

Effects of exogenous glycine betaine application on some physiological and biochemical properties of cotton (*G. hirsutum* L.) plants grown in different drought levels

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Abstract

Drought stress significantly reduces the growth and yield of cotton plants; therefore, reducing the damage caused by drought stress and improving the plant growth are highly important. The aims of this study were to investigate some of physiological and biochemical properties of cotton plants exposed to different drought levels and to determine the extent of improvement obtained by exogenous glycine betaine treatment for the damages caused by the adverse effects of drought. Three drought levels were created using PEG (Polyethylene glycol 6000) solution (0%, 10% and 20%). The drought groups were also divided into 2 subgroups by using 0 (control: glycine betaine solution was not applied) and 2 mM glycine betaine solution. The pot experiment was established in a growth chamber with 3 replications and the experiment repeated twice. The changes in the contents of chlorophyll a and b, carotenoid, total dissolved protein, total dissolved carbohydrate and some nutrients were investigated. Chlorophyll a and b, calcium, magnesium, sodium and phosphorus concentrations were decreased, while carotenoid, total dissolved protein and carbohydrate, potassium contents increased with the increasing drought level. The results revealed that adverse effects of drought stress on cotton plants were alleviated by exogenous application of glycine betaine.

Keywords: Chlorophyll-a, Chlorophyll-b, Protein Content, Nutrient Accumulation

Introduction

Cotton plant (*G. hirsutum* L.) is originated from the genus *Gossypium* of the Malvaceae family, and is a product of great economic importance for humanity with its widespread and compulsory use, and for the producer countries with the added value and employment opportunities created. The population growth and rising living standards increase the demand for cotton. Cotton is an important raw material of the gin industry for the processing, the textile industry with its fiber, the oil and feed industry with its seed, and the paper industry with its lint. Oil obtained from the cotton seeds is increasingly used as a raw material in the production of biodiesel as an alternative to petroleum (GTB, 2020). In 2019/2020 growing season, 24.4% of cotton production in the

world was carried out in India, 22.2% in China, 17.42% in United States of America, 6.1% in Pakistan, 9.3% in Brazil and 3.3% in Turkey (USDA, 2019). The amount of cotton produced in Turkey during 2019/2020 period was 814 thousand tons of fibers while 2.2 million tons of the cotton production was seed cotton. Cotton fiber yield in 2019/2020 season was 1870 kg ha⁻¹. Eighty eight percent of cotton production in Turkey is carried out in Sanliurfa, Aydin, Hatay, Diyarbakir, Adana and Izmir provinces.

The share of Şanlıurfa province in cotton production is 42% in the country (UPK, 2019; Özüdoğru, 2019).

The drought stress is one of the most serious abiotic stress factors that restrict the growth and

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development of the plant and decrease the yield (Pandey et al., 2012). A series of reactions occur in plant metabolism due to drought stress. Reactive oxygen species (ROS) are produced in the plant cell with the exposure to drought stress. These reactive substances cause stress in the photosynthesis mechanism and cause oxidation in the cell membrane and deformation in its structure (Foyer and Noctor, 2009; Dietz and Pfannschmidt, 2011). In addition, nucleic acids negatively affect plant metabolism due to the oxidation in carbohydrate and protein metabolism (Clemens, 2001; Ali et al., 2012). Drought affects carbon and nitrogen metabolisms of the plant, causing decreases in photosynthesis production; thus, negatively affect the plant growth and development (Lawlor and Tezera, 2009). The decline in the photosynthesis mechanism causes stomata closure (Chaves et al., 2009; Aranjuelo et al., 2011). Stomatal closure mechanism directly affects the carbon metabolism of plants; therefore, the severity of drought significantly affects the stomatal closure mechanism (Bota et al., 2004). Plants generally increase their antioxidant contents in response to drought stress and alter total soluble sugar, total amino nitrogen and polyphenol levels (Başal and Aydın, 2006; Chaves et al., 2009). Loka and Oosterhuis (2014) stated that the total dissolved carbohydrate content and glutathione reductase level of the pistils increased, while photosynthesis and respiration of the cotton plants significantly decreased during the flowering stage at different drought levels. Plants develop aforementioned mechanisms to survive and to have the least damage from the stress. Synthesis of osmolite is one of the mechanisms developed to prevent from drought stress damages. The osmotic pressure changes when plants are under drought stress, and plants produce non-toxic, low molecular weight and highly soluble organic materials called osmolites to compensate for the changing osmotic pressure (Serraj and Sinclair, 2002). The most common compatible osmotic preservatives are betaines, polyols and sugars (mannitol and trehalose) and amino acids (proline). The osmolites improve the resistance of plants to drought, heavy metals and stress factors (McNeil et al., 1999).

