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PHYSIOLOGICAL MARKERS MITIGATE DROUGHT STRESS IN *PANICUM TURGIDUM* FORSSK. BY ARBUSCULAR MYCORRHIZAL FUNGI

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Abstract

Drought stress is one of the primary abiotic stresses that inhibits the rehabilitation of degraded rangelands in Saudi Arabia, however, studies on the ability of arbuscular mycorrhizal fungi (AMF) to improve stress tolerance are lacking. *Panicum turgidum*, (Poaceae) is widely distributed in the Sahara of Saudi Arabia and represents a relatively good source for crude protein as fodder. An experiment was conducted in pots to evaluate the ability of AMF to improve drought-stress tolerance in *P. turgidum*. Results indicated that drought stress reduced the growth of *P. turgidum* and affected AMF colonization by reducing the number of spores, arbuscules, vesicles, and mycelia. Inoculation of the soil with AMF significantly enhanced root growth and ameliorated the negative effects of drought stress. AMF upregulated the antioxidant system in *P. turgidum* which prevented the accumulation of hydrogen peroxide and thus reduced the rate of lipid peroxidation. In addition, AMF also enhanced the synthesis of indole acetic acid (IAA); thereby promoting growth under both normal and drought-stress conditions. In summary, soil inoculation with AMF improved drought stress tolerance in *P. turgidum* by enhancing the antioxidant system in host tissues.

Key words: Antioxidants, Lipid peroxidation, Drought, AMF, *Panicum turgidum.*

Introduction

Drought is a global problem resulting from a shortage of adequate water and becomes severe when the rate of transpiration exceeds the rate of water absorption. Drought is a naturally-occurring abiotic stress that causes considerable perturbation in the metabolism of crops resulting in significant yield losses (Ahanger *et al*., 2014; Alwhibi *et al*., 2017). The impact of drought stress is becoming more pervasive due to the elevated temperatures associated with climate change, infrequent rains, and the lack of irrigation availability. Drought stress inhibits root growth which hampers plant growth by restricting a plant's access to water (Harb *et al*., 2015; Assaha *et al*., 2016). Drought stress also induces the excessive generation of reactive oxygen species (ROS) resulting in oxidative stress (Ahanger *et al*., 2014; Li, 2015; Mo *et al*., 2016). Oxidative stress in drought-stressed plants is evidenced by increased peroxidation of membrane lipids, proteins, and nucleic acids; resulting in increased leakage of cellular components, altered cellular functioning, and genotoxicity (Vurukonda *et al*., 2016). The major types of ROS that become elevated in response to drought stress include superoxide, hydrogen peroxide, and hydroxyl radicals (Ahanger *et al*., 2014). In addition to having a direct impact on the physiology of plants, drought stress has a negative impact on other aspects of normal growth, such as nutrient uptake and root proliferation (Assaha *et al*., 2016). Drought can be described as being a multidimensional stress that affects growth through its impact on various subcellular compartments, cell organs, and the whole plant (Rahdari *et al*., 2012; Ali *et al*., 2014); collectively affecting the quantity and quality of plant growth. Therefore, efficient and effective mitigation of drought stress is a high priority for securing global food security.

A variety of tolerance mechanisms initiated at the physiological, biochemical, or molecular level, work in coordination to ameliorate the negative effects of drought. Tolerance mechanisms triggered in plants in response to drought stress include the up-regulation of the antioxidant system, the synthesis of transport proteins that function in roots, and the accumulation of osmotic constituents (Tapia *et al*., 2016; Alwhibi *et al*., 2017). Enhancing these mechanisms or improving their efficiency can provide significant improvements in growth and yield under drought stress conditions. However, due to a lack of understanding about these mechanisms, it has become imperative to utilize alternative methods to improve stress tolerance. For example, both enzymatic and non-enzymatic antioxidants can neutralize the oxidative stress induced by excessive levels of ROS; thus reducing oxidative injury such as the peroxidation of membranes and proteins.

Arbuscular mycorrhizal fungi (AMF) are beneficial symbiotic organisms that can form an association with most of the plants species and provide a significant improvement in the structure and stability of the rhizospheric soil (Jing *et al*., 2014; Deepika & Kothamasi, 2015). AMF also elevate the physiological, biochemical, and molecular response of plants to stress; ultimately improving the growth performance of plants under both normal and stress conditions (Zhou *et al*., 2015; Mirshad & Puthur, 2017). AMF act as bio-ameliorators of stress can allay stress-induced deleterious changes in plant growth and metabolism (Ahanger *et al*., 2014; Mirshad & Puthur, 2017). Yang *et al*., (2015) demonstrated that

AMF-inoculated plants exhibit increased protection of photosynthesis in *Robinia pseudoacacia* L. by improving the phytostabilization and antioxidant system. AMF can improve the appearance, physiology, and nutritional aspects of plants (Mo *et al*., 2016). AMF inoculation can also help to maintain ion homeostasis in plants which enables greater metabolic activities (Jing *et al*., 2014; Deepika & Kothamasi, 2015; Nath *et al*., 2016; Mirshad *et al*., 2017).

