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Morphological and Molecular Identification of Fungi Associated with Sesame Diseased Plants of the Three Agroclimatic Zones of Burkina Faso

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Abstract

Sesame is Burkina Faso's second essential agricultural export after cotton. It's consequently a supply of income for producers and foreign exchange for the country. However, sesame production is characterized by low average yields of about 538 kg·ha^{-1} at the farmer's field as compared to the potential yield of the improved varieties (1500 - 2000 kg·ha^{-1}). Fungal diseases are some of the major constraints to sesame production in Burkina Faso. The present study contributes to the development of means to control pathogenic fungi of this crop, which are responsible for significant losses. The objective is to identify the fungi associated with diseased sesame plant samples. To this end, 149 samples of diseased sesame plants were collected from different production sites located in three agro-climatic zones of the country. The analysis of the samples according to the blotting paper method, based on the morphological characteristics of the fungi, allowed the identification of 18 genera with prevalence rates from 2.68% to 97.98%. The most frequently identified genera were *Macrophomina* (97.98%), *Cercospora* (86.57%), *Fusarium* (85.23%), *Phoma* (62.41%) and Colletotrichum (61.07%). The results also showed a variable distribution of fungi according to the agro-climatic zone with the predominance of *Macrophomina* in all three zones. Molecular identification by DNA sequencing of 120 isolates belonging to the different fungi detected allowed the identification of 25 species of which the most representative were *Macrophomina phaseolina*, *Cercospora sesami*, *Corynespora cassiicola*, *Alternaria simsimi*, *Alternaria porri*, *Fusarium oxysporum*, *F. fujikuroi*, *F. equiseti*, *Colletotrichum capsici*, and *C. gloesporiodes*. The present study showed that diseased sesame plants collected from different production sites in Burkina

Faso housed several species of fungi. The fungi presence in diseased plants indicates the need to inform and raise the stakeholders' awareness about the phytosanitary problems of sesame, but also to develop effective and appropriate control methods against these crop pathogens in Burkina Faso.

Keywords

Burkina Faso, Fungi, Molecular Identification, Morphological Identification, Sesame

1. Introduction

Sesame (*Sesamum indicum* L.) is an important annual legume cultivated throughout the world and mainly in the tropics [1]. Due to the rate of 50% edible oil content of its seeds [2], sesame is considered the queen of oil crops. Sesame oil is appreciated in Africa, Asia and even worldwide for its high quality and stability, [3] as well as for its therapeutic virtues [4]. An Iranian study on the benefits of sesame oil focused on metabolic syndrome (MetS), also known as insulin resistance. MetS is defined by the World Health Organization (WHO) as a group of symptoms including obesity, type 2 diabetes, dyslipidemia and hypertension that together increase the risk of coronary heart disease, stroke and other serious health problems. This study found the beneficial effects of sesame oil enriched with vitamin E supplementation on cardiometabolic factors in people with MetS [5].

The main sesame-producing countries in the world are Sudan (1,525,104 t), Myanmar (740,000 t), Tanzania (710,000 t), India (658,000 t) and Nigeria (490,000 t) [6]. With about 63% of world production, Africa is the leading sesame-producing continent, followed by America [6] [7]. Burkina Faso is the second largest producer in the West African sub-region after Nigeria.

Sesame is a crop generally adapted to the dry climate of the world's tropical regions, which can also be cultivated in humid zones of tropical and subtropical areas [8]. It is produced throughout the three agroclimatic zones of Burkina Faso by mostly poor farmers whose production constitutes an important source of income. Sesame is a cash crop of which extra than 80% of the production is for sale and export particularly [9]. It is the second most important agricultural export after cotton and is a source of foreign exchange for the country. Sesame production has become a tool in fighting against poverty because it allows producers to increase and diversify their income sources. Sesame yield in Burkina Faso is improved in recent years but remains, at around 723 kg·ha^{-1} [6] compared to the potential yield (1500 to 2000 kg·ha^{-1}) of improved varieties popularized in the country. These low yields are the result of poor access to inputs by the smallholder farmers, irregular rainfall, and biotic constraints, notably insect attacks and diseases caused by microorganisms that seriously constrain sesame

production in Burkina Faso. Sesame cultivation is subject to fungal diseases that occur at all stages of the plant's growth. These diseases generally manifest in the field as leaf blights and necrosis, stem and root rot, wilting and plant mortality. Very little or no work has been done on the formal identification of fungal diseases of sesame in Burkina Faso. However, the main symptoms observed in the field could be attributed to charcoal rot of *Macrophomina phaseolina* [10] [11], Cercospora leaf spot of *Cercospora sesami* [12] and fusarium wilt of *Fusarium oxysporum* [13].

According to Langham *et al.* [14], the major diseases of sesame are downy mildew, leaf spots due to *Cercospora* and *Alternaria*, and root and stem rots caused mainly by the soil-borne fungi of *Fusarium*, *Macrophomina*, and *Phytophthora* genera. In addition to these genera, *Colletotrichum* and *Corynespora* are also present, with some species attacking all parts of the sesame plant. The effective management of such diseases will contribute to yield increase and the country's economy. Accurate identification of the fungi responsible for the main fungal diseases is a prerequisite for the development of efficient control strategies against this important sesame constraint.

The present study aims to identify at morphological and molecular levels, the fungi associated with diseased sesame plants from the three agroclimatic zones of Burkina Faso.

2. Methodology

2.1. Collection of Diseased Sesame Plant Samples

Samples of diseased sesame plants were collected from the three agroclimatic zones of Burkina Faso during the 2017, 2018 and 2019 rainy seasons. These samples consisted of whole plants or organs showing symptoms of necrosis, decay, wilting, blight, or partial and total mortality were collected from sesame fields near national roads and then placed in Craft paper bags. The bags were labelled with the name and geographic coordinates of the collection site. **Figure 1** below shows the collection site in the three agroclimatic zones.

2.2. Morphological Identification of Fungi

The collected samples were treated separately according to plant organs. The diseased plants were washed with tap water to remove soil residues and other inert particles. The different organs were cut into small symptom-bearing particles and disinfected with 70% ethanol for 45 seconds. These plant fragments were then placed in Petri dishes (90 mm Ø) previously lined with three layers of blotting paper soaked in sterile distilled water. The dishes were then incubated for 5 - 7 days in a chamber at a temperature of 22˚C ± 3 and an alternating cycle of 12 hours per day of darkness and near-ultraviolet light.