Glycine betaine, one of the osmolite types, occurs naturally in many plant species and living organisms. The glycine betaine is a substance that can be synthesized in high quantity in the chloroplast, regulates the photosynthesis and thylakoid membrane structure of the cell and helps maintain the integrity of the cell membrane structure (Allakhverdieva, 2001). The glycine betaine is synthesized in some plants and accumulated against stress factors (Yancey, 1994; Subbarao et al., 2001), while they may accumulate at a very small amount in some plants or may not accumulate. The researchers indicated that even very small amount of accumulation in plants may help alleviate the stress

damage (Agboma et al., 1997a, 1997b; Yang and Lu, 2005; Zhang et al., 2013).

Materials and Methods

Plant material

Drought tolerant CANDIA cotton variety of Bayer Company was used in the study. This variety is widely used in the Southeast regions of Turkey due to better yield and fiber quality criteria.

Sterilization of Seeds and Planting

Cotton seeds were pre-treated with 70% ethanol for 30 seconds to ensure sterilization. Then, the seeds were soaked in 10% NaOCl solution for 10 minutes. The seeds were washed 3 times with double distilled water (ddI -H₂O) to prevent the possible adverse effect of NaOCl (Wu et al., 2011; Can, 2013).

The pots were washed with distilled water and sterilized, super coarse perlite (0-5 mm) was filled into the pots. Cotton seeds were germinated in magenta boxes. Five cotton seedlings were transplanted in each pot.

Setting The Growing Conditions in The Growth Chamber

The ambient temperature of the plant growth chamber was adjusted to a daily average of 27± °C (30 °C/26 °C) (Reddy et al., 2004; Salvucci and Crafts-Brandner, 2004). The lighting of the growth chamber was set to 14 hours light and 10 hours dark. Fluorescent lamps designed for plants were used in the light phase. Light intensity was measured as 350-400 μmol m⁻² sec⁻¹. The humidity level of the growth chamber was set to 65-70% (Rahman et al., 2004).

For 30 days when the plants reached the 5-6 leaf stage, ½ Hoagland nutrient solution was applied (Hoagland, 1920). Drought stress treatments were started on the 31st day. The drought treatments (0 and 10, 20%) were carried out by adding PEG 6000 solution to the Hoagland nutrient solution. Stress treatments contained 0 mM (glycine betaine not given: control group) and 2 mM glycine betaine solution, which was added to the nutrient solution and applied with the drought stress treatment.

Elemental Analysis of Plant Samples

Plant samples were separated into the parts and dried in an oven at 70 °C until the weight becomes a constant value. After reaching a constant weight, the plants were weighed and ground in a plant grinding mill (Can, 2013). Then, 0.3 g of plant samples were weighed, and 5 ml HNO₃ was added over the plant samples. The samples were filtered into polystyrene tubes using a filter paper and allowed to cool. Total volume of filtrates were completed to 15 ml with deionized water. The concentration of sodium (Na), magnesium (Mg), potassium (K), phosphorus (P) and calcium (Ca) in solution was determined using an ICP-AES (Inductively Coupled Plasma Atomic Emission Spectrometer) (Varian-Vista, axial) instrument in the Elemental Analysis Laboratory.

Photosynthetic Pigments in Leaf Tissues

Chlorophyll a, chlorophyll b and carotenoid contents of leaves were determined using the

methods described by Lichtenthaler (1987). Absorbance values of the supernatants at 663, 645 and 470 nm wavelengths were measured with a spectrophotometer. Calculations were performed using the following equations;

$$\text{Chlorophyll-a} = \Delta A_{663} \cdot 12.7 - \Delta A_{645} \cdot 2.69$$

$$\text{Chlorophyll-b} = \Delta A_{645} \cdot 22.9 - \Delta A_{663} \cdot 4.68$$

$$\text{Carotenoid} = \Delta A_{480} + 0.114 \cdot \Delta A_{663} - 0.638 \cdot \Delta A_{645} / 112.5$$

