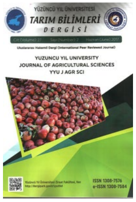
Yuzuncu Yil University
Journal of Agricultural Sciences
https://dergipark.org.tr/en/pub/yyutbd



Research Article

Effects of Severe Drought Stress on Some Physiological and Biochemical Parameters of AMF Inoculated *C. arietinum*

Sertan ÇEVİK[*1]

[1]Mersin Üniversitesi, Mut MYO, Bitkisel ve Hayvansal Üretim Bölümü, 33600, Mersin, Türkiye
[1]https://orcid.org/0000-0003-1259-7863
*Sorumlu yazar e-posta: srtncvk@gmail.com

Article Info

Received: 28.01.2021
Accepted: 28.05.2021
Online Published: 15.09.2021
DOI: 10.29133/yyutbd.870384

Keywords
Drought,
Glomus mosseae,
Cicer arietinum

Abstract: In this study, physiological and biochemical changes caused by mycorrhizal symbiosis in chickpea plants under drought conditions were investigated in both root and leaf. Drought stress reduced leaf water potential, but mycorrhizal symbiosis caused a significant increase in leaf water potential. However, the application of mycorrhiza under drought stress caused an increase in the amount of elements that are very important for the development of the plant in the root and leaf. In our study, drought increased the proline concentration and MDA content, while mycorrhiza application decreased them in both leaf and root. In addition, while mycorrhizal application increased the activity of catalase, it decreased the activity of superoxide dismutase. In general, enzyme activities were found to be higher in the leaf, but no distinct pattern was obtained between root and leaf in other analyzes. The study shows that the responses of mycorrhizal symbiosis in chickpea plants may change depending on the severity of the drought. Especially antioxidant enzyme activities and proline content patterns reveal that more comprehensive studies should be conducted on these issues. However, continuing studies until determining the effects of AMF symbiosis on grain yield under drought may provide more comprehensive results.

Şiddetli Kuraklık Koşulları Altındaki *Cicer arietinum* (Nohut) Bitkisinde Mikoriza Aşılamasının Bazı Fizyolojik ve Biyokimyasal Parametreler Üzerine Olan Etkileri

Makale Bilgileri

Geliş: 28.01.2021
Kabul: 28.05.2021
Online Yayınlanma: 15.09.2021
DOI: 10.29133/yyutbd.870384

Anahtar Kelimeler
Kuraklık,
Glomus mosseae,
Cicer arietinum

Öz: Bu çalışmada kuraklık koşulları altındaki nohut bitkilerinde mikorizal simbiyozisin meydana getirdiği fizyolojik ve biyokimyasal değişiklikler hem kök hem de yaprakta araştırılmıştır. Kuraklık stresi ile birlikte yaprak su potansiyeli azalmışken, mikorizal simbiyozis yaprak su potansiyelinde belirgin bir artışa neden olmuştur. Bununla birlikte kuraklık stresi altında mikoriza uygulaması bitkinin gelişimi için oldukça önemli olan elementlerin miktarında kök ve yaprakta artışa neden olmuştur. Çalışmamızda kuraklık ile birlikte yükselen prolin konsantrasyonu ve MDA içeriği mikoriza uygulamasıyla birlikte azalmıştır. Ayrıca antioksidan enzimlerden katalazın aktivitesi mikoriza uygulamasıyla birlikte artarken, süperoksit dismutaz aktivitesi ise düşmüştür. Genel olarak enzim aktiviteleri yaprakta daha yüksek bulunmuşken, diğer analizlerde kök ve yaprak arasında belirgin bir desen elde edilmemiştir. Yapılan çalışma kuraklığın şiddetine bağlı olarak mikorizal simbiyozisin nohut bitkisinde meydana getirdiği yanıtların değişebileceğini göstermektedir, özellikle mikorizal simbiyozis ile antioksidan enzim aktiviteleri ve prolin içerik desenleri arasındaki ilişki konularında daha fazla çalışma yapılması gerektiğini ortaya koymaktadır. Bununla birlikte bu simbiyozisin kuraklık altında tane verimine etkilerini belirleyene kadar çalışmaların sürdürülmesi daha kapsamlı sonuçların elde edilmesini sağlayabilir.

