

# Changes in biochemical characteristics and Na and K content of caper (*Capparis spinosa* L.) seedlings under water and salt stress

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## Abstract

In order to investigate the effect of water and salt stress on caper (*Capparis spinosa* L.) seedlings, a randomized complete block design with five replications was carried out in 2013 at Shiraz University, Iran. Water stress had three levels: 100 % (control), 75 %, and 50 % field capacity (FC), and five levels of salinity were applied: 0 (control), 4, 8, 12, and 18 dS m<sup>-1</sup>. The results indicated that salinity had a significantly negative effect on chlorophyll content of caper seedlings, while drought increased this content. The carotenoid content in caper seedlings under water and salinity stress was significantly increased. Proline and total protein content increased also under both salinity and water stress. Antioxidant enzyme activity; superoxide dismutase (SOD), catalase (CAT), peroxidase (POD) and ascorbate peroxidase (APX) also increased in response of salinity and drought. Salinity stress significantly increased the content of Na<sup>+</sup> in cells but decreased K<sup>+</sup> content. It seems that caper seedlings could tolerate a salinity level up to 4–8 dS m<sup>-1</sup> as well as water stress of 75 % FC, no significant differences were observed between these two salinity levels, the water stress level and the control. The interaction effect of water stress and salinity had a significant effect on biochemical characteristics of caper. The highest content of carotenoid, proline and total protein content were obtained in 50 % FC and 18 dS m<sup>-1</sup>. The results of biochemical characteristics and leaf content of K<sup>+</sup> and Na<sup>+</sup> suggest that caper plant is a very tolerant species to salinity and drought stress which make it a suitable crop for most arid and semi-arid regions of Iran.

**Keywords:** caper, catalase, peroxidase, salt stress, superoxide dismutase

## 1 Introduction

Caper (*Capparis spinosa* L.), is a multi-purpose shrub native to the Mediterranean regions and (semi-)arid tropics (Legua *et al.*, 2013). Due to the recent severe droughts in Iran and most arid and semi-arid regions in the world, farmers have attempted to cultivate drought and saline resistant plants (such as caper) instead of plants with high water requirements (Sadeghi & Rostami, 2016). Cultivation of an alternative crop (such as caper) can increase income of poor and marginal land holding farmers in arid regions and can prevent them from rural to urban migration. Commercial cultivation

of caper in Iran is still in its infancy with the possibility of future expansion owing to its economic importance which can contribute to the livelihoods of many small farmers due to its low cultivation requirements and its tolerance to adverse environmental conditions.

Caper has a deep root system, is resistant to drought conditions, and can tolerate temperatures exceeding 40 °C (Suleiman *et al.*, 2012). Because of its vegetative canopy, caper gives an excellent soil cover, thus preserving soil water (Saifi *et al.*, 2011; Rostami *et al.*, 2016). *C. spinosa* grows wild in different parts of Iran, especially in dry and arid regions, and has a variety of economic, ecological, and medicinal uses in Iran. This plant is further considered to be excellent for wind screens and sandy soil stabilisation, and its introduction

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in arid and semi-arid environments could help to prevent the disruption of the equilibrium of those fragile ecosystems (Sozzi, 2001). Recently, the cultivation of this plant has been initiated to reduce the negative effects of dust phenomenon in south and southwest of Iran.

Salinity and drought are the most common abiotic stresses that induce a significant reduction in photosynthesis, which depend on photosynthesizing tissue and photosynthetic pigments (Saed-Moocheshi *et al.*, 2014a). During stress, active solute accumulation such as soluble carbohydrates, proteins, and free amino acids is claimed to be an effective stress-tolerance mechanism. Certain plant species adapt to high salt concentrations in soil by lowering their tissue osmotic potential and by accumulating these osmotic solutes (Saed-Moocheshi *et al.*, 2014b). Salinity affects plant growth in two ways, by increasing osmotic pressure of the soil solution and/or by the specific effect of the salt ions, mainly  $\text{Na}^+$  and  $\text{Cl}^-$ . The increased osmotic pressure of the soil solution resulting from increased salt content impairs the ability of plants to absorb water by lowering leaf water potential. Under osmotic stress plants need to maintain water potential below that of the soil and maintain turgor and water uptake for growth. This requires an increase in the osmoregulators, either by accumulation of inorganic solutes (e.g.,  $\text{K}^+$ ,  $\text{Na}^+$  and  $\text{Cl}^-$ ) or by synthesis of organic solutes (e.g., proline and glycine betaine).