Total Soluble Carbohydrate Content

Total soluble carbohydrate content of leaves was determined using the phenol-sulfuric method (Dubois et al., 1956). 0.05 g of dry samples were weighed and placed into new tubes, and 70% ethyl alcohol was added to the tubes. The solution was kept in a hot water bath at 80 °C for 60 minutes. After that, the tubes were centrifuged at 3500 rpm for 20 minutes. After the centrifugation, 1000 µl of supernatant was transferred to new test tubes, 300 µl 5% phenol and 2000 µl concentrated sulfuric acid (H₂SO₄) were added and vortexed. The absorbance values of the solutions were determined by a spectrophotometer at 490 nm wavelength. Glucose solution at different concentrations was used as the standards. Total soluble carbohydrate content was calculated by creating a standard curve. Soluble total carbohydrate was determined as dry weight (mg mL⁻¹).

Total Protein Content

Total protein content was determined by the method of Bradford (1976) using BSA (Bovine Serum Albumin) standards. The absorbance of the solutions were measured at 595 nm using Shimadzu UV spectrophotometer instrument against blank. The curve was created using different concentrations as the standards. Soluble total protein content was determined as wet weight (mg mL⁻¹).

Statistical Analysis

The study was repeated twice, and only the leaf samples were analyzed in the study. Growth parameters were determined 2 times (n=10). Other analyzes were replicated 3 times. The third analysis was carried out by combining the leaves of the 1st and 2nd experiments. Statistical evaluation of the data was performed using the SPSS software. The effects of exogenous glycine betaine treatment on physiological and biochemical properties of cotton plants under drought stress levels were assessed by variance analysis (ANOVA). When ANOVA indicated significant difference, a post hoc test was used to group the treatments.

Results and Discussion

Results

Chlorophyll a, b and Carotenoids Content

The chlorophyll a content in the glycine betaine not applied group, decreased as drought severity increased. However, chlorophyll a content increased with glycine betaine (2 mM) application along with the exogenous drought treatment. The highest chlorophyll a content was obtained in the control (7.58 µg g⁻¹) when glycine betaine was not exogenously applied. The highest chlorophyll content in this group was 7.12 µg g⁻¹

The chlorophyll b content in the glycine betaine not applied group, decreased as drought severity that was created by application of 0, 10 and 20% PEG 6000 increased. However, chlorophyll b content increased with glycine betaine (2 mM) application along with the exogenous drought treatment. The highest chlorophyll a content in drought stress group was obtained in the control (8.05 µg g⁻¹) when glycine betaine was not exogenously applied. The highest chlorophyll content in this group was 7.99 µg g⁻¹ (p < 0.05) when exogenous glycine betaine was applied.

The carotenoid content in the glycine betaine not applied group, increased compared to the control as drought severity that was created by application of 0, 10 and 20% PEG 6000 increased. However, carotenoid content decreased with glycine betaine (2 mM) application along with the exogenous drought treatment. The highest carotenoid content in drought stress group was obtained in the 20% PEG 6000 (1.53 µg g⁻¹) treatment when glycine betaine was not exogenously applied. The highest carotenoid content in this group was 1.53 µg g⁻¹ (p < 0.05) when exogenous glycine betaine was applied.

Total Dissolved Carbohydrate and Protein Content:

Total dissolved carbohydrate content in the glycine betaine not applied group, increased compared to the control as drought severity that was created by application of 0, 10 and 20% PEG 6000 increased. However, total dissolved carbohydrate content decreased with glycine betaine (2 mM) application along with the exogenous drought treatment. The highest total dissolved carbohydrate content in drought stress group was obtained in the 20% PEG 6000 (19.26 µg g⁻¹) treatment when glycine betaine was not exogenously applied. The highest total dissolved carbohydrate content in this group was 17.6 µg g⁻¹ (p < 0.05) when exogenous glycine betaine was applied.

Total dissolved protein content in the glycine betaine not applied group, increased compared to the control as drought severity that was created by application of 0, 10 and 20% PEG 6000 increased. However, total dissolved protein content decreased with glycine betaine (2 mM) application along with the exogenous drought treatment. The highest total dissolved protein content in drought stress group was obtained in the 20% PEG 6000 (4.37 µg g⁻¹) treatment when glycine betaine was not exogenously applied. The highest total dissolved protein content in this group was 3.21 µg g⁻¹ (p < 0.05) when exogenous glycine betaine was applied.

