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CLIMATE CHANGE: APPLICATION OF ENDOPHYTIC FUNGAL CONSORTIA FROM XEROPHYTIC TREES FOR INDUCING DROUGHT TOLERANCE IN RICE

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ABSTRACT

Climate change has exacerbated drought and desertification thereby intensifying their frequency and duration, leading to crop failures and famine. Endophytic fungal consortia from xerophytic trees in Jigawa state, Nigeria were identified and applied to induce drought tolerance in rice. Root samples were collected from *Hyphaene thebaica*, *Acacia albida*, *Piliostigma reticulatum*, and *Ziziphus mauritiana* while rice seeds were collected from International Institute of Tropical Agriculturein Kano using standard methods. Classical method was used to culture and identify fungi with appropriate guides while their colony diameter and total biomass were measured under Polyethylene glycol PEG-8000 (8%) induced drought. Fungal inoculated rice seedlings were cultivated under drought conditions, their growth and yield parameters were measured and their differences were computed using analysis of variance while fungal consortia contributions were determined using principal component analysis. The highest number of species was five in *Aalbida* while the lowest was two in *Z. mauritiana*. The largest colony diameter and total biomass were 112.17±24.11mm and 6.01±1.12mgg^{-1} in *A. niger* and *A. aculeatus* both from *Aalbida.* Rice height was highest (71.10±16.73cm) and lowest (49.36±10.34cm) while grain yield was highest (314.40±51.50 kgha^{-1}) and lowest (149.72±38.48 kgha^{-1}) in *A. albida* consortium and the control respectively which differed significantly (P<0.05). Harvest index was highest (0.36) in *A. albida* treatment and lowest (0.30) in the control. Two principal components accounted for 100% of total variations in growth and yield parameters with PC1 (*A. albida*=99.75%)and PC2 (*P. reticulatum*=0.26%) indicating the significance of *A. albida* fungal consortia.

Key words: Endophytes, Fungi; Xerophytes, Rice, Consortium, Climate change

INTRODUCTION

Climate changehas exacerbated the impact of drought and desertification thereby intensifying their frequency and duration in many parts of the world, leading to reduction in soil quality and productivity as well as disasters of various types and magnitudes (Karolina and Agnieszka, 2023).Climatic drought is a highly unpredictable constraint and a severe threat to crop cultivation and agro-forestry worldwide, particularly in the arid zones where harsh weather conditions interfere with plant growth from germination through maturity, leading tofamines, economic losses etc. (Rehman *et al*., 2022).Food security is said to happen when all people, at all times, have physical, social and economic access to sufficient, safe and nutritious food that meets their dietary needs and food preferences for an active and healthy life (Acheampong *et al*., 2022).Sub–Saharan Africa is suffering from endemic poverty, ecosystem degradation and limited access to capital, markets as well as infrastructure and technologywhich is further aggravated by climate change, leading to shortfall in precipitation and low productivity for a region that about 63% of its population depends on agriculture (Fadina and Barjolle, 2018). The Global Food Security Index (GFSI) ranked Nigeria 107th out of 113 countries and 25th out of 28 sub-Saharan African countries in 2022 while the 2023 Global hunger index (GHI) rating scored Nigeria 28.3/50, indicating a serious level of hunger in the country (Otekunrin *et al*., 2023).

The per capita consumption of rice in Nigeria is about 47kg, making it the second most important food security crop but only 57% of the demands is locally produced, hence the need for a boost (Diagi *et al.,* 2021). The country was described as the 10th most susceptible nation to climate alterations (Onyeneke, 2021), with a risk of losing about 6–30 percent of its gross domestic product by 2050 if nothing is done (World Bank, 2021), hence the need for climate change adaptation to maintainoptimum agricultural production levels.