1. Introduction

Legumes (Fabaceae) are a very valuable plant group both agriculturally and economically. Besides being used for nutritional purposes, free nitrogen digestion in the soil also increases the ecological value of this group (Pandey, 2008). According to FAO (2019), Turkey is the most chickpea-producing (630.000 tonnes) country in the world after India. Chickpea, which is one of the most grown legume products in the world and Turkey, is generally grown in semi-arid and arid areas. Although chickpeas have developed mechanisms that can cope with drought, it is known that this stress causes serious product loss in chickpeas (Canci and Toker, 2009).

Plants are continuously exposed to abiotic and biotic stress factors throughout their life in nature. Drought, one of the most important abiotic stress, affects fields, and cause serious yield losses (Sadak et al., 2021). It has been estimated that drought-induced inefficient soil levels for crop production reach up to 28 % of the world's cultivated land (Aroca et al., 2008). Drought stress is one of the most limiting factors for chickpea growth during vegetative and reproductive development stages (Günes et al., 2006). Chickpea is known to be resistant to drought, but the yield loss due to drought is around 45-50 % for chickpeas (Devasirvatham and Tan, 2018; Shah et al., 2020). Studies conducted on this subject have shown that the morphological, physiological and biochemical mechanisms of chickpea are negatively affected by drought stress, resulting in crop losses (Rani et al., 2020).

There are many strategies developed by plants against drought stress, one of which is symbiotic interactions. Many symbiotic interactions occur between plants and other organisms in nature. One of these interactions is between the plant and mycorrhiza fungi, which was established approximately 400 million years ago (Diagne et al., 2020). Arbuscular mycorrhizal fungi (AMF) colonize within the root cortex, producing large amounts of hyphae (mycelia), increasing the surface area of the infected root. This allows the nutrients and water in the form and amount that the plant cannot take from the soil, away from the root, through the mycorrhiza hyphae and transmit it to the upper parts of the plant. Thus, a symbiotic life is established where the mycorrhizal fungus provides water and minerals to the plant and the plant carbon to the mycorrhizal fungus (Wu et al., 2008).

Increasing the surface area of plant roots infected with AMF provides a great advantage for the plant to cope with stress, especially in drought stress conditions (Ortaş, 2012). This advantage is not limited to taking water and mineral substances from the soil; It also includes many physiological and biochemical events such as the mycorrhizal promoting root regeneration, accelerating plant growth, promoting intracellular soluble substance concentration, activating the antioxidant system (Kaya et al., 2009). The symbiotic relationship between AMF and plants is an important topic that has been studied for a long time. Within the scope of these studies, the role of symbiotic relationships under stressful and/or non-stressful conditions is attempted to be understood. In this study, some physiological and biochemical responses caused by drought stress in chickpea plants were investigated. It has been observed that the plant creates different stress responses with the increase of stress intensity.

2. Materials and Methods

2.1. Plant material and drought treatment

Cicer arietinum (ILC482) seeds were sterilized by soaking in 2.5 % sodium hypochlorite solution for 10 minutes, then washed thoroughly and soaked in distilled water for 1 day. Then they were transferred to plastic pots (2 L) and filled with mineral-poor soil. The soil was autoclaved at 121 °C for 2 hours before use. Half of these seeds are infected with mycorrhiza (*Glomus mosseae*), approximately 1000 spores of *G. mosseae* were used for each seed. 4 seeds were planted in each of the pots. According to the plant output, 2 plants were developed in each pot. In addition, there were three pots in each group. All pots were watered to %85 of field capacity before sowing. After sowing, all pots were also watered 75 mL every 4 days. Plants were grown at 24 ± 2 °C, 16/8 h photoperiod, irradiance 480 µmol $m^{-2}s^{-1}$, 65 ± 5 relative humidity under controlled conditions for 21 days in the plant growth room. At the end of this period, half of the seedlings were not watered for 12 days and the other half were irrigated as control plants. Afterwards, the leaves and roots were harvested and taken into liquid nitrogen quickly and stored in a freezer at -80 °C until analysis day.

2.2. Leaf water potential

The leaf water potential was measured by using a pressure chamber (PMS Instrument Co., Model 1000).

2.3. Determination of root and leaf element contents

Leaf samples (0.5 g) were extracted in a 3:1:1 ratio nitric acid/perchloric acid/hydrochloric acid solution in an oven at 200 °C. These samples were then diluted with 50 mL of ultrapure water and analyzed by Inductively Coupled Plasma-Mass Spectrometry (ICP-MS, Agilent 7500).

