Using Fungal Endophytes for Increasing Water Productivity and Tolerance of Wheat Plants to Drought Stress

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ABSTRACT

Abiotic stresses are dramatically affecting plant growth. The research was conducted to understand the effect of fungal endophytes as a soil amendment to alleviate abiotic stress (drought) on wheat plant. Five fungal endophytes were isolated from semi-arid areas and applied to sandy soil. Pot experiments had been achieved under three water treatments well irrigated 100% of FC, moderate stress 60% of FC and severe stress 40% of FC. The physiological parameters had been measured (shoot and root fresh weights, shoot and root dried weights, plant height, grain weight, chlorophyll content, and proline content). The results showed all physiological plant parameters decreased with increasing water stress because it is adversely affected plant growth. Except proline content that increased with decreasing of water content. Results showed that Rhizopus sp and Curvularia sp enhanced plant drought tolerance through significant increasing in plant growth parameters and decreasing in proline content. Also, these funguses increased irrigation water productivity for wheat plant under stressed condition. So, fungal endophytes have a potential effect that increases water productivity and wheat tolerance to drought stress.

Keywords: wheat; drought; fungal endophytes; water productivity; water stress

INTRODUCTION

Climatic change is the main reason in decreasing precipitation and appearing of dried areas (IPCC, 2013). In Mediterranean regions the drought increases in both frequency and intensity (Naumann et al., 2018). Drought stress is a vital abiotic stress that effect on crop production. It eliminates plant growth especially in arid and semi-arid areas (Bukhari et al., 2019). Agriculture sector in Egypt is consuming the largest amount of water resources about 80% (Fuglie et al., 2021). Increasing in population and decreasing of water availability, so there is a challenge to increase crops production and save more water, so scientist is now expressing crops per drop of water. Water is a limiting factor for plant growth where most plant growth parameters (leaf water content, shoot weight, dry weight, chlorophyll content, leaf area) are reduced with water deficit (Khalil and Eldin, 2021), Also there is a need to conserve more water and increase irrigation water productivity. Irrigation water productivity is the ratio between crop yield to the applied irrigation. Deficit irrigation is a needed to manage water use and also is a water strategy to conserve water through decrease applied water to plant on a ratio less than crop water requirement through subject the plant to water stress (Pereira et al., 2002). Deficit irrigation affected plant growth and productivity. Wheat is a major cereal crop that widely used and its production strongly affects with water stress (Elseehy and El-Shehawi, 2018). Endophytes are any microorganisms inhabit plant it could be bacteria or fungi. Fungal endophytes form a relationship with plants, and it give them some benefits such as promote plant growth (Rana et al., 2019), increase nutrient deficiency through solubilize nutrient (N, P, K) and increase availability to plant (White et al., 2019) and alleviate environmental stress (drought and salinity stresses) (Morsy et al., 2020). Under drought stress fungal endophytes that colonize the rhizosphere reduces the water stress and increase plant growth through produces hormones that promote growth (Rustamova et al., 2022). Also, fungal endophytes can produce antioxidant defense that ameliorate drought plant tolerance as reported by Moghaddam et al. (2021). Hubbard et al. (2012), found that fungal endophytes improved the percent of germination, enhanced wheat tolerance to heat and drought as measured by fresh weight of seedlings. The seeding growth parameters were increased in the colonized wheat seeds exposed to heat stress than of uncolonized seeds. Yandigeri et al. (2012), should that wheat seeds have treated with fungal endophytes improved the plant growth and yield in comparison to untreated. Direct coating of cultures on seeds yielded better performance than cell-free extract coated on seeds. Also, production of phytohormones, plant growth promotion traits combined with water stress tolerance potential in these endophytic actinobacteria played a cumulative synergistic role that supported enhanced plant growth promotion of wheat in the stressed soil. Hubbard et al. (2014), found that

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inoculation altered plant responses to oxidative stress in drought conditions. These findings showed new way on mechanisms involved in plant-endophyte the associations, of wheat coincides with epigenetic differences in the plant host. Hosseini et al. (2017) interaction of the stresses showed that mechanical stress primarily controls plant water status and physiological responses. However, fungal endophyte alleviated the adverse effects of individual and combined stresses on plant growth. Colonized plants were better adapted and had greater root length and volume, relative water content and chlorophyll contents under stressful conditions due to higher absorption sites for water and nutrients compared with uncolonized plants. Wheat (Triticum aestivum) plant was selected and used as a model system to evaluate the impact of fungal endophytes under different water regimes in greenhouse experiments. So, the aim of this research was studying the effect of water stress on growth parameters and water productivity of wheat plants treated with fungal endophytes isolated from dried area.