Endophytes are bacteria or fungi that live intercellularly and/or intracellularly in healthy plant tissues without causing any apparent symptoms of disease(Nair and Padmavathy, 2014).Endophytic microbes are ubiquitous and play pivotal roles in plant development and responses to pathogens and abiotic stress while others produce important secondary metabolites that promote growth of plants and help them adapt better to the environment (Das and Varma, 2009). A symbiotic relationship was discovered between fungal endophytes and maize roots which was found to increase fresh and dry biomass as well as photosynthetic parameters, including chlorophyll and carotenoid pigments compared with their control counterparts (Bakhshi *et al*., 2023). The endophytes were reported to significantly reduce electrolyte leakage and sub-stomatal CO_2 concentrations of their hosts during higher levels of drought stress and a combination of endophytes isolated from plants in harsh regions was more effective than individual inoculation of each fungus. Abo Nouh *et al*. (2024)identified *Trichoderma atroviride*, *Fusarium acutatum* and *Aspergillus terreus* as endophytic fungi associated with wild plants of Egyptian deserts which were used to induce abiotic stress tolerance and concluded that these microbes have thepotential to improve agriculture and horticulture on dry soils which are common in semi-arid environments. Endophytes have the ability to colonize a variety of plant species and tackle all abiotic stress including drought, salt, and high temperatureswhile being used a biofertilizers (Rodriguez *et al*., 2009). Rice crops have been found to be associated with and thrive well in the presence of endophytic fungi(Subham *et al*., 2020), hence this research was carried out to identify endophytic fungi from xerophytic trees in Jigawa state, Nigeria and apply them to induce drought tolerance in rice as a strategy for climate change adaptation.

MATERIALS AND METHODS

Study Area

Jigawa State of Nigeria is located between latitudes 11.00°N and 13.00°N and longitudes 8.00°E and 10.15°E, it has a total land area of approximately 22,410 square kilometers and a semi-arid climate, characterized by long dry season and short wet season which starts late May and ends early October (Yakubu *et al.,* 2021). The map of the study area is presented in figure 1. The vegetation cover is sparse and characterized by the presence of xerophytic plants which include woody and thorny trees such as *Acacia* species, Doulm palm, Jackalberry etc as well as drought tolerant shrubs and grasses. Jigawa state is one of the major rice production centers in Nigeria where forecasting revealed that in 2030, 417950ha of land is required with 1343mm of rainfall to produce 2550000TN of rice so as to meet the demand of the state which is bedevilled by climatic drought, desertification and flood among others (Abdulmumini *et al*., 2019; Shuaibu *et al*., 2021).

