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ENDOPHYTIC FUNGI ISOLATED FROM NEEM (Azadirachta indica) INDUCED DROUGHT RESILIENCE IN RICE (Oryza sativa)

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ABSTRACT

Climate variability and extreme weather events such as droughts, floods, and heat waves are major constraints on rice production in many regions. These can cause crop damage, yield losses, and reduce the quality of rice grain, and for this, a better way needs to be found to solve these consequences, among them the use of endophytic fungi. This study aimed to isolate and identify endophytic fungi capable of increasing drought resilience in rice (Oryza sativa) from the Neem (Azadirachta indica) tree. The plant sample was sourced from Jigawa State Polytechnic Dutse, washed with 5% sodium hypochloride, and the fungal isolates were screened using PDA-agar. The inoculation was done using the stabbing method, and the growth morphology was recorded. The fungi found in this study include Meyerozyma gullieirmondi, Aspergillus aculeatus, and Carthamus oxycantha. Researchers suggest that these endophytic microorganisms synthesize bioactive chemicals within their host plant tissues, providing benefits to the plant. The results show that out of the varying watering treatments to test the impact of the isolates on the physiology as well as the chlorophyll contents of the treatment, test four has the highest values in terms of shoot lengths, roots, and average chlorophyll of 15.2 cm, 2.3 cm, and 10.4 cm, respectively, compared with the highest watering treatment (control) with 12.5 cm, 1.8 cm, and 2.5 cm, respectively. The chlorophyll contents increased due to isolates of Meyerozyma gullieirmondi, Aspergillus aculeatus, and Carthamus oxycantha, which suggests the healthy physiology of the testing plants as well as the good physiology for them to synthesize their food. This means that the bioactive component of these fungi can increase drought tolerance.

Key words: *drought resilience, neem tree, and chlorophyll*

INTRODUCTION

Climate variability and extreme weather events such as droughts, floods, and heat waves are major constraints on Rice production in many regions (Alsafadi et al., 2023). These can cause crop damage, yield losses, and reduce the quality of Rice grain. Water scarcity is a major constraint on Rice production in many regions, as Rice is a water-intensive crop (Khedwal et al., 2023). And endophytic fungi have the potential to improve Rice yields and resilience in drought-prone and nutrients depleted soils by enhancing plant growth and nutrient uptake, and helping plants tolerate environmental stresses (Fadiji et al., 2023). Developing practical applications of Endophytic fungi for improving Rice yields and resilience could help to reduce the need for chemical fertilizers and high cost of irrigation, and promote sustainable agricultural practices. Furthermore, this research could lead to the development of new products such as biofertilizer and services that could create economic opportunities and improve livelihoods for farmers and rural communities, particularly in developing countries such as Nigeria. Endophytes are microbes that spend all or a stage of their life cycle inside plant tissue. Endophytes are harmless to their host plants even though they reside within the tissue of the plant. It is suggested that endophytic microorganisms synthesise bioactive chemicals within their host plant tissues, which is beneficial to the plant. This has sparked extensive studies concerning the plant-microbe relationship (Vishwakarma et al., 2020).

Endophytes interfere with plant metabolism and obtain their nutrient from the host plant, while the plant regulates the metabolic pathways of the microorganisms for the secretion of molecules that may have defensive functions for the endophytes (Asif et al., 2023). Endophytes use chemical signals to associate with other species, which might be vital for the host's survival and adaptability to varying environmental and biological challenges (Dhayanithy et al., 2015).

Since the effects of the endophytic fungi-plant associations could be beneficial to humans by supporting the plant to overcome biotic and abiotic stress, research interest in under- standing, explaining and exploiting the plant/microbe relationship has increased in recent years (Aswani et al., 2020).

Endophytes cling to their host plants, which results in a symbiotic relationship where the microbe benefits from the plant through structural security and abundance of metabolic products from the plant, which serves as a nutrient. In contrast, host plants accumulate a diverse range of metabolic compounds from the microorganism (Gianluca et al., 2020). It is proposed that the symbiosis between a plant and the endophyte results in a situation where the plant and the microbes biosynthesize the same biochemical. Such examples include myrtucommulones, camptothecin, paclitaxel, and deoxypodophyllotoxin from Myrtus communis, Camptotheca acumi- nata, Taxus brevifolia, and Juniperus communis, respectively. This adaptation is believed to improve the microbe's survival rate within the microenvironment of plant tissues (Gianluca et al., 2020).

