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Using Mannitol as Drought Stress Agent on Globe Artichoke [*Cynara cardunculus var. scolymus* (L.) Fiori]

Enginarda [*Cynara cardunculus var. scolymus* (L.) Fiori] Kuraklık Stresi Ajanı Olarak Mannitol Kullanımı

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USING MANNITOL AS DROUGHT STRESS AGENT ON GLOBE ARTICHOKE [*Cynara cardunculus* var. *scolymus* (L.) FIORI]

ABSTRACT

The globe artichoke [*Cynara cardunculus* var. *scolymus* (L.) Fiori], one of the special members of the Asteraceae family, has been consumed by people for their culinary and health advantages since ancient times. Global agricultural output and production efficiency are both hampered by adverse environmental conditions, notably drought. In order to promote breeding studies, it is crucial to use plants that are adaptable to drought stress, which negatively impacts plant productivity. It is crucial to understand the changes taking place in plants under drought stress. In the present study, artichoke seeds were initially kept in mannitol, which is a commonly used drought stress agent, in three different concentrations (50, 100, and 250 mg L^{-1}) for 8 hours and later on obtained seedlings were subjected to same mannitol concentrations with 15 days intervals. Later on some seed germination and vegetative growth parameters, such as number of leaves, root length, stem height, plant height, chlorophyll, proline, leaf-related water contents, and total phenol-antioxidant contents in three globe artichoke cultivars (Sakız OP, Bayrampaşa OP, and Olympus F_1) were investigated under drought stress. Results revealed that the impact of drought stress on artichoke seeds and seedlings varied depending on cultivars and pre-treatment and treatment concentrations

Keywords: Abiotic Stress, Stress Agent, Growth Parameters, Total Phenol, Content, Total Antioxidant.

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ENGİNARDA [*Cynara cardunculus var. scolymus* (L.) FIORI] KURAKLIK STRESİ AJANI OLARAK MANNİTOL KULLANIMI

ÖZ

Asteraceae familyasının önemli üyelerinden biri olan enginar [*Cynara cardunculus* var. *scolymus* (L.) Fiori], eski çağlardan beri mutfak ve sağlık açısından faydaları nedeniyle insanlar tarafından tüketilmektedir. Küresel tarımsal üretim ve üretim verimliliği, başta kuraklık olmak üzere olumsuz çevre koşulları nedeniyle sekteye uğramaktadır. Bitki verimliliğini olumsuz etkileyen kuraklık stresine dayanıklı bitkilerin kullanılması, ıslah çalışmalarının teşvik edilmesi açısından büyük önem taşımaktadır. Kuraklık stresi altında bitkilerde meydana gelen değişiklikleri anlamak çok önemlidir. Bu çalışmada enginar tohumları ilk olarak yaygın olarak kullanılan kuraklık stres ajanı olan mannitol içerisinde üç farklı konsantrasyonda

(50, 100 ve 250 mg L^{-1}) 8 saat süreyle bekletilmiş ve daha sonra elde edilen fideler 15 gün aralıklarla aynı mannitol konsantrasyonlarına tabi tutulmuştur. Daha sonra üç enginar çeşidinde (Sakız, Bayrampaşa ve Olympus F_1) yaprak sayısı, kök uzunluğu, gövde uzunluğu, bitki boyu, klorofil, prolin, yaprağa bağlı su içeriği ve toplam fenol-antioksidan içeriği gibi bazı tohum çimlenmesi ve vejetatif büyüme parametreleri kuraklık stresi altında araştırılmıştır. Sonuçlar, kuraklık stresinin enginar tohumları ve fideleri üzerindeki etkisinin çeşitlere, ön işleme ve uygulama konsantrasyonlarına bağlı olarak değiştiğini ortaya koymuştur.

Anahtar Kelimeler: Abiyotik Stres, Stres Ajanı, Büyüme Parametreleri, Toplam Fenol İçeriği, Toplam Antioksidan İçeriği.

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1. INTRODUCTION

The globe artichoke [*Cynara cardunculus* var. *scolymus* (L.) Fiori] is an important perennial species and a crop alternative medicine. It has been extensively cultivated, particularly in the various countries of the Mediterranean basin.

The alterations that arise in the environmental circumstances of the plants and the changes that impact all developmental phases in an unfavorable sense are expressed as 'biological stress'. Stress factors are categorized as 'biotic' such as bacteria and viruses, or 'abiotic' such as salinity, drought, etc., and may have serious negative effects on plants at all stages of their lifespan. Stress factors not only have a detrimental impact on plant growth and development, but they also reduce production and yield. Abiotic stress factors are thought to have alarming dangers that threaten the sustainability of agriculture and the ability to meet future generations' food needs (He et al., 2018). While 20% of irrigated fields and more than 6% of the world's cultivated land struggle with salinity challenges, the fact that 45% of them are under drought stress and it makes the severity of the situation quite clear (Ashraf and Foolad, 2007; Munns and Tester, 2008; Kuşvuran, 2010).

Plants are subjected to a drought stress restricts plant growth and causes a variety of physical, physiological, biochemical, and molecular problems in plants (Gong et al., 2005; Martinez et al., 2007; Sankar et al., 2008). The photosynthetic process of plants is negatively influenced by drought stress, and various qualitative and quantitative variations in the components of chlorophyll occur. Therefore, while the accumulation of reactive oxygen species (ROS) increases and the balance in the cells' defense mechanisms is upset, the plant-water relationship deteriorates as a result of the disruptions in the plants' photosynthetic order and the irregularity of their nutrient intake (Munns and Tester, 2008; Kuşvuran, 2010; Choudhury et al., 2017). All of these circumstances hinder plants growing and developing nor-

mally, cause yield and quality losses, and, probably more crucially, threaten food safety (Mancosu et al., 2015; Cao et al., 2018; Dawood et al., 2021).

