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Fungal endophytes of sorghum in Burkina Faso: Occurrence and distribution

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A survey was conducted to assess the natural occurrence and distribution of fungal endophytes in sorghum in relation to plant performance in two distinct agro-ecological zones in Burkina Faso. Sorghum farm-saved seeds were sown in 48 farmers' fields in Sahelian and North Sudanian zones to produce sorghum plants. In each field, leaf samples were collected from five well-developed (performing) and five less-developed (non-performing) plants at 3-5 leaf stage, while at plant maturity leaf, stem and root samples were collected from the same plants and fungal endophytes were isolated. A total of 39 fungal species belonging to 25 genera were isolated. The most represented genera included *Fusarium*, *Leptosphaeria*, *Curvularia*, *Nigrospora* and *Penicillium*. The genera *Fusarium* and *Penicillium* occurred significantly higher in performing plants as compared to non-performing plants while the genera *Colletotrichum* and *Alternaria* were most represented in non-performing plants. Among the *Fusarium* species identified, *Fusarium moniliforme* was the most common fungus isolated from the plants. *Fusarium* spp. and *Penicillium* sp. were significantly present in a higher number of performing plants than in non-performing plants, while *Colletotrichum sublineolum* was more encountered in non-performing plants than in performing plants. Distribution of fungi varied based on the tissue and root accounting for the majority of the fungi isolated. This work represents the first description of the diversity of fungal species and the fungal community in sorghum, and the first report attempts to document endophytic fungal presence in Burkina Faso.

Key words: Endophytes, bio-resource, *Sorghum bicolor*, fungi.

INTRODUCTION

Sorghum (*Sorghum bicolor* L.) is the fifth most important grain crop in the world after maize, rice, wheat and barley and is on average the second most produced grain in the African continent in 2004-2013 (http://faostat.fao.org) (2014). Drought tolerance makes sorghum particularly important in the dry regions of North-East Africa, which is recognized as the centre of diversity of sorghum, where agricultural and environmental conditions are unfavourable for other cereal crops (Paterson et al., 2009). It is the major food crop used in rural populations within the

semi-arid area in Africa, and in 2013 25.7 million tonnes of grain sorghum was produced as compared to 23.5 and 8.8 million tonnes in America and Asia, respectively, making Africa the largest sorghum producer in the world (http://faostat.fao.org) (2014). However, despite high production levels in Africa, average yield is often low (0.957 t/ha) in comparison with average yields in America (3.525 t/ha) which is due to a combination of agronomic and environmental factors as well as the use of inferior sorghum varieties in Africa. In 2013, sorghum production in Burkina Faso was 1.9 million tonnes with an average yield of 1.078 t/ha. Even though total sorghum production has been increasing in recent years, this has only been achieved through cultivation of more land (Belton and Taylor, 2004).

Sorghum production is menaced by abiotic factors such as drought in the semi-arid regions of Africa. Furthermore, biotic factors such as insect pests and pathogenic fungi which are either present in the soil or are transmitted by sorghum seeds, represent other major threats to sorghum production (Chandrashekar and Satyanarayana, 2006). These biotic threats lead to significant crop damage, contributing to the severe yield losses mentioned above. Pathogenic fungi such as *Phoma*, *Curvularia*, *Fusarium* and *Colletotrichum* spp. are known threats to sorghum, and sorghum grains are also susceptible to colonization by *Aspergillus* spp. during wet periods after harvest, which can result in the accumulation of the unwanted mycotoxin, aflaxotin, in the grain (Chandrashekar and Satyanarayana, 2006). Not all species of *Curvularia* and *Fusarium* are pathogenic on sorghum, but those that are pathogenic mainly affect the stem (*Curvularia*) or the stem and leaf (*Fusarium*). Penetration and infection by pathogenic *Phoma* spp. are restricted by the thickness of the mesocarp (Kumari et al., 1992), and the research data accumulated over many years indicate innate differences among sorghum grains in their ability to resist fungal colonization (Chandrashekar and Satyanarayana, 2006). *Colletotrichum sublimeolum* is mainly a pathogen of sorghum leaves, infecting the sorghum seed head, and is the causative agent of sorghum anthracnose disease (Chandrashekar and Satyanarayana, 2006).

Several methods currently exist for the control of pathogenic fungi in sorghum tissues, most notably the use of chemical fungicides. The use of chemical control methods is for many African farmers challenged by the economic cost and the physical unavailability of fungicides. Furthermore, environmental concerns about potential adverse effects from the use of chemical fungicides call for alternative methods to control pathogenic fungi within this area. The use of botanicals against crop pathogenic fungi is a strategy currently under development in many countries. Application of an aqueous extract of *Eclipta alba*, a weed, as seed treatment was reported to inhibit sorghum seed-borne *Leptosphaerella sacchari* (*Phoma sorghina*) and increase yield in Burkina Faso (Zida et al., 2012). One currently unexploited approach towards reducing fungal diseases of sorghum is the potential use of endophytes as biocontrol agents against pathogenic microbes (Clay, 1989; Schardl et al., 2004; Schardl et al., 1991).

