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AVALIAÇÃO DOS EFEITOS DE MITIGAÇÃO DE *GLOMUS MOSSEAE* EM *TRITICUM AESTIVUM* L., CV. CHAMRAN SOB ESTRESSE SECA

EVALUATION OF MITIGATION EFFECTS OF *GLOMUS MOSSEAE* ON *TRITICUM AESTIVUM* L., CV. CHAMRAN UNDER DROUGHT STRESS

ارزیابی اثرات بهبود دهنده *GLOMUS MOSSEAE* روی *TRITICUM AESTIVUM* L., CV. CHAMRAN تحت تنش خشک

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RESUMO

O objetivo principal deste estudo foi avaliar a resposta fisiológica e de tolerância à seca associadas ao crescimento de *T. aestivum* L., cv. Mudas de Chamran com *G. mosseae* em folhas e raízes. As mudas micorrízicas arbusculares (MA) ou não-MA sob condições normais ou em stress hidrico foram avaliadas quanto aos parâmetros de crescimento, teor relativo de água (TRA), classificação do soluto, peroxidação lipídica e antioxidantes enzimáticos e não enzimáticos. Os resultados refletiram a crescente influência da MA nas mudas sob estresse hídrico: micorrização de zero e 32,25% em mudas não-MA e MA, respectivamente, peso fresco e peso seco das mudas (28,5 e 27,34%, respectivamente) e raízes (28,86 e 31,68%, respectivamente), TRA (55,15%), concentração de fósforo nas brotações e raízes (69,25 e 95,36%), teor total de proteínas solúveis e carboidratos nas brotações (52,63 e 15,80%, respectivamente) e raízes (30,65 e 9,80%, respectivamente), catalase (28,66 e 31,43%), superoxidase dismutase (28,44 e 15,27%), glutationa redutase (44,62 e 18,84%), ascorbato peroxidase (15,58 e 39,49%) e redução no acúmulo de prolina de parte aérea e raiz (45,01 e 44,03%) e peroxidação lipídica (52,27 e 57,26%) em comparação com plântulas estressadas não inoculadas. Pigmentos fotossintéticos, incluindo clorofila a (77,28%), clorofila b (51,70%), clorofila total (66,76%), carotenóides (51,75%) e metabólitos secundários antioxidantes, como compostos fenólicos totais (36,25%), teor total de flavonóides (30%) e o teor total de antocianina (29,52%) aumentou adicionalmente nas folhas bandeira de plantas MA estressadas pela água. Considerando todos os resultados deste estudo, é possível concluir que a inoculação MA de plantas de trigo pode aliviar consideravelmente os efeitos nocivos do estresse por déficit hídrico.

Palavras-chave: *mudas de trigo, déficit hídrico, micorriza arbuscular, antioxidantes enzimáticos, estresse oxidativo*.

ABSTRACT

The main aim of this study was to evaluate physiological and growth-associated drought tolerance responses of mycorrhiza inoculated *T. aestivum* L., cv. Chamran seedlings with *G. mosseae* in flag leaves and roots. The arbuscular mycorrhizal (AM) or non-AM wheat seedlings under normal or water-stressed conditions were assessed for growth parameters, relative water content(RWC), solute aggradation, lipid peroxidation, and enzymatic and non-enzymatic antioxidants. Outcomes reflected the enhancing influence of AM on seedlings

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under drought stress: mycorrhization of zero and 32.25% in non-AM and AM seedlings, respectively, fresh weight and dry weight of shoots (28.5 and 27.34%, respectively) and roots (28.86 and 31.68%, respectively), RWC (55.15%), phosphorus concentration in shoots and roots (69.25 and 95.36%), total soluble protein and carbohydrate content in shoots (52.63 and 15.80%, respectively) and roots (30.65 and 9.80%, respectively), catalase (28.66 and 31.43%), superoxidase dismutase (28.44 and 15.27%), glutathione reductase (44.62 and 18.84%), ascorbate peroxidase (15.58 and 39.49%) and reduction in proline accumulation of shoot and root (45.01 and 44.03%) and lipid peroxidation (52.27 and 57.26%) by comparison to non-inoculated stressed seedlings. Photosynthetic pigments including chlorophyll a (77.28%), chlorophyll b (51.70%), chlorophyll total (66.76%), and carotenoid (51.75%) and antioxidative secondary metabolites such as total phenolic compounds (36.25%), total flavonoid content (30%) and total anthocyanin content (29.52%) additionally increased in flag leaves of water-stressed AM plants. Taking into account all the results of this study, it can be concluded that AM inoculation of wheat plants may considerably alleviate the harmful effects of water deficit stress.

Keywords: *wheat seedlings, water deficit, arbuscular mycorrhiza, enzymatic antioxidants, oxidative stress.*

