ARBUSCULAR MYCORRHIZAL SYMBIOSIS ALLEVIATES DROUGHT STRESS IMPOSED ON WHEAT PLANTS (*TRITICUM AESTIVUM* L.)

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Absract. The aim of this study was to determine the contribution of native arbuscular mycorrhizal fungi (AMF) inoculation to growth, pigmentation and grain yield of wheat plants (*Triticum aestivum* L.) grown under different levels of water deficiency [D0, 100% Field Capacity (FC); D1, 75% FC; D2, 50% FC and D3, 25% FC]. The results suggested that AMF inoculation has a beneficial effect on plant drought tolerance and effectively improved biomass and crop productivity of wheat plants grown under drought. Mycorrhizal symbiosis alleviates the inhibitory effect of drought stress via improving water status and chlorophyll biosynthesis of wheat plants. Mycorrhizal colonization increased gradually and was higher at the maturity stage under a low level of drought (D1). The mycorrhizal wheat plants had higher shoot phosphorous than non-mycorrhizal plants at all samplings regardless of levels of drought stress. In general, with all treatments, the content of photosynthetic pigment fractions was inhibited as the level of drought increased in the soil pot experiment. However, the photosynthetic pigment contents of mycorrhizal wheat plant leaves were significantly ($p<0.05$) greater than those of non-mycorrhizal ones. The study/ concluded that the native mycorrhizae alleviate the drought stress by enhancement of the process of phosphorus uptake, pigment biosynthesis and accumulation of plant metabolites and may be used as a biofertilizer.
Keywords: *arbuscular mycorrhizal, drought stress, biofertilizer, phosphorus, wheat*

Introduction

Water shortage is considered one of the most significant environmental factors that affect plant growth and limit plant development and productivity in many arid and semiarid regions of the world. Also, seasonal water deficiency sometimes occurs in non-arid regions. Drought has a sharp decline in crop productivity, although many of these crops have many improved characteristics to withstand water shortage conditions (Bohnert et al., 1995; Zhu et al., 2012). Water deficit negatively influences the growth and metabolism of many plants, the response of plants differs depending on plant genotype, developmental stage, severity and duration of the stress (El-Enany et al., 2013, 2014).

Soil microorganisms are a very important component in the plant/soil system (Abd-Alla et al., 2014a,b; Kannenberg and Phillips, 2017). Symbionts can improve plant resistant to abiotic stresses by enhancing both plant nutrition and protection against the oxidative damage produced by the water deficiency (Ruíz-Lozano, 2003) and heavy metals (Rivera-

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Becerril et al., 2005). The symbiotic relation between arbuscular mycorrhizal fungi (AMF) and most plants provides nutrients, stimulates plant growth and increases the tolerance of plants against the stress (Barea et al., 2005; Abdel Latif and Chaoxing, 2014; Kyriazopouls et al., 2014; Shinde and Thakur, 2015). Arbuscular mycorrhizal fungi (AMF) can form mutualistic symbiotic associations with the roots of 80% of all terrestrial plant species (Smith and Read, 2008; Patale and Shinde, 2014). The AM symbiosis induced a higher improvement of physiological parameters in drought-sensitive plants than in drought-tolerant plants and drought-sensitive plants obtained higher physiological benefit from the AM symbiosis (Quiroga et al., 2017). Plants grown under water deficiency have a lower stomatal conductance in order to conserve water. Previous studies have indicated that drought stress severely affects plant growth through various mechanisms, such as reduced leaf water potential, reduced rate of cell division, and altered plant water and nutrient relationships. Consequently, CO_2 fixation is inhibited and photosynthetic rate reduced, resulting in less assimilate production for growth and yield of plants (Celebi et al., 2010; Farooq et al., 2012; Shinde and Singh, 2017).

There is considerable evidence suggesting that AMF has the potential to increase the tolerance of their host plants to water deficit stress (Asrar et al., 2012; Lazcano et al., 2014; Xiao-Qing et al., 2017). Studies have shown that the extraradical mycelia of AM fungi transfer water to their host plants under low soil moisture conditions and the AM fungi improve plant growth, development and yield (Augé et al., 2007), enhanced nutrient uptake (Michalis et al., 2013), an increase in the root hydraulic conductance (Bárzana et al., 2012), alterations in the soil's water retention properties (Augé, 2001), or improved osmotic adjustment (Aroca et al., 2007) and antioxidant activity (Bompadre et al., 2014). The beneficial functions of AMF may be of great importance to climate change, particularly with respect to water shortage and to the revegetation of degraded ecosystems; including coal mine spoil banks (Khalvati et al., 2010).

