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Genetic diversity of *Fusarium* endophytes strains from sorghum (*Sorghum bicolor* L.) tissues in Burkina Faso

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The diversity and genetic differentiation of populations of *Fusarium* species associated with sorghum fields, both endophytes obtained from sorghum performing and non performing plants and isolates obtained from two sampling periods were investigated. *Fusarium* specific Internal Transcribed Spacer 2 (FITS2) primers set were used to assess genetic variability of 32 isolates from susceptible *Fusarium* spp. endophytes from *Sorghum* tissues. *Fusarium thapsinum* (*Gibberella thapsina*) with 68.75% of the isolates constituted the majority of *Fusarium* spp. isolated in performing plants. *Gibberella thapsina* species identified are described as non-pathogenic and associated to performing plant of sorghum. Previously, some species of *Fusarium thapsinum* have been recognized as pathogenic and responsible for yield losses in several cereal crops including *Sorghum bicolor* produced in Burkina Faso. The other *Fusarium* spp. identified in this study including *Fusarium subglutinans*, *Fusarium chlamydosporum*, *Gibberella intermedia*, *Fusarium dlaminii*, *Fusarium oxysporum*, *Fusarium proliferatum*, and *Fusarium* spp. An additional unknown fungi species were also identified. A diverse population of 10 sequence types was found, although 8 sequence types represented nearly two-thirds of the isolates studied. The sequence types were placed in different phylogenetic clades within *Fusarium* spp., and endophytic isolates were not monophyletic. Phylogenetic analysis from Neighbor-Joining/UnWeighted Neighbor-Joining showed a high genetic relationship among these 32 isolates of *Fusarium* spp. and high variation in FITS sequence of them. The use of specific phylomarker of the genus *Fusarium* allowed to identify the endophytic species of this genus and to establish the phylogenetic relationships between the endophytic species of *Fusarium*. The phylogenetic analysis revealed three groups of the fungi. However, no relationship between these groups and the geographical origins of these fungi has been established.

Key words: *Fusarium thapsinum,* endophyte, FITS2 marker, sorghum.

INTRODUCTION

Sorghum (*Sorghum bicolor*) is the fith most important grain crop in the world and the main cereal crops grown in sub-Saharan Africa in terms of cultivated area, production and consumption (FAOSTAT, 2015). In Burkina Faso, sorghum is the main staple crop in terms of annual production, which is grown for human food

nutrition. Sorghum production is subject to abiotic stresses including drought, and various biotic agents such as the soilborne and seedborne fungal diseases which frequently lead to significant crop yield and grain density losses (Katilé et al., 2010). One of the major diseases of sorghum is grain mould. The disease is caused by several fungal genera, including *Fusarium*, *Leptosphaeria*, *Cochliobolus* and *Cladosporium* (Pak et al., 2016). These fungi are capable of producing mycotoxins in grains which are harmful for human and animal consumption (Agriopoulou et al., 2020). *Fusarium moniliforme* (*Fusarium thapsinum*) is one of the most important fungal species that colonize sorghum plant tissues and are mostly considered as pathogens. Some species of *F. moniliforme* isolated from farmer's fields were associated with sorghum plant performance under drought conditions and may be a potential beneficial endophyte. The term "endophyte," originally introduced by De Bary (1866), refers to any organisms occurring within plant tissues, distinct from the epiphytes that live on plant surfaces. Carroll (1986) defines endophytes as mutualists, those fungi that colonize aerial parts of living plant tissues and do not cause symptoms of disease. Therefore, latent pathogens known to live symptomlessly inside the host tissues and organisms that have an epiphytic phase in their life cycle are also endophytes (Schulz and Boyle, 2005, 2006). Endophytes are thought to play multiple physiological and ecological roles in the mutualistic association with their host plants (Ilis et al., 2017). These symbiotic associations are characterized by the early formation of particular of organs and new tissues for the signaling and nutrient communications between plants and microorganisms (Hiruma et al., 2016; Zipfel and Oldroyd, 2017). Subsequently, considerable evidence indicated endophytic associations to be important for the plant immune system (Soliman et al., 2015), disease suppression (Terhonen et al., 2016), nutrient acquisition (Hiruma et al., 2016), plant fitness (Khare et al., 2018) and tolerance to abiotic stresses (Chagas et al., 2018; Shahzad et al., 2017; Silva, 2017). Many endophytes are known to be an important source of secondary metabolites and plant hormones (Hardoim et al., 2015; Muria-Gonzalez et al., 2015; Teimoori-Boghsani et al., 2020) and have the potential to synthesize various bioactive metabolites that may be used as therapeutic agents against numerous diseases (Aharwal et al., 2016; Duan et al., 2019).

