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GENETIC MAPPING OF FUSARIUM STRAINS IN RICE SEEDS FROM THE WEST AFRICAN REGION

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Abstract: Rice (Oryza sativa L.) is a vital global food crop and economic resource, particularly in Africa. Burkina Faso, with rice cultivation in all 13 administrative regions, exhibits notable production concentrations in Hauts Bassins (16.97%), the East (15.02%), Centre-Est (14.82%), Boucle du Mouhoun (14.44%), and Cascades (10.91%). Fusarium species pose a significant threat to rice, with Fusarium-induced diseases affecting 80% of global crop plants. This study delves into the prevalence of fungal diseases, specifically those within the Gibberella fujikuroi species complex, including F. verticillioides, F. sacchari, F. subglutinans, F. proliferatum, F. andiyazi, and F. fujikuroi. Such diseases have a widespread presence, impacting agricultural crops globally.

Fusarium species like F. graminearum, F. poae, and F. avenaceaum cause severe damage to cereals. Rice, in particular, is susceptible to various fungal diseases, including the emerging threat of Bakanae disease, known for inflicting substantial yield losses in Pakistan (10-50%), India (15-25%), and Nepal (40%). Bakanae disease is attributed to one or more Fusarium species and manifests with seedling blight, root and crown rot, stunting, and distinctive symptoms induced by gibberellin, a pathogen-produced hormone. However, the presence of multiple Fusarium species within the Gibberella fujikuroi species complex complicates their differentiation.

Keywords: Rice Diseases, Fusarium Species, Bakanae Disease, Crop Health Burkina Faso Agriculture Fusarium, Single spore, Molecular identification, Rice seeds, Burkina Faso

1. Introduction

Rice (Oryza sativa L.) is an important food crop and source of income for farm households worldwide, and particularly in Africa. In Burkina Faso, it is grown in almost all of the 13 administrative regions, although there are regions with very high production, such as the Hauts Bassins (16.97% of national production), the East (15.02%), the Centre-Est (14.82%), the Boucle du Mouhoun (14.44%) and the Cascades (10.91%) (DGSS, 2020). As with rice, Leslie and Summerell (2006) report that at least 80% of the world's crop plants are associated with at least one disease caused by a *Fusarium* species. Fungal diseases caused by *Fusarium* species in the *Gibberella fujikuroi* species complex, including *F. verticillioides*, *F. sacchari*, *F. subglutinans*, *F. proliferatum*, *F. andiyazi*, and *F. fujikuroi*, are among the most common diseases reported on agricultural crops worldwide (Hsuan et al., 2011). Various *Fusarium* species including *F. graminearum*, *F. Poae*, *F. avenaceaum*, cause destructive diseases on

cereals (Goswami and Kistler, 2004; Xu et al., 2005). Rice is attacked by a large number of fungal diseases, including gigantism (Bakanae), a widespread and sometimes emerging disease in several rice-producing countries (Bashyal et al., 2016; Raghu et al., 2018). Bakanae disease is known to cause yield loss of 10-50% in Pakistan, 15-25% in India, and 40% in Nepal (Sunder et al., 2014). This disease is caused by one or more *Fusarium* species and exhibits a symptom complex that includes seedling blight, root and crown rot, stunting, and the classic symptoms of wilting and abnormal plant elongation that are induced by gibberellin, a hormone produced by the pathogen. The *Gibberella fujikuroi* species complex comprises an assemblage of *Fusarium* species with similar and overlapping morphological traits, which complicates their differentiation (Kvas et al., 2009).

Most species produce inocula that are adapted to warm, humid conditions and are highly pathogenic to cereals (Doohan et al., 2003). Infection of cereal grains such as rice by *Fusarium* species can cause contamination with mycotoxins that affect human and animal health. Fumonisins cause fatal diseases of livestock and are considered potentially carcinogenic mycotoxins for humans (Munkvold, 2017). Warth et al. (2012) detected various mycotoxins including moniliformin, in various human and animal foods in Burkina Faso. High levels of mycotoxins have also been reported in samples of infant food and cereals produced in Burkina Faso (Waré et al., 2017).

Gigantism is primarily seed-borne, and high levels of seed infection with *Fusarium* species have been found in rice seed samples produced in Burkina Faso (Mathur and Manandhar, 2003; Ouédraogo et al., 2016; Nikiema et al., 2020). These authors reported that 81-100% of rice seed samples were contaminated with *Fusarium* fungi with attack rates ranging from 1 to 41%. Despite the fact that accurate knowledge of the identity of a pathogen responsible for a given disease remains the first step toward establishing adequate disease control measures (O'Donnell and Cigelnik, 1997), knowledge of the exact identity of *Fusarium* species associated with rice seed in Burkina Faso has been little investigated. The only works are those of Mathur and Manandhar (2003), and Ouédraogo et al., (2016) that relied entirely on morphological characters to distinguish species. However, the identification of *Fusarium* species is made difficult because of the frequent lack of discriminatory cultural or morphological characters. For the last decades, the approach used has been to describe species based on DNA sequence analysis. Molecular tools, especially DNA sequencing, have emerged as a key means to identify pathogenic fungi regardless of their developmental stage and morphology. The approach has greatly reduced the number of *Fusarium* species (about 300) and grouped the species under the designation of phylogenetic clade or species complex (Geiser et al., 2013). Despite the importance of rice as a food grain and the high presence of *Fusarium* fungi in rice seeds in Burkina Faso, the available information appears insufficient to reveal the exact identity and assess the prevalence, importance and distribution of *Fusarium* species present on rice seeds in Burkina Faso. Our objective in this study is to accurately identify the *Fusarium* species associated with rice seeds in Burkina Faso.

