*	NA	NA	MN389580	MN783318
Panama	CBS 120.817	*S. oryzae*	NA	NA	MN389579	MN783319
Australia	CBS 485.80	*S. oryzae*	Sheath	1980	MN389598	MN783320

3.2. Phylogenetic Analysis of Fusarium and Sarocladium-like spp.

3.2.1. Fusarium Species

Partial sequences of *TEF-1a* gene revealed the identity of all the 46 *Fusarium* isolates used. Similarities to DNA sequences in the Fusaroid-ID and GenBank database ranged from 99–100%. Members of four species complexes were identified: *F. fujikuroi* species complex (FFSC – 48%), *F. incarnatum-equiseti* species complex (FIESC – 35%), *F. oxysporum* species complex (FOSC – 13%) and *F. sambucinum* species complex (FSAMSC – 4%) (Table 2). The phylogenetic analysis of the 46 *Fusarium* isolated is presented in Figure 3. The origin and Genbank accession numbers of the reference isolates used in given in Table A1.

FFSC isolates from Rwanda clustered with *F. andiyazi* (7 isolates), *F. madaense* (6 isolates) and *F. casha* (1 isolate). FFSC isolates from Mali were identified as F. *nygamai* (5 isolates) and *F. annulatum* (1 isolate), while in Nigeria, 2 *F. marum* isolates were found.

FIESC isolates were found in Nigeria and Rwanda and belong to five different species (*sulawesiense, pernambucatum, tanahbumbuense, hainanense, and incarnatum*) previously classified from rice, cereals, insects, and human samples [31], [35], [36], [50–53] (Figure 3). Eight of our FIESC isolates were nested within the *F. sulawesiense* clade including 7 isolates from Ibadan and an isolate from Katcha. One isolate from Ibadan clustered with members of *F. pernambucatum*, two isolates from Katcha nested within the *F. tanahbumbuense* clade, and two isolates from Ibadan nested within the *F. hainanense* group. Three isolates from Rwanda were found in *F. incarnatum* (Figure 3). None of our isolates clustered with the *F. equiseti* species clade (Figure 3).

The FOSC was found in Rwanda and Mali with members belonging to *F. callistephi* (5 isolates from Rwanda), and *F. triseptum* (1 isolate from Mali). Two isolates belonging to *F. acasia-mearnsii* in the FSAMSC were obtained from Rwanda.

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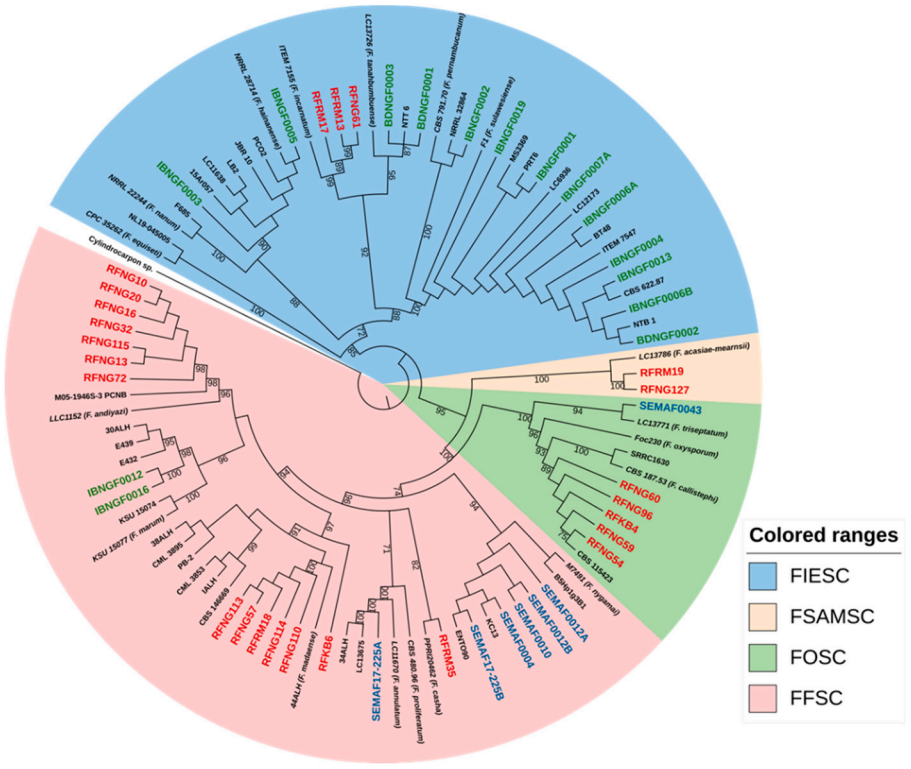

Figure 3. Phylogenetic tree based on maximum-likelihood inferred from partial *EF-1α* sequences of four *Fusarium* species complexes using IQ-Tree with GTR + G + I model and annotated using the iTOL software. *Cylindrocarpon* sp. AC2011 strain CPC 13531 was used as an outgroup. Isolates in colour and bold were obtained in this study (Blue – Mali, Green – Nigeria and Red – Rwanda). FIESC: *Fusarium incarnatum-equiseti* species complex; FSAMSC: *Fusarium sambucinum* species complex; FOSC: *Fusarium oxysporum* species complex; FFSC: *Fusarium fujikuroi* species complex.