The present study was performed to determine the changes in biochemical characteristics and leaf content of  $\text{K}^+$  and  $\text{Na}^+$  under drought and salinity stress in caper plant in order to evaluate the tolerance and adaptability of this plant under water and salt stress conditions.

## 2 Materials and methods

### 2.1 Experimental procedure

Seeds of the caper plant were gathered from Farashband belonging to Fars province of Iran., separated, washed with deionized water and sterilised with 70 % ethanol for five minutes. The seeds were placed in Petri dishes containing filter paper with 5 mL polyethylene glycol (PEG) 6000 for dormancy breaking and kept in a germinator at 4 °C for a period of four weeks. After germination, the seeds were transported to 5 L pots filled with soil. Ten germinated seeds were sown in each pot.

Treatments were arranged in a randomized complete block design with two factors, water and salinity stress, and five replications. The first factor water stress had three levels of 100 % (control), 75 %, and 50 % FC. For the determination of field capacity, pots with dry soil

were weighed, soaked, and after total drain of the water, weighed again. Maximum water holding capacity (approximately 20 %) was determined by the difference between dry and soaked soil weights. The determination of water refill for all field capacities was calculated in relation to this difference. Drought treatment levels were applied based on the weighting method by daily weighting of pots (Sadeghi & Rostami, 2016). The second factor salinity stress had five levels 0 (control), 4, 8, 12, and 18  $\text{dS m}^{-1}$ . For salinity treatments, sodium chloride and calcium chloride with the same ration were applied. The plants were grown at day/night temperatures of  $28/22 \pm 2$  °C. Directly after the sowing of germinated seeds in the pots, drought and salinity treatments started. After an experimental period of forty days, the leaves of all plants were separated from the plant, frozen in liquid nitrogen and transported to the laboratory for measurements.

Total chlorophyll, chlorophyll *a*, chlorophyll *b*, and carotenoid contents were determined for the samples according to the Arnon (1949) method. Subsequently, the content of pigments was determined based on the following standard formulas (Lichtenthaler & Buschmann, 2001):

$$\begin{aligned} \text{Total chlorophyll (mg/mL)} &= 20.2(A_{645}) + 8.02(A_{663}) \\ \text{Chlorophyll } a \text{ (mg/mL)} &= 12.7(A_{663}) - 2.69(A_{645}) \\ \text{Chlorophyll } b \text{ (mg/mL)} &= 22.9(A_{645}) - 4.68(A_{663}) \\ \text{Carotenoid (mg/mL)} &= (1000A_{470} - 3.27[\text{Chl } a] \\ &\quad - 104[\text{Chl } b]) / 227 \end{aligned}$$

where *A* is the recorded number in the spectrophotometer and Chl *a* and Chl *b* denote chlorophyll *a* and chlorophyll *b* content, respectively.

Free proline was extracted from fresh leaves according to the method described by Bates *et al.* (1973).

Frozen leaves were ground to fine powder with a mortar and pestle in liquid nitrogen and were extracted with ice-cold 0.1 M Tris-HCl buffer (pH 7.5) containing 5 % (w/v) sucrose and 0.1 % 2-mercaptoethanol (3 : 1 buffer volume / fresh weight). The homogenate was centrifuged at  $12\,000 \times g$  for 20 minutes at 4 °C and the supernatant was used to measure protein content and enzyme activity.

The protein content was estimated according to the method of Bradford (1976), using bovine serum albumin (BSA) as a standard and observance of 595 nm.

