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Endophytic *Fusarium proliferatum* Reprogrammed Phytohormone Production and Antioxidant System of *Oryza sativa* under Drought Stress

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Abstract: The aim of the current study was to isolate endophytes from the roots of *Rhazya stricta* and assess their potential to improve the growth of drought-stressed sunflower seedlings. The potential role of the isolated endophytic fungus was initially screened by using two rice varieties (mutant rice cultivar *Waito-C*: Gibberellins deficient; and *Dongji byeo*: Gibberellins-producing normal cultivar). A significant ($p < 0.05$) increase in various growth attributes of both rice varieties associated with one of the isolates i.e., N4 was noticed. Furthermore, the N4 isolate was tested for its role in improving the agronomic attributes of sunflowers under drought stress. The symbiotic association significantly ($p < 0.05$) improved the host growth and protection from PEG-induced drought stress. The drought mitigation in N4-associated sunflower seedlings can be linked with the regulation of phytohormones, stress-related metabolites, low-molecular-weight proteins and sugars, and scavenging of reactive oxygen species (ROS). Microscopic analysis revealed that the potent endophytic fungal strain consisted of thin and hyaline hyphae, forming dense olive-green mycelia (4–5 cm in dm) with black flask-shaped fruiting bodies. Based on the ITS sequence homology and phylogeny, the strain was identified as *Fusarium proliferatum* (MG251448). The results of this study concluded that this phytohormone-secreting endophyte can improve crop productivity in dry areas where drought stress is the main challenge faced by crops.

Keywords: drought; *Fusarium proliferatum*; sunflower plants; phytohormones; biostimulant

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1. Introduction

Food security is a great challenge for agriculturists due to increasing population [1] and environmental stresses such as drought stress [2–5]. Drought stress can negatively affect a plant species right from germination until production [6,7]. This stress in combination with other abiotic stressors can inhibit growth, biomass production, and yield by interfering with a variety of physiological, structural, and biochemical attributes of the plant species [8,9]. Physiological changes lead to the destruction of the plant cell membrane and cell wall structure, causing electrolyte leakage, and inhibition of cell division and photosynthesis [10]. Also, chlorophyll concentration declines significantly during drought due to chlorophyll degradation and photo-oxidation of plant pigments [11]. It was noticed previously that induced water-deficient conditions can negatively affect percent germination rate and early seedling development in plant species [12]. Additionally, polyethylene glycol is one of the best options to serve as an effective surrogate to mimic drought stress in plants, including rice and sunflowers [13].

The majority of endophytes are symbiotic bacteria and fungi that live within plant bodies and promote plant growth under stressful situations [14–18]. Research on plant–microbe

interactions relies heavily on endophyte-mediated plant stress responses [19–21]. In fact, the stimulation of stress response genes and the control of reactive oxygen species (ROS) are the two components that direct endophyte-mediated stress responses [22]. Fungal endophytes can influence mineral content, phytohormone balance, root exuded metabolites, and soil texture to promote host-plant development under stress [23]. To reduce the detrimental effects of drought on commercially significant plant species, the use of endophytes is the environmentally viable solution [24]. Sunflower (*Helianthus annuus*) is the fourth-largest oilseed crop on Earth and its cultivation is growing day by day. In Pakistan, it is grown over an area of 319,743 acres, which yields 420,487 tonnes per year [25]. Although sunflower has the capacity to tolerate the effects of drought to some extent, exposure to continuous stress severely affects its vigor and production [26].

Drought not only limits food production, it also lowers water quality, aggravates soil erosion, and increases the spread of disease, fire, and flooding. According to the 2018 United Nations report, global drought issues impacted the lives of 55 million people [27]. For instance, the estimated financial losses in the United States of America during 2014 and 2016 due to drought were 5.3 and 0.57 billion dollars, respectively [28]. To solve this issue, several approaches (development of drought-resistant cultivars, genetically engineered crops, adjusting crop plant calendar, and wise management of resources) have been adopted over the years; however, implementation of such approaches are tiring and time consuming. Moreover, the use of genetically modified plants is generally unacceptable, demands high investment in terms of money and time, and the desired genes of the host plants may be lost [29].

Certainly, global agricultural practices are focusing on sustainability, food security, and environmental protection; therefore, the use of plant symbiotic microbes from nature may be desirable. The overwhelming potential of plant growth-promoting endophytic fungi for drought tolerance has been tested in the current study. We believe that endophytic fungi might help in controlling the issues of crop production in drought-affected areas. In the present study, it was aimed to; (a) isolate and identify a fungal endophyte from *Rhazya stricta* with multi-trait plant growth-promoting activities; (b) inoculate the sunflower plants with selected potent fungal endophyte(s) to evaluate their role in the mitigation of drought stress.

2. Materials and Methods

2.1. Collection of Plant Samples

Rhazya stricta plants were collected from the Tehsil Takht-e-Nasratti in the district of Karak for the isolation of endophytic fungal species. The selected plants were dug out in whole, wrapped in sterilized bags, tagged, and transferred to the Plant Microbe Interaction Laboratory, Department of Botany, Abdul Wali Khan University Mardan-Pakistan. To minimize the possibility of infection, the samples were either evaluated right away or held at 4 °C overnight for later processing.