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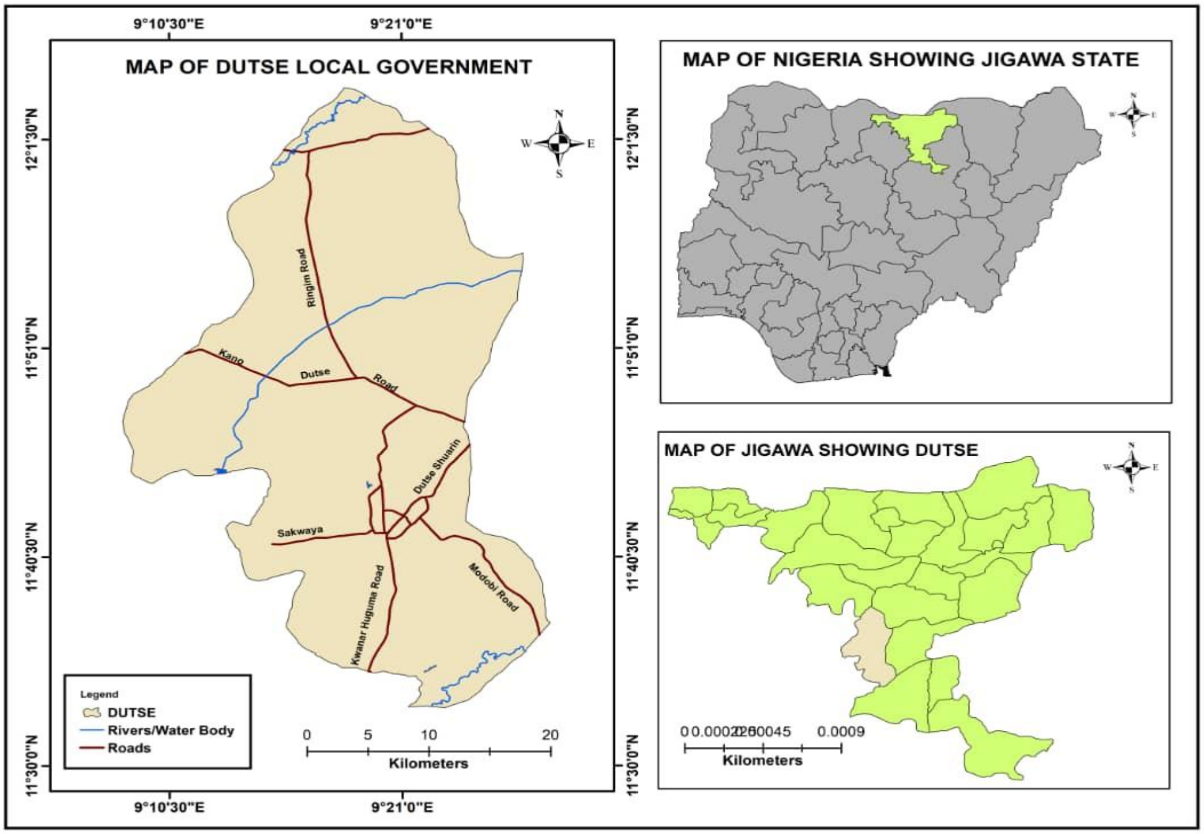

Figure 1: Map of Jigawa State showing the Study Area

Sample Collection

Plant root tissue samples were collected from the rhizosphere zones of healthy, good looking plants including *Hyphaene thebaica, Acacia albida, Piliostigma reticulatum,* and *Ziziphus mauritiana* using scalpels after surface sterilization with 70% ethanol solution followed by a 2% sodium hypochlorite solution and rinsed with sterile distilled water to remove residual disinfectants according toYong *et al*., (2017) and Pramod *et al*., (2022). FARO 48 rice seeds were collected from the International Institute of Tropical Agriculture (IITA) in Kano, Nigeria and all samples were transported to the central laboratory, Department of Environmental Sciences, Federal University Dutse, Jigawa state for experiment.

Isolation and Identification of Endophytic Fungal Consortia

Classical method was used to identify fungi where about 5mm sized pieces of the rhizospheric plant roots were poured into plates of potato dextrose agar (PDA) supplemented with Chloramphenicol (200 mg/l) and incubated in the dark at room temperature (25-28°C) for 7–14 days according to Lü *et al.* (2023).Emerging fragments of mycelia from the plants were transferred to new PDA plates without Chloramphenicol to obtain pure cultures for identification, slides were stained with lactophenol cotton blue reagent after

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preparation with different fungal cultures and examined under a bright-field and phase contrast microscope according to Sadananda *et al*. (2014). The fungi were identified based on colony morphology, such as color, texture, growth rate, aerial mycelium, sporulation or other reproductive structures in line with appropriate identification keys and taxonomic notes from various standard manuals according to Lakshangy and Sandeep (2019) andSamapti *et al*. (2022).

Assessment and Selection of Endophytic Fungal Consortia Drought Tolerance

Water deficit was generated in the isolated endophytic fungi from different xerophytic trees by supplementing polyethylene glycol PEG-8000 (8%) using 50 mL of Czapek's agar as osmoticum to imitate the osmotic pressure of fungal cells according to Rehman *et al*. (2022).Growth performance of fungi under artificial drought conditions were determined by measuring the colony diameter and total biomass of the identified fungal consortia from different trees using acalibrated microscope eyepieceand vacuum filtration methods respectively, according to Daly *et al*. (2020) and Zorn *et al*. (2020).

