DGfM

ORIGINAL ARTICLE

Effect of climate and phenological stage on fungal endophytes community in *Sorghum bicolor* leaves

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Abstract

Endophytic fungi comprise a group of microorganisms of great diversity, which can be modulated by biotic and abiotic factors. The present study aims to understand how environmental factors, blooming and cultivars change the diversity of the endophytic fungal communities in sorghum leaves in two forest areas in Brazil. Our results showed that *Sorghum bicolor* has a rich and diverse community of endophytic fungi distributed in *Ascomycota*, *Basidiomycota* and *Mucoromycota*, with members of *Dothideomycetes* and *Sordariomycetes* as dominant groups. We found 581 endophytic fungi (filamentous and yeasts) identified in 55 species of 37 genera. A greater diversity and richness of endophytic fungi were found in sorghum leaves from the Atlantic Forest (diversity = $H' = 2.68$ and richness = 46), differing significantly from the Caatinga forest (diversity = $H' = 2.51$ and richness = 25). No difference in richness or diversity was observed between the two crops or between the phenological stages. We detected different species indicator for the two areas, nine taxa were associated with sorghum in the Atlantic Forest (*Colletotrichum* sp. 2, *Epicoccum sorghinum*, *Curvularia* sp., *Colletotrichum* sp. 1, *Fusarium thapsinum*, *Nigrospora oryzae*, *Phyllosticta capitalensis*, *Fusarium oxysporum* and *Amesia nigricolor*) and five taxa were indicators of sorghum in the Caatinga Forest (*Meyerozyma* sp., *Talaromyces pinophilus*, *Metschnikowia* sp., *Rhodosporidiobolus* sp. and *Acremonium pinkertoniae*). One species was an indicator of the cultivar IPA 2502 and no species were related to the cultivar IPA SF15. In relation to pre-blooming and post-blooming stages, two species were considered indicators for the former while six were considered for the latter. We observed that the number of filamentous fungi increases with the post-blooming stage and higher rainfall, while the diversity of endophytic yeasts was influenced by smaller rainfall and pre-blooming stage. Our data improves the understanding of factors that may influence the endophytic fungal community in ecologically and economically important grass species. The endophytic fungal diversity associated with sorghum is important to the mycodiversity estimation and may be useful in studies of biocontrol of pathogens and promotion of crops growth.

Keywords *Sorghum* · Cultivars · Endophytic fungi · Diversity · Tropical forest

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Section Editor: Claus Baessler

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Introduction

Sorghum bicolor (grain sorghum) is the fifth most produced cereal in the world (Venkateswaran et al. 2019). A typical crop of tropical climate presents characteristics of xerophilic plants (Shakoor et al. 2014), and it is well adapted to hot and dry conditions, being mainly cultivated in the arid and semiarid regions of the world (Menz et al. 2002). Brazil is the 9th largest world producer, with 2.8 million tons grown in an area of 0.82 million hectares (Foreign Agricultural Service 2019). Cultivation of sorghum is extremely important in semiarid regions of Asia, Africa and Central America, mainly for human consumption (Shakoor et al. 2014; Proietti et al. 2015). In Brazil, the production of sorghum is destined for animal (forage and sweet sorghum, other sorghum type, not grain sorghum, used also to animal feed) feed and biofuels production (Taleon et al. 2012). The productivity of sorghum grain is susceptible to abiotic factors and attacks by various pathogens such as bacteria, viruses and mainly fungi; fungal contamination constitutes an important restriction for the increase of sorghum production worldwide (Astoreca et al. 2019; Choi et al. 2021; Poudel et al. 2022).

The beneficial symbiotic microbes, such as endophytic fungi, have been increasingly appreciated for the useful impact on plant physiology (Dastogeer 2018). Endophytes can promote plant growth and induce resistance to adverse abiotic and biotic effects, such as drought and nutrient deficiency in semiarid regions, and even the capacity to resist against pathogens, thus having potential to protect agricultural crops (Harman and Uphoff 2019; Quach et al. 2022). Among the endophytic fungal diversity, yeasts represent an important component and share a unique niche along with filamentous fungi (Joubert and Doty 2018). Due to capacity of endophytes act in the promotion of plant growth, these microorganisms are considered potential to applications in agriculture (Joubert and Doty 2018). Studies on fungal endophytes and their interaction with different hosts have recently shown important information about the benefits of this ecological relationship (Giauque and Hawkes 2013; Collinge et al. 2022). However, studies on the community composition of endophytic fungi in sorghum crops are still scarce (Zida et al. 2014; Rodrigues et al. 2018). To the best of our knowledge, no study has simultaneously investigated the contributions of environmental factors, phenological stages and cultivars of sorghum in the establishment of the endophytic fungal community in sorghum leaves.

Brazil has different biomes and among the tropical forests, the Atlantic Forest is considered a region with high biodiversity and endemism; however, it is highly threatened and intensely fragmented (Rodrigues et al. 2009). Another Brazilian Forest threatened by anthropic impacts is the Caatinga (da Silva and Barbosa 2017), a tropical dry forest with high richness and endemic species (Fernandes and Queiroz 2018). Environmental conditions and plant composition in the Atlantic Forest and in the Caatinga can have a significant influence on the endophytic community of plants cultivated in their areas. Several factors, such as temperature, humidity, precipitation, nutritional characteristics of the plant (Pieterse et al. 2018), host species (Saikkonen 2007) and phenological stage (Dalal and Kulkarni 2014) can change the structure of the endophytic fungal community. The understanding of the correlation between the endophytic community and plant growth stages is also considered an important factor in ecological studies of these fungi in plants of agronomic interest (Zida et al. 2014; Schlemper et al. 2018).

Phenological stage of the plants and different cultivars are significant factors that may influence the abundance, diversity and composition of endophytic fungi (da Silva et al. 2016; Schlegel et al. 2018). In order to better understand the structure of the endophytic fungal community in sorghum leaves cultivated in different forests in Brazil, we analysed the diversity and distribution of endophytes and correlated their communities with environmental factors, plant phenological stages and cultivars. Our objective was to understand how these factors change the endophytic fungal communities in sorghum leaves. We focused on three main hypotheses: (I) the distribution of fungal endophytes is modulated by the phenological stages of the plant, (II) the community of endophytic fungi in sorghum is influenced by the change of rainfall and temperature in areas with different climatic conditions and (III) the species of endophytic fungi varies among different cultivars of sorghum.