Panicum turgidum (family Poaceae) is a C_4 halophytic grass that grows in dense bushes. It is a stress tolerant plant that is commonly found in the Sahara, Pakistan, Arabia, and other desert climates (Hashem *et al*., 2015). Due to its high nutritive value and ease of cultivation, the grains of *Panicum turgidum* are considered as a potential supplementary food source to common cereals. Grains of *P. turgidum* are rich in proteins, however, the levels of these proteins are significantly affected by edaphic factors (Heneidy and Halmy 2009). In Saudi Arabia, it is found along the seashore, interior desert plains, sand dunes, and mountain ranges. Since it has the potential to endure harsh growth conditions, it has been considered as a potential nurse plant (Chaudhary & Al-Jowaid, 1999; Hashem *et al*., 2015).

The present study was conducted to investigate the impact of drought stress on the growth and antioxidant metabolism of *P. turgidum*, and to examine the potential role of AMF in improving the drought stress tolerance.

Materials and Methods

Plant seeds and arbuscular mycorrhizal fungi: Seeds of *Panicum turgidum* Forssk. were collected from plants growing in the Thomama region (N 25° 336,291 E 46° 533,651), Riyadh, Saudi Arabia. Seeds were disinfected, rinsed with distilled H_2O, and subsequently germinated in Petri dishes containing double layers of wet blotter paper. Seeds were germinated in the dark at 28 ºC for 5 days. Healthy germinated seedlings with uniform growth were used in the pot experiment (one seedling/pot). AMF used in the present study were *Claroideoglomus etunicatum* (syn. *Glomus etunicatum*), *Rhizophagus intraradices* (syn. *Glomus intraradices*), and *Funneliformis mosseae* (syn. *Glomus mosseae*) which were previously isolated and identified (Hashem *et al*., 2016) as described by Daniels & Skipper (1982) and Utobo *et al*., (2011). The identification was based on the characters of asexual spore structures as reported in a manual prepared by the International Culture Collection of Vesicular and Arbuscular Mycorrhizal Fungi (Anon., 2014). The trap culture method was used to increase the population of AMF which entailed the establishment of a trap plant (*Zea mays* L.) as described by Stutz & Morton (1996). The AMF inoculum for the pot experiment was trap soil (approx. 750 spores/20 g trap soil)/pot and soil which was not inoculated with AMF, was used as a control.

Experimental design and treatments: The study was conducted in a growth chamber, Plant Production Department, College of Food & Agricultural Sciences, King Saud University, Riyadh, Saudi Arabia. The experimental design was a complete randomized block with three replicates per treatment. The pots were filled with 2 kg of a 1:1:1 mixture of peat, perlite, and sand (v/v/v). The drought stress was induced by regulating the amount of Jensen's nutrient solution (Jensen & Collins, 1985) supplied to each pot as described by Fatokun *et al*., (2012). The procedure was described in detail in our previous study (Jemo *et al*., 2017).

The pots were arranged in four treatments

1. Control treatment: No treatments. The pots were watered with a modified Jensen's nutrient solution to maintain a 60% water holding capacity.

2. AMF treatment: Soil inoculated with AMF as described above before seeds were planted and pots were watered as described in the control treatment (non-stressed plants).

3. Drought treatment: Soil was not inoculated with AMF and the plants were subjected to a drought stress by regulating the amount of Jensen's nutrient solution added to the pots to maintain a 30% water holding capacity. The water holding capacity was reduced to 30% over one week to avoid sudden death of the treated plants.

4. AMF + Drought treatment: Soil was inoculated with AMF as described in treatment 2 and the plants were subjected to a drought stress as described in treatment 3.

The plants were grown for eight weeks in a growth chamber (26 ± 2 °C, relative humidity of 70–75%, and an average photoperiod of 18 h light/6 h dark, 350 µmol photon m-2 s^{-1} light intensity) after beginning the drought stress. At end of the experiment, the plants were carefully harvested to evaluate the above ground and root morphology. Root samples were also taken to characterize their mycorrhizal status. Shoot samples were collected to conduct the biochemical and physiological analyses.