Some of Nutrient Contents:

Total Ca content in the glycine betaine not applied group, decreased compared to the control as drought severity that was created by application of 0, 10 and 20% PEG 6000 increased. However, total Ca content increased with glycine betaine (2 mM) application along with the exogenous drought treatment. The highest Ca content in drought stress group was obtained in the control (427.53 µg g⁻¹) treatment when glycine betaine was not exogenously applied.

The highest Ca content in this group was 425.53 $\mu\text{g g}^{-1}$ ($p < 0.05$) when exogenous glycine betaine was applied.

Total K content in the glycine betaine not applied group, increased compared to the control as drought severity that was created by application of 0, 10 and 20% PEG 6000 increased. However, total K content decreased with glycine betaine (2 mM) application

along with the exogenous drought treatment. The highest K content in drought stress group was obtained in the control ($633.45 \mu\text{g g}^{-1}$) treatment when glycine betaine was not exogenously applied. The highest K content in this group was $629.13 \mu\text{g g}^{-1}$ ($p < 0.05$) when exogenous glycine betaine was applied.

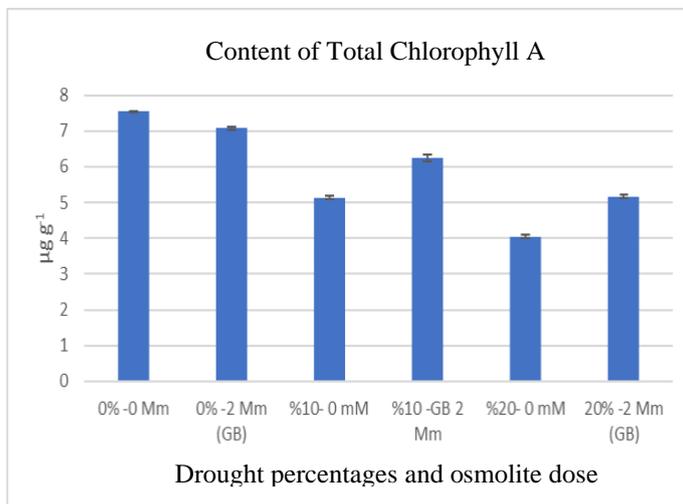


Figure 1. Effect of exogenous glycine betaine application (2 mM) to cotton plants grown in different drought severity on chlorophyll a content (error bars show standard deviation) (N=9)

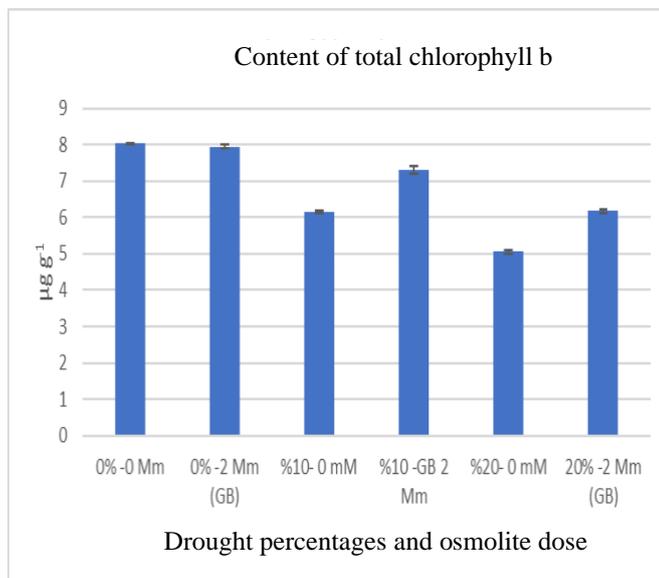


Figure 2. Effect of exogenous glycine betaine application (2 mM) on cotton plants grown at different drought severity on chlorophyll b content (error bars show standard deviation) (N=9)