چکیده

هدف اصلی این مطالعه ارزیابی واکنش‌های فیزیولوژیکی و رشدی-مرتبط با مقاومت به خشکی در برگ پرچم و ریشه گیاهچه‌های *T. aestivum* L., cv. Chamran تلقیح شده با مایکوریزا *G. Mosseae* بود. گیاهچه‌های تیمار شده گندم با آربسکولار مایکوریزا (AM) و غیره-AM تحت شرایط عادی و تنش آب از نظر پارامترهای رشدی، محتوای نسبی آب (RWC)، تجمع مواد محلول، پراکسیداسیون چربی و آنتی‌اکسیدان‌های آنزیمی و غیر آنزیمی مورد ارزیابی قرار گرفتند. نتایج منعکس کننده اثر افزایشی و بهبود دهنده AM روی گیاهچه‌های تحت تنش آبی نسبت به شاهد به قرار زیر بود: مایکوریزایی شدن ریشه‌ها صفر و 32/5% به‌ترتیب در گیاهچه‌های با-AM و بدون AM، وزن تر و خشک شاخساره (28%/5 و 27%/34، به ترتیب) و ریشه‌ها (28%/86 و 31%/68، به ترتیب)، RWC (55%/15، به ترتیب)، غلظت فسفر در شاخساره و ریشه (69%/25 و 95%/36، به ترتیب)، کل پروتئین‌های محلول و محتوای کربوهیدرات در شاخساره (52%/63 و 15%/80، به نرنیب) و ریشه (30%/65 و 9%/80، به ترتیب)، کاتالاز (28%/66 و 31%/43، به ترتیب)، سوپر اکسید دیسموتاز (28%/44 و 15%/27، به ترتیب)، گلوتاتیون ریدوکتاز (44%/62 و 18%/84، به ترتیب)، آسکوربات پروکسیداز (15%/58 و 39%/49، به ترتیب)، کاهش در برخی پارامترها از جمله کاهش تجمع پرولین در شاخساره و ریشه (45%/01 و 44%/03، به ترتیب) و مالون آلدئید (52%/27 و 57%/26، به ترتیب)، نسبت به گیاهچه‌های تلقیح نشده مشاهده گردید. بعلاوه رنگریزه‌های فتوسنتزی، شامل کلروفیل a (77%/28)، کلروفیل b (51%/70)، کلروفیل کل (66%/76) و کارتنوئید (51%/70) و متابولیت‌های ثانویه آنتی‌اکسیدانی مانند ترکیبات فنولی کل (36%/25)، محتوای فلاونوئید کل (30%) و متحوای آنتوسیانین کل (29%/52) در برگ پرچم گیاهچه‌های مورد تنش آبی واقع شده تلقیح شده با AM افزایش نشان داد. با مد نظر قرار دادن نتایج مطالعه کنونی، می‌توان نتیجه گرفت که تلقیح گیاهچه‌های گندم با AM می‌توان به نحوه موثری اثرات منفی کمبود آب را جبران نماید.

کلیدواژه‌ها: *گیاهچه گندم، کمبود اب، آرباسکولار مایکوریزا، آنتی‌اکسیدان‌های آنزیمی، تنش اکسیداتیو*

1. INTRODUCTION

Environmental abiotic stresses are of the most important performance-reducing factors for agricultural products (Hameed *et al*., 2014; Mathur *et al*., 2019). Among them drought stress as the most ubiquities abiotic stress along with the lack of soil nutrients, in many semi-arid regions of the world imposing serious restriction on yield at a world-scale of wheat (*T. aestivum*) as a critically important staple crop that provides over 20% protein and calories for over half of the world's population (Kumaraswamy and Shetty, 2016; Smith, 2017).

Further, to a large extent, in semi-arid areas where wheat is the main crop, drought stress is a constant threat that could occur at any growth stage (Wang *et al*., 2015; Saeidi *et al*., 2017). To this end, strategies to assist crops in coping with oxidative stress-driven by drought and ameliorate its impacts involve the utilization of symbiosis fungi species are essential. Drought stress besides a considerable reduction in wheat growth and yield negatively affects a wide range of other critical physiological and biochemical mechanisms (Kumaraswamy and Shetty 2016; Antoniou *et al*., 2017). Water deficit stress evokes osmotic and oxidative stress, in addition to a decrement in growth rate, biosynthesis of protein, and photosynthetic potential (Wang *et al*., 2018).

Moreover, generating a great deal of reactive oxygen species (ROS) under drought stress is more than common which is majorly responsible for peroxidation of lipids in the membrane that has disruption of cell membrane integrity as aftermath (Hameed *et al*. 2014; Jajic *et al*., 2015; Wang *et al*., 2019). The intensity of drought stress tolerance is more depends on the developmental phase and genetics (Ashraf and Foolad, 2007). Numerous metabolical strategies utilized by plants to develop important modifications at the molecular and biochemical levels and properly react drought stress generally by fortifying the enzymatic and non-enzymatic

defensive antioxidant mechanisms. In the case of enzymatic antioxidants, a rich literature exists that shows an enhance in activities of ascorbate peroxidase (APX), catalase (CAT), glutathione reductase (GR) or superoxide dismutase(SOD) to eliminate the harmful ROS generated under drought stress (Götz *et al*., 1999; Hajiboland *et al*., 2010; Amiri *et al*., 2017; Chiappero *et al*., 2019). Additionally, incrementing in non-enzymatic antioxidants, including accumulation of amino acids particularly proline as a crucial proteinogenic and plant secondary compounds such as phenolics and anthocyanin (Tattini *et al*., 2004; Khalil *et al*., 2018; Chiappero *et al*. 2019). Also, biosynthesis of some substances, namely generally known as regular responses, water-stressed plants (Bandurska 2000; Szabados and Savoure 2010).

Improving the drought resistance of wheat plants can indeed have a profound influence on their growth and grain yield under water-deficit stress. The results of current studies have shown that soil inoculation with arbuscular mycorrhizal (AM) fungi can cause a significant improvement in the nutrition absorption of plants (Alvarez *et al*., 2009; Hajiboland *et al*., 2010; López-Ráez 2016; Mathur *et al*., 2019). Which is possible by creating a symbiotic relationship through a vast network of hyphae in the soil and the rhizosphere surrounding which aim to improve the absorption of phosphorus, nitrogen, and transport of those elements to the host plant, enhancing water uptake, reducing the negative impact of environmental pollution, incrementing resistant to pathogens and positively contributing in growth and function of host plants in sustainable agricultural systems (Evelin *et al*., 2009; Santander *et al*., 2017; Mathur *et al*., 2019). Further, AM inoculation, as an applicable economically efficient, might mitigate the destructive impacts of drought through improving the enzymatic antioxidant and aggregation of compatible solutes (Jiang and Zhang 2002; Laxa *et al*., 2019). AM has been observed to enhance the drought stress tolerance in plants by increasing photosynthetic pigments, RWC, protein accumulation, and myriad other stress tolerance-related modifications (Xu *et al*., 2009; Mathur *et al*., 2019).