Determination of root morphological traits: Root samples from each treatment were collected and stored in 20%, alcohol in water (v/v) as described by Bouma *et al*., (2000), and later scanned using a suitable background in an Epson Scanner (Epson Perfection 7000 scanner with resolution of 600 dots per inch). Digital images were captured and various attributes of root morphology were analyzed using WinRHIZO Reg 2012b software (http://regent.qc.ca/assets/winrhizo_about.html). The measured root parameters included root length, root total surface area, root volume, root diameter, and the number of root tips.

Determination of arbuscular mycorrhizal colonization: Root samples from each treatment were stained with trypan blue-lactophenol (Phillips & Hayman, 1970; Zubek *et al*., 2012) and examined with a digital computerized microscope (model DP-72, Olympus) at 20 × magnification to assess AMF colonization of roots. AMF colonization was recorded as the quantity of arbuscules, vesicles, and mycelia as described by Hashem *et al*., (2016).

Percent colonization was calculated as follows:

$$\% \text{ Colonization} = \frac{\text{Total no. of AMF positive segments}}{\text{Total no. of segments studied}} \times 100$$

Measurement of photosynthetic pigments, stomatal conductance (gs) and transpiration rate (E): For the estimation of photosynthetic pigments, fresh leaves (100 mg) were extracted in acetone and the absorbance of the supernatant was recorded at 622, 664, and 440 nm using a spectrophotometer (Lichtenthaler & Wellburn, 1983). Leaf gas exchange measurements viz., stomatal conductance (*gs*) and transpiration rate (*E*) were measured on fully expanded horizontal leaves in full and bright sunlight between 10:00 h and 12:00 h using IRGA (LCA-4 model Analytical Development Company, Hoddesdon England).

Determination of lipid peroxidation (MDA) and hydrogen peroxide (H_2O_2): Lipid peroxidation was analyzed by estimating the formation of a thiobarbituric acid (TBA) reactive substance, malonaldehyde (MDA). Briefly, fresh tissue (0.2 g) was homogenized in trichloroacetic acid (TCA) (0.1% w/v) and centrifuged at 10000 × g for 5 min. Subsequently, 1 mL extract was reacted with 4 mL of 5% TBA prepared in 20% TCA in a water bath at 100 ºC for 30 min. The mixture was then cooled and centrifuged again for 10 minutes at 10000 ×g and absorbance was read at 532 nm and 600 nm (Hodges *et al*., 1999). An extinction coefficient of 155 $mM^{-1}cm^{-1}$ was used for the calculation and expressed as µmol MDA g^{-1} FW. Levels of hydrogen peroxide (H_2O_2) were estimated by homogenizing fresh leaf tissue (0.5 g) in TCA (0.1% w/v), followed by centrifugation at 12000 × g for 15 min. Subsequently, 0.5 mL of supernatant was mixed with 0.5 mL of 10 mM potassium phosphate buffer (pH 7.0) and 1 mL of 1 M potassium iodide. Absorbance was recorded at 390 nm and the concentration of H_2O_2 was determined utilizing a standard curve and expressed as µmol g^{-1} FW (Sergiev *et al*., 1997).

Determination of reduced glutathione: To estimate the amount of reduced glutathione (GSH), 500mg fresh tissue was homogenized in phosphate buffer and the extract was centrifuged at 3000 ×g for 15 min. The resulting supernatant (500µL) was then mixed with 5, 5-dithiobis-2-nitrobenzoic acid and after 10 minutes, absorbance was recorded at 412nm (Ellman 1959). The concentration of GSH was determined using a standard curve generated from GSH standards and expressed as µmol g^{-1} FW.

Extraction and quantification of indole acetic acid (IAA): To determine endogenous levels of IAA, leaves were extracted in 80% aqueous acetone (4:1, v/v) containing butylated hydroxytoluene (10 mg/l) and the resulting extract was purified using EtOAc and $NaHCO_3$ (Kusaba *et al*., 1998). The method of Kelen *et al*., (2004) was used to quantify the level of IAA. Purified extract residue was subjected to HPLC on a column of PEGASIL ODS (6 mm i.d.×150 mm, Senshu Kagaku, Tokyo, Japan) and quantification of IAA was based on a standard curve generated using IAA standards ranging from 10 to 200 ng/ml.

Statistical analysis

Data presented represent the mean and standard deviation of three replicates. Statistical significance was determined using analysis of variance (ANOVA) and a Least Significant Difference (LSD) test using SPSS software (version 15). Significance was calculated at $p<0.05$. The degree of correspondence (Pearson's coefficient) was evaluated by pairwise Spearman rank correlation coefficients using SAS 9.4.

Results

The effect of drought on the percent colonization and sporulation of AMF are presented in Table 1. Drought reduced the number of AMF spores by 72.32% and the percent colonization as measured by the number of mycelia, vesicles, and arbuscules was reduced by 34.07, 21.93 and 61.28%, respectively (Table 1).