Soil and Rice Seed Preparation and Inoculation

Field experiments were conducted under the natural atmospheric condition of Jigawa state with uniform soil type and watering regimes where rice was grown in sterile plots arranged in a randomized complete block design (RCBD) with three replications to eliminate all formsof bias according to Olejeme *et al*. (2023). Fungal consortia from different trees were counted using a counting chamber and10^6, 10^4 and10^2 spores per mlof distilled water suspension were prepared. Sterilized rice seeds were germinated for 24 hours after which they were incubated with 10 ml of eachfungal spore suspension and shaken at 30 rpm for 72 hours and then transplanted into the prepared beds where they werewatered with sterilized tap water which was reduced after 14 days to mimic drought conditions while control treatments received same amount of autoclaved distilled water without any fungal consortium treatment according to Airin *et al.* (2023). The fungal consortia treated rice seedlings and control were cultivated under drought conditions until harvest while their growth and yield parameters were measured.

Determination of Rice Growth and Yield Parameters

Rice growth and yield parameters including plant height, root length, number of leaves, growth period, grain number per panicle, grain yield, 1000-grain-weight and straw yield were determined by direct counting or measurement with their respective meters according to Gbadamosi and Daniel (2014). Harvest index (HI) of rice was calculated by dividing the economic yield by the biological yield according to Sangita *et al*. (2018), thus;

$$HarvestIndex(HI) = \frac{Economicyield\ (Grainyield)}{Biologicalyield\ (Grainyield + Strawyield)} \quad (1)$$

Statistical Analysis

Differences in rice growth and yield parameters treated with endophytic fungal consortia from different xerophytic trees and

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the control were computed using one way analysis of variance (ANOVA) while the fungal consortia contributions on rice growth and yield parameters were determined using principal component analysis (PCA).

RESULTS

Endophytic Fungal Consortia from Xerophytic Plants

The identified fungal consortia associated with the rhizosphere zones of all the sampled xerophytic trees during this study are presented in figure 2 while their morphological identifying features are presented in table 2 with *Aspergillus niger* occurring in all trees.

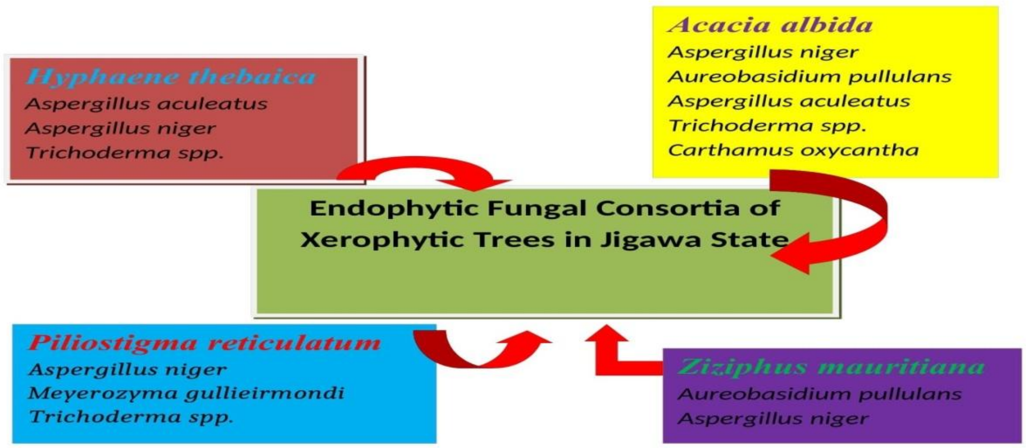
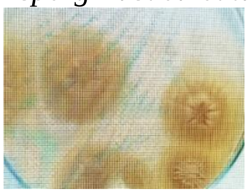
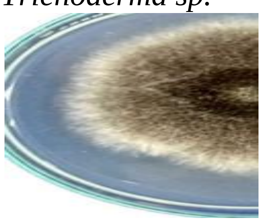
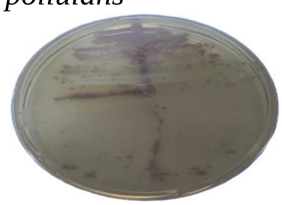