At the end of the incubation, the Petri dishes were observed under a stereomicroscope and a microscope to identify the fungi that had grown on the plant fragments. The identification was done based on macroscopic (color and aspect

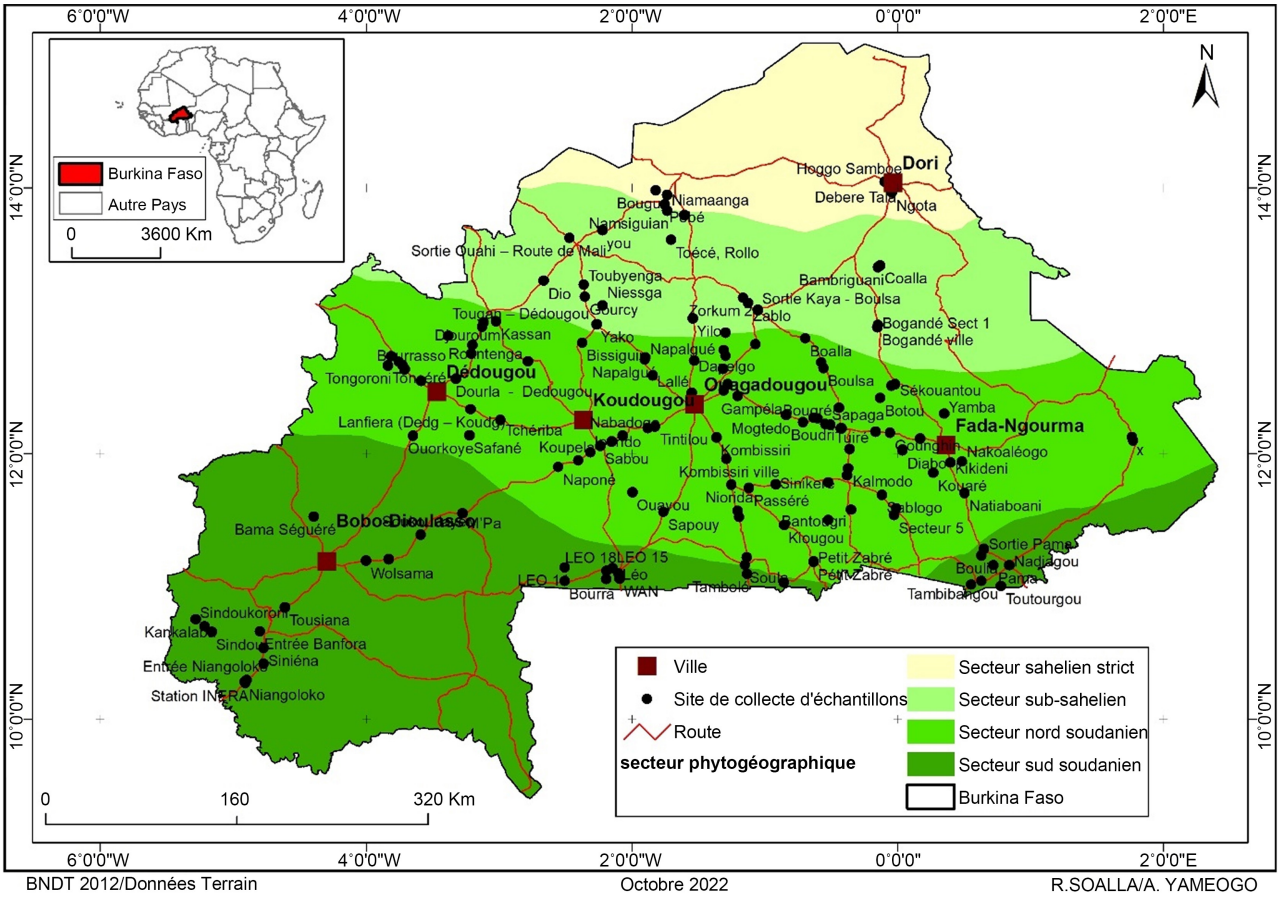

Figure 1. Collection sites of diseased sesame plants in the agroclimatic zones of the country.

of mycelium) and microscopic (shape and structure of conidia and mycelium) characteristics as described in the identification manual of [15]. For most fungi, identification was limited to the genus level.

The identified fungi were reported on an identification sheet according to the infected organ. The prevalence rates of the different fungi associated with diseased plants were calculated according to the formula below:

$$Pi = \frac{Ni}{N} \times 100$$

with *Pi* = Prevalence of fungus i; *Ni* = Number of samples of diseased sesame plants infected with fungus i; *N*: Total number of samples of diseased sesame plants examined

The prevalence rates according to the plant organ from which the fungi were detected were also calculated by the formula below:

$$Pio = \frac{Noi}{No} \times 100$$

where *Pio* is the prevalence of fungus i detected on plant organ o (leaf, stem, root or capsule); *Noi* the number of samples of organ o infected by fungus i and *No*, the total number of organ o samples examined.

2.3. Isolation of Fungi

Morphologically identified fungi were isolated in Eppendorf tubes containing sterile distilled water to form isolates and stored in the refrigerator at 4˚C as conidial or mycelial suspension. One drop of each suspension was spread on a Petri dish containing agar medium and incubated at laboratory conditions (25˚C ± 3) for 12 to 24 hours. Five germinating spores were then isolated and transferred to new Petri dishes containing Potato Dextrose Agar (PDA) for one spore per dish and placed in the incubation chamber for growth under the same conditions described above. The resulting pure single-spore isolates were stored for further study.

2.4. DNA Extraction

Single-spore isolates were grown in Potato Dextrose Broth (PDB) liquid medium. A 4-mm diameter mycelial explant of each isolate from a 7-day-old culture on PDA medium was aseptically collected and deposited into a PDB medium contained in a 250-ml Erlenmeyer flask. The inoculated media were incubated at laboratory room temperature (25˚C ± 3), with shaking at the speed of 100 oscillations per minute, for two to five days. At the end of the incubation, the Erlenmeyer contents were filtered with a vacuum pump and the mycelium was collected in an Eppendorf tube and dried in an oven at 27˚C for 48 to 76 hours.