AMF inoculation had a significant impact on root attributes (Table 2). Relative to non-inoculated control plants, AMF-inoculated plants exhibited a significant increase in root length (37.31%), root surface area (48.13%), root volume (57.14%), root diameter (17.47%), and the number of observed root tips (14.72%). The non-inoculated *P. turgidum* plants were exposed to drought exhibited reduced root length, root surface area, root volume, root diameter, and the number of root tips by 61.4, 56.6, 44.8, 7.1, and 51.8%, respectively. The negative impact of drought stress on root attributes, however, was ameliorated by inoculation of plants with AMF. This was especially true for root diameter and the number of root tips, where the impact of drought stress was completely mitigated (Table 2).

Drought stress resulted in a considerable reduction in the synthesis of photosynthetic pigments (Table 3). In contrast AMF-inoculation of plants increased their synthesis in non-stressed plants and also ameliorated the negative impact of drought stress (Table 3). Relative to the non-stressed control, drought-stressed *P. turgidum* plants exhibited a 32%, 38%, 37% and 54 % reduction in the level of chlorophyll a, chlorophyll b, total chlorophylls, and carotenoids, respectively (Table 3). Importantly, AMF-inoculated plants when exposed to a drought stress only exhibited a 22.6, 28.8, 23.5, and 28.9% reduction in chlorophyll a, chlorophyll b, total chlorophylls and carotenoids, respectively. These data once again indicated that AMF inoculation partially mitigated the negative impact of drought stress on photosynthetic pigments. Under normal growth conditions AMF-inoculated plants exhibited an increase of 29% in chlorophyll a, 23% in chlorophyll b, 26% in total chlorophylls, and 25% in carotenoids, relative to the non-inoculated control plants. The positive impact of AMF inoculation was consistent on other photosynthetic attributes, including transpiration rate and stomatal conductance, which was increased by 29 and 21%, respectively. Drought stress resulted in a decline of 74.5 and 74.5% in transpiration rate and stomatal conductance, respectively, while AMF-inoculated plants exposed to a drought stress exhibited only a 49.3 and 44.2% reduction in transpiration rate and stomatal conductance, respectively (Table 3).

Table 1. Effect of drought stress on the spore number, total colonization (%) measured interms of number of mycelium, vesicles, and arbuscules in *Panicum turgidum*. Poor (P), medium (M) and A (abundant).

Treatments	*Number of spores	Mycorrhizal status (%)											
		Total colonization			Mycelium			Vesicles			Arbuscules		
		Mycelium	Vesicles	Arbuscules	P	M	A	P	M	A	P	M	A
AMF	63.6a	54.73a	31.59a	21.35a	35.6b	41.4a	38.8a	70.0a	17.4b	13.2b	36.6b	59.6a	45.4a
Drought + AMF	17.6b	36.08b	24.66b	8.26b	64.8a	35.2b	16.8b	71.6a	24.0a	27.0a	55.2a	38.2b	20.4b
LSD at: 0.05	10.369	10.882	18.734	14.938	11.94	4.67	12.32	1.03	4.81	10.03	10.72	8.91	12.38

*Number of spores/ 100 g soil

Data presented is mean of three replicates. Data followed by same letter are significantly different at p<0.05

Table 2. Effect of drought stress on the root length (cm), root surface area (cm^2), root volume (cm^3), root diameter (mm) and number of root tips in *Panicum turgidum* with and without AMF inoculation.

	Root length (cm)	Surface area (cm^2)	Root volume (cm^3)	Root diameter (mm)	Number of root tips
C	46.20b	24.854b	1.074b	1.70c	33.6c
AMF	73.70a	47.918a	2.506a	2.06a	39.4a
Drought	17.84c	10.786d	0.5926d	1.83b	16.2d
Drought + AMF	32.89c	17.628c	0.7588c	1.70c	37.4b
LSD at 0.05	3.9863	5.2566	0.4687	0.3932	8.3743

Data presented is mean of three replicates. Data followed by same letter are significantly different at p<0.05

Table 3. Effect of drought stress on the chlorophylls (Chl a, b and total chl), carotenoids (mg g^{-1} FW), transpirations rate (*E*, mmol H_2O m^{-2} S^{-1}) and stomatal conductance (*gs*, mmol CO_2 m^{-2} S^{-1}) of *Panicum turgidum* with and without AMF inoculation.