Rice is a vital crop that provides a significant source of food for people worldwide. However, biotic and abiotic stresses, such as drought and nutrient depletion, can limit Rice production, leading to lower yields and lower quality crops (Grote et al., 2021). Climate change is exacerbating these challenges, as more frequent and prolonged drought periods are becoming a reality in many regions worldwide (Asif et al., 2023).

MATERIALS AND METHODS

Sample collection and surface sterilization

Sample from selected xerophytic plant species of Adzadhirata indica plants were collected from Faculty of Science (11.700N and 9.370E); behind Microbiology Laboratory, Fedral University Dutse, Jigawa State-Nigeria based on the protocol adapted by Zuo et al. (2022). The plant tissue samples will be collected from healthy, symptom-free plants. The plant samples were surface sterilized using 70% ethanol solution followed by a 2% sodium hypochlorite solution. The sterilized sample was then processed for fungal isolation.

Fungal isolation and identification

Endophytic fungi from the processed plant samples was isolated adopting tissue segment through direct plate method onto potato dextrose agar (PDA) and incubated at room temperature (25-28°C) for 7-14 days (Zuo et al., 2022). Once fungal growth is visible on the plates, individual fungal colonies were sub-cultured to obtain pure isolate for further characterization. Preliminary identification was based on colony morphology, such as color, texture, and growth rate. Other morphological and physiological characteristics such as spore morphology, growth patterns, and biochemical tests were used for the fungal identification.

Rice seed preparation and inoculation

Healthy and uniform Rice seeds from some cultivars were selected and surface sterilized with 70% ethanol for 10 minute and 4% sodium hypochlorite for 5 minutes (Sharma et al., 2023b). The seeds were rinsed thoroughly with sterile distilled water and air-dried for 2-3 hours. The surface-sterilized Rice seeds were inoculated with the isolates of each species of endophytic fungi by soaking the seeds in a spore suspension or by coating the seeds with fungal mycelia according to procedure described by Tehri et al., (2022).

Control seeds were treated with sterile water. Different soil samples were prepared to mimic the drought and nutrient-depleted conditions by adding appropriate amounts of sand, vermiculite, and low-nutrient soil mixtures (Pogmala et al., 2022).

Growth and yield determination

Growth parameters such as plant height, and length of the root biomass, and chlorophyll content were determined at regular intervals of 3 days during the experiment.

Preparation of the leaves Chlorophyll

One gram of leaf sample were finely cut and grinded using a clean pestle and mortar. To this homogenized leaf material, 20ml of 80% acetone and 0.5gm $MgCO_3$ powder were added. The materials were further grinded gently. The sample was put into a refrigerator at 40C for 4 hours. Thereafter, the sample was centrifuged at 500 rpm for 5 minutes. The supernatant were transferred to 100ml volumetric flak. The final volume was made up to 100 ml with addition of 80% acetone. The color absorbance of the solution was scanned using UV-spectrophotometer at 645 and 663nm against the acetone (80%) as a blank (APHA, 1989).

Chlorophyll Analysis

The analysis of chlorophyll of the sampled was done using the formula below;

Formula

$$\text{Chl a} = 11.75 \times A_{662.6} - 2.35 \times A_{645.6}$$

$$\text{Chl b} = 18.61 \times A_{645.6} - 3.96 \times A_{662.6}$$

Where, Ca and Cb are the chlorophyll a and chlorophyll b, A is absorbance.

RESULTS AND DISCUSSION

RESULTS

4.1. Endophytic Fungal Isolation

A total of six strains of endophytic fungi were isolated from the various parts of the xerophytic plant; Azadrachta indica (Figure 4.1-4.2a and b) and the treatment were shown in 4.3.

