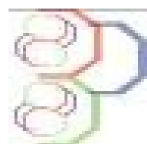

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Molecular characterization of the main fungi associated to Bambara groundnut foliar diseases in Burkina Faso

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ABSTRACT

Objective: This study aims to update the database of fungi associated to Bambara groundnut foliar diseases in Burkina Faso using both molecular and morphological identification approaches.

Methodology and Results: In this study, molecular approach based on the sequencing of ITS (Internal Transcripted Spacer) region of fungi and morphological approach were used to identify the main fungi associated to Bambara groundnut foliar diseases. The study was performed with universal polymerase chain reaction (PCR) primer ITS1/ITS4. BlastN comparisons between 19 fungal isolates contigs of the 16 major fungi were produced by their DNA sequences assembly and GenBank sequences yielded identity scores of 99 to 100 % with all of them. The degrees of similarity between these contigs and the loci sequences of classified fungi in GenBank indicate that our fungal isolates are the same species with those in Genbank, particularly the first of the list show after the blastN. It is the first report of molecular characterization of the main fungi infecting Bambara groundnut in Burkina Faso.

Conclusion and Application of results: Nineteen fungi associated to Bambara groundnut foliar diseases were identified and can be taken as targets in varietal improvement of Bambara groundnut for resistance to fungal diseases in Burkina Faso.

Key words: Bambara groundnut, fungi, molecular characterization, PCR primer ITS1/ITS4, Burkina Faso.

INTRODUCTION

Bambara groundnut *(Vigna subterranea* (L) Verdc.) is an indigenous African food legume and has advantages over more favoured species in terms of nutritional value and tolerance to adverse environmental conditions (Mkandawire, 2007; Berchie *et al.,* 2012). The crop is the third most

important food legume in Africa after groundnut and cowpea (Odongo *et al.,* 2015). It is a potential crop to provide food security in the dry areas of Africa. Its cultivation outside the African continent is very limited and the main world crop production (45 to 50%) is provided by West Africa (Baudoin et Mergeai, 2001; Brink *et al.,* 2006, Hillocks *et al.,* 2012). Africa-wide production is estimated to be over 330,000 t annually (Hillocks *et al.,* 2012) and Burkina Faso is one of the largest producers of Bambara groundnut which is the second most important food legume after cowpea (Cirad-Gret, 2002; Ouédraogo *et al.,* 2008; Hillocks *et al.,* 2012). Although Bambara groundnut is considered to be generally less affected by diseases and pests than groundnut or cowpea, several diseases and pests can cause serious damage to the crop (PROTA, 1980; Baudoin et Mergeai 2001). The fungal diseases are a major constraint on Bambara groundnut production in Burkina Faso and can cause yield losses of up to 83% in the event of severe attack (Sérémé *et al.,* 1991; Sérémé, 1992). Around sixteen fungal species have been associated with foliar diseases on Bambara groundnut in Africa six of them have been reported from Burkina Faso since for a long time (Table 1).

Table 1. List of Bambara groundnut-infecting fungi present in Africa

Fungi/Diseases	Country	Reference
Fusarium sp/ wilt	Botswana, Togo, Zambia, Zimbabwe	Heller *et al.,* 1997; Mkandawire, 2007; Hillock *et al.,* 2012
Cercospora sp /leaf spot	Botswana, Togo	Heller *et al.,* 1997; Hillock *et al.,* 2012
Phoma sorghina	Burkina Faso	Heller *et al.,* 1997
Phomopsis sojae	Burkina Faso	Heller *et al.,* 1997
Fusarium oxysporum	Burkina Faso, Tanzania	Heller *et al.* 1997; Baudoin et Mergeai, 2001
Rhizoctonia solani	Burkina Faso, Sierra Leone, Tanzania	Heller *et al.,* 1997; Baudoin et Mergeai, 2001; Hillock *et al.,* 2012
Macrophomina phaseolina	Burkina Faso	Heller *et al.,* 1997
Puccinia sp	Nigeria	Heller *et al.,* 1997; Hillock *et al.,* 2012
Colletotrichum sp	Nigeria	Heller *et al.,* 1997; Hillock *et al.,* 2012
Colletotrichum capsici	_	Baudoin et Mergeai, 2001
Cercospora anescens/leaf spot	Tanzania, Zimbabwe	Heller *et al.,*1997; Baudoin et Mergeai,2001;Mkandawire,2007; Hillock *et al.,* 2012
Erysiphe sp/powdery mildew	Tanzania	Heller *et al.,* 1997
Cercospora leaf blight	Zambia	Heller *et al.,* 1997; Mkandawire,2007
Phyllosticta voandzeia/ Leaf spot	Zimbabwe	Heller *et al.,* 1997
Sclerotium rolfsii	Zimbabwe	Heller *et al.,* 1997; Mkandawire, 2007, Hillock *et al.,* 2012
Phomopsis sp/ Leaf blotch	Burkina Faso, Zimbabwe	Kiwallo, 1991; Heller *et al.,* 1997, Mkandawire, 2007