In their study, Możdżeń et al. (2015) examined the impact of mannitol, a polyhydric alcohol that is used to induce osmotic stress in plants as a result of drought in maize. According to the findings of their investigation, mannitol adversely affected corn plant germination, decreased water intake, and delayed plant growth and development. It was also noted that it affected the amount of chlorophyll and gas exchange in the leaves.

The present study is focused on revealing the effects of different dosages of mannitol as drought stress agent on seed germination and some vegetative growth parameters (number of leaves, root length, stem and plant heights, contents leaf-related water, chlorophyll, proline, and total phenol-antioxidant in three globe artichoke cultivars.

2. MATERIALS AND METHODS

2.1. Plant Materials, Growing Conditions and Treatments

The present study was carried out at Akdeniz University, Faculty of Agriculture, and Department of Horticulture. Seeds of two open-pollinated (OP) globe artichoke cultivars namely Sakız and Bayrampaşa and, one F_1 hybrid cultivar, Olympus, were employed as plant material in the current study. For these objective, plastic pots with 35 cm diameter – 13 cm lengths were used. As growing medium, a combination of peat: perlite (2:1, v/v) was utilized. Starting 2 days before the seed sowing, the peat: perlite mixture was irrigated with tap water. Irrigation was continued every day in order to keep soil moisture at field capacity. Five seeds of three artichoke cultivars were sown in each plastic growing pot, and then as the first true leaf emerged, plants were reduced to two plants in each plastic pot.

2.2. Pre-Treatment Process of Seeds

The seeds of artichoke cultivars were subjected to surface sterilization with 0.1% mercury (II) chloride (mercuric chloride) for 5 minutes, and then rinsed 4 times with sterile distilled water (Dawood et al., 2021). For the pre-treatment of drought stress, after the sterilization process, the seeds were kept in 50 mg L^{-1} (M 50), 100 mg L^{-1} (M 100), and 250 mg L^{-1} (M 250) concentrations of mannitol for 8 hours. Then, pre-treated seeds and control group seeds were sown in plastic pots. After sowing seeds, all pots were treated with 100 mL of semi-strength Hoagland nutrient solution (Dawood et al., 2021) and the same solution was applied on the

10th and 30th days during the growth processes of the plants. The prepared nutrient solution pH was adjusted to 5.5 with 0.1 mM KOH. The measurements of the seedlings in the pots were made in accordance with the determined parameters and recorded as the first measurement values.

2.3. Treatment of Mannitol to Seedlings and Measurements

Following the seed sowing, mannitol treatments to seedlings were conducted on the plants with three or four true leaves. A total of 200 mL mannitol solution was given at 50 mg L^{-1} (M 50), 100 mg L^{-1} (M 100), and 250 mg L^{-1} (M 250) concentrations, with 15 days intervals (day 0, day 15, day 30), while the control group was treated with only distilled water. At the end of the 45th day, observations about some growth parameters (length of shoot and root, shoot diameter, fresh and dry weights of leaves) were recorded. The evaluation of the obtained data as a result of the treatments was recorded as a second measurement.

After sowing, seed emergence time, emergence rates, first true leaf emergence time, and germination percentages of seeds were recorded. At the end of the mannitol treatments, measurements were taken regarding growth parameters. Accordingly, from 10 randomly selected plants; length of shoots and roots, shoot diameters, fresh weights of the leaves were measured. Finally, after keeping the leaves in an oven set at 70 °C for 72 hours, dry weights were scaled. To measure the amount of chlorophyll in the leaves, a portable chlorophyll meter called the SPAD (SPAD-502, Minolta Corporation, Ltd., Osaka, Japan) was employed. To ascertain the SPAD values, measurements were conducted at the leaf center. Twenty random readings were performed for each application, averaged, and then transformed into a single SPAD value. Using a digital scale (0.0001 g), the weights of the leaves, while they were fresh and dried, were calculated.

2.4. Determination of the Leaf Relative Water Content (LRWC)

The fresh weights of leaf samples taken randomly from plants under mannitol treatments and control groups were scaled. Removed leaves were transferred to petri dishes containing pure water, kept under low light intensity for 4 hours, and turned into turgor. At the end of the 4 hours, the weight of the turgor leaves was measured and recorded as "turgid weight". Afterward, the turgid leaves were kept in an oven set at 65 °C for 48 hours, then weighed and their "dry weight" was expressed in g. Accordingly, the relative water contents (%) of these leaf samples were calculated based on down stated formula (1) (Sanchez et al., 2004; Kuşvuran, 2010);

$$\text{LRWC (\%)} = [(\text{Fresh weight} - \text{Dry weight}) / (\text{Turgid weight} - \text{Dry weight})] \times 100 \quad (1)$$

2.5. Biochemical Analyses

2.5.1. Quantification of Chlorophyll (by spectrophotometer)

A total of 200 mg fresh leaf samples were grounded in 80% acetone prepared in dark and homogenized. After filtering the homogeneous leaf samples acetone was added to extract to make it up to 10 mL, then chlorophyll amounts of leaf samples were measured at 652 nm with a spectrophotometer device (Kuşvuran, 2010).

