



RESEARCH PAPER

OPEN ACCESS

Influences of ascorbic acid and gibberellin on alleviation of salt stress in summer savory (*Satureja hortensis* L.)

Elham Nikee¹, Alireza Pazoki^{2*}, Hossein Zahedi¹

¹Department of Agronomy and Plant Breeding, Eslamshahr Branch, Islamic Azad University, Tehran, Iran

²Department of Agronomy, Yadegar-e-Imam Khomeini (RAH) Branch, Islamic Azad University, Tehran, Iran

Key words: Salinity, ascorbic acid, gibberellin, savory, growth.

<http://dx.doi.org/10.12692/ijb/5.4.245-255>

Article published on August 30, 2014

Abstract

Soil salinity is one of the most important constraints that limit crop production in arid and semi arid regions. This research was carried out in order to investigate the effects of exogenous ascorbic acid (0 and 4 mM), gibberellin (0 and 4 mM) and different salinity levels (0, 25, 50 and 75 mM NaCl) on some growth parameters and physiological attributes in summer savory (*Satureja hortensis* L.). The results showed that growth parameters such as root weight, leaf weight, shoot weight as well as leaf area decreased due to salinity stress. In addition, salinity stress decreased membrane stability and chlorophyll *a* content. On the other hand, application of 4 mM ascorbic acid considerably increased growth parameters, relative water content, membrane stability and chlorophyll *a* content. Similar results were obtained when 4 mM gibberellin was applied. Under conditions of salinity stress 4 mM ascorbic acid and 4 mM gibberellin could increase transpiration rate, relative water content, chlorophyll *b*, total chlorophyll and xanthophyll content. In general, it was concluded that synergistic interaction between ascorbic and gibberellin could alleviate the adverse effects of salinity on summer savory plants.

* Corresponding Author: Alireza Pazoki ✉ pazoki_agri@yahoo.com

Introduction

Summer savory (*Satureja hortensis* L.) is a genus of aromatic plants of the family Lamiaceae. There are about 30 different species called savories, which summer savory and winter savory are the most important in cultivation. *Satureja* species are native to warm temperate regions and may be annual or perennial. They are low-growing herbs and sub-shrubs, reaching heights of 15–50 cm. The leaves are 1 to 3 cm long, with flowers forming in whorls on the stem, white to pale pink-violet. The main constituents of the essential oil of savory are phenols, carvacrol and thymol, as well as p-cymene, β -caryophyllene, linalool and other terpenoids (Sefidkon *et al.*, 2006). The essential oil and oleoresin are used in food industries. In addition, the essential oil of savory has been used in the perfume industries, either alone or with other essential oils (Sefidkon *et al.* 2004). As a medicinal plant, savory has been traditionally used as a stimulant, stomachic, carminative, expectorant and aphrodisiac. The essential oil has demonstrated antimicrobial and anti-diarrheic activities because of phenols in the oil (Sefidkon and Jamzad, 2005).

Salinity is one major environmental determinant of plant growth and productivity. Salinization is rapidly increasing on a global scale and effect more than 10% of arable land, which results in a decline of the average yields of major crops greater than 50% (Wang *et al.*, 2009). NaCl is the predominant salt causing salinization, and it is not surprising that plants have evolved mechanisms to alleviate its adverse effects (Munns and Tester, 2008). An important aspect of salinity tolerance studies is production of reactive oxygen species under stress conditions which are considered as signal molecules at non-toxic concentrations but at toxic concentrations they are also capable of injuring cells (Meloni *et al.*, 2003). Increased levels of enzymatic and non-enzymatic antioxidants are one of the strategies adopted by the plants to overcome damage imposed by reactive oxygen species. There are many studies indicating the positive correlation between levels of antioxidants and plant stress tolerance (Munir and Aftab, 2009;

Munir and Aftab, 2011). Non-enzymatic factors such as alpha-tocopherol (vitamin E), ascorbic acid (vitamin C) and carotenoids (Sairam *et al.*, 2002), also play an important role in enhancing salt stress tolerance of plants. Ascorbic acid is an important factor involved in biological defence mechanisms (Noctor and Foyer, 1998). Many workers have reported that exogenous application of ascorbic acid and other biomolecules such as sorbitol, mannitol, auxin and gibberellic acid is a 'short-cut method' for increasing the stress tolerance in different plants (Ashraf *et al.*, 2003; Munir and Aftab, 2009). Ascorbic acid has a great potential in ameliorating and modifying the salt stress-induced changes in plants (Hamada, 1998). Generally, its concentration is higher in leaves than that in other plant parts and it is 5–10 times higher than that of glutathione (Smirnoff, 2000a). The role of ascorbic acid as an antioxidant has been shown by Muller-Moule *et al.* (2004). Thus, high endogenous ascorbic acid in plants is necessary to counteract oxidative stress in addition to regulating other processes of plant metabolism. Endogenous ascorbic acid can be increased by exogenous application of ascorbic acid through the rooting medium as a foliar spray or as seed priming. Despite its role in scavenging reactive oxygen species, ascorbic acid is also involved in regulating photosynthetic capacity by controlling stomatal movement (Chen and Gallie, 2004). Ascorbate is also an important co-factor of some enzymes or protein complexes that are involved in the regulation of photosynthesis (Davey *et al.*, 2000). In general, effects of ascorbic acid in mitigating the adverse effects of salt stress have been ascribed to activation of some of the enzymatic reactions (Kefeli, 1981). Furthermore, such positive effects of ascorbic acid in overcoming the adverse effects of salt stress were attributed to the stabilization and protection of photosynthetic pigments and the photosynthetic apparatus from oxidative damage (Hamada, 1998).