The limited knowledge of fungi infecting Bambara groundnut in Burkina Faso hinders the control of fungal diseases and the creation of resistant varieties. In the event of climate changes which have an influence on epidemiology and inoculum survival (Boland *et al.,* 2004), the main objective of this study is to update the database of Bambara groundnut fungal diseases in Burkina Faso. The Molecular approach was used for completing the morphological identification to characterize the main fungi associated to Bambara groundnut diseases in Burkina Faso. The ITS (Internal Transcribed Spacer) region of fungi rDNA has been investigated for species identification and phylogenetic relationships (Talarmin, 2007; Karsch-Mizrachi *et al.,* 2012). Here, universal polymerase chain reaction (PCR) primer ITS1/ITS4 (White *et al.,* 1990) was used to characterize the main fungi associated to Bambara groundnut foliar diseases in Burkina Faso.

MATERIAL AND METHODS

Plant sampling: One hundred and sixty-six (166) leaf samples of Bambara groundnut plants infected by fungal diseases were randomly collected from farmer's fields in forty-four (44) sites (fig 1) and experimental plots. The sampling was carried out through the three agro-climatic zones (humid Sudan zone, sub-humid Sudan-Sahel zone and dry Sahel zone) of Burkina Faso (fig 1) from September to October, 2015. Eighteen (18) samples were collected in the humid Sudan zone, one hundred and twenty-nine (129) in the sub-humid Sudan-Sahel zone and nineteen (19) from the dry Sahel zone.

Fig 1. Sites of collection of leaf samples of Bambara groundnut in 2015 in Burkina Faso

Isolation of fungi associated with infected leaves of Bambara groundnut: The collected fresh leaves were surface cleaned in sterilized distilled water followed by immersion in 70% (v/v) ethanol for 1 min. The samples were left to air dry for 30 min. Three to four fresh leaflets of each sample with diseases symptoms were transferred into Petri dishes containing two layers of blotting papers humidified with sterile distilled water. Petri dishes containing samples were incubated at 22 to 25°C under alternating 12 h of light and 12 h of dark for 7 days. After incubation, fungi were identified based on the examination of the acervuli and conidia produced on the infected tissues on the blotter papers under the stereomicroscope and compound microscopes based on the identification key established by Marthur and Kongsdal (2003). A prevalence rate was calculated for each fungus identified as a percentage of samples infected by this fungus.

Single spore production of the main fungi: The choice of the main fungi has been based on their prevalence (≥25%) in each agro-ecological zone. So, using a sterile loop, isolates were further placed onto potato dextrose agar (PDA) containing streptomycin (0.3 µg/L of PDA). The plates were incubated at 22°C to 25°C for 7 days under ultraviolet (UV) light of alternating 12 h light and darkness to obtain pure culture. For single spore culture production, distilled water (100 ml) was added to Petri dishes containing culture of fungal isolates. Then 200 µl of the suspension was diluted according to its concentration in spores and 200 µl of diluted suspension were spread on AGAR medium and incubated for 24 to 48 h. Five single cells from each isolate were again transferred onto fives new Petri dishes containing PDA. After seven days of growth, single spore pure cultures were obtained and stored at 20°C until used.