Material and methods

Study areas

The study was carried out in two sorghum crops located in the Experimental Station of the Agronomic Institute of Pernambuco (IPA) in Serra Talhada and Goiana municipalities, Pernambuco state, Northeast region, Brazil. The two areas studied are about 450 km distant from each other. The Serra Talhada area (7°55′48″S 38°17′18″W) is located in one of the Brazilian tropical dry forest (Caatinga). The climate tropical semiarid (BSh) according to the classification of Köppen (Koppen 1923) characterized by long hot and arid periods. The rainy season begins in November and ends in April. The mean annual temperature is 25.8 °C, mean annual precipitation is 640 mm and the predominant soils are Argisols (Pereira et al. 2017). The Goiana area (7°38′20″S 34°57′22″W) is located in the Atlantic Forest biome. The climate in the region is

Isolation and morphological identification of endophytic fungi

Sorghum leaves were collected in August and October 2014 in the Atlantic Forest domain area (Goiana), and in December 2014 and March 2015 in the Caatinga domain area (Serra Talhada). In each area, the leaves were collected from individuals of two different cultivars of *S. bicolor*, IPA SF15 and IPA 2502. The cultivar IPA SF15 has a high dry matter yield, besides adapting to different climatic and soil situations (Tabosa et al. 2002), and the IPA 2502 has stems rich in fermentable sugars, high production of biomass, resistant to water stress, adapting well to the scarcity of rainfall in the semiarid regions (Durães 2011).

The experimental design consisted of two areas, two sorghum cultivars and two plant phenological stages: before reproductive differentiation (pre-blooming) and after grain differentiation (post-blooming). For each area, 48 random points were delimited, and three individuals of sorghum were chosen at each point, from which nine leaves were collected, totalizing 864 leaves (432 from sorghum growing in the Caatinga forest area and 432 from the Atlantic Forest area). The leaves were washed in tap water, fragmented into 5 mm discs, using a sterile perforator, and under aseptic conditions the fragments were disinfested using 70% alcohol for 30 s and 2% sodium hypochlorite (NaClO) for 120 s. The fragments were then washed twice in sterile distilled water (Araújo et al. 2002; modified). Overall, 1728 leaves discs were placed in Petri dishes containing malt extract agar (MEA) supplemented with chloramphenicol (50 mg L^{-1}), incubated at room temperature (28 $\pm$ 2 °C) and observed daily during 15 days for the fungal isolation. After isolation and purification of endophytes, a previous morphological identification of endophytic fungi was performed using microcultures and macro- and micro-morphological analyses and reproductive structures observation using several mycological literatures (e.g. Ellis 1971, 1976; Sutton 1980; Domsch et al. 1993; Seifert et al. 2011). For endophytic fungi without sporulation in culture, it was necessary to use different culture media to stimulate the fungal sporulation, such as potato-dextrose agar (PDA), wheat germ agar, oatmeal agar and V8-juice agar. For the relative frequency, the number of isolates of each species was divided by the total number of isolates (Bezerra et al. 2013).

DNA extraction, PCR and sequencing

After previous morphological identification, at least one representative of each fungal species was used to perform DNA extraction. All filamentous isolates that did not sporulated in culture were also included to the molecular study, as well as all yeasts. The fungal biomass was obtained from cultures grown on MEA after 7 days, the mycelium was transferred to 2 mL micro-tubes with screw cap, and to each tube was added 0.5 g of glass beads of two different diameters on the proportion of 1:1 (150–212 μm and 425–600 μm). The material was crushed by stirring at high speed in a FastPrep homogenizer.

The extraction of the genomic DNA was performed by the CTAB method based on the protocol described by Oliveira et al. (2016). For amplification of the rDNA ITS region, the primers ITS1 and ITS4 were used (White et al. 1990). Polymerase chain reaction (PCR) that contained PCR reactions were carried out in a volume of 25 μL containing 75 mM Tris-HCl (pH8.8), 200 mM (NH4)2SO4, 0.01% Tween 20, 2 mM MgCl2, 200 μM each dNTPs, 1 μM each primer and 2 units Taq DNA polymerase. Thermal cycling parameters were temperature an initial preheat 95 °C for 3 min; followed by 39 cycles of denaturation at of 94 °C for 45 s, annealing at 58 °C for 1 min and extension 72 °C for 1 min; 72 °C for 7 min. The products of DNA extractions and reactions (5 μL) of PCR were visualized under UV light in 1% agarose gel stained with GelRed. Amplification products were purified with the PureLink PCR Purification Kit (Invitrogen), following the manufacturer's instructions, and the sequences were obtained using the Multipurpose DNA Sequencing Platform of the Bioscience Center/UFPE.

Phylogenetic analyses

The ITS rDNA sequences obtained in this study were used to search for similar sequences deposited in the GenBank database of the NCBI using the BLASTn tool. The sequences selected along the sequences from endophytes were aligned using the MAFFT v.7 interface (Katoh and Standley 2013) and adjusted using MEGAv.7 (Kumar et al. 2016). For the phylogenetic analyses, a matrix for filamentous fungi and another for yeasts was constructed. Bayesian inference (BI) and maximum likelihood (ML) analyses were conducted with MrBayes on XSEDE and RAxML-HPC BlackBox (8.2.8), respectively, both in the CIPRES science gateway (https://www.phylo.org/) as described by Silva et al. (2019). The best nucleotide model for each matrix was obtained using MrModelTest v.2.3 (Nylander 2004) which proposed GTR+I+G for both datasets. The ITS sequences were deposited in GenBank (Table 1).

Table 1 Endophytic species from leaves of *Sorghum bicolor* crops in Atlantic Forest and Caatinga forest areas in Brazil

Proposed taxa (GenBank accession numbers)	Closest blast match (GenBank accession No.)	Identity (%)	Atlantic Forest				Caatinga				N	Fr (%)
			Cultivar IPA2502 Pre	Cultivar IPASF15 Post	Cultivar IPA2502 Pre	Cultivar IPASF15 Post	Cultivar IPA2502 Pre	Cultivar IPASF15 Post	Cultivar IPA2502 Pre	Cultivar IPASF15 Post		
Acremonium pinkertoniae (OP485629)	*Acremonium pinkertoniae* (NR159611)	100					1	12			13	2.24
Arthrinium sorghi (MK371706)	*Arthrinium* sp. (MH498515)	99.81		1							1	0.17
Aspergillus terreus (OP485631)	*Aspergillus terreus* (LC496504)	100	2		1						3	0.52
Amesia nigricolor (OP485630)	*Amesia nigricolor* (MH861594)	100	1		4						5	0.86
Bifusisporella sorghi (NR164042)	*Magnaporthales* sp. (KJ572179)	96.58	2								2	0.34
Candida sp.1 (OP485667)	*Candida pseudointermedia* (NR_155007)	95.91							3		3	0.52
Candida sp.2 (OP485668)	*Candida floricola* (KX811203)	99.52			1						1	0.17
Candida parapsilosis (OP485669)	*Candida parapsilosis* (NR_130673)	99.77			1						1	0.17
Colletotrichum sp.1 (OP485634)	*Colletotrichum fructicola* (MK041495)	99.91	11	9	13	6					39	6.71
Colletotrichum sp.2 (OP485635)	*Colletotrichum falcatum* (NR_144793)	99.81		31	2	35					68	11.70
Cladosporium sp. (OP485632)	*Cladosporium globisporum* (NR_111534)	100			3		3				6	1.03
Curvularia sp. (OP485633)	*Curvularia hawaiiensis* (HG778988)	99.12	3	23		50			1		77	13.25
Daldinia sp. (OP485636)	*Daldinia clavata* (AM749931)	97.87					1				1	0.17
Diaporthe sp.1 (OP485637)	*Diaporthe passifloricola* (NR_147595)	98.21	2								2	0.34
Diaporthe sp.2 (OP485638)	*Diaporthe yunnanensis* (NR_152472)	99.02	2	2							4	0.69
Diaporthe sp.3 (OP485639)	*Diaporthe pseudomangiferae* (NR_111858)	99.21			1						1	0.17
Diaporthe sp.4 (OP485640)	*Diaporthe ueckerae* (NR_147543)	96.08	2			2	2				6	1.03
Dirkmeia sp. (OP485670)	*Dirkmeia churashimaensis* (AB704895)	98.45	1				1				2	0.34
Epicoccum sorghinum (OP485641)	*Epicoccum sorghinum* (FJ427067)	99.78	5	35		28					68	11.70