Superoxide dismutase (SOD) inhibits the photochemical reduction of nitro-blue-tetrazolium (NBT) (Beauchamp & Fridovich, 1973), and this ability was

**Table 1:** Analysis of variance (ANOVA) for measured traits.

Source	Degree of freedom	Mean squares								
		Proline	Protein	Sodium content	Potassium content	Carotenoid	Ascorbate peroxidase	Superoxide dismutase	Catalase	peroxidase
Drought	2	409.1**	203.15**	12.08**	204.01**	0.17**	0.47**	0.6**	0.18**	2.51**
Salinity	4	50.26**	99.15**	1.06**	101.15**	0.11**	0.18**	0.44**	0.16**	0.19**
Drought * Salinity	8	11.84**	4.1**	0.05**	7.02**	0.01 <sup>ns</sup>	0.02 <sup>ns</sup>	0.03 <sup>ns</sup>	0.05 <sup>ns</sup>	0.03 <sup>ns</sup>
Error	60	2.2	3.25	0.10	11.02	0.03	0.002	0.02	0.03	0.09
Coefficient of variation		8.14	10.12	14.23	13.06	15.19	11.24	12.61	16.06	14.06

<sup>1</sup> \*\*, \*, and <sup>ns</sup> are representation of significant in 1 % level, significant in 5 % level, and not significant, respectively.

used to determine its activity. For SOD assay, the reaction mixture contained 50 mM K-phosphate buffer (pH 7.8), 13 mM methionine, 75  $\mu$ M NBT, 0.1  $\mu$ M EDTA, 4  $\mu$ M riboflavin, and extracted enzyme. The reaction started by adding riboflavin, after which the tubes were placed under two 15 W fluorescent lamps for 15 minutes. A complete reaction mixture-lacking enzyme, which gave the maximal colour, was considered as control. A non-irradiated complete reaction mixture was used as a blank. One unit of SOD activity was defined as the amount of enzyme required to cause 50 % inhibition of the reduction of NBT as monitored at 560 nm (Giannopolitis & Ries, 1977).

Peroxidase (POD) activity was assayed (Polle *et al.*, 1994) at 436 nm by its ability to convert guaiacol to tetraguaiacol ( $\epsilon = 26.6 \text{ mM cm}^{-1}$ ). The reaction mixture contained 100 mM K-phosphate buffer (pH 7.0), 20.1 mM guaiacol, 10 mM  $\text{H}_2\text{O}_2$ , and enzyme extract. The increase in absorbance was recorded by adding  $\text{H}_2\text{O}_2$  at 436 nm for 5 minutes. The activity of catalase (CAT) was determined by monitoring the disappearance of  $\text{H}_2\text{O}_2$  at 240 nm ( $\epsilon = 40 \text{ mM cm}^{-1}$ ). The reaction mixture contained 50 mM K-phosphate buffer (pH 7.0), 33 mM  $\text{H}_2\text{O}_2$ , and enzyme extract.

For measuring ascorbate peroxidase (APX) activity, 50 mm sodium phosphate buffer (pH = 6), 0.1 mM EDTA, 0.1 mM  $\text{H}_2\text{O}_2$ , and 0.5 mM ascorbate were mixed and 0.2 mL enzyme extract was added. After that, the absorption of the light was measured at 290 nm wave length and the enzyme activity was estimated (Nakano & Asada, 1981).

For the determination of leaf sodium (Na) and potassium (K) contents the samples were dry ashed at 550 °C, then 2 mol HCl solution was used for extraction (Chapman & Pratt, 1961). Subsequently, Na and K content were determined by atomic absorption by spectrophotometer (Varian model Spectera AA 220, Australia).

Univariate normality test was carried out on residuals of the ANOVA model for all measured traits for test-

ing hypothesis related to normal distribution of the data using SAS 9.3 software. The main effects of factors and their interactions were tested using analysis of variance (ANOVA) by GLM procedure of SAS. Least significant difference (LSD) was used for mean comparison of main treatment factors and their interactions at the significant level of 5 %.