2.2. Isolation and Screening of Endophytic Fungi

The plant samples were first washed with tap water to eradicate dust and sand particles and were then superficially disinfected with 70% ethanol for 30 s. Traces of ethanol from the disinfected plants were removed by washing with sterilized distilled water (ddH_2O). Superficially disinfected roots of plants were sliced thinly with a sterile blade (size = 1–2 cm). For the isolation of fungal endophytes, eight to ten wounded root segments were cautiously placed in Petri dishes containing Hagem medium. The plates were covered with sterile polyethylene bags and incubated at 28 ± 2 °C for 7 days. After a week of incubation, morphologically different fungal colonies were re-cultured on potato dextrose agar (PDA) plates and the process was repeated until a single colony was obtained [30].

2.3. Isolation and Estimation of Metabolite Production

For the collection of secondary metabolites, the fungal isolates were inoculated in a flask containing Czapek broth and incubated in a shaking incubator at 30 °C and 120 rpm for 7 days [31]. The fungal culture filtrate and biomass were separated by passing the contents of the flask through a filter paper. The fungal culture filtrate (CF) was stored at 4 °C until further analysis.

2.4. Screening of IAA- and GAs-Producing Fungal Strains

The isolated endophytic fungal strains (21 species) were initially screened on the gibberellins (GAs) deficient *Waito-C* rice cultivar to check the plant growth-promoting capabilities of the isolated fungal CF. Prior to the experiment, rice seeds were superficially disinfected for 1 min with 70% aqueous ethanol, and traces of ethanol were removed by washing the seeds with ddH_2O. The seeds were then incubated for 4 days at 28 °C to germinate. After incubation for 4 days, the uniformly sized seedlings were transferred to pots containing 0.8% *w*/*v* water agar medium [31]. The pots were placed in LabTech's growth chamber (Model; LGC-5101 G) under controlled conditions (i.e., photoperiod of day/night cycle, 14 h–28 °C ± 0.2; 10 h–25 °C ± 0.2; relative humidity 65–70%) for 7 days. Approximately 3 mL of CF was then applied as foliar spray to the rice seedlings with or without drought stress (PEG was used to induce drought stress). The root and shoot lengths, fresh and dry weights of rice seedlings from drought, and −ive (DDW) and +ive (Czapeck broth) control treatments were measured after 7 days. The fungal CF that performed well under the drought-stressed conditions was selected for further analysis.

2.5. Quantification of GAs and IAA in Culture Broth

Bioactive endophytes were inoculated in 120 mL of Czapek broth for 7 days at 30 °C (shaking incubator at 120 rpm) in order to estimate GAs in the fungal CF [31]. GAs were extracted and purified from the CF as described by Hamayun et al. [32]. Prior to partitioning, deuterated internal standards of GAs (20 ng; [17, 17-2H2] GA1, GA3, GA4, GA8, GA12, GA15, GA19, GA20, and GA24) obtained from Prof. Lewis N. Mander of the Australian National University in Canberra, Australia were mixed with the CF. The CF was then subjected to gas chromatographic/mass spectrometry for the detection and measurement of GAs. The data were calculated in ng/mL and the analysis was repeated three times.

High-performance liquid chromatography (HPLC) was used for the analysis of IAA in the supernatant of the potent fungal isolate. The fungal supernatant (20 mL) was loaded on a 5 mm reverse phase column (mBondapak C18, 250 mm × 4 mm) with the help of an HPLC micro syringe. The sample was fractioned under isocratic conditions with methanol and water (80:20 *v*/*v*) as a mobile phase at a flow rate of 1.0 mL/min. Eluates were detected by a differential ultraviolet detector at 254 nm. Pure IAA was used as a standard to quantify IAA in the fungal supernatant [33].

2.6. Extraction and Quantification of ABA in Culture Broth

ABA was extracted and quantified from fungal isolates by the method of Cohen et al. [34]. Briefly, fungal isolates were grown in Czapeck media for seven days and the obtained pure culture filtrate was then supplemented with [(+)-3, 5, 5, 7, 7, 7 d]-ABA as an internal standard. The sample was then subjected to gas chromatography–mass spectrometry with selected ion monitoring (GC-MS/SIM) and ABA was quantified.

2.7. Molecular Identification of Fungal Endophyte

Using the Khan et al. [35] standard procedure, genomic DNA was isolated from fungal isolates. Selected endophytic fungal isolates were identified by amplifying their ITS region of

18 S rDNA with universal primers, ITS-1 (5′-TCC GTA GGT GAA CCT GCG G-3′) and ITS-4 (5′-TCC TCC GCT TAT TGA TAT GC-3′). The gene sequence homology of the ITS

region of allied fungi was compared using the NCBI BLAST tool (http://blast.ncbi.nlm.nih.gov) accessed on 4 August 2022.

The closely related sequences obtained were aligned through CLUSTAL W using MEGA version 7.0 software [36]. The maximum parsimony tree was constructed using the same software. The bootstrap replications (1000) were used as a statistical support for the nodes in the phylogenetic tree.