Treatments	Photosynthetic pigments ((mg g^{-1} FW)						Transpiration rate (*E*)	Stomatal conductance (*gs*)
	Chl a	Chl b	Carotenoids	Chl a/b	Chl a+b	Total pigments		
C	1.022b	0.6006b	0.3186b	1.70285	1.623	1.941b	2.707b	190.46b
AMF	1.435a	0.7796a	0.4246a	1.84361	2.215	2.639a	3.795a	239.74a
Drought	0.6993d	0.3756d	0.1463d	1.86987	1.075	1.221d	0.689d	48.51d
Drought + AMF	0.7913c	0.4663c	0.2266c	1.69954	1.257	1.484c	1.373c	106.34c
LSD at 0.05	0.2789	0.026	0.0377	0.3917	0.2763	0.638	0.2483	6.4744

Data presented is mean of three replicates. Data followed by same letter are significantly different at p<0.05

Non-stressed, AMF-inoculated plants of *P. turgidum* exhibited a 9.7 and 19.0% increase in the endogenous concentration of IAA, relative to non-inoculated plants, in shoot and root tissues, respectively (Fig. 1). Drought stress, however, reduced shoot and root concentrations of IAA by 46.8 and 38.3%, respectively. The negative effect of drought stress on IAA concentrations in shoots and roots was partially mitigated by AMF-inoculation and only a 27.6% and 23.4% reduction in IAA levels was observed in drought-stressed shots and roots, respectively (Fig. 1).

P. turgidum plants when subjected to drought stress exhibited a considerable increase in hydrogen peroxide which resulted in higher levels of lipid peroxidation. Relative to non-inoculated, non-stressed plants, drought stress increased hydrogen peroxide levels by 70.5 and 68.3% in non-inoculated shoots and roots, respectively and a 56.6 and 53.6% increase in lipid peroxidation. In contrast to non-inoculated, drought-stressed plants, AMF inoculated, drought-stressed plants exhibited a relatively lower increase in hydrogen peroxide (33.7 and 34.7% in shoots and roots, respectively) and lower levels of lipid peroxidation (23.6 and 21.2 in shoots and roots, respectively). AMF decreased the generation of hydrogen peroxide and lipid peroxidation by 10.9 and 6.8%, respectively, in shoots, and 31.7 and 18.9%, respectively, in roots; preventing oxidative damage (Figs. 2A and B).

The physiological markers of stress resistance induced in *P. turgidum* and the Pearson correlation values are presented in Table 4. The physiological markers for mycorrhizal status (MS) exhibited a positive but non-significant correlation with chlorophyll a, while a significant and positive correlation was observed for chlorophyll b. MS also exhibited a significant, positive correlation with carotenoid levels. A significant positive correlation was observed between MS and the concentration of IAA in roots. The analysis also revealed a significant positive correlation between chlorophyll a and the concentration of IAA in shoots. In contrast, a positive but non-significant correlation was observed in roots. All of the physiological parameters displayed a significant positive correlation between MS and chlorophyll a, chlorophyll b, and carotenoids; in addition to IAA in roots and shoots.

Table 4. The correlation matrix between mycorrhizal status and physiological markers of stress induced in *P. turgidum*.

	Mycelium	Chl a	Chl b	Caot.	Total pig.	IAA/Shoot	IAA/Root
Mycelium	1.00000	0.80651 0.0525	0.95932 0.0024	0.94015 0.0053	0.90460 0.0132	0.92843 0.0075	0.97273 0.0011
Chl a		1.00000	0.78792 0.0627	0.79115 0.0609	0.97188 0.0012	0.84494 0.0342	0.78704 0.0632
Chl b			1.00000	0.96632 0.0017	0.90892 0.0121	0.98746 0.0002	0.9980 <.0001
Caot.				1.00000	0.90612 0.0128	0.96648 0.0017	0.97092 0.0013
Total pig.					1.00000	0.94401 0.0046	0.90747 0.0124
IAA/Shoot						1.00000	0.98117 0.0005
IAA/Root							1.00000

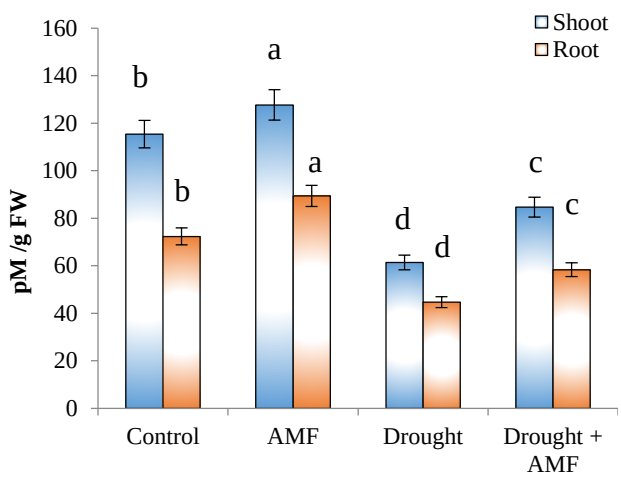

Fig. 1. Effect of drought stress on the endogenous concentration of IAA in shoot and root of *P. turgidum* with and without AMF inoculation. Data presented is mean of three replicates. Data followed by different letter are significantly different at $p<0.05$. LSD at 0.05: Shoot, 3.9006; Root, 3.5434).