On the other hand, attempts are being made to ameliorate salt stress by using phytohormones (Rao *et al.*, 2002). Gibberellins are a class of phytohormones that control many aspects of plant

growth and development, including seed germination, leaf expansion, stem elongation, flower initiation and development and fruit development (Li *et al.*, 2010). Previous studies have suggested the possible involvement of gibberellin in stress adaptation in some plants (Achard *et al.*, 2006; Rodriguez *et al.*, 2006; Magome *et al.*, 2008; Maggio *et al.*, 2010). Reports are available that the external application of gibberellin can alleviate deleterious effects of salinity. Ashraf *et al.* (2002) showed that gibberellin application increased the nutrient uptake, dry weights, plant height, leaf area and yield of wheat under saline conditions. There is also evidence that gibberellin can significantly relieve NaCl-induced growth inhibition in rice (Wen *et al.*, 2010).

Previous studies revealed that supplying low levels of ascorbic acid and gibberellin could alleviate the detrimental effects of NaCl in many plants species. However, few studies have focused on *Satureja* species. Therefore, the major objective of this study was to investigate the effects of ascorbic acid and gibberellin on the number of physiological aspects of summer savory under saline conditions, and to determine how ascorbic acid and gibberellin can ameliorate the adverse effects of salt stress on this plant.

Materials and methods

Plant material and growth conditions

The pot experiment was carried out in a greenhouse located in Pakdasht region, Varamin, Tehran, Iran in 2013. The savory seeds were surface sterilized for 5 min in sodium hypochlorite solution and then in 96% ethanol for 30 s and then rinsed with distilled water. Twenty sterilized seeds were sown in plastic pots (30cm height and 25cm diameter) filled with sandy loam soil (soil properties are given in Table 1) and placed in greenhouse adjusted at 28/20°C day/night temperature cycles, relative humidity 40%, 16/8 h day/night photoperiod and complementary light intensity 250 $\mu\text{M m}^{-2} \text{s}^{-1}$. Watering was performed immediately after seed sowing with tap water and repeated every other day until salt stress induction.

Experimental design and treatments

The experimental design consisted of a sixteen treatments, arranged in factorial based on completely randomized design with four replications, giving a total of 64 pots. Five-week old savory plants were sprayed with ascorbic acid (0 and 4 mM) and gibberellin (2 and 4 mM) before inducing salinity stress with different concentrations of saline water (0 mM, 25mM, 50mM and 75mM). Control plants were sprayed with distilled water. Saline water was delivered to plants twice a week and last for three weeks.

Data collection

Nine weeks after germination, stomatal resistance and transpiration was measured using Li-6400, Li-Cor, Lincoln, USA. Afterwards, uniform and fully expanded leaf samples were collected and frozen in liquid nitrogen for pigments analysis and membrane stability assay. At this time, fresh leaf samples were taken to measure leaf area and leaf relative water content. In addition, ten plants from each pot were harvested in order to measure leaf weight, root weight and above ground biomass weight. For this purpose, fresh plant materials were weighted and then oven dried at 75°C for 72 h for measuring dry weights.

Chlorophyll was extracted in 80% acetone from the leaf samples according to the method of Arnon (1949). Extracts were filtrated and content of total chlorophyll was determined by spectrophotometry at 645 and 663 nm, respectively. The content of chlorophyll *a*, *b* and total was expressed as $\mu\text{g ml}^{-1}$ (Arnon, 1949).

Carotene and xanthophyll contents were estimated according to the method of Mukherji and Biswas (1979) and data were expressed in terms of optical density $\mu\text{g ml}^{-1}$.

Relative water content was measured in each plot. Leaf discs were taken and weighted (fresh weigh, FW). The discs were then placed in distilled water for 5 h at 25°C and then their saturated weights (SW) were measured. The discs were then dried in oven at

70 °C for 24 h to calculate dry weight (DW). Relative water contents were calculated by following formula:

$$RWC = \frac{(FW - DW)}{SW - DW}$$

Membrane stability was measured as described by Lutts *et al.* (2004) with a few modifications. Plant material (0.3 g) was washed with deionised water, placed in tubes with 15 ml of deionised water and incubated for 2 h at 25°C. Subsequently, electrical conductivity of the solution (L_1) was determined. Samples were then autoclaved at 120°C for 20 min and the final conductivity (L_2) was measured after equilibration at 25°C. Membrane stability was defined as follows: Membrane stability (%) = (L_1/L_2) × 100.