2.4. Antioxidant enzyme assays

The same extraction method was used for SOD and CAT enzymes. Leaf and root tissues (0.5 g) were homogenized with phosphate buffer (5 mL, pH 6.8) and centrifuged at +4 °C, 5 min, 16.000 g and supernatant was used for measurements. Total SOD activity was determined according to Beyer and Fridovich (1987). One unit of SOD activity was defined as the amount of enzyme that was required to cause 50% inhibition of the reduction of NBT as monitored at 560 nm. CAT activity was determined by measuring the rate of decomposition of H2O2 at 240 nm, as described by Aebi (1983).

2.5. Determination of Lipid peroxidation

Lipid peroxidation was determined by measuring the malondialdehyde (MDA) level, according to Ohkawa et al. (1979). Firstly, leaf and root tissues (0.2 g) were homogenized in trichloroacetic acid (5 %) (TCA) solution and centrifuged at 12.000 rpm. The supernatant, thiobarbituric acid (TBA) and 20 % TCA solutions were transferred to the tubes in equal volumes and incubated at 96 °C for 25 min. After that, the tubes were centrifuged at 12.000g for 5 min and the supernatant was measured at 532 and 600 nm. The MDA content was calculated using the extinction coefficient.

2.6. Determination of free proline content

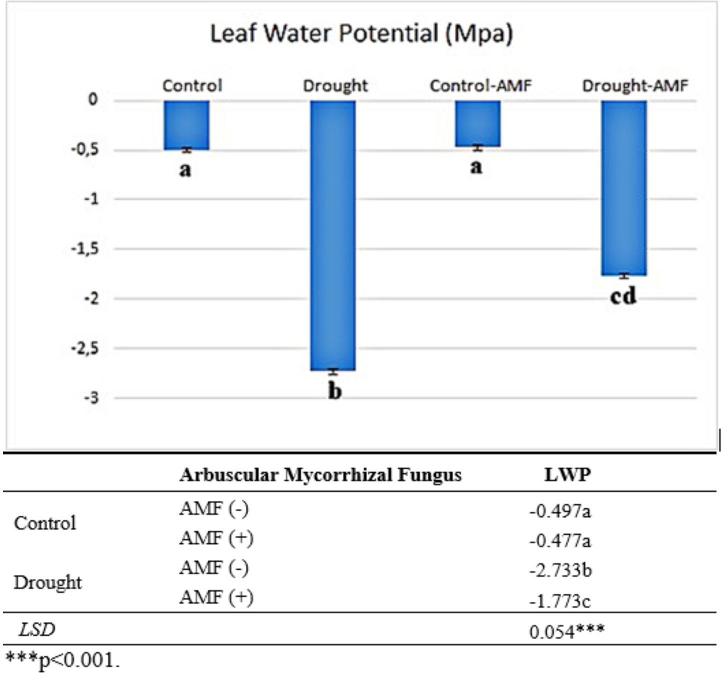
Free proline content was determined according to the method of Bates et al. (1973). Leaf and root samples were homogenized in sulfosalicylic acid (3 %) and centrifuged at 3.000 rpm, then the supernatant, acetic acid and ninhydrin were mixed well and boiled for 1h. Then, cold toluene was added to this mixture and the toluene phase was measured at 520 nm. The proline concentration was calculated by using a calibration curve and expressed as μmol proline g^{-1} FW.

2.7. Statistical analysis

Stress and mycorhiza treatments were carried out completely randomized experimental design with two factors. Treatments had three replications with three plants each. Data were subjected to ANOVA and the means were separated using the LSD multiple range test at $P<0.05$. All the statistical analyses were performed using the JMP8 Software package).

3. Results

Drought stress significantly reduced leaf water potential. However, AMF inoculation enhanced leaf water potential under drought stress (Figure 1).



	Arbuscular Mycorrhizal Fungus	LWP
Control	AMF (-)	-0.497a
	AMF (+)	-0.477a
Drought	AMF (-)	-2.733b
	AMF (+)	-1.773c
LSD		0.054***

***p<0.001.

Figure 1. Changes in leaf water potential under drought and/or AMF inoculation.

