DESERT

Desert
Online at http://desert.ut.ac.ir

Desert 21-1 (2016) – 49-55

Evaluating the physiological and hormonal responses of caper plant (*Capparis spinosa*) subjected to drought and salinity

H. Sadeghi*, L. Rostami

Department of Natural Resources and Environmental Engineering, College of Agriculture, Shiraz University, Shiraz, Iran

Received: 15 September 2015; Received in revised form: 13 March 2016; Accepted: 14 May 2016

Abstract

In order to investigate the effect of drought and salinity stress on caper plant (*Capparis spinosa*), a factorial experiment including two factors consisting of drought and salinity levels based on randomized complete block design (RCBD) with five blocks were carried out at College of Agriculture, Shiraz University, Shiraz, Iran. After pretreatment, seeds were germinated in petri dishes and then transported to pots, to grow out of the glasshouse under natural conditions (same as environmental condition). Measuring morphological (Plant height, number of leaves, length of greatest leaf, root length, shoot fresh weight, shoot dry weight, root fresh weight and root dry weight, and chlorophyll (pad) and also physiological traits (relative water content (RWC) and water saturated deficit (WSD) for both root and shoot, root water content (RooWC) and leaf water content (LWC) showed that salinity and drought stresses as well as ABA concentration had negative effects on the plant. ABA content was significantly higher in salt-treated plants than in drought treated ones. The injury effect of salt stress was lower than drought. The results also indicated that the changes in response to the two stresses were low and so that this plant could be a suitable candidate for sowing and using in arid and semi-arid regions and also in saline condition.

Keywords: Chlorophyll; ABA; Arid regions; Root dry weight; Drought

1. Introduction

Adoptable and multipurpose plants can provide a valuable opportunity to enhance greenery and prevention of soil erosion in harsh climatic area. Due to the harsh climatic conditions in arid and semi-arid area, the erosion of the soil is a great problem. Caper Plant (*Capparis spinosa* L.) a multipurpose plant requires a semiarid or arid climate. Caper plant is a shrub native to the Mediterranean regions and tropics that grows wild on walls or in rocky coastal areas (Legua *et al.*, 2013). This plant has a deep root system and is resistant to drought condition. It can tolerate the temperatures exceeding 40 °C (Suleiman *et al.*, 2012). Because of its vegetative canopy, caper plant covers soil surfaces making soil water to be reserved (Saifi *et al.*, 2011). It grows in a wide range of soils condition and different situation, and could be used as culinary, medicinal purposes, and ornamental shrub. Using its deep root system and high resistance in harsh condition, it could be used for the prevention of soil erosion in arid and semi-arid areas (Musallam *et al.*, 2012). Caper plant as a shrub well adapted to water deficit conditions having less than 200 mm annual rainfall, but for commercial uses and plant production, this crop cannot be grown under such conditions without any irrigation (Legua *et al.*, 2013).

Plants have developed different mechanisms to adapt to drought and salinity stress, involving complex physiological and biochemical changes. In the process of stress adaptation, hormones, especially abscisic acid (ABA), play important roles (Amjad *et al.*, 2013). It has been proposed that ABA acts as a mediator and the major internal signal in plant response to abiotic stresses (Javid *et al.*, 2011). ABA concentration increases proportionally with salt stress related to leaf water potential suggesting that increased ABA concentration is due to water deficit created by salts rather than a salt specific effect (Lovelli *et al.*, 2012). This higher ABA concentration reduces the loss of water as transpiration by the closure of stomata under stressful conditions (Zhang *et al.*, 2014; Hariadi *et*

* Corresponding author. Tel.: +98 713 2287159,
Fax: +98 713 2287159.
E-mail address: Sadeghih@shirazu.ac.ir

al., 2011). Due to the recent severe drought in Iran and most arid and semi arid regions in the world, farmers have attempted to growth drought and saline resistant plant (such as caper) instead of plant with high water requirements. Caper plant is native in the Mediterranean landscape, from Portugal to Egypt and today is cultivated in southern parts of Iran, some regions of Italy as well as Morocco and Jordan as popular crop. There is a high interest for caper cultivation for several reasons among them, are the possible use of marginal lands, adapted to dry areas receiving less than 200 mm annual rainfall, low initial cost, higher profit margins as compared to other local crops, synergisms of crops with tourism activities and alternative use of capers in cosmetics and pharmacology but very little is known about its responses stress-related morpho-physiological and hormonal characteristics. This research aimed to evaluate changes in some morphological, physiological and hormonal properties of caper plant in response to drought and salinity stresses to measure tolerance and adoptability of this plant under these conditions.