Considering the growing risk of drought stress in semi-arid wheat farms which has experienced significant reductions in growth and yield as the aftermath, and the essentiality of research in this area to find breakthroughs, in this study we aimed to examine the influence of inoculated AM fungus (*Glomus mosseae*) on alleviating the negative effects imposed by drought stress on *T. aestivum* cv. Chamran under greenhouse condition.

2. MATERIALS AND METHODS

Wheat (*T. aestivum* cv Chamran) seeds (obtained from Seed and Plant Improvement Institute, Karaj, Iran) using 1% (v/v) sodium hypochlorite for 5 min disinfected then Benomyl 10%(fungicide) was applied on seeds for further sterilization and washed with distilled water three times. Then, the seeds were sown in plastic pots (20 seeds per pot, thinned and uniform ones selected) filled with 1 kg of 3-time autoclaved sterilized soil mixture (perlite/field soli 2:1 (v/v) ratio). At the first irrigation, all pots received 200 cc of water than the 16 pots divided into two groups including eight were non-inoculated which a half received normal irrigation (100 cc of water, two times a week, considered as control) and the rest subjected to water stress (50cc of water, two times a week), the other eight pots were inoculated and had the same treatments as the first group. For mycorrhization of each pot, 12.5 g of G. mosseae spores (acquired from Agriculture Department of Islamic Azad University Rafsanjan Brench, Iran) was blended with the soil mixture before sowing seeds. Pots placed at Germinator with a light density of roughly 100 $lmol \cdot m^{-2} \cdot s^{-1}$ day/night temperatures of 25 ± 1/ 18 ± 1 °C under a 16 h photoperiod in a greenhouse. After germination of seeds, pots received Hoagland solution containing: 5 ml/L KNO_3, 5 ml/L $Ca(NO_3)_2$, 2 ml/L $MgSO_4$, 1 ml/L KH_2PO_4, 1 g/L $MnCl_2$, 1 g/L $ZnSO_4$, 1 g/L $CuSO_4$, 1 g/L Na_2MoO_4, 2 g/L Fe-EDDHA, and 1 g/L H_3BO_3 two times, once one week after sown seeds and another three weeks later. The duration of the experiment completed in 7 weeks, afterward, the samples of flag leaves and roots were taken.

2.1 Growth parameters

The fresh weight (FW) and dry weight of flag leaf and underground parts were determined using a digital scale to weigh the fresh plant tissues individually then oven-dried them at 70 °C throughout 72 h.

2.2 RWC

To evaluate the water status fresh weight (FW) of leaves, four mature leaves were obtained instantly. Afterward, the leaves were soaked in distilled water for four h under 25 °C to measure turgid weight(TW). Then, to obtain dry weight (DW), specimens were dried by the oven for 24 h at 80 °C. Equation 1 was used to calculate RWC (Tofighi *et al*., 2017).

RWC = (FW − DW) / (TW − DW) (Eq. 1)

2.3 Phosphorus concentration

A mixture of 5 mL of extracted material and 10 ml of Barton reagent with a final volume of 50 mL. After keeping samples at room temperature, phosphorus (P) contents were evaluated using a spectrophotometer (Perkin Elmer, Lambda 25, UV/VIS) at 420 nm using a standard curve. The preparation of Barton reagent was based on the method introduced by He and Dijkstra (2014).

2.4 Estimation of AM colonization

To study the symbiosis and to obtain Arbascular mycorrhization (AM%) of *G. mossea*, one-centimeter fragments of the wheat root was bleached at 10% KOH solution for seven min, then using a 5% solution of the vinegar and dye the root segments stained (Vierheilig *et al*., 1998). To evaluate the colonization percentage, 40 fragments of 1 cm stained roots on slides and using a light microscope(Norris and Ribbons 1972). Ultimately, the percentage of mycorrhization was determined using a method described by Beltrano and Ronco (2008).

2.5 *Total carbohydrate*

The determination of soluble carbohydrates of flag leaves was carried out based on the method previously described by Lee *et al*. (2008). To this end, samples were taken from flag leaf oven-dried at 80 °C for 48h. Five mL distilled water added to 1 gram of derived tissue then placed in a 90 °C warm water bath for one h. Afterward, eliminate the residual, the samples were centrifuged for 15 min at 5000 rpm, then, 5 mL of the solution added to a mixture of 95 mL distilled water and 2 mL of resorcinol acid 0.1% composed of 0.5 g of resorcinol, 300 ml of pure ethanol, and 100 mL of concentrated hydrochloric acid. The solution was incubated in a warm water bath at 80 °C for 5 min then immediately placed at cold water. For standard solution, inulin was used. Using spectrophotometer (Perkin Elmer, Lambda 25, UV/VIS) at wavelength 520 nm, soluble carbohydrate concentration was determined.

2.6 Estimation of photosynthetic pigments

To estimate pigments involved in the photosynthetic process including, chlorophyll (Chl) a, Chl b, total Chl and carotenoids (Car) contents employing spectrophotometric procedure as Parry *et al*., (2014) described. To extract the photosynthetic pigments, fresh leaf tissues were homogenized with 95% ethyl alcohol in a test tube at 5000 rpm for 5 min at 60 °C. Then the final volume adjusted into 10 ml with 95% ethyl alcohol and at the absorbance of 663, 644, and 452 nm (Perkin Elmer, Lambda 25, UV/VIS), the concentration of chlorophylls and Car were measured and expressed as mg/g FW.