An endophyte can be defined as any microorganism, typically bacterial or fungal, that lives within a plant (Clay and Schardl, 2002). There is now a substantial amount of literature regarding beneficial endophytes, mostly related to the ascomycete endophytes of the fungal genera *Neotyhodium* and *Epichloë*, which are associated with temperate grasses (*Poöideae*). There is a well-accepted notion that grass endophytes have mutualistic relationships with their hosts, and this has led to claims that they co-evolve with their hosts (Faeth, 2002; Porras-Alfaro and Bayman, 2011). The growing list of beneficial effects imparted by endophytes to their hosts includes tolerance to drought (Clay and Schardl, 2002; Hahn et al., 2008; Malinowski and Belesky, 2000; Redman et al., 2002; Sherameti et al., 2008), improved salt tolerance (Baltruschat et al., 2008; Redman et al., 2011), enhanced growth (Bae et al., 2009; Mucciarelli et al., 2003; Waller et al., 2005) and increased tolerance to pathogens (Porras-Alfaro and Bayman, 2011). Furthermore, endophytic fungi have been noted for benefits to the consumers of their plant hosts, such as reduction of mycotoxins produced by mycotoxinogenic fungi (Danielsen and Jensen, 1999). Currently, endophytes have a well-recognized potential as biocontrol agents in a wide variety of plants, and the potential for endophytes as biocontrol agents in cereals has recently been reviewed (O'Hanlon et al., 2012). Investigating and harnessing the potential of endophytes expands the possibility for developing biocontrol strategies to control sorghum pathogens as well as enhancing stress tolerance through artificially inoculated stable endophytes. To date, no study has been undertaken on the tissue-specific prevalence of endophytic fungi in sorghum, and therefore it is conceivable that this is a resource which should be explored for its potential use as biocontrol agents to manage fungal diseases as well as to enhance stress tolerance.

The objective of this study was to isolate and identify the endophytic fungal diversity within different tissues of sorghum plants originating from farm-saved sorghum seeds grown in two agroecological zones of Burkina Faso as a starting point for further investigations into the potential of endophytes to control fungal pathogens in this important cereal crop.

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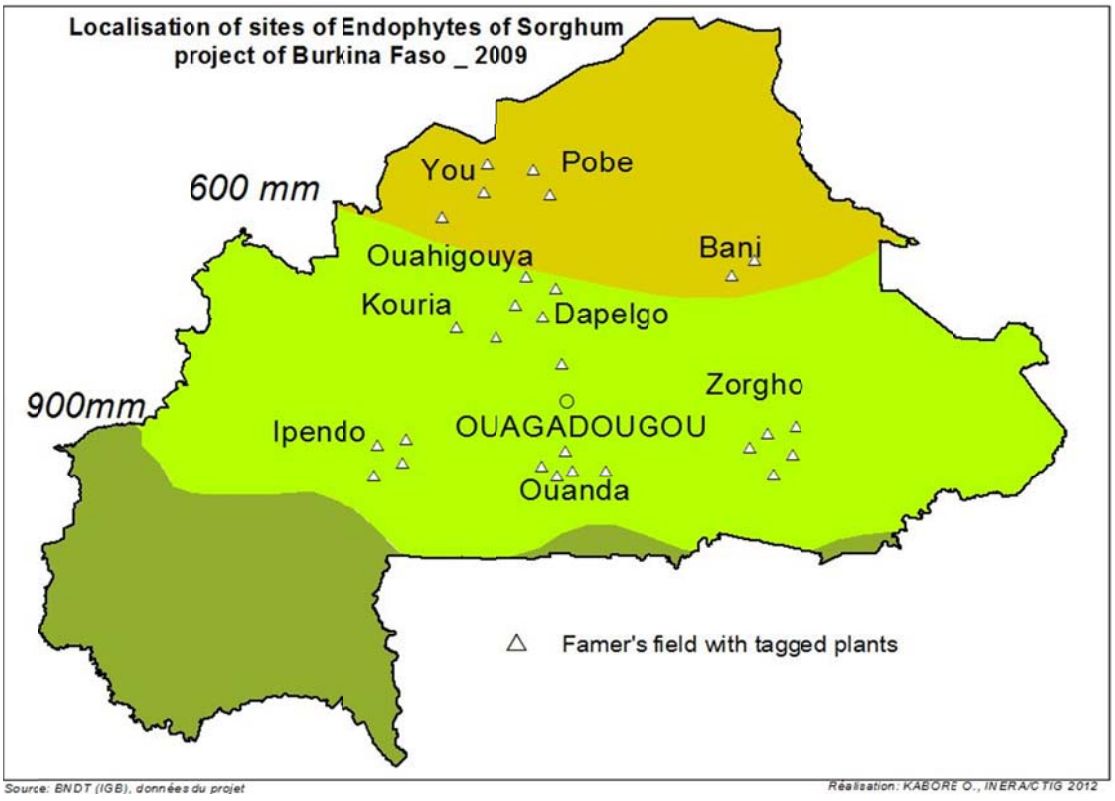

Figure 1. Distribution of sites for collection of endophytic fungi from sorghum plants in Burkina Faso in 2009.