2. Materials and Methods

2.1. Experimental procedure

The seeds of the caper plant were gathered from Farashband belonging to Fars province in Iran (Fig. 1), Voucher specimen (No. 32658) was deposited at the Herbarium of Fars Research Center for Agriculture, Shiraz, Iran. The seeds were separated, washed with deionized water, and sterilized with ethanol, and after that, they were placed in petri dishes consisting of filter paper and 5 mL polyethylene glycol 6000. For a period of four weeks, the petri dishes were kept in a germinator having a temperature of −4°C in order to cold stratification on seed dormancy. After germination, the seeds were transported to pots filled with soil (**Error! Reference source not found.**). Ten germinated seeds were sown in each pot. The experiment was carried out as a factorial arranged randomized complete block design with five replications.Total numbers of pots were 75 and the pots were kept out of the glasshouse at the natural temperature of the region in spring (2014). Drought stress had three levels of 100, 75, and 50 of FC (Field Capacity). 10 days after sowing salinity and drought treatments were started. Drought treatment levels were applied based on the weighting method by daily weighting of pots. Five levels of salinity containing 0, 4, 8, 12, and 18 ds m^{-1} were also used. For salinity treatments, sodium chloride and calcium chloride with the same ratios were applied. Forty days after sowing, the leaf samples were cut from the plant and were frozen for transporting to the lab for measuring traits.

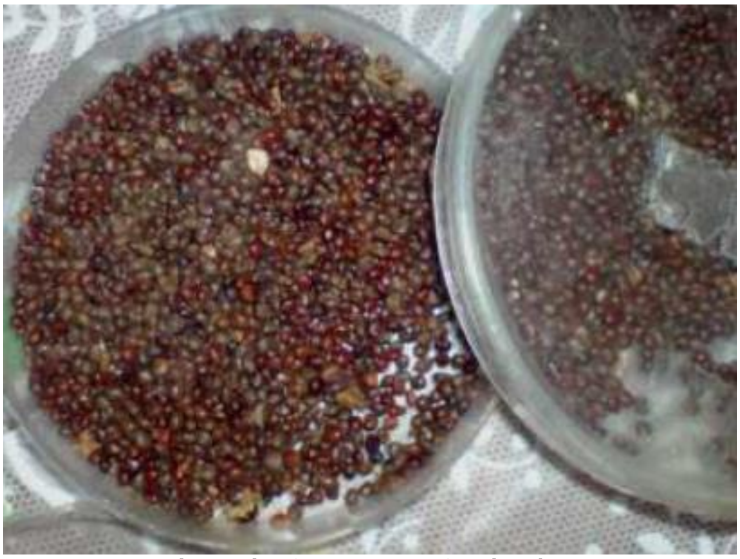

Fig. 1. Used caper plant (*Capparis spinosa*) seeds in the experiment

Table 1. Physical and chemical properties of soil

Clay(%)	Silt(%)	Sand(%)	PH	OC(%)	soil texture
25.3	67.1	7.6	7.5	0.78	silty-loam
PWP	FC	Total N (%)	K(mg kg^{-1})	P(mg kg^{-1})	EC(dSm^{-1})
0.09	0.19	0.08	462	16.3	0.55

OC: Organic Carbon; PWP Permanent Wilting Point in percent by volume; FC: Field Capacity in percent by volume

2.2. Measuring the traits

Morphological traits consist of plant height, number of leaves, length of greatest leaf; root length, shoot fresh weight, shoot dry weight, root fresh weight and root dry weight were measured in this study. Greenish index (Spad chlorophyll) was also measured as an index for chlorophyll content.

Physiological traits containing relative water content (RWC) and water saturated deficit (WSD) for both root and shoot and, root water content (RooWC) and leaf water content (LWC) were measured using following equations.