2.8. Assessment of Potent Fungal Endophytes in Alleviation of Drought Stress in Host

The symbiotic association between the fungal endophyte and host plant species was examined by setting up an experiment with a completely randomized design. The experimental setup was divided into 4 groups:

- Group 1: Sunflower + 1/2 strength of Hoagland's solution + 0% PEG
- Group 2: Sunflower + 1/2 strength of Hoagland's solution + endophytes + 0% PEG
- Group 3: Sunflower + 1/2 strength of Hoagland's solution + endophytes + 8% PEG
- Group 4: Sunflower + 1/2 strength of Hoagland's solution + 8% PEG + no endophytes

The bioactive endophytic fungal strains (based on the results obtained by screening bioassays and biochemical characterizations) were grown in Czapek broth (50 mL) as mentioned previously. Similarly, disinfected sunflower seedlings of the same size and vigor were shifted to pots (containing 300 g/pot of autoclaved sand). Fungal biomass and half-strength Hoagland's solution were added to the seedlings in the group 2 and 3 pots. The control plants received only 50 mL/pot of autoclaved distilled water with half-strength Hoagland's solution. The fungal-associated and non-associated sunflower plants were allowed to grow for twelve days in an environmentally controlled growth chamber (photoperiod: 14 h/10 h, temperature: 28–25 °C ± 0.3; humidity levels 65–70%). To induce drought, sunflower plants from group 3 and 4 received 8% PEG solution (50 mL/pot) at an interval of 3 days. The agronomic attributes, i.e., root and shoot lengths, and fresh and dry weights were noted after harvest of the seedlings. A chlorophyll meter (SPAD-502 Minolta, Japan) was used for the determination of total chlorophyll content. The chlorophyll a, chlorophyll b, and carotenoid contents were estimated according to the protocol of Maclachlan and Zalik [37]. The dry weights of the roots and shoots of the sunflower seedlings were measured after drying in an oven at 70 °C for 72 h. For the evaluation of accumulated metabolites, the samples were macerated in liquid nitrogen, lyophilized in a Virtis Freeze Dryer (Gardiner, NY, USA) and stored at −80 °C until further use.

2.9. Phenolics, Flavonoids, Proline, Antioxidants, and Lipid Peroxidation

Total phenolic contents were determined by following the protocol of Castrica et al. [38]. The total flavonoids were estimated by the aluminum chloride ($AlCl_3$) colorimetric method [39]. Proline determination was carried out by the method of Bates and medicine [40]. The antioxidant activity was evaluated using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) and, 2-Azino-Bis-3-Ethylbenzothiazoline-6-Sulfonic Acid (ABTS) assays [41,42]. Lipid peroxidation was estimated by measuring malondialdehyde (MDA) content according to the method of Reiznerová et al. [43]. MDA was measured by 2-thiobarbituric acid (TBA) assay [44].

2.10. Light and Scanning Electron Microscopy

Sunflower roots inoculated with fungal endophyte were surface-sterilized as described previously. The roots were then treated with 20% KOH (10 mL) for 24 h. After 24 h, the roots were rinsed with distilled water, followed by acidification with 10% HCl (10 mL). The roots were dyed with 0.4% trypan blue and 95% lactic acid for the next 24 h and finally treated/bleached with 95% lactic acid for the next 24 h [45]. The fragments were then studied under compound light microscope (IM-910 Germany). For scanning, fungal hyphae and spores from slide culture were prepared by the standard methods of Ketjarun et al. [46]. Fungal hyphae were transferred from agar block to metallic stub, coated with gold (150 Å or 10^{-12} m) in a sputter chamber (Ion-sputter JFC-1100), and observed under scanning

electron microscopy (Jet microscope JSM-T200). The microstructure of sunflower roots from all treatments was analyzed to verify the fungal endophyte colonization in the host plants under drought (0.4%) and normal conditions. The spore characteristics of the fungi—such as shape, hypha structure, and sporangia—were also studied.

2.11. Statistical Analysis

Means of different treatments were compared for significance through analysis of variance (ANOVA) and the Duncan test as a post-hoc test. The software SPSS for Windows (Ver 20.0) was used for statistical analysis.

3. Results

3.1. Effect of Fungal CF on Waito-C Rice Growth

A total of 60 root pieces of *R. stricta* were inoculated on Hagem medium, out of which 57 were colonized by endophytic fungi. On the basis of colonial shape, base color, aerial mycelium height and coloring, surface characteristics, growth rate, and depth of growth into medium, a total of 21 distinct fungal endophytes were isolated. The potential of the isolated endophytes to promote growth of gibberellin-deficient mutant rice (*Waito-C*) was examined. A substantial growth enhancement was noticed in *Waito-C* rice seedlings of all treatments after applying 10 µL of fungal culture filtrate to the apices (Table 1 and Figure 1). Although all the strains significantly (p = 0.05) promoted root and shoot lengths, root and shoot weights, and chlorophyll content, the strain N4 was the most potent and was selected for further analysis (Table 1 and Figure 1).

Table 1. Growth-promoting effect of culture filtrate of different endophytic fungal isolates on *Waito-C* seedlings.