DNA extraction: DNA of each isolate was extracted using the cethyltrimethylammonium bromide (CTAB) method used by Sadfi-Zouaoui et al. (2008) with some minor modifications. After adding 600 µl of CTAB extraction buffer [1.4 M NaCl, 2% CTAB (w / v), 0.1 M Tris-Base pH8, 20 mM EDTA and 0.2% (v / v). v) β-mercaptoethanol] and introducing two beads into each tube containing 100 mg of mycelium, the tubes were vortexed and stirred for five to ten minutes. The beads were then removed and the tubes were placed in a water bath at 65 ° C for fifteen minutes. A volume of 450 µl of phenol and 450 µl of alcohol-iso-amyl chloroform (consisting of 49 ml of chloroform and 1 ml of iso-amyl alcohol) was added to each tube and then mixed by inversion of the tubes until the content becomes milky. The mixture was then centrifuged at 13,000 x g for five minutes at 25 ° C. The supernatant (approximately 500 µl) was recovered in a new 1.5 ml tube. After adding 400 µl of alcohol-isoamyl chloroform, the tubes were again centrifuged at 13000 g for two minutes at 25 ° C. The supernatant was further transferred to a new 1.5 ml tube and 0.7 volume (350 µl) of isopropanol was added to the solution to precipitate the DNA. Further centrifugation was performed for 20 minutes at 13000 g at 25 ° C to obtain a DNA pellet at the bottom of the tube. The pellet was rinsed with 500 µl of 96% alcohol and centrifuged for three minutes at 13000 g at 25 ° C. This rinsing step was repeated three times. The pellet was dried under the hood and then dissolved in 30 µl of sterile distilled water. The DNA thus obtained was stored at -20 ° C.

DNA concentration measurement: The concentration of DNA obtained for each isolate was measured by spectrophotometer (Nanodrop 2000) and the initial concentration was evaluated in nanograms per microliter (ng / µl). The different concentrations were diluted case by case so that the DNA volume taken for the PCR contains about 40 ng of DNA.

Polymerase chain reaction (PCR): The amplification reaction was carried out in 25 µl containing 40 ng of DNA (4 µl), 2.5 µl of stained buffer Taq DNA polymerase 10X (Promega), 0.5 µl of dNTP (10 mM), 0.5 µl of each primers (ITS1 at 10 µM and ITS4 at 10 µM), 0.1 µl of Taq DNA polymerase (5 U/ µl) (Promega) and sterile distilled H_2O. The amplification reaction was carried out in a thermal cycler. The PCR program was as follows: pre-denaturation at 94 ° C for 5 minutes followed by 30 consecutive cycles of denaturation at 94 ° C for 30 seconds, specific primer hybridization at 58 ° C for 30 minutes and an elongation at 72 ° C for 5 minutes and a post-elongation at 72 ° C for 10 minutes.

Agarose gel electrophoresis: A 1% (W / V) agarose gel was prepared with TAE buffer 0.5 X (Tris, acetic acid, EDTA pH 8). A volume of 2 µl of ethidium bromide was added to the agarose once dissolved in the microwave and then cooled. The gel thus prepared was cast in a mini electrophoresis plate in which the buffer TAE (0.5 X) was added after solidification of the gel. The gel wells were then loaded with 12 µl of the PCR product of each sample. A molecular weight marker (100 bp), a water-control and a positive control were separately loaded into the wells of the gel. Electrophoresis was performed at 100 V for 20 minutes. The amplified products were visualized under UV light.