RWC= FW-DW / TW-DW (Barrs and Kozlowski, 1968)

WSD =TW-FW / TW-DW(Barrs and Kozlowski, 1968)

LWC = FW-DW / FW (Claeke and McCaig, 1982)

Where FW, DW and TW are fresh weight, dry weight and turgid weight, respectively. Leaves were sampled for ABA determination by using an aliquot of 250 mg of leave tissue was ground into powder with liquid nitrogen with amortar and pestle, and put in a tube. 2.5 mL extraction solvent (2-propanol/H2O/HCl 37%; 2:1:0.002, v/v/v) was added to each tube. The tubes were shaken at a speed of 100 rpm for 30 min at 4 ◦C. 2.5 mL of dichloromethane was added to each tube, and then the samples were shaken for 30 min at 4 ◦C and centrifuged at 13,000×g for 5 min. After centrifugation, two phases were formed, with plant debris between the two layers, so 1.0 mL of the solvent from the lower phase was transferred using a Pasteur pipette into a screw-cap vial, and the solvent mixture was concentrated using an
) showed significant effect of salinity and drought stress on plant height, leaf number, leaf length, root length, shoot fresh weight, shoot dry weight, root fresh weight, root dry weight, and chlorophyll index (spad). The interaction effect of drought by salinity was just significant for plant height and shoot dry weight. Table 3 shows mean comparison for different levels of drought and salinity stress. The highest value for plant height, leaf number, leaf length, chlorophyll (spad), and root length were

evaporator with nitrogen flow. Finally, the samples were re-dissolved in 0.1 mL methanol and stored at −20 ◦C before quantitative analysis. The quantitative determinations of abscisic acid (ABA) were carried out by a competitive enzyme linked immunosorbent assay (ELISA) using the Phytodetek® ABA Test Kit (Agdia Biofords, Evry, France) 40 days after sowing as described by Lovelli *et al.*, (2012)

2.3. Statistical analysis

Univariate normality test was carried out on residuals of the ANOVA model for all measured traits for testing hypothesis of normal distribution of the data using SAS9.3 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

3.1. Morphological traits

The results of analysis of variance (

observed in 100% field capacity related to drought stress and control related to salinity stress. In both stresses by increasing the severity of stress, the values of the traits were decreased. Chlorophyll (spad) of 100 and 75 % field capacity were not different. Root length of salinity levels containing 4, 8, 12, and 18 ds m^{-1} were different. Difference between 12 and 18 ds m^{-1} related to leaf number, leaf length, and chlorophyll (spad) were not significant.

Table 2. Analysis of variance (ANOVA) for morphological measured traits

Source	Degree of freedom	Mean squares								
		Plant height (cm)	leaf number	leaf length (cm)	Root length (cm)	Shoot fresh weight (gr)	Shoot dry weight (gr)	Root fresh weight (gr)	root dry weight (gr)	Chlorophyll (Spad)
Drought	2	2469.1**	2333.85**	19.98**	404.81**	0.37**	0.77**	0.9**	0.39**	1402.51**
Salinity	4	151.26**	109.15**	1.16**	121.35**	0.21**	0.27**	0.74**	0.26**	880.19**
Drought* Salinity	8	15.84**	6.1ns	0.08ns	7.82ns	0.02ns	0.01**	0.09ns	0.06ns	24.83ns
Error	60	3.1	5.15	0.11	16.09	0.05	0.003	0.08	0.06	251.79
Coefficient of variation		9.09	14.82	16.63	21.56	17.39	12.34	13.71	19.46	23.66

**, *, and ns are representation of significant in 1% level, significant in 5% level, and not significant, respectively

Table 3. Means comparison for some measured traits related to drought and salinity stress

	Plant height (cm)		leaf number		leaf length (cm)		Chlorophyll (spad)		Root length (cm)	
Drought (%FC)										
100	28.7	A	25.08	A	2.82	A	34.49	A	11.36	C
75	20.5	B	15.08	B	2.08	B	33.27	A	14.60	B
50	8.92	C	5.76	C	1.04	C	20.95	B	19.36	A
Salinity(ds/m)										
0	23.67	A	19.07	A	2.27	A	40.87	A	10.33	B
4	20.8	B	16.73	B	2.17	A	32.45	AB	15.03	A
8	19.567	B	15.07	C	2.08	A	29.15	B	15.67	A
12	17.5	C	13.4	D	1.75	B	24.09	B	17.07	A
18	15.33	D	12.27	D	1.62	B	21.3	B	17.43	A

