J. Appl. Environ. Biol. Sci., 8(3)95-101, 2018

ISSN: 2090-4274
Journal of Applied Environmental and Biological Sciences
www.textroad.com

Endophytic Fungi Promote Growth of *Zea Mays* L. Under PEG Induced Drought Stress

Nighat Seema[a], Muhammad Hamayun[a,*], Anwar Hussain[a], Mohib Shah[a], Muhammad Waqas[b] and Ayaz Ahmad[c]

[a]Department of Botany, Abdul Wali Khan University Mardan 23200, Pakistan
[b]Department of Agriculture Extension, Govt of Khyber Pakhtunkhwa Buner 19290, Pakistan
[c]Department of Biotechnology, Abdul Wali Khan University Mardan 23200, Pakistan

Received: November 5, 2017
Accepted: January 31, 2018

ABSTRACT

Plants are sometimes exposed to extreme environmental conditions. Drought stress is one of the major abiotic constraint limiting crop growth and productivity worldwide. It is reported that endophytic fungi can benefit the host plants under biotic and abiotic stress conditions. In the current study, we isolated 46 endophytic fungi from three xerophytes and screened them on *Waito*-C rice, gibberellins (GAs) deficient dwarf rice cultivar, for their growth promoting capacity. It was observed that 27 fungal isolates promoted growth attributes of *Waito*-C rice. These growth-promoting fungal endophytes were further bio-assayed on maize (*Zea mays*L.) under normal and 8% polyethylene glycol (PEG) induced drought stress. The fungal endophytes promoted maize growth attributes and chlorophyll contents. The best results were recorded for isolates 1-3-1, 1-5-2, 2-1-1, 2-1-2, 3-1-2 and 3-1-3.Based on our finding, it was concluded that endophytic fungi can ameliorate the adverse effect of drought stress on the growth of plants growing in dry environments.
KEYWORDS: Drought stress; Endophytic fungi, Plant growth; Maize.

INTRODUCTION

The plants of almost all the species of the Plantae kingdom have shown that they are inhabited by endophytic microbes. These unique associations may have positive effects on plant growth and tissue differentiation, as well as on biotic and abiotic stresses to which plants are exposed without any damage[1]. In addition, endophytic fungi can help plants providing them nutrients and make them immune to pathogenic microbes[2]. Being very diverse in distribution and association, these endophytes are a rich source of biologically active secondary metabolites[3]. In fact, in recent years, many biologically active compounds such as anticancerous, cytotoxic, antimicrobial and antioxidant compounds have been isolated from endophytic fungi[4].

Plants are sometimes exposed to extreme environmental conditions. Drought stress is one of the major abiotic constraint limiting crop growth and productivity worldwide. Fungal endophytes are asignificantly important component of terrestrial ecosystems, which enable host plants to flourish in severe environments. Studies show that fungal endophytes can enhance tolerance of their host plant and also increase resistance against a variety of other stressors[5]. By influencing plant morphology, development, and physiological and biochemical responses to stress, fungal endophytes can induce mechanisms of drought tolerance in their hosts[6].

Plants and fungi in the natural ecosystem have a symbiotic relationship. Plants are subjected to many stresses (biotic and abiotic stresses) in their natural environment, and commensal fungi contribute to and adapt plants to these environmental stresses[7]. These fungi are important for the structure, function, and health of plant communities[8, 9]. Endophytic fungi are the major part of plant-associated fungal symbionts present in plant tissue and may be associated with roots, stems and/or leaves, without causing any disease symptoms[10]. These fungi can serve as a defender of predators[11], growth promoters[12] and competitors to microbial pathogens[13].The promotion of plant growth can be attributed to the secretion of secondary metabolites (gibberellin, auxins, cytokinins) by endophytic fungi in the rhizosphere[14, 15]. These fungi express symbiotic, parasitic and other symbiotic ways of life[16].