MATERIAL AND METHODS

The experiments were conducted in green house in Faculty of Agriculture; Egypt. Wheat seeds Giza 171 obtained from Agriculture Research Center in Egypt. Seeds were planted in pots filled with sandy soil, the coarse fractions determine by dry sieving according to Gee and Bauder (1979) and the fine fractions determine by international pipette method according to Klute and Dirksen (1986). Field capacity (FC) and wilting points (WP) were measured by pressure plate apparatus at 0.1 and 15 bar respectively according to Israelsen and Hansen (1962). Table (1) displayed the soil characteristic determined according to Black (1965). The wheat was planted in November 2019 and harvested in March 2020. The average values of high and low temperatures were 22 °C and 15 °C respectively and with an average relative humidity was 69%.

Water Treatment:

There were three water treatments that wheat was subjected to it. Irrigation treatments were well-irrigated treatment I1 (100% of FC), medium-stressed treatment I2 (60% of FC) and severe-stressed treatment I3 (40% of FC). The plants were well irrigated for two weeks after planting date. Then plants were subjected to water treatments till harvesting. The water treatments were 320 ml at I1, 192 ml at I2 and 128 ml at I3. Water was added to each pot to fill the losses in water through evapotranspiration. The loss in water was calculated by weighed the pots twice a week.

Soil Physical characteristic	Value
Sand	90%
Silt	3%
Clay	7%
Soil Texture	Sandy
Bulk density (gm/cm ³)	1.7
Field capacity (%)	10
Permanent Wilting point (%)	2
Soil chemical characteristic	
pH	7.6
EC (ds/m)	1
OM (%)	0.2

Table 1. Initial soil physical and chemical properties

The irrigation water productivity (IWP, kg/m³):

IWP was calculated according to Jensen (1983) as follows:

$$IWP = \frac{Ya}{AW}$$

Where, Ya is the seed yield of various treatments (kg/ha), and AW is seasonal applied water (m^3/ha) .

Selection and Isolation of endophytic fungi

Fresh healthy leaves of Tribulus terrestris were collected from semiarid area, Alexandria, Egypt. Samples were washed with tap water then distilled water and sterilized by 3.0% sodium hypochlorite (NaOCl) for 3 min. After that washed 3 times by sterile distilled water for 1 min and the sterilized leaves were cut into small pieces 5 mm² and placed on potato dextroze agar Petri dishes media with 50 mg/L of ampicillin to stop the bacterial growth and incubated at $25\pm2^{\circ}$ C. The dishes were checked until the fungal endophytes emerged. The endophytes were sub cultured into plates containing PDA (Sunitha et al., 2013).

Morphological Identification of Fungal Endophytes

Isolated fungal were kept at $28^{\circ}C \pm 2^{\circ}C$ in dark for 10 days then visually examined for their morphological characterization regarding macroscopic and microscopic characters (Barnett and Hunter, 1998).

Fermentation and Preparation of endophyte bioinoculate

Erlenmeyer flasks (250 mL) containing 100 ml of Potato Dextrose Broth (PDB) were inoculated with three 10 mm disks endophytic fungus. The flasks were placed on a shaker incubator (120 rpm) at $25 \pm 2^{\circ}$ C. The liquid cultures were filtered using double layer sterile Whatman filter paper. The separated fungal mycelium was refrigerated untill using in pot experiment.

Pot experiment

The five isolated fungal were treated with soil. The fungal mycelium (30 gm) was mixed with of soil (1 Kg) in pots prior to planting date. Wheat seeds were surface sterilized with 0.1% sodium hypochlorite, rinsed by distilled water and were sown in surface sterilized plastic pots (10 x15 cm) containing 2 kg of sandy soil.