Fungal Isolates	Shoot Lengths (cm)	Root Lengths (cm)	Fresh Weights (g/Seedling)	Dry Weights (g/Seedlings)	CC (SPAD)	Growth Status
Control	5.92 ± 2.27	6.67 ± 2.98	0.07 ± 0.02	0.004 ± 0.003	35.60 ± 13.15	
CZPK	6.50 ± 0.45	6.58 ± 1.68	0.06 ± 0.01	0.006 ± 0.003	34.70 ± 9.09	
1-1-1	7.50 ± 0.71	6.00 ± 1.05	0.08 ± 0.03	0.010 ± 0.003	35.10 ± 9.23	Promoted
1-1-2	7.42 ± 1.53	7.00 ± 2.60	0.08 ± 0.02	0.012 ± 0.003	32.36 ± 4.20	Promoted
1-1-3	8.25 ± 1.60	8.25 ± 3.19	0.11 ± 0.02	0.011 ± 0.003	32.23 ± 7.54	Promoted
1-1-4	7.92 ± 0.86	5.83 ± 2.40	0.12 ± 0.02	0.010 ± 0.003	24.83 ± 1.09	Promoted
1-2-2	7.50 ± 0.84	7.67 ± 2.25	0.05 ± 0.04	0.011 ± 0.003	27.13 ± 4.17	Promoted
1-2-3	7.50 ± 1.22	8.17 ± 2.46	0.08 ± 0.03	0.010 ± 0.003	26.30 ± 3.33	Promoted
1-2-4	7.58 ± 0.80	5.75 ± 1.92	0.11 ± 0.03	0.010 ± 0.003	30.33 ± 10.88	Promoted
1-2-5	7.92 ± 1.32	7.33 ± 1.13	0.12 ± 0.02	0.011 ± 0.003	28.23 ± 3.76	Promoted
1-3-1	7.90 ± 1.14	7.67 ± 1.66	0.11 ± 0.03	0.011 ± 0.003	43.73 ± 2.11	Promoted
1-3-2	7.67 ± 0.41	6.42 ± 2.54	0.08 ± 0.02	0.011 ± 0.003	43.90 ± 2.22	Promoted
1-3-3	8.50 ± 1.00	7.25 ± 1.04	0.07 ± 0.01	0.011 ± 0.003	42.07 ± 1.19	Promoted
1-4-2	7.58 ± 1.07	7.75 ± 3.25	0.06 ± 0.01	0.011 ± 0.003	42.70 ± 3.48	Promoted
1-4-6	7.50 ± 0.84	7.67 ± 2.25	0.05 ± 0.04	0.011 ± 0.003	53.26 ± 5.49	Promoted
1-5-2	8.33 ± 0.75	8.00 ± 3.03	0.05 ± 0.01	0.012 ± 0.003	57.20 ± 5.05	Promoted
1-5-3	7.83 ± 0.52	6.42 ± 1.36	0.08 ± 0.02	0.011 ± 0.003	31.40 ± 9.28	Promoted
2-1-1	8.42 ± 0.80	8.42 ± 2.28	0.07 ± 0.03	0.012 ± 0.003	34.63 ± 6.40	Promoted
2-1-2	8.42 ± 0.97	9.33 ± 2.44	0.04 ± 0.02	0.011 ± 0.003	44.10 ± 3.19	Promoted
2-1-3	8.42 ± 0.58	8.33 ± 0.82	0.07 ± 0.02	0.012 ± 0.003	45.76 ± 4.94	Promoted
2-2-1	7.50 ± 1.22	8.17 ± 2.46	0.08 ± 0.03	0.010 ± 0.003	26.07 ± 3.09	Promoted
2-2-2	7.58 ± 1.07	7.75 ± 3.25	0.06 ± 0.01	0.011 ± 0.003	30.50 ± 2.18	Promoted
3-1-1	7.67 ± 0.75	7.67 ± 2.80	0.06 ± 0.01	0.011 ± 0.003	29.06 ± 6.38	Promoted
3-1-2	8.00 ± 0.84	8.08 ± 2.41	0.13 ± 0.03	0.011 ± 0.003	30.50 ± 6.87	Promoted
3-1-3	8.50 ± 1.14	6.83 ± 1.91	0.12 ± 0.02	0.011 ± 0.003	33.90 ± 10.79	Promoted
3-2-2	8.08 ± 1.39	6.67 ± 2.66	0.09 ± 0.04	0.011 ± 0.003	31.10 ± 6.09	Promoted
3-3-2	7.67 ± 1.03	6.08 ± 2.13	0.14 ± 0.02	0.011 ± 0.003	27.93 ± 4.08	Promoted
3-4-1	7.92 ± 0.58	5.50 ± 1.30	0.14 ± 0.03	0.011 ± 0.003	46.93 ± 0.98	Promoted
3-5-2	8.00 ± 0.63	7.33 ± 2.42	0.04 ± 0.01	0.011 ± 0.003	46.03 ± 2.02	Promoted