Table 1 (continued)

Proposed taxa (GenBank accession numbers)	Closest blast match (GenBank accession No.)	Identity (%)	Atlantic Forest				Caatinga				N	Fr (%)
			Cultivar IPA2502 Pre	Cultivar IPASF15 Post	Cultivar IPA2502 Pre	Cultivar IPASF15 Post	Cultivar IPA2502 Pre	Cultivar IPASF15 Post	Cultivar IPA2502 Pre	Cultivar IPASF15 Post		
Exserohilum rostratum (OP485642)	*Exserohilum rostratum* (LT837823)	99.80	1	1	1	1	3		3	1	11	1.89
Fusarium oxysporum (OP485643)	*Fusarium oxysporum* (U28161)	99.52		6		5					11	1.89
Fusarium dlaminii (OP485644)	*Fusarium dlaminii* (KU680355)	99.77		1		1	3	1			6	1.03
Fusarium thapsinum (OP485645)	*Fusarium thapsinum* (MH865738)	100	10	19	4	15				3	51	8.78
Hanseniaspora guilliermondii (OP485671)	*Hanseniaspora guilliermondii* (NR_138192)	99.38								1	1	0.17
Hypoxylon griseobrunneum (OP485647)	*Hypoxylon griseobrunneum* (KC968928)	99.38	2					1		1	4	0.69
Hypoxylon sp. (OP485646)	*Hypoxylon submonticulosum* (MG004779)	92.81		1							1	0.17
Metschnikowia sp. (OP485678)	*Metschnikowia lopburiensis* (AB697761)	99.46	2				14		16		32	5.51
Metschnikowia saccharicola (OP485679)	*Metschnikowia saccharicola* (AB697760)	94.31						1			1	0.17
Meyerozyma sp. (OP485680)	*Meyerozyma guilliermondii* (HQ693803)	98.74			2		6	1	17	4	30	5.16
Mucor irregularis (OP485650)	*Mucor irregulares* (JX976252)	98.93	3								3	0.52
Mucor indicus (OP485648)	*Mucor indicus* (MH861293)	95.28		1	1						2	0.34
Mucor merdicola (OP485649)	*Mucor merdicola* (KT960373)	99.64	1								1	0.17
Nigrospora oryzae (OP485651)	*Nigrospora oryzae* (KX986054)	100	1	10	1	16					28	4.82
Paraphaeosphaeria sp. (OP485652)	*Paraphaeosphaeria verruculosa* (NR_163521)	99.78	1	2	1			1			5	0.86
Parengyodontium album (OP485653)	*Parengyodontium album* (LC092879)	99.81						1			1	0.17
Penicillium citrinum (OP485654)	*Penicillium citrinum* (NR_121224)	100				1	3				4	0.69
Periconia macrospinosa (OP485655)	*Periconia macrospinosa* (JX981482)	99.78	1								1	0.17
Purpureocillium lilacinum (OP485657)	*Purpureocillium lilacinum* (MH865306)	100			2			1			3	0.52

Table 1 (continued)

Proposed taxa (GenBank accession numbers)	Closest blast match (GenBank accession No.)	Identity (%)	Atlantic Forest — Cultivar IPA2502 Pre	Atlantic Forest — Cultivar IPASF15 Post	Atlantic Forest — Cultivar IPA2502 Pre	Atlantic Forest — Cultivar IPASF15 Post	Caatinga — Cultivar IPA2502 Pre	Caatinga — Cultivar IPASF15 Post	Caatinga — Cultivar IPA2502 Pre	Caatinga — Cultivar IPASF15 Post	N	Fr (%)
Phyllosticta capitalensis (OP485656)	*Phyllosticta capitalensis* (FJ538348)	100		3	7	3					13	2.24
Pyrenochaetopsis indica (OP485659)	*Pyrenochaetopsis indica* (NR_160058)	100	1	2							3	0.52
Pyrenochaetopsis sp. (OP485658)	*Pyrenochaetopsis terrícola* (MH697394)	97.98	1								1	0.17
Pseudozyma sp. (OP600004)	*Pseudozyma thailandica* (NR_155686)	88.23	1								1	0.17
Papiliotrema sp.1 (OP485672)	*Papiliotrema terrestres* (KT253546)	99.18							3		3	0.52
Papiliotrema sp.2 (OP485674)	*Papiliotrema wisconsinensis* (NR_160324)	93.70	2								2	0.34
Papiliotrema sp.3 (OP485675)	*Papiliotrema laurentii* (KC485478)	99.58	5						8		13	2.24
Papiliotrema rajasthanensis (OP485673)	*Papiliotrema rajasthanensis* (AM262325)	100			1						1	0.17
Rhodosporidiobolus sp. (OP485676)	*Rhodosporidiobolus fluvialis* (KY104696)	100	1			1	3	4	2	1	12	2.07
Sordariomycetes (OP485661)	*Sordariomycetes* sp. (KM519289)	98.97		1							1	0.17
Sordariomycetes (OP485662)	*Fungal endophyte* (KF435633)	100	1			1					2	0.34
Saitozyma sp. (OP485677)	*Saitozyma flava* (NR_073218)	98.92	1								1	0.17
Sarocladium implicatum (OP485660)	*Sarocladium implicatum* (FN706553)	99					1				1	0.17
Talaromyces pinophilus (OP485663)	*Talaromyces pinophilus* (KM066204)	100		1	1		6	11	6		25	4.30
Xylaria feejeensis (OP485665)	*Xylaria feejeensis* (GU322452)	100				1					1	0.17
Xylariales (OP485664)	*Xylariales* sp. (KU747888)	96.91				1					1	0.17
Xylaria sp. (OP485666)	*Xylaria* sp. (FJ799952)	99.08								1	1	0.17
Total of isolates			66	151	48	165	41	35	62	13	581	100

Information: sorghum cultivars (IPA2502 and IPASF15), phenological stage (Pre blooming and Post blooming), number of isolates (N), relative frequency (Fr)

Statistical analysis

To evaluate the species of endophytic fungi between areas, analyses of cultivars and phenological stages were carried out of the diversity index of Shannon-Weiner and richness per sample. The richness (S) was the number of species in each sample and the Shannon-Weiner diversity index was calculated through to the equation $H' = -\Sigma(Xi/Xo) \times \log(Xi/Xo)$, Xi = number of endophytic isolates of each species and Xo = total number of endophytic isolates of all species. To test whether there was statistical significance between richness and diversity, the Kruskal-Wallis non-parametric test was used ($p \leq 0.05$). This test was used because the samples did not reach normality.