Measurements

All physiological parameters were measured through the experiment and after harvesting. The experiments were achieved in three replicates using split plot randomized design. The chlorophyll content index was measured by a Minolta SPAD chlorophyll meter (SPAD-502 plus, Konica Minolta Sensing, Japan) according to Yadava (1986) through the growing season. Ghorophyll content was measured in fully expanded leaves. After harvesting, planted were removed from soil to determine growth parameters (fresh shoot weight, fresh root weight, plant height and proline content. Drying of shoot and root under 70 °C in oven, then dry weights were recorded. Proline content was measured according to Bates et al. (1973). Weighed 0.1 g from the dry leaf and mixed with 10 mL aqueous sulfosalicylic acid (3%). Taking 2 ml of the filtrate were mixed with 2 ml glacial acid and 2 ml ninhydrin acid, they were heated in a water bath for 1 hr. at 100° C. after which they were cooled in ice bath. The mixture was extracted with 4 mL toluene then shaked for 20 sec. The chromophores containing toluene was aspirated from the aqueous phase the samples leaved under room temperature then measured the absorbance using spectorophotometer at 520 nm.

Statistical analysis:

Statistical analysis was performed for the data by split plot design in two ways analysis of variance (ANOVA) (Steel et al., 1997) and LSD for three replicates. The factors were three irrigation regimes (100%, 60%, and 40% F.C.) and five fungal endophytes. Statistical analysis was achieved by ANOVA, F-test, and LSD procedures available within the statistix version 10 software (Copy right 2005, Analytical Software, USA).

RESULTS AND DISCUSSION

Isolation and identification of endophytic fungi

Five endophytic fungi were isolated from *Tribulus terrestris* leaves. The fungal stains were identified to the genus level based on taxonomical structures including the colony growth rate and color, the shape and the type of conidia under light microscopes. The fungal endophytes were classified to five genera; *Alternaria* sp, *Aspergillus* sp, *Chaetomium* sp, *Rhizopus* sp and *Curvularia* sp according to the previous observations.

Effect of water treatment on plant growth parameter:

Figure 1 and 2 showed significant relation between three water treatment (No-stress condition 100% FC, Moderate stress condition 60% and sever stress condition 40%) with fungal-free on wheat physiological parameters. Figure 3 showed a photo for three irrigation levels on wheat plant growth.

Chlorophyll content: There was a significant decrease in chlorophyll content with water deficit. The chlorophyll contents were significantly decreasing with increasing of water stress. It reduced from 51.03 SPAD at 100% FC to 47.99 SPAD at 40% FC. Because abiotic stress could destroy the pigment protein complexes then chlorphyll content is reduced (Lai et al., 2007) or reduction of enzymes which produce photosynthetic pigment (Murkute et al., 2006).

Proline content: water deficit significantly increased proline content in plant. Proline accumulation is considering a sign to water stress. The accumulation of proline in plant considered a mechanism strategy to cope water stress. Where increasing in proline level reduces leaves water potential (Tatar and Gevrek, 2008). In our study proline was increased from 12.5 µmoles proline/gm dry weight at well irrigated pots 100% FC to 23.1 µmoles proline/gm dry weight in sever stressed condition 40% FC. The same trend was found by Nio et al. (2011), Mwadzingeni et al. (2016), Chowdhury et al. (2021) and Maralian et al. (2010). So increasing of proline in plant subjected to water deficit is a mechanism to tolerate drought.

Plant height: The plant height was decreased from 77.3 cm height at well-irrigated to 61.7 cm in stressed condition. Analysis of variance showed a significant decrease between the Irrigation treatments.

Total fresh and dry weights: there was non-significant in fresh and dry weights between treatments 60% FC and 40% FC but there was a significant decrease compare them with treatment 100% FC. The total fresh weight dropped from 17.9 gm at well-irrigated to 8.8 gm weight in sever-condition (40% FC). Also the total dry weights decreased from 9.3 gm in 100% FC to 3.7 gm in sever condition. This reduction of biomass is physiological adaptation to water stress. This finding with in a good agreement with Nowsherwan et al. (2018) who found decreasing in wheat Fresh and dry weights, chlorophyll content and plant heights with increasing water stress.