### 3 Results

The growth parameters for this study are presented by Sadeghi & Rostami (2016). The results of analysis of variance (Table 1) showed significant effect of salinity and drought stress on proline, protein, sodium and potassium content, as well as on carotenoid and antioxidant enzyme activity. The interaction effect of drought by salinity was only significant for proline, protein, sodium and potassium content. The results showed that the content of chlorophyll *a*, chlorophyll *b*, and total chlorophyll decreased with increase in salinity (Table 2). The decrease rate of chlorophyll *a* was higher than chlorophyll *b*. Chlorophyll *a* content was not affected by 4 dS  $\text{m}^{-1}$ , and 8 dS  $\text{m}^{-1}$  treatments, but it was significantly reduced at 12 dS  $\text{m}^{-1}$  and 18 dS  $\text{m}^{-1}$ . Salinity level of 18 dS  $\text{m}^{-1}$  was the only level which showed significant difference in chlorophyll *b* from other salinity levels. Total chlorophyll content showed similar results to chlorophyll *a*. Carotenoid content increased with an increase in salinity level. The highest content of carotenoid was observed at 18 dS  $\text{m}^{-1}$  salinity level, while the lowest content was obtained in the control. In contrast to salinity, the content of chlorophyll *a* and total chlorophyll increased with increase in severity of water stress (Table 2). However, chlorophyll *b* was not affected by different water stress levels. Carotenoid content increased with drought. The highest content of carotenoid was obtained in 50 % FC and the lowest content in 100 % FC irrigation.

**Table 2:** Pigment content of caper seedlings under different irrigation and salinity levels at 40 days after seeding.

	Chlorophyll a (mg/mL)	Chlorophyll b (mg/mL)	Total chlorophyll (mg/mL)	Carotenoid (mg/mL)
<i>FC (%)</i>				
100	11.20 <sup>b</sup>	3.10 <sup>a</sup>	14.30 <sup>b</sup>	1.87 <sup>c</sup>
75	12.50 <sup>a</sup>	2.90 <sup>a</sup>	15.40 <sup>a</sup>	2.45 <sup>b</sup>
50	12.70 <sup>a</sup>	3.10 <sup>a</sup>	15.80 <sup>a</sup>	2.90 <sup>a</sup>
<i>Salinity levels (dS m<sup>-1</sup>)</i>				
0	12.34 <sup>a</sup>	3.40 <sup>a</sup>	15.74 <sup>a</sup>	1.56 <sup>d</sup>
4	12.00 <sup>ab</sup>	3.30 <sup>a</sup>	15.30 <sup>ab</sup>	1.90 <sup>c</sup>
8	12.40 <sup>a</sup>	3.20 <sup>a</sup>	15.60 <sup>a</sup>	2.11 <sup>c</sup>
12	11.70 <sup>b</sup>	3.00 <sup>ab</sup>	14.70 <sup>b</sup>	2.45 <sup>b</sup>
18	11.01 <sup>c</sup>	2.80 <sup>b</sup>	13.81 <sup>c</sup>	2.99 <sup>a</sup>

Means with the same letters in each column are not significantly different (least significant difference at 5% level of probability).  
FC: field capacity

The contents of free proline and total protein increased under salinity and water stress treatments (Figs. 1 and 2). Highest contents of proline and total protein were observed under the highest level of salinity (18 dS m<sup>-1</sup>). The 12 and 18 dS m<sup>-1</sup> salinity levels showed a significant difference compared to the other levels (Fig. 1). Similar to salinity stress, water stress also caused an increase in proline and protein content (Fig. 2). Highest amounts of free proline and total protein were measured for 50 % FC, while the lowest amount was recorded for 100 % FC.

### 3.1 Antioxidant enzymes activity

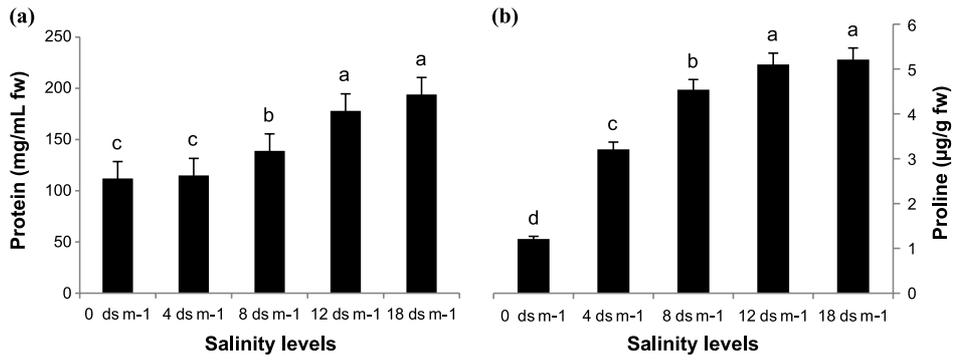
Superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), and ascorbate peroxidase (APX) activities increased with increase in salinity (Fig. 3). The rate of increase in response to salinity was highest for SOD, while lowest for APX. Control, 4, 8, and 12 dS m<sup>-1</sup> salinity levels showed no significant difference for APX, but 18 dS m<sup>-1</sup> treatment had the highest APX activity. Also, no significant differences in POD and SOD were observed between control and 4 dS m<sup>-1</sup> treatment and in CAT among the control, 4, and 8 dS m<sup>-1</sup> treatments. These results indicate the salinity threshold and its tolerance to salinity levels of about 4-8 dS m<sup>-1</sup> because these levels showed no significant difference for antioxidant enzyme activity in this experiment. Meanwhile, drought increases the activity of enzymatic antioxidant in caper plant as well (Fig. 4). Highest activity of enzymatic antioxidant was obtained in 50 % FC and the lowest in the 100 % FC.