Discussion

Increasing episodes of drought throughout the world has highlighted the urgent need to identify alternative strategies for maintaining plant growth and yield for sustainable food security. Drought alters root architecture and development by hampering cell division; resulting in reduced cellular expansion and hence tissue elongation. In the current study, inoculation of plant root systems with AMF significantly improved root growth which was evidenced as increases in root length, surface area, volume, diameter, and number of root tips. Therefore, it could be inferred that AMF may have significantly impacted cellular division of root cells by elevating the level of expansin proteins (Mohanty *et al*., 2018). The results of our study are in agreement with the results reported by Aroca *et al*., (2012), Hashem *et al*., (2014) and Alwhibi *et al*., (2017). AMF-colonized plants exhibit increased resilience to stress and the beneficial impact of AMF on overall plant performance has been widely accepted (Alqarawi *et al*., 2014). AMF activates tolerance mechanisms in plants and AMF-colonized plants exhibit greater water and nutrient uptake (Wu *et al*., 2010).

In addition to reduce the growth of *P. turgidum*, drought stress also impeded the colonization of roots by AMF, as measured by the number of colonized mycelia, arbuscules, and vesicles. The reduction in AMF colonization thus reduced the beneficial impact of AMF on the host plant (Deepika & Kothamasi, 2015). Similar to these results, Jing *et al*., (2014) and Mirshad & Puthur (2017) also reported a reduction in the percent colonization due to drought stress in Sainfoin (*Onobrychis viciifolia*) and *Saccharum spontaneum*. Improved growth due to AMF colonization under normal growth conditions occurs primarily due to enhanced phosphorous solubilization which has been reported to be negatively impacted by reduced soil moisture (Deepika & Kothamasi, 2015). Maintaining a high percentage of AMF colonization can directly influence the water holding capacity of a soil (Wu *et al*., 2013), thus facilitating the optimal uptake of water, even under low water availability. These positive effects are mainly due to secretion of a compound, glomalin, by extra radical hyphae (Fagbola *et al*., 2001). Prolonged exposure to drought stress affects the growth, as well as the potential colonization of AMF, by restricting the optimal moisture content required for their establishment (Oyewole *et al*., 2017). Reduced water availability has been reported to reduce the expression level (transcript number) of the AMF large ribosomal subunit in sorghum (Symanczik *et al*., 2015).

AMF-inoculation of *Panicum turgidum* resulted in a significant elevation in the synthesis of chlorophyll pigments and the associated photosynthetic parameters, transpiration rate and stomatal conductance; parameters that were drastically reduced by exposure to drought stress. It has been estimated that drought stress inhibits the synthesis of photoassimilates by reducing the synthesis of chlorophyll pigments and the activity of ferredoxin $NADP^+$ oxidoreductase, thus reducing the fixation of carbon and the generation of ATP and NADPH (Nikolaeva *et al*., 2010). Under both normal and drought stress conditions, increased chlorophyll synthesis in AMF-inoculated plants, relative to non-inoculated plants, has been ascribed to a greater uptake of magnesium; which is the central structural component of chlorophyll (Tadayyon *et al*., 2017). Khayatnezhad &

Gholamin (2012) reported a reduction in chlorophyll in *Zea mays* due to drought stress that resulted in a significant decrease in yield. In the present study, AMF inoculation of *P. turgidum* promoted chlorophyll synthesis under both normal and drought-stress conditions; confirming its beneficial role in improving photosynthetic efficiency. Drought stress reduces the transcript levels of glutamate 1-semialdehyde aminotransferase, protoporphyrinogen oxidase, and Fe chelatase2, which is reflected in a reduction in the synthesis of 5-aminolevulinic acid and protoporphyrin IX (Phung *et al*., 2011). AMF inoculation may have induced the activity of chlorophyll biosynthesizing enzymes and also helped to maintain the photo-transformation of Pchlide to chlide. Inoculation of watermelon with AMF protected chloroplasts from drought-induced disaggregation of grana and thylakoid membranes by reducing the formation of osmiophilic globules in the chloroplast stroma; thus helping to maintain the photosynthetic efficiency (Mo *et al*., 2016). Previously, Zhou *et al*., (2015), while investigating the effect of AMF on *Triticum aestivum*, observed a significant improvement in the transpiration rate, stomatal conductance, and the rate of photosynthesis rate under both normal and drought-stress conditions. Results of the present study confirmed and support the protective role of AMF on photosynthesis and the metabolism of plants.