The resistance of host plants to drought stress has been known as a completely documented feature of abiotic stress tolerance in endophyte-infected grasses[6]. In fact, a few investigations have given confirmation that the endophyte can enhance tolerance to drought stress[17-19]. Variety of plants treated with endophytes, under low

***Corresponding Author:** Muhammad Hamayun, Department of Botany, Abdul Wali Khan University, Mardan 23200, Pakistan. Email: hamayun@awkum.edu.pk

water accessibility, indicated more prominent development and biomass formation[20], as compared to uninfected plants.

In general, drought stress occurs when available soil moisture decreases and atmospheric conditions cause transpiration or evaporation to cause loss of water[21]. *Otostegialimbata* (Benth) belonging to family *Lamiaceae* used to treat gum disease, dental disease, wound healing, high blood pressure, eye inflammation, and most importantly as an anti-cancer agent. Many plants belonging to the family *Labiatae* have been screened because they also have potential pharmaceutical uses for antimicrobial activity[22]. .*Gymnosporiaroyleana* (Wall) commonly called "surazghay" belonging to Celastraceae family while *Rhazyastricta* (Decne) is a common evergreen poisonous shrub of the Apocynaceae.

Waito-c rice is a known dwarf rice cultivar with reduced GA biosynthesis. Thus, shoot elongation of these seedlings can be effectively associated with the activity of plant-propagating secondary metabolites from the applied fungal culture filtrate. Recent studies have shown that plant growth promotion may be attributed to the secretion of gibberellins (GAs) by the endophytic fungi in the rhizosphere[23, 24].Application of polyethylene glycol-induced drought stress in combination with host plants and endophytic fungi significantly increased plant biomass and related growth parameters as compared to control plants. Thus, fungal endophytes promote the survival of their host plants under stress conditions by the secretion of favorable secondary metabolites. The purpose of this study was to investigate endophytic fungi that promote plant growth by producing secondary metabolites.

MATERIALS AND METHODS

Collection of Plant Samples

Two plants (*otostagialimbata* & *gymnosporiaroyleana*) were collected from the Kot Manzary baba Dargai, Malakand agency, Khyber Pakhtunkhwa, Pakistan. Malakand agency is located 341, 35 north latitude and 711, 57 east longitude. the plants collected were identified, dug out as a whole, sealed in sterile bags, labeled and transported to laboratory. the samples were processed immediately to reduce the risk of contamination. *rhazyastricta* was collected from tehsil Takht-e-nasratti, district Karak, Khyber Pakhtunkhwa, Pakistan. The total area of the tehsil is about 613.66km^2. Majority of the area consists of rigged dry hills and rough field areas.

Isolation and purification of fungal isolates from xerophytes

The plant samples were first washed with tap water than surface sterilized by 70% ethanol for 30 sec and washed with autoclaved double distilled water and make a thin slice of each surface sterilized part of the plant (size= 5mm) [25].

For fungal endophyte isolation, five to six plant segments (0.5 cm) of each part of the plants were carefully placed in Petri-plates, containing Hagem media (0.5% glucose, 0.05% MgSO4, 0.05% KH2PO4,.7H2O, 0.05% NH4Cl, 0.1% FeCl3, 80 ppm streptomycin and 1.5% agar; pH 5.6 ± 0.2). The sterilized plant segments were also imprinted on separate Hagem plates to ensure the effectiveness of surface sterilization[26, 27]. The Petri dishes were sealed with aluminum foil and incubated at 27±2^{0}C for a week under the dark condition and checked every day for the emergence of endophytic fungi.

The initially emerged fungal spots from the plant segments were isolated and subculture on potatodextrose agar medium (PDA) under sterilized conditions[27, 28].After purification, PDA medium plates/slants were inoculated by the fungal isolates, and finally, pure grown cultures were stored for further experiments.

For secondary metabolites production, the fungal isolates were inoculated on Czapek broth medium (pH 7.3±0.2) and incubated in a shaking incubator set at 30ºC with 120 rpm for 7 days. The composition of Czapek medium was: 1% Glucose, 1% Peptone, 0.05% KCl, 0.05% MgSO4.7H2O, and 0.001% FeSO4.7H2O[27].