3.2.2. Sarocladium Species

Thirty-one *S. oryzae*-like isolates were used for phylogenetic analysis (Nigeria=16, Mali=2, Rwanda=6, reference isolates =7). Partial sequences and concatenation of both ITS and ACT regions showed the identities of all the isolates. They were further subjected to BLASTn comparison with isolates in GenBank. Results revealed that all the 31 isolates had 98 -100% identity with *Sarocladium* species. A concatenated tree, in which reference sequences from GenBank were included (see Table A2), clearly delineated the *Sarocladium* isolates into three distinct phylogenetic groups with high bootstrap values (Figure 4). Most isolates from Nigeria (11 of 16), one isolate from Mali (SEMA0013A) and one isolate from Rwanda (RFRG2) clustered together with reference isolates CBS 180.74 from India, CBS 361.75 from Kenya, and CBS120.817 from Panama and were identified as *S. oryzae*. The second Mali isolate (SEMA0029) and five of the six Rwandan isolates clustered with reference isolates CBS 101.61 from Mexico and CBS 399.73 from India and belong to the *S. attenuatum* lineage. Five isolates from Nigeria clustered with reference isolate CBS 414.81 from Nigeria and the S. *sparsum* isolate 18042 from Taiwan (Figure 4). They mostly occurred in Ibadan-Nigeria (DS) except for an isolate (BDNG0025) found on infected seed in Katcha (SGS). Finding a substantial number of this group in Ibadan was not strange because it clustered with a reference isolate CBS 414.81 of Ibadan origin, collected, and reported [21], and later deposited into the GenBank [42], and recently reclassified as *S. sparsum*.

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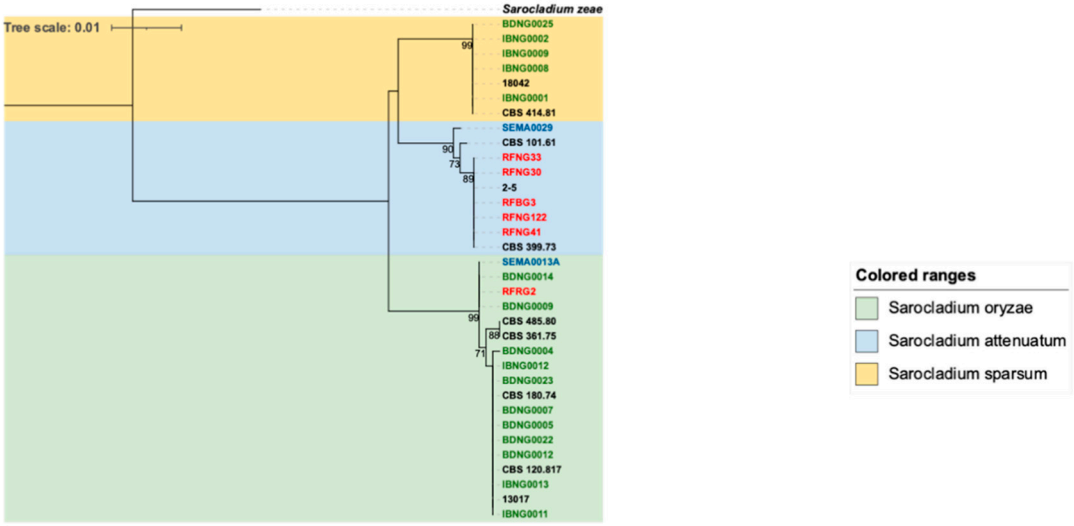

Figure 4. Phylogenetic tree based on the concatenation of both ITS and Actin region of *Sarocladium* species obtained in this study. The tree was generated using the Jukes Cantor model and the maximum likelihood method in MEGA. *Sarocladium zeae* strain CBS 800.69 was used as an outgroup. Sequences in colour and bold were obtained in this study (Blue – Mali, Green – Nigeria and Red – Rwanda).