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

Mean comparison for shoot fresh and dry weight, root fresh and dry weight related to drought stress are presented in Table 4. The highest values for shoot fresh weight and shoot dry weight were observed in 100% FC and their lowest values were observed in 50%FC, while the lowest values for root fresh and dry weight were obtained in 100% FC and their highest values were obtained in 50% FC. Between 75 and 50% FC related to shoot fresh weight and root dry weight significant difference were not observed. Root fresh weight showed no significant difference between 100 and 75% FC. Effects of salinity levels on shoot fresh and dry weight showed that salinity decreased these traits (Figure 2). For shoot fresh weight, no significant differences were observed among 0, 4, 8, and 12 ds m^{-1}, but 18 ds m^{-1} showed a significant difference with other levels. The difference between 12 and 18 ds m^{-1} were also not significant. Figure 3 shows root fresh and dry weight mean comparison for different salinity levels. Salinity decreased the weight of fresh and dry root. Between 0, 4, and 8 ds m^{-1} related to both root fresh and dry weight significant difference were not observed.

Table 4. mean comparison for some morphological and physiological measured traits related to drought stress

%FC	Shoot fresh weight(gr)		Shoot dry weight(gr)		Root fresh weight(gr)		Root dry weight(gr)		Shoot RWC		leaf water content		WSD-Shoot	
100	1.42	A	0.653	A	1.857	B	1.154	B	0.623	A	0.738	A	0.506	A
75	1.283	B	0.475	B	1.958	B	1.298	A	0.595	A	0.624	B	0.404	B
50	1.176	B	0.303	C	2.224	A	1.404	A	0.493	B	0.537	C	0.377	B

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

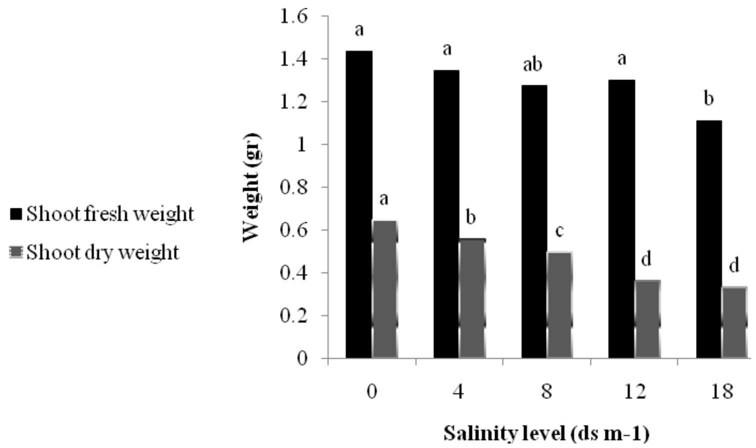

Fig. 2. Effect of salinity on shoot fresh/dry weight. Means with the same letters are not significantly different (least significant difference at 5% level of probability)

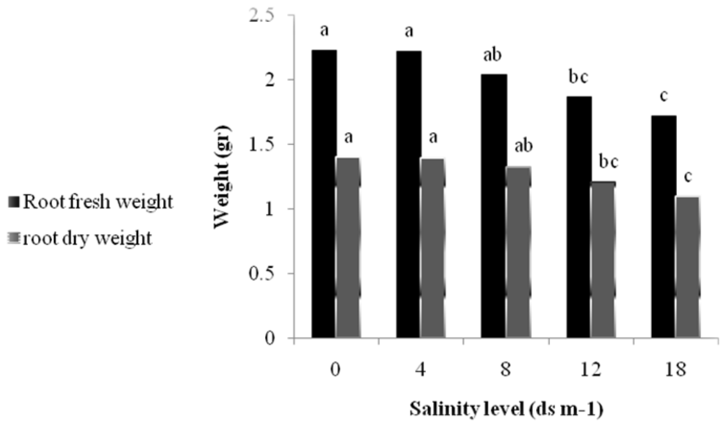

Fig. 3. Effect of salinity on root fresh/dry weight. Means with the same letters are not significantly different (least significant difference at 5% level of probability)