2.7 Total soluble protein

The total protein concentration of extracts obtained from flag leaves by homogenizing them with liquid nitrogen then centrifuged at 14,000 rpm for 15 min at 4 °C, measured by Coma colorant Brillant Blue-G reagent 250 in 95% ethanol and 85% orthophosphoric acid. Bovine plasma gamma globulin protein (BSA; Merck, Germany) was used as a standard protein curve. For this purpose, the enzymatic surfactant liquid extracted, added to 2.5 ml of Bradford reagent (Merck, Germany) and the mixture of tube contents and placed at dark for 15 min. The absorbance of samples at wavelength 595 nm employing spectrophotometer (Perkin Elmer, Lambda 25, UV/VIS) was read, and the total protein concentration was calculated by comparing it to the standard curve.

2.7 Free proline

Using Bates *et al*., (1973) method, the free proline concentration was measurement. 250 mg of plant tissue frozen in liquid nitrogen, then 5 ml of sulfosalicylic acid 3% was added. Using Whatman filter paper, the blend filtered. One mL of the mixture was added to the equal volume of acetic acid Glacial and ninhydrin reagent (Sigma-Aldrich, Germany) and kept in a hot water bath at 100 °C. Reactions have begun by placing test tubes in an ice bath. 2 ml toluene mixed with that solution and Vertex for 15 seconds. After keeping samples at 25 °C, two separate layers were formed. Finally, the absorbance of the upper colored layer (containing free proline) was read at 520 nm with the aim of a spectrophotometer (Perkin Elmer, Lambda 25, UV/VIS). The standard curve for measuring proline concentration was prepared using standard solutions.

2.8 Enzymatic antioxidants

An amount of half a gram of fresh tissue of flag leaf was homogenized in 2 ml of 50 mM potassium phosphate buffer (pH 7) in ice-cold condition, afterward, at 12,000 for 20 min at 4 °C the mixture was centrifuged. The obtained supernatant was utilized to measure different antioxidant enzymes; APX, CAT, SOD, and GR. APX: its activity determined using Nakano and Asada (1981) spectrophotometric method at 290 nm. To extract APX, 25 mM phosphate buffer with seven pH containing 0.1 mM EDTA (Ethylenediaminetetraacetic acid; Merck,

Germany) was used. The results expressed as mg^{-1} protein min^{-1}. The determination of CAT activity was based on Aebi (1984) procedure. The reaction mixture prepared using 50 mM potassium phosphate buffer (pH 7.0), 30% (w/v) H_2O_2, and 100 µL of enzyme extract. Increment in absorbance rate at 420 nm for 1 min in a single unit was considered as enzyme activity, which expressed as mg^{-1} protein min^{-1}. SOD: for extraction of SOD at 4 °C, 50 mM phosphate buffer with seven pH, including 0.1 mM EDTA was used. SOD activity was evaluated according to the Kaushal and Wani (2016) method by spectrophotometer at 560 nm and expressed as mg^{-1} protein min^{-1}. GR: evaluation of GR was based on the modified method of Foyer and Halliwell (1976). An amount of 75 µL extracts procured from leaf tissue in addition to 1.5 mL KNa-phosphate buffer solution (0.05 M, pH 7.8), 0.75 mL 3 mM 5,5′-dithio-bis-(2-nitrobenzoic acid) (DTNB, Merck, Germany), 0.15 mL NADPH (Nicotinamide adenine dinucleotide phosphate; Merck, Germany) (2 mM), and 0.15 mL oxidized glutathione (GSSG; 20 mM; Sigma-Aldrich Chemie, Steinheim, Germany) prepared. GSSG was mixed with the reaction and the increment in absorbance was monitored at 412 nm for 3 min. The non-GR related NADPH oxidation during the evaluation was considered. The final enzymatic activity mentioned as µmol of oxidized NADPH per 1 gram of FW for 1 min. These indicators were all determined using a spectrophotometer (Perkin Elmer, Lambda 25, UV / VIS).

2.9 Lipid peroxidation

To determine the concentration of malondialdehyde (MDA) in the flag leaves, the method described by Xu *et al*. (2013) was used. First 0.5 g fresh leaves homogenized in 20% thiochloroacetic acid (TCA; Sigma-Aldrich Chemie, Steinheim, Germany) solution contained 0.5% thiobarbituric acid for four then the mixture for 25 min at 95 °C placed in the Ben Marie bath. Afterward, the mixture cooled in an ice bath and using a spectrophotometer (Perkin Elmer, Lambda 25, UV / VIS) the absorbance of the solutions was read at 532 nm. The target matter to absorb in this wavelength is the red complex (TBA-MDA), the absorbance of the other nonspecific pigments was measured at 600 nm and subtracted. To calculate the concentration of MDA the extinction coefficient (155 mM^{-1} cm^{-1}) and determined with Equation 2.

$$MDA\ (\mu mol\ g^{-1}\ Fw) = [A532\text{-}A600/155]\times 1000 \quad \text{(Eq. 2)}$$

2.10 Total Phenolic Content

Using the Folin–Ciocalteu method, total phenolic content (TPC) was evaluated (Singleton *et al*., 1999). 125 µl of methanol extract mixed with 375 µl of water and 2.5 ml of Folin-Ciocalteu reagent 10% (Sigma-Aldrich Chemie, Steinheim, Germany). After 6 min, after 2 ml, 5.7% sodium carbonate was added. The absorbance of the mixture after incubation for 90 min at the dark condition was measured at a wavelength of 765 nm using the Spectrophotometer (Perkin Elmer, Lambda 25, UV / VIS). Finally, TPC is calculated using the standard curve and expressed as Mg of Gallic acid equivalent gram of DW (mg GAE/g DW).

2.11 Total Flavonoid Content

The aluminum chloride colorimetric method was used to determine total flavonoid content (TFC) Lamaison and Carnet (1990). To 0.5 ml of each extract (10 mg/ml), 1.5 ml of methanol, 0.1 ml of aluminum chloride solution in 10% ethanol, 0.1 ml of 1 M potassium acetate and 2.8 ml of distilled water were added. The mixture was read 30 min after incubation at room temperature at 415 nm (Perkin Elmer, Lambda 25, UV/VIS). Quercetin (Sigma-Aldrich Chemie, Steinheim, Germany) was used for the calibration curve, and the final results are expressed as mg of quercetin equivalents (QE) per gram of DW (mg QE/g DW).