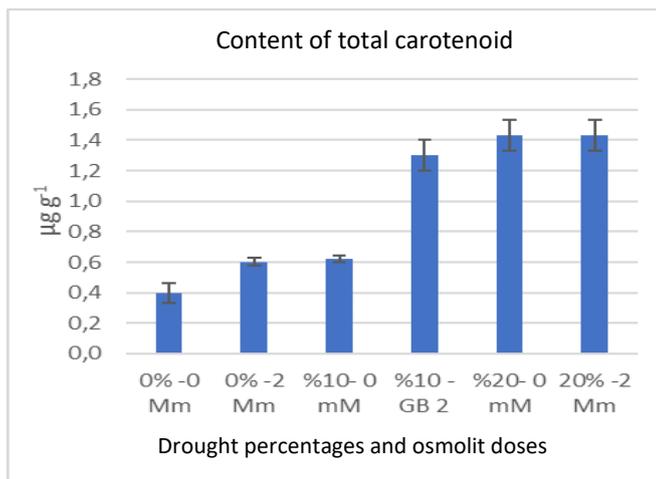


Figure 3. Effect of exogenous glycine betaine application (2 mM) on total carotenoid content of cotton plants grown at different drought severity (error bars show standard deviation) (N=9)

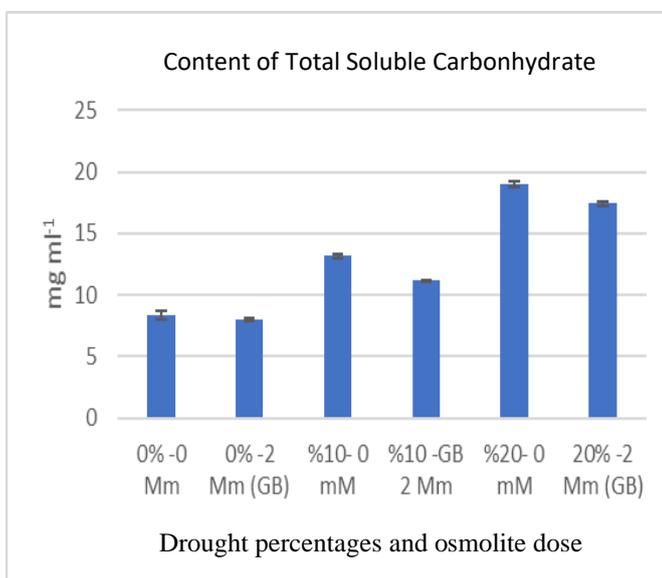


Figure 4. Effect of exogenous glycine betaine application (2 mM) on total dissolved carbohydrate content of cotton plants grown at different drought severity (error bars show standard deviation) (N = 9)

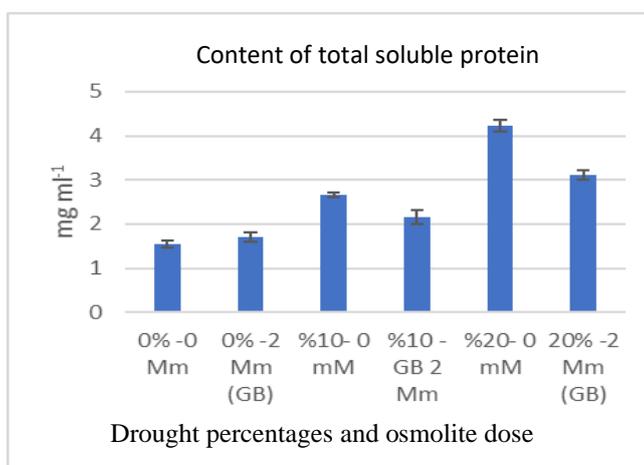


Figure 5. Effect of exogenous glycine betaine application (2 mM) on total dissolved protein content of cotton plants grown at different drought severity (error bars show standard deviation) (N = 9)