Wheat (*Triticum aestivum* L.) is the major food crop plants, are known to be commonly associated with AM fungi, which inhabit in agricultural soils contributing physiologically and ecologically to the health of both plants and soils (Li et al., 2012). Colonization of roots by AM fungi has been shown to increase the drought resistance of wheat (Al-Karaki and Clark, 1998). Therefore, our aim in the present study was to evaluate the potential of native species of AMF to mitigate the adverse effects of drought stress on wheat growth and for improved grain yield.

Material and Methods

Production of mycorrhizal inoculum

Native adapted AM fungi were isolated from the arid environment [Al-Uqair coast, Al-Ahsa, Saudi Arabia, (25°39’35.8”N 50°11’25.6”E)] and propagated for inoculums production. The most abundant mycorrhizal species selected and morphologically identified according to Schüessler and Walker (2010). Native AM fungi were propagated on maize (*Zea mays.* L) as host plant on the sterilized clay-sand mixture (50% soil and 50% sand). Plants were cultured in the greenhouse for 3 months. The roots colonized by AM fungi were checked during the culture, and the presence of spores was confirmed by sieving. One hundred grams of soil containing a mixture of mycorrhizal spores, extra-radical hyphae, and roots fragments applied as mixture culture inoculum. Non-mycorrhizal maize roots and sterilized soil used for the control treatment.

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Experimental design and growth conditions

Wheat grains (*Triticum aestivum* L.) were surface sterilized and grown in plastic pots filled with 4 kg sterilized soil at different levels of drought (D0, 100% FC-D1, 75% FC-D2, 50% FC and D3, 25% FC). The seedlings were thinned to 10 plants one week after germination. All pots were fertilized with 50 mg N-KNO_3 g^{-1} soil. Solutions of K_2HPO_4 were prepared and P was added at 30 or 60 mg^{-1} g soil. A control treatment was also included. The experiment in a completely randomized design was performed by fifty- gram inoculum of AMF mixture and placed in pots below the grain of the tested plant (approximately contain 90–100 spores/10 g soil). Pots of the control treatments received the same volume of autoclaved inoculums. All pots contain 10 seedlings were kept in the greenhouse with day and night temperatures of 27 and 15˚C, respectively, day and night relative humidity of 70 and 80%, respectively, and a photoperiod of 14 h. Each treatment was replicated three times.

Growth parameters and grain yield

After 42 days of planting, some wheat plants were removed gently from the pots, washed with water. Then, plants were separated into shoots and roots and part of fresh roots and shoots were immediately frozen for analysis. The dry weights of the plants were recorded by placed the samples in an oven at 80°C until the dry weight was constant. At maturity, yield components [spike length, the number of grains per spike, grain yield per plant and 100- grain weight (seed index)] were determined on 3 plants for each treatment. Also, days to flowering and days to maturity were recorded for all treatments.

Estimation of mycorrhizal colonization

Root segments were separated from the plant, washed and then cut into one centimeter long pieces. The segments were cleared with 10% (w/v) KOH at 70^{o}C for 20 min and stained with 0.5% (w/v) Trypan blue. The stain was prepared by mixing water, glycerin and lactic acid in proportions 1:1:1 (v/v/v). Mycorrhizal colonization was assessed by the method of Brundrett et al. (1984). Frequency of mycorrhizas (F%), the intensity of mycorrhizal colonization in the root (M%), and arbuscule frequency in roots (A%) was calculated according to Trouvelot et al. (1986) using the MYCOCALC (http://www.dijon. inra.fr/mychintec/Mycocalc-prg/download.html) program.

Quantification of phosphoros mycorrhizal dependency

According to Plenchette et al. (1983), the dependency of mycorrhizal plants growth and Phosphorous (P) uptake were calculated as:

$$\frac{[\text{P content (M)} - \text{P content (NM)}]}{\text{P content (M)}} \times 100 \qquad \text{(Eq.1)}$$

where M, mycorrhizal plants and NM, non-mycorrhizal plants.