MATERIALS AND METHODS

Sample collection

In general, in sub-Saharan Africa, vegetation, soil, etc. are strongly linked to the annual precipitation. Sorghum plant samples were collected in farmer's fields in two agro-ecological zones during the raining season, 2009: the Sahelian zone with an average annual precipitation ranging from 300 to 600 mm and the North Sudanian zone with 600 to 900 mm precipitation. At the first sampling period, rains were regular and the relative humidity was relatively high (> 70%) and temperature was in the interval of 22-35°C. At plant maturity (second sampling period), rains were rare or completely absent, temperature was high (30-39°C) and relative humidity <50°C. Nine villages (Bani, Pobe, You, Ouahigouya from the Sahelian zone and Kouria, Dapelgo, Ipendo, Ouanda and Zorgho from the North Sudanian zone) were considered as sampling sites (Figure 1). In each village, samples were collected in five fields belonging to farmers who establish their crop from farm-saved seed. The agronomic management of the sampling sites was carried out according to the farmers' capacity (soil cultivation, plant establishment, fertilizer application, etc.). None of the farmers used fungicides. In each field within an area of approximately 3 x 3 m, ten plants (five performing plants (P) (well-growing, vigorous plants) and five neighbouring non-performing plants (NP) (less vigorous, without disease symptoms) were identified and tagged in early summer (Figure 2). Within each field, P and NP plants were at the same developmental stage and either performing or non-performing plants showed disease infection. The first sampling was carried out non-destructively when sorghum plants had 3-5 leaves, while the second sampling was carried out on the same plants at maturity. The first sampling was restricted to the leaves, while during the second sampling leaf, stem and root fragments were collected for endophyte isolation from the tagged plants. Fungal occurrence was subsequently compared within these groups of plants and within plant tissues. A total of 330 from the 450 labelled plants in the two zones were still available for investigation. The difference was due to loss of labels (unintended removals of various kinds) or plants had already been harvested before sampling. At the 3-5 leaf stage, 250 plants (125 P plants and 125 NP plants) were investigated, while at maturity stage 280 plants (140 P and 140 NP plants) were subject to investigation. Sampling and transport to the villages was time consuming and some plants were developed further than the 3-5 leaf stage before sampling. Therefore, the total number of plants sampled was higher in sampling 2 than in sampling 1.

Samples were transported in paper bags to the laboratory and stored in the refrigerator. Within one to two days after collection, samples were surfaced-sterilized and incubated on PDA medium for nine days. Analysis, which primarily involved fungal isolation and identification, was carried out in the Laboratory of Phytopathology of Kamboinsé Research Station in Burkina Faso. Furthermore, agronomic data (stem diameter, plant height, plant weight, panicle length and weight, grain weight and total number of grains) were recorded for these plants. These data are to be presented in a follow-up paper.

Isolation and identification of endophytic fungi

Fungal endophytes were isolated from samples of leaf, stem and root collected from individual field plants according to the protocol described by Petrini (1986) with modifications. Sorghum leaf, stem and root tissues were cut into 12-15 mm pieces prior to sterilization. All fragments were surface-sterilized in 70% (v/v) ethanol for one

Figure 2. Pictures of performing (P) plant (right) and a non-performing (NP) plant (left).

minute followed by immersion in 3% (v/v) sodium hypochlorite (NaOCl) for four minutes and then in 70% (v/v) ethanol for 30 s. Tissue fragments were rinsed three times in sterilized distilled water. The fragments were subsequently plated in Petri dishes containing potato dextrose agar (PDA) (3.9% w/v), which was aseptically supplemented with streptomycin antibiotic (0.2% v/v) to inhibit bacterial growth. Plates were incubated in the dark for nine days at 28°C. All colonies observed were sub-cultured onto fresh PDA without streptomycin and incubated at 24°C for seven days under a cycle of 12 h UV light/12 h darkness. Fungal isolates were primarily identified based on fungal morphology and compared with the current published identification keys (Hyn et al., 2004; Mathur and Kongsdal, 2003; Singh et al., 1991).

Statistical analysis

One-way analysis of variance (ANOVA) and least significant difference (LSD) were performed on the data recorded. Fungal occurrence was compared within the two agro-ecological zones, within the two groups of plants (P and NP plants) and within plant tissues.

RESULTS

Endophytic fungal biodiversity in sorghum in two agro-ecological zones in Burkina Faso

From the potential 450 tagged plants (9 villages x 5 farmers x (5 P + 5 NP)), a total of 330 plants were investigated. In total, 39 fungal species, belonging to 25 genera, were isolated from sorghum plants in the two agro-ecological zones in Burkina Faso during two sampling periods (Table 1). *Fusarium moniliforme*, *Fusarium* spp., *Leptosphaerella sacchari*, *Nigrospora oryzae*, *Curvularia* spp. and *Penicillium* sp. were frequently encountered in plants in both zones. About 15.38% of these fungal species were mainly associated with sorghum plants in the Sahelian zone, while 28.20% were most abundant in the Sudanian zone. All the other fungi were invariably present in each of the two zones.

Occurrence of endophytic fungi in performing and non-performing plants

The major genera identified included *Fusarium*, *Leptosphaeria*, *Curvularia*, *Penicillium, Nigrospora*, *Alternaria*, *Rhizoctonia*, *Colletotrichum* and *Exserohilum*. At both sampling times, the percentages of performing plants colonized by these genera were generally higher than those of non-performing plants, except for *Colletotrichum* (Figure 3). The genus *Colletotrichum* seemed to be most abundant within non-performing plants. Statistical analysis showed that among the major genera, the presence *in plantae* of *Fusarium* spp. ($p = 0.0077$ at first and second samplings) and *Penicillium* spp. ($p = 0.011$ at first sampling) was significantly associated with plant performance.

Among the fungal species identified, only 27 species were isolated from leaf samples at the first sampling (3-5 leaf stage), whereas all of the 39 fungal species were isolated at the second sampling (at maturity) when fungal isolation was performed on leaf, stem and root samples (Table 2). The results showed that at the 3-5 leaf stage, significant differences were observed between performing and non-performing plants colonized by *F. moniliforme* ($p = 0.0099$), *Fusarium* spp. ($p = 0.0102$), *Penicillium* sp. ($p = 0.0116$) and *Colletotrichum sublineolum* ($p = 0.0174$). *Fusarium* sp. *F. moniliforme* and *Penicillium* spp. were significantly associated with

Table 1. Occurrence of fungal endophytes in sorghum plants in two agro-ecological zones in Burkina Faso.