Grain yield: grain yield is significantly decreased with water stress. The same finding was by Ahmadi and Siose Mardeh (2004). There was a significant reduction in grain weight between 100% FC and (60% FC & 40% FC). But there was no significant between 60% FC and 40% FC in grain weight.



Fig. 1. The effect of water treatment chorophyll (SPAD), Plant height (cm) and proline content for endophyte-free plant



Fig. 2. The effect of water treatment on total fresh weight, total dry weight and grain weight for endophyte-free plant



Fig. 3. wheat plant under well irrigated (100% FC), moderate irrigated (60% FC) and severe condition (40% FC) with endophytes-free



Fig. 4. effect of fungal endophytes on wheat plant severe water stress condition (40% FC) using five fungal and fungal-free

Water deficit causes decreasing the water flow transport from xylem to other cells which leads to reduce in plant growth. Also, it causes modifications in metabolic and physiological activities and negative effect on reproductive organs. (Taiz and Zeiger, 2006).

The interaction between fungal endophytes and water treatment on plant growth parameter:

Figure 4 showed the fungal endophytes effect on severe water condition. We noticed that Rhizopus sp (F4) and Curvularia sp (F5) increased the growth of wheat plant under sever condition in comparison to control with endophyte-free.

Table (2) summarized the interaction effect between water amounts and fungal endophytes application. Fungal endophytes had a significant effect on morphological wheat parameters. ANOVA results showed that there was a significant effect of fungal endophytes to some physiological parameters. A significant increasing in Total shoot and root dry weight was found in Rhizopus sp and curvularia sp in sever conditions (40% FC). It could be because these fungus compounds produces as antibiotic production, phytohormones, nutrient mineralization., etc., that enhanced growth in drought conditions (Yung et al., 2021).

The total water applied water (AIW) and irrigation water productivity (IWP) under three water treatments:

Table (3) showed the total applied irrigation water quantities were 1344, 806 and 538 m3/fed for I1, I2 and

13 irrigation treatments, respectively. According the average water productivity (IWP) were 2.4, 2.9 and 3.4 kg/m³. The IWP increased with increasing water stress. The maximum value with high water stress (40% FC) and combination with Curvularia sp, it was 4.65 kg/m³ water, but the minimum one with no stress (100% FC) and Aspergillus sp, it was 2.02 kg/m³ water applied. The highest water productivity was observed with stressed

water level 40% FC with *Curvularia* sp fungi. So treatment soil with these fungal endophytes alleviated water stress, increased wheat tolerance to drought and increase water productivity. These results were in agreement with the results of Noreldin and Mahmoud (2017).

Table 2. Mean	values of	some	physiological	wheat	parameters	of	shoot	dry	weight,	root	dry	weight,	proline
content and pla	nt height												

Water	Fungi	Chorophyll (SPAD)	dry shoot weight (gm)	dry root weight (gm)	Proline
	Endophyte-free	50.32a	7.62abc	3.20b	13.512fgh
11	Alternaria sp	40.33b	8.10ab	3.83ab	12.75fghi
	Aspergillus sp	50.0a	5.20bcd	2.77b	12.45ghij
	Chaetomium sp	48.23ab	4.93bcd	2.3b	10.93j
	Rhizopus sp	52.0a	7.10bcd	3.93ab	11.40ij
	<i>Curvularia</i> sp	52.7a	6.10bcd	1.57b	12.27hij
	Endophyte-free	44.8ab	3.26bcd	1.25b	19.15c
	Alternaria sp	46.2ab	5.73bcd	2.13b	14.14efg
	Aspergillus sp	46.9ab	5.40bcd	2.23b	15.20e
12	Chaetomium sp	44.43ab	5.17bcd	3.07b	15.20e
	Rhizopus sp	48.2ab	2.73cd	1.17b	14.32ef
	<i>Curvularia</i> sp	47.8ab	2.93cd	0.97b	13.65efgh
	Endophyte-free	42.7ab	3.71bcd	1.00b	23.12a
	Alternaria sp	48.4ab	3.83bcd	1.47b	21.63ab
12	Aspergillus sp	48.9ab	2.53d	1.07b	23.12a
13	Chaetomium sp	52.1a	4.27bcd	1.13b	23.12a
	Rhizopus sp	52.5a	11.43a	7.93a	17.06d
	<i>Curvularia</i> sp	52.7a	7.80abc	4.27ab	20.36bc