The concentration of P in the shoot was determined after digestion in a mixture of concentrated nitric and perchloric acids (4:1). Each sample was placed in 50 mL measuring flasks and 10 mL of the acid mixture was added. The mixture was first heated on fry pans to 90°C for 30 min, and then temperature increased to 140°C to remove excess nitric acid. De-ionized water was added to make up to definite volume of

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the primary extract. An aliquot of this primary extract was diluted for determining phosphorus shoot concentration spectrophotometrically (Olsen and Sommers, 1982).

Determination of photosynthetic pigments

The fractions of pigments (chlorophyll a, chlorophyll b and carotenoids) were estimated using the spectrophotometric method recommended by Lichtenthaler (1987). The photosynthetic pigments were extracted from a definite weight of fresh leaf samples in 5 ml of 95% ethyl alcohol at 60˚C, until colorless. Then the total volume was completed to 10 ml with 95% ethyl alcohol and absorbance readings were followed with a spectrophotometer (Unico UV-2100 spectrophotometer. The extinction was measured against a blank of pure 95% ethyl alcohol at three wavelengths of 452, 644 and 663 nm. The concentration of chlorophylls and carotenoids were calculated as mg/g FW using the following equations:

$$\text{Chlorophyll a} = (13.36 \times A_{663}) - (0.918 \times A_{644}) \quad \text{(Eq.2)}$$

$$\text{Chlorophyll b} = (27.49 \times A_{664}) - (3.87 \times A_{663}) \quad \text{(Eq.3)}$$

$$\text{Carotenoids} = (4.2 \times A_{452}) - [(0.0264 \times \text{Chl.a}) + (0.46 \times \text{Chl.b})] \quad \text{(Eq.4)}$$

Statistical analysis

The data were subjected to one-way ANOVA using the SPSS 10.0 software program. Means and standard errors were calculated for 3 replicate values. Means were compared by Duncan's multiple range test and statistical significance was determined at a 5% level.

Results

A total number of 7 morphotypes of native species of AMF were recovered from rhizosphere plants grown in a harsh environment. Based on the number of spores the seven species in stressed soils were *Acaulospora* sp., *A. bireticulata* (Rothwell & Trappe), *A. capsicula* Blaszk., *Glomusaggregatum* Schenck and Smith, *G. clarum* Nicolson & Schenck, *G. geosporum* (Nicol. & Gerd.) Walkerand *G. mosseae* (Nicol. & Gerd.) Gerd. & Trappe. The most common native species (*Acaulospora capsicula*,*Glomus aggregatum* ,and *Glomus geosporum*) were propagated and used as mixture inoculum for further experiments.

The experiment was conducted on *Triticum aesativum* L. under different levels of drought (D0, D1, D2, and D3) and inoculated with or without a mixture of native AM fungi to grow for 3 months. Under well-watered conditions, mycorrhizal inoculation significantly increased all the growth parameters. The fresh and dry weights of mycorrhizal plants increased significantly than non-mycorrhizal plants. The drought treatments significantly reduced the growth criteria of both the mycorrhizal and nonmycorrhizal plants (*Table 1*). The data indicated that drought treatments were significantly ($p > 0.05$) reduced fresh or dry weights of wheat plant roots especially at the highest levels of drought (D2 and D3) as compared by reference control (D0). Inoculation of wheat plants with AM fungi stimulated the growth, especially the plants under drought. The data presented in *Table 1* shows that all water deficit levels exerted an inhibitory effect on the root and shoot dry yield of non-mycorrhizal wheat plants. On

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the other side, the fresh and dry shoot weights of mycorrhizal plants showed an increase at all drought levels as compared with control plants.

Analysis of the data revealed that drought significantly affected days to flowering of wheat plants of which delayed as compared with control. Days to the flowering of mycorrhizal wheat plants grown under drought stress were significantly early flowering than those of their respective non-mycorrhizal counterparts. The period of maturity of wheat spike plants showed the effectiveness of mycorrhizal inoculation as shown in *Table 2*.