2.5.2. Determination of Proline Content

The acid-ninhydrin method and the spectrophotometric approach were both employed to calculate the proline concentration. The following protocol has been employed to serve the purpose. A total 100 mg fresh leaf sample weighed, and crushed in liquid nitrogen. The powdered materials were extracted in 2 mL of 40% methanol. One mL of glacial acetic acid, 6 M orthophosphoric acid and 25 mg of ninhydrin were added on top of the 1 mL obtained extract. This mixture was incubated at 100 °C for one hour. After adding 5 mL of toluene, the tubes were thoroughly mixed, and then were allowed to cool down. After cooling down two distinct phase forms were obtained as a consequence. The upper phase was examined with a spectrophotometer at a wavelength of 528 nm to determine the proline quantity. After drawing calibration curves and expressing them as mmol g^{-1} proline fresh weight, the proline quantity in the leaves was calculated using the L-proline standard.

2.5.3. Determination of Total Phenol-Antioxidant Contents

The leaf sample's total phenol content was calculated using the Singleton et al. (1999) approach. The Folin-Ciocalteu reagent (FCR) was used to accomplish the goal. Ethanol was used to dilute the leaf sample extracts to a 500 g mL concentration before adding 0.5 mL of FCR. After shaking the extracts for three minutes to homogenize, 0.5 mL of saturated Na_2CO_3 solution was added, which was followed by adding 5 mL of diluted de-ionized water. After completing these steps, the mixture was placed in the dark for two hours, and then absorbance readings were calculated using a spectrophotometer set at 760 nm. The outcomes were presented as gallic acid equivalent (mg GAE/g extract), which was done by making a calibration curve for gallic acid.

The following method was used for estimating the total antioxidant content of leaf samples. One mL of each buffer solution of copper (II), neocuproin, and ammonium acetate were added to each test tube containing the leaf sample extracts. Then, antioxidant solution and distilled water were added to each test tube to complete the total volume to 4.1 ML. A spectrophotometer was used to determine the absorbance values at 450 nm after they had been well agitated and kept at room temperature for 30 minutes.

2.6. Statistical Analyses

An entirely randomized factorial design with three replications was used to conduct the current experiment and the statistical software JMP version 5.0.1 (SAS Institute Inc., Cary, NC, USA) was used to analyze the data.

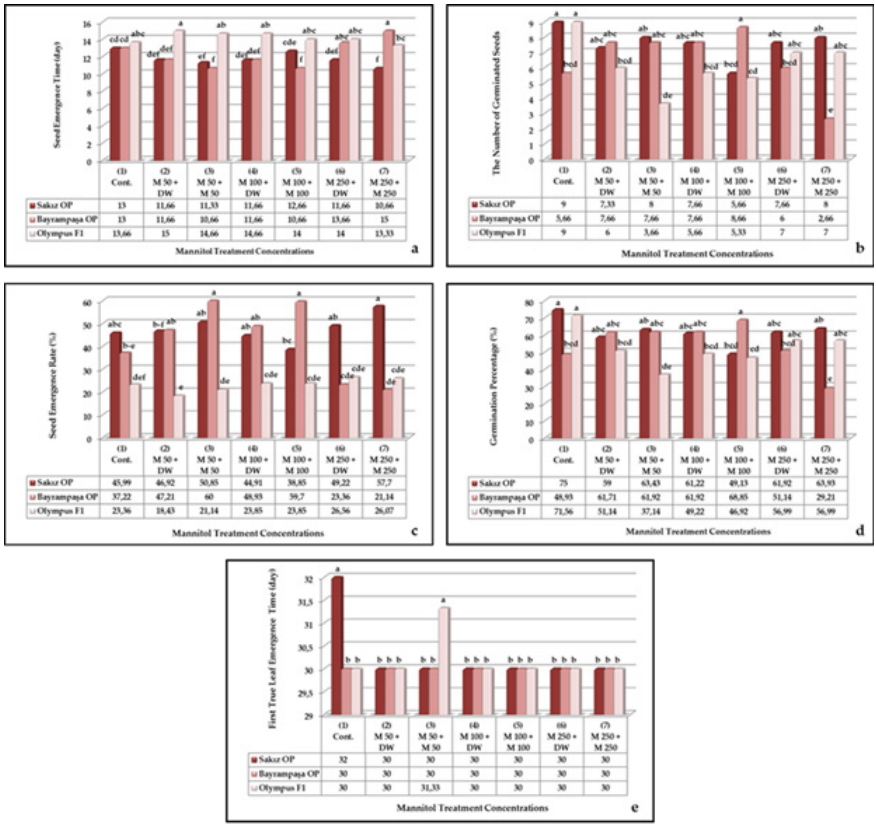
3. RESULTS AND DISCUSSION

3.1. Effects of Mannitol on Seeds From Emergence to Germination

There were statistically significant differences among cultivars and mannitol treatment concentrations regarding their respond to mannitol applications based on data obtained on seed emergence times, number of germinated seeds, seed emergence rates (%) and germination percentage (%). Application of 50 mg L^{-1} mannitol solution ended with statistically significant differences on seed emergence times of Sakız OP and Bayrampaşa OP cultivars in a comparison to control treatment (Figure 1a). It was determined that the applied mannitol concentrations suppressed the number of germinated seeds, and the seeds in the control group germinated better than all other mannitol applications (Figure 1b). Results revealed that seed emergence rate of Sakız OP cultivar was not adversely affected by increasing mannitol concentration. In many studies, it has been reported that the regression detected in seed development was due to the increase in the dose of mannitol (Sadeghian and Yavari, 2004; Younis et al., 2010). In the present study, it was determined that the treatment Bayrampaşa OP cultivar seeds with 50 mg L^{-1} and 100 mg L^{-1} of mannitol solutions did not have any negative affect on seed emergence rate. However, in present study at the highest mannitol application concentration (M 250 + M 250) resulted with the highest seed emergence rate for Sakız OP cultivar, clearly indicating cultivar response to mannitol treatments may vary based on the cultivar used (Figure 1c).