Data analysis

The results were submitted to statistical analysis using the software SAS System for Windows 9.1. The analysis of variance (ANOVA) was carried out, and based on the level of significance in the F test (p

<0.05). Mean values were compared using Duncan's Multiple Range Test.

Results and discussion

Analysis of variance indicated that salinity stress and ascorbic acid foliar application had significant effect on all studied traits (Table 2). Similarly, all savory traits except for membrane stability were affected by gibberellin foliar application (Table 2). Interaction between salinity and ascorbic acid was significant on transpiration rate, chlorophyll *b*, total chlorophyll and xanthophyll content (Table 2). In addition, transpiration rate, relative water content, chlorophyll *b*, total chlorophyll and xanthophyll content were affected by salinity and gibberellin interaction (Table 2). Interaction between gibberellin and ascorbic acid was significant on shoot dry weight, chlorophyll *b* and xanthophyll content (Table 2). Among studied traits, stomatal conductance and carotene content affected by triple interaction of salinity, ascorbic acid and gibberellin (Table 2).

Table 1. Chemical and physical soil properties at depth of 30 cm.

pH	EC dS m ⁻¹	Organic carbon (%)	Clay (%)	Silt (%)	Sand (%)	Texture	N (%)	P (mg kg ⁻¹)	K (mg kg ⁻¹)	Soluble Na (mg l ⁻¹)
7.8	35.8	4.2	13	20	67	Sandy loam	0.4	511	5905	2809

Table 2. Analysis of variance on some savory traits affected by salinity, ascorbic acid and gibberellin.

S.O.V	df	Root fresh weight	Root dry weight	Leaf fresh weight	Leaf dry weight	Shoot fresh weight	Shoot dry weight	Leaf area	Stomatal resistance	Transpiration	Relative water content	Membrane stability	Chlorophyll <i>a</i>	Chlorophyll <i>b</i>	Total chlorophyll	Carotene	Xanthophyll
S	3	**	**	**	**	**	**	**	**	**	**	*	**	**	**	**	**
A	1	**	**	**	**	**	**	**	**	**	**	*	**	**	**	**	**
G	1	**	**	**	**	**	**	**	**	**	**	ns	**	**	**	**	**
S×A	3	ns	ns	ns	ns	ns	ns	ns	*	*	ns	ns	ns	**	*	ns	*
S×G	3	ns	ns	ns	ns	ns	ns	ns	**	**	*	ns	ns	*	*	**	**
G×A	1	ns	ns	ns	ns	ns	*	ns	**	ns	ns	ns	ns	*	ns	ns	*
S×A×G	3	ns	ns	ns	ns	ns	ns	ns	**	ns	ns	ns	ns	ns	ns	*	ns
Error	48	0.67	0.0003	0.006	0.0004	0.09	0.0007	45.96	48966.86	0.3	15.20	175.12	0.02	0.02	0.05	0.02	0.02
C.V (%)		9.63	22.52	16.19	17.96	17.05	12.22	17.56	2.71	13.48	4.82	28.77	5.88	9.15	6.14	9.64	7.89

S: salinity, A: Ascorbic acid; G: gibberellin; *, ** and ns: significant at 0.05, 0.01 probability level and not significant, respectively.

Salinity stress significantly decreased savory growth so that root and shoot weight (either fresh weight or dry weight) decreased with increasing salinity level. Furthermore, leaf weight and leaf area decreased due to salinity stress (Table 3). Salinity which is result of

osmotic pressure leads reduction in water absorbance so cell division and differentiation reduce and reduction of plant growth will be explainable. Etesami and Galeshi (2008) reported that salinity is the cause of reduction in dry weight of barley (*Hordeum*

vulgare L.) seedling. Massai *et al.* (2004) have found that salinity postpones plant growth under reduction of photosynthesis effects, it is cause of closing stomata and reduction of water entrance into the plant and so that it cause duplicate reduction in plant

weight. Younis *et al.* (2010) reported that the growth reduction caused by salinity stress is due to inhibited apical growth in plants as well as endogenous hormonal imbalance.

Table 3. Main effect of salinity on some savory traits.

Salinity (mM)	Root fresh weigh (g)	Root dry weight (g)	Leaf fresh weight (g)	Leaf dry weight (g)	Shoot fresh weight (g)	Shoot dry weight (g)	Leaf area (cm ²)	Membrane stability (%)	Chlorophyll <i>a</i> (µg ml ⁻¹)
0	0.32a	0.09a	0.56a	0.12a	1.998a	0.24a	29.45a	54.66a	3.08a
25	0.29ab	0.07b	0.47b	0.11b	1.84ab	0.22b	24.48b	47.32ab	2.92b
50	0.26bc	0.07bc	0.44b	0.10bc	1.73b	0.20b	23.25b	42.64b	2.02c
75	0.23c	0.06c	0.41b	0.09c	1.45c	0.17c	20.93b	39.34b	1.53d

Values within the each column and followed by the same letter are not different at $P < 0.05$ by an ANOVA protected Duncan's Multiple Range Test.