Fungi	Sahelian zone	Sudanian zone	Average	LSD (5%)
Fusarium moniliforme	37.32[a]	18.82[b]	28.50	5.31
Fusarium pallidoroseum	0.35[a]	0.78[a]	0.56	0.90
Fusarium equiseti	1.78[a]	0.19[b]	1.02	1.21
Fusarium culmorum	0.71[a]	0.39[a]	0.56	0.90
Fusarium spp.	13.92[a]	8.43[b]	11.30	3.86
Leptosphaeria sacchari	17.85[b]	32.15[a]	24.67	5.11
Phoma sp.	0.17[a]	0.00[a]	0.09	0.37
Macrophomina phaseolina	0.17[a]	0.39[a]	0.28	0.64
Cladosporium sphaerospermum	1.07[b]	3.52[a]	2.24	1.77
Colletotrichum sublineolum	1.25[b]	8.62[a]	4.76	2.52
Colletotrichum gloerosporioides	0.17[a]	0.98[a]	0.56	0.90
Colletotrichum spp.	0.17[b]	1.56[a]	0.84	1.09
Exserohilum rostratum	4.28[a]	2.15[a]	3.27	2.13
Nigrospora oryzae	17.69[a]	15.88[a]	16.82	4.00
Gloeocercospora sorghi	0.35[b]	1.96[a]	1.12	1.26
Rhizopus sp.	4.10[a]	2.54[a]	3.36	2.17
Curvularia lunata	0.53[b]	4.90[a]	2.61	1.90
Curvularia penniseti	0.00[a]	0.19[a]	0.09	0.37
Curvularia spp.	26.25[a]	22.74[a]	24.57	5.20
Acremonium strictum	1.25[a]	1.96[a]	1.40	1.41
Acremonium sp.	0.89[a]	0.78[a]	0.37	0.73
Penicillium sp.	11.60[b]	31.37[a]	21.02	4.75
Trichothecium sp.	0.71[a]	0.39[a]	0.56	0.90
Epicoccum purpurascens	0.17[a]	0.78[a]	0.74	1.04
Bipolaris spicifera	0.53[a]	0.00[a]	0.28	0.63
Bipolaris sorghicola	0.53[a]	0.39[a]	0.46	0. 82
Bipolaris spp.	1.25[a]	0.58[a]	0.93	1.16
Melanospora zamiae	1.60[a]	0.39[a]	1.02	1.21
Alternaria alternata	0.17[b]	2.94[a]	1.49	1.45
Alternaria longissima	0.71[b]	2.15[a]	1.40	1.41
Alternaria spp.	1.25[a]	0.19[b]	0.74	1.03
Ascochyta sp.	0.17[a]	0.00[a]	0.09	0.37
Botryodiplodia theobromae	1.60[a]	0.19[b]	0.93	1.15
Cercospora sp.	0.17[b]	2.35[a]	1.21	1.31
Rhizoctonia solani	5.00[a]	5.49[a]	5.23	2.68
Myrothecium sp.	0.17[a]	0.00[a]	0.09	0.37
*Diplodia*sp.	0.00[a]	0.58[a]	0.28	0.63
Peronoslerosporasorghi	0.00[a]	0.19[a]	0.09	0.37
Phaeoisariopsis griseola	0.17[a]	0.19[a]	0.18	0.52

Means within the same line followed by the same letter are not significantly different at the level of 5%, according to LSD test.

performing plants, while *C. sublineolum* was associated with non-performing plants. At plant maturity and for each fungus, performing and non-performing plants presented similar levels of infection.

Potential beneficial/pathogenic fungal endophytes isolated from sorghum plants in Burkina Faso

Despite the high occurrence of certain fungal species in performing plants, these plants were well developed and looked healthy in comparison with their/the neighbouring non-performing plants. The genus *Fusarium* with five fungal species was the most common fungus associated with sorghum plants. *F. moniliforme* and *L. sacchari* infecting 28.50 and 24.67% respectively of sorghum plants most highly represented fungal species (Table 1). Our observations allow us to identify only one species of *Exserohilium*: *Exserohilum rostratum*.