The DNA extraction concerned 120 isolates of fungi including 20 belonging to the genus *Macrophomina*, 30 to the genus *Fusarium*, 20 to the genus *Cercospora*, 11 to the genus *Alternaria*, 10 to the genus *Colletotrichum*, 6 to the genus *Phoma*, 5 to the genus *Curvularia* and 18 to other genera including *Nigrospora* (2), *Cladosporium* (2), *Exserohilum* (1), *Pestalotia* (1), *Phomopsis* (1), *Rhizoctonia* (1), *Melanospora* (1), *Myrothecium* (1) and *Botryodiplodia* (1), *Aspergillus* (2) and the unknowns (5).

Mycelium samples contained in 2 ml Eppendorf tubes were then ground using Tissue Lyser II and subjected to DNA extraction following the Cetyl-Trimethyl-Ammonium Bromide (CTAB) method of Ford *et al.* [15] with some modifications. A volume of 600 µl of CTAB solution (1.4 M NaCl; 2% CTAB (w/v); 0.1 M Tris-Base pH8; 0.02 M EDTA pH8; 0.2 B-Mercaptoethanol (v/v)) was added to 75 - 100 mg of conidial powder and incubated at 65˚C for 10 minutes. Then 450 µl of chloroform: isoamyl alcohol (49:1) was added to the tube containing the sample, vortexed gently, and centrifuged at 13,000 g for 5 minutes at 25˚C. The same process with lsoamyl alcohol was repeated with the top part of the first step. The top part of the second step was transferred into a 1.5 ml tube to which 0.7 volume of isopropanol solution was added to precipitate the DNA at −20˚C for 30 minutes. After centrifugation at 13,000 g for 10 minutes, the liquid in the tube was removed and the pellet was rinsed with 500 µl of Ethanol at 70% by centrifugation for 3 minutes at 13,000 g at 25˚C. The rinsing process was repeated a 2nd time. The DNA contained in the tubes was dissolved by adding 50 µl of sterile distilled water.

DNA concentrations were then determined using the NanoDrop 2000 spec-

trophotometer.

2.5. Amplification of DNA from Isolates

The DNA samples were amplified by PCR, targeting regions 1 and 2 of the ITS sequences and the 5.8S rDNA sequence with primers ITS1 and and ITS4 [16]. Amplification reactions were performed in a 20 µl reaction mixture consisting of Solis BioDyne's HOT FIREPol® DNA Polymerase enzyme 4 µl, 1 µl of each primer and 13 µl of water.

The PCR program was adapted to that of the enzyme supplier as follows. An initial denaturation at 95˚C for 12 minutes, followed by 35 cycles of denaturation at 95˚C for 30 seconds, hybridization at 58˚C for 30 seconds and elongation at 72˚C for one minute, and final elongation at 72˚ for 5 minutes.

After amplification, the PCR products are revealed by electrophoresis on 10% agarose gel previously incorporated with Ethidium Bromide and illuminated with ultraviolet light (UV).

2.6. Sequencing

The PCR products of 111 DNA samples of fungal isolates revealed by agarose gel electrophoresis were sequenced by the biotechnology company Macrogen in the Netherlands.

2.7. Sequence Analysis

The sequencing results were first processed with BioEdit software for sequence alignment and cleaning. Using BLAST, the generated consensus sequence was then compared to other DNA sequences in the National Centre for Biotechnology Information (NCBI) non-redundant nucleotide database

3. Results

3.1. Samples Collected

Sesame plant samples were collected from sesame production sites in 33 of the 45 provinces of Burkina Faso and across the three agroclimatic zones. Of a total of 149 diseased plant samples collected, 103 were from the Sudano-Sahelian zone, 28 were from the Sudanian zone and 18 were from the Sahelian zone.

3.2. Prevalence of Fungi Associated with Sesame in Burkina Faso

In general, depending on the organ of the diseased plant, the top five fungi encountered were as follows (**Table 1**):

- Roots: *Macrophomina* (96.40% of prevalence rate), *Fusarium* (22.30%), *Phoma* (9.35%), *Cercospora* (5.03%), *Alternaria* (4.32%);
- Leaves: *Cercospora* (79.16%), *Macrophomina* (56.25%), *Fusarium* (54.86%); *Colletotrichum* (46.52%), *Phoma* (41.66%);
- Stems: *Macrophomina* (87.83%), *Fusarium* (62.16%), *Colletotrichum* (45.27%), *Cercospora* (43.91%), *Phoma* (31.08%);

Table 1. Prevalence of fungal genera identified on samples and different plant organs of sesame collected in Burkina Faso.

N°	Fungi	Fungal prevalence rate (%)				
		Wole Samples (N = 149)	Leave (N = 144)	Stems (N = 148)	Roots (N = 149)	Capsules (N = 126)
1	*Alternaria*	48.32	31.25	8.78	4.32	25.40
2	*Botryodiplodia*	26.85	8.33	13.51	4.32	9.52
3	*Cercospora*	86.58	79.17	43.92	5.04	42.86
4	*Colletotrichum*	61.07	46.53	45.27	4.32	7.94
5	*Curvularia*	31.54	25.00	9.46	1.44	19.05
6	*Exserohilum*	14.09	6.25	4.73	0.72	4.76
7	*Fusarium*	85.23	54.86	62.16	22.30	41.27
8	*Macrophomina*	97.99	56.25	87.84	96.40	65.87
9	*Myrothecium*	2.68	1.39	0.68	0.00	0.00
10	*Nigrospora*	26.85	19.44	2.04	0.72	9.52
11	*Pestalotia guepini*	5.37	3.47	0.00	1.44	1.59
12	*Phoma*	62.42	41.67	31.08	9.35	21.43
13	*Phomopsis*	6.04	0.70	3.38	0.72	2.38
14	*Rhizoctonia solani*	10.07	6.94	4.73	0.72	7.94
15	*Inconnus*	34.90	9.72	8.16	2.16	16.67
16	*Cladosporium*	20.13	-	-	-	-
	Aspergillus	-	-	-	-	-
17	*Melanospora*	2.68	-	-	-	-

- Capsules: *Macrophomina* (65.87%), *Cercospora* (42.85%), *Fusarium* (41.26%), *Alternaria* (25.39%), *Phoma* (21.43%).