Results of ANOVA for physiological traits consist of root and shoot relative water content, root and shoot water saturated deficit, and root and leaf water content are presented in Table 5. Effect of drought and salinity were significant for shoot RWC, leaf water content, and shoot WSD, while they were not significant for root RWC, root water content, and root WSD. The interaction effect of drought by salinity was not significant for all physiological traits (Table 5). The highest amounts of shoot RWC, leaf water content, and shoot WSD were obtained in 100% FC, while the lowest amounts were obtained in 50% FC (Table 4). Levels of 100 and 75% FC showed no significant difference for related to shoot RWC; on the other hand, shoot WSD of 75 and 50% FC was not significant. Figures 5 shows changes of shoot RWC, leaf water content, and shoot WSD in response to salinity levels. All of these mentioned traits decreased in line with increase in severity of salinity. For shoot RWC salinity levels of 0, 4, and 8; for leaf water content salinity levels of 0, and 4; and finally for shoot WSD salinity levels of 0, 4, and 8 showed no significant difference one another.

Table 5. Analysis of variance (ANOVA) for physiological measured traits

Source	Degree of freedom	Mean squares					
		Shoot RWC	leaf water content	Shoot-WSD	Root- RWC	Root water content	Root-WSD
Drought	2	0.12**	0.26**	0.12**	0.032ns	0.38ns	0.026ns
Salinity	4	0.09**	0.09**	0.09**	0.029ns	0.43ns	0.025ns
Drought*Salinity	8	0.02ns	0.01ns	0.02ns	0.042ns	0.33ns	0.039ns
Error	60	0.03	0.01	0.03	0.022	0.39	0.019
Coefficient of variation		22.88	12.82	23.37	24.66	21.03	24.81

**, *, and ns are representation of significant in 1% level, significant in 5% level, and not significant, respectively

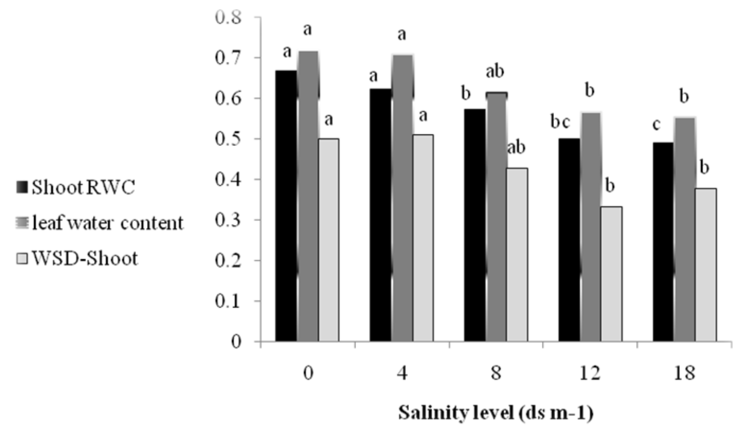

Fig. 4. Effect of salinity on relative water content (RWC), leaf water content, and water saturated deficit of shoot. Means with the same letters are not significantly different (LSD at 5% level of probability)

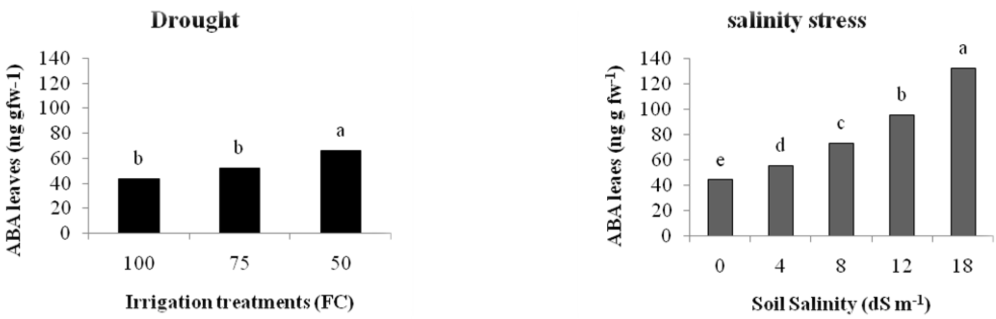

Fig. 5. Leaves ABA concentration of *C. spinosa* in response to increasing salinity and drought level
Means with the same letters are not significantly different (LSD at 5% level of probability)

Drought and salinity stress both induced ABA accumulation in *C. spinosa* (Figure 5). ABA concentrations increased with increasing drought and salinity level, but it was always higher in salt stress-treated plants than in plants exposed to drought (Figure 5). The difference was significant for salt stress-treated plants and drought treated plants at 50% FC compared to control. The highest quantity of ABA was found at 18 dS m^{-1} of soil salinity.