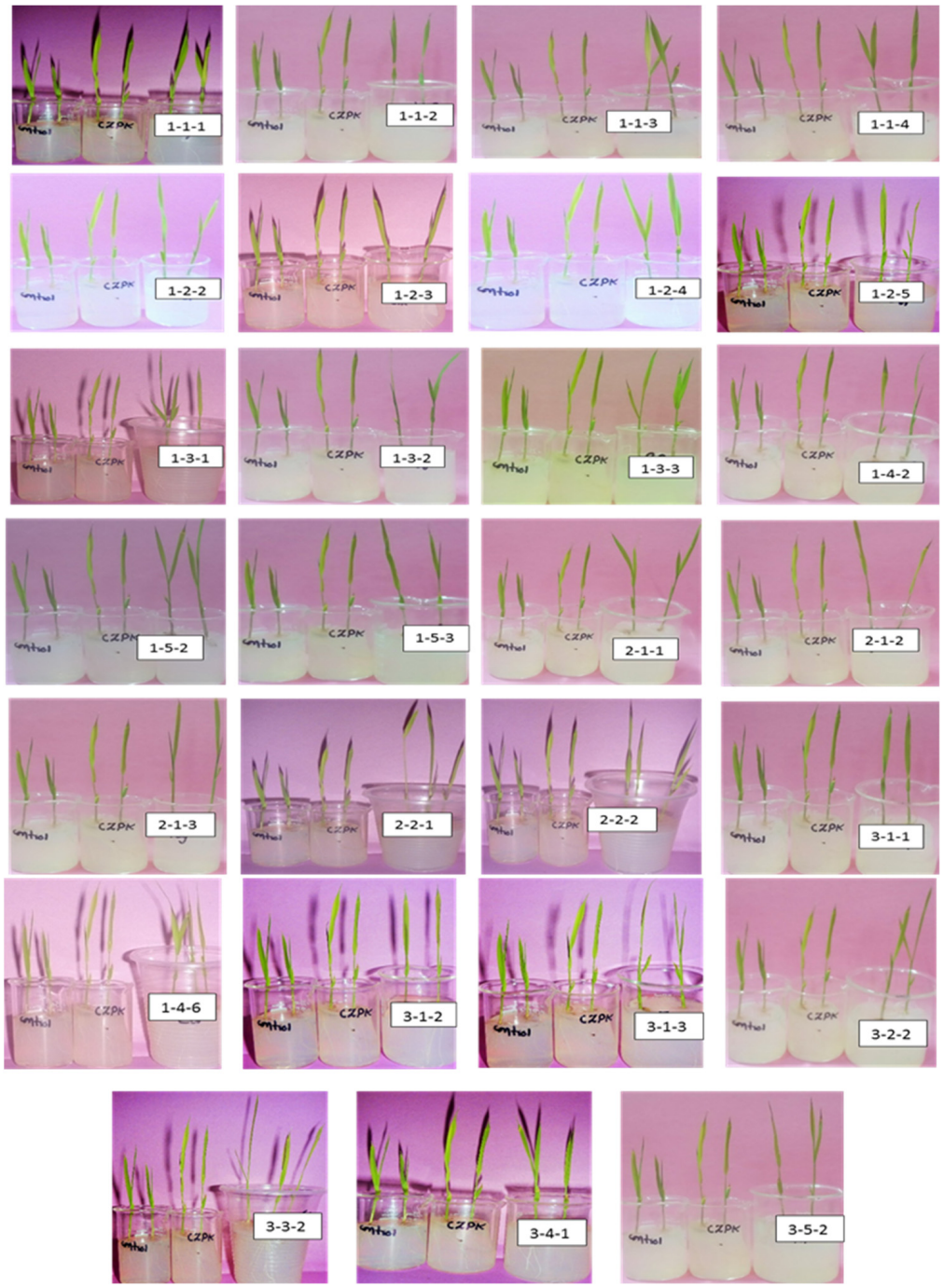

Figure 1. Screening bioassay of fungal culture filtrates on *Waito-C* rice seedlings. Twenty-seven fungal isolates were screened; all had a growth-promotion effect on seedlings (after 7 days of incubation), after being treated with 10 µL of concentrated culture filtrates of respective fungi.

3.2. Screening of Potential Fungal Endophytes for Phytohormones

The culture filtrate of N4 showed the presence of physiologically active GAs in appreciable amounts. The concentration of GA1 in CF of N4 was 1.321 ng/mL, GA3 was 1.101 ng/mL, and GA4 was 1.696 ng/mL. Among physiologically inactive GAs, GA12 (2.993 ng/mL), GA15 (0.218 ng/mL), and GA20 (0.473 ng/mL) were the most prominent ones in the CF of N4. Bioactive GA4 and GA12 were present in much greater amounts than the other GAs. In addition to GAs, IAA and ABA were also present in higher amounts. The

amount of IAA was 0.340 ± 1.21 µg/mL, while that of ABA was 10.27 ng/mL in the CF of N4.

3.3. Identification and Phylogenetic Analysis of a Bioactive Endophyte

According to the results of a BLAST search, *Fusarium proliferatum* and fungal strain N4 have a 48% sequence identity with 48% bootstrap support in MP dendrogram (Figure 2). Based on phylogenetic analysis and sequence similarities, the N4 strain was identified as *F. proliferatum* KU847857.1. The sequence was submitted to the NCBI GenBank under accession number MG251448.