Morphological identification of *Fusarium* endophytic species was previously performed and several *Fusarium* spp. Including *F. moniliforme* (*Gibberella thapsina*), *Fusarium subglutinans*, *Fusarium chlamydosporum*, *Fusarium proliferatum*, *Fusarium oxysporum*, and *Fusarium solani* were identified (Bacon et al., 2001; Demers et al., 2015). Both morphological and molecular identifications are essential for elucidating the fungal species of fungus and establishing genetic relationships within species (Laura et al., 2010). Internal transcribed spacer (ITS) markers are successfully used for characterization of molecular or genetic diversity of many organisms including plants, fungi, and bacteria (Cros et al., 1993). Some ITS markers notably ITS2 are used as phylomarkers for detection of intra and interspecific relationships within populations (Banerjee et al., 2007; Lei et al., 2012) and for validation of species status (Dabert, 2006). The focus of the study is highly relevant as a follow up on our previous finding that many *Fusarium* spp. was significantly associated with well growing young plants of sorghum in Burkina Faso (Zida et al., 2014). This study actually identifies the benefit of *Fusarium* endophyte species associated in performing plant used as specific PCR primer set of *Fusarium* spp. by amplification of the ITS2 region. The research also established phylogenetic relationship of the 32 endophytic *Fusarium* spp. identified.

MATERIALS AND METHODS

Site of sorghum tissues collection in Burkina fields

Sorghum plant tissues were collected in farmer's fields in Burkina Faso. A total of 9 sites under two agro-ecological zones; the sahelian zone with an average annual precipitation ranging from 300 to 600 mm and the north sudanian zone (Soudano-Sahelian) with 600 to 900 mm precipitation were considered (Figure 1). In each site, 5 fields arbitrary chosen were investigated for sorghum plant tissue (leaves, stems and roots) sampling. Sorghum tissues were collected during two sampling periods, first sampling (S1, during the three leaves stage) and harvested sampling collected at maturity (S2). Two types of plants divided into performing plant (PP) and non-performing plants (NP) were collected according to their vigor and behavior to drought in farmers' fields. For each field, 10 plants, 5 performing plants and 5 non-performing plants were considered for tissues sampling.

Fungal endophyte isolation and morphological identification

Sorghum endophytic fungi were isolated according to the protocols described by Petrini (1992). Sorghum leaf, stem and root tissues were cut into 12 to 15 mm pieces. The fragments were surface sterilized in 70% ethanol (v/v) for 1 min, immersed in sodium hypochlorite (NaOCl) 3% for 4 min and, then in 70% ethanol for 30 s and finally washed three times successively in sterilized distilled water. The growth media, potato dextrose agar (PDA) was used for fungal isolation. After drying under the laminar flow hood, pieces were transferred to Petri dishes containing autoclaved PDA previously aseptically supplemented with streptomycin in order to suppress bacterial growth. A total of 450 sorghum plants were investigated. Sorghum fragments (leaf, stem, and root) were plated in Petri dishes, 12 from each of the 450 investigated plants.