Colonization Frequency

The colonization frequency of isolated endophytic fungi from root parts of the plants can be calculated following the established protocol[29, 30].

$$\% \text{ Colonization frequency} = \frac{\text{Total twig segments colonized}}{\text{Total number of segments in that sample}} \times 100 \quad (01)$$

$$\text{Dominant fungi } \% = \frac{\text{No.of isolates collected from the sample}}{\text{Total no.of twig segment in sample}} \times 100 \quad (02)$$

Screening for Plant Growth Promoting Capacity on Waito-C rice

Presence or absence of plant growth promoting metabolites in fungal CF was confirmed by performing screening bioassays on gibberellins biosynthesis deficient mutant rice Waito-C. Waito-C has a dwarf phenotype. For bioassay experiment, rice seeds were surface sterilized with 2.5% sodium hypochlorite for 30 minutes or 70% ethanol, rinsed with autoclaved DDW and then incubated for 4days to obtain equally germinated seeds[31]. Then pre-germinated

Waito-C seeds were transferred to small beakers having water: agar medium (0.8% w/v) under aseptic conditions[27]. The rice seedlings were grown in growth chamber (day/night cycle: 14 hr- 28°C ± 0.3;10 hr - 25°C ± 0.3; relative humidity 70%;) for 1 week. 10µl of fungal CF was applied at the apex of the rice seedlings. One week after treatment, the shoot length, chlorophyll content and shoot fresh weight were recorded and compared with negative (autoclaved DDW) and positive controls (Czapek broth).Upon screening results, bioactive fungal strains were selected for further experiments and identification.

Bioassay of Fungal Isolates on Zea Mays Growth under Drought Stress

Experiments were conducted with a completely randomized block design in order to assess the endophytic fungus relationship with host-plants. Experiments comprised of maize plants (*Zea mays* L, Azam variety), with (i) fungal inoculation, (ii) without inoculation, (iii) fungal inoculation with drought stress (8% PEG), and (iv) without inoculation and with drought stress. On the basis of results obtained in Waito-C rice, fifteen bioactive endophytic fungal isolates were selected to assess their effects in mitigating drought stress. Fungal isolates were inoculated in Czapek broth (50 ml) as described previously. Then sterilized pre-germinated maize seedswere grown in pots containing soil (300 g/ pot). The fungal mycelia and culture filtrate (20 ml for each pot containing ten propagules) were added to thepot containing soil. The control plants only received 20 ml/pot autoclaved distilled water(DDW). The endophytic fungi and maize plants were grown together for ten days in agrowth chamber (day/night cycle: 14 hr- 28°C ± 0.3;10 hr - 25°C ± 0.3; relative humidity 60-65%;12 plants per treatment) and irrigated with distilled water. After ten days, three doses of 8% PEG solution (20-50 ml/ pot) was applied to maize plants at three days intervals for one week in order to assess the effect of drought stress on these plants[32]. The growth parameters i.e. shoot length, root length, shoot fresh and dry weights and root fresh and dry weights were measured for harvested maize plants, while chlorophyll content of fully expanded leaves were analyzed[33]with the help of chlorophyll meter (SPAD-502 Minolta, Japan). Dry weights were measured after drying the plants at 70°C for 72 h in oven[33, 34].

RESULTS AND DISCUSSION

Isolation of Endophytic Fungi from Xeric Flora

We isolated 46 endophytic fungi from 160 root pieces of three xeric plants. These fungi were grown on Hagem media plates for seven days. The pure culture plates were grouped on the basis of colony shape, height and color of aerial hyphae, base color, growth rate, margin characteristics, surface texture and depth of growth into medium[5]. The morphological trait analysis reveals that 27 endophytic fungi were different.A maximum number of endophytic fungi were isolated from *Rhazyastricta* (21), with 95% infection rate. The colonization frequency of *O. limbata* and *G. royleana* was 95%, and 85% respectively. The dominant frequency of *R. stricta, O. limbata,*and*G. royleana* was 35%, 22% and 30% respectively (Table 1).Little attention has been given to endophytic fungal association in extreme environmental conditions because of their slow growth rate and strenuous handling, however endophytic diversity of these microbes is of great interest because of producing secondary metabolites. The microbial population decreases with an increase in the altitude[5]. Endophytic fungi interact with the host plant by producing secondary metabolites that induce resistance and protection against various pathogens. They protect the plant in drought as well as in cold climatic conditions[35].