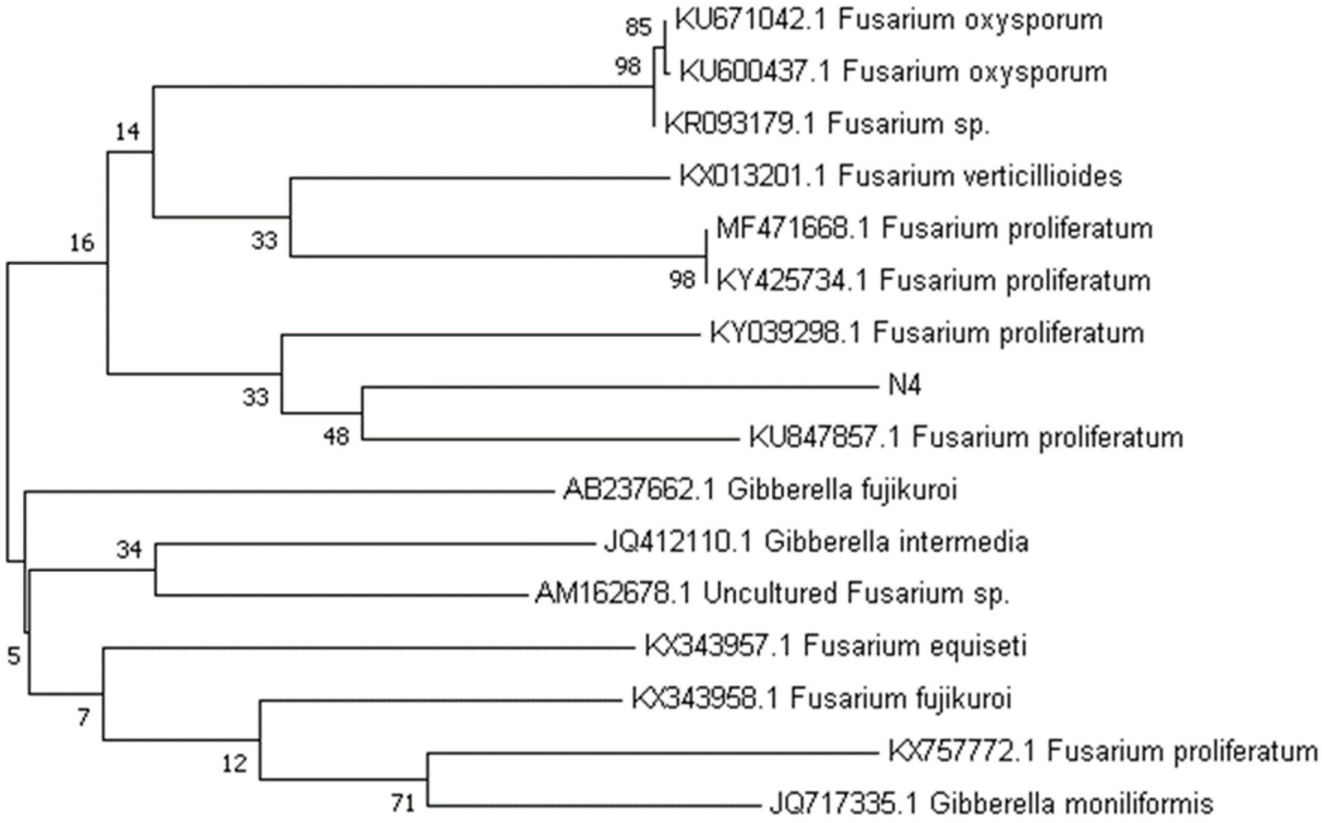

Figure 2. Identification of fungal isolate N4 by phylogenetic analysis. Neighbor joining tree (550 bootstrap replications) was constructed using 16 taxa. Bootstrap support of 48% for subclade of N4 with *Fusarium proliferatum* strongly suggests this fungal isolate as a new strain of *Fusarium proliferatum* (KU847857.1).

3.4. Effect of F. proliferatum Association on Sunflower Growth in Drought Stress

3.4.1. Fresh and Dry Weight

The PEG-induced drought provoked a substantial dose-dependent reduction ($p < 0.0$) in biomass (fresh/dry) of the host plants. After inoculating the host plants with selected endophytes, a prominent increase in the growth attributes were noticed in plants under drought stress as well as in unstressed plants (Figure 3). Plants treated with *F. proliferatum* (22.64 ± 2.57) exposed to 8% PEG stress had an equal increase in shoot length as compared to the control plant. Similarly, a 34% increase in dry weight was noted in fungal-associated plants treated with 8% PEG.

Figure 3. Effect of endophytic *F. proliferatum* on the growth of sunflower seedlings under normal (optimum water) and drought (8% PEG) conditions. Seedlings were grown for 12 days and then exposed to different treatments of 8% PEG at three-day intervals.

3.4.2. Chlorophyll Content (a, b)

Both chlorophyll a (16.80 ± 0.85) and chlorophyll b (11.51 ± 0.58) were found in higher concentrations in sunflower plants inoculated with *F. proliferatum* and exposed to drought stress as compared to the control plants (chlo a 3.60 ± 0.19 and chlo b 2.49 ± 0.17).