Plates were incubated in darkness for 9 days at 28°C. Each

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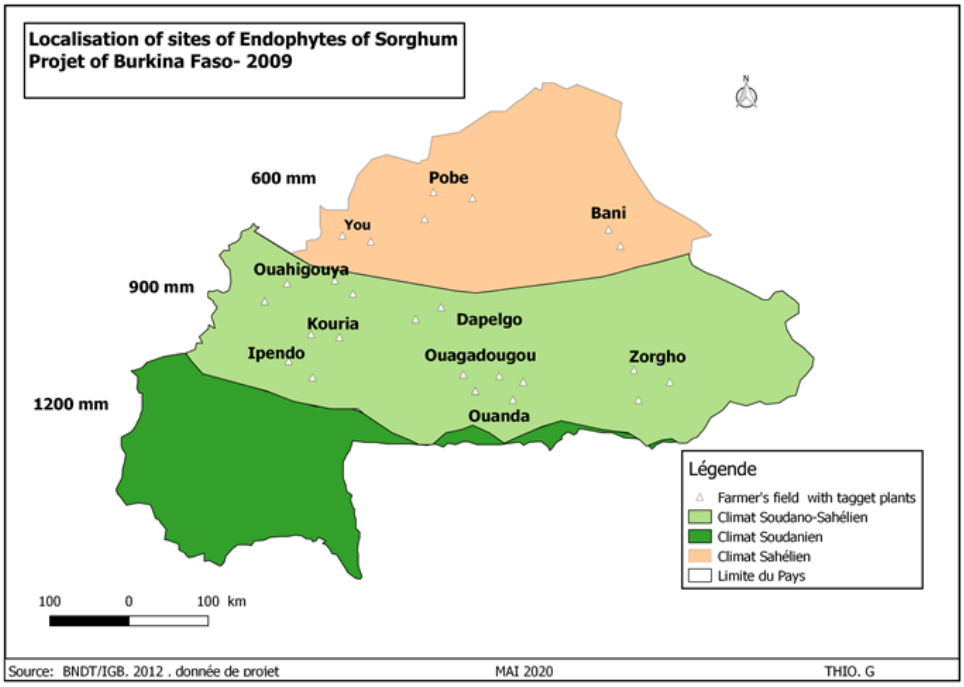

Figure 1. Map of Burkina Faso showing sampling sites and agro-ecological zones.

isolated fungus was placed into a new PDA culture without streptomycin and incubated at 24°C for 7 days under UV light for 12 h and darkness for 12 h. The identification of fungi was based on macroscopic and microscopic structures observed under the stereomicroscope and compared to compound and/or fungi identification manual published descriptions (Marthur and Kongsdal, 2003). Isolates of each fungal species identified were transferred to Eppendorf tubes containing 2 ml of sterile distilled water and stored at -20°C. The fungal isolates were brought to the Danish Seed Health Centre (DSHC, Denmark) for molecular identification, PCR and sequencing.

Isolates (32) of susceptible *F. moniliforme* were used for molecular characterization (Table 1). 200 µl of each isolate were retransferred to new PDA medium aseptically supplemented with streptomycin antibiotic and incubated at 24°C on a 12-h light/dark cycle for 5 to 7 days. One 5 mm diameter disk of each isolate were sampled and transferred into 50 mL potato dextrose broth (PDB) liquid medium. After 3 to 5 days of growth on orbital shaker, mycelia from each isolate were harvested by vacuum filtration and lyophilized until dry.

Molecular identification: DNA extraction, amplification, sequencing and data analysis

Mycelia from each isolate were ground in nitrogen liquid using mortar and pestle. DNA of each susceptible *F. moniliforme* was extracted with the Qiagen DNeasy Plant Mini Kit. To characterize *Fusarium* strains, *Fusarium*-ITS (FITS) primers, FITS-F2 (5**'**-ACCAGCGGAGGGATCATTAC-3') and FITS-R2 (5'-CTGGGGCAATCCCTGTTGGTT-3') provided by DSHC were used.

PCR was performed using a Master Cycler Gradient thermocycler. The PCR mixture total volume of 21.3 µl contained 1 µl DNA sample (10-100 ng DNA), 18 µl Buffer mix (860 µl MilliQ water, 100 µl Buffer 10X, 20 µl $MgCl_2$ 100 mM, 20 µl DNTP 10 nM), 1 µl of each FITS primer (10 pmol/µl), 0.3 µl Taq DNA polymerase (2.5 U/µl, Fermentas, EU). The PCR condition include 94°C for 5 mn for initial denaturation, followed by 34 cycle of denaturation at 94°C for 1 min, primer annealing at 61°C for 1 min and extension at 72°C for 1 min. The final extension was set at 72°C for 10 min.

Gel electrophoresis and bands analysis

PCR products (5 µl) were analyzed on 0.7% agarose gel in Tris/Borate/EDTA electrophoresis buffer and stained with ethidium bromide solution (14 µl for 1 L of buffer). DNA ladder was used as molecular weight markers to determine the size of bands. After approximately 45 min at 100 mV, the gel was visualized and documented using the UTP-Bio Doc system. Data were analyzed by comparing FITS-2 profiles in terms of presence or absence of each reproducible DNA fragment.