Table 1. *Growth parameters of wheat plants in response to mycorrhizal inoculation grown under different levels (D0, D1, D2, and D3) of drought stress*

Parameters		Treatments							
		D0		D1		D2		D3	
		NM	M	NM	M	NM	M	NM	M
Shoot	Fresh Weight (g)	7.08 ± 0.27 **e**	8.38 ± 0.39 **f**	6.28 ± 0.34 **d**	7.12 ± 0.44 **e**	5.12 ± 0.31 **ab**	5.95 ± 0.52 **cd**	4.59 ± 0.28 **a**	5.54 ± 0.24 **bc**
	Dry Weight (g)	1.87 ± 0.18 **cd**	2.24 ± 0.14 **e**	1.58 ± 0.15 **bc**	1.90 ± 0.13 **d**	1.43 ± 0.11 **ab**	1.72 ± 0.16 **cd**	1.23 ± 0.08 **a**	1.69 ± 0.14 **cd**
	Length (cm)	81.67 ± 3.51 **cd**	89.33 ± 6.03 **d**	76.67 ± 6.03 **bc**	81.33 ± 3.05 **cd**	64.33 ± 3.50 **b**	72.67 ± 4.50 **bc**	56.33 ± 4.10 **a**	75.33 ± 6.02 **bc**
Root	Fresh Weight (g)	0.70 ± 0.07 **cd**	0.90 ± 0.13 **e**	0.64 ± 0.11 **bc**	0.79 ± 0.06 **de**	0.51 ± 0.04 **ab**	0.63 ± 0.03 **bc**	0.45 ± 0.04 **a**	0.59 ± 0.05 **bc**
	Dry Weight (g)	0.17 ± 0.03 **bcd**	0.20 ± 0.02 **d**	0.16 ± 0.01 **abc**	0.18 ± 0.01 **cd**	0.14 ± 0.01 **ab**	0.17 ± 0.01 **bc**	0.13 ± 0.02 **a**	0.16 ± 0.01 **abc**
	Length (cm)	51.33 ± 5.51 **bcd**	56.33 ± 7.37 **d**	44.00 ± 6.56 **abc**	63.00 ± 4.58 **d**	42.00 ± 7.93 **ab**	54.67 ± 7.76 **cd**	34.67 ± 4.50 **a**	52.67 ± 4.50 **bcd**

*NM nonmycorrhizal wheat plants; M, mycorrhizal wheat plants. Means in each column followed by the same letter are not significantly different ($P < 0.05$) as determined by Duncan's test. Values are the means of three replications

Table 2. *Yield components of wheat plants in response to mycorrhizal inoculation grown under different levels (D0, D1, D2, and D3) of drought stress*

Parameters	Treatments							
	D0		D1		D2		D3	
	NM	M	NM	M	NM	M	NM	M
Days to Flowering	96.67 ± 1.52 **b**	86.33 ± 3.06 **a**	95.67 ± 3.06 **b**	88.00 ± 2.00 **a**	101.67 ± 1.53 **ab**	89.00 ± 2.65 **a**	104.33 ± 1.15 **c**	94.33 ± 3.15 **b**
Days to Maturity	130.33 ± 2.52 **c**	121.67 ± 1.53 **a**	131.00 ± 3.61 **c**	124.67 ± 1.53 **ab**	135.67 ± 1.32 **d**	127.33 ± 2.08 **bc**	138.00 ± 2.65 **da**	128.33 ± 1.45 **bc**
Spike Length (cm)	8.67 ± 0.32 **cd**	9.70 ± 0.56 **e**	7.93 ± 0.51 **bc**	9.37 ± 0.15 **de**	7.43 ± 0.71 **ab**	8.93 ± 0.25 **de**	6.77 ± 0.40 **a**	8.10 ± 0.20 **bc**
Number of Grains/Spike	42.67 ± 4.51 **de**	48.6 ± 4.21 **e**	35.00 ± 3.71 **bc**	38.00 ± 3.78 **cd**	29.00 ± 2.52 **ab**	36.00 ± 4.04 **cd**	24.67 ± 2.00 **a**	33.33 ± 1.53 **bc**
Grain Yield /Plant (g)	14.27 ± 2.25 **e**	16.99 ± 0.29 **f**	13.88 ± 0.30 **de**	14.47 ± 0.49 **e**	12.12 ± 0.47 **b**	13.19 ± 0.69 **cd**	11.12 ± 0.33 **a**	12.38 ± 0.24 **bc**
Seed Index (100 grain weight in g)	31.75 ± 0.27 **cd**	37.18 ± 1.15 **bc**	30.15 ± 1.22 **d**	32.02 ± 2.13 **cd**	26.87 ± 2.90 **b**	30.42 ± 1.37 **bcd**	21.72 ± 1.82 **a**	27.31 ± 2.01 **b**