Table 1: Dominant fungi (%) and colonization frequency (%) of the fungal isolates

Plant names	Fungal Isolates	Segments screened	Segments with fungi	Colonization frequency	Dominant fungi
Rhazyastricta	21	60	57	95%	35%
Otostagialimbate	13	60	57	95%	22%
Gymnosporiaroyleana	12	40	34	85%	30%

Screening of Fungal Isolates on Waito-C Rice

After careful removal of duplication, 27 endophytic fungi were selected from atotal of 46 isolates. The culture filtrate (10µl) of these endophytic fungi were applied on Waito-C rice seedlings, under growth chamber conditions. It gave significant growth promotion results, which was possibly induced by gibberellins present in fungal culture filtrate. The shoot & root lengths, fresh weights and dry weights of rice seedlings were checked and compared with positive and negative control (autoclaved distilled water, DDW) after one week of culture filtrate application. On the basis of shoot length, all the screened fungal isolates were growth promoter (Figure 3). Since Czapek medium contains nutrients, we used it as a positive control in order to observe the level of growth promotion imparted by it. The seedlings survived easily in water-agar media in the absence of nutrient solution and gave shoot elongation

results in 2 weeks. No two fungal isolates with similar morphological growth pattern and/or colonial morphology were selected to avoid unnecessary repetition (Table 2). Best growth promoter was selected for further study. We used screening bioassays of endophytic fungal culture filtrate in order to identify bioactive fungal strains because endophytic fungi have been known as an important source of various kinds of bioactive secondary metabolites[20]. It has been known recently that some of the strains of endophytic fungi can produce plant hormones especially gibberellins (GAs). GAs are ubiquitous substances that elicit various metabolic functions required for plants growth particularly shoot length. To characterize GAs secreted in the pure fungal culture of bioactive endophyte, it was inoculated in Czapek broth (50 ml) for 7 days at 30°C (shaking incubator-120 rpm) as described previously[25]. The filtrate and mycelium were separated by centrifugation (10,000rpm at 4°C for 10min). In screening bioassays, rice cultivars were used as rice can easily grow under controlled and sterilized conditions using an autoclaved water-agar hydroponic medium that can help in the assessment of culture filtrate obtained from endophytic fungi.

Table 2: Screening of fungal isolates on Waito-C for their plant growth promoting capacity