3.3. Pathogenicity Testing

Representative isolates from the dominant *Fusarium* groups recorded in Nigeria, Rwanda and Mali were used for pathogenicity testing on the FARO 44 rice variety. All the four *Fusarium* species complexes could induce rice sheath rot symptoms on the rice cultivar, but the degree of virulence of all the *Fusarium* species tested showed significant variations on the rice cultivar (Figure 5). Specifically, one of the two isolates of *F. marum* IBNGF0016 from Ibadan in Nigeria had the highest disease severity on FARO 44 followed by the second *F. marum* isolate IBNGF0012 and an FIESC isolate *F. sulawesiense* BDNGF0002 from Katcha in Nigeria. On the contrary, *F. nygamai* originated from Mali and one of the FIESC isolate *F. tanahbumbuense* BDNGF0001 from Ibadan-Nigeria were the least virulent isolates.

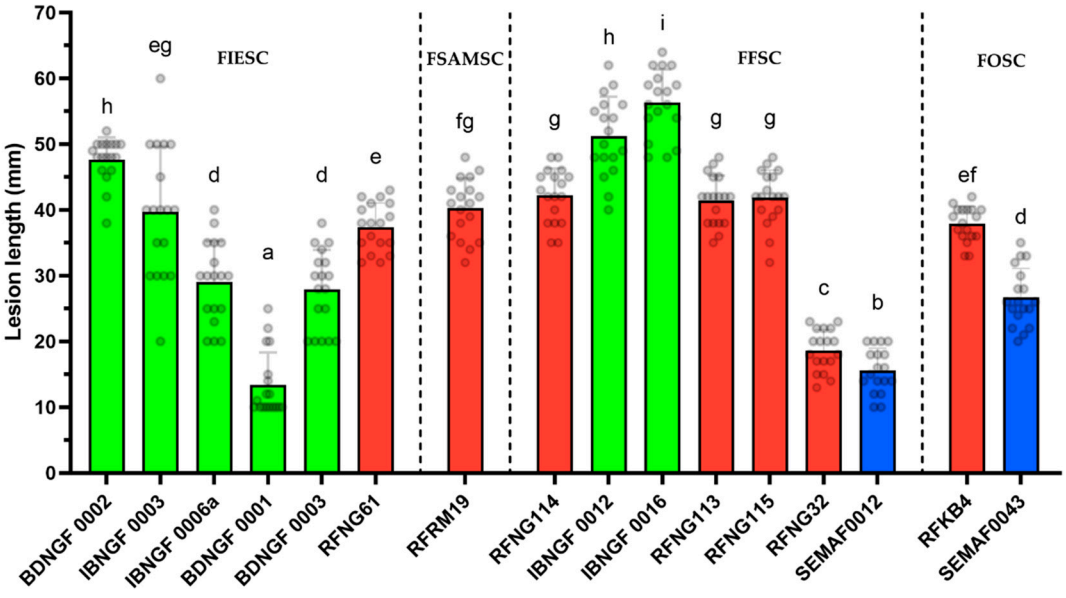

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Figure 5. Mean lesion length (millimeters) at 8 dpi on FARO44 rice cultivar inoculated with isolates from four Fusarium species 6 weeks after planting. Different letters indicate statistically significant differences based on Kruskal-Wallis Rank Sum test followed by a post hoc Mann-Whitney test. Statistical significance was defined as P= 0.05.

For the pathogenicity tests with *Sarocladium* species, disease evaluation at 8 days post inoculation (DPI) showed that all the thirteen isolates tested could induce typical sheath rot symptoms on FARO 44 albeit with varying disease development levels (Figure 6). Isolates affiliated with *S. sparsum*, all of Nigeria origin, were the most aggressive ($p < 0.05$). In contrast, isolates affiliated with *S. oryzae* and *S. attenuatum* were less virulent (Figure 6). *S. oryzae* isolate IBNG0011 from Nigeria is the most aggressive among the group while SEMA0013A from Mali appears to be the least virulent.