In summary, these results show that *Macrophomina*, *Fusarium*, *Cercospora*, *Colletotrichum*, *Phoma* and *Alternaria* were the major fungi associated with diseased sesame plants in Burkina Faso.

Regarding the distribution of fungi among the climatic zones (Table 2), four (4) fungi, namely *Cercospora*, *Colletotrichum*, *Macrophomina* and *Phoma*, were widespread in all three climatic zones, contaminating between 50% and 100% of the samples collected in each zone. The fungi with the lowest occurrence in the zones were *Myrothecium* (0% - 3.88%), *Phomopsis* (0% - 10.71%) and *Melanospora* (0% - 11.11%). In general, all the fungi were invariably distributed among the three zones except *Cercospora*, *Exserohilum* and *Cladosporium* which were diversely distributed according to the climatic zones. *Cercospora* was more present in plants from the Sudano-Sahelian and Sudanian zones (91.26% - 92.86%) than in those from the Sahelian zone (55.56%). On the other hand, *Exserohilum*, and to a lesser extent, *Cladosporium* were more frequently found in samples collected in the Sahelian zone (38.89% each) than in those collected in the other two zones (9.71% - 14.29% and 14.56% - 28.57%, respectively).

Table 2. Prevalence of fungal genera identified on samples of diseased sesame plants according to the different agroclimatic zones.

N°	Genus of fungi	Probability (5%)	Prevalence of fungi by climatic zone (%)		
			Sudanese-Sahelian	Sudanian	Sahelian
1	*Alternaria*	0.4832	46.60a	60.71a	38.89a
2	*Botryodiplodia*	0.2014	24.27a	25.00a	50.00a
3	*Cercospora*	0.0001	91.26a	92.86a	55.56b
4	*Colletotrichum*	0.8713	62.14a	64.29a	50.00a
5	*Curvularia*	0.6066	32.04a	25.00a	38.89a
6	*Exserohilum*	0.0042	9.71b	14.29b	38.89a
7	*Fusarium*	0.2500	88.35a	85.71a	77.78a
8	*Macrophomina*	0.0977	99.03a	96.43a	100.00a
9	*Myrothecium*	0.4046	3.88a	0.00a	0.00a
10	*Nigrospora*	0.9698	26.21a	25.00a	33.33a
11	*Pestalotia*	0.2699	4.85a	10.71a	5.56a
12	*Phoma*	0.1450	58.25a	82.14a	61.11a
13	*Phomopsis*	0.3304	5.83a	10.71a	0.00a
14	*Rhizoctonia*	0.1718	7.77a	10.71a	22.22a
15	*Cladosporium*	0.0274	14.56b	28.57ab	38.89a
16	*Melanospora*	0.0528	1.94a	0.00a	11.11a
17	unknown	0.7207	35.92a	28.57a	38.89a

3.3. Molecular Identification of Fungi Associated with Diseased Sesame Plant Samples

Revealing PCR products by 10% agarose gel electrophoresis with a 100 base pair molecular weight marker yielded bands between 500 and 600 base pairs (**Figure 2**).

The results of the ITS sequence alignment followed by their comparison with the National Centre for Biotechnology Information non-redundant nucleotide database were presented in **Table 3**. The percentages of identity and coverage of the sequences were respectively between 97.13% and 100% and between 85% and 100%. As for the locus size of the corresponding closest accession, it ranged from 523 to 1120 base pairs. The results identified 25 species of fungi belonging to 13 genera which are *Macrophomina*, *Fusarium*, *Cercospora*, *Corynespora*, *Alternaria*, *Colletotrichum*, *Nigrospora*, *Exerohilum*, *Lasiodiplodia*, *Curvularia*, *Phoma*, *Cladosporium* and *Didymela.*

Sequence analysis revealed that all 20 isolates of the genus *Macrophomina* were close to those of the species *Macrophomina phaseolina* with percentages of identity and coverage between 95% and 100%, and between 97.47% and 100% respectively. In addition to these 20 isolates, one isolate identified molecularly as belonging to the genus *Rhizoctonia* was found to be very close to the reference

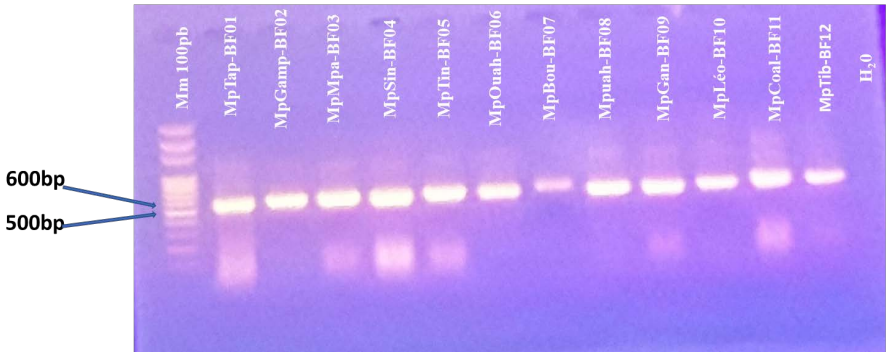

Figure 2. Some PCR products revelation by electrophoresis on agarose gel of 10%.

Table 3. DNA sequences analysis results by blast on NCI.