In terms of germination percentage (%), the seeds of Sakız OP and Olympus F_1 cultivars had the highest germination percentage in the control application, while the seeds of Bayrampaşa OP cultivar demonstrated the highest germination percentage at 100 mg L^{-1} mannitol pre-treatment (Figure 1d). It clearly shows that although there are differences in germination percentages in terms of their response to pre-treatment on the basis of cultivars, Bayrampaşa OP cultivar is more tolerant to drought conditions compared to the other two cultivars. The negative effect of germination by applied mannitol as a drought agent has been reported in previous studies. The rate of germination decreased when drought stress was introduced. The study's findings are not in line with those of other studies (Gholamin and Khayatnezhad, 2010; Jorenush and Rajabi, 2015), maybe due to cultivar differences.

Considering the first true leaf emergence times were negatively affected for Sakız OP cultivar with the control group and Olympus F_1 cultivar with 50 mg L^{-1} mannitol pre-treatment (Figure 1e). This clearly shows that there are differences between the first true leaf emergence times in terms of their response to pre-treatment on the basis of cultivars, and some cultivars do not positively respond to low mannitol solution. As it is known, water is an essential component of photosynthesis, a fundamental transporter of nutrients, and has a role in ensuring the optimal plant growth and development. Water stress is often defined as either abundance or an absence of water that significantly affects the biochemical and physiological functions of plants. It becomes apparent in the early stages of the life cycle of plants and inhibits seed germination (Możdżeń et al., 2015). However, in the current study, it was determined that drought pre-treatments on seeds did not have negative effects at certain concentrations, but on the contrary, showed positive effects in some growth parameters such as first true leaf emergence. So, it is thought that the negative effect of drought stress on the life cycles of plants is related to the severity of drought stress and the tolerance levels of varieties.



(1): Different letters among cultivars and mannitol treatments denote significant differences (LSD test, $p<0.05$).

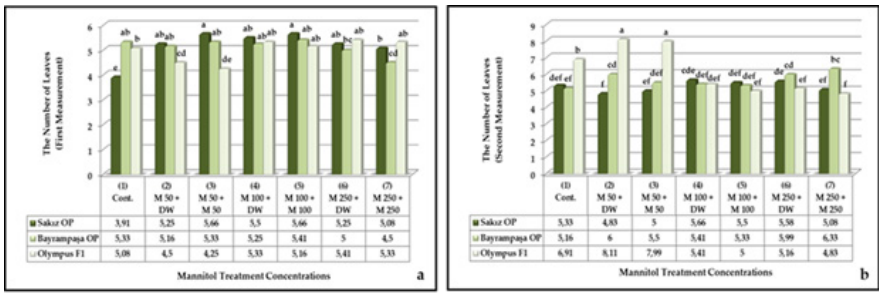
(2): **(a)** LSD cultivars* = 0,620; LSD treatments = 0,947; LSD cult. x treat.* = 1,641; **(b)** LSD cultivars* = 0,901; LSD treatments = 1,376; LSD cult. x treat.* = 2,384; **(c)** LSD cultivars* = 5,954; LSD treatments = 9,095; LSD cult. x treat.* = 15,753; **(d)** LSD cultivars* = 5,966; LSD treatments = 9,114; LSD cult. x treat.* = 15,785; **(e)** LSD cultivars = 0,312; LSD treatments* = 0,476; LSD cult. x treat.* = 0,825.

(3): Abbreviations: M50 = 50 mg L^{-1} mannitol, M100 = 100 mg L^{-1} mannitol, M250 = 250 mg L^{-1} mannitol; DW = Distilled water; Cont. = Control.

Figure 1. Effect of mannitol as drought agent on; (a) seed emergence times, (b) the number of germinated seeds, (c) seed emergence rates (%), (d) germination percentage (%), (e) first true leaf emergence times.

3.2. Effects of Mannitol on Growth Parameters

When the effects of mannitol treatment on the number of leaves of the cultivars were evaluated, there was no statistical difference among the cultivars in the first measurement, although there was in the second, while there were statistically important differences among the mannitol treatments, for both measurements. As can be seen from the Figure 2, Sakız OP cultivar had the highest number of leaves in the first measurements of 50 mg L^{-1} and 100 mg L^{-1} mannitol concentrations. However, in the second measurement, the response of the Olympus F_1 cultivar to 50 mg L^{-1} mannitol solution was found to be better than the other cultivars and among mannitol treatment doses. Results clearly show that although there was no negative effect of drought pre-treatment in early stage of life cycles, those pre-treatments may lead negativities in later stage of life cycles.



(1): Different letters among cultivars and mannitol treatments denote significant differences (LSD test, $p<0.05$).

(2): (a) LSD cultivars = 0,203; LSD treatments* = 0,311; LSD cult. x treat.* = 0,539; **(b)** LSD cultivars* = 0,261; LSD treatments* = 0,399; LSD cult. x treat. * = 0,692.