The analysis of Na<sup>+</sup> and K<sup>+</sup> showed that salinity increased the sodium ion content of caper plant leaves, while it decreased its potassium content (Fig. 5). Lowest content of Na<sup>+</sup> was obtained in control, which showed no significant difference with 4 and 8 dS m<sup>-1</sup> salinity levels. Highest content of Na<sup>+</sup> was observed in 18 dS m<sup>-1</sup> salinity level. Conversely, lowest content of K<sup>+</sup> was observed in 12 dS m<sup>-1</sup> salinity level, while no significant differences with 4 and 8 dS m<sup>-1</sup> salinity levels occurred. The ratio of Na<sup>+</sup> / K<sup>+</sup> showed no significant difference between control, 4, and 8 dS m<sup>-1</sup> salinity levels, while this ratio was highest at 18 dS m<sup>-1</sup> level and significant different with the other levels. FC of 100 % showed the lowest content of Na<sup>+</sup> and also Na<sup>+</sup> / K<sup>+</sup> ratio, while it showed the highest content of K<sup>+</sup>. On the other side, 50 % FC had the highest Na<sup>+</sup> and Na<sup>+</sup> / K<sup>+</sup>, but the lowest K<sup>+</sup> in caper plant leaves (Fig. 5).

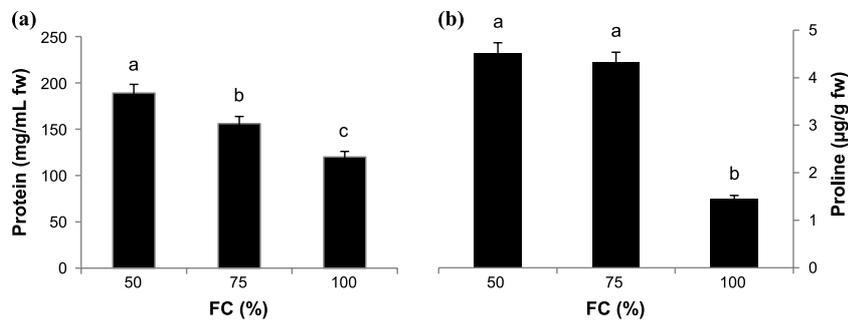
## 4 Discussion

The results of this study showed that the content of chlorophyll *a*, chlorophyll *b*, and total chlorophyll decreased under salinity stress, while, carotenoid content increased. Water stress increased the chlorophyll contents measured. Azooz *et al.*, (2011) have argued that the reduction in photosynthetic pigment contents under salinity stress is related to pigment destruction and the instability of pigment complex. This occurrence is probably related to the interference of salt ions with the chlorophyll structural component, and protein synthesis, rather than the interruption of chlorophyll (Jaleel *et al.*, 2008). Moreover, drought increased chlorophyll content of caper plant, which may be due to an increase in the concentration of chlorophyll. Similar results were obtained in sour orange (García-Sánchez *et al.*, 2002), olive (Mousavi *et al.*, 2008), and maize (Saed-Moocheshi *et al.*, 2014a) under salinity stress.