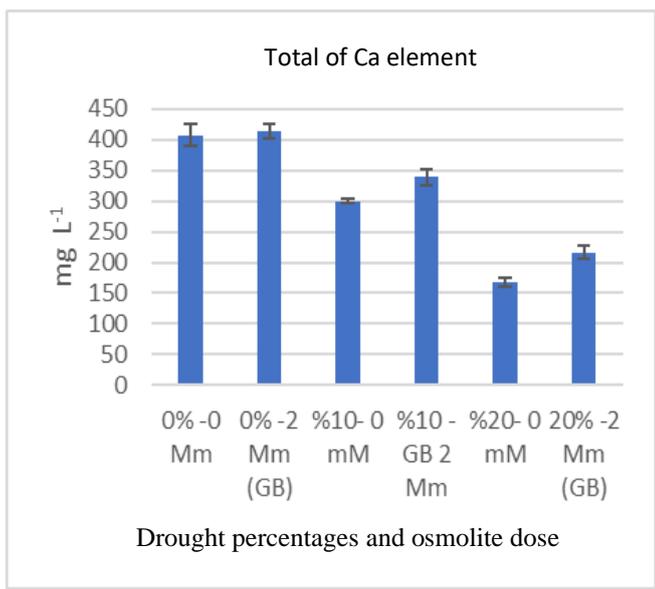


Figure 6. Effect of exogenous glycine betaine application (2 mM) on total Ca content of cotton plants grown at different drought severity (error bars show standard deviation) (N = 9)

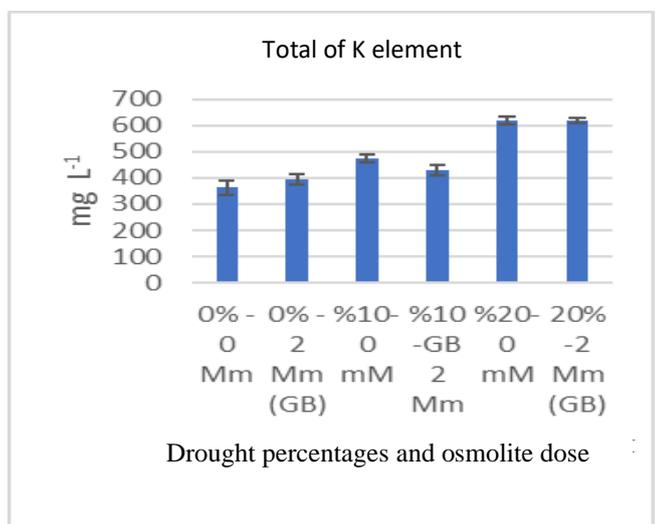


Figure 7. Effect of exogenous glycine betaine application (2 mM) on total K content of cotton plants grown at different drought severity (error bars show standard deviation) (N = 9)

Magnesium Content

Total Mg content in the glycine betaine not applied group, decreased compared to the control as drought severity that was created by application of 0, 10 and 20% PEG 6000 increased. However, total Mg content increased with glycine betaine (2 mM) application along with the exogenous drought treatment. The highest Mg content in drought stress group was obtained in the control (195.5 µg g⁻¹) treatment when glycine betaine was not exogenously applied. The highest Mg content in this group was 196.73 µg g⁻¹ (p<0.05) when exogenous glycine betaine was applied.

Sodium Content

Total Na content in the glycine betaine not applied group, decreased compared to the control as drought severity that was created by application of 0, 10 and 20% PEG 6000 increased. However, total Na content increased with glycine betaine (2 mM) application along with the exogenous drought treatment. The highest Na content in drought stress group was obtained in the control (149.8 µg g⁻¹) treatment when glycine betaine was not exogenously applied. The highest Na content in this group was 171.17 µg g⁻¹ (p<0.05) when exogenous glycine betaine was applied.

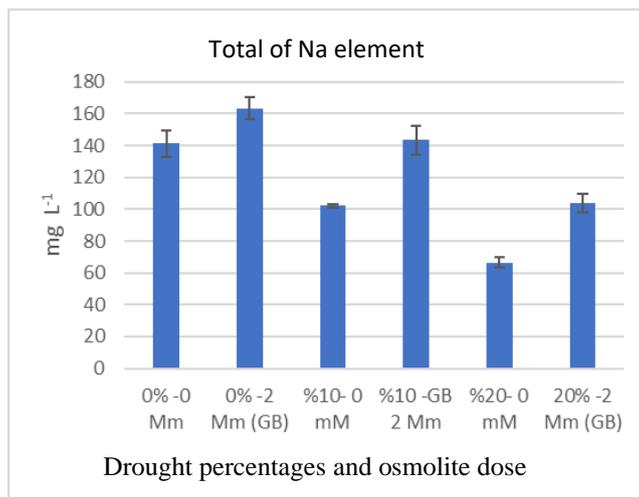


Figure 9. Effect of exogenous glycine betaine application (2 mM) on total Na content of cotton plants grown at different drought severity (error bars show standard deviation) (N = 9)

Total P content in the glycine betaine not applied group, decreased compared to the control as drought severity that was created by application of 0, 10 and 20% PEG 6000 increased. However, total P content increased with glycine betaine (2 mM) application along with the exogenous drought treatment. The

highest P content in drought stress group was obtained in the control (238.4 $\mu\text{g g}^{-1}$) treatment when glycine betaine was not exogenously applied. The highest P content in this group was 272.12 $\mu\text{g g}^{-1}$ ($p < 0.05$) when exogenous glycine betaine was applied.