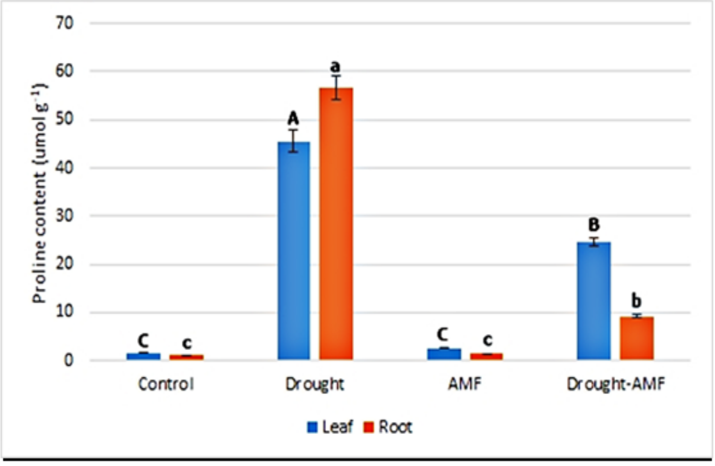
In the present study, AMF inoculation significantly increased the element contents in the root especially under drought conditions (Table 1).

Table 1. Effects of mycorrhizal symbiosis on some element content of *C.arietinum* roots under drought stress

	Arbuscular Mycorrhizal Fungus	**Al (ppb)**	**P (ppb)**	**Ca (ppm)**	**Mn (ppb)**	**Fe (ppm)**	**Ni (ppb)**	**Cu (ppb)**	**Zn (ppb)**
Control	AMF (-)	178.54c	5.92c	4.95c	10.15c	0.48c	3.45c	<1.0d	3.58c
	AMF (+)	203.05b	14.97b	6.52b	11.39b	0.49bc	3.71b	1.44b	6.26b
Drought	AMF (-)	160.05d	4.85c	4.83c	9.21d	0.52b	3.55bc	1.20c	6.25b
	AMF (+)	333.16a	19.85a	10.15a	19.22a	0.80a	5.75a	2.27a	34.78a
	LSD	3.785***	1.202***	0.382***	1.998***	0.038***	0.236***	0.181***	1.428***

***p<0.001.

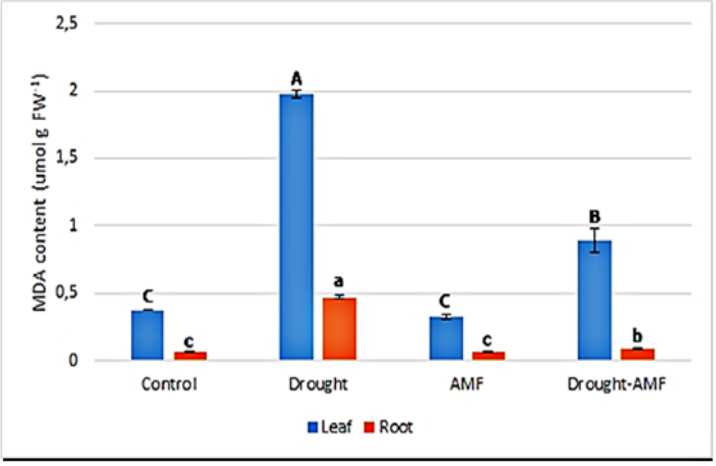
Proline (Figure 2) and MDA (Figure 3) contents of *C.arietinum* increased with drought stress compared to the control group in both leaf and root. AMF inoculation of *C.arietinum* resulted in a significant decrease in proline and MDA content in both leaf and root under drought. While the proline content was higher in the roots under drought conditions compared to the leaves, this situation reversed with AMF inoculation under drought. MDA content was found higher in leaves at all conditions.



	Arbuscular Mycorrhizal Fungus	Proline -Root-	Proline -Leaf-
Control	AMF (-)	1.153c	1.542c
	AMF (+)	1.385c	2.542c
Drought	AMF (-)	56.516a	45.593a
	AMF (+)	9.225b	24.672b
LSD		2.899***	2.760***

***$p<0.001$.

Figure 2. Proline content in leaves and roots of chickpea plants under drought and arbuscular mycorrhizal fungal inoculation.