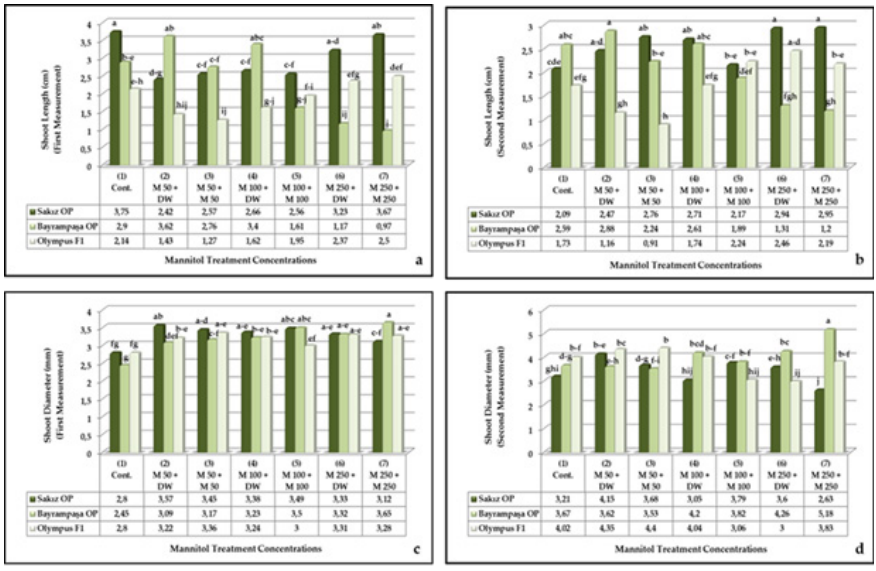
(3): Abbreviations: M50 = 50 mg L^{-1} mannitol, M100 = 100 mg L^{-1} mannitol, M250 = 250 mg L^{-1} mannitol; DW = Distilled water; Cont. = Control.

Figure 2. Effect of mannitol as drought agent on the number of leaves: (a) first measurement, (b) second measurement.

Considering the shoot lengths of the seedlings of the cultivars, statistical differences were determined among cultivars and mannitol concentrations in both measurements taken. In Figure 3a and 3b, although there were statistically significant differences among cultivars and application concentrations, it was seen that pre-treatment did not have a positive effect on shoot length, except for 250 mg L^{-1} mannitol concentrations in Sakız OP cultivar and 50 mg L^{-1} in Bayrampaşa OP cultivar. However, looking at the second measurement data, it is clearly seen that 250

mg L^{-1} of mannitol application or the addition of distilled water positively affects the shoot length in the 250 mg L^{-1} pre-treatment Sakız OP cultivar.

Considering shoot diameters of the seedlings, no difference was determined among the cultivars in the first measurement, but all treatments were found to be effective. In the second measurement, statistical differences were recorded among the cultivars and treatments. Bayrampaşa OP cultivar with 250 mg L^{-1} mannitol treatment came to the fore regarding shoot diameter among other treatments and cultivars (Figure 3c and d).



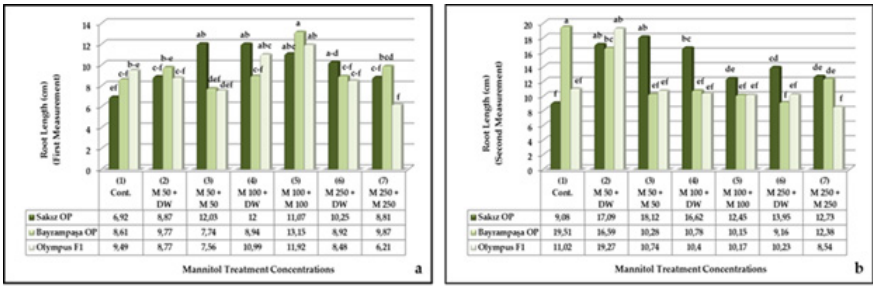
(1): Different letters among cultivars and mannitol treatments denote significant differences (LSD test, $p<0.05$).

(2): (a) LSD cultivars* = 0,316; LSD treatments* = 0,484; LSD cult. x treat. * = 0,838; **(b)** LSD cultivars* = 0,228; LSD treatments = 0,349; LSD cult. x treat. * = 0,605; **(c)** LSD cultivars = 0,149; LSD treatments* = 0,228; LSD cult. x treat.* = 0,395; **(d)** LSD cultivars* = 0,216; LSD treatments = 0,330; LSD cult. x treat. * = 0,573.

(3): Abbreviations: M50 = 50 mg L^{-1} mannitol, M100 = 100 mg L^{-1} mannitol, M250 = 250 mg L^{-1} mannitol; DW = Distilled water; Cont. = Control.

Figure 3. Effect of mannitol as drought agent on shoot lengths; (a) first measurement, (b) second measurement; shoot diameters (c) first measurement, (d) second measurement.

Regarding the root lengths, there was no difference among the cultivars in the first measurement, but a statistically significant difference was determined in the second measurement and the Sakız OP cultivar had the longest root length. Accordingly, 100 mg L^{-1} mannitol pre-treatment on Bayrampaşa OP seeds had positive effect on root length, while there were no positive effects of various mannitol concentrations on root length in the all second measurements (Figure 4). Due to the fact that roots are in direct contact with the soil and absorb water from the soil, root length is one of the most significant drought stress features. Because of this, root length is a crucial indicator of how a plant will react to drought stress (Mostafavi et al., 2011).