*NM nonmycorrhizal wheat plants; M, mycorrhizal wheat plants. Means in each column followed by the same letter are not significantly different ($P < 0.05$) as determined by Duncan's test. Values are the means of three replications

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The data showed that amongst all the treatments, days to maturity significantly ($P<0.05$) delayed under drought stress. Yield and its component consider the main target for the activity of plants. So the data recorded in *Table 2* show that wheat plants inoculated with AMF caused a marked effect on the seed yield per plant and its components (spike length, grains of a spike, grains per plants and grains index, the weight of 100 seeds) in comparison to the treated plants. The data in *Table 2* demonstrated that/ spike length of water deficit at all levels was significantly decreased with increased water deficit level in soil, especially at high water stress levels. The highest values of spike length obtained from wheat plants inoculated with AMF, while the minimum spike length recorded from non-mycorrhizal plants grown at high water deficit treatment soil. These results showed a general trend of an increase in grain per spike with inoculation of treated plants (*Table 2*). Seed index weight of 100 grains (g) was recorded and the data revealed that grain index was significantly increased in mycorrhizal wheat inoculated plant than those uninoculated wheat plants. The root colonization rate was affected by substrate, inoculation, and the interaction of the substrate with drought stress or inoculation. In general, different levels of drought stimulated development the frequencies of root colonization (F%), the intensity of root cortex colonization (M%), and arbuscular development (A%) by *Acaulospora capsicula*, *Glomus aggregatum*, and *Glomus geosporum* at different growth stages in wheat plants as shown in *Table 3*.

Table 3. *Mycorrhizal colonization of wheat plants under different levels (D0, D1, D2, and D3) of drought stress*

Growth stages	Mycorrhizal colonization (%)	Drought levels			
		D0	D1	D2	D3
Vegetative	F %	48.0	52.0	43.0	36.0
	M %	21.3	20.0	17.0	15.3
	A %	16.7	13.0	8.0	5.3
Flowering	F %	63.0	69.0	60.0	49.0
	M %	54.0	58.0	49.0	43.3
	A %	36.3	42.3	35.6	29.0
Maturity	F %	83.3	85.0	75.0	62.0
	M %	57.2	62.0	53.0	55.0
	A %	53.3	58.0	44.3	48.0

*F%: Frequency of mycorrhizal root segments, M%: the intensity of mycorrhizal colonization in the root, A%: Arbuscule frequency in roots

The microscopic assessment confirmed that plants of non-inoculation treatment were not colonized by AMF. As is evident from *Table 3*, at the vegetative stage, the highest value of frequency of mycorrhizal root segments recorded under a low level of drought. At the flowering stage, mycorrhizal colonization increased as compared with those of vegetative stage, and F%, M %, and A% recorded a maximum value under a low level of drought.

It was observed that the highest mycorrhizal colonization was recorded at the maturity stage (as compared with those of vegetative and flowering stage). Frequency percentage of mycorrhizae in roots reduced at the vegetative stage by about 4%, whereas increased by about 8% and 5% in flowering stage and maturity, respectively at low drought level (D1). The moderate (D2) and high (D3) drought levels obviously reduced the percentage of arbuscular mycorrhizae frequency in roots at the different

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stages of plant growth. The mycorrhizae in roots of wheat plants consisted of arbuscules, vesicles, as well as intra-and extraradical hyphae. The arbuscules and vesicles were patchily distributed along the roots examined. The intraradical hyphae were evenly distributed and frequently formed coils.

The phosphorus content of wheat plant shoots inoculated with AMF grown at different levels of the drought was represented in *Fig. 1A*. The data revealed that P uptake was significantly reduced by drought treatments. The P contents of wheat shoots were used as indicators of mycorrhizal activity in the soil. Mycorrhizal inoculation of the wheat plant significantly raised the P uptake especially at low drought level (D1) and shoots of mycorrhizal wheat plants had significantly higher concentrations of P than those of nonmycorrhizal wheat plants.