Fungal Isolates	Shoot length (cm/ plant)	Root length (cm/ plant)	Fresh weight (g/plant)	Dry weight (g/plant)	Growth status
Control	5.92±2.27	6.67±2.98	0.07±0.02	0.004±0.003	
CZPK	6.50±0.45	6.58±1.68	0.06±0.01	0.006±0.003	
1-1-1	7.50±0.71	6.00±1.05	0.08±0.03	0.010±0.003	Promoted
1-1-2	7.42±1.53	7.00±2.60	0.08±0.02	0.012±0.003	Promoted
1-1-3	8.25±1.60	8.25±3.19	0.11±0.02	0.011±0.003	Promoted
1-1-4	7.92±0.86	5.83±2.40	0.12±0.02	0.010±0.003	Promoted
1-2-2	7.50±0.84	7.67±2.25	0.05±0.04	0.011±0.003	Promoted
1-2-3	7.50±1.22	8.17±2.46	0.08±0.03	0.010±0.003	Promoted
1-2-4	7.58±0.80	5.75±1.92	0.11±0.03	0.010±0.003	Promoted
1-2-5	7.92±1.32	7.33±1.13	0.12±0.02	0.011±0.003	Promoted
1-3-1	7.90±1.14	7.67±1.66	0.11±0.03	0.011±0.003	Promoted
1-3-2	7.67±0.41	6.42±2.54	0.08±0.02	0.011±0.003	Promoted
1-3-3	8.50±1.00	7.25±1.04	0.07±0.01	0.011±0.003	Promoted
1-4-2	7.58±1.07	7.75±3.25	0.06±0.01	0.011±0.003	Promoted
1-4-6	7.50±0.84	7.67±2.25	0.05±0.04	0.011±0.003	Promoted
1-5-2	8.33±0.75	8.00±3.03	0.05±0.01	0.012±0.003	Promoted
1-5-3	7.83±0.52	6.42±1.36	0.08±0.02	0.011±0.003	Promoted
2-1-1	8.42±0.80	8.42±2.28	0.07±0.03	0.012±0.003	Promoted
2-1-2	8.42±0.97	9.33±2.44	0.04±0.02	0.011±0.003	Promoted
2-1-3	8.42±0.58	8.33±0.82	0.07±0.02	0.012±0.003	Promoted
2-2-1	7.50±1.22	8.17±2.46	0.08±0.03	0.010±0.003	Promoted
2-2-2	7.58±1.07	7.75±3.25	0.06±0.01	0.011±0.003	Promoted
3-1-1	7.67±0.75	7.67±2.80	0.06±0.01	0.011±0.003	Promoted
3-1-2	8.00±o.84	8.08±2.41	0.13±0.03	0.011±0.003	Promoted
3-1-3	8.50±1.14	6.83±1.91	0.12±0.02	0.011±0.003	Promoted
3-2-2	8.08±1.39	6.67±2.66	0.09±0.04	0.011±0.003	Promoted
3-3-2	7.67±1.03	6.08±2.13	0.14±0.02	0.011±0.003	Promoted
3-4-1	7.92±0.58	5.50±1.30	0.14±0.03	0.011±0.003	Promoted
3-5-2	8.00±0.63	7.33±2.42	0.04±0.01	0.011±0.003	Promoted

Bioassay of Fungal Isolates on *Zea Mays* Under Drought Stress

On the basis of screening results of *Waito*-C rice seedlings, fifteen bioactive endophytic fungi were selected to assess their effects in mitigating drought stress. Drought stress was induced by applying 8% PEG to plants. Three doses of drought stress were given at 3 days intervals. On the completion of the third dose, growth parameters i.e. shoot lengths, root lengths, plants fresh and dry weights were measured for harvested maize plants, while chlorophyll content of fully opened leaves was analyzed with the help of chlorophyll meter (SPAD-502 Minolta, Japan). Dry weights were measured after drying the plants at 70°C for 72 h in an oven. All the growth parameters were compared with the controlled plants receiving only autoclaved distilled water (DDW). Non-inoculated and stressed plants had lower shoot length (18.98±0.78) and dry weight (0.21±0.02). Fifteen fungal isolates were applied in order to assess their drought tolerance ability. Eight bioactive fungal isolates showed an increase in shoot lengths and dry weights and also enhance the chlorophyll contents of the plants under stress conditions (Table 3).

Table 3: Bioassay of endophytic fungal isolates on *Zea mays* L. under normal conditions