2. Materials and Methods

2.1. Collection *of rice seed samples*

Rice seed samples were collected during the 2017-2018 season from farmers at a rate of 500-1000g of seed per sample. The sampling was done in 59 sites located in 31 provinces of the country. These provinces are cutting across the country's 13 administrative regions and the three main agroclimatic zones (Sahelian, Sudanese and Sudanian) of Burkina Faso. Samples were collected on well-known seven rice varieties, including six improved varieties (FKR19, FKR45N, TS2, FKR56, FKR78, NERICA4) and one local variety.

2.2 *Isolation and purification of Fusarium isolates from rice seeds*

The fungal isolates used in this study were isolated from rice seeds. The standard blotting paper method was used to detect fungi of the genus *Fusarium* capable of growing on seeds in the presence of moisture. For this purpose, 400 seeds of each sample were placed in Petri dishes (90 mm diameter) containing three layers of moistened blotting paper, at a rate of 25 seeds per dish. The plates were placed in the incubation chamber at 25°C and for seven (7) days under alternating light cycles of 12 hours of near ultraviolet light and 12 hours of darkness per day. Incubated seeds were examined individually under a stereoscopic microscope and identification was confirmed by examination of the mycelium and/or conidia under a light microscope and using the Fungal Identification Manual of Mathur and Kongsdal (2003). The *Fusarium* fungi present on the seeds were collected and subcultured on PDA (Patato Dextrose Agar) medium composed of one liter of distilled water, 4 g of potato infusate, 20 g of D(+) - glucose and 15 g of agar. Successive subcultures allowed the purification of the different fungi in culture, then their conservation at a temperature of 4 °C.

2.3 *Production of single spore isolates*

Single spore cultures were produced from pure cultures of *Fusarium* isolates (population isolates). For each population isolate, two drops of conidial suspension containing approximately 100 spores/ml of suspension prepared from a 10-day-old pure culture were spread on Agar medium contained in a Petri dish. Two days after seeding, germinating spores were transferred to Petri dishes containing PDA medium using a single germinating spore per dish. On the basis of morphological characteristics (colony color, conidial shape and size), one to four single-spore isolates were selected per isolate population for use in the study. At the end of the operation, 125 single-spore isolates were obtained for biomolecular analysis.

2.4 *Sequencing of the 5,8S-ITS region*

One strain was selected from each restriction profile group for sequencing. PCR products were purified (NEW ENGLAND Biolabs® Inc.) under the combined action of two hydrolytic enzymes (Exonuclease I and Shrimp Alkaline Phosphatase).

2.5 *Genomic DNA extraction and ITS amplification*

Genomic DNA was extracted from mycelia grown on liquid Sabouraud medium for two days. DNA extraction was performed using the CTAB (Cetyltrimethylammonium bromide) method (Kumar et al., 2014). The amplification reaction was performed in a 25 µl reaction volume containing 5 µL of DNA, 7.5 µL of sterile distilled H2O, 5 µL of Buffer (5X), 3 µL of MgCl2 (25 mM), 2 µL of dNTPs (10 mM), 1 µL of each of the primers (ITS1 at 10 µM and ITS4 at 10µM), and 0.5 µl of Taq DNA polymerase). The

primers used were ITS1: TCC GTA GGT GAA CCT GCG G and ITS4: TCC TCC GCT TAT TGA TAT GC (White et al., 1990).

The amplification reaction was performed in a MJ Research thermal cycler (PTC-200) according to the following program: pre-denaturation at 95°C for 5 minutes followed by 35 consecutive cycles of denaturation at 94°C for 1 minute, primer-specific hybridization at 55°C for 1 minute and elongation at 72°C for 1 minute. Final elongation was performed at 72°C for 10 minutes and the amplicons were kept at 4°C in the thermal cycler. The amplified DNA had been separated on a 1.25% agarose gel in the presence of a molecular weight marker (100 bp), a negative control (water) and a positive control (yeast DNA). The PCR products were visualized and photographed under UV light.

2.6 *RFLP analysis*

Enzymatic digestion was performed in a 25 µl reaction volume containing 6µL of PCR product, 16 µL of sterile distilled H2O, 2.5 µL of RE- Buffer (10X), 0.5µL of HhaI enzyme. The program was as follows: incubation at 37°C for 15 minutes followed by 80°C for 5 minutes. The enzymatic reaction product has been visualized after electrophoresis on 2% agarose gel. The resulting products were reamplified using the Genomelab DTCS Quick Start Kit (Beckman Coulter, USA) following the cyclic sequencing program of 30 cycles of denaturation (96°C, 20 s), hybridization (50°C, 20 s) and extension (60°C, 4 min). Cycle sequencing products were purified by ethanol precipitation and then separated using a CEQ TM8000 gene analyzer sequencer.

2.7 *Sequence analysis*

Fungal species identification and phylogenic tree construction were performed using Geneious Prime software (Version 2020.0.3). Cliqs software version 1.4.097 was used to determine the size of the different fragments. The ITS nucleotide sequences for each species were then compared to the NCBI (National Center for Biotechnology Information: www.ncbi.nih.gov) databases using the Basic Local Alignment Search for Nucleotide

Sequences (BLASTN) tool. The phylogenetic tree was constructed using MEGA 7.0.14 software (Kumar et al., 2016) using the UPGMA (Unweigted Pair Group Method with Arithmetic Mean) method (Backeljau et al., 1996). Reliability was estimated using the bootstrap method with 1000 permutations.