Figure 2: Endophytic Fungal Consortia from Different Xerophytic Trees in Jigawa State, Nigeria

Table 1: Some Endophytic Fungi and their Morphological Features during the Research

Endophytic Fungi	Colony Colour	Shape	Texture	Conidia Arrangement

 *Aspergillusaculeatus*	Dark brown to gray	Globose to subglobose	Granular	Irregular
 *Aspergillus niger*	White to dark yellow	Globose	Smooth	Regular in basipetal chains
 *Trichoderma sp.*	Green to pale yellowish	Globose to ellipsoidal	Smooth	Irregular
 *Aureobasidium pollulans*	Black to cream or pink-colored	Ovoid	Smooth and slimy	Regular
 *Meyerozyma guilliermondii*	White to creamy	Hat-shaped or elongated ovoid	Smooth, waxy or greasy	Regular, chained or clustered

Drought Tolerance Status of Endophytic Fungal Consortia

The averagecolony diameter of treated fungal species in the consortia obtained from different trees and the untreated(control) species are presented in Table 2. There was a significant difference ($P<0.05$) in colony diameter among treated species for drought tolerance of the fungal consortium from different trees and the control.

Table 2: Colony Diameter (mm) of Endophytic Fungi from Different Trees under Drought Conditions

Fungi	*H. thebaica*	*A. albida*	*P. reticulatum*	*Z. mauritiana*	Control	F–value	P–test
A. aculeatus	69.21±19.28^{a}	93.46±21.72^{b}	–	–	128.33±28.29^{e}	8.96	P<0.05
A. niger	98.31±18.46^{a}	112.17±24.11^{b}	73.57±16.41^{c}	81.35±15.34^{d}	119.36±31.51^{e}	4.71	P<0.05
Trichoderma spp.	51.33 ±16.72^{a}	69.41±21.30^{b}	56.55±19.17^{c}	–	96.48±21.38^{d}	4.55	P<0.05
A. pollulans	–	71.32±19.51^{a}	–	34.52±9.86^{b}	73.82±20.40^{c}	7.48	P<0.05
C. oxycantha	–	38.33±9.08^{a}	–	–	52.71±11.62^{b}	4.14	P<0.05
M. gullieimondi	–	–	75.39±16.20^{a}	–	74.86±18.63^{a}	1.09	P>0.05

Note: Values are$\bar{x} \pm \sigma$,– represents values not observed, Rows withouta common superscript differ significantly (P<0.05).

In the PEG-8000 drought induced condition, the highest total biomass was 6.01±1.12 mgg^{-1}) in *Aspergillus aculeatus* from *Acacia albida* while the lowest was 3.01±0.95 mgg^{-1} in *Aureobasidium pollulans* from *Ziziphus mauritiana.* Total biomass among treated fungal species of the consortia from different trees and the control differed significantly (P<0.05) which are presented in Table 3.

Table 3: Total Biomass (mgg^{-1})of Endophytic Fungi from Different Trees under Drought Conditions

Fungi	*H. thebaica*	*A. albida*	*P. reticulatum*	*Z. mauritiana*	Control	F–value	P–test
A. aculeatus	3.80±1.01^{a}	6.01±1.12^{b}	–	–	6.22±1.40^{c}	8.51	P<0.05
A. niger	4.02±1.28^{a}	5.67±1.21^{b}	5.61±1.02^{c}	4.53±1.11^{d}	5.54±1.03^{e}	4.73	P<0.05
Trichoderma spp.	3.69±0.98^{a}	5.47±1.13^{b}	4.62±0.95^{c}	–	5.53±1.04^{d}	3.48	P<0.05
A. pollulans	–	4.52±1.08^{a}	–	3.01±0.95^{b}	5.24±1.03^{c}	4.81	P<0.05
C. oxycantha	–	3.68±1.06^{a}	–	–	4.11±1.02^{b}	3.11	P<0.05
M. gullieimondi	–	–	3.21±0.88^{a}	–	5.01±1.11^{b}	5.26	P<0.05

Note: Values are$\bar{x} \pm \sigma$,– represents values not observed, Rows withouta common superscript differ significantly (P<0.05).