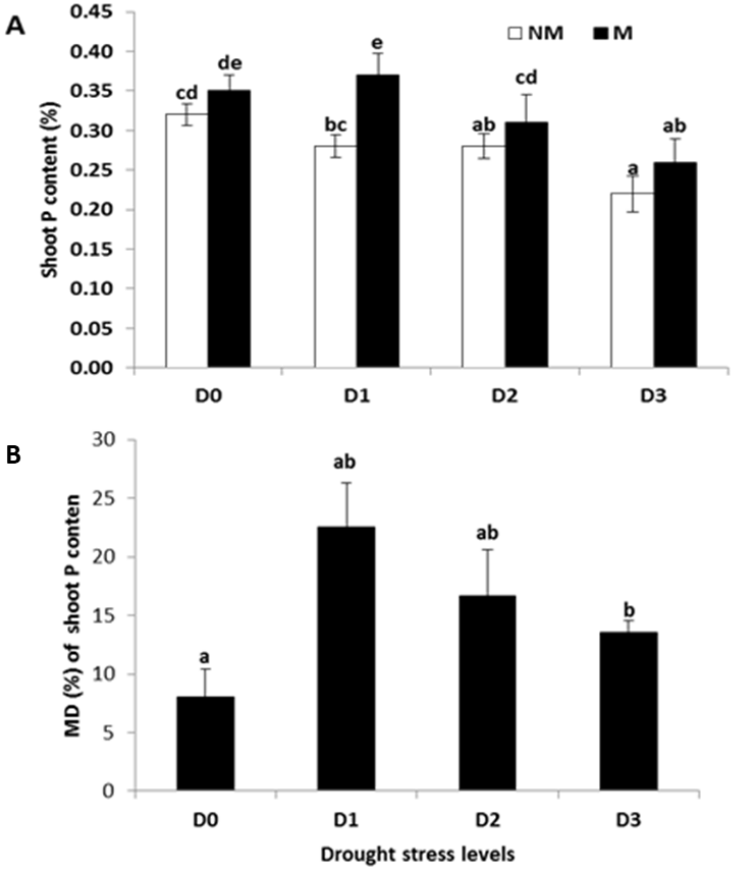

Figure 1. *Effect of different levels (D0, D1, D2, and D3) of drought on/ (A) wheat shoot phosphorus content (%); (B) Mycorrhizal dependency of P wheat shoot content (%). Means in each column followed by the same letter are not significantly different (P < 0.05) as determined by Duncan's test. Values are the means of three replications*

Photosynthetic pigments

The content of the photosynthetic pigments (chlorophyll a, chlorophyll b and carotenoids) of mycorrhizal and non-mycorrhizal in wheat plant leaves are presented as mg g^{-1} leaf fresh weight in *Figure 2*. The current study shows that photosynthetic pigment fractions are significantly affected water deficit and mycorrhizal inoculation as indicated by significant two-way interaction based on ANOVA. In general, with all

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treatments, the content of photosynthetic pigment fractions was decreased as the level of drought increased in the soil pot experiment.

However, the contents of photosynthetic pigments of mycorrhizal wheat plant leaves were significantly ($p<0.05$) greater than those of non-mycorrhizal ones. In non-mycorrhizal plants a significant reduction in total pigments was recorded at high water deficit (D3).