3. Results

3.1 *Rice seed samples and Fusarium isolates collected*

Fifty-nine (59) seed samples were collected from 59 localities in the three agro-ecological zones of the country. Among these samples, 36 samples (i.e. 61.02%) came from the Sudano-Sahelian zone, 18 samples (i.e. 30.51%) from the Sudanian zone and 5 samples (i.e. 8.47%) from the Sahelian zone. It is noted that the majority of rice seed samples came from the Sudano-Sahelian and Sudanian zones with 61.02% and 30.51% respectively, while in the Sahelian zone, the number of samples collected was relatively low. Twenty-eight (28) *Fusarium* isolates were successfully isolated and purified from *Fusarium* spp. infected rice seeds. From the 28 core isolates or "population isolates", genomic DNAs of 118 single-spore isolates were obtained and submitted for molecular identification.

3.2 *Molecular identification based on ITS*

The total size of the ITS1 and ITS4 regions of all single spore isolates, including the 5.8S rDNA gene ranged from 544 to 579 bp. The amplicons obtained (Fig.1) and the restriction fragments (Fig. 2) indicated four profiles. Profiles 1 and 4 correspond to isolates with an amplicon of 556 bp and 579 bp, respectively. Profiles 2 and 3 correspond to isolates with an amplicon of 544 bp each,

Analysis of the amplicons obtained after enzymatic restriction of the HhaI enzyme reveals that each isolate produced 2 characteristic bands between 172- 290 bp (Fig. 2). Profile 1 isolates show bands at 290-172 bp while profile 2 isolates display bands at 200-265 bp. Profile 3 and profile 4 isolates show respective characteristic bands located at 247-290 bp and 177-200 bp.

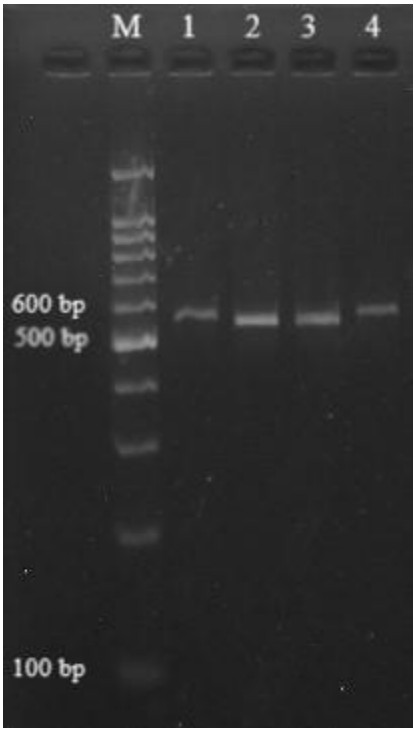

Fig. 1. Size of fragments obtained after amplification of the ITS-5.8S-rDNA region of single spore isolates. M: 100 bp DNA marker; Line 1 to 4: *Fusarium thapsinum*, *Fusarium equiseti*, *Fusarium oxysporum*, *Fusarium chlamydosporum*

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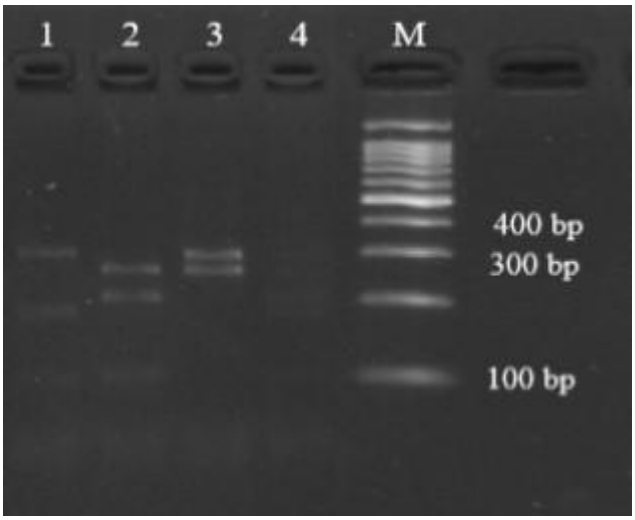

Fig. 2. Restriction profiles obtained after digestion of the HhaI enzyme.
M: 100 bp DNA marker; Line 1 to 4: *Fusarium thapsinum*, *Fusarium equiseti*, *Fusarium oxysporum*, *Fusarium chlamydosporum*

3.3 *ITS sequence analysis*

Sequence identification of all *Fusarium* isolates isolated from rice seeds in Burkina Faso in the NCBI database is presented in Table 1. The BLAST search for similarity to the sequences of the reference *Fusarium* species in the database showed percentages of similarity of isolates ranging from 94.16 to 100%. The ITS sequences of *Fusarium* isolates 001-C-1 to 125-CS-1 were aligned with the consensus region using the CLUSTAL W program. Sequence analysis and comparison with sequences in the NCBI database revealed that the single spore isolates in profile 1 correspond to *Fusarium thapsinum* species while the isolates in profile 2 correspond to *Fusarium equiseti* species. The isolates in profile 3 and profile 4 correspond to *Fusarium oxysporum* and *Fusarium chlamydosporum* species respectively. Thus, out of 118 single-spore isolates studied, the identification indicates 67 *Fusarium thapsinum* with 99.20% similarity, 21 *Fusarium equiseti* with 100% similarity (or exact match), 20 *Fusarium oxysporum* isolates with 94.16% similarity and 10 *Fusarium chlamydosporum* with 97.67% similarity. Phylogenetic tree analysis reveals a distribution of Fusarium isolates into four main clades named I, II, III and IV. (Fig. 3). A total of 1000 bootstrap replications were performed and high percentages of bootstrap replications were given on the internal nodes of the tree.