2.12 Anthocyanin

For the assay, extracts obtained from methanol solvent: hydrochloric acid (98: 2 v / v) was centrifuged at 1000 rpm for 20 min. 0.5 ml of solution with 49.5 ml of 1mM 2N-morpholino ethane sulfonic acid buffer with a pH of 1 and 4.5 in 50 ml balloons, and after 30 min absorbance measured with a spectrophotometer (Perkin Elmer, Lambda 25, UV / VIS) at 510 nm. Concentrations of anthocyanins were reported in milligrams of cyanidin-3-glucoside per gram fresh weight *(*mg cyanidin-3-glucoside/*g* FW) (Farooq *et al*., 2009).

2.2 Statistical analysis

The experimental design used in this experiment was a completely randomized factorial design with four replicates. All other assays were conducted in triplicate. Variance analysis was carried out utilizing the SAS statistical package (version 9.4; SAS Institute, Cary, NC, USA). Tukey's range test assessed differences among treatments at the level of 1% and 5%.

3. RESULTS AND DISCUSSION:

3.1 Mycorrhization

Mycorrhizal inoculation (AM%) of G. mosseae in roots of inoculated wheat seedlings was assessed (Table 1). The seedlings grown under N+M and D+M that were AM-inoculated showed high mycorrhization (73.46 and 38.25%, respectively). The mycorrhization in water-stressed seedlings decreased almost to half of its value in well-irrigated pots. Since the soil mixture overall autoclaved to eliminate the possible effect of other factors, therefore, the non-AM plants under water stress and control showed no mycorrhization in their roots.

3.2 Growth indicators and RWC of leaves

Imposing drought stress considerably changed the intensity of stress-associated physiological responses in *T. aestivum* cv. Chamran plants. The decline in growth parameters observed in plants under drought stress which could be attributed to the inhibitory impact of water shortage on cell enlargement and photosynthesis potential of the plant (Xu *et al*., 2009; Wang *et al*., 2018). The results indicated the negative influence of drought stress on growth as it reflected in shoot and root FW as compared to controls (Table 1). Whereas, Mycorrhizal inoculation had positive impacts on improving growth parameters in which FW of shoot and root were 28.5% and 28.68%, respectively, and showed significant difference with those subjected to drought stress with no G. mosseae inoculation. Similarly, the DW content of shoots and roots also followed the same paradigm (Table 1; 27.34% and 31.68%). However, plants inoculated with G. mosseae either under normal conditions or drought stress had higher values in comparison to the other two treatments. Inoculation of wheat plants with G. mosseae significantly contributed to improving the RWC % of plants subjected to the drought stress in comparison with non-inoculated plants. In N+M treatment, the highest RWC observed with a significant difference at $P<0.05$ Tukey's test. While RWC of leaves of seedlings under drought with no AM decreased to 40.75% and raised significantly in water-stressed AM-plants up to 70.95% (Figure 1). Notable mitigation in negative effects of drought stress on plant growth was observed in inoculation with AM. Through positively influencing the availability of nutrients for the roots (data not shown) and improving water uptake, *G. mosseae* inoculation generates several metabolical improvements which increase in biosynthesis of proteins as critical aftermath prevents from growth reduction in plants subjected to drought stress (Nadeem *et al*., 2014; López-Ráez 2016; Zhang *et al*., 2019).

Of the renowned effect of AM on plants is the improvement of nutrient content in particular P, which is the cornerstone of resistance against stresses, specifically drought (Augé 2001; Giri *et al*., 2007). Further, the results of this experiment showed a markedly decrease in water content of leaf of water-stressed plants, probably owing to a decline in the osmotic potential of cells. By positively affecting the availability of water, AM fungi found to be contributed in enhancing RWC of leaves of plants subjected to water stress, besides, rectified intergradation of the membrane and influencing the biosynthesis of phytohormones, namely declining ABA which causes improvement of leaf water status, are other potential possibilities. Similar findings previously reported by other scholars (Ortiz *et al*., 2015; Zhou *et al*., 2015; Rahimi *et al*., 2017; Chandrasekaran *et al*., 2019).

3.3 P concentration

The concentration of P in shoots and roots (69.25% and 95.36%, respectively) of AM-plants under drought stress were significantly higher compared with water-stressed non-inoculated plants at $P<0.05$ Tukey's test (Table 1). AM inoculation considerably increased P content of shoots and roots of normally irrigated by over 100% in comparison to those under drought stress. In this study also the P content in inoculated and non-inoculated wheat plants notably enhanced, possibly due to the fact that under drought stress the mobility of nutrients and soil moisture decreased which ends up with limiting available P(Danielsen and Polle 2014; He and Dijkstra 2014) and its where that mycorrhiza with extended hyphae can aim to keep the nutrition supply continue(Alvarez *et al*. 2009; Evelin *et al*. 2009; Danielsen and Polle 2014).

3.4 Total soluble proteins, carbohydrates and proline

Concentration changes in compounds involved in osmoregulation of the cell, including total soluble proteins, carbohydrates, and proline content of wheat flag leaf and root under conditions of D, D+M, N, and control (Table 1) were studied. Comparison of the means of the constituents mentioned above indicated that except for proline they all followed a similar pattern of physiological response in which their content enhanced considerably (total soluble proteins: 52.63% and 30.65%; total soluble carbohydrates: 15.80% and 9.45% in leaf and root, respectively) in both leaf and root of wheat plants inoculated

with *G. mosseae* under drought stress when compared to the water-stressed non-AM plant. Whereas, the values of proline observed in AM-plants were significantly lower (45.01% and 44.03%, leaf and root, respectively) than non-AM plants. Additionally, the concentration of total soluble proteins, carbohydrates, and increment in proline was higher in aerial parts than roots.