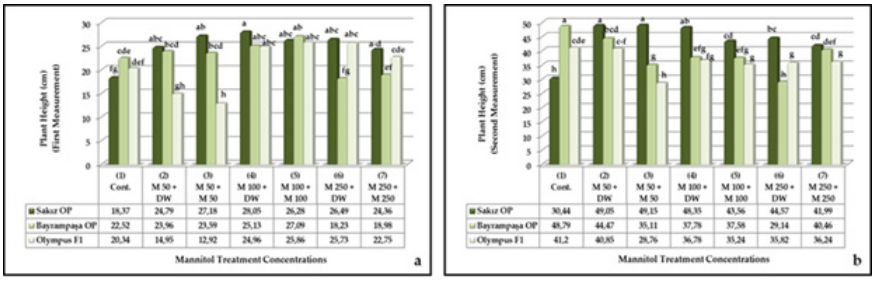
(1): Different letters among cultivars and mannitol treatments denote significant differences (LSD test, $p<0.05$).

(2): (a) LSD cultivars = 1,105; LSD treatments* = 1,688; LSD cult. x treat.* = 2,923; **(b)** LSD cultivars* = 1,041; LSD treatments* = 1,590; LSD cult. x treat.* = 2,755.

(3): Abbreviations: M50 = 50 mg L^{-1} mannitol, M100 = 100 mg L^{-1} mannitol, M250 = 250 mg L^{-1} mannitol; DW = Distilled water; Cont. = Control.

Figure 4. Effect of mannitol as drought agent on root lengths; (a) first measurement, (b) second measurement.

Considering plant heights, statistically significant differences were found among cultivars and mannitol treatments. Mannitol pre-treatment concentrations did not produce positive results on plant height, excluding pre-treatment of 100 mg L^{-1} mannitol concentration with Sakız OP cultivar. However, it is clearly seen from Figure 5 that the responses of cultivars to mannitol treatments were different. Applying 50 mg L^{-1} of mannitol or distilled water to the 50 mg L^{-1} mannitol pre-treated Sakız OP cultivar resulted with satisfactory results on plant height.



(1): Different letters among cultivars and mannitol treatments denote significant differences (LSD test, $p<0.05$).

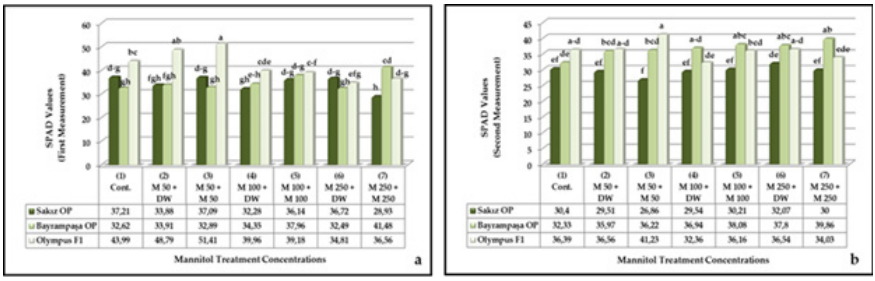
(2): (a) LSD cultivars* = 1,514; LSD treatments* = 2,313; LSD cult. x treat.* = 4,00; **(b)** LSD cultivars* = 1,546; LSD treatments* = 2,361; LSD cult. x treat.* = 4,090.

(3): Abbreviations: M50 = 50 mg L^{-1} mannitol, M100 = 100 mg L^{-1} mannitol, M250 = 250 mg L^{-1} mannitol; DW = Distilled water; Cont. = Control.

Figure 5. Effect of mannitol as drought agent on plant heights; (a) first measurement, (b) second measurement.

One of the primary abiotic stress factors that may limit plant growth as well as affect the biochemical structure of plants is drought stress (Anjum et al., 2012; Grand et al., 2014). Due to its high foliage yield and lengthy production cycle, artichokes demand a lot of water, but once they develop after the first year, they are quite drought tolerant and can endure low water conditions (Fernández et al., 2006). The reduction or absence of nutrition transfer from seed-storage tissues to embryos during drought circumstances is one of the mechanisms that led to a drop in shoot length. Additionally, during drought stress, seeds absorb less water, resulting in with a decrease on hormone and enzyme output, which consequently hinders seedling development on root and shoot development of seedlings. Regarding the findings of the current study on seedling growth parameters, it was seen that applied mannitol concentrations negatively affected seedling growth and development. However, it has been clearly revealed that the pre-treatment of artichoke seeds with 50 mg L^{-1} or 100 mg L^{-1} of mannitol solution positively affected seedling growth and development.

Related to SPAD values of leaves, it was determined that there were statistical differences among cultivars and mannitol treatments in both measurements. Olympus F_1 cultivar showed the highest SPAD values with 50 mg L^{-1} mannitol application in both pre-treatment of seeds and further mannitol treatment (Figure 6).



(1): Different letters among cultivars and mannitol treatments denote significant differences (LSD test, $p<0.05$).

(2): (a) LSD cultivars* = 2,145; LSD treatments* = 3,276; LSD cult. x treat.* = 5,675; **(b)** LSD cultivars* = 1,880; LSD treatments = 2,873; LSD cult. x treat.* = 4,976.

(3): Abbreviations: M50 = 50 mg L^{-1} mannitol, M100 = 100 mg L^{-1} mannitol, M250 = 250 mg L^{-1} mannitol; DW = Distilled water; Cont. = Control.

Figure 6. Effect of mannitol as drought agent on; SPAD values, (a) first measurement, (b) second measurement.