Clade I consisted of profile 2 isolates with strong similarities to each other and *Fusarium equiseti*, referred (MT123101.1), with a bootstrap support of 94%. Clade II consisting of profile 3 isolates also gave strong similarities to *Fusarium oxysporum* (HF548706.1) and supported by the bootstrap value of 93%. Profile 4 isolates were grouped into clade III and showing strong similarities to the control strain *Fusarium chlamydosporum* (MZ604122.1) with the highest bootstrap value of 100%. Clade IV groups profile 1 isolates showing strong similarities gave the bootstrap value of 98% which refers to *Fusarium thapsinum* (HF566402.1). The rDNA sequences 019-HB-2, 073-CE-1, 009-C-1, 005-C-1, and

080-S-1 were deposited in the GenBank database under accession numbers OP580496, OP578220, OP574199, OP574092, and OP456398 respectively.

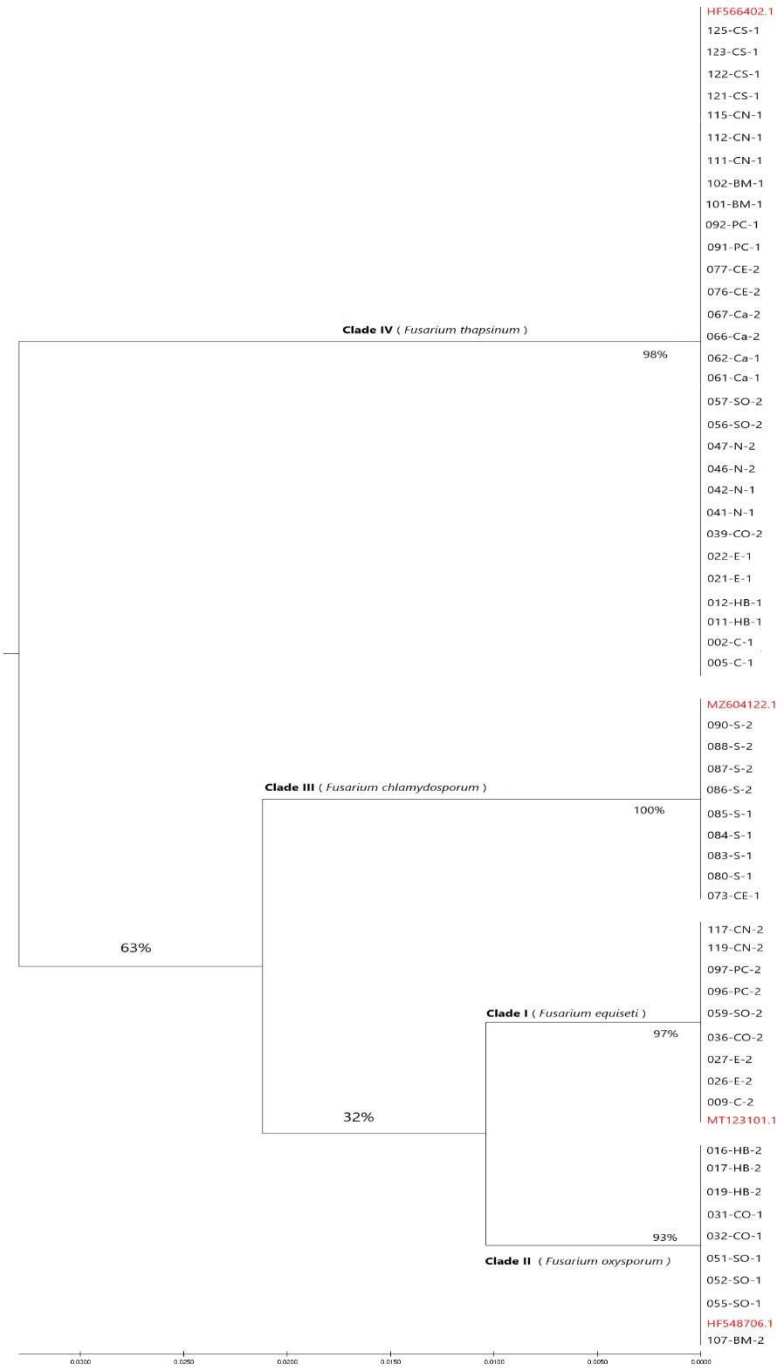

Fig. 3. Phylogenetic tree generated using nucleotide sequence information from the ITS region of conserved ribosomal DNA of *Fusarium* isolates.

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Clade I: isolates related to *Fusarium equiseti* (MT123101.1). Clade II: isolates related to *Fusarium oxysporum* (HF548706.1). Clade III: isolates related to *Fusarium chlamydosporum* (MZ604122.1). Clade IV: isolates related to *Fusarium thapsinum* (HF566402.1).

3.4 *Distribution of Fusarium species according to agro-ecological zones*

The study revealed that all single spore isolates belonged to 4 *Fusarium* species and were distributed throughout the territory according to the different agro-ecological zones of Burkina Faso (Fig. 4).

- *Fusarium chlamydosporum* comprises 10 isolates, i.e. 8.47% of the isolates studied. Eighty-six percent (90%) of the isolates of this species are located in the Sahelian zone compared to 10% in the Sudano-Sahelian zone. This species is absent in the Sudanian zone.
- *Fusarium oxysporum* comprises 20 isolates, i.e. 16.95% of isolates. These isolates are equally distributed in the Sudanian zone (50%) and the Sudano-Sahelian zone (50%).
- *Fusarium equiseti*, with 21 isolates (17.80%), was encountered mainly in the Sudano-Sahelian zone (80.95% of the total), followed by the Sahelian zone (14.29%) and to a lesser extent in the Sudanian zone (4.76%).
- *Fusarium thapsinum* accounts for more than half of the isolates collected (67 isolates or 56.78%). This species is also strongly represented in the Sudano-Sahelian zone (56.72% of the total number), followed by the Sudanian zone (35.82%) and finally in the Sahelian zone (7.47%).