Sequencing of amplified ITS: PCR products (amplified ITS) were purified using the MPBiomedicals Geneclean Turbo Kit. The purified DNA was eluted in 30 µl of sterile distilled water and 18 µl was removed for sequencing. Sequencing was performed by Genewiz http://www.beckmangenomics.com/ with primers ITS1 and ITS4

Sequences analysis: The sequences were processed, cleaned and aligned with the Bioedit software. The consensus sequences obtained from the treatments and cleanings were compared to those of the nucleotide sequence database of NCBI (National Center for Biotechnology Information) on the site: https: //blast.ncbi.nlm.nih. gov / Blast.cgi

RESULTS

Several species of fungi are associated with Bambara groundnut diseases in Burkina Faso. The analyses of leaf samples of diseased plants (fig. 2), based on morphological characters, detected 35 species of fungi (Table 1) belonging to 19 genera (*Macrophomina, Alternaria, Cladosporium, Phoma, Cercospora, Rhizoctonia, Fusarium, Curvularia, Myrothecium, Colletotrichum, Melanospora, Exserohilum, Leptosphaerulina, Didymella, Nigrospora, Aspergillus, Pestalotia, Ulocladium* and *Microdochium*). Fungi were more represented in the Sudan-Sahel zone with 32 species encountered, followed by the Sahel zone with 23 species and finally the Sudan zone with 13 species. The prevalence of fungi varied from 0.77%for *Microdochium oryzae* and *Fusarium moniliforme* in Sudan Sahel zone to 83.33% for *Cladosporium sphaerospermum* and *Cercospora sp* in Sudan zone (Table 1). Among the 35 fungal species identified using morphological identification approach, sixteen species were the most prevalent (prevalence ≥25%). The fungal species such as *Macrophomina phaseolina, Alternaria sp* and *Curvulria sp* had their prevalence higher than 25% in each climatic zone. While *Cladosporium sphaerospermum* and *Cercospora sp* were the most prevalent (83.33%) *in* Sudan zone, *Curvularia sp was* the most prevalent (69.77 %,) in sudan-sahel zone. In Sahel zone, *Fusarium equiseli* and *Exserohilum rostiatum* were the most prevalent (68.42%). Nineteen isolates constituted with the most prevalent species were identified using molecular identification approach (Table 2).BlastN comparisons between the 19 isolates contigs of the 16 major fungi that were produced by their DNA sequences assembly and GenBank sequences yielded identity scores of 99 to 100 % with all of them(Table 2). The degrees of similarity between these contigs and the loci sequences of classified fungi in GenBank indicate that our fungal isolates are the same species with those in Genbank, particularly the first of the list show after the blastN. Of the nineteen (19) fungi isolates subjected to molecular analysis, fifteen (15) species were identified (Table 2).

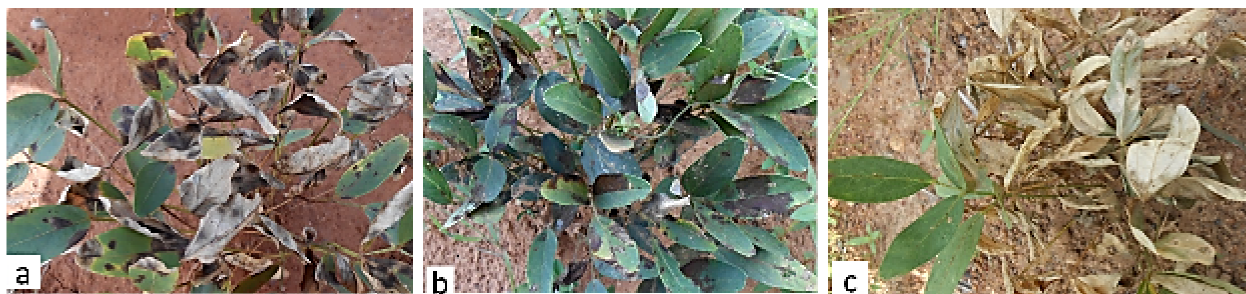

Fig 2. Some symptoms of Bambara groundnut leaf diseases observed in the field
a): Light brown spots with or without a yellow halo, b): Reddening spots, c): wilting and drying of the plant. Fungi associated to symptoms about morphological identification a): *Cercospora* sp., *Alternaria* sp et *Phoma* sp., b): *Cercospora* sp, *Fusarium equiseti* et *Phoma lingam*, c): *Macrophomina phaseolina*, *Colletotrichum capsici*, *Phoma sorghina, Fusarium culmorum* et *Curvilaria sesami*.