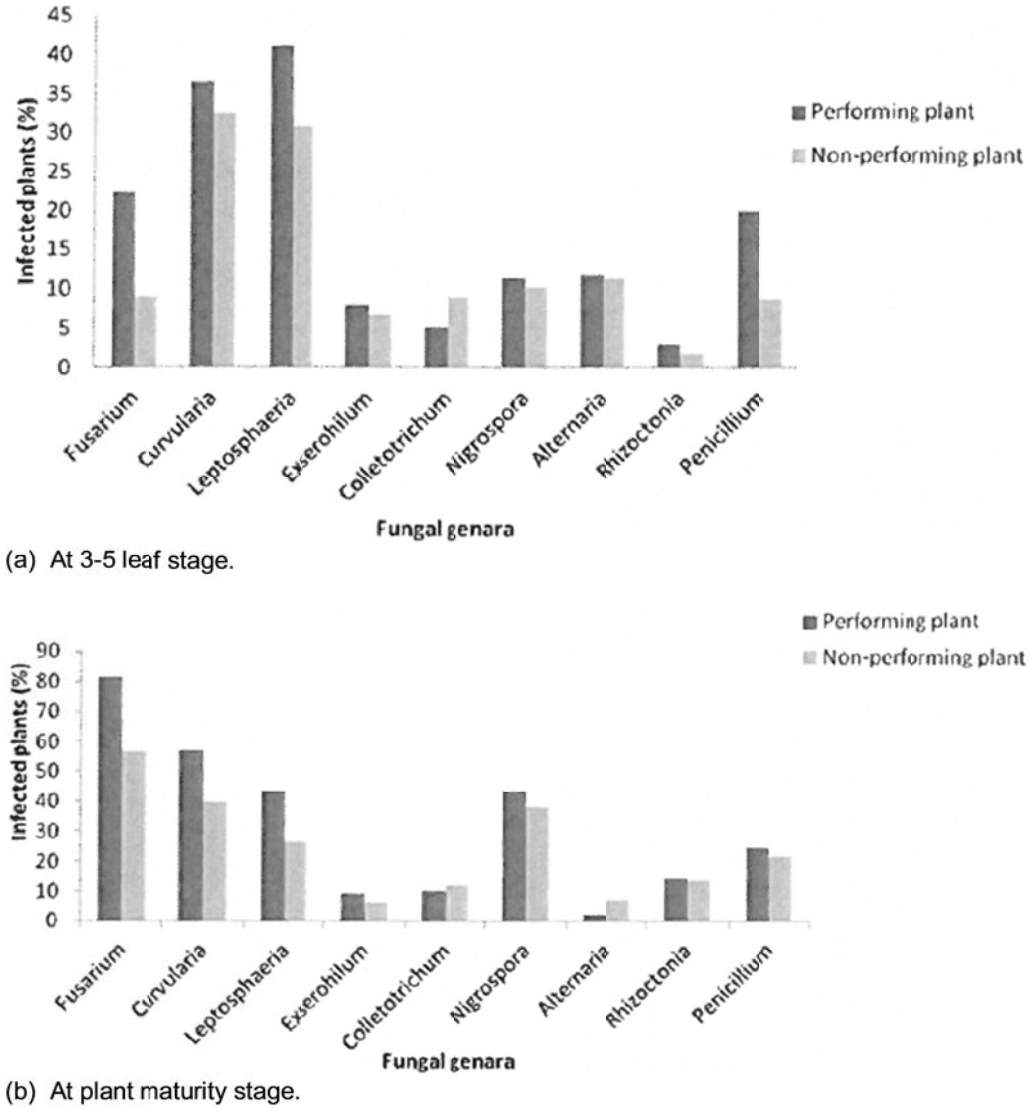

Figure 3. Percentages of performing and non-performing sorghum plants colonized by nine major genera of endophytic fungi in Burkina Faso at 3-5 leaf (a) and maturity (b) stages.

In the Sudanian zone, only *F. moniliforme* significantly colonized a higher number of performing plants than non-performing plants ($p = 0.0085$ and $p = 0.0105$ respectively). In the Sahelian zone, *Fusarium* spp. ($p = 0.0407$), *Nigrospora oryzae* ($p = 0.0407$) and *Penicillium* sp. ($p = 0.0431$) colonization was significantly more abundant in performing plants than in non-performing plants (Table 3). According to the hypothesis that beneficial endophytic fungi could be strongly associated with performing plants, while pathogenic endophytic fungi could be strongly associated with non-performing plants, the fungal species *F. moniliforme*, *Fusarium* spp., *Nigrospora oryzae* and *Penicillium*sp. were considered potential beneficial endophytes (Table 3). In contrast, *C. sublineolum*, occurring in higher numbers of non-performing plants than performing plants in the Sudanian zone ($p = 0.0298$), was considered a potential pathogenic endophyte. Potentially beneficial endophytic *F. moniliforme* mainly occurred in the Sudanian zone, while *Fusarium* spp. and *Penicillium* sp. were most abundant in the Sahelian area. Potentially pathogenic *C. sublineolum* was most encountered in the Sudanian zone.

According to the results presented in Table 3, the potential beneficial/pathogenic endophytes (*F. moniliforme*, *Fusarium* spp., *Nigrospora oryzae*, *C. sublineolum* and *Penicillium* sp.) were isolated from plants at the 3-5 leaf stage, while only *F. moniliforme* was also detected in plants at maturity. At plant maturity and for each fungus, performing and non-performing plants presented similar levels of colonization. These results indicated that plant growth stage might be the best indicator for the isolation of potentially beneficial

Table 2. Occurrence of endophytic fungi within performing and non-performing plants of sorghum at plant growth and plant maturity stages in Burkina Faso.