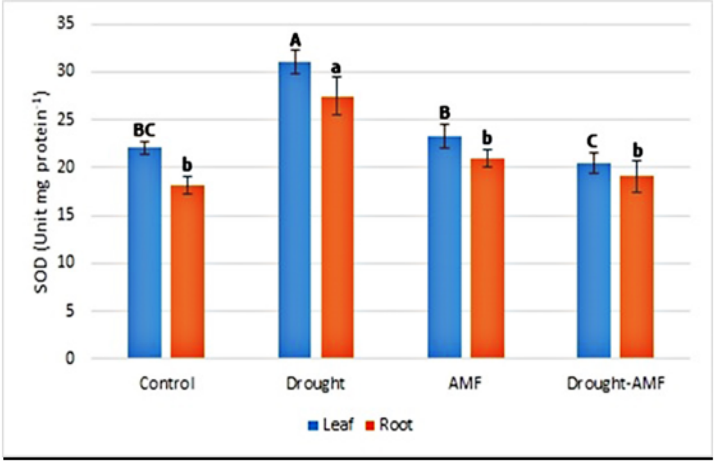


	Arbuscular Mycorrhizal Fungus	MDA -Root-	MDA -Leaf-
Control	AMF (-)	0.065c	0.378c
	AMF (+)	0.063c	0.326c
Drought	AMF (-)	0.472a	1.973a
	AMF (+)	0.092b	0.895b
LSD		0.017***	0.109***

***$p<0.001$.

Figure 3. MDA content in leaves and roots of chickpea plants under drought and arbuscular mycorrhizal fungal inoculation.

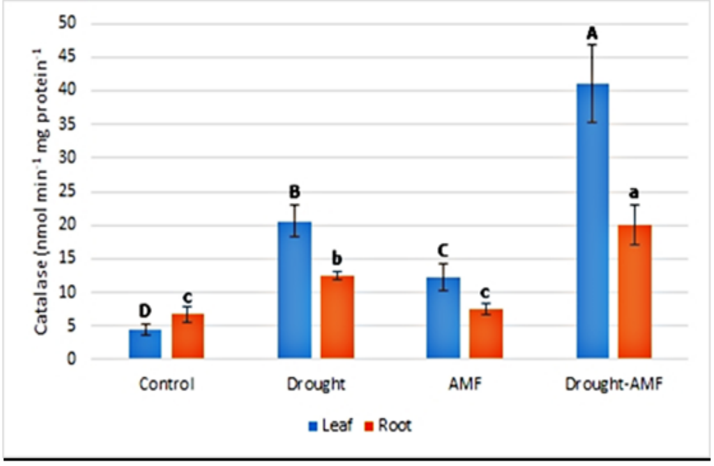
Antioxidant enzymes (SOD and CAT) activities increased under drought stress in both leaf and root. However, AMF inoculation decreased the SOD activity (Figure 4) while increasing the CAT activity (Figure 5) compared to drought stressed group. Also interestingly, AMF increased enzyme activities compared to control.



	Arbuscular Mycorrhizal Fungus	SOD -Root-	SOD -Leaf-
Control	AMF (-)	18.153b	22.110bc
	AMF (+)	20.978b	23.227b
Drought	AMF (-)	27.459a	31.056a
	AMF (+)	19.096b	20.453c
LSD		3.295***	2.544***

***p<0.001.

Figure 4. Superoxide dismutase activity in leaves and roots of chickpea plants under drought and arbuscular mycorrhizal fungal inoculation.



	Arbuscular Mycorrhizal Fungus	CAT -Root-	CAT -Leaf-
Control	AMF (-)	6.803c	4.487d
	AMF (+)	7.552c	12.261c
Drought	AMF (-)	12.483b	20.618b
	AMF (+)	19.984a	41.062a
LSD		3.802*	7.692*

***p<0.05.

Figure 5. Catalase activity in leaves and roots of chickpea plants under drought and arbuscular mycorrhizal fungal inoculation.

4. Discussion and Conclusion

In this study, it was determined that inoculation of chickpea plants with *G. mosseae* improved plant tolerance for drought stress. Although this situation has been shown in the literature in general, some data obtained from this study have the potential to provide new information to the literature and some issues related to mycorrhizal symbiosis should be studied in more detail.