Isolate	Morphological Identification	Isolate sequence size (bp)	Correspondant species in the NCBI database	coverage (%)	Similarity rate (%)	Accession size (BP)	**Accession**
MpTap-BF01	*Macrophomina phaseolina*	556	*Macrophomina phaseolina*	100%	99.82%	583	MT186826.1
MpGamp-BF02	*Macrophomina phaseolina*	548	*Macrophomina phaseolina*	100%	100.00%	583	MT186826.1
MpMpa-BF03	*Macrophomina phaseolina*	559	*Macrophomina phaseolina*	99%	100.00%	583	MT186826.1
MpSin-BF04	*Macrophomina phaseolina*	557	*Macrophomina phaseolina*	99%	100.00%	583	MT186826.1
MpTin-BF05	*Macrophomina phaseolina*	565	*Macrophomina phaseolina*	99%	98.45%	583	MZ502501.1
MpOuah-BF06	*Macrophomina phaseolina*	568	*Macrophomina phaseolina*	99%	99.65%	1120	OM106520.1
MpBou-BF07	*Macrophomina phaseolina*	558	*Macrophomina phaseolina*	99%	100.00%	583	MT186826.1
MpOuah-BF08	*Macrophomina phaseolina*	573	*Macrophomina phaseolina*	99%	99.48%	572	MW045603.1
MpGan-BF09	*Macrophomina phaseolina*	638	*Macrophomina phaseolina*	99%	97.47%	693	OM341626.1
MpLéo-BF10	*Macrophomina phaseolina*	556	*Macrophomina phaseolina*	99%	100.00%	583	MT186826.
MpCoal-BF11	*Macrophomina phaseolina*	553	*Macrophomina phaseolina*	100%	98.73%	596	MH864182.1
MpTib-BF12	*Macrophomina phaseolina*	554	*Macrophomina phaseolina*	100%	100.00%	693	OM341626.1
MpLéo-BF13	*Macrophomina phaseolina*	581	*Macrophomina phaseolina*	95%	97.49%	583	MT186826.1
MpNou-BF14	*Macrophomina phaseolina*	548	*Macrophomina phaseolina*	100%	98.72%	583	MT186826.1
MpHar-BF15	*Macrophomina phaseolina*	555	*Macrophomina phaseolina*	99%	99.10%	596	MH864182.1
MpSan-BF16	*Macrophomina phaseolina*	554	*Macrophomina phaseolina*	99%	100.00%	596	MH864182.1
MpKal-BF17	*Macrophomina phaseolina*	558	*Macrophomina phaseolina*	99%	99.28%	596	MH864182.1
MpSap-BF18	*Macrophomina phaseolina*	551	*Macrophomina phaseolina*	100%	100.00%	596	MH864182.1
MpPô-BF19	*Macrophomina phaseolina*	556	*Macrophomina phaseolina*	100%	99.82%	558	KF951702.1
MpTous-BF20	*Macrophomina phaseolina*	552	*Macrophomina phaseolina*	99%	99.82%	596	MH864182.1
MpGna-BF119	*Rhizoctonia solani*	555	*Macrophomina phaseolina*	99%	99.82%	596	MH864182.1
FusTin-BF21	*Fusarium sp.*	577	*Fusarium incarnatum*	92%	99.25%	543	MK192051.1
FusNiag-BF23	*Fusarium sp.*	569	*Fusarium incarnatum*	91%	99.61%	548	MT563420.1
FusMpa-BF22	*Fusarium sp.*	557	*Fusarium fujikuroi*	99%	99.46%	595	MT742817.1
Fus-Kamb-BF25	*Fusarium sp.*	599	*Fusarium fujikuroi*	98%	97.13%	592	MN565957.1
Fus-Mpa-BF24	*Fusarium equiseti*	617	*Fusarium equiseti*	85%	99.06%	551	MK764999.1

Continued

Fus-San-BF27	*Fusarium equiseti*	624	*Fusarium equiseti*	85%	99.07%	551	MK764999.1
Fus-Pô-BF29	*Fusarium equiseti*	709	*Fusarium equiseti*	100%	100.00%	571	MN498032.1
Fus-Kour-BF52	*Fusarium equiseti*	517	*Fusarium equiseti*	99%	100.00%	586	MT560375.1
FuSs-BF28	*Fusarium sp.*	617	*Fusarium proliferatum*	89%	99.27%	565	MT372093.1
Fus-Nia-BF36	*Fusarium sp.*	531	*Fusarium proliferatum*	99%	99.25%	572	OL873221.1
Fus-Bama-BF49	*Fusarium sp.*	523	*Fusarium proliferatum*	98%	99.61%	563	MT560218.1
Fus-Pô-BF33	*Fusarium sp.*	533	*Fusarium proliferatum*	99%	100.00%	572	OL873221.1
Fus-Nou-BF48	*Fusarium sp.*	527	*Fusarium proliferatum*	99%	99.62%	576	MT560212.1
Fus-Kom-BF38	*Fusarium sp.*	530	*Fusarium penzigii*	99%	99.62%	548	MN548457.1
Fus-Pam-BF40	*Fusarium sp.*	541	*Fusarium penzigii*	98%	99.44%	548	MN548457.1
Fus-Tap-BF47	*Fusarium sp.*	532	*Fusarium penzigii*	99%	98.68%	548	MN548457.1
Fus-Kour-BF30	*Fusarium sp.*	575	*Fusarium oxysporum*	98%	97.19%	687	KU671036.1
Fus-Sin-BF43	*Fusarium sp.*	708	*Fusarium oxysporum*	99%	99.62%	557	MN726603.1
Fus-Yam-BF41	*Fusarium solani*	537	*Fusarium solani*	100%	99.81%	553	MN545491.1
Cer-Mpa-BF59	*Cercospora sp.*	511	*Cercospora sesami*	99%	100.00%	545	MK764999.1
Cer-Pô-BF63	*Cercospora sp.*	514	*Cercospora sesami*	100%	98.81%	545	MN498032.1
Cer-Mpa-BF66	*Cercospora sp.*	1005	*Cercospora sesami*	100%	99.80%	545	MT560375.1
Cer-Pam-BF67	*Cercospora sp.*	1021	*Cercospora sesami*	99%	100.00%	545	MT372093.1
Cer-Kom-BF68	*Cercospora sp.*	512	*Cercospora sesami*	100%	100.00%	545	OL873221.1
Cer-Ded-BF70	*Cercospora sp.*	545	*Cercospora sesami*	99%	100.00%	545	MT560218.1
Cer-Tin-BF71	*Cercospora sp.*	707	*Cercospora sesami*	99%	100.00%	545	OL873221.1
Cer-Komb-BF122	*Cercospora sp.*	507	*Cercospora sesami*	100%	99.80%	545	MT560212.1
Cer-Tib-BF54	*Cercospora sp.*	509	*Cercospora kikuchii*	100%	100.00%	550	MN548457.1
Cer-Kom-BF55	*Cercospora sp.*	1000	*Cercospora kikuchii*	99%	100.00%	544	MN548457.1
Cer-Sak-BF56	*Cercospora sp.*	876	*Cercospora kikuchii*	99%	100.00%	550	MN548457.1
Cer-Kom-BF57	*Cercospora sp.*	505	*Cercospora kikuchii*	99%	99.80%	545	KU671036.1
Cer-Léo-BF58	*Cercospora sp.*	511	*Cercospora kikuchii*	99%	99.80%	550	MN726603.1
Cer-Bama-BF72	*Cercospora sp.*	637	*Cercospora canescens*	99%	99.61%	545	MN545491.1
Cor-Gan-BF53	*Cercospora sp.*	538	*Corynespora cassiicola*	99%	99.81%	547	MW165772.1
Cor-Nia-BF61	*Cercospora sp.*	527	*Corynespora cassiicola*	100%	99.24%	570	MH762895.1
Cor-Komb-BF64	*Cercospora sp.*	1136	*Corynespora cassiicola*	99%	99.43%	561	MN945374.1
Cor-Bama-BF65	*Cercospora sp.*	756	*Corynespora cassiicola*	99%	99.81%	561	MN945374.1
Cor-Pô-BF69	*Cercospora sp.*	1130	*Corynespora cassiicola*	99%	100.00%	570	MH762895.1
Cor-Pô-BF63	*Cercospora sp.*	514	*Corynespora cassiicola*	99%	100.00%	561	MN945374.1
Cor-Léo-BF108	*.Inconnu*	529	*Corynespora cassiicola*	100%	99.81%	561	MN945374.1
Alt-Kamb-BF73	*Alternaria sp.*	663	*Alternaria porri*	99%	99.63%	582	MT554514.1
Alt-Léo-BF76	*Alternaria sp.*	666	*Alternaria porri*	99%	99.63%	582	MT554514.1
Alt-Boul-BF80	*Alternaria sp.*	542	*Alternaria porri*	99%	100.00%	582	MT554514.1