Positive PCR products amplified by the FITS-F2 primers were purified by and desalted using QIAquick PCR purification kit (Qiagen). PCR products were cloned and sequenced using the Eurofins MWG Operon's sequencing service (Eurofins Genomics LLC). The sequences corresponding to the 32 *Fusarium* spp. isolates were processed by the BLAST program integrated into the BioEdit Alignment software for the molecular identification.

Phylogenetic reconstruction

Sequence alignment was carried out using the ClustalW Multiple alignment and a phylogenetic tree was constructed using DARwin6.0.4 software (Thompson et al., 1994).

RESULTS

PCR products analysis

Electrophoresis and analysis of amplified PCR products

Table 1. Identity and origin of susceptible *Fusarium* spp. strains used in this study.

Accession number	Isolates No.	Tissues	Plant type	Localities	Agroecological zone
1082/48.801	1	Leaf.2	PP*	You	Sahelian
1137/48.792	2	Root	PP	Bani	Sahelian
1136/48.791	3	Leaf.2	PP	Bani	Sahelian
1104/48.808	4	Leaf.2	PP	Ouahigouya	Sahelian
1170/48.828	5	Leaf.1	PP	Ouanda	North soudanian
1090/48.805	6	Leaf.2	PP	You	Sahelian
1153/48.823	8	Leaf.1	PP	Ipendo	North soudanian
1087/48.804	9	Root	PP	You	Sahelian
1092/48.796	10	Root	PP	Pobe Mengao	Sahelian
1143/48.795	11	Stem	PP	Bani	Sahelian
1066/48.827	12	Leaf.2	PP	Ouanda	North soudanian
1096/48.798	13	Root	PP	Pobe Mengao	Sahelian
1139/48.793	16	Stem	PP	Bani	Sahelian
1090/48.805	18	Stem	PP	You	Sahelian
1122/48.813	20	Stem	PP	Kouria	North soudanian
1171/48.829	23	Leaf.1	PP	Zorgho	North soudanian
1176/48.833	25	Leaf.1	PP	Zorgho	North soudanian
1153/48.823	27	Leaf.1	PP	Ipendo	North soudanian
1069/48.825	28	Leaf.1	PP	Dapelgo	North soudanian
1117/48.812	30	Leaf.1	PP	Kouria	North soudanian
1149/48.821	32	Leaf.1	PP	Ipendo	North soudanian
1176/48.833	33	Leaf.1	PP	Zorgho	North soudanian
1153/48.823	34	Leaf.1	NP**	Ipendo	North soudanian
1082/48.801	35	Leaf.2	NP	You	Sahelian
1086/48.803	36	Leaf.2	NP	You	Sahelian
1082/48.801	37	Leaf.2	NP	You	Sahelian
1122/48.813	38	Root	NP	Kouria	North soudanian
1148/48.819	39	Root	NP	Ipendo	North soudanian
1139/48.793	40	Stem	NP	Bani	Sahelian
1122/48.813	41	Root	PP	Kouria	North soudanian
1103/48.807	43	Stem	PP	Ouahigouya	Sahelian
1092/48.796	092	Leaf.1	PP	Pobe Mengao	Sahelian

*Performing plant; **Non-performing plant.

revealed the presence of a polymorphic band corresponding to the 28S rDNA gene. The different sizes indicated the presence of 2 groups of fungi. The first group corresponds to the *G. thapsina, F. chlamydosporum, F. oxysporum, Fusarium proliferatum, Fusarium dlaminii* and *Gibberella* species located at 400 bp. The second group with band size of approximately 380 bp corresponds to the *F. subglutinans* species (Figures 2 and 3).

The species-specific sequence analysis and clone's identification

The present study aims to characterize thirty two isolates of susceptible *Fusarium* spp. species by using molecular approaches, identify the specific sequence of FITS-2 regions from isolates as markers and to establish the relationship between these fungal strains. Majority of the isolates (68.75%) were identified as *G. thapsina*. Only, 12.5% of the isolates were identified as *F. subglutinans.* The six other species identified have each one isolate. Eight specific sequences corresponding to the 8 *Fusarium* spp. identified have been reported in this work. These *Fusarium* spp. were benefit or pathogens to sorghum plant. FITS2 sequences length varies from 138 to 319 bp in *Fusarium* spp. and maximum length being 138 bp and minimum of 319 bp for *Fusarium intermedia* and *G. thapsina*, respectively. Table 2 shows different clone's specific sequences of *Fusarium* species. Twenty five (25) clones of *Fusarium* have been associated to sorghum performing plants and considered as potential endophytes.