The comparison of identities of main fungi with morphological approach between their identities with molecular approach, revealed a concordance with the fungi species *Macrophomina phaseolina, Colletotrichum capsici, Exserohilum rostratum, Curvularia lunata and Phoma* sp. However molecular approach brought more precisions on the fungi strains. For species such as *Curvularia eragrostidis*, *Fusarium* sp, and *Cladosporium cladosporioides*, compliance was limited to the genus level. For others, the morphological results did not agree with those at the molecular level. This is the case of the species *Corynespora sp., Thielavia terricola* and *Macrophomina phaseolina* previously identified morphologically as *Alternaria* sp., *Fusarium* sp. and *Rhizoctonia solani*, respectively.

Table 2. Fungi associated with diseased Bambara groundnut plants and their prevalence according to climatic zones of Burkina Faso

N°	Fungi isolates	Prevalence (%) according to climatic zones		
		Sahel zone	Sudan-sahel zone	Sudan zone
1	****Macrophomina phaseolina*	31,58	30,23	27,78
2	****Alternaria sp*	31,58	38,76	44,44
3	***Cladosporium sphaerospermum*	10,52	65,88	83,33
4	***Phoma sp*	5,26	58,14	77,78
5	***Cercospora sp*	10,53	43,41	83,33
6	*Alternaria alternata*	10,53	22,48	11,11
7	**Phoma sorghina*	63,15	14,72	00
8	**Rhizoctonia solani*	47,37	15,5	16,67
9	**Fusarium equiseli*	68,42	19,38	00
10	**Curvularia lunata*	52,63	22,48	11,11
11	*Myrothecum roridum*	26,31	15,5	00
12	**Colletotrichum capsici*	52,63	22,48	11,11
13	*Phoma lingam*	10,52	11,62	00
14	**Melanospora zamia*	21,05	31,01	00
15	**Exserohilum rostiatum*	68,42	10,85	00
16	**Leptosphaerulina crassiaca*	21,05	46,51	00
17	***Fusarium oxysporum*	42,1	54,26	00
18	*Colletotrichum graminicola*	10,52	2,32	00
19	****Curvularia sp*	26,31	69,77	55,56
20	*Didymella bryoniae*	5,26	00	00
21	*Fusarium sp*	10,53	14,73	22,22
22	*Phoma exigua*	5,26	00	00
23	*Curvularia eragrolides*	5,26	00	00
24	*Fusarium moniliforme*	00	0,77	00
25	*Nigrospora oryzae*	00	6,97	11,11
26	*Fusarium culmorum*	00	1,5	00
27	*Cercospora sesami*	00	3,1	5,55
28	*Curvularia pallescens*	00	0,77	00
29	*Aspergilus niger*	00	1,55	00
30	*Aspergilus flavus*	00	1,55	00
31	*Alternaria sesamicola*	00	1,55	00
32	*Alternaria brassicicola*	00	3,1	00
33	*Pestalotia guepini*	00	2,32	00

34	*Ulocladium consortiale*	00	3,87	00
35	*Microdochium oryzae*	00	0,77	00

*(prevalence ≥25 in one climatic zone); ** (prevalence ≥25 in two climatic zones); *** (prevalence ≥25 in three zone climati zones)

Table 3. Results of sequence comparisons of isolates with those available in the NCBI database