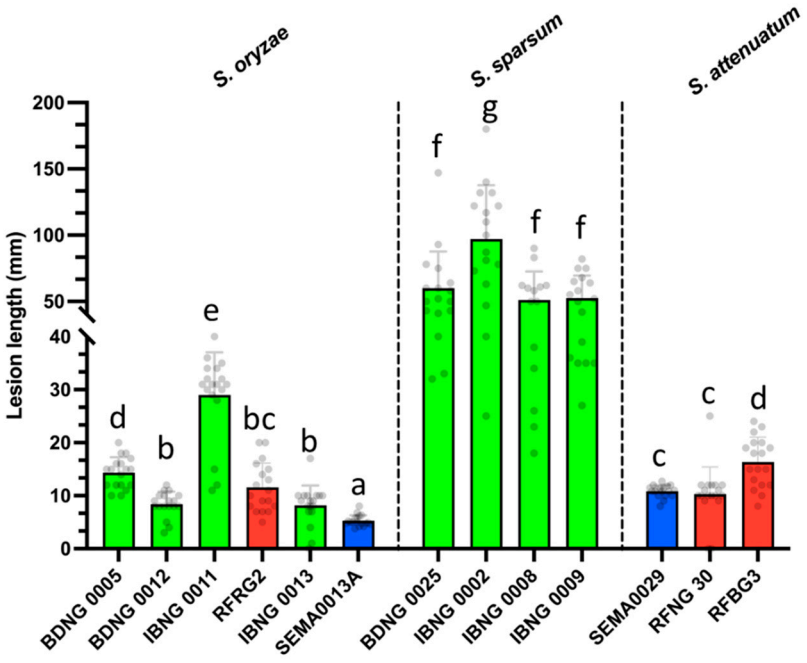

Figure 6. Mean lesion length (millimeters) at 8 dpi on FARO44 rice cultivar inoculated with Sarocladium species after 6 weeks of planting. Different letters indicate statistically significant differences based on Kruskal-Wally's Rank Sum test followed by a post hoc Mann-Whitney test. Statistical significance was defined as P= 0.05.

3.4. Mycotoxin Profiling (In vitro)

Forty-six *Fusarium* and thirty-one *Sarocladium* species obtained from rice with sheath rot symptoms were investigated for multi-mycotoxin production using LC-MS/MS . None of the *Sarocladium* isolates produced mycotoxins. Our results revealed that the *Fusarium* species were able to produce eight mycotoxins. The most detected mycotoxins include type A (DAS and NEO) and B (NIV and FUS-X) trichothecenes which were produced by FIESC isolates obtained across the two regions of Nigeria albeit at different concentrations (Table 4). Furthermore, zearalenone (ZEN) at a very high concentration of 26,173 and 32,529 µg/kg was detected in the two Ibadan-Nigerian *F. hainanense* isolates IBNGF0005 and IBNGF0003, respectively. Two Rwandan isolates *F. madaense* RFRM18 and *F. acasiae-mearnsii* RFRM19 produced ZEN at the concentration of 1349 and 329 µg/kg, respectively. The predominant mycotoxins detected from Malian *F. nygamai* isolates were fumonisins (FB1, FB2, and FB3), which occurred at a very high concentration. FB1 concentrations ranged from 53,118 – 141,102 µg/kg followed by FB2 ranging from 3,391 - 5,122 µg/kg while FB3 occurred at a lower concentration which ranged between 355 – 692 µg/kg. Besides *F. nygamai*, *F. annulatum* SEMAF17-225A from Mali produced DAS, FUS-X, NIV, and NEO at different concentrations (Table 4).

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Table 4. Mycotoxins produced by the *Fusarium* spp *in vitro*. The values are concentration in (µg/kg) using LC-MS/MS. All strains were tested, only mycotoxins producers are reported.

Fusarium sp.	Strain code	Mycotoxin (µg/kg)							
		NIV	NEO	FX	DAS	FB1	FB2	FB3	ZEN
FIESC									
F. hainanense	IBNGF0003								32432
F. hainanense	IBNGF0005								29681
F. sulawesiense	IBNGF0004	73		106					
F. sulawesiense	IBNGF0006A	115	20	196					
F. sulawesiense	IBNGF0006B	95							
F. sulawesiense	IBNGF0007A	56							
F. sulawesiense	IBNGF0013	47	16	211					
F. sulawesiense	IBNGF00019	2575	118	1370	53				
F. sulawesiense	BDNGF0002	81							
F. pernambucanum	IBNGF0002	122	25	355					
F. tanahbumbuense	BDNGF0001				12				
F. tanahbumbuense	BDNGF0003	53							
FFSC									
F. annulatum	SEMAF17-225A	567	34	159	77				
F. madaense	RFRM18	195		1120					1349
F. nygamai	SEMAF0010					69679	4234	573	
F. nygamai	SEMAF0012A					118024	9325	702	
F. nygamai	SEMAF0012B					53118	3389	355	
FOSC									
F. triseptatum	SEMAF0043	40							
FSAMSC									
F. acasiae-mearnsii	RFRM19	82		178					330

4. Discussion

This study provides new insights into the genetic, pathogenic, and toxigenic diversity of *Fusarium* and *Sarocladium* species associated with rice sheath rot disease in SSA. Molecular characterization using the *EF-1α* gene enabled the delineation of *Fusarium* isolates into four distinct *Fusarium* species complexes, whereas concatenation of *ITS* and *ACT* sequences delineated *Sarocladium* into three species. It was discovered that *Fusarium* species are the dominant species associated with rice sheath rot disease in Mali and Rwanda, while in Nigeria, *Fusarium* and *Sarocladium* species were equally represented. Phylogenetic analysis showed that isolates grouped differently according to their geographical location (Figure 3 & 4).