Table 1. Sequence identification of 118 *Fusarium* isolates isolated from rice seeds in Burkina Faso in the NCBI database.

Isolates	ITSSIZE (bp)	Species accession number	Identity (%)	Species	Administrative regions	Agro Ecological zones
001-C-1	556	KU856647.1	99.20%	*Fusarium thapsinum*	Centre	S-S
002-C-1	556	KU856647.1	99.20%	*Fusarium thapsinum*	Centre	S-S
003-C-1	556	KU856647.1	99.20%	*Fusarium thapsinum*	Centre	S-S
004-C-1	556	KU856647.1	99.20%	*Fusarium thapsinum*	Centre	S-S
005-C-1	556	KU856647.1	99.20%	*Fusarium thapsinum*	Centre	S-S
006-C-2	544	MT123101.1	100%	*Fusarium equiseti*	Centre	S-S
007-C-2	544	MT123101.1	100%	*Fusarium equiseti*	Centre	S-S
008-C-2	544	MT123101.1	100%	*Fusarium equiseti*	Centre	S-S
009-C-2	544	MT123101.1	100%	*Fusarium equiseti*	Centre	S-S
010-C-2	544	MT123101.1	100%	*Fusarium equiseti*	Centre	S-S
011-HB-1	556	KU856647.1	99.20%	*Fusarium thapsinum*	Haut-Bassins	So
012-HB-1	556	KU856647.1	99.20%	*Fusarium thapsinum*	Haut-Bassins	So
013-HB-1	556	KU856647.1	99.20%	*Fusarium thapsinum*	Haut-Bassins	So
014-HB-1	556	KU856647.1	99.20%	*Fusarium thapsinum*	Haut-Bassins	So

015-HB-1	556	KU856647.1	99.20%	*Fusarium thapsinum*	Haut-Bassins	So
016-HB-2	544	KJ439145.1	94.16%	*Fusarium oxysporum*	Haut-Bassins	So
017-HB-2	544	KJ439145.1	94.16%	*Fusarium oxysporum*	Haut-Bassins	So
018-HB-2	544	KJ439145.1	94.16%	*Fusarium oxysporum*	Haut-Bassins	So
019-HB-2	544	KJ439145.1	94.16%	*Fusarium oxysporum*	Haut-Bassins	So
020-HB-2	544	KJ439145.1	94.16%	*Fusarium oxysporum*	Haut-Bassins	So
021-E-1	556	KU856647.1	99.20%	*Fusarium thapsinum*	Haut-Bassins	So
022-E-1	556	KU856647.1	99.20%	*Fusarium thapsinum*	Est	So
023-E-1	556	KU856647.1	99.20%	*Fusarium thapsinum*	Est	So
024-E-1	556	KU856647.1	99.20%	*Fusarium thapsinum*	Est	So
025-E-1	556	KU856647.1	99.20%	*Fusarium thapsinum*	Est	So
026-E-2	544	MN589627.1	100%	*Fusarium equiseti*	Est	S-S
027-E-2	544	MN589627.1	100%	*Fusarium equiseti*	Est	S-S
028-E-2	544	MN589627.1	100%	*Fusarium equiseti*	Est	S-S
029-E-2	544	MN589627.1	100%	*Fusarium equiseti*	Est	S-S
030-E-2	544	MN589627.1	100%	*Fusarium equiseti*	Est	S-S
031-CO-1	544	MN309858.1	94.16%	*Fusarium oxysporum*	Centre-Ouest	S-S
032-CO-1	544	MN309858.1	94.16%	*Fusarium oxysporum*	Centre-Ouest	S-S
033-CO-1	544	MN309858.1	94.16%	*Fusarium oxysporum*	Centre-Ouest	S-S
034-CO-1	544	MN309858.1	94.16%	*Fusarium oxysporum*	Centre-Ouest	S-S
035-CO-1	544	MN309858.1	94.16%	*Fusarium oxysporum*	Centre-Ouest	S-S
036-CO-2	544	MN498032.1	100%	*Fusarium equiseti*	Centre-Ouest	S-S
037-CO-2	556	KU856647.1	99.20%	*Fusarium thapsinum*	Centre-Ouest	S-S
039-CO-2	556	KU856647.1	99.20%	*Fusarium thapsinum*	Centre-Ouest	S-S
040-CO-2	556	KU856647.1	99.20%	*Fusarium thapsinum*	Centre-Ouest	S-S
041-N-1	556	KU856647.1	99.20%	*Fusarium thapsinum*	Nord	S-S
042-N-1	556	KU856647.1	99.20%	*Fusarium thapsinum*	Nord	S-S
043-N-1	556	KU856647.1	99.20%	*Fusarium thapsinum*	Nord	S-S
044-N-1	556	KU856647.1	99.20%	*Fusarium thapsinum*	Nord	S-S
045-N-1	556	KU856647.1	99.20%	*Fusarium thapsinum*	Nord	S-S
046-N-2	556	KU856647.1	99.20%	*Fusarium thapsinum*	Nord	S-S
047-N-2	556	KU856647.1	99.20%	*Fusarium thapsinum*	Nord	S-S
048-N-2	556	KU856647.1	99.20%	*Fusarium thapsinum*	Nord	S-S
049-N-2	556	KU856647.1	99.20%	*Fusarium thapsinum*	Nord	S-S
050-N-2	556	KU856647.1	99.20%	*Fusarium thapsinum*	Nord	S-S
051-SO-1	544	MN309858.1	94.16%	*Fusarium oxysporum*	Sud-Ouest	So