Isolates	Size of contig sequences (bp)	Results of comparisons with NCBI database				
		Accession numbers	Corresponding species in the NCBI database	Loci (bp)	Identity	Query cover
Macrophomina phaseolina (S)	522	KF951768.1	*Macrophomina phaseolina* souche CPC 21498	536	100%	99%
Macrophomina phaseolina (SS)	544	KF951780.1	*Macrophomina phaseolina* souche CPC 21519	554	100%	100%
Macrophomina phaseolina (SD)	511	KU856652.1	*Macrophomina phaseolina* isolat 171	566	99%	100%
Rhizoctonia solani (S)	544	KU856652.1	*Macrophomina phaseolina* isolat 171	566	100%	100%
Curvilaria lunata (S)	477	KU856618.1	*Curvularia eragrostidis* isolat 206	572	99%	100%
Colletotrichum capsici (SS)	510	KY052773.1	*Colletotrichum truncatum* souche JW-WG-03	531	100%	100%
Fusarium equiseti (S)	535	KU571535.1	*Fusarium sp.* BAB-4826	559	99%	100%
Phoma sorghina (S)	528	KF493958.1	Uncultured Phoma clone TVD_ITS1F ITS4_58	579	99%	99%
Exserohilum rostratum (S)	573	KU856642.1	*Exserohilum rostratum isolat* 204	585	100%	100%
Alternaria sp. (S)	538	KU898065.1	*Corynespora sp.* isolat OLS1	826	99%	100%
Alternaria sp. (SS)	510	KM979947.1	*Phoma sp.* F162	552	100%	100%
Curvularia sp. (SS)	552	KP131939.1	*Curvilaria lunata* souche IP 2328.98	574	100%	100%
Phoma sp. (SS)	507	KX758542.1	*Phoma sp.* isolat Guangxi-1	541	99%	100%
Cercospora sp. (SD)	543	KU856634.1	*Curvularia lunata* isolat 214	554	100%	100%
Cladosporium sphaerospermum (S)	521	KX664414.1	*Cladosporium cladosporioides* isolat F47-03	1087	100%	100%
Cladosporium sphaerospermum (SD)	520	KX664414.1	*Cladosporium cladosporioides* isolat F47-03	1087	100%	100%
Cladosporium sphaerospermum (SS)	522	KX664414. 1	*Cladosporium cladosporioides* isolat F47-03	1087	100%	100%
Alternaria alternata (SS)	538	KU898065.1	*Corynespora sp.* isolat OLS1	826	100%	100%
Fusarium sp. (S)	539	GU966509.1	*Thielavia terricola* souche QD02	567	100%	100%