Fungal Isolates	SL(cm)	RL(cm)	PFW(g)	PDW(g)	Chl. (SPAD)
Control	21.40±1.01	12.53±0.94	1.32±0.16	0.24±0.03	35.60±13.15
1-1-2	20.00±1.73	17.57±1.69	1.80±0.27	0.25±0.03	35.10±9.23
1-1-3	19.47±1.24	16.90±0.96	1.65±0.10	0.25±0.02	32.23±7.54
1-1-4	21.53±1.12	17.00±2.01	1.53±0.13	0.23±0.02	27.13±4.17
1-2-3	23.62±0.90	19.08±1.69	2.02±0.31	0.24±0.03	30.33±10.88
1-3-1	**22.75**±1.19	**17.18**±1.32	**1.65**±0.10	**0.30**±0.02	**43.73**±2.11
1-3-3	**25.53**±1.48	**11.80**±0.92	**1.71**±0.17	**0.30**±0.02	**42.07**±1.19
1-5-2	**23.77**±1.82	**12.87**±1.64	**1.64**±0.24	**0.27**±0.03	**53.26**±5.49
1-5-3	21.67±2.29	15.67±1.51	1.55±0.09	0.24±0.02	31.40±9.28
2-1-2	**22.48**±1.85	**18.93**±1.25	**1.66**±0.22	**0.28**±0.03	**44.10**±3.19
2-1-3	24.75±1.76	11.83±0.89	1.75±0.22	0.28±0.03	26.07±3.09
2-3-1	20.52±1.82	12.73±1.14	1.14±0.09	0.20±0.03	29.06±6.38
2-3-2	22.85±1.91	10.80±1.74	1.43±0.16	0.26±0.01	33.90±10.79
3-1-1	23.32±1.09	13.55±1.30	1.30±0.09	0.26±0.04	28.60±4.77
3-1-2	24.20±2.41	12.35±0.75	1.47±0.20	0.27±0.04	28.77±2.22
3-1-3	**23.72**±2.50	**14.33**±1.42	**1.69**±0.07	**0.26**±0.02	**46.93**±0.98

Shoot length(19.13±2.11) and dry weight (0.21±0.03) of fungal isolate 1-1-2 was at par with the control (Table 4) under drought stress. On the basis of results obtained five fungal isolates, 1-3-3 (27.25±1.28), 3-1-3 (25.95±2.08), 2-1-2 (24.28±1.69), 1-5-2 (25.42±1.80 and 1-3-1(23.58±1.31) showing a maximum increase in shoot lengths and dry weights. These fungal isolates were marked as N1, N2, N3, N4, and N5 respectively. Endophytic partner residing in root tissues and secreting plant growth regulating compounds are of great interest to enhance crop yield and quality. Such growth regulating compounds can influence plant growth in stressful environments. Endophytic fungi offer an important role in the protection of plants and making plants more fit to cope with biotic and abiotic stress tolerance, decreasing water consumption and increasing biomass[34, 35].

Table 4: Bioassay of fungal isolates on Zea mays L. under PEG-induced drought stress

Fungal Isolates	SL(cm)	RL(cm)	PFW(g)	PDW(g)	CC (SPAD)
Control	21.40±1.01	12.53±0.94	1.32±0.16	0.24±0.03	35.60±13.15
Stress (8%PEG)	18.98±0.78	16.85±1.43	1.34±0.26	0.21±0.02	31.70±9.09
1-1-2 +8%PEG	19.13±2.11	16.62±1.14	1.29±0.38	0.21±0.03	32.36±4.20
1-1-3 +8%PEG	20.33±0.59	14.90±1.25	1.61±0.14	0.26±0.01	24.83±1.09
1-1-4 +8%PEG	24.42±1.53	19.08±0.88	1.93±0.33	0.32±0.02	26.30±3.33
1-2-3 +8%PEG	24.78±1.36	18.00±1.55	1.81±0.28	0.28±0.04	28.23±3.76
1-3-1 +8%PEG	**23.58**±1.31	**18.67**±0.58	**2.14**±0.22	**0.32**±0.02	**43.90**±2.22
1-3-3 +8%PEG	**27.25**±1.28	**14.57**±0.91	**1.94**±0.15	**0.35**±0.03	**42.70**±3.48
1-5-2 +8%PEG	**25.42**±1.80	**17.05**±1.30	**1.94**±0.22	**0.29**±0.05	**57.20**±5.05
1-5-3 +8%PEG	21.48±1.25	17.23±0.77	1.94±0.12	0.24±0.02	34.63±6.40
2-1-2 +8%PEG	**24.28**±1.69	**17.25**±0.87	**1.75**±0.22	**0.30**±0.03	**45.76**±4.94
2-1-3 +8%PEG	24.40±1.24	10.57±1.56	1.60±0.16	0.28±0.03	30.50±2.18
2-3-1 +8%PEG	21.88±1.57	12.57±1.55	1.38±0.14	0.26±0.03	30.50±6.87
2-3-2 +8%PEG	23.88±0.63	12.38±1.34	1.57±0.10	0.26±0.03	31.10±6.09
3-1-1 +8%PEG	22.27±2.78	12.03±1.67	1.22±0.10	0.24±0.04	27.96±2.31
3-1-2 +8%PEG	23.55±1.63	14.03±1.35	1.49±0.16	0.25±0.02	27.93±4.08
3-1-3 +8%PEG	**25.95**±2.08	**14.20**±0.88	**1.77**±0.18	**0.33**±0.04	**46.03**±2.02