Table 4.1 Plates morphology and microscopic identification of the isolates

Plates	Colony appearance	Microscopic morphology	Isolates
SDA1	Dark brown fluffy mycelium	Spore non-septate mycelium with cross wall	*Meyerozyma gullieirmondi*
SDA2	White fluffy mycelium with reverse pigmentation of ash	Spore mycelium	*Aspergillus aculeatus*
SDA3	Brown fluffy mycelium with reverse pigmentation of white	Septate non-spore mycelium	*Carthamus oxycantha*
SDA4	Brown fluffy mycelium with reverse pigmentation of white	Septate non-spore mycelium	*Penicillia spp.,*

Table 4.2: Growth and physiological parameters of *O. sativa* after 20 days of treatment

Parameters	10ml (Control)	8ml (test 1)	6ml (test 2)	4ml (test 3)	2ml (test 4)
Length of shoots	12.5cm	11.8cm	11.6cm	15.2cm	13.7cm
Length of roots	1.8cm	1.9cm	2cm	2.3cm	2.1cm
Average chlorophyll contents	2.488	0.145	6.904	10.368	2.291

DISCUSSION

Isolation and identification of fungi

The isolates obtained from the study was incubated at 300C to isolates those that can withstand the extra temperature addition as it's usually grown at 250C. The resistant strains were allowed to grow for 7 days and the growth of isolates were evaluated in terms of fresh weight by harvesting the fungal biomass from culture through filtration. The resistant strains were then checked for various growth parameters and applied to Study plant (Rice) under mediated drought stress pattern of SDA treatment. Among all the isolated strains, the Meyerozyma gullieirmondi, Aspergillus aculeatus, Carthamus oxycantha, has the drought tolerance as described by Khan et al., (2013) while Penicillia spp.,is normal fungi with no drought tolerance at all. In this study, Meyerozyma gullieirmondi, Aspergillus aculeatus, Carthamus oxycantha were selected for further experiments based on their resistance response to the induced drought stress. Based on visual features, preliminary identification of chosen strains of fungal endophytes was performed and the texture and shape of the colony, growth pattern, hyphae color, and spores, the fungal isolates helped to recognized them to at least the genus level (Table 4.1).

Drought resistance in Endophytic fungi

The drought resistance in this study was tested based on the method of Madira et al., (2016) from which the train of the isolated endophytes was inoculated into every study group excluding the control for three days. And the watering was measured based on the study volume (in ml) and later the chlorophyll contents were determine as the factor that distinguish the drought resilience due to inoculum or not. The one with highest chlorophyll was considered as the resilience when compared to control test.

Endophytic fungi influence on drought resistance as well as the chlorophyll contents in Rice (Oryza sativa)

Under field conditions, the ability of fungal endophytes in the promotion of different growth parameters i.e., length of the shoot, and root length of O. sativa plants, were recorded at 20 days after 3days of inoculation treatment with isolated endophytes. All fungal isolates individually were significantly tested for promoting the growth parameters. The results shows that out of the varying watering treatment to test the impact of the isolates on the physiology as well as the chlorophyll contents of the treatment, test four has the highest values in terms of shoot lengths, roots and averages chlorophyll of 15.2cm, 2.3cm and 10.4cm, respectively when compared with the highest watering treatment (control) with 12.5cm, 1.8cm and 2.488 respectively (Table 4.2). The figure displayed how treatment three is more developed despite the fact that it's receiving only 4ml of water in a day. Also the chlorophyll extract was shown in fig. 4.4. This study was in line with the finding of Choi et al., (2019).

Influence of Endophytic fungi on Rice chlorophyll contents

Data from the table 4.2 shows that the chlorophyll contents of each treatment where treatment three (test 3) has the chlorophyll contents which suggest the healthy physiology of the testing plants as well as good physiology for them to synthesized their food. The second in ranking in terms of chlorophyll contents is treatment 2 (test 2) with 6.903, then test 4 (2.291) and lastly test 1 and control with 0.145 and 2.488 respectively. Although the last treatment has the least water application but it's very closely in chlorophyll value in comparable with the control that received 10ml every day due to the presence of Aspergillus aculeatus. According to Andreas (2012), chlorophyll is the pigment responsible for capturing sunlight and converting it into energy through photosynthesis. Therefore, chlorophyll contents always indicate the overall health of the plants, as the decrease in the chlorophyll suggests the lack of adequate nutrients. On this note, treatment number three produced the healthiest plant with highest chlorophyll that give it the ability to synthesize its own food using carbon dioxide and also shows the information about the nutrient contents of plants. This means that the endophytic fungi inoculated in the study enhance the nutrient contents as it increase the chlorophyll pigment to the tested plant. The present study is in line with the research of John and Hussain et al., (2015) which test the effectiveness of the same fungi on tomatoes.