Rice Growth and Yield Parameters

Rice crops grown with endophytic fungal consortium from *Acacia albida* had the highest mean height of 71.10±16.73 cm and the lowest was 49.36±10.34 cm in the untreated rice, a trend followed by all measured growth and yield parameters culminating intoharvest index, the highest and lowest of which were0.36 and 0.30 respectively. All growth and yield parameters differed significantly (P<0.05) among treatments and the control which are presented in table 4.

Table 4: Growth and Yield Parameters of Rice Treated with Endophytic Fungi from Xerophytic Trees

Parameters	*H. thebaica*	*A. albida*	*P. reticulatum*	*Z. mauritiana*	Control	F–value	P–test
Height (cm)	54.39±13.88^{a}	71.10±16.73^{c}	56.20±10.37^{b}	50.52±8.26^{d}	49.36±10.34^{e}	7.53	P<0.05
Root length (cm)	11.20±2.81^{a}	16.48±3.61^{c}	13.50±3.10^{b}	10.35±3.57^{d}	9.58±2.72^{e}	13.79	P<0.05

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Number of leaves	49.65±8.90[a]	66.54±10.22[c]	50.40±8.51[b]	50.51±9.12[d]	46.50±8.81[e]	9.22	P<0.05
Grain yield (kgha^{-1})	201.34±48.43[a]	314.40±51.50[c]	189.25±46.51[b]	166.39±41.83[d]	149.72±38.48[e]	11.30	P<0.05
1000 grain weight(g)	26.33±5.31[a]	34.06±7.22[b]	27.31±5.81[c]	24.38±6.60[d]	22.58±5.25[e]	6.46	P<0.05
Straw yield (kgha^{-1})	389.94±91.31[a]	559.50±86.55[c]	394.51±71.40[b]	368.41±58.32[d]	347.80±68.93[e]	9.18	P<0.05
Harvest Index	0.34	0.36	0.32	0.31	0.30		

Note: Values are$\bar{x} \pm \sigma$,– represents values not observed, Rows withouta common superscript differ significantly (P<0.05).

Endophytic Fungal Consortia Contributions to Rice Growth and Yield Parameters

Total variations in growth and yield parameters of rice during this study were accounted for by two principal components (PCs), with PCs 1 and 2 accounting for about 99.74% and 0.26% respectively. Straw yield had the highest contributionto PC1 while root length in PC2 making the two PCs account for 100% growth and yield parameters variations in the cultivated rice. Figure 2 shows the contribution of endophytic fungal consortia to total variations in rice growth and yield parameters.