An indicator species analysis was performed to verify which species are related to each area, phenological stages and sorghum cultivars. The indicator value (IndVal) was calculated and the significance was determined by the Monte Carlo test. The species with IndVal $\geq 25\%$ and $p < 0.05$ were considered to be indicators. Venn diagrams were constructed to verify the shared and exclusive species between forest types and cultivars using the tool http://bioinformatics.psb.ugent.be/webtools/Venn/.

Number of isolates and species richness of the samples were modelled using generalized linear models, using rainfall, temperature, pre- and post-blooming and sorghum variety as explanatory variables. Number of isolates and richness are count data, then Poisson regressions were performed, but signs of overdispersions were detected (i.e. residual deviance much larger than the degrees of freedom) affecting the interpretability of the models coefficients, which is resulted from the response variable variance to be larger than the mean (up to 4.7 times larger in the acquired data) (Dean and Lawless 1989). To circumvent the problems caused by overdispersion, negative-binomial models were constructed for count data instead (Gardner et al. 1995). Collinearity was assessed with variance inflation factor analysis, and high values (higher than 4) between rainfall and area, and between pre-post blooming and mean temperature were observed. Rainfall was chosen instead of the area, and pre- and post-blooming instead of temperature, as mean temperature variation in the studied areas are very low (23.9–25.7 °C), and probably biologically with low significance. Goodness-of-fit of the negative binomial models were performed using Pearson's chi-squared test of the probability of the residual deviance, given the degrees of freedom. The best model with a good fit were chosen based on the lowest Akaike Information Criterion (AIC) value.

To infer if the endophytic fungal community composition changes is related to any of the measured variables, a principal coordinate analysis (PCoA) was performed, with the resulting sample scores correlated with the measured variables. Numerical variables were fitted to the sample scores as vectors, and categorical variables as factors using the "envfit ()" function of the "vegan" package (Oksanen et al. 2016). The p value for each variable was accessed with 999 permutations. The test of indicator species was performed with the command "multipatt" function in the "indicspecies" package (Cáceres and Legendre, 2009) using RStudio (R Core Team, 2017).

Results

Fungal isolation rate and identification

From 581 endophytes isolated, 477 were filamentous fungi and 104 were yeasts. Members of the phylum *Ascomycota* (540 isolates, 93%) were dominant, followed by members of *Basidiomycota* (35 isolates, 6%) and *Mucoromycota* (6 isolates, 1%) which had fewer representatives. The ascomycetous isolates were represented by four classes, *Dothideomycetes* (185 isolates, 31.84%), *Eurotiomycetes* (32 isolates, 5.51%), *Saccharomycetes* (69 isolates, 11.88%), *Sordariomycetes* (254 isolates, 43.72%) and 11 orders, *Botryosphaeriales* (13 isolates) *Capnodiales* (6), *Diaporthales* (13), *Eurotiales* (33), *Glomerellales* (107), *Hypocreales* (86), *Magnaporthales* (2), *Pleosporales* (166), *Saccharomycetales* (69), *Sordariales* (5), *Xylariales* (37) and members classified as *Ascomycota incertae sedis* (3 isolates). The basidiomycetous endophytes were represented by three classes, *Microbotryomycetes* (12 isolates, 2%), *Tremellomycetes* (20 isolates, 3.4%), *Ustilaginomycetes* (3 isolates, 0.5%), distributed in *Sporidiobolales* (12 isolates), *Tremellales* (20) and *Ustilaginales* (3), and the mucoromycetous endophytic fungi were represented by *Mucorales* (6 isolates, 1%) (Table 1). The largest number of endophytes (430 isolates, 74%) was found in sorghum cultivated in the Atlantic Forest area while in the Caatinga Forest 151 (26%) fungal were recovered. The endophytic species most abundant were *Curvularia hawaiiensis* (77 isolates, 13.25%), *Colletotrichum* sp. 2 (68 isolates, 11.7%) and *E. sorghinum* (68 isolates, 11.7%). From all species found, 34 were represented by one, two or three isolates and were considered as rare isolation.

Diversity and species composition

Fifty-five endophytic species distributed in 37 genera, only 16 taxa were shared between the two crop areas (Fig. 1A). Greater richness of endophytic fungi was found in sorghum leaves from Atlantic Forest (46 species) compared to the Caatinga (25 species) and differed significantly between areas ($p < 0.01$). We also observed statistical differences in the diversity of endophytic fungi in sorghum leaves between the different areas ($p < 0.01$), the Atlantic Forest showed greater diversity ($H' = 2.68$) compared to the Caatinga ($H' = 2.51$).
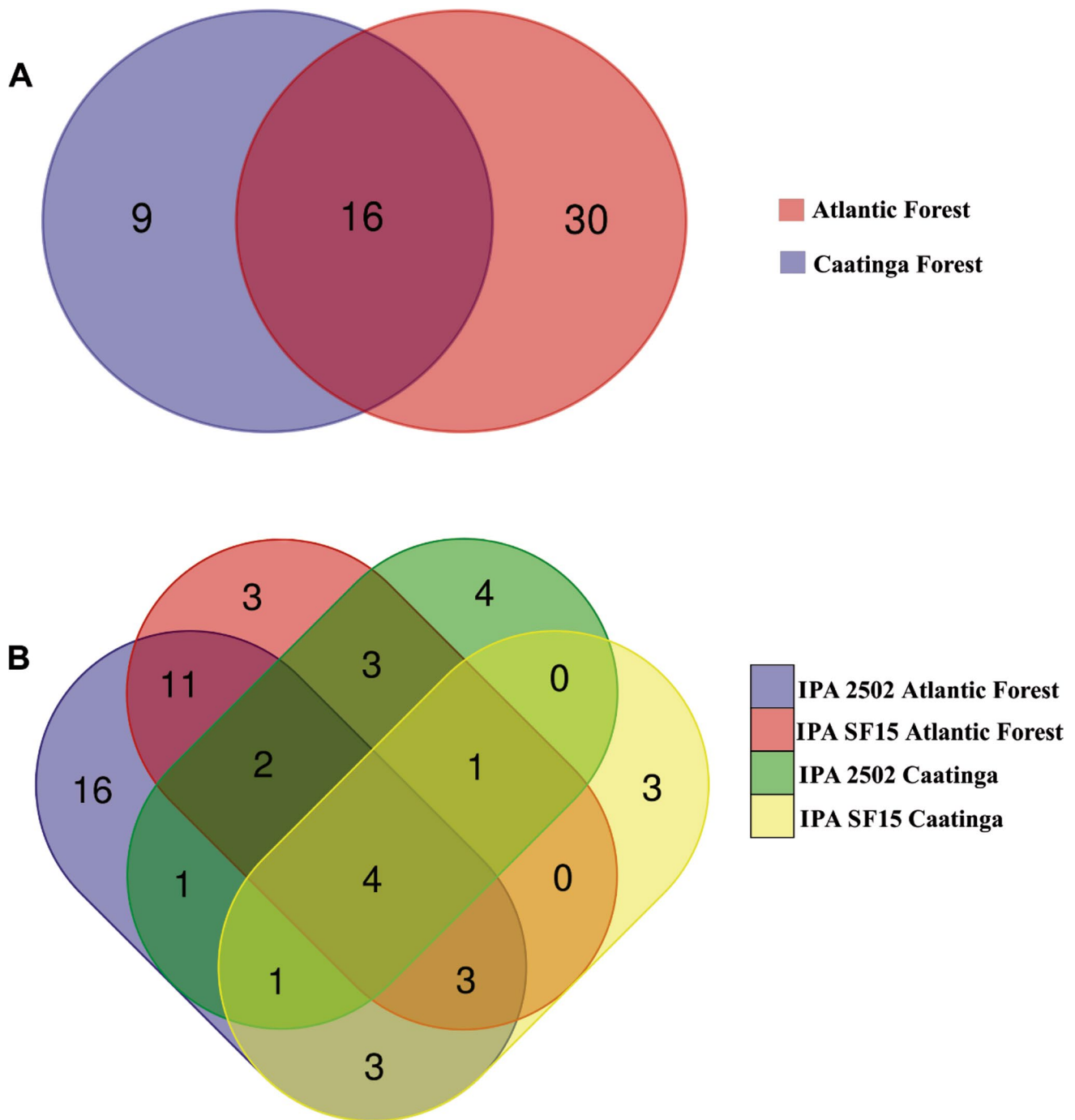
Fig. 1 **A** Venn diagrams represent the number of shared fungal taxa among between samples for Caatinga and Atlantic Forest. **B** Venn diagrams represent the number of shared fungal taxa among between samples for each cultivar (IPA 2502, IPA SF15 (Atlantic Forest) and IPA 2502 e IPA SF15 (Caatinga))