The water-stressed wheat plants had a dramatically decreased level of total soluble protein possibly owing to the reduction in photosynthetic activity of leaves under stress condition (Sara *et al*., 2012), whereas, inoculated plants with AM under normal or stressed conditions had a higher but statistically insignificant concentration of total soluble protein, which mirrors the positive effects of AM on preventing the decrease of biosynthesis mechanisms under stress through improving the content of photosynthetic pigments. Reduction in the uptake of mineral elements essential for the biosynthesis process or proteolysis under drought stress leads to a decrease in soluble proteins (El-Komy *et al*., 2003; Abdelmoneim *et al*., 2014; Delshadi *et al*., 2017).

Of the essential osmoprotectants in plants subjected to abiotic stress is proline which its accumulation is a fundamental reaction with the antioxidant capability and effective in tranquilizing homeostasis under stress condition (Bandurska 2000; Szabados and Savoure 2010; Antoniou *et al*. 2017). In the present study, the proline content in non-inoculated water-stressed plants notably increased while otherwise observed in inoculated plants under either normal irrigation or stressed. A similar pattern has been reported in previous studies (Abdelmoneim *et al*. 2014; Chiappero *et al*. 2019), the effect of mycorrhizal inoculation by *G. mosseae* on the accumulation of proline can be a good indication that plants inoculated with AM were experienced less drought stress-associated metabolical modifications, therefore, required a lower quantity of proline for osmotic adjustment in compare to non-treated plants with AM (Asrar *et al*., 2012). Consequently, it can be concluded that elevation in proline content is not needlessly associated with improving resistance to drought stress (Lutts *et al*., 1999), at least in the case of cv. Chamran. In accordance to our results, Porcel and Ruiz-Lozano (2004) recorded a decrease in proline concentration in AM-inoculated *Glycine max* L. Additionally, similar results have been reported by Asrar *et al*. (2012) in *Antirrhinum majus* L. and Amiri *et al*. (2017) in *Pelargonium graveolens* L. There is some ambiguity in the actual association of proline with tolerance to stresses (Ashraf and Foolad 2007; Benitez *et al*., 2016), while some scholars postulated that increase in proline level is indeed related to a specific level of tolerance, while others have found the proline aggregation as a signal of injury(Hien *et al*., 2003; Silvente *et al*., 2012). Carbohydrates are of the critical primary compounds to promote various physiological tolerant processes under abiotic stresses (Amiri *et al*. 2017). Induction the biosynthesis of soluble carbohydrates under water deficit has been commonly reported, particularly in AM-inoculated plants which are possible owing to the positive influences on improving photosynthetic performance that results in higher assimilation and synthesis of free carbohydrates (Lee *et al*. 2008) in addition to enhancing carbon fixation and enzymatic functions (Hameed *et al*. 2014; López-Ráez 2016). Thus, the aggregation of soluble carbohydrates may have contributed in enhancing the physiological capability of cv. Chamran to tolerate drought stress.

3.5 MDA

Lipid peroxidation intensity decreased significantly by 52.27% and 57.26%, respectively, in shoots and roots of AM-inoculated plants under drought stress in comparison with water-stressed non-AM plants (Table 1). Mycorrhizal inoculation resulted in decrementing the MDA level in shoots and roots (significant at $P<0.05$ Tukey's test**).** Control of AM on MDA seems to be higher in roots as compared to flag leaves.

3.6 Enzymatic activities

A marked enhancement in enzymatic activity of CAT, SOD, GA, and APX in roots and flag leaves of wheat plants as a result of mycorrhizal inoculation by *G. mosseae* observed (CAT: 28.66% and 31.43%; SOD: 28.44; GR: 44.62% and 18.84%; APX: 39.49% and 15.58%, respectively) in comparison to non-inoculated plants under water stress (Table 1). These increases for enzymatic antioxidants were always equal or higher in shoots than roots, by comparison. Also, the activity of these antioxidant enzymes was much lower in well-irrigated AM-plants when compared to AM and non-AM plants under stress.

The major source of negative effects of drought stress and abiotic stress overall is the generation an excessive level of ROS that leads to lipid peroxidation which has MDA as a byproduct and perfect indication of membrane degradation, dysfunctioning or peroxidation of lipids in the membrane (Xu *et al*. 2013; Laxa *et al*. 2019; Wang *et al*. 2019). The concentration of MDA in shoot and root of water-stressed wheat plants

significantly increased while remarkably lessened in AM inoculated plants under normal or drought stress conditions in consistent to Porcel and Ruiz-Lozano (2004), Chiappero *et al*. (2019). Also, the MDA in roots was lower than shoots, which reflects less oxidative damage to the roots, which was in agreement with Zhu *et al*., (2011). The abundancy of ROS in particular hydrogen peroxide (H_2O_2), and superoxide radicals (O_2^-) under normal condition constantly suppressed by antioxidants while when plant subjected to drought stress the ROS generation radically increases to a level that harms the vital organs such as photosynthesis system, lipids of membranes and cause mutation in DNA (Lushchak 2014; Jajic *et al*. 2015; Chiappero *et al*. 2019). Generally, in response to such an increase, the level of antioxidant enzymes (i.e., SOD, APX, GR, and CAT) rises significantly. The activities of these enzymes have been shown to be elevated even under moderate water stress (Jiang and Zhang 2002). The participation of the aforementioned antioxidants, CAT, APX, and GR, is through the simultaneous act to the production of water and oxygen from H_2O_2 (Gratão *et al*., 2005) or SOD as a defensive initiative mechanism against ROS, by dismutating O_2– to H_2O_2 and O_2. The AM inoculation found to be effective in increasing the antioxidant enzymes in wheat seedlings subjected to drought stress or those grown under normal conditions. Improving antioxidant activity in AM inoculated plants under drought stress also observed by Khalafallah and Abo-Ghalia (2008) in water-stressed wheat seedlings or in *Pistacia vera* L. Abbaspour *et al*., (2012) inoculated with AM.