All of the 22 *G. thapsina* species have a common

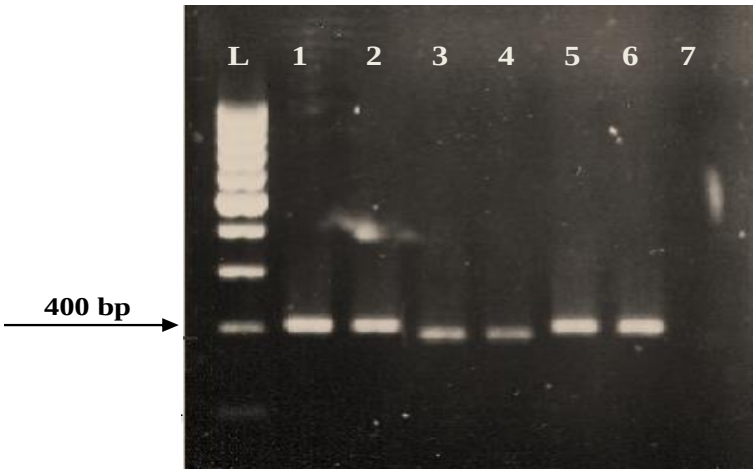

Figure 2. PCR patterns of six (6) endophytic fungal of 28S rDNA, ITS2 primers. Lane L: DNA Ladder; 1: 1 (*Gibberella thapsina*); 2: 2 (*Gibberella thapsina*); 3: 3 (*Fusarium subglutinans*); 4: 4 (*Fusarium subglutinans*); 5: 5 (*Gibberella thapsina*); 6: 6 (*Gibberella thapsina*); 7: control (Sterile distilled water).

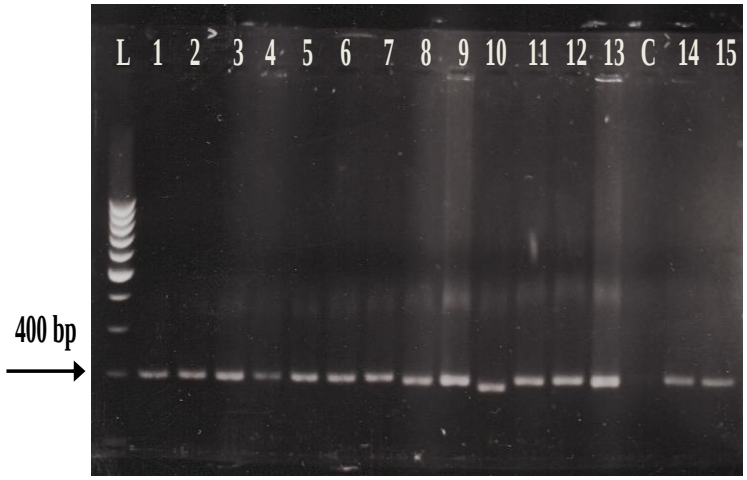

Figure 3. PCR patterns of sixteen (16) *Fusarium* endophytic fungal of 28S rDNA, FITS primes. Lane 1=L: DNA Ladder; 1: 25 (*Gibberella thapsina, G.t*); 2: 27 (*G. intermedia*); 3: 28 (*Gt*); 4: 30 (*Gt*); 5: 32(*Gt*); 6: 33 (*Gt*); 7: 1 (*Gt*); 8: 10 (*Gt*); 9: 12 (*Gt*); 10: 13 (*Fusarium subglutinans*); 11:37 (*Gt*); 12: 41 (*F. dlamani*); 13: 20 (*Gt*); C: control (Sterile distilled water); 15: 36 (*Gt*); 16: 39 (*Gt*).

specific sequence Seq1 in the genome region 1 (Gr1) as described in Table 2. This DNA region Gr1 is a promise for species specific primer designation in *Fusarium* spp. A second genome region (Gr2) distinguished two groups among the 22 *G. thapsina* isolates. The first group with 11 *G. thapsina* isolates has the sequence set GGGGTAC (Seq9) and the others 11 isolates of the second group do not show this sequence (Table 2). Therefore, the results also indicate that the second group of *G. thapsina* (none Seq9) from the first sampling period in leaf tissue (leaf1). The sequence set Seq9 has also been identified in *F. dlaminii* isolate. All of the *F. subglutinans* isolates and *F. chlamydosporum* species are characterized by the sequence set GGGACT (Seq10).