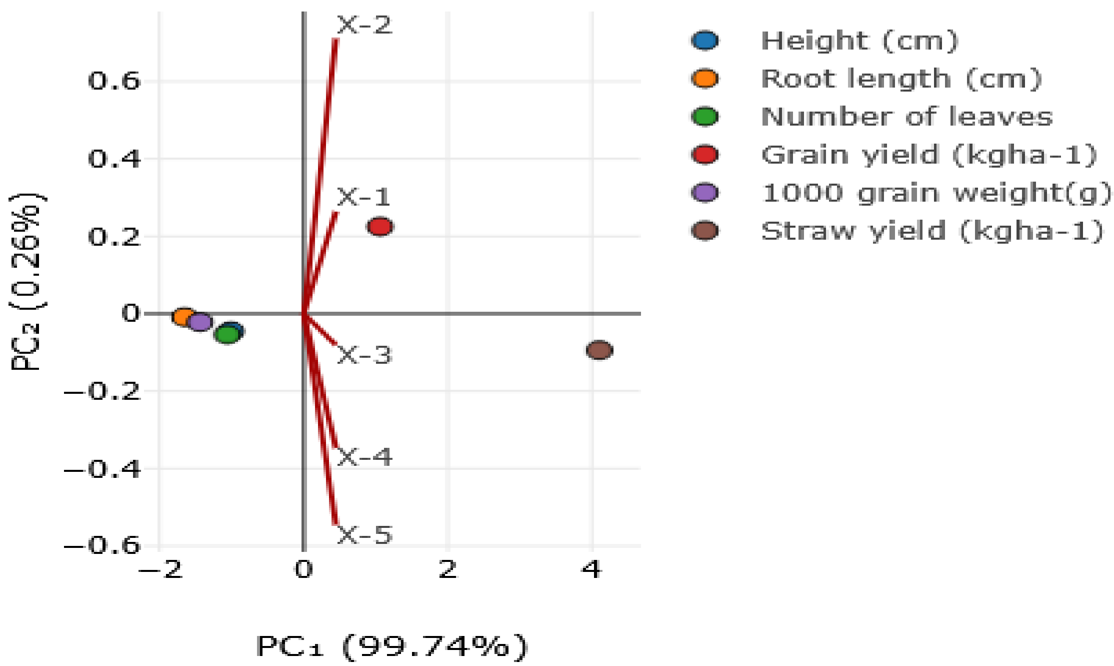

Figure 3:Principal Components showing the Contribution of Endophytic Fungal Consortia to Total Variations in Rice Growth and Yield Parameters

DISCUSSION

Endophytic Fungal Consortia and their Drought Tolerance

The endophytic fungal consortia had the highest number of species in *Acacia albida* with about five species including *Aspergillus niger* which was found in all the

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studied xerophytic trees in this research. This could be attributed to the *A. albida*'s ability to secrete exudates including organic acids and proteins in the rhizosphere which initiate and facilitate host plants and fungal endophytes communication leading to the colonization process (Minz *et al*., 2013). *A. albida* is a widely distributed species throughout the tropical African dry zones and has an inverted phenology unique to it where its leaves are shed during the rainy season which makes it a perfectagroforestry candidate with significant positive influence on ecosystem crops and services (Djim *et al*., 2023). The tree was considered as one of the 14 priority species foragricultural environment and rural living improvement by the Food and Agricultural Organization (FAO, 1977) and has been usedwidelyto increase soil fertility, crop yields and provide fodderin traditional farming systems, hencein many West African countries, the tree was held in high esteem and protected by law (Lu*et al.,* 2022). The fungal consortia in *Piliostigma reticulatum* and *Hyphaene thebaica* consisted of only three species. These tree species are drought tolerant, produce antioxidants and secondary metabolites including phenols, tannins, flavonoids, steroids, glycosides, terpenes, terpinoids etc and are very useful in agroforestry thereby constituting islands of soil fertility with better water availability and more intense biological activity in their rhizosphere (El-Beltagi*et al*., 2018; Fall *et al.,* 2021). *Ziziphus mauritiana* has methanolic extracts, saponins, tannins, alkaloids and flavonoids which are associated with endophytes and have been used in agriculture, healthcare etc since prehistorical times (Mesmar *et al*., 2022).

The ubiquitous presence of *A. niger* could be due to the fungus' ability to secrete secondary metabolites including low molecular weight organic acids such as citric, gluconic, oxalic, itaconic, malic, lactic, acetic etc, enzymes such as cellulase, amylase, β-glucosidase, aryl-phosphatase, lipase etc and other products which promote the invasion of plant species by changing the soil microbial community structure and carbon biomass as well as enzyme activity, which improves soil properties and processes ((Elsheikh *et al*., 2021; Šimonovičová *et al*., 2021). *Aureobasidium pollulans* has been identified as a potential biostimulant which enhances the drought tolerance of plants while increased photosynthetic performance was associated with *Aspergillusaculeatus*. *Trichodermaspp* suppresses *soil-borne plant pathogens and Meyerozymagullieirmondii*is known for the overproduction of vitamin B2 (riboflavin) *(*Romi *et al*., 2014; Niaz *et al*., 2024).