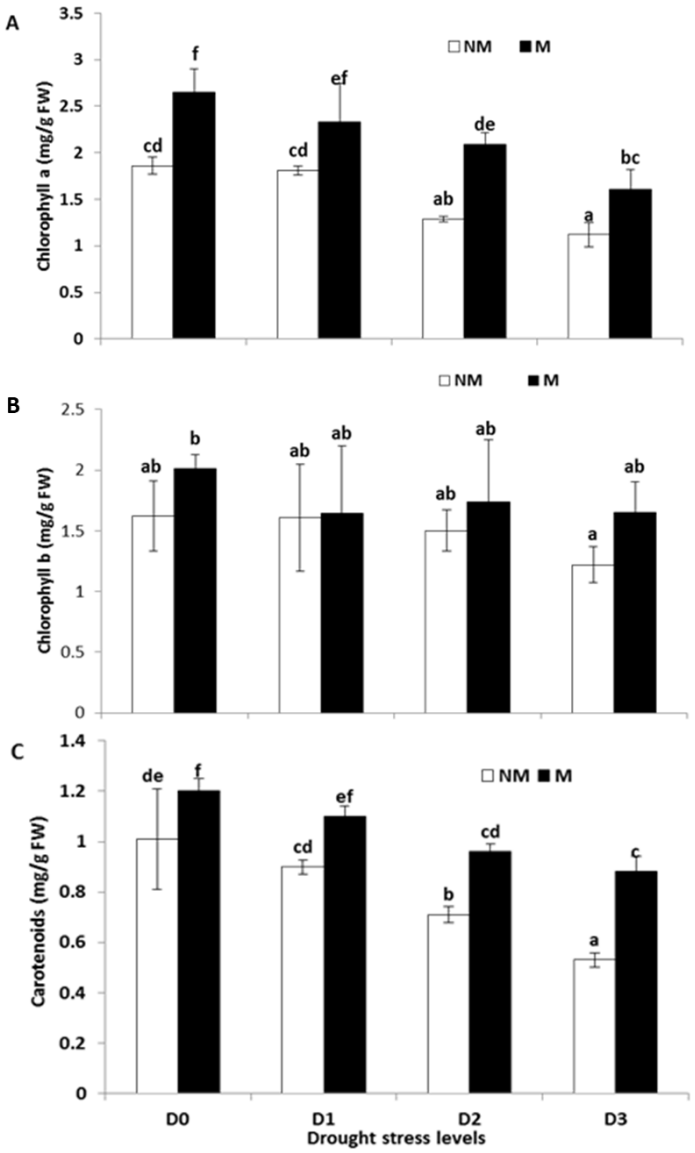

Figure 2. *Chlorophyll a (A); chlorophyll b (B); and carotenoids (C) contents in leaves of wheat plants inoculated with mycorrhizal fungi grown under different levels (D0, D1, D2 ,and D3) of drought stress. Means in each column followed by the same letter are not significantly different ($P < 0.05$) as determined by Duncan's test. Values are the means of three replications*

Analysis of variance of chlorophyll contents showed that mycorrhizal colonization significantly improved chlorophyll concentrations. Also, the interaction of drought and mycorrhizal inoculation exhibited a stimulatory effect on the different types of photosynthetic pigments (*Fig. 2*).

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Discussion

In the present study, drought decreased the fresh and dry weights of wheat plants which are recorded by many studies (Auge, 2001; Celebi et al., 2010; Kyriazopouls et al., 2014). Previous reports have indicated that drought stress severely affects plant growth through various mechanisms, such as reduced leaf water potential, reduced rate of cell division, and altered plant water and nutrient relationships (Boomsma and Vyn, 2008; Kilic and Yagbasanlar, 2010; Farooq et al., 2012). The positive effect of AMF on wheat growth under different levels of drought suggested that the application of AMF technology may be a sustainable method for improving plant performance under the adverse conditions (Kyriazopouls et al., 2014; Malik et al., 2017). AM symbiosis drives an increase in the leaf area and coarse root mass and improves the plant-soil water relationship under drought stress, which can affect various physiological processes. The improvement of biomass in mycorrhizal plants may also be due to the enhanced water uptake (Subramanian et al., 2006; Habibzadeh et al., 2013). Our results in *Table 2* are in accordance with the current tendency for reduced use of agrochemicals, research is currently aimed at crop yield improvement and at yield sustainability; thus, microbial-based approaches have been proposed to improve crop yield (Covacevich et al., 2007). In this respect, numerous researchers have reported improvement in biomass production and grain yield in the grain of cereals after inoculation with AMF (Tarafdar and Marschner, 1994). The fungal filaments outside the root system can spread and explore soil areas not reachable by plant roots. One centimeter of colonized roots might produce 50 to 150 cm of extraradical hyphae (Harley, 1989). Through this mechanism, AMF associations may improve crop yield by increasing the capacity of plants to obtain nutrients that are relatively immobile in the soil such as phosphorus (P).

Our results in accordance with most studies which indicated that drought stress decreased the colonization rates (Kohler et al., 2009; El-Mesbahi et al., 2012) and may be due to the adaptation of AMF isolates to different substrates and different behaviors of plants under drought stress (Gholamhoseini et al., 2013). The decrease of root colonization by drought stress is due to reducing spore germination and plant photosynthetic capacity (Wu et al., 2013; Xiao-Qing et al., 2017). Michalis et al. (2013) concluded that soil moisture can have various effects on AMF spore germination and thus root colonization. Moreover, drought modifies various features of the root system which, in turn, may influence the degree of colonization and the frequency of different AM fungal structures (Fusconi and Berta, 2012). Some results opposite to our studies and showed that mycorrhizal colonization increased with increasing intensity of drought stress (Zhao et al., 2015). They concluded that under drought stress, watering caused lighter compaction, better pore structure and soil aeration, which benefits the development of mycorrhizae.

