



Effect of ascorbate and gibberellin on some morphological traits and relative water content in fenugreek (*Trigonella foenum graecum* L.) under different levels of salinity stress

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ABSTRACT

To evaluate the effect of ascorbate and gibberellin on some morphological traits and relative water content of medicinal plant fenugreek in different levels of salinity, an experiment was carried out in Shahr- e - Rey branch of Islamic Azad University in October 2012. The experiment was arranged as factorial experiment in the basis of completely randomized design with four replications. The experimental factors included four salinity levels (0, 30, 60 and 90 mM), three ascorbate levels (0, 3 and 6 mM) and two gibberellin levels (0 and 2 mM). The results showed that, the simple effect of salinity, gibberellin and ascorbate on experimented traits were significant. Except double effects of gibberellin and ascorbate on stem length, leaf area interaction effects of salinity and ascorbate on relative water content, all double and triple interaction effects on experimented traits were not significant. The findings of the salinity simple effects indicated that, increase in salt concentration reduced the amount of concerned traits. So that the lowest biomass, vegetative growth and RWC were obtained in salinity by 90 mM. Gibberellin and ascorbate application could decrease adverse effects of stress, so that, by spraying these compounds on the plant, an increase was observed in leaf vegetative growth and relative water content.

Key words: Fenugreek, Ascorbate, Gibberellin, Salinity stress, Morphological traits.

INTRODUCTION

Fenugreek (*Trigonella foenum-graecum* L.) is an herbaceous and annual plant which belongs to legumes. Fenugreek is a plant which its leaf and seed have medicinal value and in Iran, its leaves are used fresh or

dried (Omidbeigi, 2004; Najafpoornavaei, 1994). The plant is originated in the Mediterranean which extends in Western Asia, Ukraine and also from India to China. Fenugreek has a major role in nitrogen fixation therefore; it is suitable for using in crop rotation. The plant is self-fertile and is pollinated by wind and sometimes insects. The basic chromosome number of this species is eight and is as diploid (Omidbeigi, 2004). For protection against oxidative damages, the plant cells are equipped with a vacuum system of free radicals such as ascorbic acid. Ascorbate acts as an antioxidant by doing removal of hydrogen peroxide. Hydrogen peroxide is generated from optical oxygen reduction in photosystem I. This process is catalyzed by ascorbate peroxidase which traps created hydrogen peroxide once being formed (Hasibi et al. 2008). Also it interferes as reducing in reproduction – tocopherol, Xanthophyll cycle and protection of enzymes with prosthetic groups of transition elements (Smirnoff & Wheeler, 2000). Gibberellin has an important role in different processes of the life cycle of higher plants and leads to growth and development of plant organs (Rademacher, 2000). In addition to growth, gibberellin makes nodes to increase in the plant. Also, it nowadays is considered as an efficient hormone in stimulation of dormancy in many horticultural crops (Hedden & Proebsting, 1999).

MATERIALS AND METHODS

In order to investigate the effect of ascorbate and gibberellin on some morphological traits of medicinal plant fenugreek, an experiment was carried out in Shahr-e Rey branch of Islamic Azad University in October 2012. The experiment was arranged as factorial experiment in the basis of completely randomized design with four replications. The experimental factors included four salinity levels (0, 30, 60 and 90 mM), three ascorbate levels (0, 3 and 6 mM) and two gibberellin levels (0 and 2 mM). Fenugreek potted planting was carried out using measured agricultural soil considering essential elements. The pots floor was filled by coarse sand for suitable drainage by 5 cm height, and 6 kg of considered soil. The pots irrigation was done since planting until stress application equally in each pot and as one time within two days. After the beginning of stress, irrigation was carried out two times a week equally by distilled water. Ascorbate and gibberellin were given the plant with mentioned concentrations for retrofitting plants to salinity stress. The plants were exposed to stress 5 weeks after generation for 3 weeks, and after 9 weeks, sampling was conducted to do experiments. Once harvesting, 10 plants of each pot were selected, roots and aerial parts were separated, and initially, root length and shoot length were measured. Then, a leaf area meter was used to measure leaf area. Fresh weight of roots stems and shoots were also measured. After putting all mentioned parts in a 75 °C oven for 48 hours, their dry weight was calculated. The data obtained from measurement were analyzed by SAS software, and mean comparison at error level of 1% and 5% was done by Duncan test, and graphs were drawn using Excel software.