Four *Fusarium* species complexes (FIESC, FFSC, FOSC, and FSAMSC) were found to be associated with rice sheath rot in SSA. Previous research has resolved FIESC species as a mere complex indicated by numbers, but current studies further updated them according to the new nomenclature well elucidated from numbers to names [49–51]. Additionally, they were characterized using the recently updated *Fusarium* ID database (Fusarioid-ID). Dominant in our findings were members of the FIESC previously classified from rice, cereals, insects, and human samples [29,33,52–55] which include *F. sulawesiense* (FIESC 16), *F. pernambucatum* (FIESC 17), *F. tanahbumbuense* (FIESC 24), F. *hainanense* (FIESC 26), and *F. incarnatum* (FIESC 38). This is similar to those found associated with rice sheath rot in Indonesia [19], India [17] and the USA [18]. Larger part of the isolates

clustered with *F. sulawesiense* which supports the findings in Brazil and China [29,56], while the abundance of *F. hainanense* and few others such as *F. pernambucatum*, *F. tanahbumbuense*, were among the FIESC reported on Brazilian rice. There is a wide variation among the species obtained within the FIESC complex and the observed variation could be correlated with variation in agro-ecological zones. Notable is the fact that some of the *F. sulawesiense* isolates from Ibadan are closely related to strains NTB 1 (rice sheath rot, Indonesia), LC6936 (rice, China), F1 (Sweet potato, US), BT48, and PRT6 (oil palm, Indonesia), and ITEM7547 (*Musa*, Bahamas) all of which originated from climates characterised by high temperature and humidity. Isolate F1 originated from Louisiana, USA that is known for its humid subtropical climate with long, hot, and humid summer, similar to the climate of ITEM7547 from Bahamas [57,58]. Based on our findings we can hypothesize that environmental factors could be the driving forces to be considered in the distribution of the FIESC isolates. Notable differences in climate and farming practice could not be underestimated as the three *F. incarnatum* isolates from a higher altitude in Rwanda formed part of recently classified FIESC 38 isolates from a similar altitude and climate in Brazil [29]. Within the FIESC clades, none of the studied isolates was found among *F. equiseti* clade which disagrees with the previous studies of [54,55,59] whereby variable percentage of both *F. equiseti* and *F. incarnatum* were reported from rice samples. Members of *F. equiseti* clade are frequent in cereals grown in Western Europe, Turkey, and North America [34].

FFSC species have been implicated as another causal agent of various rice diseases including sheath rot [51,60]. *F. andiyazi* and *F. madaense* represent the principal species recovered in Rwanda; *F. nygamai* was the dominant species isolated from Mali, while *F. marum* was recovered from Nigeria. An isolate each of *F. casha* (Rwanda) and *F. annulatum* (Mali) were also found among our FFSC. The peculiarity in the clustering of *F. andiyazi*, *F. madaense* and *F. marum*, clades observed in our studies is comparable to the typical phylogenetic pattern observed in [61]. *F. andiyazi* and *F. madaense* are typically associated with tropical grasses including sorghum, maize, millet, and rice in various parts of the world [61] The two virulent *F. marum* isolates from Nigeria clearly resolved into a separate clade and clustered with two *F. marum* isolates obtained from sorghum in Cameroon [61]. Isolates clustering with *F. nygamai*, the most dominant species in Mali, are closely related to isolates obtained from cereals from regions with similar warm and dry climate (Fig 4), such as Australia [62], Italy [28], Mexico [63], and Tunisia [36]. These FIESC and FFSC findings are consistent with the recent report of rice sheath rot disease in Indonesia, [19], and rice disease in China [56,64].

Furthermore, isolates clustering with *F. callistephi* were found in Rwanda, while *F. triseptatu*m was obtained in Mali. Both are members of the FOSC. *F. callistephi* is mainly known as a wilt pathogen on ornamentals from the Asteraceae family [65]. Lastly, members of FSAMSC which includes two isolates clustering with *F. acasiae-mearnsii* of Rwanda origin were also identified (Fig. 4). *F. acasiae-mearnsii* isolates have previously be found in Australia and South-Africa and can cause head blight on wheat [66].

Reports on mycotoxins produced by *Fusarium* species isolated from rice sheath rot disease in sub-Saharan Africa are very limited. In consequence, this is the first study to investigate the toxigenic potentials of *Fusarium* isolates from rice sheath rot disease in Mali, Nigeria, and Rwanda. The predominant mycotoxins found in FIESC isolates from Nigeria were trichothecenes, while the three *F. incarnatum* isolates from Rwanda did not produce mycotoxins. Among the 12 FIESC isolates collected from Ibadan (derived savannah region) in Nigeria, type A (DAS, NEO) and B (NIV, FUS-X) trichothecenes were detected in 7 samples. This confirms the previous mycotoxins findings on cereals [29,59,67]. In addition, a huge ZEN production of 32,529 and 26,173 µg/kg was detected from the two *F. hainanense* isolates collected from Ibadan. Similar results for *F. hainanense* were obtained in Brazilian rice [29]. This also corroborates the study of [68] which demonstrated that ZEN production by *Fusarium* species is greater in moldy samples which is favoured by wet climates with high rainfall and high humidity. Within the FFSC, *F. andiyazi* isolates did not produce mycotoxins, while the *F. annulatum* isolate and 3 out of the 5 *F. nygamai* isolates from the dry and hot Sudan Guinea Savannah of Mali produced trichothecenes and fumonisins (FB1, FB2, and FB3), respectively. This is consistent with the findings of [69] reporting high levels of toxins for *F. nygamai* while *F. andiyazi* isolates produced little or no mycotoxins. Moreover, fumonisin-producers were not detected among the