Continued

Alt-Ded-BF82	*Alternaria sp.*	542	*Alternaria porri*	99%	100.00%	582	MT554514.1
Alt-Pô-BF77	*Alternaria sp.*	553	*Alternaria simsimi*	98%	99.09%	571	JF780938.1
Alt-Sou-BF78	*Alternaria sp.*	551	*Alternaria simsimi*	99%	99.27%	571	JF780938.1
Alt-Bama-BF79	*Alternaria sp.*	559	*Alternaria simsimi*	98%	99.09%	571	JF780938.1
Alt-Komb-BF81	*Alternaria sesami*	554	*Alternaria simsimi*	98%	99.82%	571	JF780938.1
Alt-Nia-BF83	*Alternaria sesami*	549	*Alternaria simsimi*	99%	99.63%	571	JF780938.1
Alt-Pô-BF62	*Alternaria sesami*	544	*Alternaria simsimi*	100%	99.08%	571	JF780938.1
Col-Tin-BF88	*Colletotrichum gloeosporioides*	544	*Colletotrichum gloeosporioides*	100%	99.63%	583	MW603454.1
Col-Gan-BF90	*Colletotrichum capsici*	572	*Colletotrichum truncatum*	98%	98.58%	586	KX685450.1
Col-Nou-BF91	*Colletotrichum capsici*	554	*Colletotrichum capsici*	99%	93.87%	592	MT012102.1
Cur-Léo-BF95	*Curvularia lunata*	582	*Curvularia lunata*	99%	99.48%	576	KU715116.1
Cur-Ded-BF96	*Curvularia lunata*	544	*Curvularia lunata*	99%	100.00%	562	LC317566.1
Cur-Bou-BF97	*Curvularia lunata*	594	*Curvularia lunata*	100%	98.66%	603	MH010914.1
Cur-Léo-BF98	*Curvularia sp.*	544	*Curvularia lunata*	100%	99.45%	573	MN173127.1
Pho-Sin-BF99	*Phoma lingam*	518	*Phoma multirostrata*	99%	100.00%	523	MT635199.1
Pho-Léo-BF100	*Phoma lingam*	531	*Phoma multirostrata*	99%	99.06%	547	KJ767077.1
Pho-Sap-BF102	*Phoma lingam*	518	*Phoma multirostrata*	99%	100.00%	547	KJ767077.1
Pho-Pô-BF103	*Phoma sorghina*	520	*Phoma multirostrata*	99%	99.81%	524	KM659039.1
Pho-Nia-BF104	*Phoma sorghina*	526	*Phoma multirostrata*	99%	99.23%	524	KM659039.1
Nig-Gan-BF112	*Nigrospora oryzae*	529	*Nigrospora sphaerica*	99%	92.67%	559	MW081353.1
Nig-Har-BF114	*Nigrospora oryzae*	534	*Nigrospora oryzae*	99%	95.68%	532	MT672515.1
Exs-Sin-BF105	*Exserohilum rostratum*	580	*Exserohilum rostratum*	100%	100.00%	603	MN960317.1
Exs-Kom-BF118	*Exserohilum rostratum*	580	*Exserohilum rostratum*	100%	99.59%	627	MN599590.1
Las-Sap-BF116	*Botryodyplodia theobromae*	518	*Lasiodiplodia theobromae*	99%	99.81%	550	MK530050.1
Cla-Tib-BF120	*Cladosporium sp.*	524	*Cladosporium sphaerospermum*	99%	99.81%	530	MF467891.1
Did-Kak-BF74	*Inconnu*	6542	*Didymella americana*	99%	99.24%	550	MK646045.1

Mp = *Macrophomina phaseolina*; Fus = *Fusarium*; Cer = *Cercospora*; Cor = *Corynespora*; Alt = *Alternaria*; Col = *Colletotrichum*; Cur = *Curvularia*; Pho = *Phoma*; Nig = *Nigrospora*; Exs = *Exserohilum*; Las = *Lasidiodiplodia*; Cla = *Cladosporium*; Did = *Didymella*; BF = Burkina Faso; 2nd three correspond to the collecting site name.

isolate of *M. phaseolina* (MH864182.1) with a percentage identity of 99.82%. Eight (8) of the *Macrophomina* isolates showed perfect similarity (100% identity) with the *M. phaseolina* isolate MH864182.1 in the database.