052-SO-1	544	MN309858.1	94.16%	*Fusarium oxysporum*	Sud-Ouest	So
053-SO-1	544	MN309858.1	94.16%	*Fusarium oxysporum*	Sud-Ouest	So
054-SO-1	544	MN309858.1	94.16%	*Fusarium oxysporum*	Sud-Ouest	So
055-SO-1	544	MN309858.1	94.16%	*Fusarium oxysporum*	Sud-Ouest	So
056-SO-2	556	KU856647.1	99.20%	*Fusarium thapsinum*	Sud-Ouest	So
057-SO-2	556	KU856647.1	99.20%	*Fusarium thapsinum*	Sud-Ouest	So
058-SO-2	556	KU856647.1	99.20%	*Fusarium thapsinum*	Sud-Ouest	So
059-SO-2	544	MN498032.1	100%	*Fusarium equiseti*	Sud-Ouest	So
060-SO-2	556	KU856647.1	99.20%	*Fusarium thapsinum*	Sud-Ouest	So
061-Ca-1	556	KU856647.1	99.20%	*Fusarium thapsinum*	Cascades	So
062-Ca-1	556	KU856647.1	99.20%	*Fusarium thapsinum*	Cascades	So
063-Ca-1	556	KU856647.1	99.20%	*Fusarium thapsinum*	Cascades	So
064-Ca-1	556	KU856647.1	99.20%	*Fusarium thapsinum*	Cascades	So
065-Ca-1	556	KU856647.1	99.20%	*Fusarium thapsinum*	Cascades	So
066-Ca-2	556	KU856647.1	99.20%	*Fusarium thapsinum*	Cascades	So
067-Ca-2	556	KU856647.1	99.20%	*Fusarium thapsinum*	Cascades	So
068-Ca-2	556	KU856647.1	99.20%	*Fusarium thapsinum*	Cascades	So
069-Ca-2	556	KU856647.1	99.20%	*Fusarium thapsinum*	Cascades	So
070-Ca-2	556	KU856647.1	99.20%	*Fusarium thapsinum*	Cascades	So
073-CE-1	579	MF754105.1	97.67%	*Fusarium chlamydosporum*	Centre-Est	S-S
075-CE-1	544	MN498032.1	100%	*Fusarium equiseti*	Centre-Est	S-S
076-CE-2	556	KU856647.1	99.20%	*Fusarium thapsinum*	Centre-Est	S-S
077-CE-2	556	KU856647.1	99.20%	*Fusarium thapsinum*	Centre-Est	S-S
078-CE-2	556	KU856647.1	99.20%	*Fusarium thapsinum*	Centre-Est	S-S
079-CE-2	556	KU856647.1	99.20%	*Fusarium thapsinum*	Centre-Est	S-S
080-S-1	579	MF754105.1	97.67%	*Fusarium chlamydosporum*	Sahel	Sa
082-S-1	579	MF754105.1	97.67%	*Fusarium chlamydosporum*	Sahel	Sa
083-S-1	579	MF754105.1	97.67%	*Fusarium chlamydosporum*	Sahel	Sa
084-S-1	579	MF754105.1	97.67%	*Fusarium chlamydosporum*	Sahel	Sa
085-S-1	579	MF754105.1	97.67%	*Fusarium chlamydosporum*	Sahel	Sa

086-S-2	579	MF754105.1	97.67%	*Fusarium chlamydosporum*	Sahel	Sa
087-S-2	579	MF754105.1	97.67%	*Fusarium chlamydosporum*	Sahel	Sa
088-S-2	579	MF754105.1	97.67%	*Fusarium chlamydosporum*	Sahel	Sa
089-S-2	579	MF754105.1	97.67%	*Fusarium chlamydosporum*	Sahel	Sa
090-S-2	556	KU856647.1	99.20%	*Fusarium thapsinum*	Plateau-Central	S-S
091-PC-1	556	KU856647.1	99.20%	*Fusarium thapsinum*	Plateau-Central	S-S
092-PC-1	556	KU856647.1	99.20%	*Fusarium thapsinum*	Plateau-Central	S-S
093-PC-1	556	KU856647.1	99.20%	*Fusarium thapsinum*	Plateau-Central	S-S
094-PC-1	556	KU856647.1	99.20%	*Fusarium thapsinum*	Plateau-Central	S-S
095-PC-1	556	KU856647.1	99.20%	*Fusarium thapsinum*	Plateau-Central	S-S
096-PC-2	544	MN498032.1	100%	*Fusarium equiseti*	Plateau-Central	S-S
097-PC-2	544	MN498032.1	100%	*Fusarium equiseti*	Plateau-Central	S-S
098-PC-2	544	MN498032.1	100%	*Fusarium equiseti*	Plateau-Central	S-S
099-PC-2	544	MN498032.1	100%	*Fusarium equiseti*	Plateau-Central	S-S
100-PC-2	544	MN498032.1	100%	*Fusarium equiseti*	Plateau-Central	S-S
101-BM-1	556	KU856647.1	99.20%	*Fusarium thapsinum*	Boucle du Mouhoun	S-S
102-BM-1	556	KU856647.1	99.20%	*Fusarium thapsinum*	Boucle du Mouhoun	S-S
103-BM-1	556	KU856647.1	99.20%	*Fusarium thapsinum*	Boucle du Mouhoun	S-S
104-BM-1	556	KU856647.1	99.20%	*Fusarium thapsinum*	Boucle du Mouhoun	S-S
105-BM-1	556	KU856647.1	99.20%	*Fusarium thapsinum*	Boucle du Mouhoun	S-S
106-BM-2	544	MN258900.1	94.16%	*Fusarium oxysporum*	Boucle du Mouhoun	S-S
107-BM-2	544	MN258900.1	94.16%	*Fusarium oxysporum*	Boucle du Mouhoun	S-S
108-BM-2	544	MN258900.1	94.16%	*Fusarium oxysporum*	Boucle du Mouhoun	S-S
109-BM-2	544	MN258900.1	94.16%	*Fusarium oxysporum*	Boucle du Mouhoun	S-S