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isolates collected from Nigeria and Rwanda. Thus, fumonisin contamination may be expected to be higher in samples collected in the Sahel with a warm and dry climate. The development of fumonisins in cereal crops prior to harvest might increase due to heat and water stress that characterized the environmental drought [70]. Only two of the 23 *Fusarium* isolates from Rwanda produced mycotoxins: trichothecenes (NIV and Fus-X) and ZEN were detected in a *F. madaense* isolate and a *F. acaciae-mearnsii* isolate, both obtained from the Rwamagana district. It has been shown before that *F. acacia-mearnsii* isolates can produce NIV [71] and ZEN [72].

According to the research of [73], which elucidated the presence and absence of biosynthetic gene clusters responsible for the synthesis of mycotoxins and secondary metabolites in FIESC, further studies are necessary to investigate if the mycotoxin production potentials of our isolates agree with their genetic profile or assess if there are differences in expression level. In conclusion, mycotoxin production is common in rice-derived *Fusarium* isolates from Nigeria (12 out of 15) and Mali (5 out of 7), but rare in Rwanda (2 out of 25).

In contrast to the heterogeneity observed among *Fusarium* species, three clearly delineated *Sarocladium species* were recovered from the three countries of study, however with lower frequency of occurrence in Mali and Rwanda. Following the characterization of *Sarocladium* species causing rice sheath rot in Taiwan by [12] and using a concatenation of two genes, we were able to resolve our isolates into three species namely *attenuatum, oryzae* and *sparsum* (Fig. 4). Isolates belonging to *S. sparsum* were only found in Nigeria and mostly originated from Ibadan (DS) except for an isolate (BDNG0025) from an infected seed in Katcha (SGS). Finding larger part of this group in Ibadan was not strange because it clusters with an Ibadan origin reference isolate CBS 414.81, collected and reported as *S. attenuatum* [21], and later deposited into the Genbank [42]. Our results clearly show, however, that isolate CBS 414.81 belongs to *sparsum* species. Surprisingly, this group was not found in Mali and Rwanda. There occurs a notable correlation between this group and the collection region, which proved that geographical area and climate are the most crucial factors that influence occurrence of these pathotypes and their virulence. This agrees with the hypothesis that isolates from different locations may also vary in their level of aggressiveness [74] [75]. *S. oryzae* isolates (Fig. 4) showed a strong intra-species similarity that is not phylogeographic based. Isolates in this species were the most predominant and widely distributed. It consists of 18 similar isolates from nearly all the rice-growing regions in the world. Most isolates from Nigeria (11 of 16) belongs to this group. An isolate from Mali (SEMA0013A) generated from this study, isolates from previous studies on rice such as 13017 from Taiwan [12] CBS180.74 from India, African isolates CBS361.75 from Kenya [76] RFRG2 from Rwanda (this study), Central American CBS120.817 from Panama and CBS485.80 Australia [77] are part of this group. They were found in two agro-ecological zones of Nigeria, although more frequently from Katcha than Ibadan.

The presence of *S. oryzae* in all the rice growing regions of the world is a signal of its flexibility to adjust to various agro-ecological zones. It also suggests a link between its dispersal, rice movement and international trade. A potential quarantine threat is of a great concern with the rapid distribution of this group. This might also imply that the origin of the isolate may be connected to Asia. It should be noted that African countries including Mali, Nigeria and Rwanda are major importers of rice from Asia despite Nigeria being the highest producer in the continent.

A third distinct group, *S. attenuatum*, was dominated by Rwandan isolates, the second Mali isolate, SEMA0029 also formed part of the group.

It is important to note that the two *Sarocladium* isolates obtained from Mali formed two of the three species found in this study despite the small sample size. Several reasons might be responsible, from accession variability, as local rice is mostly cultivated in Mali, [78], to toxigenic variability among the competing *Fusarium* species, and multiple cropping system variation, among others. Larger scale surveys across the country are necessary to have a wider knowledge of genetic diversity and distribution.