Phylogenetic analysis using DNA sequencing of FITS

Cluster analysis with FITS-2 profiles formed 3 groups at root 63% (Figure 4). Groups 1 and 2 are homogenious

Table 2. Molecular identification of 32 strains of *Fusarium* spp.

Clones No.	NCBI species identification	Plant type	Variation in genome region 1 (Gr1)	Variation in Gr2
1.FITS	*G. thapsina*	PP*	AAATACAGTGGCGGTCTCG (Seq1)	GGGGATC (Seq9)
2.FITS	*G. thapsina*	PP	Seq1	Seq9
3.FITS	*F. subglutinans*	PP	AAATTGATTGGCGGTCACG (Seq2)	GGGACT (Seq10)
4.FITS	*F. subglutinans*	PP	Seq2	Seq10
5.FITS	*G. thapsina*	PP	Seq1	None (-)
6.FITS	*G. thapsina*	PP	Seq1	Seq9
8.FITS	*F. chlamydosporum*	PP	AAATCGATTGGCGGTCACG (Seq3)	Seq10
9.FITS	*G. thapsina*	PP	Seq1	-
10FITS	*G. thapsina*	PP	Seq1	-
11.FITS	*G. thapsina*	PP	Seq1	Seq9
12.FITS	*G. thapsina*	PP	Seq1	Seq9
13.FITS	*F. subglutinans*	PP	Seq2	Seq10
16.FITS	*G. thapsina*	PP	Seq1	Seq9
18.FITS	*G. thapsina*	PP	Seq1	Seq9
20.FITS	*G. thapsina*	PP	Seq1	Seq9
23.FITS	*G. thapsina*	PP	Seq1	-
25.FITS	*G. thapsina*	PP	Seq1	-
27.FITS	*G. intermedia*	PP	Seq4	-
28.FITS	*G. thapsina*	PP	Seq1	-
30.FITS	*G. thapsina*	PP	Seq1	-
32.FITS	*G. thapsina*	PP	Seq1	-
33.FITS	*G. thapsina*	PP	Seq1	-
34.FITS	*G. thapsina*	NP**	Seq1	-
35.FITS	*G. thapsina*	NP	Seq1	-
36.FITS	*G. thapsina*	NP	Seq1	-
37.FITS	*Gibberella sp.*	NP	AAATAACGTGGAGGTCTCG (Seq5)	-
38.FITS	*F. proliferatum*	NP	A-ATACAGTGGCGGTCTCG (Seq6)	-
39.FITS	*F. oxysporum*	NP	AAATTACAGTGGCGGTCTCG (Seq7)	-
40.FITS	*G. thapsina*	NP	Seq1	Seq9
41.FITS	*F. dlaminii*	PP	AAATCTAGTGGCGGTCTCG (Seq8)	Seq9
43.FITS	*G. thapsina*	PP	Seq1	Seq9
092.FITS	*F. subglutinans*	PP	Seq2	Seq10

*Performing plant; **Non-performing plant; Seq: Specific sequence set.

group and consisting only of the isolates *G. thapsina* species. Group 3 is heterogenious and includes isolates from *G. thapsina, F. subglutinans, F. intermedia, F. oxysporum, F. chlamydosporum, F. proliferatum, F. dlamini*i and *Gibberella* spp. isolate. All of the *Fusarium* spp. analysed are used in this study as closely related and from the common ancestor. Phylogenic analysis and relationship indicate that the four isolates of *F. subglutinans* and *F. chlamydosporum* isolate formed a sub-group and belong to clade 2 (boostrap value at 59%). Intraspecific diversity was observed among the species *G. thapsina* showed the highest level of intraspecific diversity by forming 3 groups (G1-G3).

Analysis of sequence identity matrix reveals a high penalty for closely related sequences. The sequence similarity analysis within *Fusarium* endophytes isolates indicates values ranging from 0.976 to 0.302. Thus, the highest degree of sequence identity was observed, respectively between the strains 12 *t* and 16 *Gt* with 0.976, 12 and 20 *Gt* (0.971), and 12 and 43 *Gt* (0.971). However, the lowest degree of most diversity was observed between strains 27 *F.int*. and 39 *Gt* (0.302). The analysis of sequence identity between *Fusarium sub.* strains ranged from 0888 to 0.932 (Table 3). The results show the high relationship of 13 strains of *Fusarium* spp. among the 32 isolates.