Fungal species	Plant growth stage		Plant maturity stage	
	Performing plants (%)	Non-performing plants (%)	Performing plants (%)	Non-performing plants (%)
Fusarium moniliforme	14.40*	4.80	12.43	10.40
Fusarium pallidoroseum	2.40	0.80	0.16	0.00
Fusarium equiseti	0.80	0.00	0.48	0.32
Fusarium culmorum	0.00	0.00	0.40	0.08
Fusarium spp.	8.80*	1.60	4.22	4.55
Leptosphaeria sacchari	42.40	32.00	7.15	6.74
Phoma sp.	0.00	0.00	0.00	0.08
Macrophomina phaseolina	0.80	0.80	0.08	0.00
Cladosporium sphaerospermum	3.20	3.20	0.73	0.48
Colletotrichum sublineolum	0.80*	6.40	1.62	1.78
Colletotrichum gloerosporioides	0.80	2.40	0.00	0.16
Colletotrichum spp.	0.80	0.80	0.16	0.40
Exserohilum rostratum	4.00	4.80	1.13	0.81
Nigrospora oryzae	13.60	9.60	6.34	5.93
Gloeocercospora sorghi	0.80	4.00	0.24	0.24
Rhizopus sp.	0.80	0.00	1.46	1.38
Curvularia lunata	4.00	7.20	0.65	0.48
Curvularia penniseti	0.00	0.00	0.08	0.00
Curvularia spp.	28.80	30.40	7.64	7.80
Acremonium strictum	0.00	0.00	0.89	0.32
Acremonium sp.	0.00	0.00	0.16	0.16
Penicillium sp.	20.00*	8.80	8.13	7.23
Trichothecium sp.	0.80	0.00	0.24	0.16
Epicoccum purpurascens	0.80	0.80	0.24	0.24
Bipolaris spicifera	0.00	0.00	0.16	0.08
Bipolaris sorghicola	0.80	0.00	0.16	0.16
Bipolaris spp.	0.80	0.00	0.32	0.40
Melanospora zamiae	0.80	0.00	0.56	0.24
Alternaria alternata	5.60	4.80	0.16	0.08
Alternaria longissima	3.20	4.80	0.24	0.16
Alternaria spp.	0.00	0.00	0.08	0.56
Ascochyta sp.	0.00	0.00	0.24	0.00
Botryodiplodia theobromae	0.00	0.00	0.16	0.65
Cercospora sp.	3.20	0.80	2.27	2.19
Rhizoctonia solani	0.80	0.00	0.00	0.08
Myrothecium sp.	0.00	0.00	0.00	0.24
*Diplodia*sp.	0.00	0.00	0.08	0.00
Peronoslerosporasorghi	0.00	0.00	0.24	0.40
Phaeoisariopsis griseola	0.00	0.80	0.00	0.24

*: At the same sampling stage, % of colonized performing plants by a fungus is significantly different from % of colonized non-performing plants by the same fungus at the level of 5% according to LSD test.

endophytic fungi in sorghum plants.

Localization of endophytic fungi in different parts of the sorghum plant

Fungal isolation from plant leaf, stem and root material at maturity stage aimed to localize the part of the sorghum plant that would be useful for isolating endophytic fungi. The results of the present study indicated that eight fungal species (*Fusarium* spp., *F. culmorum*, *N. oryzae*, *Rhizopus* sp., *Melanospora zamiae*, *Alternaria* spp., *Cercospora* sp. and *Rhizoctonia solani)* were mainly

Table 3. Distribution of endophytic fungal species and their association with sorghum plant performance in two agro-ecological zones of Burkina Faso in 2009.

Fungal species	At 3-5 leaf stage				At plant maturity			
	Sudanian zone		Sahelian zone		Sudanian zone		Sahelian zone	
	P	NP	P	NP	P	NP	P	NP
Fusarium moniliforme	15.29*	3.52	12.50	7.50	9.80*	5.88	14.30	13.61
Fusarium pallidoroseum	2.35	1.17	2.50	0.00	0.19	0.00	0.13	0.00
Fusarium equiseti	1.17	0.00	0.00	0.00	0.00	0.00	0.83	0.55
Fusarium culmorum	0.00	0.00	0.00	0.00	0.39	0.00	0.41	0.13
Fusarium spp.	8.23	2.35	10.00*	0.00	3.13	3.52	5.00	5.27
Leptosphaeria sacchari	58.82	44.70	7.50	5.00	8.43	6.47	6.25	6.94
Phoma sp.	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.13
Macrophomina phaseolina	1.17	1.17	0.00	0.00	0.00	0.00	0.13	0.00
Cladosporium sphaerospermum	2.35	4.70	5.00	2.50	1.56	0.78	0.13	0.27
Colletotrichum sublineolum	1.17*	8.23	0.00	2.50	3.52	3.52	0.27	0.55
Colletotrichum gloerosporioides	1.17	3.52	0.00	0.00	0.00	0.19	0.00	0.13
Colletotrichum spp.	1.17	1.17	0.00	0.00	0.39	0.78	0.00	0.13
Exserohilum rostratum	4.70	4.70	2.50	5.00	0.39	0.19	1.66	1.25
Nigrospora oryzae	15.29	14.11	10.00*	0.00	5.88	5.09	6.66	6.52
Gloeocercospora sorghi	1.17	5.88	0.00	0.00	0.39	0.39	0.13	0.13
Rhizopus sp.	1.17	0.00	0.00	0.00	1.37	0.98	1.52	1.66
Curvularia lunata	5.88	9.41	0.00	2.50	1.37	0.57	0.13	0.13
Curvularia penniseti	0.00	0.00	0.00	0.00	0.19	0.00	0.00	0.00
Curvularia spp.	36.47	40.00	12.50	7.50	4.90	5.09	9.58	9.72
Acremonium strictum	0.00	0.00	0.00	0.00	1.37	0.58	0.55	0.13
Acremonium sp.	0.00	0.00	0.00	0.00	0.39	0.39	0.00	0.00
Penicillium sp.	20.00	10.58	20.00*	5.00	13.92	12.35	4.02	3.61
Trichothecium sp.	1.17	0.00	0.00	0.00	0.00	0.19	0.41	0.13
Epicoccum purpurascens	1.17	0.00	0.00	2.50	0.19	0.39	0.27	0.13
Bipolaris spicifera	0.00	0.00	0.00	0.00	0.00	0.00	0.27	0.13
Bipolaris sorghicola	1.17	0.00	0.00	0.00	0.19	0.00	0.13	0.27
Bipolaris spp.	1.17	0.00	0.00	0.00	0.39	0.00	0.27	0.69
Melanospora zamiae	1.17	0.00	0.00	0.00	0.19	0.00	0.83	0.41
Alternaria alternata	8.23	7.05	0.00	0.00	0.19	0.19	0.13	0.00
Alternaria longissima	4.70	7.05	0.00	0.00	0.19	0.00	0.27	0.27
Alternaria spp.	0.00	0.00	0.00	0.00	0.00	0.19	0.13	0.83
Ascochyta sp.	0.00	0.00	0.00	0.00	0.00	0.00	0.41	0.00
Botryodiplodia theobromae	0.00	0.00	0.00	0.00	0.00	0.19	0.27	0.97
Cercospora sp.	4.70	1.17	0.00	0.00	0.39	0.98	0.13	0.00
Rhizoctonia solani	1.17	0.00	0.00	0.00	2.94	2.35	1.08	2.08
Myrothecium sp.	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.13
*Diplodia*sp.	0.00	0.00	0.00	0.00	0.00	0.58	0.00	0.00
Peronoslerosporasorghi	0.00	0.00	0.00	0.00	0.00	0.19	0.00	0.00
Phaeoisariopsis griseola	0.00	1.17	0.00	0.00	0.00	0.00	0.00	0.41