It is a well-known phenomenon that several factors such as variation in climate, topography, farming practices among others are drivers of variation in pathogen populations. To verify this claim, this study has revealed a strong ecology driven diversification among the *Sarocladium species* used. It

also exposed how environmental variation was able to influence the genetic and virulence relatedness. The *S. sparsum* isolates that are mainly found in Ibadan (DS) are clearly more aggressive on the rice FARO44 cultivar used in this study than the *S. oryzae* and *S. attenuatum* strains, which are more common in the savannah region in Katcha-Badeggi and in Rwanda. This confirms previous work showing that group 3 strains (= *S. sparsum*) are more aggressive on the rice *japonica* cultivar Kitaake than group 1 (= *S. oryzae*) or group 2 (= *S. attenuatum*) strains. *S. sparsum* isolates also produce high amounts of the toxin helvolic acid *in planta* which is clearly correlated with disease severity [79].

The results showed that isolates of *Sarocladium* species were able to induce sheath rot symptoms and they are the most aggressive pathogens on FARO44 cultivar used in this study with higher pathogenicity index. We also observed a noticeable correlation between isolate's country of origin and virulence, which tends to agree with earlier findings where isolates were found to be widely diverse in virulence within countries and within the same population. The aggressiveness of *Sarocladium* species collected from the two locations in Nigeria, and Selingue in Mali seems to be influenced by their location. The same trend of aggressiveness was reported in the study of [21] who used four isolates (presumably *S. sparsum*) collected from the southern region (DS and Humid Forest) of Nigeria. The similarity between isolates from Katcha (SGS) in Nigeria and the two Mali (NGS) isolates that were classified as moderate and low virulence may not be unconnected with the similarity in their ecological origin.

5. Conclusion

In conclusion, our study showed that diversity occurs within the East (Rwanda) and West African (Mali and Nigeria) isolates of *Fusarium* and *Sarocladium*. Notable correlation was observed between the virulent group and collection area. The toxigenic profile of both pathogens was elucidated, and we found that most *Fusarium* isolates from Nigeria and Mali were able to produce one or more mycotoxins. In contrast, only two out of 24 isolates from Rwanda were able to produce mycotoxins. Further investigations with a broader geographic scope and a larger collection of samples are necessary to examine pathogenic variability and the population genetic structure. Meanwhile, based on the distinct groups of isolates from different regions, breeders in various agroecological regions should take note of the variations in virulence. This information can serve as a basis for selecting strains useful for identification and selecting effective sources of resistance for local rice breeding programs.

Moreover, additional studies are required to determine whether *Sarocladium* and *Fusarium* individually contribute to the observed symptoms in the field or if there is an interplay between both pathogens in the rice sheath rot complex. To the best of our knowledge, this research provides the first comprehensive dataset on the distribution, genetics, pathogenicity, and toxigenic profile of *Fusarium* species associated with rice sheath rot disease in sub-Saharan Africa.

Author Contributions: Conceptualization,V.B.; O.A.; O.I.N; and M.H.; methodology, O.A.; V.B.; M.D.V, F.O. and G.K.H.H.; validation, O.A.; V.B.; formal analysis, O.A. investigation, O.A.; V.B.; L.B, and G.K.H.H.; Resources, O.A.; V.B.; J.O.; O.E.O.; A.O.; curation, O.A; writing—original draft preparation, O.A. writing, review, and editing, M.H.; visualization, O.A.; V.B.; supervision, M.H, and S.D.S project administration, O.A.; V.B.; M.H.; funding acquisition, O.A.; V.B.; S.D.S.; and M.H.; All authors have read and agreed to the published version of the manuscript.

Funding: V.P. Bigirimana received a doctoral grant from the Belgian Technical Cooperation (BTC) (project reference: 10RWA/0018). This work was funded by a grant from the Special Research Fund of Ghent University (GOA 01GB3013) and by the Fund for Scientific Research Flanders (FWO G031317N).

Acknowledgments: The authors wish to appreciate Christ'l Detavernier, Frédéric Dumoulin (UGent, Faculty of Pharmaceutical Sciences) for their help with the LC-MS/MS analysis. We also acknowledged the contributions of Aderonke Oludare and Opeyemi Ogedengbe (AfricaRice) for their coordination of rice sampling and transportation to Belgium.

Conflicts of Interest: The authors declare no conflict of interest.

Table A1. Reference strains of *Fusarium* obtained from GenBank used for building phylogenetic tree.