AMF inoculation increased leaf water potential of *C.arietinum* under drought stress. When the roots of plants infected with AMF, the surface areas of the roots increase, resulting in the potential for the plant to absorb more water from more areas (Bagyaraj et al., 2015). This gives the plant a great advantage, especially under drought stress conditions.

Under drought conditions, amount of some important macroelements such as phosphorus (P), calcium (Ca) and microelements such as iron (Fe), manganese (Mn), nickel (Ni), copper (Cu) and zinc (Zn) (Table1) increased by mycorrhizal symbiosis. Chen et al. (2020) explained these results by extraradical hyphal network formed in the soil upon AMF colonization. P and Ca are the main mineral elements for plant growth. Increasing the amount of these elements under drought stress leads to an increase in root growth, leaf area, photosynthesis rate, higher membrane stability, and water content (Ahanger et al., 2016). Zn, Mn and Fe are also important micronutrients, which have several vital roles for plants. Babaeian et al. (2011) showed that foliar application of these elements enhanced the yield components and alleviate the effects of drought. Peuke and Rennenberg (2011) also emphasized that Fe, Mn and Zn are important ligands for more than 1500 proteins that have catalytic, (co-)activating and/or structural functions. Ahanger et al. (2016) also well discussed in detail the importance of all these mineral elements in drought tolerance mechanisms. By mycorrhizal symbiosis, the increase in the amount of these elements under drought indicates that this symbiosis will provide an advantage for the *C.arietinum* plants to cope with stress.

Proline concentration increased with drought stress. Proline is known as a good osmolyte, and accumulation of proline under drought stress is well documented in various plant species in the literature. (Chun and Chandrasekaran, 2018; Çevik et al., 2019). Inoculation of *C.arietinum* by AMF decreased proline concentration under drought stress. There are different results related to AMF inoculation and proline accumulation under environmental stress in the literature. Some researchers reported that AMF inoculation increased proline content (Begum et al., 2019; Garg and Baher, 2013) while others reported no significant difference (Sohrabi et al., 2013) or decrease (Abdelmoneim et al., 2014) in different plants under stress conditions. Accumulation of proline in plants under drought known as a basic response to stress (Abdelmoneim et al., 2014). There is a good correlation between the increase of proline content and the intensity of the drought. As the severity of the drought increases, the proline content also increases (Keyvan, 2010). In this study, the decrease in proline content with AMF inoculation may indicate that the severity of the drought was reduced with AMF treatment.

Malondialdehyde (MDA), one of the end products of lipid peroxidation, is a good indicator of the level of oxidative stress (Gawel et al., 2004). The data of the present study showed that lipid peroxidation in chickpea plants significantly increased under drought stress. However, AMF treatment decreased lipid peroxidation compared to the drought group. Some researchers determined that the amount of MDA also increased due to the increase of radicals, especially H_20_2 (Ibrahim and Jaafar, 2012; Hasanuzzaman et al., 2020). As seen in Figure 6, AMF treatment increased the catalase activity. Catalase catalyzes the oxidation of hydrogen peroxide to water and oxygen. Increased activity of catalase with AMF treatment may have caused the scavenging of H_20_2. This situation may have caused a decrease in lipid peroxidation.

Studies have shown that antioxidant enzyme activities increase under various stresses with AMF application (Chang et al., 2018; Duc et al., 2018). However, the decrease in SOD activity may be explained by reducing the intensity of stress with AMF inoculation, but this situation cannot explain the increase in catalase activity. Total enzyme activity analyzes may occasionally lead to such contradictions. Therefore, detailed isoenzyme analyzes can contribute to the solution of this problem. However, in this study, increases in enzyme activities also occurred regardless of the stress conditions. This situation may indicate that different signalling mechanisms are stimulated with the treatment of AMF. Although some studies claimed this may be related to the increase in nutrient intake, more studies should be conducted to clarify this issue.

In conclusion, this study showed that with the inoculation of AMF, the leaf water content and the amounts of some important mineral elements increased, and the proline and MDA content decreased under drought conditions. In addition, while there was a general tendency to increase antioxidant enzyme activities with AMF treatment, the increases in enzyme activities in the control groups also suggested that the antioxidant system could be stimulated in a stress-independent way. Especially the relationships between AMF-proline and AMF-antioxidant system, which have conflicting results in the literature, should be investigated with advanced molecular techniques. In particular, proteomic analysis can provide more data on this subject.