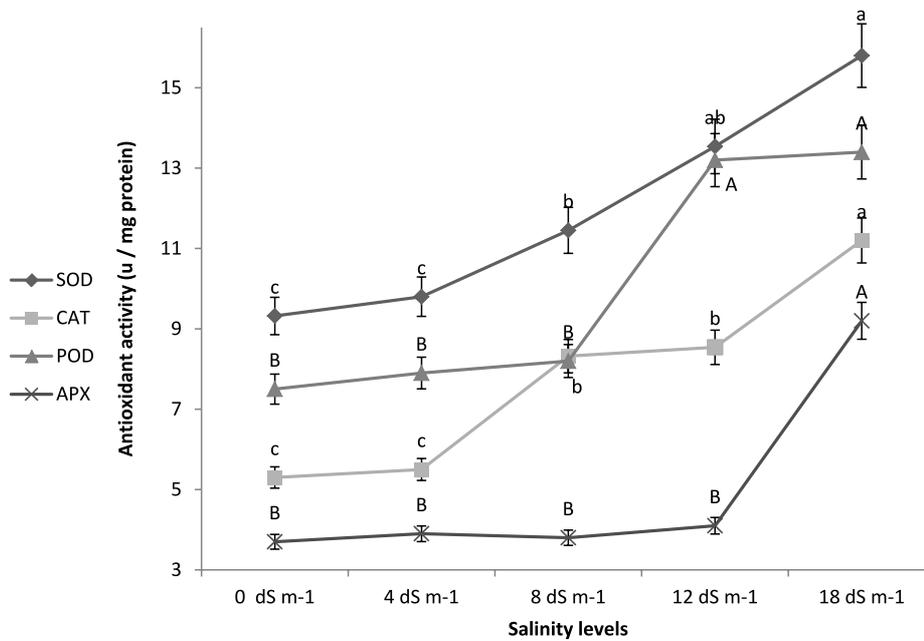
The contents of free proline and total protein were increased under both salinity and drought stress conditions. Similar results were also reported for sugar beet and wild beet (Bor *et al.*, 2003), rice (Türkan & Demiral, 2009), maize (Saed-Moocheshi *et al.*, 2014c), and broad bean (Azooz *et al.*, 2011). Ascorbate peroxidase is the key enzyme for scavenging hydrogen peroxide in chloroplast and cytosol of plant cells (Amako *et al.*, 1994). Numbers of different reports have shown an enhanced expression of APX in plants in response to different abiotic stress such as drought and salinity (Saed-Moocheshi *et al.*, 2014c). Over expression of APX in tobacco chloroplasts enhanced plant tolerance to salt and water deficit (Hebelstrup & Møller, 2015). As we have



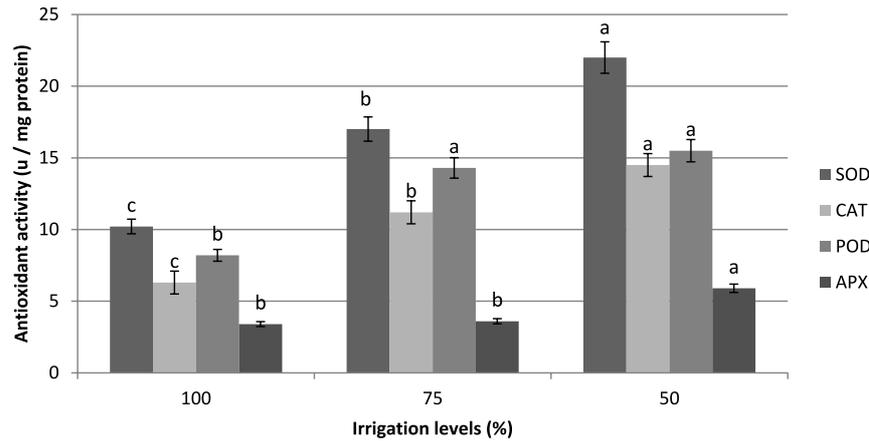
**Fig. 1:** Proline and total protein content under different salinity levels. Means with the same letter(s) are not significantly different (5% level).