3.5. Phenolics, Flavonoids, Proline, Antioxidants, and Lipid Peroxidation

The amount of TPC decreased with the exposure of PEG (8%) in the medium. However, inoculation of *F. proliferatum* tended to enhance the production of TPC in sunflower seedlings exposed to normal and drought-induced conditions (Figure 4a). The amount of TFC in non-associated sunflower plants (31.93 ± 1.56) under stress was very low as compared to the plants associated with *F. proliferatum* (39.53 ± 2.88) (Figure 4b). A similar result was also recorded for total proline production, where a significant (p = 0.05) increase in the production of proline (13.08 ± 0.82) was observed in the host plants treated with *F. proliferatum* and exposed to drought stress (Figure 4c). In the ABTS and DPPH assays, the antioxidant activity decreased when the samples were treated with fungus alone (Figure 4d). *F. proliferatum* (719.63 ± 1.49) showed lower free radical scavenging ability as compared to the control (734.41 ± 1.10). The sunflower plants inoculated with *F. proliferatum* (237.35 ± 2.91) under drought stress produced the lowest amount of MDA as compared to the non-inoculated plants (281.07 ± 1.81) (Figure 4e).

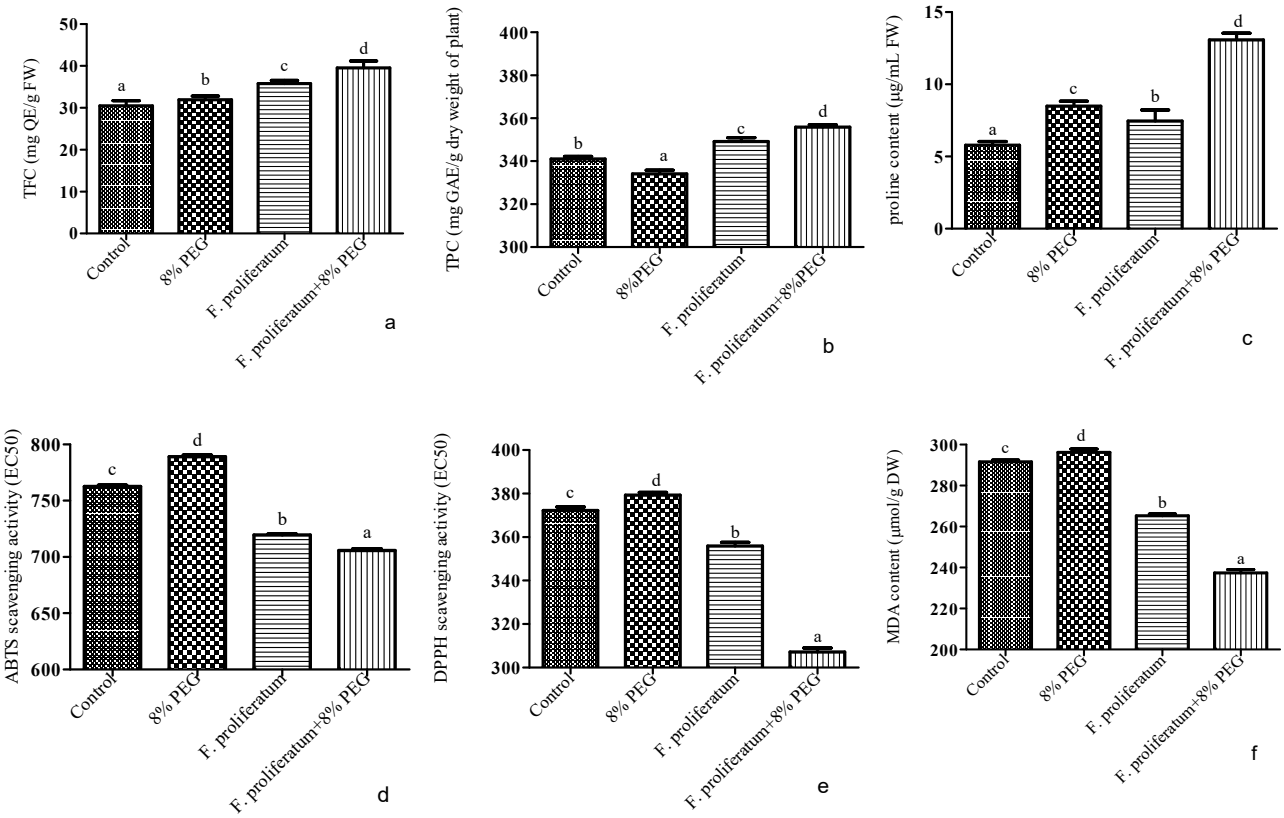

Figure 4. Effects of *F. proliferatum* on (**a**) TFC, (**b**) TPC, (**c**) proline, (**d**) ABTS, (**e**) DPPH, and (**f**) MDA content in sunflower seedlings grown under normal and drought conditions for 21 days. Bars represent mean of triplicates with SE±. The alphabets represent significance at the level of $p < 0.05$.

3.6. Light and Scanning Electron Microscopy

The septate hyphae of fungal isolate N4 (Figure 5) appeared to be coiled with curved micro-conidia when examined under light and electron microscope (Figure 5). A light pink woolly mycelium of about 7–8 cm producing light to deep purple pigments was noticed under light and electron microscope.