References

Abdelmoneim, T. S., Moussa, T. A. A., Almaghrabi, O. A., Alzahrani, H. S., & Abdelbagi, I. (2014). Increasing plant tolerance to drought stress by inoculation with arbuscular mycorrhizal fungi. *J. Life Sci., 11,* 10-17.

Aebi, H. E., Bergmayer, J., & Grabl, M. (1983). Catalase in: Methods of enzymatic analysis. Eds. *Verlag Chemie, Weinheim, 3,* 273-286.

Ahanger, M. A., Moad-Talab, N., Abd-Allah, E. F., Ahmad, P., & Hajiboland, R. (2016). P. Ahmad, & W. Blackwell (Eds), *Plant Growth Under Drought Stress: Significance of Mineral Nutrients* (pp. 649–668). In "Water stress and crop plants: a sustainable approach.

Aroca, R., Vernieri, P., & Ruiz-Lozano, J. M. (2008). Mycorrhizal and non-mycorrhizal Lactuca sativa plants exhibit contrasting responses to exogenous ABA during drought stress and recovery. *Journal of Experimental Botany, 59,* 2029-2041.

Babaeian, M., Piri, I., Tavassoli, A., Esmaeilian, Y., & Gholami, H. (2011). Effect of water stress and micronutrients (Fe, Zn and Mn) on chlorophyll fluorescence, leaf chlorophyll content and sunflower nutrient uptake in Sistan region. *Afri. J. of Agric. Res*., *6,* 3526-3531.

Bagyaraj, D. J., Sharma, M. P., & Maiti, D. (2015). Phosphorus nutrition of crops through arbuscular mycorrhizal fungi. *Curr. Sci., 108*(7), 1288-1293.

Bates L. S., Waldren R. P., & Teare, I. D. (1973). Rapid determination of free proline for water-stress studies. *Plant and Soil, 39,* 205-207.

Begum, N., Ahanger, M. A., Su, Y. Y., Lei, Y. F., Mustafa, N. S. A., Ahmad, P., & Zhang L. X. (2019). Improved drought tolerance by AMF inoculation in maize (*Zea mays*) involves physiological and biochemical implications. *Plants, 8,* 579.

Beyer, W. F., & Fridowich, I. (1987). Assaying for superoxide dismutase activity: Some large consequences of minor changes in conditions. *Analytical Biochemistry, 161,* 559-566.

Canci, H., & Toker, C. (2009). Evaluation of yield criteria for drought and heat resistance in chickpea (*Cicer arietinum* L.). *Journal of Agronomy and Crop Science, 195,* 47-54.

Chang, W., Sui, X., Fan, X., Jia, T., & Song, F. (2018). Arbuscular mycorrhizal symbiosis modulates antioxidant response and ion distribution in salt-stressed *Elaeagnus angustifolia* seedlings. *Front. Microbiol. 9*, 652.

Chen, W., Meng, P., Feng, H., & Wang, C. (2020). Effects of arbuscular mycorrhizal fungi on growth and physiological performance of Catalpa bungei CA Mey. under drought stress. *Forests 11*(10), 1117

Chun, S .C., & Chandrasekaran, M. (2018). Proline accumulation influenced by osmotic stress in arbuscular mycorrhizal symbiotic plants. *Front. Microbiol. 9,* 2525.

Çevik, S., Güzel Değer, A., Yıldızlı, A., Gök, A., & Unyayar, S. (2019). Proteomic and physiological analyses of dl-cyclopentane-1,2,3-triol-treated barley under drought stress. *Plant Mol Biol Rep 37,* 237-251.

Devasirvatham, V., Tan, D. K. Y. (2018). Impact of High Temperature and Drought Stresses on Chickpea Production. *Agronomy*, *8*(8), 145.

Diagne, N., Ngom, M., Djighaly, P., Fall, D., Hocher, V., & Svistoonoff, S. (2020). Roles of Arbuscular Mycorrhizal Fungi on Plant Growth and Performance: Importance in Biotic and Abiotic Stressed Regulation. *Diversity, 12,* 370.

Duc, N. H., Csintalan, Z., & Posta, K. (2018). Arbuscular mycorrhizal fungi mitigate negative effects of combined drought and heat stress on tomato plants. *Plant Physiol. Biochem. 132,* 297–307.