Salinity stress decreased membrane stability, however; there was no significant difference between salinity levels (Table 3). Membrane stability reflects the changes of cell membrane structure under stress. The results of the present study are in agreement with Bayat *et al.* (2012) who determined that membrane

stability of *Calendula officinalis* plant was intensively decreased by salt stress treatment. These results suggested cell membrane structure of summer savory leaves under salinity stress received damage after treatment with NaCl.

Table 4. Main effect of ascorbic acid on some savory traits.

Ascorbic acid (mM)	Root fresh weigh (g)	Root dry weight (g)	Leaf fresh weight (g)	Leaf dry weight (g)	Shoot fresh weight (g)	Shoot dry weight (g)	Leaf area (cm ²)	Relative content (%)	Membrane stability (%)	Chlorophyll <i>a</i> (µg ml ⁻¹)
0	0.24b	0.06b	0.39b	0.09b	1.56b	0.20b	20.12b	82.48b	42.30b	2.24b
4	0.30a	0.08a	0.55a	0.13a	1.94a	0.28a	28.94a	79.16a	49.68a	2.53a

Values within the each column and followed by the same letter are not different at $P < 0.05$ by an ANOVA protected Duncan's Multiple Range Test.

There was linear reduction in chlorophyll *a* content with increasing salinity level. El-Tayeb (2005) found that chlorophyll *a*, *b* and carotenoids decreased significantly in NaCl treated plants in comparison to controls of barley plants. Based on the theory of Schutz and Fangmeir (2001), the reduction of chlorophyll due to stress is related to the increase of production of free oxygen radicals in the cell. These free radicals cause peroxidation, disintegration and reduction of chlorophyll content in plant under stressful conditions.

Ascorbic acid foliar application increased root weight, leaf weight as well as shoot fresh weight (Table 4). Leaf growth and expansion increased on account of

ascorbic acid foliar application (Table 4). The beneficial effect of ascorbic acid on plant growth may be due to the fact that ascorbic acid is involved in the regulation of root elongation, cell vacuolation and cell expansion (Smirnoff, 1996). Ascorbic acid induced increase in growth under non-saline conditions may have been due to a double action of ascorbic acid on cell growth by modifying the cell cycle and stimulating quiescent cells to divide, and by accelerating cell elongation (Saeidi-Sar *et al.*, 2013). In addition, ascorbate is a co-factor for prolyl-hydroxylase that post-translationally hydroxylates proline residues in cell wall hydroxyprolinerich glycoproteins required for cell division and expansion (Smirnoff and Wheeler, 2000b). Moreover, ascorbic

acid increases the content of auxine, which stimulates cell division and/or cell enlargement and this, in turn, improves plant growth (Khan *et al.*, 2011).

Ascorbic acid foliar application had positive effect on leaf relative water content and cell membrane stability (Table 4). It has been reported that exogenous application of ascorbic acid enhances potassium concentration in plants (Saeidi-Sar *et al.*, 2013). This increase may be attributed to the positive effect of ascorbic acid on the root growth, which

consequently increases the absorption of different nutrients and alleviates the harmful effects of salinity. In addition, ascorbic acid would inhibit a stress-induced increase in the leakage of essential electrolytes following peroxidative damage to plasma membranes (Khan *et al.*, 2011). Moreover, the increase of trans-membrane electron transport via cytochrome *b* using ascorbic acid depolarizes the plasma membrane, and activates the H⁺-ATPase resulting in increased ion uptake (Smirnoff and Wheeler, 2000b).

Table 5. Main effect of gibberellin on some savory traits.

Gibberellin (mM)	Root fresh weigh (g)	Root dry weight (g)	Leaf fresh weight (g)	Leaf dry weight (g)	Shoot fresh weight (g)	Leaf area (cm ²)	Chlorophyll <i>a</i> (µg ml ⁻¹)
0	0.25b	0.06b	0.42b	0.09b	1.54b	20.25b	2.31b
4	0.30a	0.08a	0.52a	0.12a	1.96a	28.81a	2.46a

Values within the each column and followed by the same letter are not different at $P < 0.05$ by an ANOVA protected Duncan's Multiple Range Test.

Chlorophyll *a* content increased because of ascorbic acid foliar application (Table 4). Ascorbic acid has a major role in photosynthesis, acting in the Mehler peroxidase reaction with ascorbate peroxidase to regulate the redox state of photosynthetic electron carriers and as a co-factor for violaxanthin de-

epoxidase, an enzyme involved in xanthophyll cycle-mediated photoprotection (Smirnoff and Wheeler, 2000b). Consequently, in ascorbic acid treated plants, high level of pigments can synergistically function with ascorbic acid to provide an effective barrier against oxidation under salinity stress.

Table 6. Interaction between salinity and ascorbic acid on some savory traits.