3.7 Photosynthetic pigments

The photosynthetic pigments in leaves as a critical measure to understand the effects of drought stress in wheat plants were evaluated (Table 2). The content of Chl a, b, total and Car in flag leaves of wheat inoculated with G. mosseae and subjected to drought stress almost all followed a similar paradigm and incremented compared to non-inoculated plants under drought stress (Chl a: 77.28%; Chl b: 51.70%; total: 66.76% and Car: 51.75%), statistically significant at $P<0.05$ Tukey's test. In non-AM plants subjected to drought stress experienced a considerable reduction in photosynthetic pigments.

A major part of dry weight in wheat produced by flag leaf, therefore, enhancing its photosynthetic potential can have a substantial impact on growth and yield as it has become of the interest of plant breeders in wheat (Yang *et al*., 2007; Xu *et al*. 2013). In this investigation, the content of pigments: Chl a, b, and total in wheat plants inoculated with AM was significantly higher in comparison to non-inoculated AM. Given the importance of a high concentration of Chl in photosynthesis, it can be postulated that AM inoculation enhancing the growth rate in inoculated or non-inoculated through increasing or stabilizing the content of Chl in plants under normal or stress condition. The outcomes of the current study are inconsistent with published researches claiming the growth improvement of mycorrhizal plants under drought stress (Garmendia *et al*., 2017; Hao *et al*., 2019; Mathur *et al*. 2019). Car is potent photoprotection compounds in which intensification of its biosynthesis can effectively help plants to cope with stress through detoxification of reactive oxygen species (ROS) (Götz *et al*. 1999; Hashimoto *et al*., 2016; Paliwal *et al*., 2017). In accordance with that argument, the result of this study strongly indicated the improvement in Car concentration in the flag leaf of wheat plant inoculated with AM under water stress condition. Photosynthetic apparatus heavily relies on Car to protect them from the attack of ROS, which otherwise the photosynthesis rates in drought stress conditions drop dramatically. Our investigation revealed that photosynthetic pigments content in cv. Chamran enhanced considerably with the aim of AM inoculation. In numerous similar assessments, photosynthetic pigments, more often than not, increased with AM inoculation in comparison with non-inoculated plants under water stress (Al-Karaki *et al*., 2004; Kaya *et al*., 2009; Hajiboland *et al*. 2010; Hao *et al*. 2019).

Phenolic compounds as a large group of secondary plant metabolites have long known for their significant contribution in plant tolerance under various stresses which often is due to their high antioxidant activity and quenching ROS (Amiri *et al*. 2017; Khalil *et al*. 2018; Chiappero *et al*. 2019). Evaluation the phenolic, flavonoid and anthocyanin compounds in this study revealed the increase of these valuable photo protectors in both inoculated and non-inoculated wheat seedlings under drought stress in which the content of TPC, TFC, and TAC as a notable source of antioxidant activity remarkably enhanced in flag leaves of wheat inoculated with mycorrhizal fungus G. mosseae (Table 2). The improvement in those abovementioned bioactive constituents in water-stressed AM-plants was 36.35%, 30%, and 24.52% higher in comparison to non-inoculated plants. The difference of TAC content was not statistically significant between treatments, while TPC and TFC showed the highest increase in all treatment when compared to control with D+M

treatments, which in agreement with the results of previous studies on various species inoculated with AM such as incrementing TFC in *Ligustrum vulgare* L. (Tattini *et al*. 2004), TAC in *Oryza sativa* L. (Farooq *et al*. 2009) or TPC in *Mentha pulegium* L. (Oueslati *et al*., 2010) and Thymus vulgaris L. (Khalil *et al*. 2018). The information on ATC of wheat under drought stress is rare, in this respect, our results can greatly contribute to encouraging scholars to consider this essential secondary chemical in further relevant studies.

4. CONCLUSION

The threat of abiotic stresses, drought, in particular, is ubiquitous and is annually responsible for a large portion of crop losses, therefore, addressing this challenging issue through efficient strategies as exploiting the considerable potential of AM is pivotal especially for main food crops which in this study mycorrhizal inoculation an important widely cultivated versatile cultivar of wheat in Iran, cv. Chamran observed to be significantly positive in improving the capability of seedlings to cope with osmotic stresses imposed by water deficiency and enhanced traits associated with growth, osmoprotectants, and strongly inhibited MDA, notably improved enzymatic antioxidants as well as secondary metabolites that possess antioxidant activity. Considering the capability of cv. Chamran to provide high yield in either well-watered or arid farming, the results of this study. Indeed, for the first time presented an insight into physiological responses of this cultivar to drought and remarkable benefits of AM inoculation, which can be important from an economic perspective. Additionally, further investigations urgent by using various irrigation regimes or inoculation with other mycorrhizal bacteria. It's critical to conduct a complimentary, carefully designed field experiment to assess the quality and quantity of grain yield.

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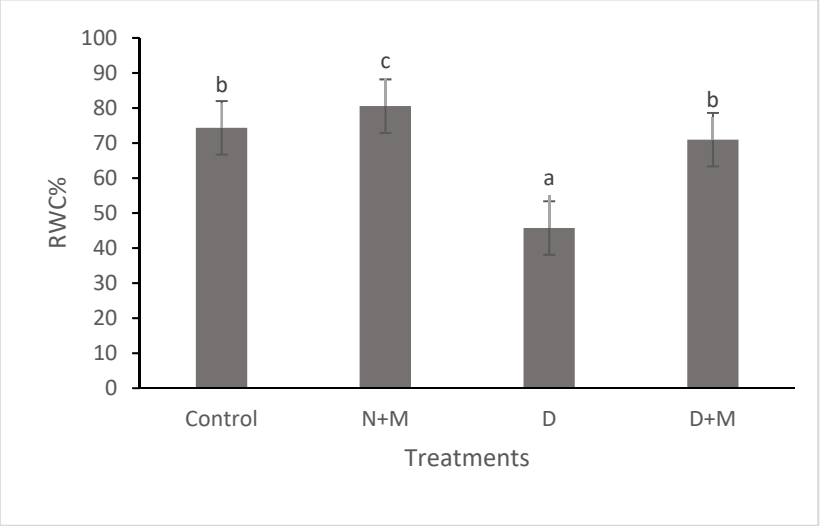