SL = Shoot Length, RL= Root Length, PFW = Plant Fresh Weight, PDW =Plant Dry Weight. For each set of treatment, the different letter indicates significant differences at $p < 0.05$ levels as estimated by Duncan's Multiple Range Test (DMRT).

Conflict of Interests

The authors declare that they have no conflict of interest.

REFERENCES

1. Saikkonen, K., et al., *Fungal endophytes: a continuum of interactions with host plants.* Annual Review of Ecology and Systematics, 1998. **29**(1): p. 319-343.
2. Singh, L.P., S.S. Gill, and N. Tuteja, *Unraveling the role of fungal symbionts in plant abiotic stress tolerance.* Plant signaling & behavior, 2011. **6**(2): p. 175-191.
3. Strobel, G.A., *Endophytes as sources of bioactive products.* Microbes and infection, 2003. **5**(6): p. 535-544.
4. Aly, A.H., et al., *Fungal endophytes from higher plants: a prolific source of phytochemicals and other bioactive natural products.* Fungal diversity, 2010. **41**(1): p. 1-16.
5. Rodriguez, R., et al., *Fungal endophytes: diversity and functional roles.* New phytologist, 2009. **182**(2): p. 314-330.
6. Malinowski, D.P. and D.P. Belesky, *Adaptations of endophyte-infected cool-season grasses to environmental stresses: mechanisms of drought and mineral stress tolerance.* Crop Science, 2000. **40**(4): p. 923-940.
7. Herman, P., *Biodiversity and evolution in mycorrhizae of the desert.* Microbial Endophytes. Marcel Dekker, New York, USA, 2000: p. 141-160.
8. Clay, K. and J. Holah, *Fungal endophyte symbiosis and plant diversity in successional fields.* Science, 1999. **285**(5434): p. 1742-1744.
9. Rodriguez, R. and R. Redman, *Fungal life-styles and ecosystem dynamics: biological aspects of plant pathogens, plant endophytes and saprophytes*, in *Advances in botanical research*. 1997, Elsevier. p. 169-193.
10. Arnold, A.E., *Endophytic fungi: hidden components of tropical community ecology.* Tropical forest community ecology, 2008: p. 178-188.
11. Siegel, M. and L. Bush, *Toxin production in grass/endophyte associations*, in *Plant relationships*. 1997, Springer. p. 185-207.
12. Bacon, C.W. and J. White, *Physiological adaptations in the evolution of endophytism in the Clavicipitaceae.* Microbial endophytes, 2000: p. 237-261.
13. Scannerini, S., A. Fusconi, and M. Mucciarelli, *The effect of endophytic fungi on host plant morphogenesis*, in *Symbiosis*. 2001, Springer. p. 425-447.
14. Rim, S.-O., et al., *Fusarium proliferatum KGL0401 as a new gibberellin-producing fungus.* Journal of microbiology and biotechnology, 2005. **15**(4): p. 809-814.
15. Ahmad, W., et al., *A novel hybrid decision support system for thyroid disease forecasting.* Soft Computing, 2018: p. 1-7.
16. Lewis, D., *Symbiosis and mutualism: crisp concepts and soggy semantics.* 1985.
17. Arachevaleta, M., et al., *Effect of the tall fescue endophyte on plant response to environmental stress.* Agronomy journal, 1989. **81**(1): p. 83-90.
18. Bacon, C.W., *Abiotic stress tolerances (moisture, nutrients) and photosynthesis in endophyte-infected tall fescue.* Agriculture, ecosystems & environment, 1993. **44**(1-4): p. 123-141.
19. West, C.P., C. Bacon, and J. White, *Physiology and drought tolerance of endophyte-infected grasses.* Biotechnology of endophytic fungi of grasses, 1994: p. 87-99.