4. Discussion

In this research the injury effect of drought stress on capper plant was greater than salinity stress because rate of decreasing in morphological traits were higher under drought stress. These results indicate that capper plant can tolerate salinity more than drought. On the other hand, the difference between 100 and 75% FC for morphological traits were significant and, between 75 and 50% FC were not significant. These results show that moderate drought stress have a little negative effect on morphological response of capper plant. Salinity levels of 0, 4, and 8 ds m^{-1} have little effects on most of the morphological traits. Physiological responses of plants in different situations constitute stress are varied (Pessarakli, 2014; Saed-Moocheshi *et al*., 2014). All metabolic activities of plants are affected regularly by environmental changes (Auge *et al.*, 2015). Drought stress when accompanied with very high constraint or fluctuations results in injury, abnormal physiological changes, etc. (Suzuki *et al.*, 2014). The changed physiological condition tends to change the equilibrium and results in stress and strain (physical and chemical) (Nemali *et al.*, 2014). Drought and salinity stresses decreased the amount of ch *a* and the lowest ch *a* was recorded for sever salinity stress (Pirasteh Anosheh *et al*., 2011).

ABA was indeed accumulates at higher amounts in salt-treated plants than in drought-treated ones. The same results have been found by Ben Hassine and Lutts (2010) who reported that salt treated *Atriplex halimus* contain more ABA concentration than in drought treated ones. In addition to hormonal compounds, the injury effect of drought stress was higher under drought stress with lower ABA content. Concerning to abscisic acid, many reports have shown its involvement in physiological and biochemical processes related to salinity tolerance of plants (Lovelli *et al*., 2012; Sadeghi and Robati, 2015). Our results suggested that ABA plays important roles in reducing the injury effect of salt stress in *C. spinosa*. Under salt stress, by increasing the salinity level, ABA levels increased dramatically (Figure 5), suggesting that ABA acts as a signal in caper salt response. ABA controls many stress adaptation responses, including stomatal closure, activation of genes involved in osmotic adjustment, ion compartimentation, regulation of shoot versus root growth and modifications of root hydraulic conductivity properties (Zhang *et al.*, 2006; Antoni *et al.*, 2011; Javid *et al*., 2011; Hu *et al*., 2006; Jiang *et al*., 2002).

5. Conclusion

Caper Plant is a multipurpose plant adapted to semiarid or arid climate. Physiological properties of caper plant root showed no significant differences for salinity and drought stress levels. Also, physiological response of shoot showed a bit changes in response to salinity and drought stress. All of these results are indicated that caper plant is a suitable plant for to be used in arid and semi-arid and also in saline condition to obtain different benefit from this plant such as greenery benefits and using its fruit for adorable foods.

Refferences

Amjad M., J. Akhtar, M. Anwar-ul-Haq, A. Yang, S.S. Akhtar, S.E. Jacobsen, 2014. Integrating role of ethylene and ABA in tomato plants adaptation to salt stress. Scientia Horticulture, 172; 109–116.

Antoni, R., L. Rodriguez, M. Gonzalez-Guzman, G.A. Pizzio, P.L. Rodriguez, 2011. News on ABA transport, protein degradation, and ABFs/WRKYs in ABA signaling. Current Opinion. Plant Biology, 14; 1–7.

Auge, RM., H.D. Toler, A.M. Saxton, 2015. *Arbusculr mycorrhizal* symbiosis alter stomatal conductance of host plants more under drought than under amply watered conditions: a meta-analysis. Mycorrhiza, 25; 13-24.

Barrs, H., T. Kozlowski, 1968. Determination of water deficit in plant tissues. Water Deficit and Plant Growth, 1; 235-268.

Ben Hassine, A., S. Lutts, 2010. Differential responses of saltbush *Atriplex halimus* L. exposed to salinity and water stress in relation to senescing hormones abscisic acid and ethylene. Journal of Plant Physiology, 167; 1448–1456.

Claeke, J.M., T.N. McCaig, 1982. Excised leaf water retention capability as an indicator of drought resistance of *Triticum* genotypes. Canadian Journal plant Science, 62; 571-578.