AMF inoculation of *P. turgidum* inhibited the drought-stress-induced formation of free radicals, such as H_2O_2, inhibiting the peroxidation of membrane lipids as measured by malonaldehyde levels. These findings support the results of Mirzaee *et al*., (2013) in *Brassica napus* and Zhou *et al*., (2015) in *Triticum aestivum*. Giannakoula & Ilias (2013) reported that drought stress in tomato caused a considerable increase in H_2O_2 production, resulting in increased lipid peroxidation. Reactive oxygen species (ROS), such as H_2O_2, potentially diffuse large distances via membrane aquaporins causing damage to multiple sites; resulting in altered cellular structural stability (Bienert *et al*., 2007). AMF-mediated reduction in the generation of H_2O_2, however, inhibits peroxidation and thus helps to maintain the structural and functional stability of a cell. Alwhibi *et al*., (2017) and Hashem *et al*., (2016) also reported a strong positive effect obtained from beneficial microorganisms in tomato and *Cassia italica* where there was a considerable reduction in oxidative stress and improved growth. Stress-induced generation of ROS often has an impact on the polyunsaturated component of membranes hampering their fluidity and hence functioning. AMF colonization of *Triticum aestivum* (Zhou *et al*., 2015) and watermelon (Mo *et al*., 2016) inhibited drought-induced ROS generation and increased photosynthetic efficiency relative to non-inoculated plants. In the current study, AMF inoculation of *P. turgidum* mitigated the negative impact of drought stress by inhibiting peroxidation and preventing damage to cellular structures. Drought stress up-regulates lipoxygenase activity (Sofo *et al*., 2004) which may have been down-regulated in AMF-inoculated plants, however, further studies are required to confirm above statement.