We detected different species indicator for some studied area; nine species were associated with the sorghum cultivation in the Atlantic Forest area: *Colletotrichum* sp. 2, *Epicoccum sorghinum*, *Curvularia* sp., *Colletotrichum* sp. 1, *Fusarium thapsinum*, *Nigrospora oryzae*, *Phyllosticta capitalensis*, *Fusarium oxysporum* and *Amesia nigricolor*

(Table 2). While five species were considered indicators of sorghum cultivation in the Caatinga Forest area: *Meyerozyma* sp., *Talaromyces pinophilus*, *Metschnikowia* sp., *Rhodosporidiobolus* sp. and *Acremonium pinkertoniae*. Seven genera were considerate indicators of the Atlantic Forest: *Colletotrichum*, *Curvularia*, *Epicoccum*, *Fusarium*,

Table 2 Species and genus indicator endophytes of some forest type, stage phonological (pre and post-blooming) or sorghum cultivars (IPA 2502) (IV ≥ 25%, $p < 0.05$)

Indicator taxa	Forest			Blooming/Cultivar		
	IV	p		IV	p	
Species						
Amesia nigricolor	32.3	0.04	Atlantic Forest			
Colletotrichum sp. 2	73.6	0.001	Atlantic Forest			
Curvularia sp.	70.3	0.001	Atlantic Forest	65.9	0.001	Post
Colletotrichum sp. 1	67.7	0.001	Atlantic Forest	65.2	0.001	Post
Epicoccum sorghinum	70.7	0.001	Atlantic Forest	62.1	0.001	Post
Fusarium thapsinum	67.2	0.001	Atlantic Forest	53.6	0.009	Post
Nigrospora oryzae	57.7	0.001	Atlantic Forest	52.0	0.001	Post
Fusarium oxysporum	45.6	0.003	Atlantic Forest	45.6	0.001	Post
Phyllosticta capitalensis	52.0	0.001	Atlantic Forest			
Acremonium pinkertoniae	35.4	0.027	Caatinga	35.4	0.022	IPA 2502
Meyerozyma sp.	50.3	0.004	Caatinga			
Metschnikowia sp.	39.5	0.023	Caatinga	45.6	0.002	Pre
Rhodosporidiobolus sp.	39.5	0.038	Caatinga			
Talaromyces pinophilus	48.0	0.01	Caatinga			
Papiliotrema sp. 3				38.2	0.012	Pre
Genus						
Colletotrichum	87.8	0.001	Atlantic Forest			
Epicoccum	70.7	0.001	Atlantic Forest	62.1	0.001	Post
Curvularia	70.3	0.001	Atlantic Forest	65.9	0.001	Post
Fusarium	69.7	0.001	Atlantic Forest	59.9	0.002	Post
Nigrospora	57.7	0.001	Atlantic Forest	52.0	0.002	Post
Phyllosticta	52.0	0.001	Atlantic Forest			
Mucor	35.4	0.023	Atlantic Forest			
Meyerozyma	50.3	0.002	Caatinga	44.9	0.004	Pre
Talaromyces	48.0	0.001	Caatinga			
Metscnikowia	42.0	0.021	Caatinga			
Rhodosporidiobolus	39.5	0.043	Caatinga			
Acremonium	35.4	0.029	Caatinga	35.4	0.024	IPA 2502
Papiliotrema				46.6	0.001	Pre

Nigrospora, *Phyllosticta* and *Mucor*; five genera were indicators of the Caatinga which are *Acremonium*, *Meyerozyma*, *Metscnikowia*, *Rhodosporidiobolus* and *Talaromyces*.

When comparing the two cultivars in the study (IPA SF15 and IPA 2502), no difference in richness or diversity was observed ($p > 0.05$) between the two crops (IPA SF15: $H' = 2.85$ and IPA 2502: $H' = 3.08$). In general, a total of 38, 27, 16 e 15 taxa were present in the cultivars IPA 2502, IPA SF15 (Atlantic Forest), IPA 2502 e IPA SF15 (Caatinga), respectively. Higher number of unique species was found in the cultivar IPA 2502 in the Atlantic Forest area (Fig. 1B). Venn diagrams showed that the cultivars (IPA 2502 and IPA SF15) in Atlantic Forest shared exclusively 11 taxa; however, there were no taxa shared exclusively between cultivars (IPA 2502 and IPA SF15) in the Caatinga (Fig. 1B). Only four species were shared between all cultivars: *Exserohilum rostratum*, *Fusarium dlaminii*, *Talaromyces pinophilus* and *Rhodosporidiobolus* sp. Only the species

Acremonium pinkertoniae was an indicator of the cultivar IPA 2502. *Acremonium* was indicative of the cultivar IPA 2502 and no genus was related to the cultivar IPA SF15.

There was no statistical difference ($p > 0.05$) between the average richness and diversity per sample between the phenological stages: pre-blooming ($H' = 3.17$) and post-flowering stage ($H' = 2.48$). However, indifferent of the site of cultivation, in the pre-blooming stage we observed a high richness of endophytic fungi. In relation to abundance, the Atlantic Forest showed a greater number of isolates in the post-blooming period, while in the Caatinga, greater abundance was found in the pre-blooming stage.