Generally, the untreated endophytic fungal consortium exhibited the highest colony diameter and total biomass which implies the efficacy of polyethylene glycol PEG-8000 as an artificial treatment to mimic drought conditions as it *simulates dehydration* thereby decreasing the osmotic potential of the water solution, making it less available to plants (Bukan *et al*., 2024). This was followed by the consortium in *A. albida* with a significant difference among trees which could be attributed to the number of fungal species per consortia. Endophytic fungal consortia enhance their drought tolerance by upregulating the antioxidant enzyme (APX) through heat shock factors (Javed *et al*., 2022) which is multiplied by the number of individuals and fungal species type, leading to their survival and increased colony diameter as well as total biomass in the harsh conditions.

Rice Growth and Yield Parameters and Endophytic Fungal Consortia Contributions

The plant–fungi symbiosis and their functional relationships helped build protective regulatory mechanisms against drought stress which showed in the growth and yield parameters of rice in this research as fungal consortia treatments were significantly better than the control. Endophytic fungi are reportedly capable of altering plant physiology and change root morphogenetic characters of plants through their extensive network of hyphae which absorb water and nutrients from the smallest pore spaces where plant root hairs cannot reach (Wen *et al*., 2022). Pérez-Moncada *et al*. (2024) reported that improved morphological, physiological, nutritional, and yield variables were observed as a result of co-inoculation of *Bradyrhizobium liaoningense* and *Ambispora leptoticha* in three soybean cultivars. Growth and yield parameters were significantly higher in *A. albida* treated rice which could be attributed to the number of endophytic microbes in the consortium and their combined activities. Ghulam *et al*. (2018) reported the effect of consortium application to be more prominent and significantly different from the application of a single species of bacteria and fungus which improvedgrain yield and physiological attributes of wheat.

PC1 represents contributions from the fungal consortium in *Acacia albida* which exhibited high loadings for all the growth and yield parameters of rice in this research while PC2 represents contributions from *P. reticulatum* suggesting that the number of fungal species in the consortia play significant role in influencing rice growth and yield parameters (Doni *et al.,* 2022). PCA Variables are narrowed down into few significant components using PCA which explains their variance based on multivariate relationships and reduces dimensionality while creating linear combinations of the original variables (Barde *et al.*, 2024).

CONCLUSION

This research demonstrated practically the presence of endophytic fungi as consortia in the rhizosphere zones of different xerophytic tree plants from Jigawa state, Nigeria with *Acacia albida* having the highest number of fungal species. *Aspergillus niger* occurred in all the consortia and had the largest colony diameter while *Aspergillus aculeatus*had the highest total biomass under drought condition, both from *A. albida*. Field experiments showed significant influence of endophytic fungal consortia on growth and yield parameters of rice with *A. albida* consortium generally having the highest performance and the control having the lowest. Principal components analysis (PCA) revealed two PCs representing endophytic fungal consortium from *A. albida* (PC1) and *P. reticulatum* (PC2) accounting for 100% of the total variations in growth and yield parameters which were strongly associated with the number and activities of endophytic fungal species. This study however, did not cover the identification of major chemical constituents in active endophytic fungal extracts which is strongly suggested for development of bioinoculants to increase the growth of specific crops under drought stress. It is also suggested that more of *A. albida* should be planted and employed in agroforestry and endophytic fungal consortia from these trees be isolated and applied for crop improvement in the face of drought and climate change.

Conflict of Interest

There is no any conflict of interest regarding the publication of this manuscript.

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