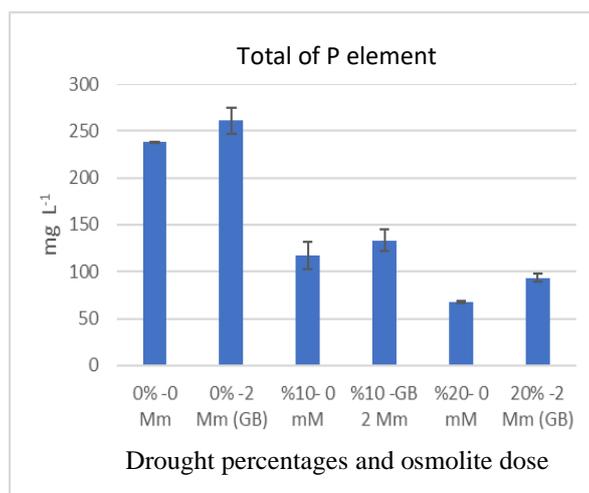


Figure 10. Effect of exogenous glycine betaine application (2 mM) on total phosphorus content of cotton plants grown at different drought severity (error bars show standard deviation) (N = 9)

Discussion

Photosynthesis mechanism is slowed down and chlorophyll structure is decreased under stress conditions (Pettigrew et al., 2005). In addition, the drought stress causes a decrease in water content and stomata closure. This situation results in a decrease in CO_2 concentration, and led to photooxidation and photoinhibition (Flexas and Medrano, 2002). The results are in agreement with the Thimmanaik et al. (2002), Parida et al. (2007), Guerfel et al. (2009) and Nikolaeva et al. (2010) who reported that chlorophyll was degraded and photosynthetic pigment content decreased due to the indirect chain reaction.

Increasing the severity of drought may reduce the chlorophyll content; thus, the plant become more sensitive to drought stress (Anju et al., 1994). The presence of glycine betaine in chloroplasts protects the thylakoid membranes, and maintains photosynthetic efficiency by providing membrane integrity (Yokoi et al., 2002; Yang and Lu, 2005).

The findings indicated reductions in chlorophyll a and b contents with the drought severity, however, exogenous glycine betaine application improved the damage caused by the drought stress. The results revealed that cotton plants maintain the photosynthesis mechanism resilient against the drought stress.

Carotenoid is an antioxidant that is bound with weak bonds to proteins in the cell, and prevents the pigments from being oxidized and degraded (Çınar, 2003; Kalefetoğlu and Ekmekçi, 2005). The antioxidant content generally increases as the drought severity increases. Increasing carotenoid content during the drought stress activates the antioxidant mechanism of the plant and reduces the negative impact of drought.

The photosynthesis mechanism of plants exposed to drought stress decelerates, which affects the carbohydrate mechanism and causes deterioration (Pelleschi et al., 1997; Kim et al., 2000). Kim et al. (2000) reported that metabolic processes such as photosynthesis and respiration are important to provide intracellular carbohydrate content. The findings of Kerepesi and Galiba (2000) who reported that increasing drought severity increases the dissolved carbohydrate content in plant cells coincides with the findings of Kim et al. (2000), Sanchez et al. (2004) and Zali and Ehsanzadeh (2018). The findings indicated that cotton plant has a deteriorated carbohydrate mechanism due to drought, and this metabolism can be improved by application of exogenous glycine betaine.