RESULTS AND DISCUSSION

Root length

The results of variance analysis showed that, simple effects of salinity, ascorbate and gibberellin on root length became significant at level of 1%. In double and triple interactions of this trait, there was no significant difference (Table 1). comparison of mean simple salinity effects explains that, salinity causes to decrease root length so that, the minimum amount of root length was obtained in treatment of 90 MM salinity equal with 8.51 cm and its maximum was observed in treatment of no stress equal with 10.04 cm. Gibberellin and ascorbate application decreased adverse effects of stress and increased root length in the plant so that, the maximum amount of root lengthways observed in treatment with 9.72 cm gibberellin equal with 9.72 cm and 9 MM ascorbate equal with 10.58 cm (Table 2). Stress application in the plant led

to anatomical and morphological changes and consequently decrease of growth and efficiency. Use of Gibberellic acid leads to improve growth traits including root length, wet and dry weight in plant (Siripornadulsid et al., 2002). An experiment was carried out on morphological traits of cumin and valerian (Salami et al., 2004). The results showed that, by increasing sodium concentration, root length has been reduced in cumin genotypes. Ascorbate causes to increase the plant's ability to absorb water which has been explained as fundamental mechanism in coping with stress (Kusaka et al., 2005).

Stem length

Results of the research indicated that, there is a significant difference between different salinity level, Ascorbate and gibberellin in terms of stem length. Except interaction of ascorbate and gibberellin, no significant difference other double and triple interactions on stem length was observed (Table 1). The maximum root length was in conditions of no stress by 27.87 cm and its minimum was for the salinity of 90 MM by 23.45 cm (Table 2). In an experiment conducted by Habib Elahi et al., 2012), the results demonstrated a significant reduction in aerial parts length under salinity stress. The maximum length of aerial part belonged to the control plants. Although gibberellin application caused to increase root length, but decreased the stem length. Simple effect of ascorbate showed that, by increasing ascorbate concentration, stem length also increased so that, the maximum amount of stem length was for applying 6 MM ascorbate by 27.76 cm and its minimum was obtained in treatment without ascorbic acid by 23.15 cm (Table 2). According to Pastori et al. (2003), increase of abscisic acid is a result of ascorbic shortage so; ascorbic acid can prevent inhibitory effects of abscisic acid on growth, by preventing the increase of abscisic acid level increase. Results of the comparison of mean of interaction of ascorbate and gibberellin showed that, simultaneous use of these two compounds caused to increase stem length compared with control plant so that, the maximum amount of stem length belonged to the treatment of 6 MM ascorbate and gibberellin by 31.23 cm (Table 4). Gibberellins lead to increase the growth in complete plants. Aerial organs elongation which occurs by the presence of gibberellin in aerial organs is resulted from increase of meiosis by cells elongation, or both together. Gibberellins increase the capability of expansion of the cell wall that results in softening of the cell wall which lets the cell to be elongated (Betrand & Ernstsens, 2001).

Dry weight of root

Obtained results of comparing the data mean of this trait showed that, there was a significant difference at the level of 1% between various salinity level and ascorbate and gibberellin. Double and triple interactions of experimental factors on dry weight of root were not significant (Table 1). Comparison of the mean of simple salinity effects showed that, salinity caused to decrease dry weight of root, so that, the minimum amount of dry weight in root belonged to the 90 MM salinity treatment by 0.07 gr (Table 2). In an experiment on sunflower conducted by Mostafa et al. (2012), the results showed that, gibberellin application leads to increase dry weight, so that, it's maximum amount was achieved in the treatment of gibberellin application by 0.09 gr. The results of mean comparison of simple effect of ascorbate states that, the maximum amount of dry weight of root was found in the treatment of 6 mM ascorbate by 0.11 gr and its minimum was for the treatment with absence of ascorbate (Table 2).

Dry weight of aerial organs

Results of variance analysis showed that, simple effects of salinity, ascorbate and gibberellin on dry weight of aerial organs were significant at the level of 1%. Except double interaction of ascorbate and gibberellin, there was no significant in all double and triple interactions on this trait (Table 1). Results of mean comparison of salinity simple effects indicates that, the minimum amount of dry weight of aerial organs was obtained in salinity treatment of 90 mM by 0.1 and its maximum was found in treatment with

absence of salinity by 0.15 gr (Table 2). In an experiment conducted on safflower under stress conditions (Sheydaei et al., 2010), the results showed that, salinity causes to decrease dry weight of aerial organs and root. Decrease of dry weight of plant resulted from salinity also has been reported by some other researchers for safflower (kaya & Ipek, 2003) and barley (Janzen, 1988). Results from gibberellin simple effect indicated that, gibberellin application caused to increase dry weight of aerial organs relative to the treatment without application of this hormone, so that, its maximum amount was obtained in in gibberellin application treatment by 0.14 gr. Also, ascorbate could properly cause to increase dry weight of aerial organs by decreasing adverse effects of stress, so that, the maximum amount of dry weight was related to the treatment of 6 MM ascorbate by 0.17 gr (Table 2). In an experiment carried out on thyme, the results demonstrated that, gibberellin application leads to increase dry weight of aerial organs and root (Pazoki et al., 2012). Interaction of ascorbate and gibberellin also increased dry weight of aerial organs, so that, it's maximum amount was related to the treatment of 6 MM ascorbate and gibberellin application by 0.19 gr (Table 4). Based on current studies, the ability of external ascorbate in increasing the plant growth and decreasing harmful effects of stress can be imagined so that, these harms are resulted from antioxidant level decrease (Alscher et al., 1977).