DISCUSSION

The current study provides strong evidence of existence of non pathogenic fungal endophytes in sorghum plant.

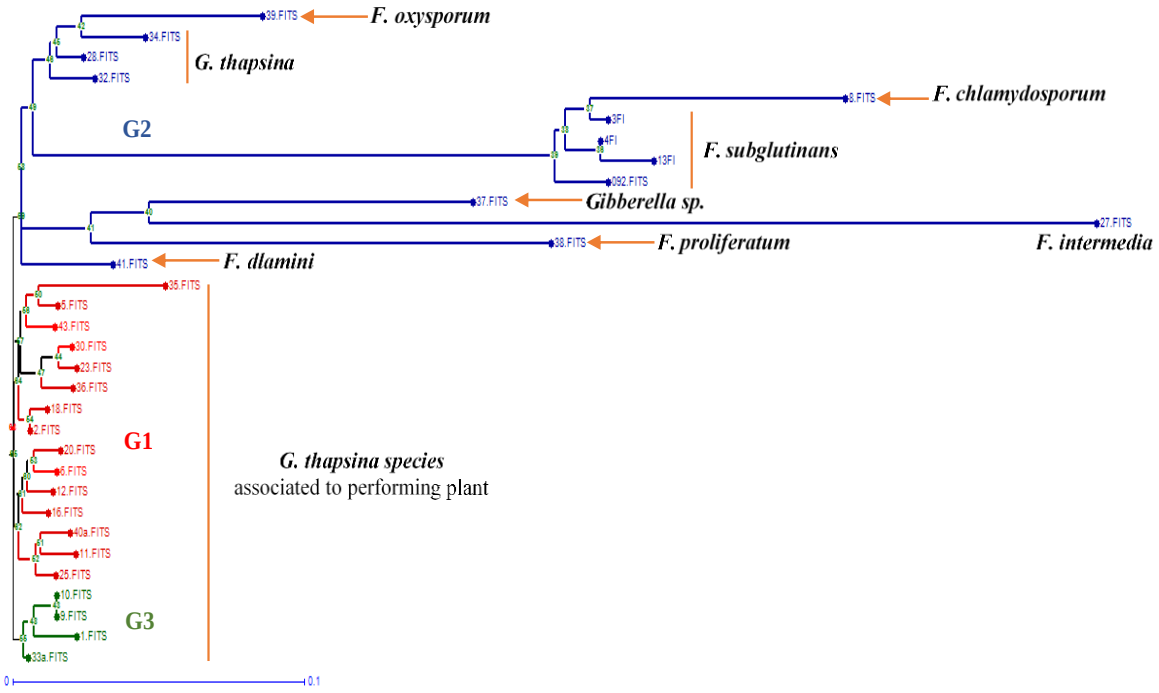

Figure 4. Molecular diversity of 32 isolates of *Fusarium spp*. strains obtained by using Neighbor-joining/UnWeighted Neighbor-Joining. DARwin6.0.4 software. 0.1 distance.

Table 3. Sequence Identity Matrix of 13 strains of *Fusarium* (among a total of 32 strains).

Seq->	1.Gt	2.Gt	3.Fs	4.Fs	5.Gt	6.Gt	8.Fc	9.Gt	10.Gt	11.Gt	12.Gt	13.Fs	92.Fs
1.Gt	ID												
2.Gt	0.948	ID											
3.Fs	0.685	0.689	ID										
4.Fs	0.683	0.721	0.931	ID									
5.Gt	0.905	0.947	0.674	0.705	ID								
6.Gt	0.911	0.953	0.663	0.693	0.937	ID							
8.Fc	0.644	0.651	0.846	0.838	0.633	0.627	ID						
9.Gt	0.906	0.937	0.654	0.683	0.948	0.96	0.614	ID					
10.Gt	0.9	0.92	0.663	0.675	0.904	0.932	0.623	0.926	ID				
11.Gt	0.942	0.959	0.691	0.695	0.92	0.932	0.648	0.91	0.915	ID			
12.Gt	0.965	0.959	0.696	0.693	0.921	0.927	0.67	0.911	0.911	0.959	ID		
13.Fs	0.685	0.679	0.921	0.932	0.664	0.654	0.819	0.655	0.641	0.668	0.674	ID	
92.Fs	0.672	0.668	0.925	0.888	0.653	0.643	0.795	0.645	0.634	0.661	0.666	0.92	ID

Gt= *G. thapsina*, Fs= *F. subglutinans*, Fc= *F. Chlamydosporum*.