P: Performing plant; NP: non-performing plant. *: From the same zone and at the same sampling stage, % of colonized performing plants by a fungus is significantly different from % of colonized non-performing plants by the same fungus at the level of 5% according to LSD test.

encountered in sorghum roots, while four species (*L. sacchari*, *G. sorghi*, *Acremonium* sp. and *Bipolaris* spp.) were most commonly isolated from the sorghum leaf (Table 4). The following fungi were easily isolated simultaneously from two different parts of the plant: *F. equiseti*, *E. rostratum*, *Curvularia* spp. and *A. longissima* were mainly isolated from leaf and root; *C. sublineolum* and *Penicillium* sp. from leaf and stem and *F. moniliforme* and *Trichothecium* sp. from stem and root. The remaining fungi were invariably encountered in sorghum leaf, stem

Table 4. Distribution of endophytic fungi in different parts (leaf, stem and root) of sorghum plant at maturity stage.

Fungal species	Colonized plants by fungi (%)		
	Leaf	Stem	Root
Fusarium moniliforme	27.14b	38.57a	37.30a
Fusarium pallidoroseum	0.71a	0.00a	0.00a
Fusarium equiseti	2.50a	0.00b	1.15ab
Fusarium culmorum	0.00b	0.00b	2.30a
Fusarium spp.	12.50b	8.57b	18.84a
Leptosphaeria sacchari	33.21a	6.42c	23.07b
Phoma sp.	0.35a	0.00a	0.00a
Macrophomina phaseolina	0.35a	0.00a	0.00a
Cladosporium sphaerospermum	2.14a	2.50a	0.76a
Colletotrichumsublineolum	8.21a	5.00ab	1.92b
Colletotrichum gloeosporioides	0.71a	0.00a	0.00a
Colletotrichum spp.	1.42a	1.07a	0.00a
Exserohilum rostratum	3.57a	0.35b	5.00a
Nigrospora oryzae	20.71b	6.07c	29.23a
Gloeocercospora sorghi	2.14a	0.00b	0.00b
Rhizopus sp.	3.57b	1.78b	7.69a
Curvularia lunata	1.78a	0.07a	2.30a
Curvularia penniseti	0.00a	0.35a	0.00a
Curvularia spp.	25.35a	13.57b	31.15a
Acremonium strictum	2.50a	1.42a	1.53a
Acremonium sp.	1.42a	0.00b	0.00b
Penicillium sp.	25.00a	26.78a	16.92b
Trichothecium sp.	0.00b	0.35ab	1.53a
Epicoccum purpurascens	0.35a	0.35a	1.53a
Bipolaris spicifera	0.35a	0.00a	0.76a
Bipolaris sorghicola	0.35a	0.00a	1.15a
Bipolaris spp.	2.85a	0.00b	0.38b
Melanospora zamiae	0.35b	0.35b	3.07a
Alternaria alternate	0.35a	0.00a	0.78a
Alternaria longissima	0.35ab	0.00b	1.53a
Alternaria spp.	0.35b	0.35b	2.30a
Ascochyta sp.	0.00a	0,35a	0.00a
Botryodiplodia theobromae	0.00b	2.50a	1.15ab
Cercospora sp.	0.35b	0.35b	2.30a
Rhizoctonia solani	6.78b	2.50c	11.15a
Myrothecium sp.	0.00a	0.00a	0.38a
Diplodia sp.	0.71a	0.35a	0.00a
Peronoslerospora sorghi	0.35a	0.00a	0.00a
Phaeoisariopsis griseola	0.35a	0.00a	0.00a

Means within the same line followed by the same letter are not significantly different at 5% level, using the LSD test.

and root at low levels of infection.