3.3. Leaf Relative Water Content (LRWC)

Turgid fresh (TFLW) and dry leaves (TDLW) were recorded, and the changes in the weights were presented in the charts below (Figure 7a). The relative water content (%) of the leaves belonging to the cultivars was presented in the following graphic (Figure 7b). The relative water content, which depicts the water status of plant tissues under water stress, is another stress level indicator. The relative water content of artichoke leaves was affected by different mannitol concentrations applied. Although relative water content is known to decrease in conditions of water scarcity or extreme stress, it was shown that this drop was cultivar-specific in the current study. The relative water content relates to cell volume and represents the balance between absorbed water and water consumed by transpiration (Hassanzadeh et al., 2009). The relationship between retaining high relative water content and tolerance to drought stress is the key factor that provides better conditions for a plant's metabolic functions via osmotic regulation (Nouraei et al., 2018).

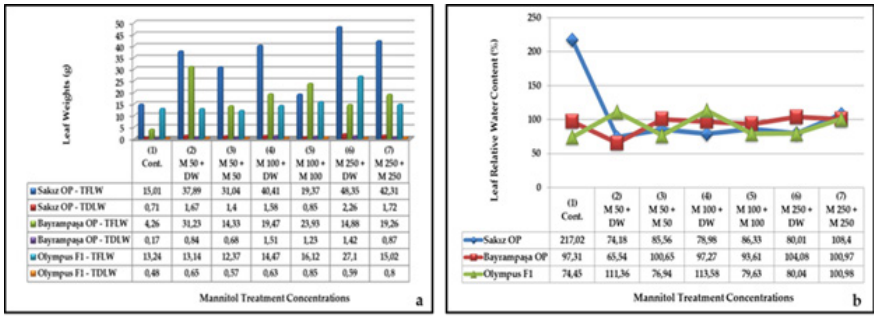


Figure 7. Effect of mannitol as drought agent on; (a) Turgid fresh (TFLW) and dry leaf (TDLW) weights; (b) Leaf relative water content.

3.4. Biochemical Analyses

After the measurements, the fresh and dry weights of the leaves (fresh leaf weight 'FLW', dry leaf weight 'DLW') were recorded, and the changes in the weights were presented in the Figure 8.

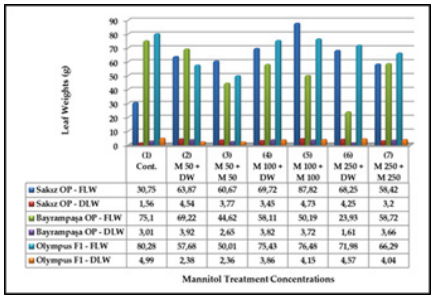
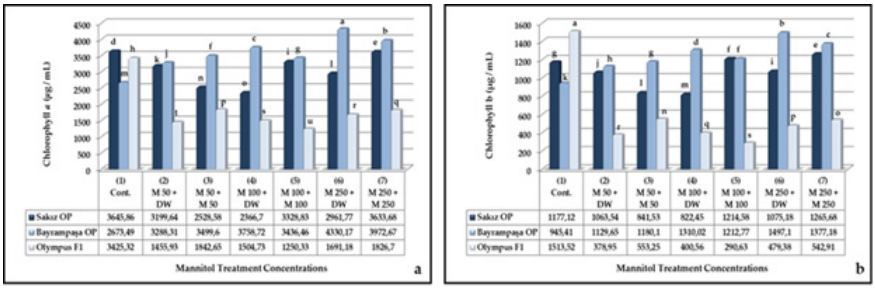


Figure 8. Effect of mannitol as drought agent on; artichoke fresh leaf weight 'FLW', dry leaf weight 'DLW'.

In order to reveal the effects of different doses of mannitol applied to the seedlings, biochemical analyses were carried out after determining the fresh and dry weights of the leaf samples of the cultivars.

Considering the effects of mannitol treatments in terms of chlorophyll *a* and *b* contents in artichoke leaves, statistically significant differences were determined among both cultivars and mannitol treatment doses (Figure 9). In light of the obtained data, the highest amount of chlorophyll *a* was recorded in 250 mg L^{-1} mannitol pre-treated Bayrampaşa OP cultivar, while the highest chlorophyll *b* obtained in the control group of Olympus F_1 cultivar. Considering drought stress, the redu-

ctions in chlorophyll levels in the leaves of artichokes and chickpeas were determined by Mafakheri et al. (2010) and Nouraei et al. (2018), respectively, while the chlorophyll content of potatoes has not changed according to Masoudi-Sadaghiani et al. (2011). Results of present study clearly shows there was no positive effect of mannitol treatments on chlorophyll *a* formation while, there was a positive effect of mannitol pre-treatment at high volume concentrations. Regarding chlorophyll *b*, there was no increase on chlorophyll *b* formation for both mannitol pre-treatment and treatments.



(1): Different letters among cultivars and mannitol treatments denote significant differences (LSD test, $p<0.05$).

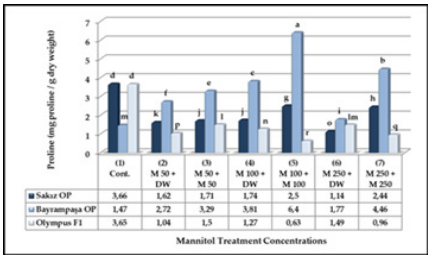
(2): (a) LSD cultivars* = 2,631; LSD treatments* = 4,02; LSD cult. x treat.* = 6,962; **(b)** LSD cultivars* = 1,901; LSD treatments* = 2,904; LSD cult. x treat.* = 5,030.