Species complex	Species	Isolate name	Host	Origin	Accession number	References
FIESC	*F. equiseti*	NL19-045005	Soil	Netherlands	MZ921835	[50]
		CPC 35262	Human toenail	Czech republic	QED42271	[49]
	F. hainanense (26)	LC11638	*Oryza sp*	China	MK289581	[80]
		15Ar057	Rice	Brazil	MK298120	[29]
		LB2	*Oryza sativa*	Philippines	JF715935	[31]
		PCO2	Oil palm	Indonesia	HM770725	[58]
		NRRL 28714	Clinical samples	USA	GQ505604	[54]
		JBR 10	*Oryza sativa* sheath	Indonesia	MT138474	[19]
	F. nanum (25)	F685	Wheat	Spain	KF962950	[81]
		NRRL 22244	Clinical samples	USA	GQ505596	[54]
	F. tanahbumbuense (24)	LC13726	*Digitaria* sp	China	MW594396	[80]
		NTT 6	*Oryza sativa* sheath	Indonesia	MT138460	[19]
	F. incarnatum (38)	ITEM 7155	*Trichosanthe dioica*	Malawi	LN901581	[34]
	F. pernambucanum (17)	NRRL 32864	Clinical samples	USA	GQ505613	[54]
		CBS 791.70	*Musa sampientum*	Netherlands*	MN170491	[49]
	F. sulawesiense (16)	CBS 622.87	*Bixa orellana*	Brazil*	MN170503	[49]
		ITEM7547	*Musa sampientum*	Bahamas	LN901580	[34]
		LC12173	*Luffa aegyptica*	China	MK289605	[80]
		MS3369	Wild rice	Brazil	MT682685	[82]
		LC6936	*Oryza sativa*	China	MK289621	[80]
		F1	Sweet potato	USA	KC820972	[83]
		BT48	Oil palm	Indonesia	HM770722	[58]
		PRT6	Oil palm	Indonesia	HM770723	[58]
		NTB 1	Indonesia	*Oryza sativa* sheath	MT138458	[19]

doi:10.20944/preprints202308.2064.v1

FFSC	*F. andiyazi*	LLC 1152	*Striga hermonthica* seed	Ethiopia	OP486864	[84]
		MO5-1946S-3_PCNB	Sorghum grain	USA	KM462919	[85]
	F. marum	KSU 15077	Sorghum	Cameroun	MT374735	[61]
		KSU15074	Sorghum	Cameroun	MT374736	[61]
		E432	Rice seeds	Italy	GU827420	[86]
		E439	Rice seeds	Italy	GU827419	[86]
		30ALH	*Oryza sativa* seed	China	FN252387	[15]
	F. madaense	CBS 146669	*Arachis hypogaea*	Nigeria	MW402098	[40]
		44ALH	*Oryza sativa* seed	Tanzania	FN252390	[15]
		IALH	*Oryza sativa* seed	Burkina Faso	FN252388	[15]
		CML3853	*Sorghum bicolor*	Nigeria	MK895723	[61]
		CML3895	*Sorghum bicolor*	Tanzania	MK895727	[61]
		PB-2	Sugarcane	China	KP314282	[87]
		38ALH	*Oryza sativa* seed	India	FN252389	[15]
	F. casha	PPRI20462	*Amaranthus cruentus*	South Africa	MF787262	[88]
	F. nygamai	B5Hp1g3B1	Barley	Tunisia	MG452941	[36]
		KC 13	Tomato	Kenya	KT357537	[89]
		ENTO90	Wild rice	Australia	MG873156	[62]
		M7491	Rice	Italy	HM243236	[28]
	F. annulatum	LC11670	*Oryza sativa*	China	MW580517	[64]
		34ALH	*Oryza sativa* seed	China	FN252396	[15]
		LC13675	*Syzygium samarangense*	China	MW580542	[64]
	F. proliferatum	CBS 480.96	Soil	Papua New Guinea	MN534059	[90]
FOSC	*F. triseptatum*	LC13771	Deep sea sediment	China	MW594358	[64]
	F. oxysporum	Foc230	Banana	Nigeria	AY217161	Upublished
	F. callistephi	CBS 187.53	*Callistephus chinensis*	Netherlands	MH484966	[84]

doi:10.20944/preprints202308.2064.v1

		SRRC1630	Cooked rice	Nigeria	KT950251	[91]
		CBS 115423	*Agathosma betulina*	South Africa	MH484996	[84]
FSAMSC	*F. acaciae-mearnsii*	LC13786	*Musa nana*	China	MW620091	[64]

Table A2. Sequences of reference strains of *Sarocladium oryzae* on rice obtained from GenBank used for building phylogenetic trees.

Genus	Species	Isolate	Origin	Accession number ITS	Reference ITS	Accession number ACT	Reference ACT
Sarocladium	*Attenuatum*	CBS 399.73	India	HG965027	[42]	HG964979	[42]
		2-5	Taiwan	LC461444	[12]	LC464336	[12]
	oryzae	CBS 180.74	India	HG965026	[42]	HG964978	[42]
		13017	Taiwan	LC461506	[12]	LC464380	[12]
	sparsum	CBS 414.81	Nigeria	HG965028	[42]	HG964980	[42]
		18042	Taiwan	LC461520	[12]	LC464308	[12]
Sarocladium	*zeae*	CBS 800.69	USA	FN691451	[92]	HG965000	[42]

doi:10.20944/preprints202308.2064.v1

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