Drought stress may reduce nutrient mineralization by lowering nutrient availability (Heidari and Karami, 2014). Mycorrhizal symbiosis may improve plant nutrition, which is generally regarded as an important drought tolerance mechanism (Smith and Read, 2008; Li et al., 2012). Hijikata et al. (2010) noticed that the activity of high-affinity P transporters on the plasma membrane of extraradical hyphae is most likely directly involved in enhanced drought tolerance in plants. In the present study, the P contents in mycorrhizal plants were consistently higher than those in non-mycorrhizal plants, regardless of the intensity of drought stress. The increase in P concentrations of mycorrhizal plants may provide more RNA to meet the protein synthesis needs, resulting in a higher plant growth rate (Matzek and Vitousek, 2009). Under drought conditions, the RNA-directed synthesis of some protein or enzymes may be of physiological importance in helping the plants withstand drought stress (Fan and Liu, 2011).

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The results obtained showed that wheat plants under drought stress were strongly mycorrhizal dependent (*Fig. 1B*). The lowest mycorrhizal dependency recorded at the control drought level. SchiiBler et al. (2001) stated that arbuscular mycorrhiza is formed in about 80% of land plants with soil fungi belonging to *Glomeromycota*. Arbuscular mycorrhizal plants can take up more soil nutrients especially phosphate (P), micronutrients such as copper and zinc, than non-mycorrhizal plants (Smith and Read, 1997). They concluded that the growth of a host plant can be improved by mycorrhizal colonization provided that soil available P is a limiting factor for plant growth. The degree of growth improvement is affected by factors such as host plant species, fungal species, and soil conditions.

Analysis of variance of chlorophyll contents (*Fig. 2*) revealed that mycorrhizal colonization significantly improved chlorophyll concentrations and the interaction of drought and mycorrhizal inoculation exhibited a stimulatory effect on the different types of photosynthetic pigments. Chlorophyll status is a key index for evaluating plant photosynthetic efficiency and environmental stress. Augé (2001), Gemma et al. (1997) and Azooz and Youssef (2010) are in agreement with the results of other proved the differences of chlorophyll *a*, chlorophyll *b* and chlorophyll *a* + *b* concentrations were significant between AM and non-AM maize plants under drought stress conditions (Zafari et al., 2017). They suggest that drought stress interferes less with chlorophyll synthesis and/or more with chlorophyll breakdown (Evelin et al., 2009) or protects pigments against (Kyriazopouls et al., 2014) oxidative damage generated by drought (Shinde and Thakur, 2015). Drought stress caused a great decline in the chlorophyll *a*, chlorophyll *b* and the total chlorophylls content in many plant varieties investigated by some authors in non-mycorrhizal than mycorrhizal in plants (Ommen et al., 1999; Manivannan et al., 2007; Evelin et al., 2009). Also, unchanged chlorophyll level during drought stress has been reported in other species, depending on the duration and severity of drought (Kpyoarissis et al., 1995). The decrease in chlorophyll under drought stress may result in chloroplast damage caused by active oxygen species (Herbinger et al., 2002). Consequently, the rate of photosynthesis decreased (Kawamitsu et al., 2000) due to lower stomatal conductance in order to conserve water and the resistance of the stomata to CO_2 entry probably is the main factor limiting photosynthesis under drought, resulting in less assimilate production for growth and yield of plants.

Conclusion

The mycorrhizal root colonization rate was significantly affected by drought stress levels or the stages of plant growth of wheat plants. Also, it can be concluded that the AMF enhances the absorption of phosphorus and other nutritional elements and then improves the nutritional status of wheat plants during drought stress which resulted in an increase in chlorophyll contents than control plants. In spite of better performance of inoculation with mycorrhizae, there are still certain aspects that need critical consideration. One important aspect is the evaluation of this approach under natural field conditions. Most of the previous studies were conducted under controlled conditions, and the response of these organisms observed under such conditions may vary significantly in view of the variable ecology of these microorganisms in the natural environment. Additionally, the researches also have to face other harsh conditions like the toxicity of heavy metals and pathogen attacks. Therefore, the role of these microorganisms for providing relief from other stresses.

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