S= isolates from sahel; SS= Isolates from Soudan-sahel, SD=Isolates from Soudan

DISCUSSION

Nineteen (19) main fungi isolates associated with Bambara groundnut foliar diseases in Burkina Faso, were characterized using molecular approaches in this study. The identity scores given by the BlastN comparison were 99 to 100 %. According to Raja et al. (2017), molecular identification results obtained by comparing nucleotide sequences of fungi isolates to nucleotide sequences database (GenBank) by the "BLAST" search method on NCBI site, are considered reliable, if comparative sequence cover a rate of 80% at least and a similarity index of 97% at least. The coverage rates and similarity indices obtained in this study are at least 99%. Therefore, we can say that this study results are reliable. However, this study revealed some discrepancies between morphological and molecular approaches concerning the identity of some species. The discrepancies between both approaches can be explained by the flaws that morphological identification approach can include and by the gaps that ITS sequences and the search by "BLAST" on the NCBI site can contain. Indeed, morphological approaches to fungal systematics are problematic because of the lack of useful traits for aggregation and they often fail to provide a robust evolutionary framework, particularly at the species level (Geiser, 2004). Morphological characters can often be misleading due to cryptic speciation and convergent evolution (Harrington and Rizzo 1999, Olson 2002, Hughes et al. According to Raja et al. (2017), until recently, it was a common practice in mycology to make nomenclatural errors by naming the asexual and sexual forms of the same fungus differently (dual nomenclature). In addition, the possible errors of observation or appreciation of biologist could lead to errors of identification. Besides, the ITS region is not a good marker in some very specific genera, such as *Aspergillus*, *Cladosporium, Fusarium, Penicillium* and *Trichoderma*, as these taxa have no gaps in their ITS regions or when they possess them, they are narrow (Schoch *et al.,* 2012). In addition, a variation in the intra-genomic ITS region occurs in some fungal groups (Lindner and Banik, 2011) although more recent studies suggest that this is not widespread in fungi (Lindner et al. 2013). Also, it has been reported that identifications using BLAST search in the nucleotide sequence database (GenBank) should be done with caution as approximately 27% of the GenBank fungal ITS sequences were submitted with insufficient taxonomic identification (Nilsson *et al.,* 2006). At last, to the list of both identification approaches gaps cited below, we can add the fact that about 20% of the fungal sequences in GenBank may be incorrectly annotated, the fact that the taxonomic names are not up-to-date due to the rapid change in the fungal taxonomy and the fact that most some fungal species described (approximately 70%) have not been sequenced so far (Rossman and Hernandez, 2008). For the present study and for the non-concordant cases, a recovery of both morphological and molecular identification could be considered in order to confirm or invalidate the current results. Among the nineteen (19) main fungi associated to Bambara groundnut foliar diseases in Burkina Faso that this study allowed to characterize, only the species *Macrophomina phaseolina* and *Fusarium* sp were previously reported to be associated to Bambara groundnut fungal diseases in Africa (Heller *et al.,* 1997)). In Burkina Faso, only *Macrophomina phaseolina was* previously reported to be associated to Bambara groundnut fungal diseases (Heller *et al.,* 1997)). However, this study makes it clear that the three isolates of *Macrophomina phaseolina from Burkina Faso* (isolate from Sahel zone, isolate from Sudan Sahel zone and from Sudan zone) are three different strains. This specie is known to be an important soil- and seed-borne pathogen, has a broad geographic distribution and a large host range (Sarr *et al.,* 2014). *Macrophomina phaseolina* causes considerable damage in cluster bean, an important legume of arid and semiarid regions in India, Australia, Brazil, South Africa and Pakistan, as well as Oklahoma and Texas in the USA (Purkayastha *et al.,* 2006). *Fusarium* sp causes leaf blight disease on Bambara groundnut in Northern Nigeria (Tanimu *et al.,* 1997). For others fungi (*Curvularia eragrostidi, Colletotrichum capsici, Cladosporium cladosporioides, Curvularia aeria, Phoma* sp and *Setosphaeria rostrata*) characterized in our study, some literature data give some brief news about these fungi. So, *Curvularia eragrostidis* is a cosmopolitan pathogen that infects hosts from several botanical families (Ferreira *et al.,* 2014). In Brazil, this fungus causes leaf spot on *A. comosus*, infects *Allium sativum, Dioscorea alata, D. cayenensis*, *Oryza sativa*, *Sorghum bicolor*, *Vigna unguiculata*, and *Zea mays* (Ferreira *et al.,* 2014). It also causes postharvest rot disease in pineapple in this country (Ferreira *et al.,* 2014). *Colletotrichum capsici* is an important plant pathogen that has a wide host range including pepper, eggplant, muskmelon, chickpea, grapes, and many other species of plants (Diao *et al.,* , 2014). *C. capsici* have been identified as important

pathogens causing anthracnose on cowpea in Burkina Faso, chili and Andean blackberry (*Rubus glaucus*) in Thailand and Colombia (Thio *et al.,* 2016; Montri *et al.,* 2009). *Cladosporium cladosporioides* is a very common, cosmopolitan, saprobic species and often occurs as a secondary invader on necrotic parts of many different host plants (Bensch *et al.,* 2010). *Curvularia aeria* causes leaf spot diseases on tomato in Pakistan (Nayab and Akhtar, 2016). It was reported that *Phoma* sp causes leaf spot disease on *Schisandra chinensis* in Korea (Choi, 2014). *Setosphaeria rostrata* is a common plant pathogen causing leaf spot disease, affects a wide range of plant species, mainly grasses (Kusai *et al.,* 2016).

CONCLUSION

The combination of morphological identification and molecular approach based on sequencing of rDNA-ITS region of fungi, has strengthened our knowledge about fungi microflora associated to Bambara groundnut foliar diseases in Burkina Faso. This study reveals the occurrence of nineteen fungi associated to Bambara groundnut foliar diseases. These fungi can be taken as targets in varietal improvement of Bambara groundnut for resistance to fungal diseases in Burkina Faso.

Conflict of interests

The authors have not declared any conflict of interest

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