Use of fungal endophytes as beneficial bioresource to protect against plant-parasitic has previously been demonstrated (Terhomen et al., 2016; Pavithra et al., 2020). This study reveals differences between *Fusarium* spp. endophytes associated to sequence variability and plant type (performing and non performing plant). *G. thapsina* is known to be a seedling pathogen and cause of stalk rot and grain mold of sorghum (Kelly et al., 2017; Nor et al., 2019). The sequence set seq1 identified is a conserved region in *G. thapsina* rDNA independently to sorghum plant growth period. The sequence set seq9 identified in some *G. thapsina* isolate from sorghum leaves tissue in early plant growth may indicate a vertical transmission of the endophyte within the plant from leaf to grain in farmer's field. Molecular phylogenetic relationships among plant pathogenic and nonpathogenic

Fusarium strains have been studied (Fourie et al., 2011; Imazaki and Kadota, 2015). *F. thapsinum* (*F. moniliforme*) is known to exist as an endophyte and a facultative pathogen transmitting both vertically as laterally (Bacon et al., 2001). *G. thapsina* is also known to produce gibberellin mycotoxin in sorghum (Klittich et al., 1997). In this study, *G. thapsina* (*F. thapsinum*) strains were identified as a major endophyte fungi associated to sorghum performing plants in field condition. The pathogenicity of *G. thapsina* to sorghum has not been tested under field conditions, but some strains can cause lesions in sorghum stalks under greenhouse conditions (Stokholm et al., 2016). In this study, four *F. subglutinans* endophyte with a specific band at 380 bp approximately in electrophoresis gel have been identified. These *F. subglutinans* endophyte formed a sub-group with *F. chlamydosporum* isolate and characterized by the sequence set seq10. This specific sequence were associated to performing plants of sorghum in field condition. The role of *F. subglutinans* as benefit endophyte has been demonstrated (Lee et al., 1995).

Many *Fusarium* endophytes possess antifungal properties that are useful against a number of plant pathogens in different plant system (Shah et al., 2019). Molecular characterization of the endophytic and biological control mechanisms of *Fusarium* has been reported (Imazaki and Kadota, 2015; Zhao et al., 2019). The role of *Fusarium* endophytes in many plant have been described (Ilic et al., 2017). For example, *F. proliferatum*, has been employed to control grapevine downy mildew caused by *Plasmopara viticola* (Bakshi et al., 2001; Mondello et al., 2019). *F. proliferatum* is considered a mycoparasitic, cold-tolerant fungus, capable of controlling the development of *P. viticola* via secretion of extracellular glucanolytic enzymes (Bakshi et al., 2001; Pancher et al., 2012). Endophytic colonization by the fungus *F. oxysporum* can result in increased host resistance to pests and diseases, and greater biomass production (Waweru et al., 2014).

In this study, eight species of *Fusarium* endophytes have been described based on rDNA sequence analyzing and phylogenetic relationship. However, there are few studies that have assessed their effect in the field. Further studies will be necessary to prove the ability of these *Fusarium* endophyte species and the environmental conditions required to actively infect and colonize sorghum and separate them from the saprophytes. Subsequent investigations also have to determine whether mycotoxins are produced in sorghum tissue by the different species because pathogenicity and mycotoxin production of sorghum derived *Fusarium* isolates was already proven (Zida et al., 2014).

Conclusion

The *Fusarium* ITS2 marker provides a powerful tool for studies of intraspecific variation and phylogenies of closely related species of *G. thapsina* endophytes. In this study, thirty two endophytic *Fusarium* spp. isolates were molecular identified. Thus, FITS sequences successfully differentiate the species and the different sizes of the amplified products confirm the presence of a codominant and specific FITS marker. This study reveals different between *F. thapsina* endophytes associated to sequence variability and also the necessity to characterize by molecular approach clones of *Fusarium* spp.

The *Fusarium* specific ITS markers (FITS) can be used for *Fusarium* pathogenic and beneficial endophytic species and associated disease detection. These markers can be used to support traditional identification of fungi or as an alternative approach and to facilitate pathogenicity tests which can be influenced by several biotic and abiotic factors. All of the specific sequences identified could be used for primers design in *Fusarium* endophyte identification.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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