Pairwise t-test: Treatments with similar symbols are expressing no significance among them

Irrigation		Plot	Grain yield			IWP		
Treatment	Fungi	A, cm ²	g/pot	kg/fed	mm	m ³ /pot	m ³ /fed	Kg/m ³
	Endophyte-free	70 5	7.0	3721.52	320	0.003	1344	2.77
	Alternaria sp		6.7	3562.03	320	0.003	1344	2.65
	Aspergillus sp		5.1	2711.39	320	0.003	1344	2.02
(I1)	Chaetomium sp	10.5	5.2	2764.56	320	0.003	1344	2.06
	Rhizopus sp		6.1	3243.04	320	0.003	1344	2.41
	Curvularia sp		6.9	3668.35	320	0.003	1344	2.73
1	Average		6.2	3278.5	320.0	0.0	1344.0	2.4
	Endophyte-free		5.0	2658.23	192	0.002	806	3.296
	Alternaria sp	78.5	4.2	2232.91	192	0.002	806	2.769
	Aspergillus sp		4.1	2179.75	192	0.002	806	2.703
(I2)	Chaetomium sp		4.0	2126.58	192	0.002	806	2.637
	Rhizopus sp		4.5	2392.41	192	0.002	806	2.967
	Curvularia sp		4.8	2551.90	192	0.002	806	3.165
1	Average		4.4	2357.0	192.0	0.0	806.4	2.9
	Endophyte-free		3.5	1860.76	128	0.001	538	3.461
	Alternaria sp		2.3	1222.78	128	0.001	538	2.275
(I3)	Aspergillus sp	70 5	2.2	1169.62	128	0.001	538	2.176
	Chaetomium sp	/8.5	3.3	1754.43	128	0.001	538	3.263
	Rhizopus sp		4.5	2392.41	128	0.001	538	4.450
	Curvularia sp		4.7	2498.73	128	0.001	538	4.648
Average			3.4	1816.5	128.0	0.0	537.6	3.4

Table 3. Total water applied (AIW, m³/fed) and irrigation water productivity (IWP, Kg/m³, AIW)

CONCLUSION

Fungal endophytes could be a promising way to alleviate drought tolerance in wheat plant and also, applied to reduce the negative impact of climate change on water productivity. We concluded that deficit irrigation affected on plant growth parameters and reduced main parameters as fresh and dry weight, plant height, grain weight and chlorophyll content. Fungal endophytes alleviate this adversely effect of through improve plant tolerance by increasing chlorophyll content and reducing proline content under stressed condition beside that it is increased water productivity.

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الملخص العربى

استخدام الفطريات الداخلية المعيشة لزيادة إنتاجية المياه وتحمل نباتات القمح للجفاف

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تؤثر الضغوط غير الحيوية بشكل كبير على نمو النبات. تم إجراء البحث لفهم تأثير الفطريات الداخلية المعيشة كأضافة أرضية لتقليل الإجهاد الغير حيوي (الجفاف) على نبات القمح. تم عزل خمسة فطريات داخلية من المناطق شبه القاحلة .تم إجراء تجارب أصص تحت ثلاث معالجات مائية مروية بشكل جيد بنسبة ١٠٠٪ من FC ، وإجهاد معتدل الفسيولوجية (الأوزان الطازجة للنبات والجذور، الأوزان المجففة للنبات والجذور، ارتفاع النبات، وزن الحبوب، محتوى

الكلوروفيل، محتوى البرولين). أظهرت النتائج انخفاضًا في جميع قياسات النبات الفسيولوجية مع زيادة الإجهاد المائي لأنه يؤثر سلبًا على نمو النبات. باستثناء محتوى البرولين الذي يزداد مع تتاقص محتوى الماء. عزز كلا من فطر *Rhizopus sp و Curvularia sp من* تحمل النبات للجفاف من خلال زيادة معنوية في معايير نمو النبات وخفض محتوى البرولين. ايضا ازدادت الانتاجية المائية عند استخدام هذان الفطران. لذلك فان للفطريات الداخلية المعيشة لها تأثير على زيادة الانتاجية المائية وتحمل القمح لإجهاد الجفاف.