DISCUSSION

With the objective of exploring potential endophytic fungi for control of fungal pathogens in sorghum, classical endophyte isolation and morphological identification methods were employed in order to identify all culturable fungi present in leaf, stem and root tissues of sorghum collected from local farmers fields in two agro-ecological zones in Burkina Faso. Endophytic fungi isolated from

these different tissue types were compared in order to detect tissue-specific differences in the communities. A total of 39 fungal species were identified representing 25 distinct genera with the most prevalent isolates being representatives of the *Fusarium*, *Leptosphaeria*, *Curvularia* and *Penicillium* genera. In the majority of cases, it was possible to identify fungal isolates to species level based on morphological characteristics and the use of several taxonomic keys. However, in some situations, it was not possible to identify isolates with certainty beyond genus level. This is particularly relevant for isolates belonging to the *Curvularia*, *Fusarium*, *Bipolaris*, *Colletotrichum* and *Alternaria* genera, which appeared quite frequently. Several unidentified fungal species belonging to *Trichothecium*, *Penicillium*, *Rhizopus*, *Cercospora* and *Diplodia* genera were also isolated.

These findings are largely in agreement with other sorghum-related studies in that several fungal species isolated were already known to be pathogens of sorghum (Zida et al., 2008). Sorghum grain mold most likely occurs due to a combination of *Curvularia lunata*, members of the *F. moniliforme* complex, *Alternaria* spp., *Bipolaria* spp., *Cladosporium* spp. or *Phoma* spp. *L. sacchari*, *C. sublineolum*, *F. moniliforme* and other fungi isolated during this study are known to occur as pathogens of sorghum (ICRISAT, 1980). Interestingly, these fungi were isolated from performing plants, appearing healthy and showing no obvious symptoms of disease. This could be tentatively interpreted as a pathogen suppression effect as a result of the presence of other competing organisms within the plants, or could more specifically be the result of *in situ* pathogen suppression by a fungal endophyte, as has already been described. In previous studies, *Fusarium* spp. has been found in plants of maize, sorghum and soybean without causing symptoms (Leslie et al., 1990) and also *L. sacchari* (as *Phoma sorghina*) has been reported as an endophyte in rice (Fisher and Petrini, 1992).

Future investigations will be needed in order to address these hypotheses. For example, *Fusarium verticillioides* has been described as a pathogen of sorghum; this fungus has also been shown to act endophytically and to reduce the severity of corn smut caused by *Ustilago maydis* on maize following co-inoculation of endophyte and fungal spore suspensions in greenhouse experiments (Lee et al., 2009). Despite the fact that these experiments took place under greenhouse conditions, one cannot rule out the possibility of a pathogen suppression effect by *F. verticilliodes* in the field. *F. moniliforme* is known to exist as an endophyte and a facultative pathogen transmitting both vertically as laterally (Bacon et al., 2001). It is also significant that *F. moniliniforme* is known to produce fumonisin mycotoxins in sorghum in addition to being a well-known pathogen causing head mold (Shetty and Bhat, 1997).

L. sacchari, isolated during this study, is a ubiquitous and common fungus in the tropics and subtropics, causing diseases of cereals and other *Gramineae* and forage crops (White and Morganjones, 1983). *L. sacchari* is also known to cause leaf spots of minor importance in a variety of hosts including sorghum and maize and leads to seedling loss in sorghum through pre- and post-emergence death (Zida et al., 2008). Furthermore, it has recently been found as a pathogen on wheat leaves in Argentina (Perello and Moreno, 2005). The isolation of *L. sacchari* from leaves, stems and roots in this study confirms that this fungus may exist as a pathogen of sorghum in Burkina Faso. However, since most of the plants collected during this study were apparently healthy, with no visible symptoms of disease, it is possible that *L. sacchari* was not in fact acting as a pathogen in these plants. A potential correlation between *L. sacchari* and *Curvularia* is particularly interesting owing to the recent observation that *Curvularia* species were among the endophytes with the greatest ability to significantly reduce the Black Pod Rot caused by *Phytophthora palmivora* in cocoa tree pods in Brazil (Hanada et al., 2010). One could speculate that the correlation between *Curvularia* spp. and *L. sacchari* was representative of an association between these two fungi whereby both are found simultaneously in plants, but the presence of *Curvularia* prevents development of disease by *L. sacchari*. It cannot be excluded that the correlations and effects observed in this study might be in part influenced by the presence of non-culturable fungi within the sorghum tissues. The endophyte isolation method employed (Petrini, 1986) relies on the growth of fungi which can be readily cultured on laboratory media (PDA), and therefore it does not provide any information on those fungi which might not be amenable to laboratory culture conditions. Future studies could also assess the presence of these fungi by employing DNA sequencing technologies. Nevertheless, the methods employed here have yielded a considerable number of potentially beneficial endophytes and interesting observations.

In conclusion, several studies have indicated a positive effect of fungal endophytes on pathogen suppression (Arnold et al., 2003; Hanada et al., 2010; Shittu et al., 2009). Other studies have reported cases in which endophytes have no effect on fungal infection. For example, *Neotyphodium coenophialum* presence was shown to have no influence on the severity of stem rust caused by *Puccinia graminis* in tall fescue seedlings. Studies indicated that endophytes may only be beneficial to plants under certain environmental conditions (Wali et al., 2006; Welty et al., 1991). Clearly, this is a complex area, and to our knowledge, the work presented here is the first thorough report concerning endophyte isolation from sorghum plants, representing a starting point for investigation into endophytic potential within sorghum. Investigation into molecular identification and pathogenicity tests of the isolated endophytic fungi, the effects of specific fungi on sorghum health as well as screening of

isolates with the potential to increase the stress tolerance will be the topic in future studies.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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