Salinity (mM)	Ascorbic acid (mM)	Transpiration (µmol m ⁻² s ⁻¹)	Chlorophyll <i>b</i> (µg ml ⁻¹)	Total chlorophyll (µg ml ⁻¹)	Xanthophyll (µg ml ⁻¹)
0	0	5.38a	2.11b	5.11c	1.46cd
25		4.31b	1.65c	4.44d	1.53e
50		2.38cd	1.02e	2.99f	1.66e
75		1.57d	0.57g	2.07h	1.97bc
0	4	5.72a	2.15a	5.29a	1.39bc
25		5.80a	1.97b	5.03b	1.61b
50		4.33b	1.10d	3.17e	1.67de
75		3.00c	0.59f	2.18g	1.99a

Values within the each column and followed by the same letter are not different at $P < 0.05$ by an ANOVA protected Duncan's Multiple Range Test.

Gibberellin foliar application caused an increase in savory growth parameters i.e. root fresh and dry weight, leaf fresh and dry weight, shoot fresh weight and leaf area (Table 5). Exogenous application of

gibberellin might increase plant growth by enhancing the content of endogenous gibberellin as that mentioned by Rodriguez *et al.* (2006). Additionally, the enhancement of growth rate by gibberellin might

result in an enlargement of leaf area, activation of cell division or cell elongation, stimulation of photosynthetic rate, modified partitioning of photosynthates, or in their combination.

Chlorophyll *a* content positively responded to gibberellin foliar application and increased (Table 5).

The inhibitory effect of gibberellin on chlorophyll catabolism might be partly due to the down regulation of the activities of enzymes involved in chlorophyll catabolism and the alleviation of oxidative chlorophyll bleaching (Li *et al.*, 2010).

Table 7. Interaction between salinity and gibberellin on some savory traits.

Salinity (mM)	Gibberellin (mM)	Transpiration ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	Relative water content (%)	Chlorophyll <i>b</i> ($\mu\text{g ml}^{-1}$)	Total chlorophyll ($\mu\text{g ml}^{-1}$)	Xanthophyll ($\mu\text{g ml}^{-1}$)
0	0	5.18ab	86.14a	1.88b	4.78b	1.36d
25		4.50b	80.18b	1.64bc	4.36b	1.39d
50		2.41cd	75.89ab	0.95cd	2.86d	1.47d
75		1.72d	72.10b	0.41e	1.85e	1.67bc
0	4	5.91a	87.73a	2.37a	5.62a	1.59c
25		5.61a	86.13a	1.99ab	5.11a	1.68b
50		4.30b	79.29b	1.18c	3.30c	1.80ab
75		2.85c	80.10b	0.76d	2.39d	2.28a

Values within the each column and followed by the same letter are not different at $P < 0.05$ by an ANOVA protected Duncan's Multiple Range Test.

Salinity decreased transpiration rate whereas ascorbic acid foliar application improved transpiration rate in savory plants (Table 6). Salt stress is known to cause a significant reduction in the gas exchange attributed, net photosynthesis, transpiration rate and stomatal conductance of plants (Ali and Ashraf, 2011). In the present investigation, salt stress caused a marked reduction in transpiration but ascorbic acid foliar

application mitigated the adverse effects of salinity on transpiration by decreasing stomatal resistance. This could have also been due to the fact that ascorbic acid as an antioxidant has the ability to mitigate the negative effects of stress on plants by neutralizing harmful oxidants which have been reported to damage plant membranes (Miguel *et al.*, 2006).

Table 8. Interaction between gibberellin and ascorbic acid on some savory traits.

Gibberellin (mM)	Ascorbic acid (mM)	Shoot dry weight (g)	Chlorophyll <i>b</i> ($\mu\text{g ml}^{-1}$)	Xanthophyll ($\mu\text{g ml}^{-1}$)
0	0	0.15c	1.16a	1.46c
	4	0.21b	1.24b	1.76b
4	0	0.20b	1.20c	1.49c
	4	0.28a	1.48a	1.92a

Values within the each column and followed by the same letter are not different at $P < 0.05$ by an ANOVA protected Duncan's Multiple Range Test.

Moreover, application of ascorbic acid under salinity stress conditions caused significant increase in chlorophyll *b*, total chlorophyll and xanthophyll content compared with control plants which were treated with distilled water (Table 6). Ascorbic acid

can mitigate the adverse effects of salinity through increasing the content of auxine and gibberellin and decreasing abscisic acid level (Khan *et al.*, 2011), which may be involved in protecting the photosynthetic apparatus and consequently

increasing the photosynthetic pigments.

Similar results were obtained when gibberellin was applied on salt stressed savory plants. In other words, transpiration rate, relative water content, chlorophyll *b*, total chlorophyll and xanthophyll content increased on account of gibberellin application under salinity stress conditions (Table 7). It has been reposted that gibberellin plays a vital role in tolerance to salt stress by improving plant growth, pigments synthesis and photosynthesis rate (Maggio *et al.*, 2010).