Hariadi, Y., K. Marandon, Y. Tian, S.E. Jacobsen, S. Shabala, 2011. Ionic and osmotic relations in quinoa (*Chenopodium quinoa* Willd.) plants grown at various salinity levels. J. Experimental Botany, 62; 185–193.

Hu, X.L., M.Y. Jiang, J.H. Zhang, A.Y. Zhang, F. Lin, M.P. Tan, 2006. Calcium calmodulin is required for abscisic acid-induced antioxidant defense and functions both upstream and downstream of H2O2 production in leaves of maize (*Zea mays*) plants. New Phytolgist, 173; 27-38.

Javid, M.G., A. Sorooshzadeh, F. Moradi, S. Modarres, S. Sanavy, I. Allahdadi, 2011. The role of phytohormones in alleviating salt stress in crop plants. Australian Journal of Crop Science; 5; 726–734.

Jiang, M., J. Zhang, 2002. Role of abscisic acid in water stress-induced antioxidant defense in leaves of maize seedlings. Free Radical Research, 36; 1001–1015.

Legua P., J. Martinez Melgarejo, R. Martinez, F. Hernandez, 2013. Phonological growth stages of caper plant (*Capparis spinosa* L.) according to the Biologische Bundesanstalt, Bundessortenamt and chemical scale. Annals of Applied Biology, 163; 135-141.

Lovelli S, A,Scopa Perniola, T, Di Tommaso, A, Sofo, 2012. Abscisic acid root and leaf concentration in relation to biomass partitioning in salinized tomato plants. Journal of Plant Physiology, 169; 226– 233.

Mussalam, I., M. Duwayri, R. Shibi, F. Alali, 2012. Investigation of rutin content in different plant parts of wild caper (*Capparis spinosa* L.) populations from Jordan. Research Journal of Medicinal Plants, 6; 27-36.

Nemali, K.S., C. Bonin, F.G. Dohleman, M. Stephans, W.R. Reeves, D.E. Nelson, P. Castiglioni, J.E. WhitselSammons R.A. Silady, 2014. Physiological responses related to increased grain yield under drought in the first biotechnology –derived drought tolerant maize. Plant, cell & Environment, 38; 1866-1880.

Pessarakli, M., 2014. Handbook of plant and crop physiology. CRC Press.

Pirasteh Anosheh, H., H. Sadeghi, Y. Emam, 2011. Chemical Priming with Urea and KNO3 Enhances Maize Hybrids (*Zea mays* L.) Seed Viability under Abiotic Stress. Journal of Crop Science and Biotechnology, 14; 289–295.

Sadeghi, H., Z. Robati, 2015. Response of *Cichorium intybus* L. to eight seed priming methods under osmotic stress conditions. Biocatalysis and Agricultural Biotechnology, 4; 443-448

Saed-Moocheshi, A., A. Shekoofa, H. Sadeghi, M. Pessarakli, 2014. Drought and salt stress mitigation by seed priming with Kno3 and urea in various maize hybrids: an experimental approach based on enhancing antioxidant responses. Journal of Plant Nutrition, 37; 674-689.

Saifi, N., J, Ibijbijen, D. Echgadda, 2011. Genetic diversity of caper plant (*Capparis* spp) from north Morocco. Journal of food Agriculture Environment, 9; 14-20.

Suleiman, M.K., N.R. Bhat, S. Jacob, R.R. Thomas, 2012. Effect of rooting hormones (IBA and NAA) on rooting of Semi hard wood cuttings of *Capparis spinosa.* Journal of Agriculture and Biodiversity, 1; 135-139.

Suzuki, N., R.M. Rivero Shulaev, E. Blumwald, R. Mittler, 2014. Abiotic and biotic stress combinations. New Phytologist, 203; 32-43.

Zhang, J., W. Jia, J. Yang, A.M. Ismail, 2006. Role of ABA integrating plant responses to drought and salt stresses. Field Crop Research, 97; 11–9.

Zhang, L.X., J.H. Lai, Z.S. Liang, M. Ashraf, 2014. Interactive effects of sudden and gradual drought stress and foliar-applied Glycinbetaine on growth, water relations, osmolyte accumulation and antioxidant defense system in two maize cultivars differing in drought tolerance. Journal of Agronomy and Crop Science, 200; 425-433.