In the Atlantic Forest, a total of 114 endophytes were found during the host pre-blooming stage, while 316 were recorded during the host post-blooming stage. Although the pre-blooming stage had a lower number of isolates, the richness was higher (38 taxa in pre-blooming and 24 species in post-blooming). The Atlantic Forest during the

pre-blooming stage exhibited as dominant species *Colletotrichum* sp. 1 and *F. thapsinum*, whereas in post-blooming *Colletotrichum* sp. 2, *C. hawaiiensis* and *E. sorghinum* were dominant species. On the other hand, endophytic species of the Caatinga area differ from the Atlantic Forest area, mainly because it had a larger number of endophytic yeasts (85 isolates, 56%). During the pre-blooming stage were found 17 species, wherein *Metschnikowia* sp. and *Meyerozyma* sp. were dominant species, whereas in post-blooming of the 14 species, *A. pinkertoniae* and *T. pinophilus* were dominant. For the pre-blooming stage, two species and *Meyerozyma* and *Papiliotrema* were considered indicator genera while for the post-blooming stage, six species were indicators and *Epicoccum*, *Curvularia*, *Fusarium* and *Nigrospora* were the associated genera (Table 2).

Analysis of environmental factors and endophytic fungal diversity

The regression models that best described the variation in species richness and number of isolates are presented in the Table 3. All constructed models are shown in the Supplementary material S1. There was no effect of cultivar types on endophytic species; however, rainfall had a positive effect on the number of isolates and species, whereas the post-blooming period had a positive effect only on the number of isolates (Fig. 2). This pattern was driven by filamentous fungi, when analysed separately it was possible to observe the effect of these variables, where rainfall and post-blooming period have a positive effect on the number of isolates (Fig. 2, Table 4). Nevertheless, endophytic yeast responded differently, with a lower number of species and isolates on higher rainfall and post-blooming period. Sorghum cultivars have no predictive power on the number of species and isolates on both groups of fungi.

The relationship between sample's species composition is shown in the principal coordinate analysis (PCoA) ordination plots (Fig. 3). Excepting for sorghum cultivars, all variables are significantly related with the sample scores in the ordination. The stronger correlations are with rainfall followed by the blooming period (Table 5). The filamentous fungi communities displayed a similar pattern, whereas the yeast community composition seems to be affected only by the blooming period and temperature.

Discussion

Characteristics of community composition and dominant groups

In the present study, the composition, diversity and distribution of the endophytic fungal community in sorghum leaves were investigated, correlating with environmental factors, phenological stage and cultivar. Members of three phyla, eight classes, fifteen orders, twenty-eight families, thirty-five genera and fifty-five species were identified, showing a rich diversity of endophytes fungi in sorghum leaves. The rich endophytic fungal community belonging to the phylum *Ascomycota* observed in the present study is according to previous studies on fungal endophytes in grasses and other plant species (Loro et al. 2012; Sánchez Márquez et al. 2010; García et al. 2013; Zida et al. 2014; Santamaria et al. 2018, Ren et al. 2019, Kinge et al. 2019, Pambuka et al. 2021). Species of *Dothideomycetes* and *Sordariomycetes* were dominant in the present study; both were already found associated with species of *Poaceae*, including *Sorghum* (Crouch and Beirn 2009; Higgins et al. 2011). Members of these classes are cosmopolitan, and they are often found as endophytes, pathogens or saprobes in woody debris, decaying leaves or dung (Schoch et al. 2006; Hongsanan et al. 2017). Combinations of these niches suggest that it is possible that these fungi start their life cycle as endophytes and change their mode of life to a saprobic state when the plant dies (Schoch et al. 2006; Herre et al. 2007).

The isolation of 55 species distributed in fifteen taxonomic orders shows that sorghum harbours a rich and diverse endophytic mycobiota compared with data

Table 3 Negative binomial regression models that best explain the variation in the number of endophytic fungi isolates and species richness in leaves of sorghum crops samples. All models tested are in Supplementary Material S1. The "+" sign between the explanatory variables represent an additive effect, and a "x", an interaction. All models have a good fit (Pearson's χ^2 test p value > 0.05)

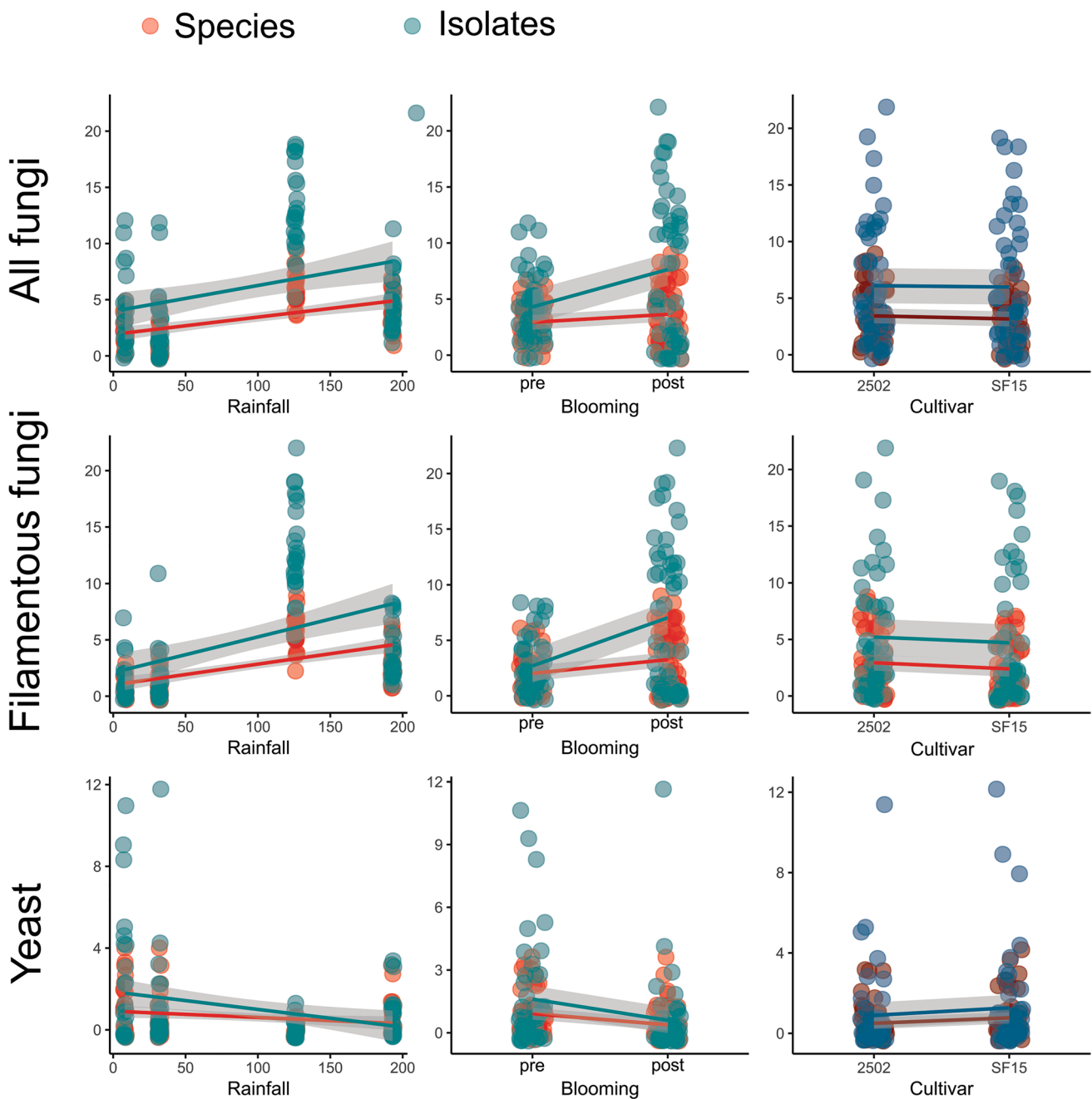| Response variable	Explanatory variables		Estimates	Std. error	Z test	p value	AIC
No. of isolates	Rainfall + Blooming	Intercept	1.5605	0.1511	10.33	< 0.001	536.07
		Rainfall	0.0047	0.0012	3.82	< 0.001	
		Pre-blooming	− 0.5809	0.1788	− 3.24	0.001	
Species richness	Rainfall x Blooming	Intercept	− 0.0641	0.2165	− 0.29	0.767	359.37
		Rainfall	0.0146	0.0019	7.63	< 0.001	
		Pre-blooming	0.7998	0.2646	3.02	0.002	
		Rainfallxpre-blooming	− 0.0118	0.0022	− 5.45	< 0.001	