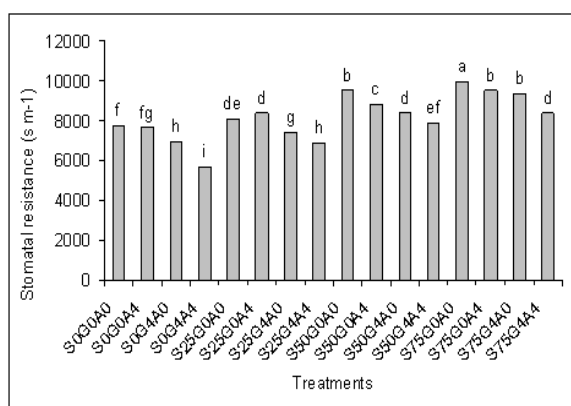


Fig. 1. Triple interaction between salinity, ascorbic acid and gibberellin on stomatal resistance of summer savory. Values within the each column and followed by the same letter are not different at $P < 0.05$ by an ANOVA protected Duncan's Multiple Range Test.

Interaction between ascorbic acid and gibberellin was significant on shoot dry weight, chlorophyll *b* and xanthophyll content (Table 8). The highest shoot dry weight, chlorophyll and xanthophyll were observed when 4 mM ascorbic acid and 4 mM gibberellin were applied on savory plants (Table 8). Previous studies revealed that supplying low levels of ascorbic acid and gibberellin could alleviate the adverse effects of abiotic stresses on plants (Saeidi-Sar *et al.*, 2007) and improve growth and development (Li *et al.*, 2010).

Significant triple interactions between salinity, ascorbic acid and gibberellin on stomatal resistance and carotene content are shown in figure 1 and 2, respectively. Salinity increased stomatal resistance whereas ascorbic acid or gibberellin application decreased stomatal resistance (Figure 1). The highest stomatal resistance was observed in high salinity

stress treatment without foliar application but the lowest resistance was related to none stressed plants treated with 4 mM ascorbic and 4 mM gibberellin (Figure 1).

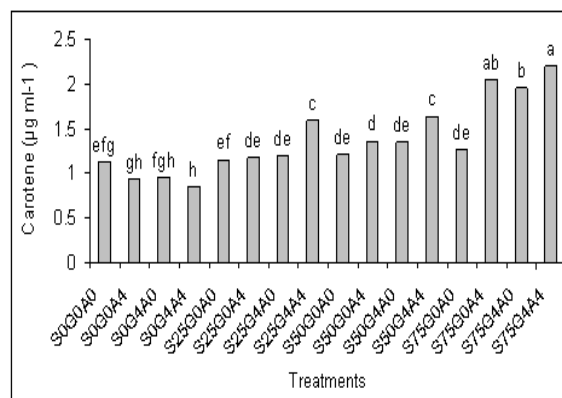


Fig. 2. Triple interaction between salinity, ascorbic acid and gibberellin on carotene content of summer savory. Values within the each column and followed by the same letter are not different at $P < 0.05$ by an ANOVA protected Duncan's Multiple Range Test.

Under salinity stress conditions, application of ascorbic acid and gibberellin increased carotene content in savory plants (Figure 2). The lowest carotene content was observed in non stressed plants and treated with 4 mM ascorbic acid and gibberellin, while the highest content was related to severe salt stress (75 mM) and 4 mM ascorbic acid and gibberellin (Figure 2). Pisal and Lele (2005) on the basis of their experiments on unicellular green algae *Dunaliella salina* suggested that the cells show unbalanced physiological conditions under the influence of various stresses leading to increased β -carotene content. They observed increased production of β -carotene in cells with increasing NaCl in the growth medium. They suggested that, β -carotene is a secondary metabolite and these molecules are produced by the cells under stress for protection. Generally, the results of this study showed that growth parameters and physiological attributes of summer savory plants were adversely affected by salinity stress. By increasing salinity level fresh and dry weight of different parts of plant decreased. Membrane stability and chlorophyll *a* content also decreased due to salt stress. In general, our study showed that although salt stress is harmful to

summer savory plants, foliar application of ascorbic acid and gibberellin could mitigate adverse effect of salinity and improve plant growth through increasing photosynthesis, relative water content, membrane stability and biosynthesis of photosynthetic pigments.