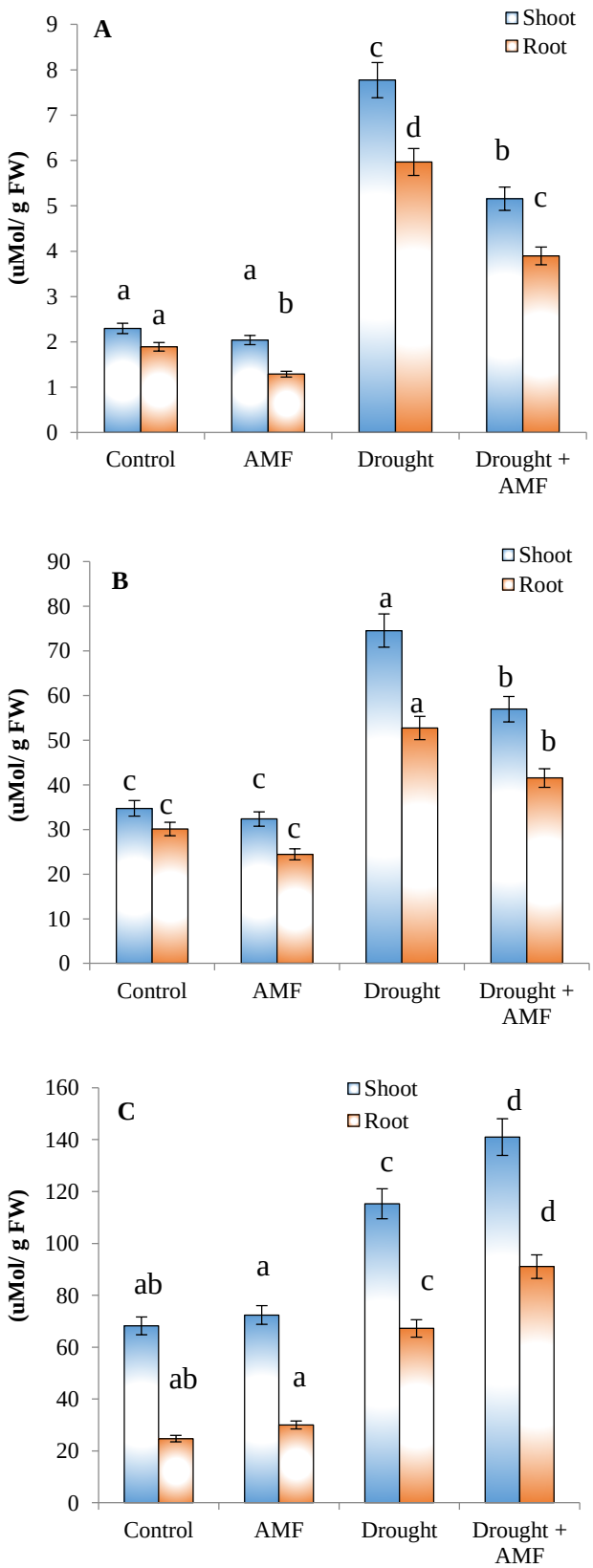

Fig. 2A-C. Effect of drought stress on the (**A**) hydrogen peroxide and (**B**) lipid peroxidation and (**C**) reduced glutathione in shoot and root of *P. turgidum* with and without AMF inoculation. Data presented is mean of three replicates. Data followed by different letter are significantly different at $p<0.05$. LSD at 0.05: Hydrogen peroxide, (Shoot: 0.3255, Root: 0.1499); Lipid peroxidation, (Shoot: 1.6158, Root: 1.1387); Reduced glutathione, (Shoot: 3.0972, Root: 2.4462).

Reduced generation of free radicals and oxidative damage in AMF-inoculated *P. turgidum* plants was correlated with the optimal functioning of the antioxidant defense system, providing the ability to neutralize toxic free radicals (Hashem *et al*., 2015, 2016). Drought stressed plants appear to optimize antioxidant activity for the protection of metabolic processes, such as photosynthesis and respiration (Alqarawi *et al*. 2014; Harb *et al*., 2015). In the present study, AMF enhanced antioxidant metabolism in host plants, thus ensuring greater protection of key metabolic pathways. Previous studies in *Hordeum vulgare* (Harb *et al*., 2015) and tomato (Alwhibi *et al*., 2017) have also reported drought-induced up-regulation of the antioxidant system. Optimization of the enzymatic and non-enzymatic components of the antioxidant system by AMF inoculation of plant hosts, helps to maintain ROS levels below a threshold level; thus, preventing a wide array of injury to cells (Nath *et al*., 2016). Plants overexpressing antioxidant system genes exhibit increased stress tolerance and yields (Weng *et al*., 2015). AMF optimization of plant antioxidant defense systems has been reported to increase with the intensity of the stress factor and that the initiation of such mechanisms results in greater photosynthetic efficiency (Yang *et al*., 2015). AMF-induced up-regulation of a plant's antioxidant system reduces lipid peroxidation. Additionally, Sofo *et al*., (2004) and Fazeli *et al*., (2007) were also reported that, in addition to reducing peroxidation and promoting growth, AMF inoculation of plants may provide greater structural stability to nucleic acids and inhibit the initiation of drought-induced genotoxic stress.

Drought stress reduced the endogenous concentration of auxin (IAA), however, this decrease was mitigated in AMF-inoculated plants. Growth hormones are key regulators of plant development and also mediate cell signaling in response to stress and participate in the activation of specific tolerance mechanisms (Bielach *et al*., 2017). A decline in auxin levels in response to abiotic stress has been reported to regulate drought adaptation in plants by up-regulating the expression of stress-related genes, including late embryogenesis abundant protein coding genes (Xie *et al*., 2003). Altered auxin concentration also negatively regulates the *DEEPER ROOTING 1* (*DRO1*) gene that controls the angle of root growth and whose expression is associated with increased drought avoidance and yield maintenance in rice (Uga *et al*., 2013). AMF inoculation of *P. turgidum* may have directly contributed to drought avoidance and growth maintenance by elevating the endogenous concentration of IAA. Abd_Allah *et al*., (2015) also reported a significant improvement in the endogenous concentration of IAA in *Phaseolus vulgaris* subjected to a salinity stress. Drought hampers cellular division, and hence growth, by altering the endogenous concentrations of auxins (Llanes *et al*., 2016). Therefore, AMF-induced increases in IAA levels may be responsible for greater growth observed in AMF-inoculated plants of *P. turgidum* under both normal and drought stress conditions.

Conclusion

Based on the results of the present study, it can be clearly stated that drought stress reduces the growth of *P. turgidum* by altering root development, chlorophyll pigment synthesis, antioxidant metabolism, and hormone production. AMF inoculation of plants triggered the initiation of tolerance mechanisms, such as enhanced root growth and increased pigment synthesis, that also beneficially supported other photosynthetic parameters, such as stomatal conductance and transpiration rates. Drought stress caused oxidative damage to membranes while AMF inoculation of plants mitigated oxidative stress by up-regulating the antioxidant system, which helped to maintain ROS levels at non-injurious levels. Therefore, the results of the present study support the premise that AMF-inoculation of plants can improve the drought tolerance of *P. turgidum* and should be considered as an effective approach for maintaining plant health and vigor under ever-increasing episodes of drought.

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