Figure 1: *Effect of water deficiency and inoculation with G. mosseae on RWC of seedlings of T. aestivum cv. Chamran. Different letters indicate significant differences at P<0.05*

Table 1. *Effect of water deficiency and inoculation with G. mosseae on Mycorrihization, FW, DW, P, total soluble protein, carbohydrates and free proline, MDA, CAT, SOD, GR and APX in flag leaves and roots of seedlings of T. aestivum cv. Chamran.*

Parameters	**Treatments**			
	Control	**N+M**	**D**	**D+M**
Mycorrhization	0±0.0a	73.46± 2.49c	0±0.0a	38.25±1. 2b
		Flag leaf		
Shoot freshweights	0.5732±0.0018b	0.6139±0.0011b	0.4283±0.0027a	0.5491±0.0019b
Shoot dry weights	0.0627±0.0011a	0.0717±0.001a	0.0596±0.0014a	0.0759±0.0015a
P concentration	2.2193±0.1263ab	2.9718±0.1794b	1.4719±0.1329a	2.4913±0.1825b
Total soluble protein	4.7166±0.879b	4.8535±0.715b	3.1874±0.366a	4.8650±0.436b
Proline	1.4250±0.686a	1.5600±0.637a	2.4825±0.896b	1.3650±0.484a
Total soluble carbohydrates	4.4862±0.208a,b	5.0222±0.482a	6.3746±0.465b,c	7.3821±0.539c
MDA	0.1339±0.002b	0.1032±0.049a	0.1828±0.008c	0.0781±0.001a
CAT	121.2050±3.611b	129.2650±5.064a	147.3225±5.813c	189.5525±7.154d
SOD	83.2400±2.014b	95.3475±7.010a	123.4325±3.175c	158.5375±11.013d
GR	0.2900±0.010b	0.3475±0.015a	0.4375±0.013c	0.6325±0.008d
APX	8.1525±1.146a	10.9300±0.914b	13.0085±1.1628c	18.1458±1.277d
		Root		
Root fresh weights	0.0938±0.0005a	0.1254±0.001b	0.0739±0.001a	0.0951±0.0013a
Root dry weights	0.0116±0.0007a	0.0186±0.0004b	0.0103±0.0006a	0.0135±0.0009b
P concentration	1.9426±0.1893b	2.3358±0.1127b	1.1832±0.1922a	2.3115±0.1327b
Total soluble protein	3.2447±0.301b	3.5211±0.765b	2.6252±0.500a	3.4301±0.448b
Proline	1.2150±0.025a	1.5350±0.707ab	1.9075±0.021b	1.0675±0.214a
Total soluble carbohydrates	3.2162±0.298a	3.7745±0.148a,b	4.2171±0.261b	4.5485±0.259b
MDA	0.1138±0.002a	0.0936±0.010a	0.1582±0.008a	0.0744±0.001a
CAT	109.3829±3.027a	112.9725±9.056b	130.4075±8.201c	171.4025±5.019 d
SOD	79.0500±5.970a,b	88.0650±5.176a	118.0925±4.062b	136.1350±7.018c
GR	0.0825±0.004a	0.1000±0.013a	0.0950±0.004a	0.1129±0.003a
APX	5.0975±0.127a	6.8732±0.119ab	7.0375±0.185b	7.9233±0.241b

Values represent the mean ± SE of four replicates. Different letters indicate significant differences at P<0.05
Note: Mycorrhization**: %;** Shoot fresh weights: g; Shoot dry weights: g; P concentration: mg g $^{-1}$DW; Total soluble protein: mg g $^{-1}$DW; Proline: mg g $^{-1}$DW; Total soluble carbohydrates: mg g $^{-1}$DW; MDA: µM g^{-1} FW; CAT: mg^{-1} protein min^{-1}; SOD: mg^{-1} protein min^{-1}; APX: mg^{-1} protein min^{-1}; GR: µmol of oxidized substrate/(g fr wt min).

Table 2. *Effect of water deficiency and inoculation with G. mosseae on photosynthetic pigments (Chl a, b, and total), phenolic compounds (phenol and flavonoid), and anthocyanin content in flag leaves of seedlings of T. aestivum L., cv. Chamran.*

Parameters	Treatments			
	CONTROL	**N+M**	**D**	**D+M**
Chl a	1.3786±0.109a	1.4085±0.046a	1.0514±0.068a	1.8648±0.126b
Chl b	0.9484±0.048a	0.9573±0.055a	0.7388±0.019a	1.1203±0.090a
Chl t	2.3270±0.096b	2.3658±0.199b	1.7902±0.216a	2.9851±0.396c
Car	0.6520±0.114a	1.0086±0.142a	0.9336±0.152a	1.4095±0.036b
TPC	16.9639±1.967a	36.5472±4.307c	34.7000±2.993b	47.2833±5.551d
TFC	12.7150±1.853a	19.0519±1.471b	27.5071±1.936c	35.7548±3.250d
TAC	0.7803±0.028a	0.6864±0.077a	0.7424±0.081a	0.9242±0.060a

Values represent mean ± SE of four replicates. Different letters indicate significant differences at $P < 0.05$ Note: Chl a, Chl b, Chl t, Car: mg/g FW; TPC: mg GA/g DW; TFC: mg QE/g DW; TAC: *mg cyanidin-3-glucoside*/*g* FW.