References

- Achard P, Cheng H, De Grauwe L, Decat J, Schoutteten H, Moritz T, Van Der Straeten D, Peng J, Harberd NP.** 2006. Integration of plant responses to environmentally activated phytohormonal signals. *Science* **311**, 91-94.
<http://dx.doi.org/10.1126/science.1118642>
- Ali Q, Ashraf M.** 2011. Induction of drought tolerance in maize (*Zea mays* L.) due to exogenous application of trehalose, growth, photosynthesis, water relations and oxidative defence mechanism. *Journal of Agronomy and Crop Science* **197**(4), 258-271.
<http://dx.doi.org/10.1111/j.1439-037X.2010.00463.x>
- Arnon DI.** 1949. Copper enzymes in isolated chloroplasts, polyphenoloxidase in *Beta vulgaris* L. *Plant Physiology* **24**(1), 1-15.
<http://dx.doi.org/10.1104/pp.24.1.1>
- Ashraf M, Karim F, Rasul E.** 2002. Interactive effects of gibberellic acid (GA₃) and salt stress on growth, ion accumulation and photosynthetic capacity of two spring wheat (*Triticum aestivum* L.) cultivars differing in salt tolerance. *Plant Growth Regulation* **36**(1), 49-59.
- Ashraf M, Kausar A, Ashraf YM.** 2003. Alleviation of salt stress in pearl millet (*Pennisetum glaucum* L.) R. Br. through seed treatments. *Agronomie* **23**, 227-234.
<http://dx.doi.org/10.1051/agro:2002086>
- Bayat H, Alirezaie M, Neamati H.** 2012. Impact of exogenous salicylic acid on growth and ornamental characteristics of calendula (*Calendula officinalis* L.) under salinity stress. *Journal of Stress Physiology & Biochemistry* **8**(1), 258-267.
- Chen Z, Gallie DR.** 2004. The ascorbic acid redox state controls guard cell signaling and stomatal movement. *Plant Cell* **16**(5), 1143-1162.
<http://dx.doi.org/10.1105/tpc.021584>
- Davey M, Montagu WMV, Inze D, Sanmartin M, Kanellis A, Smirnoff N, Benzie IJJ, Strain JJ, Favell D, Fletcher J.** 2000. Plant L-ascorbic acid, chemistry, function, metabolism, bioavailability and effects of processing. *Journal of the Science of Food and Agriculture* **80**(7), 825-860.
[http://dx.doi.org/10.1002/\(SICI\)10970010\(20000515\)80:7%3C825::AID-JSFA598%3E3.3.CO;2-Y](http://dx.doi.org/10.1002/(SICI)10970010(20000515)80:7%3C825::AID-JSFA598%3E3.3.CO;2-Y)
- El-Tayeb MA.** 2005. Response of barley grains to the interactive effect of salinity and salicylic acid. *Plant Growth Regulation* **45**(3), 215-224.
<http://dx.doi.org/10.1007/s10725-005-4928-1>
- Etesami M, Galeshi S.** 2008. Assessment of 10 barely (*Hordeum vulgare* L.) genotypes reaction to salinity in germination and seedling growth stages. *Journal of Agriculture Science and Natural Resource* **15**(5), 39-46.
- Kefeli VI.** 1981. Vitamins and some other representatives of non hormonal plant growth regulators. *Pribl Biochemistry and Microbiology* **17**, 5-15.
- Khan TA, Mazid M, Mohammad F.** 2011. A review of ascorbic acid potentialities against oxidative stress induced in plants. *Journal of Agrobiology* **28**(2), 97-111.
<http://dx.doi.org/10.2478/v10146-011-0011-x>
- Li JR, Yu K, Wei JR, Ma Q, Wang BQ, Yu D.** 2010. Gibberellin retards chlorophyll degradation during senescence of *Paris polyphylla*. *Biologia Plantarum* **54**(2), 395-399.
<http://dx.doi.org/10.1007/s10535-010-0072-5>
- Lutts S, Almansouri M, Kinet JM.** 2004. Salinity and water stress have contrasting effects on the relationship between growth and cell viability during

and after stress exposure in durum wheat callus.

Plant Science **167**(1), 9-18.

<http://dx.doi.org/10.1016/j.plantsci.2004.02.014>

Maggio A, Barbieri G, Raimondi G, De Pascale S. 2010. Contrasting effects of GA₃ treatments on tomato plants exposed to increasing salinity. Journal of Plant Growth Regulation **29**(1), 63-72.

<http://dx.doi.org/10.1007/s00344-009-9114-7>

Magome H, Yamaguchi S, Hanada A, Yuji Kamiya Y, Oda K. 2008. The DDF1 transcriptional activator upregulates expression of a gibberellin-deactivating gene, GA₂ox₇ under high-salinity stress in *Arabidopsis*. Plant Journal **56**(4), 613-626.

<http://dx.doi.org/10.1111/j.1365-3113X.2008.03627.x>

Massai R, Remorin D, Tattini H. 2004. Gas exchanges, water relation and osmotic adjustment in two scion root stock combinations of prunus under water various salinity. Plant and Soil **259**, 153-162.

Meloni DA, Oliva MA, Martinez CA, Cambraia J. 2003. Photosynthesis and activity of superoxide dismutase, peroxidase and glutathione reductase in cotton under salt stress. Environmental and Experimental Botany **49**(1), 69-76.

[http://dx.doi.org/10.1016/S0098-8472\(02\)00058-8](http://dx.doi.org/10.1016/S0098-8472(02)00058-8)

Miguel A, Rosales J, Ruiz M, Hernandez J, Soriano T, Castilla N, Romero L. 2006. Antioxidant content and ascorbate metabolism in cherry tomato exocarp in relation to temperature and solar radiation. Journal of the Science of Food and Agriculture **86**, 1525-1551.

Mukherji S, Biswas AK. 1979. Modulation of chlorophyll, carotene and xanthophylls formation by penicillin, benzyladenine and embryonic axis in mungbean (*Phaseolus aureus* L.) cotyledons. Annals of Botany **43**(2), 225-229.

Muller-Moule P, Golan T, Niyogi KK. 2004. Ascorbate-deficient mutants of *Arabidopsis* grow in high light despite chronic photooxidative stress. Plant

Physiology **134**(3), 1163-1172.