Fig. 2 Scatterplot and regression lines of the endophytic fungal species and isolates per sample along a rainfall gradient, post and pre-blooming period and between two sorghum cultivars

obtained in other studies in crop Sorghum (Zida et al. 2014; Rodrigues et al. 2018). The dominant fungal genera were *Colletotrichum*, *Curvularia* and *Epicoccum*. The presence of a large number of isolates shows to be a characteristic of the endophytic mycobiota of grasses (Neubert et al. 2006; Sánchez Márquez et al. 2010; Loro et al. 2012; Zida et al. 2014). Similar results were previously found by Zida et al. (2014) who studied *S. bicolor* in a semiarid region in Burkina Faso, Africa. Studies showed

that *Colletotrichum* endophytic species are considered biocontrol agents, confer protective benefits to plants, reduce incidence of disease and increase fertility under nutritional conditions (Arnold et al. 2003; Mejía et al. 2008; Salunkhe et al. 2011; Hiruma et al. 2016; Khiralla et al. 2018; Bengyella et al. 2019, Casas et al. 2021). Although *Colletotrichum* and *Curvularia* species are pathogens known worldwide for a wide variety of hosts, mainly grass species (Manamgoda et al. 2011; Dean et al. 2012). At present

Table 4 Negative binomial regression models describing the variation in the number of endophytic filamentous or yeasts fungi isolates and species richness in leaves of sorghum crops samples. All models have a good fit (Pearson's χ^2 test p value > 0.05)

Explanatory variable		Estimate	SD	z-value	p	Estimate	SD	z	p
Filamentous fungi richness						Filamentous fungi isolates n			
Rainfall	Intercept	0.079	0.161	0.490	0.624	0.465	0.195	2.356	0.017
	Rainfall	**0.008**	**0.001**	**6.992**	**< 0.001**	**0.010**	**0.001**	**6.740**	**< 0.001**
Blooming	Intercept	1.182	0.129	9.132	< 0.001	1.952	0.158	12.301	< 0.001
	Blooming	**− 0.478**	**0.198**	**− 2.418**	**0.015**	**− 0.960**	**0.239**	**− 4.003**	**< 0.001**
Cultivars	Intercept	1.078	0.139	7.737	< 0.001	1.650	0.180	9.148	< 0.001
	Cultivar	− 0.204	0.201	− 1.014	0.311	− 0.100	0.256	− 0.394	0.693
Yeasts richness						Yeasts isolates n			
Rainfall	Intercept	− 0.086	0.230	− 0.373	0.709	0.604	0.285	2.120	0.033
	Rainfall	**− 0.005**	**0.002**	**− 2.101**	**0.036**	**− 0.007**	**0.002**	**− 2.864**	**0.004**
Blooming	Intercept	− 0968	0.259	− 3.734	< 0.001	− 0.511	0.294	− 1.736	0.083
	Blooming	**0.877**	**0.327**	**2.683**	**0.007**	**0.986**	**0.397**	**2.481**	**0.013**
Cultivars	Intercept	− 0.693	0.247	− 2.802	0.005	− 0.110	0.294	− 0.373	0.709
	Cultivar	0.439	0.328	1.319	0.187	0.349	0.408	0.857	0.392

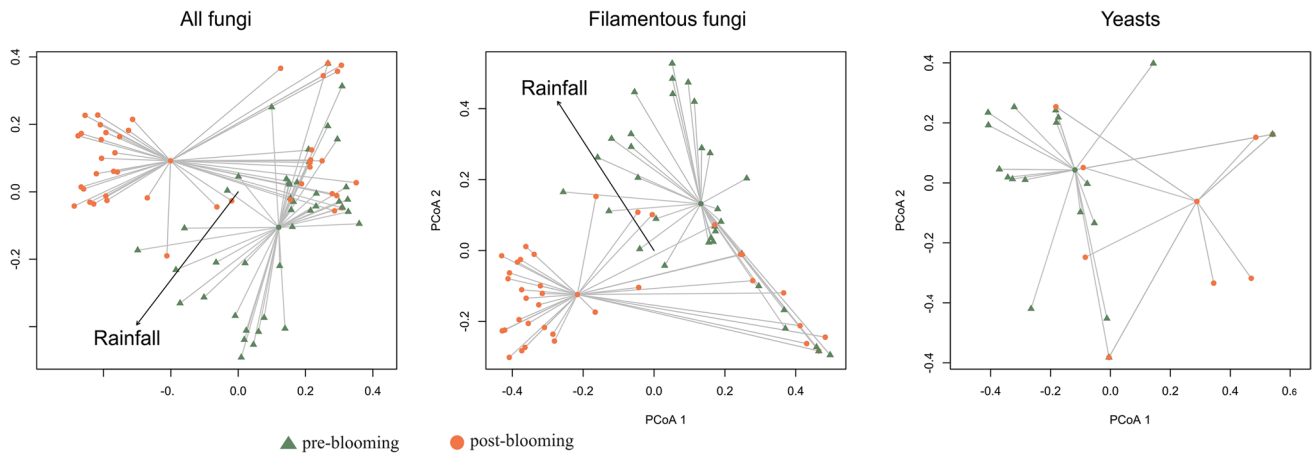Significant results in bold

Fig. 3 Principal coordinate analysis (PCoA) ordination of the endophytic fungi samples composition, based on the Jaccard's dissimilarity. Statistically significant vectors and factors of the measured variables are displayed (Table 2)

Table 5 Correlation values of the vectors and factors of the measured variables with the two-dimensional distribution of sample scores in the PCoA ordinations of yeast, filamentous and all fungal species (Fig. 2)

	All fungi		Filamentous fungi		Yeasts	
Vector	R^2	p value	R^2	p value	R^2	p value
Rainfall	**0.538**	**< 0.001**	**0.606**	**< 0.001**	0.021	0.711
Factors						
Cultivars	0.012	0.875	0.006	0.627	0.077	0.068
Blooming period	**0.207**	**< 0.001**	**0.237**	**< 0.001**	**0.131**	**0.005**

Significant results in bold

study, it apparently does not cause disease in the host, suggesting that future studies are needed to better understand the ecological function of sorghum-fungus interaction.