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110-BM-2	544	MN258900.1	94.16%	*Fusarium oxysporum*	Boucle du Mouhoun	S-S
111-CN-1	556	KF897854.1	99.20%	*Fusarium thapsinum*	Centre-Nord	Sa
112-CN-1	556	KF897854.1	99.20%	*Fusarium thapsinum*	Centre-Nord	Sa
113-CN-1	556	KF897854.1	99.20%	*Fusarium thapsinum*	Centre-Nord	Sa
114-CN-1	556	KF897854.1	99.20%	*Fusarium thapsinum*	Centre-Nord	Sa
115-CN-1	556	KF897854.1	99.20%	*Fusarium thapsinum*	Centre-Nord	Sa
117-CN-2	544	MN498032.1	100%	*Fusarium equiseti*	Centre-Nord	Sa
119-CN-2	544	MN498032.1	100%	*Fusarium equiseti*	Centre-Nord	Sa
120-CN-2	544	MN498032.1	100%	*Fusarium equiseti*	Centre-Nord	Sa
121-CS-1	600	KF897856.1	99.20%	*Fusarium thapsinum*	Centre-Sud	S-S
122-CS-1	600	KF897856.1	99.20%	*Fusarium thapsinum*	Centre-Sud	S-S
123-CS-1	600	KF897856.1	99.20%	*Fusarium thapsinum*	Centre-Sud	S-S
125-CS-1	600	KF897856.1	99.20%	*Fusarium thapsinum*	Centre-Sud	S-S

S-S: Sudano-Sahelian; So: Sudanian; Sa: Sahelian

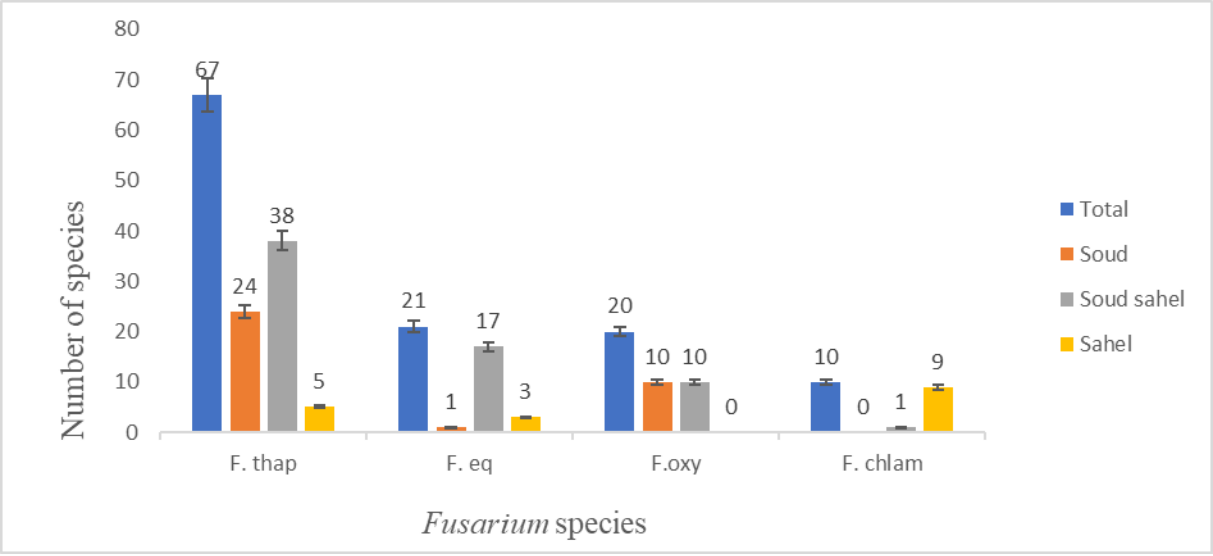

Figure 4. Distribution of *Fusarium* species according to agro -ecological zones in Burk ina Faso. Soud: sudanian zone; Soud sahel: sudano-sahelian zone; Sahel: sahelian zone ; F: *Fusarium*; thap : *thapsinum*; eq: *equiseti*; oxy: *oxysporum*; chlam : *chlamydosporum*

4. Discussion

In this study, rice samples were collected throughout the country and divided into three agro-ecological zones. The high number of samples recorded in the Sudano-Sahelian and Sudanian zones is explained by the fact that these two zones are characterized by climatic conditions that are favorable for rice

cultivation. For molecular identification, fungal isolation based on morphological characters yielded 28 basic isolates (populations isolates) from which 118 single spore isolates of *Fusarium* spp. This large number of single spore isolates suggests a high presence of *Fusarium* spp. on these rice seed samples, confirming the work of Nikiema et al. (2020) who showed that *Fusarium* spp. was found in all agro-ecological zones of Burkina Faso, with prevalence rates ranging from 78.2 to 83.87%.

Observation of the profiles showed a single clear band that, after amplification, corresponded to the expected molecular fragment size of the ITS-5.8S-rDNA region. No band was observed in the control, represented by distilled water. These PCR amplification results suggested that all strains tested belonged to the genus *Fusarium*. Our results are similar to those of Mirhendi et al. (2010) who showed that amplification of genomic DNA with ITS1-ITS4 primers yields a product of approximately 550 base pairs (bp). Studies by Hasan et al. (2016) confirm that the molecular technique, particularly the application of polymerase chain reaction (PCR), is a viable alternative because it is accurate, reliable, and reproducible.