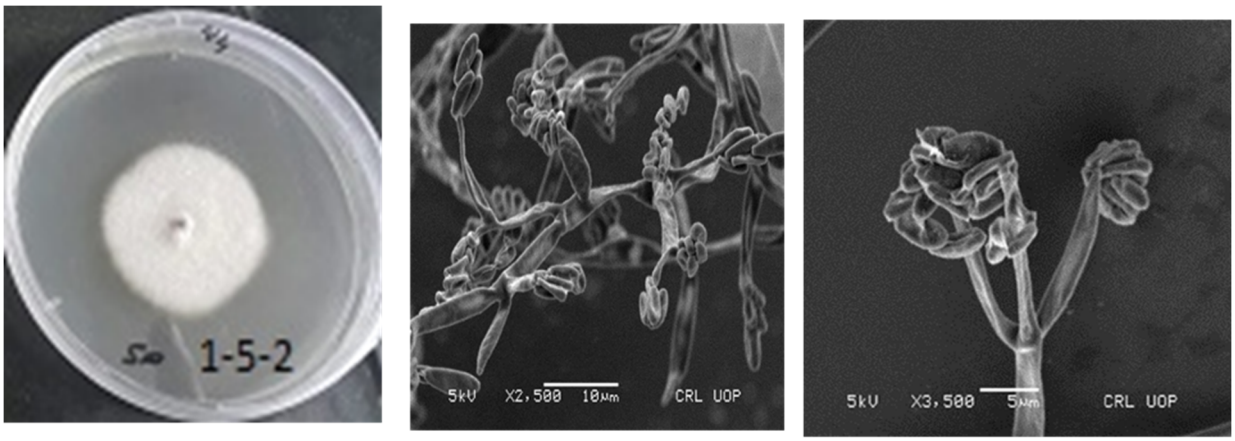

Figure 5. Selected fungal endophytes showing their mycelia, hyphae, conidiophore, and conidia/spores.

4. Discussion

In the current project, the CF of endophytic fungus *Fusarium proliferatum* N4 was screened on gibberellins (GAs) deficient rice (*Waito-C* rice) at the two leaf stage. The assay indicated that the fungus was able to promote growth of *Waito-C* rice by contributing GAs. *Waito-C* rice can't convert GA20 to bioactive GA1 because it lacks GA3 β-hydroxylase (3-oxidase) enzyme. Hence, this rice mutant can be used as an effective surrogate for screening GA-producing and plant growth-promoting endophytic fungi [47]. The ability of the isolated fungal endophyte to secret GAs was further confirmed by HPLC analysis. The fungus also produced another important plant growth-promoting hormone, IAA. Besides their role in plant growth promotion, these phytohormones are also a key determinant in plant drought responses. In the past, the overproducing auxin phenotypes of poplar and potato have shown great resistance to drought stress [48,49]. In fact, the improved root system of the plant may improve the plant's efficiency in absorbing soil solution in drought-affected areas [50]. Moreover, studies on sunflower have shown that GAs are important in drought stress tolerance, as these hormones improve proline biosynthesis and ROS scavenging [51].

Besides rice, the IAA- and GAs-producing endophytic *F. proliferatum* also improved sunflower growth under drought stress. The capacity of endophytes to improve host growth and quality through secretion of plant growth-promoting hormones makes them of significant interest. Likewise, endophytic fungus plays a significant role in protecting host plants by inducing tolerance in them against various biotic and abiotic stresses. As discussed above, production of IAA and GAs by endophytes may help the sunflower to attain normal growth under drought stress. Production of the phytohormones by the endophytes not only promoted the host growth attributes but also enhanced the ameliorative capacity under water-deficient conditions [52,53]. Previously, it was noticed that some important endophytic fungal strains such as *F. oxysporum*, *A. niger*, *P. cyclopium*, *R. stolonifer*, *P. corylophilum,* and *P. funiculosum* secrete IAA and GA to considerable levels to help their hosts under stressful conditions [54–56].

Our results also confirmed the results of previous research showing that the shoot growth of rice cultivars can be stimulated by the action of plant growth-promoting hormones and secondary metabolites released by endophytic fungi [57–59]. Secondary metabolites of the fungal endophytes might be generated to establish a symbiotic relationship with the host plant species [60]. Apart from that, the endophytes also trigger the host roots to produce substantial quantities of antioxidants that can actively scavenge free radicals. By doing this, it ensures maintenance of normal cell conditions, thereby keeping cell viability and preventing oxidative damage [61,62]. Fungal endophytes found in the root tissues of host plants can sequester substances that control plant development. Such growth-regulating substances can help plant species to flourish under challenging conditions. Sunflower, like most other plants, is highly sensitive to salinity [63,64]. Recent research revealed that injecting *F. Proliferatum* dramatically enhanced host plant development under saline conditions [65]. Since both IAA and GAs stimulate plant growth and development, their existence in the fungus's CF significantly corroborates our findings. The potential involvement of *F. proliferatum* in the host plants is strengthened by its existence in the cortex cells. *F. proliferatum's* mycorrhizal relationships with sunflower plants may have assisted the host plant in reducing the negative consequences of drought stress [66].

5. Conclusions

Fusarium proliferatum had significantly enhanced plant growth-promoting attributes in sunflower as compared to control plants. The symbiotic association of *F. proliferatum* improved the host growth and its ability to withstand high drought-stress conditions. Acclimatization of host plants to drought stress can be linked with the drought-related metabolites and phytohormones released by *F. proliferatum*. The released metabolites and phytohormones might help in reprogramming of the host antioxidant system to manage

the drought. Thus, this fungus can be used as a plant biostimulant drought-stress reliever in areas exposed to severe drought conditions.

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