20. Morse, L.J., T. Day, and S.H. Faeth, *Effect of Neotyphodium endophyte infection on growth and leaf gas exchange of Arizona fescue under contrasting water availability regimes.* Environmental and Experimental Botany, 2002. **48**(3): p. 257-268.

21. Khajeh-Hosseini, M., A. Powell, and I. Bingham, *The interaction between salinity stress and seed vigour during germination of soyabean seeds.* Seed Science and technology, 2003. **31**(3): p. 715-725.

22. Orhan, D.D., et al., *Assessment of antioxidant, antibacterial, antimycobacterial, and antifungal activities of some plants used as folk remedies in Turkey against dermatophytes and yeast-like fungi.* Turkish Journal of Biology, 2012. **36**(6): p. 672-686.

23. CHOI, W.-Y., et al., *Isolation of gibberellins-producing fungi from the root of several Sesamum indicum plants.* Journal of microbiology and biotechnology, 2005. **15**(1): p. 22-28.

24. Ahmad, W., et al., *Thyroid Diseases Forecasting Using a Hybrid Decision Support System Based on ANFIS, k-NN and Information Gain Method.* J. Appl. Environ. Biol. Sci, 2017. **7**(10): p. 78-85.

25. Khan, S.A., et al., *Plant growth promotion and Penicillium citrinum.* BMC microbiology, 2008. **8**(1): p. 231.

26. Arnold, A.E., et al., *Diversity and phylogenetic affinities of foliar fungal endophytes in loblolly pine inferred by culturing and environmental PCR.* Mycologia, 2007. **99**(2): p. 185-206.

27. Hamayun, M., et al., *Gibberellin production and plant growth enhancement by newly isolated strain of Scolecobasidium tshawytschae.* Journal of microbiology and biotechnology, 2009. **19**(6): p. 560-565.

28. Waller, F., et al., *The endophytic fungus Piriformospora indica reprograms barley to salt-stress tolerance, disease resistance, and higher yield.* Proceedings of the National Academy of Sciences of the United States of America, 2005. **102**(38): p. 13386-13391.

29. Schulz, B., et al., *Endophytic fungi: a source of novel biologically active secondary metabolites.* Mycological Research, 2002. **106**(9): p. 996-1004.

30. Ragazzi, A., et al., *Endophytic fungi in Quercus cerris: isolation frequency in relation to phenological phase, tree health and the organ affected.* Phytopathologia Mediterranea, 2001. **40**(2): p. 165-171.

31. Merzlyak, M.N., A.E. Solovchenko, and A.A. Gitelson, *Reflectance spectral features and non-destructive estimation of chlorophyll, carotenoid and anthocyanin content in apple fruit.* Postharvest Biology and Technology, 2003. **27**(2): p. 197-211.

32. Pandey, A. and L. Palni, *The rhizosphere effect in trees of the Indian Central Himalaya with special reference to altitude.* Applied Ecology and Environmental Research, 2007. **5**(1): p. 93-102.

33. Bohm, P., *Estimating willingness to pay: why and how?* The Scandinavian Journal of Economics, 1979: p. 142-153.

34. Clay, K. and C. Schardl, *Evolutionary origins and ecological consequences of endophyte symbiosis with grasses.* the american naturalist, 2002. **160**(S4): p. S99-S127.

35. Rim, S.-O., et al., *Isolation and identification of fungal strains producing gibberellins from the root of plants.* Microbiology and Biotechnology Letters, 2007. **35**(4): p. 357-363.