Out of the 30 isolates of the genus *Fusarium*, the analysis of the sequences obtained identified 19 isolats belonging to seven (7) species including *F. proliferatum* (5), *F. equiseti* (4), *F. penzigii* (3), *F. incarnatum* (2), *F. fujikuroi* (2), *F. oxyporum* (1) and *F. solani* (1). Sequence identity and coverage percentages of *Fusarium* isolates ranged from 97.18% to 100% and 85% to 100%, respectively. Two *Fusarium* isolates showed complete similarity to the reference isolate (MN498032.1) of *F. equiseti*, and one to the reference isolate (OL873221.1) of *F. proliferatum*.

Three (3) species of *Cercospora* including *C. sesami* (8 isolates), *C. kikuchii* (5) and *C. canescens* (1) were identified after sequence analysis of the isolates belonging to this genus, with percentages of identity and coverage of 99.61% - 100% and 99% - 100%, respectively. The eight (8) isolate sequences identified as those of *C. sesami* species were close to that of a single accession (MT186826.1) in the NCBI database. Of these eight isolates, five showed perfect similarity to the reference accession of *C. sesami*. Two isolates were also identical to the reference isolate MK336506.1 of *C. kikuchii*, and one was identical to the reference isolate MH777047.1 of C*. kikuchii*. Sequence analysis also revealed a new genus: *Corynespora* grouping the six other isolates initially identified morphologically as belonging to the genus *Cercospora*. These isolates all belong to the species *Corynespora cassiicola*, two of them being perfectly similar to the NCBI reference isolates MH762895.1 and MNP45374.1 of *C. cassiicola*. One isolate belonging to the genus "Unknown" was also identified as *C. cassiicola*.

All DNA sequences from *Alternaria* isolates were identified as closely related to *A. simsimi* and *A. porri*. A comparison of the sequences to the NCBI nucleotide database indicated that four were very close to the sequence of the accession (MT554514.1) of *A. porri* with a similarity rate of 100% for two of them. As for the other sequences, six (6), belonging to *A. simsimi*, the percentages of coverage were between 98% and 100% and the percentages of identity between 99.08% and 99.82%. These sequences were also close to accession JF780938.1 of the database.

Colletotrichum gloesporioides, *Colletotrichum truncatum* and *Colletotrichum capsici* were the three species identified after sequence analysis of *Colletotrichum* isolates, with coverage and identity percentages of 100% and 99.63%, 98% and 98.85%, and 99% and 93.87%, respectively with their respective reference sequences MW603454.1, KX685450.1 and MT012102.1. Molecular analysis of the remaining seven isolates of the *Colletotrichum* genus was inconclusive.

With coverage percentages of 99% and identity rates between 99% and 100%, the DNA sequences of the five isolates of the genus *Phoma* were very close to those of two reference accessions (MT635199.1, KJ767077.1) of the species *Phoma multirostrata*, with perfect similarity for two of the isolates.

Of the five isolates belonging to the genus *Curvularia*, four were identified as closely related to the species *C. lunata*, with varying percentages of identity (93.87% - 100%). Of these isolates, only one showed 100% identity with the reference accession LC317566.1 in the database. Analysis of the fifth isolates was inconclusive.

The two *Nigrospora* isolates were all identified as *Nigrospora sphaeriaca* (MW081353.1) and *Nigrospora oryzae* (MT672515.1), with reference species identity percentages of 92.67% and 95.68%.

The two isolates of the genus *Exserohilum* were all identified as closely related to two accessions (MN960317.1 and MK530050.1) of the species *Exserohilum rostratum*, with, however, one of the isolates identical to accession MN960317.1

in the database.

Three isolates initially identified morphologically as belonging to the genera *Botryodiplodia*, *Cladosporium*, and "unknown" were molecularly identified as *Lasiodiplodia theobromae*, *Cladosporium sphaerospermum* and *Didymella americana*, respectively. The sequences showed near 100% coverage and 99% similarity rates to accessions of the three respective reference species.

Molecular analysis of isolates initially identified as belonging to the genera *Pestalotia* (1 isolate), *Phomopsis* (1); *Melanospora* (1) and "unknown" (7), yielded inconclusive results.

4. Discussion

One of the advantages of sesame is that it can be produced under a variety of climatic conditions ranging from dry arid zones to humid and rainy zones [17]. Sesame is produced throughout Burkina Faso, across the country's three agro-climatic zones. However, the largest sesame-producing provinces are located in the Sudano-Sahelian zone, making this area the most important for the production of this important cash crop for the country. Thus, the collection of samples of diseased sesame plants focused on this zone with 102 collection sites without forgetting the other zones in proportion to the importance of their production.

Based on morphological characteristics, several species of fungi belonging to 16 genera were identified as associated with samples of diseased sesame plants, reflecting the diversity of potential pathogenic fungi associated with sesame in Burkina Faso. In Pakistan, the diversity of pathogenic fungi in sesame had been reported by Altaf *et al.* [18] who identified 11 species of pathogenic fungi associated with poor seed germination and diseased sesame seedlings.

The diseased plant samples were heavily contaminated by the genera *Macrophomina* (97.99%), *Fusarium* (85.23%), *Cercospora* (86.58%), *Phoma* (62.42%) and *Colletotrichum* (61.07%) with prevalence rates above 50%. These high prevalence rates reveal the importance of the species of fungi of these genera in sesame production sites in Burkina Faso. When considering the different plant organs of the samples, the predominance of these genera was variable. On roots, stems and capsules, *Macrophomina* was the dominant genus, while *Cercospora* was the most important on leaves. Species belonging to both genera have been reported as major pathogens on sesame. The genus *Macrophomina* and particularly the species *M. phaseolina* is a pathogen, responsible for root and stem rot (known as ash rot) on several crops of economic importance [19] [20] including sesame [10] [11]. One of the important leaf diseases of sesame is due to the species *Cercospora sesami* responsible for the so-called Cercospora Leaf Spot (CLF) [12] [21].

In addition to the genera *Macrophomina*, *Fusarium* and *Colletotrichum*, all sesame stems pathogens [14], *Cercospora* has also been strongly detected on sesame stems from Burkina Faso. The importance of the *Cercospora* genus on stems suggests a high severity of the disease, greater than or equal to 43.1% ac-

cording to the rating scale of Enikuomehin *et al.* [12] corresponding to the appearance of symptoms on the stem.