(3): Abbreviations: M50 = 50 mg L^{-1} mannitol, M100 = 100 mg L^{-1} mannitol, M250 = 250 mg L^{-1} mannitol; DW = Distilled water; Cont. = Control.

Figure 9. Effect of mannitol as drought agent on; (a) Chlorophyll *a* and (b) Chlorophyll *b* contents in artichoke leaves.

When the results of mannitol treatments in terms of proline amount were examined, statistically significant differences were determined among cultivars and mannitol treatments (Figure 10). It was determined that the highest amount of proline was obtained in Bayrampaşa OP cultivar with 100 mg L^{-1} mannitol treatment. The rise of the proline concentration levels in globe artichoke leaves based on mannitol treatment doses and time is in agreement with other researches on different crops such as *C. annuum*, *M. pomifera*, *A. thaliana* (Anjum et al., 2012; Sperdouli and Moustakas, 2012; Khaleghi et al., 2019). Due to proline's dual roles as an osmotic agent and a radical scavenger, its production and accumulation have been linked to plants' ability to withstand drought stress. Proline acts as an osmo-compatible substance as well as a non-enzymatic antioxidant (Gill and Tuteja, 2010; Kauer and Asthir, 2015). When cells are under stress, proline can assist

reduce their osmotic capacity and protect proteins by maintaining their chemical composition (Hossain et al., 2014). Proline may act as a free radical scavenger and also it may have performed a crucial role in the plant amelioration process after stress conditions (Khaleghi et al., 2019).



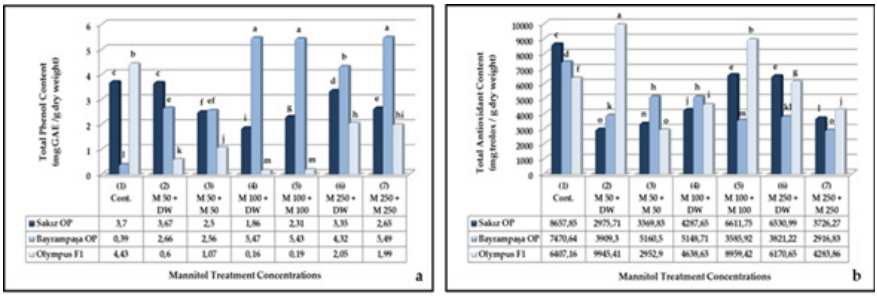
(1): Different letters among cultivars and mannitol treatments denote significant differences (LSD test, $p<0.05$).

(2): LSD cultivars* = 0,00940; LSD treatments* = 0,014; LSD cult. x treat.* = 0,024.

(3): Abbreviations: M50 = 50 mg L^{-1} mannitol, M100 = 100 mg L^{-1} mannitol, M250 = 250 mg L^{-1} mannitol; DW = Distilled water; Cont. = Control.

Figure 10. Effect of mannitol as drought agent on proline amount of artichoke leaves.

Considering the total phenol and total antioxidant contents, statistical differences were determined among cultivars and mannitol treatments, as can be seen in Figure 11. In the current study, drought stress led to an increase in phenolic content. It is thought that the increase detected in the total polyphenol content may be related to the lignification of the cell wall and the production of specific amino acids in order to provide osmotic regulation. Therefore, the increase is perceived as a biochemical response of plants when they encounter various stress conditions (Salem et al., 2014; Okunlola et al., 2017). On the other hand, in the current study, the highest total antioxidant content among mannitol treatments was recorded in the control group. Similarly, in the studies conducted by Nouraei et al. (2018) and Lucini et al. (2016), they stated that flavonoids decreased in artichoke leaves when they were subjected to stress conditions. Therefore, it was thought that it caused changes in the levels of genes that had important roles in the biosynthesis of flavonoids, which lead to a change on antioxidant content under drought conditions and also decreases the beneficial components of artichoke leaves (Yuan et al., 2012; Nouraei et al., 2018).



(1): Different letters among cultivars and mannitol treatments denote significant differences (LSD test, $p<0.05$).

(2): (a) LSD cultivars* = 0,055; LSD treatments* = 0,084; LSD cult. x treat.* = 0,146; **(b)** LSD cultivars* = 40,852; LSD treatments* = 62,403; LSD cult. x treat.* = 108,086.

(3): Abbreviations: M50 = 50 mg L^{-1} mannitol, M100 = 100 mg L^{-1} mannitol, M250 = 250 mg L^{-1} mannitol; DW = Distilled water; Cont. = Control.

Figure 11. Effect of mannitol as drought agent on; (a) Total phenol, (b) Total antioxidant contents.

CONCLUSIONS

Findings of current study clearly revealed that different mannitol treatment concentrations largely affected globe artichoke physiological and biochemical characteristics. Results of the present study also indicated that response to mannitol treatments relating to seed and seedling growth parameters, chlorophyll, proline, total phenol, and total antioxidant contents varied based on cultivar differences. Considering the results of the second measurement obtained from the treatment, it is thought that the obtained results might have been affected by the pre-treatment. Therefore, it is recommended to use different mannitol concentrations and various drought-tolerant cultivars for future studies.

Conflict of Interest

The authors declare that there is no conflict of interest.

Ethics

This study does not require ethics committee approval.

Author Contribution Rates

Design of Study: TOK (%60), ANO (%40)

Data Acquisition: TOK (%50), TT (%15), ANO (%35)

Data Analysis: TOK (%50), TT (%30), ANO (%20)

Writing up: TOK (%65), ANO (%35)

Submission and Revision: TOK (%60), ANO (%40)

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