Influence of environment on endophytic fungi

The results indicate that both factors (biotic and abiotic) drive the community of endophytic fungi in *S. bicolor*. In general, the abundance of filamentous fungi increases with higher rainfall and post-blooming period, whereas both

abundance and richness of yeasts decreases. The contrasting response of yeasts and filamentous fungi community is probably an indication of niche partitioning. However, the introduction of different fungal species into a niche increases competition for nutrients, enzyme production and niche colonization, creating competitive conditions that may have been responsible for decreasing the community of endophytic yeasts (Joubert and Doty 2018). Both occupy the same plant tissue, but they present particular physiological properties and probably they are adapted to different environments, that is, present different responses for the plant physiology, water availability, and resource allocation during reproduction. Little information is known about the ecology of endophytic yeasts, and more studies are needed to elucidate in detail the relationship which they establish with plants (see Joubert and Doty 2018).

In the present study, the rainfall was the environmental factor that had determined the density and influenced the composition of the endophytic fungi community in sorghum leaves. High humidity conditions are more favourable to sporulation and fungal infection, so it is therefore likely that the greater rainfall present in the Atlantic Forest influence the increase of endophytic colonization associated with the host species. The precipitation and the level of endophytic colonization of leave tissues are positively correlated (Rodrigues 1994; Wilson and Carroll 1994; Rajagopal and Suryanarayanan 2002; Mishra et al. 2012, Li et al. 2018). Precipitation could possibly also be associated with the greater richness and diversity of endophytic fungi in crops in the Atlantic Forest. For example, Naik et al. (2008) studying medicinal plants collected in southern India obtained more endophytes with increasing rainfall during the rainy season than during the summer season. Suryanarayanan et al. (2002) verifying the diversity of endophytic fungi in four different types of tropical forests under a precipitation gradient recovered a higher number of fungal isolates during the rainy season. Massimo et al. (2015) evaluating the endophytic fungi distributions associated with tree and shrub leaf tissues of the Sonoran Desert (Arizona) observed that the isolation frequency increased in the period characterized by high humidity and rainfall. On the other hand, when the plant is subjected to a narrow band of water potential, a wide range of responses is displayed at the molecular and cellular levels, such as inhibition of shoot growth and closure of the stomata as an attempt to maintain favourable water content in the tissues by greater possible time (Martínez-Vilalt and Garcia-Forner 2016, Lata et al. 2018). This regulation in the opening of the stomatal pore may restrict the endophytic fungal infection during the dry period.

The detection of sorghum indicator genera and species for the study areas shows that the differences in environmental conditions, including temperature and rainfall, as well as the host physiology favored the distribution of these species.

We observed that some species occurred exclusively in a cultivated area, such as *Colletotrichum* sp. 1 and *Epicoccum sorghinum* that were indicator species for sorghum crop in the Atlantic Forest and *Talaromyces pinophilus* for sorghum crop in the Caatinga area. Similarly to our study, the genera *Colletotrichum* and *Phyllosticta* were also reported as indicators for Atlantic Forest ecosystem (brejo de altitude), while *Talaromyces* was indicative for Caatinga, by Pádua et al. (2019) who investigated the fungal endophyte community of the leaves of *Myracrodruon urundeuva* in Brazil. Olmo-Ruiz et al. (2014) also reported *Colletotrichum* sp. as indicator species for *Elaphoglossum doanense* in the tropical moist forest, Costa Rica. Although the species belonging to these genera are found colonizing a variety of hosts in *Poaceae* (Wong and Hyde, 2001; Sánchez Márquez et al. 2007; Angelini et al. 2012; Manamgoda et al. 2013), the preference of these taxa can be correlated with the ideal conditions that the environment provides for growth and the function of these fungi in each environment.

Influence of host including species and flowering period

Although environmental variation between sites has a very large influence on endophytic communities, genetic differences within the same plant species can modulate fungal diversity (Ahlholm et al. 2002). However, in the present study, there was no effect of the different sorghum cultivars on the community of endophytic fungi. In despite of some species were observed only in one of the cultivars. This result was in agreement with other studies (Pancher et al. 2012; Whitaker et al. 2018; Oliveira et al. 2021). Further studies are needed to explore the ecological and evolutionary consequences of host species on the composition of the endophyte community.

We observe that the increase in the filamentous fungi abundance is correlated with the phenological stages of the plant. This result is consistent with the observations made by Martins et al. (2021) that in cultivars of *Olea europaea* an increase is recorded in the abundance of fungi along the different phenological stages. Similarly, Schlemper et al. (2018) found growth stage as a dominant factor determining the fungal community in the sorghum rhizosphere. We suggest that the influence of the phenological stage at fungal community composition occurs due to the better habitat condition with the physiological changes of the sorghum, aging of the leaf, decrease of the content of antifungal substances and increase of microscopic wounds. In addition, leaf chemistry also may influence endophytic fungal infection (Arnold and Herre 2003). Plants produce different chemical exudates during the different growth stages (Berg and Smalla 2009), which may have greater effects on the fungal community. Zahn and Amend (2019)

suggested that fungi are responsible for directly modifying the phenology of host's flowering and are capable of producing abundant secondary metabolites that may act on flowering phenology. Other researchers suggested that endophytic fungi are effective in altering hormone levels and controlling stomatal behaviour and osmotic adjustment, improving turgor maintenance (Malinowski and Belesky 2000; Mandyam and Jumpponen 2005).

Despite the growing studies of host interactions with the endophytic fungal assembly, it is not clear yet how the plants can select the endophytic mycobiota over biotic and abiotic conditions (García et al. 2013). The present study revealed that endophytic fungal communities are driven by the plant growth stages and rainfall, showing that several factors modulate the composition of the endophytic fungal community in sorghum leaves. Although the cultivar did not influence the endophytic community, the interaction between endophyte-host needs to be studied in detail to better understand the biology and ecology of these fungi associated with sorghum crops and other economically important plants. Our results improve our understanding of the dominant factors that influence the community of endophytic fungi in ecologically and economically important grass species. It is suggested that future studies test the biocontrol potential of these fungi and the potential to promote the growth of plants such as sorghum. Furthermore, these taxa can be used in future studies considering their agricultural and biotechnological potential.

Data availability The datasets generated during the current study are available in the Genbank repository.

Declarations

Ethics approval This article does not contain any studies with animals performed by any of the authors.

Consent to participate Not applicable.

Consent for publication Not applicable.

Competing interests The authors declare no competing interests.

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