Photosynthesis electron transport mechanism and photophosphorylation mechanism of chloroplasts, which were isolated from plants grown under drought conditions decreased (Smimoff, 1993). The PS II in the isolated chloroplasts was affected by the drought (He et al., 1995). The D1 and D2 regions in the PS II reaction mechanism are responsible for photoinhibition, and these regions go through transformations under stress (Baker, 1991). The increase in total protein content may be related to the proteins in the degraded region due to the impact of increased reactive oxygen species under stress conditions. In addition, drought stress not only affects photosynthesis reactions, but also affects nucleic acid and protein synthesis. Inhibition in nucleic acid synthesis also decreases the protein synthesis mechanism (Çırak and Esenal, 2006). Protein synthesis rate decreases, while protein structures change and their structures are fragmented (Chartzoulakisa, 2002; Parida et al., 2007).

Drought stress increases the soluble protein content of some plants, and proteins, stimulated by the effect of drought stress, develop an adaptation mechanism against drought (Bray, 1993; Han and Kermod, 1996; Riccardi et al., 1998; Can, 2013). The synthesis of some proteins from the dehydrin family increases with drought stress and plays an important role in protecting other proteins and their structural integrity. The results obtained are in accordance with the findings of Bray (1993) and Close et al (1996) who attributed to the increase in the dissolved protein content with the degradation and fragmentation of their structures.

Calcium uptake of plants decreases due to the drought stress. The Ca element is used in osmotic regulation of plants and plays an important role in the

signal formation mechanism against stress factors (Bartels and Sunkar, 2005). Jenne et al. (1958) attributed the decrease in Ca content to competing with P and K ions under stress conditions and stated that the intracellular P, K and Ca contents reached 40, 71 and 91% under stress conditions. The plants may have used Ca element in their metabolisms to develop drought signal.

Potassium increases the internal balance (homeostasis) by regulating the stomata to reduce the effect of drought stress in plants, regulating the intracellular osmotic pressure, protein and energy mechanism (Beringer and Trolldenier, 1978; Marscher, 1995). In addition, the K element is also used to control the transpiration mechanism and reduces stress damage by balancing the osmotic pressure (Andersen et al., 1992). Potassium production of plants in arid regions is more important than organic matter synthesis to protect against the stress. Plants reallocate the energy to increase stress tolerance and the energy released in osmotic balance increases with K uptake in stress-inducing conditions. Previous studies indicated that the K content of plants increased under arid conditions (Morgan, 1992; Yaşar et al., 2006).

Havlin et al. (1999) reported that the uptake mechanism of Mg, Fe and P ions are disrupted due to drought stress, their uptake into the cell is prevented and some toxicities start to occur in plants. These ratios under stress conditions vary between the species. Our results are similar to the findings of Can (2013) who reported that the K content increased and Ca, Mg, P and Na contents of cotton genotypes grown under different drought stress conditions decreased due to the increasing drought severity.

Conclusion

Cotton is considered the white gold of our country and is one of the vital sources of the economy. Drought is one of the major abiotic stress factors that limit the growth of many plants such as cotton and reduce crop yield. Reactive oxygen species (ROS) are produced under drought stress conditions. The ROS causes damage to the plants by changing the physiology and biochemistry of the plants. Exogenous osmolite application is an alternative mean to reduce the damage caused by ROS. Glycine betaine is the most common osmolite used against drought stress. Exogenous application of glycine betaine was reported to increase plant tolerance to heavy metals (Cao et al., 2013; Ali et al., 2015) and other stresses factors such as drought, salinity, high and low temperature (Yang et al., 2008; Iqbal et al., 2009; Islam et al., 2009; Chen and Murata, 2011).

This improvement demonstrates that glycine betaine is an alternative product that can be used to reduce crop loss during drought conditions. However, further studies are needed to investigate the effect of application before the drought in preventing the drought damage. In addition, the physiological and biochemical changes occur in exogenous glycine

betaine application at different development stages of the cotton plant can be studied. Determining the activity of enzymatic components of the antioxidant system and the isoenzymes and even investigating the gene expression will be effective in elucidating the effect of osmotic preservatives on the drought tolerance mechanism.

Compliance with Ethical Standards

Conflict of interest

The authors declared that for this research article, they have no actual, potential or perceived conflict of interest.

Author contribution

The contribution of the authors to the present study is equal.

All the authors read and approved the final manuscript. All the authors verify that the Text, Figures, and Tables are original and that they have not been published before.

Ethical approval

Ethics committee approval is not required.

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Data availability

Not applicable.

Consent for publication

Not applicable.

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