5.2 CONCLUSION

In this study, Penicillia spp., Meyerozyma gullieirmondi, Aspergillus aculeatus, and Carthamus oxycantha were isolated and Meyerozyma gullieirmondi, Aspergillus aculeatus, and Carthamus oxycantha selected for further experiments based on their resistance response to the induced drought stress. And the finding suggested that those endophytic microorganisms with exception of Penicillia spp., can synthesize bioactive chemicals like; Indole Acetic Acid, GA and Flavonoid within their host plant tissues, which is beneficial to the plant. This has sparked extensive studies concerning the plant-microbe relationship. Endophytes interfere with plant metabolism and obtain their nutrient from the host plant, while the plant regulates the metabolic pathways of the microorganisms for the secretion of molecules that may have defensive functions for the endophytes. Endophytes use chemical signals to associate with other species, which might be vital for the host's survival and adaptability to varying environmental and biological challenges.

As the chlorophyll is the pigment responsible for capturing sunlight and converting it into energy through photosynthesis. Therefore, chlorophyll contents always indicate the overall health of the plants as the decrease in the chlorophyll suggests the lack of adequate nutrients. And with this finding, more research should be done to ensure the reliability of the fungi on overcoming any biotic and abiotic stress.

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SUPPLEMENTARY MATERIALS

Fig. 4.1a: Adzrachta indica tree

Fig 4.1b: A. indica samples' leaves

Fig. 4.2a: SDA plate 1

Fig 4.2b: SDA plate 2

Fig 4.2c: SDA plate 3

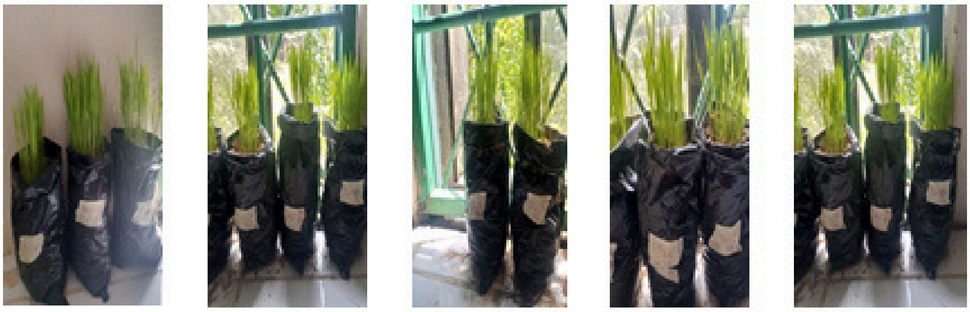

Fig. 4.3: treatment of control, test 1 to test 4 will water application of 10ml, 8ml, 6ml, 4ml and 2ml respectively.

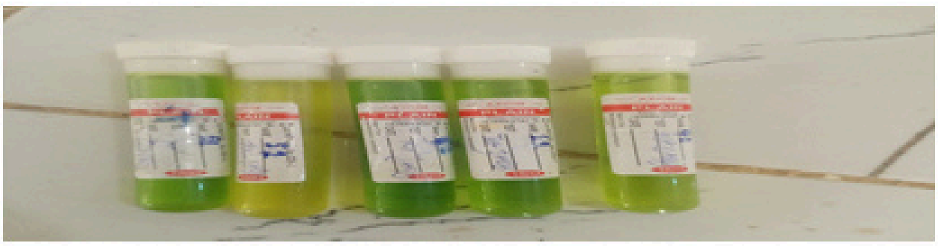

Fig. 4.4: Chlorophyll extract before calorimetric determination