According to the different agroclimatic zones of sample collection, the study revealed the strong presence of five genera (*Cercospora*, *Colletotrichum*, *Fusarium*, *Macrophomina* and *Phoma*) with incidence rates ≥ 50% in all zones. These fungi are known as causal pathogens of the destructive sesame diseases *Cercospora* Leaf Spot (*Cercospora*), anthracnose (*Colletotrichum*), fusarium wilt disease [22] root and stem rot (*Macrophomina*), leaf spot (*Phoma*) [14]. The expansion of these fungi in all areas suggests that these microorganisms adapt to a wide range of moisture and temperature conditions. It is noteworthy that all samples from the Sahelian zone were contaminated with the genus *Macrophomina*. The species *M. phaseolina* has been recognized as responsible for ash rot on sesame by several authors [10] [11]. The development of this disease would be favoured by high temperatures [23] [24] and pockets of drought, characteristic of this part of the country. *Exserohilum* and *Cladosporium* are genera that are particularly represented in the Sahelian zone characterized by annual rainfall of less than 600 mm and high temperatures, indicating that dry and hot conditions seem favorable to the development of these two genera. In general, all other genera invariably proliferate in both the Sudanian and Sudano-Sahelian zones.

Molecular tools used for the identification of fungi, due to their accuracy, are often complementary to identification based on morphological characteristics. Identification of fungi isolated from fragments of diseased sesame plants based on morphological characteristics could easily lead to misidentification. Based on the morphological characteristics of the 111 isolates obtained, were identified as belonging to 16 known genera including *Fusarium*, *Macrophomina*, *Cercospora*, *Alternaria*, *Colletotrichum*, *Phoma*, *Curvularia*, *Nigrospora*, *Cladosporium*, *Exserohilum*, *Pestalotia*, *Phomopsis*, *Rhizoctonia*, *Melanospora*, *Myrothecium*, and *Botryodiplodia*, and other unidentified genera referred as "Unknown" genus. DNA sequence analysis of these same fungal isolates identified 25 species belonging to 11 of the 16 genera initially identified morphologically, thus confirming the diversity of the mycoflora associated with sesame and revealed by the morphological identification. Three new genera were also identified. These are the genera *Corynespora*, *Lasiodiplodia*, and *Didymella*.

The 20 isolates of the genus *Macrophomina* were identified as *Macrophomina phaseolina*. Molecular analysis also showed that the isolate previously identified morphologically as belonging to the genus *Rhizoctonia* was found to be closely related to the species *M. phaseolina*. These results indicate that *M. phaseolina* species is probably the only species of the genus *Macrophomina* associated with sesame in Burkina Faso. The uniqueness of species in this genus could be explained by the use of generic primers and not specific to this genus. The work of [25] developed primers specific to three species of the genus *Macrophomina* including *M. phaseolina*.

From the 30 isolates of the genus *Fusarium*, molecular analysis confirmed 19

as belonging to the genus *Fusarium* and composed of seven different species, thus revealing a diversity of *Fusarium* species associated with diseased sesame plants in Burkina Faso. These are *F. proliferatum* (5 isolates), *F. equiseti* (4), *F. penzigii* (3), *F. incarnatum* (2), *F. fujikuroi* (2), *F. oxysporum* (2) and *F. solani* (1). All species identified, except *F. penzigii* have been previously reported as pathogens of sesame [14]. These include *F. proliferatum* [27], *F. oxysporum* [13] [22] [27], *F. solani* [28], *F. equiseti* and *F. fujikuroi* [14]. Among these species, *F. oxysporum* responsible for fusarium rot of sesame is one of the major pathogenic fungi in the production areas of major sesame-producing countries [22] [29]. It should be noted that molecular analysis of the other 11 isolates, initially identified as belonging to the genus *Fusarium*, was inconclusive.

Molecular analysis revealed three species of *Cercospora*: *C. sesami* (8), *C. kikuchii* (5) and *C. canescens* (1). The remaining six *Cercospora* isolates and one unknown isolate were found to be *Corynespora cassiicola*. One of the most prevalent diseases of sesame in the production areas is CLF due to *Cercospora sesami* [12] [31] [32]. In addition to this species, *C. kikuchii* and *C. canescens* were identified on diseased plant samples, suggesting the presence of three probable species associated with sesame leaf necrosis in Burkina Faso.

Alternaria Leaf Spot (ALS) due to *Alternaria sesami* and *Alternaria sesaminicola* is the major leaf disease of sesame in the humid tropics. In the present study, two potential species of ALS agents of sesame were identified. These are *A. simsimi* previously reported as the cause of ALS in Korea [32] and *A. porri* reported by [33] as the cause of ALS of onion.

Apart from the genera *Fusarium*, *Cercospora* and *Alternaria* reported as the major fungal pathogens of sesame worldwide, the present study identified species of the genera *Colletotrichum* (2), *Curvularia* (1), *Phoma* (1), *Nigrospora* (2), *Exserohilum* (1) associated with sesame from Burkina Faso and previously reported by Enikuomehin *et al.* [14] as potential pathogens of sesame. Accurate molecular identification allowed the identification of the species *Corynespora cassiicola* reported to cause spots on leaves, stems, roots and flowers of several economically important plants [34]. On the sesame crop, [35] reported for the first time in China, the root rots due to *C. cassiicola*. In the present study, *C. cassiicola* was associated with all parts of the plant but particularly leaves and would be a potential foliar disease agent on sesame [36].

Based on morphological and molecular characteristics many potential pathogenic fungi belonging to many genera are identified associated with the sesame plant in Burkina Faso.

5. Conclusion

Morphological identification of fungi associated with samples of diseased sesame plants demonstrated a diversity of potential pathogen agents of sesame in Burkina Faso. This diversity varies according to the agro-climatic zones of the country and is composed of 16 genera dominated by *Macrophomina*, *Fusarium*

and *Cercospora*. Molecular identification confirmed most of the results obtained from the morphological identification, providing precision on the identity of the fungal species associated with sesame in Burkina Faso. Thus, the top three fungal genera associated with sesame in Burkina Faso are *Macrophomina*, *Fusarium* and *Cercospora*. A study of the pathogenicity of the main species identified and further investigations on the genetic diversity of the isolates by using specific primers are necessary for the development of effective protection methods against the main diseases of the crop.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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