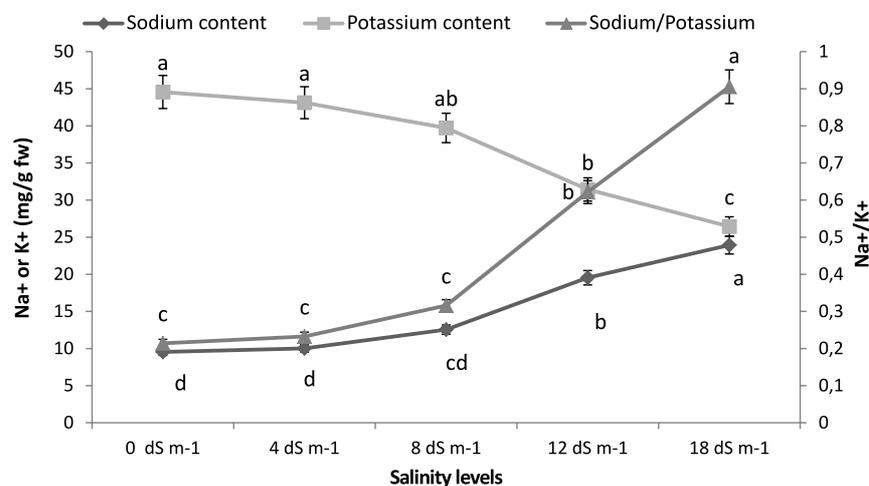
**Fig. 2:** Proline and total protein content under different FC (%). Means with the same letter are not significantly different (5% level).



**Fig. 3:** Antioxidant enzymes' activity under different salinity levels. Means with the same letter(s) are not significantly different (5% level). SOD: Superoxide dismutase, CAT: catalase, POD: peroxidase, and APX: ascorbate peroxidase.



**Fig. 4:** Antioxidant enzymes activity of caper plant under different field capacity (FC%). Means with the same letter are not significantly different (5% level). SOD: Superoxide dismutase, CAT: catalase, POD: peroxidase, and APX: ascorbate peroxidase.



**Fig. 5:** Sodium and potassium content in addition to their ration ( $\text{Na}^+ / \text{K}^+$ ) under different salinity levels. Means with the same letter(s) are not significantly different (5% level).

mentioned above, abiotic and environmental stress (such as salinity and drought) increase the amount of reactive oxygen species (ROS) which can damage other vital molecules and metabolites such as DNA, pigments, proteins, lipids (Hebelstrup & Møller, 2015). This mechanism (Antioxidant enzymes activity) can reduce ROS and in this way protect cells from further damage. The plant growth parameters showed a significant negative effect of salinity and drought stress on plant height, leaf number, leaf length, root length, shoot fresh weight, shoot dry weight, root fresh weight and root dry weight (Sadeghi & Rostami, 2016). The interaction effect of drought by salinity was only significant for plant height and shoot dry weight. The highest values for plant height, leaf number, leaf length and root length were observed in 100% field capacity. Under both stress factors while

increasing the severity of stress, the values of the traits decreased.

In the present study, the content of  $\text{Na}^+$  increased under salinity in caper plant leaves, while  $\text{K}^+$  content decreased. Salinity, which is usually caused by the effect of NaCl in the soil, causes an imbalance in the ionic equilibrium in the soil solution and thereby decreases the absorption of the mineral elements and it also decreases the content of  $\text{K}^+$  in plants (Aroca *et al.*, 2007). Higher  $\text{Na}^+$  in soil causes disequilibrium in nutrient ions in plants. The capacity of plants to absorb less  $\text{Na}^+$  and more  $\text{K}^+$  and thus maintaining a high cytosolic  $\text{K}^+ / \text{Na}^+$  ratio is likely to be one of the key determinants of plant salt tolerance. In this study, caper plant showed no significant increase in the amount of  $\text{Na}^+$  in response to salinity levels of 4 and 8  $\text{dS m}^{-1}$  and it could therefore be considered as a plant tolerant to salinity.

## 5 Conclusions

Cultivation of crops such as caper which is resilient to dry and saline conditions can minimise the production and income loss in drought periods, increase the production in areas with uncertain rainfall and on land with saline soils. The results indicated that antioxidant enzyme activity increased in response to salinity and drought in caper plant. Salinity had a negative effect on chlorophyll content of caper, while drought caused an increase in the content of chlorophyll pigments. Total protein content of caper plant under both salinity and water stress was increased.

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