<http://dx.doi.org/10.1104/pp.103.032375>

Munir N, Aftab F. 2009. The role of polyethylene glycol (PEG) pre-treatment in improving sugarcane's salt (NaCl) tolerance. Turkish Journal of Botany **33**, 407-415.

Munir N, Aftab F. 2011. Enhancement of salt tolerance in sugarcane by ascorbic acid pre-treatment. African Journal of Biotechnology **10**, 18362-18370.

Munns R, Tester M. 2008. Mechanisms of salinity tolerance. Annual Review of Plant Biology **59**(1), 651-681.

<http://dx.doi.org/10.1146/annurev.arplant.59.032607.092911>

Noctor G, Foyer CH. 1998. Ascorbate and glutathione Keeping active oxygen under control. Annual Review of Plant Physiology and Plant Molecular Biology **49**(1), 249-279.

<http://dx.doi.org/10.1146/annurev.arplant.49.1.249>

Pisal DS, Lele SS. 2005. Carotenoid production from microalgae, *Dunaliella salina*. Indian Journal of Biotechnology **4**, 476-483.

Rao SSR, Vardhini BV, Sujatha E, Anuradha S. 2002. Brassinosteroids, A new class of phytohormones. Current Science **82**, 1239-1245.

Rodriguez AA, Stella AM, Storni MM, Zulpa G, Zaccaro MC. 2006. Effects of cyanobacterial extracellular products and gibberellic acid on salinity tolerance in *Oryza sativa* L. Saline Systems **2**, 7. <http://dx.doi.org/10.1186/1746-1448-2-7>

Saeidi-Sar S, Abbaspour H, Afshari H, Yaghoobi SR. 2013. Effects of ascorbic acid and GA₃ on alleviation of salt stress in common bean (*Phaseolus vulgaris* L.) seedlings. Acta Physiologia Plantarum **35**, 667-677.

- Sairam RK, Rao KV, Srivastava GC.** 2002. Differential response of wheat genotypes to long term salinity stress in relation to oxidative stress, antioxidant activity and osmolyte concentration. *Plant Science* **163**(5), 1037-1046.
[http://dx.doi.org/10.1016/S0168-9452\(02\)00278-9](http://dx.doi.org/10.1016/S0168-9452(02)00278-9)
- Schutz M, Fangmeir E.**2001. Growth and yield response of spring wheat (*Triticum aestivum* L. cv. *Minaret*) to elevated CO₂ and water limitation. *Environmental Pollution* **114**(2),187-194.
[http://dx.doi.org/10.1016/S0269-7491\(00\)00215-3](http://dx.doi.org/10.1016/S0269-7491(00)00215-3)
- Sefidkon F, Jamzad Z, Mirsa M.** 2004. Chemical variation in the essential oil of *Satureja sahendica* from Iran. *Food Chemistry* **88**(3),325- 328.
<http://dx.doi.org/10.1016/j.foodchem.2003.12.044>
- Sefidkon F, Jamzad Z.** 2005. Chemical composition of the essential oil of three Iranian *Satureja* species (*S. mutica*, *S. macrantha* and *S. intermedia*). *Food Chemistry* **91**(1), 1-4.
<http://dx.doi.org/10.1016/j.foodchem.2004.01.027>
- Sefidkon F, Abbasi K, Bakhshi Khaniki G.** 2006. Influence of drying and extraction methods on yield and chemical composition of the essential oil of *Satureja hortensis*. *Food Chemistry* **99**(1), 19-23.
<http://dx.doi.org/10.1016/j.foodchem.2005.07.026>
- Smirnoff N.**1996. The function and metabolism of ascorbic acid in plants. *Annals of Botany* **78**, 661-669.
- Smirnoff N.** 2000a. Ascorbic acid, metabolism and functions of a multifaceted molecule. *Current Opinion in Plant Biology* **3**, 229-235.
- Smirnoff N, Wheeler GL.** 2000b. Ascorbic acid in plants, biosynthesis and function. *Critical Reviews in Plant Sciences* **19**(4), 267-290.
[http://dx.doi.org/10.1016/S0735-2689\(00\)80005-2](http://dx.doi.org/10.1016/S0735-2689(00)80005-2)
- Wang WB, Kim YH, Lee HS, Kim KY, Kwask SS.** 2009. Analysis of antioxidant enzymes activity during germination of alfalfa under salt and drought stresses. *Plant Physiology and Biochemistry* **47**(7), 570-577.
<http://dx.doi.org/10.1016/j.plaphy.2009.02.009>
- Wen F, Zhang Z, Bai T, Xu Q, Pan Y.** 2010. Proteomics reveals the effects of gibberellic acid (GA₃) on salt-stressed rice (*Oryza sativa* L.) shoots. *Plant Science* **178**(2), 170-175.
<http://dx.doi.org/10.1016/j.plantsci.2009.11.006>
- Younis ME, Hasaneen MNA, Kazamel AMS.** 2010. Exogenously applied ascorbic acid ameliorates detrimental effects of NaCl and mannitol stress in *Vicia faba* seedlings. *Protoplasma* **239**(1), 39-48.
<http://dx.doi.org/10.1007/s00709-009-0080-5>