To verify the difference in profile between *Fusarium* single spore isolates, PCR-RFLP was used. Amplification of the ITS-5.8S-rDNA region with primers ITS1 and ITS4 and subsequent digestion with the restriction enzyme HhaI produced amplicons corresponding to four (4) profiles belonging to different *Fusarium* species. The role of the enzyme would be primordial because it acts on the DNA of each *Fusarium* species according to an identification site. RFLP PCR using HhaI allowed to prove this difference at the molecular level and to discriminate the *Fusarium* species. This molecular difference could be attributed to the existence of a polymorphism between the *Fusarium* species by the size of the amplicons and then their restriction profile. Also, this confirmed the results of real-time PCR. The results obtained are in accordance with the studies of several authors in which the RFLP method was found to be useful for the differentiation of *Fusarium* species (Zarrin et al., 2016; Bateman et al., 1996; Dissanayake et al., 2009). According to Xu (2010), PCR-RFPL has been widely exploited for the study of several fungal species. It is very useful for investigations related to inter-strain variability, population evolution and species evolution.

The search for similarities by Standard Nucleotide BLAST showed that the percentage of species similarity ranged from 94.16% to 100%, with coverage rates above 80%. It can be said that differences in gene DNA sequences could be used to support morphological identification of *Fusarium* species. Comparison of the

DNA sequences with the sequences available in the NCBI database provided information on the identity of the *Fusarium* species namely *Fusarium chlamydosporum, Fusarium oxysporum*, *Fusarium equiseti* and *Fusarium thapsinum*. Only *Fusarium oxysporum* species showed a similarity rate of 94.16% reflecting a sequence divergence of 5.84%.

Raja et al. (2017) demonstrated that molecular identification results obtained by comparing nucleotide sequences of fungal isolates on the nucleotide sequence database (GenBank) by the "BLAST" search method, are considered reliable, when the comparative sequences cover a rate of at least 80% and a similarity index of at least 97%.

The use of primers ITS1 and ITS4 allowed the distinction of band profiles specific to the genus *Fusarium*. The similarities of the ITS-5.8S-rDNA sequence of the single spore isolates with the GeneBank sequences used as references were supported by bootstrap values of more than 90% as reported in this study. According to Horton and Bruns (2001) and Bridge et al. (2003), the most popular locus for DNA-based mycological studies, and thus for species identification, is the internal transcribed spacer (ITS) region of the nuclear ribosomal repeat unit. Similarly, Schoch et al. (2012) found that the ITS region was among the markers with the highest probability of correct identifications for a very large group of sampled fungi. Kelly et al (2011) and Seena et al. (2010) through additional fungal studies, confirmed the ITS region as a suitable fungal barcode.

The enumeration of *Fusarium* species in this study reveals a high number of *Fusarium thapsinum* (56.78% of isolates) compared to *Fusarium chlamydosporum* (8.47% of isolates). The geographical distribution indicates that *Fusarium thapsinum* and *Fusarium equiseti* species are found in the three agro-ecological zones (Sahelian, SudanoSahelian and Sudanian), in contrast to *Fusarium chlamydosporum* species found mainly in the Sahelian zone and *Fusarium oxysporum* found in the Sudanian and Sudano-Sahelian zones. This distribution indicates the ubiquitous nature of *Fusarium thapsinum* and *Fusarium equiseti*, which have the capacity to adapt to the different climatic conditions of Burkina Faso.

The phylogenetic tree generated allowed to confirm the similarity between *Fusarium* species through the grouping of taxa into clades. Thus, the different single spore isolates, according to their degree of similarity with their reference controls, were grouped into clades I, II, III and IV, for isolates related to *F. equiseti*, *F. oxysporum*, *F. chlamydosporum* and *F. thapsinum*, respectively. The bootstrap value indicates an assessment of the resistance of a node to data perturbation. Also, bootstrap values between 93% and 100% confirm the level of similarity and robustness of the phylogenetic tree. Harrow et al. (2010) state that phylogenetic analysis of DNA sequences was able to distinguish and assess the genetic relationship between closely related *Fusarium* species. For Hillis and Bull (1993), Zharkikh and Li (1992), a value of 70% would indicate strong support for a group.

The bootstrap has become a common feature of phylogenetic analysis. However, the interpretation of bootstrap values remains open to discussion, and phylogeneticists have used these values in multiple ways (Soltis and Soltis, 2003). The UPGMA method by iterations, gradually reduces the size of the matrix providing the set of distances between all pairs of sequences, and generates a rooted tree. Tateno et al. (1982) indicate that the UPGMA method shows the best performance when the coefficient of variation of the length of the branches is small. Nevertheless, any tree creation method is likely to make mistakes in obtaining the correct topology with high probability, unless all branch lengths of the true tree are long enough.

The development of new disease-resistant varieties and the development of strategies to control epidemics, require knowledge of the molecular structure and genetic relationships in the pathogen population. Benslimane (2016) showed that the generated markers allow a study of biodiversity directly at the genome level. Correct identification of a plant pathogenic fungus is important for the

development of integrated disease control methods and serves as a basis for making decisions to protect agricultural crops and other natural resources.

Conclusion

This molecular characterization study carried out on fungi associated with rice seeds in Burkina Faso identified *F. thapsinum* (56.78%), *F. equiseti* (17.80%), *F. oxysporum* (16.95%) and *F. chlamydosporum* (8.47%). *F. thapsinum* and *F. equiseti* species are distributed in the three agroecological zones (Sahelian, Sudano-Sahelian and Sudanian) compared to *Fusarium chlamydosporum* and *Fusarium oxysporum* species. The precise identity of a plant pathogen is important for the development of disease control methods. The DNA sequence analysis method remains the sophisticated and reliable tool for species name identification. Monitoring of fungal pathogens is necessary to understand the population dynamics and to adapt an appropriate integrated pest management strategy for the rice crop.

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Conflict of interest

The authors declare that there are no conflicts of interest.

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