Food and Agriculture Organization (FAO). (2019). FAOSTAT Statistical Database of the United Nation Food and Agriculture Organization (FAO) Istatistical division. Rome.

Garg, N., & Baher, N. (2013). Role of arbuscular mycorrhizal symbiosis in proline biosynthesis and metabolism of *Cicer arietinum* L.(chickpea) genotypes under salt stress. *J Plant Growth Regul., 32,* 767-778.

Gunes, A., Cicek, N., Inal, A., Alpaslan, M., Eraslan, F., Guneri, E. & Guzelordu, T. (2006). Genotypic response of chickpea (*Cicer arietinum* L.) cultivars to drought stress implemented at pre-and post-anthesis stages and its relations with nutrient uptake and efficiency. *Plant Soil and Environment*, *52*, 368–376.

Hasanuzzaman, M., Bhuyan, M. H. M., Zulfiqar, F., Raza, A., Mohsin, S. M., Mahmud, J. A., Fujita, M., & Fotopoulos, V. (2020a). Reactive oxygen species and antioxidant defense in plants under abiotic stress: revisiting the crucial role of a universal defense regulator. *Antioxidants, 9,* 681.

Ibrahim, M. H., & Jaafar H. Z. E. (2012). Primary, secondary metabolites, H_2O_2, malondialdehyde and photosynthetic responses of Orthosiphon stamineus Benth. to different irradiance levels. *Molecules, 17,* 1159-1176.

Kaya, C., Ashraf, M., Sonmez, O., Aydemir, S., Tuna, A.L., & Cullu, M. A. (2009). The influence of arbuscular mycorrhizal colonisation on key growth parameters and fruit yield of pepper plants grown at high salinity. *Scientia Horticulturae, 121,* 1-6.

Keyvan, S. (2010). The effect of drought stress on yield, relative water content, proline, soluble carbohydrates and chlorophyll of bread wheat cultivars. *J. Animal Plant Sci. 8*(3), 1051-1060.

Ohkawa, H., Ohishi, N., & Yagi, K. (1979). Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Analytical Biochemistry, 95,* 351-358.

Ortas, I. (2012). The effect of mycorrhizal fungal inoculation on plant yield, nutrient uptake and inoculation effectiveness under long-term field conditions. *Field Crops Res., 125,* 35–48.

Pandey, A., Chakraborty, S., Datta, A., & Chakraborty, N. (2008). Proteomics approach to identify dehydration responsive nuclear proteins from Chickpea (*Cicer arietinum* L.). *Molecular & Cellular Proteomics. 7*(1), 88-107.

Peuke, A. D., & Rennenberg, H. (2011) Impacts of drought on mineral macro-and microelements in provenances of beech (Fagus sylvatica L.) seedlings. *Tree Physiology 31,* 196–207.

Rani, A., Devi, P., Jha, U. C., Sharma, K. D., Siddique, K. H., & Nayyar, H. (2020). Developing climate-resilient chickpea involving physiological and molecular approaches with a focus on temperature and drought stresses. *Frontiers in plant science*, *10,* 1759.

Sadak, A., Akkopru, A., & Sensoy, S. (2021). Effects of Endophytic Bacteria on Some Physiological Traits and Nutrient Contents in Pepper Seedlings under Drought Stress. Yüzüncü Yıl Üniversitesi Tarım Bilimleri Dergisi, 31(1), 237-245.

Shah, T. M., Imran, M., Atta, B. M., Ashraf, M. Y., Hameed, A., Waqar, I., Shafiq, M., Hussain, K., Naveed, M., Aslam, M., & Maqbool, M. A. (2020). Selection and screening of drought tolerant high yielding chickpea genotypes based on physio-biochemical indices and multi-environmental yield trials. *BMC Plant Biol*. *20,*171.

Sohrabi, Y., Heidari, G., Weisany, W., Ghasemi Golezani, K., & Mohammadi, K. (2012). Some physiological responses of chickpea cultivars to arbuscular mycorrhiza under drought stress. *Russ. J. Plant Physiol., 59,* 708-716.

Wu, Q. S., Xia, R. X., & Zou, Y. N. (2008). Improved soil structure and citrus growth after inoculation with three arbuscular mycorrhizal fungi under drought stress. *European journal of soil biology, 44*(1), 122-128.