Dry weight of leaf

Results of variance analysis showed that, simple effects of salinity, ascorbate and gibberellin on dry weight of leaf were significant at the level of 1%. There was no significant in all double and triple interactions on this trait (Table 1). Salinity led to decrease dry weight of leaf, so that, the minimum amount of dry weight of leaf was obtained in salinity treatment of 90 mM by 0.1. In an experiment conducted on chamomile (Noori et al., 2012), same results were obtained, and salinity caused to decrease dry weight of aerial parts of the plant. Use of ascorbate and gibberellin could decrease adverse effects of stress, so that, the maximum amount of dry weight was related to the treatment of gibberellin application by 0.15 gr and also, treatment of 6 MM ascorbate by 0.17 gr (Table 2). In an experiment conducted on anise plant (Kavan et al., 2009), the results showed that, applying ascorbic acid under stress conditions led to increase the number of plant leaf. Rosales et al. (2006) found that, ascorbic acid increases meiosis, leaf area and wet and dry weight of leaf, and decreases the injury resulted from oxygen radicals which are created in stress conditions by its antioxidant property.

Leaf area

Results of variance analysis showed that, all simple effects of salinity, ascorbate and gibberellin was significant at the level of 1% (Table 1) so that, by increasing salinity concentration, leaf area was significantly decreased so that, the maximum amount of leaf area was obtained in the treatment without stress by 38.45 cm². Simple effect of ascorbate indicated that, ascorbate could increase leaf area so that, the maximum leaf area was related to the treatment of 6 MM ascorbate by 44.86 cm² and the minimum leaf area was related to the treatment without ascorbate by 20.25 cm² (Table 2). Gibberellin application also decreased stress effects on plant and increased leaf area significantly (Table 2). Double interaction of gibberellin and ascorbate was significant at the level of 5% (Table 1), so that, the maximum amount of leaf area was obtained in the treatment of gibberellin and 6 mM ascorbate application by 55.95 cm² (Table 4). Decrease of leaf area is one of the earliest reactions of plant against salinity stress. Since, dry mass accumulation and leaf area is reduced continuously by salinity; decrease of leaf area may be a reason of growth reduction by this factor. Salinity stress leads to internodes and plant height shortening, and reduction of dry weight of leaf and consequently decreases of dry weight of aerial organs through reducing cell proliferation and dry mass accumulation time. Some references have considered the reduction of tillers number in the plant as the most important factor of leaf weight reduction (koffi & Stewart, 2001). Non-biological stress leads to create active oxygen in plant organs so that, it has been

observed that, in leaf tissue of *Prunus*, by water progress, oxidative stress (H_2O_2 production) increases and by ascorbate presence in these conditions, aerial organs have more growth and the high ratio of root to aerial organs has been reduced (Billaud et al., 2001). Hayashi (1961) conducted an experiment on tomato and proved that, leaf application of gibberellin increases photosynthesis activity in this plant. He found that, increase of photosynthesis is not due to photosynthesis activity but also, it is due to leaf area increase.

Relative leaf water content

Results of variance analysis showed that, except of double interaction of salinity and gibberellin, gibberellin and ascorbate and triple interaction, simple effect at the level of 1% and double effect of ascorbate and salinity at the level of 5% were significant for the tested trait (Table 1). Mean comparison of the effect of various salinity levels on relative leaf water content showed that, increase of salinity concentration caused significantly to reduce RWC, so that, the minimum amount of salinity was obtained in 90 mM by 77.94% (Table 2). Relative leaf water content is considered as a reliable criterion to measure water in plant tissues because, relative leaf water content can show better the balance between plant water and transpiration rate through direct relation with cell volume (Schonfield et al., 1988). In another experiment conducted on safflower, direct relation between increase and decrease of water potential of different varieties was shown so that, by increasing salinity concentration, relative leaf water content was decreased (Javadipoor et al., 2012). Mean comparison of ascorbate simple effect demonstrated that, ascorbate led to increase this trait, so that, the maximum amount was related to 6 mM ascorbate by 86.49 (Table 2). In investigation of the results of mean comparison in various stress levels, a significant decreasing trend is observed which shows a significant increase in the simple effect of gibberellin by spraying this hormone that demonstrates the role of gibberellin in decreasing the adverse effects of stress (Table 2). In an experiment carried out on thyme (Pazoki et al., 2012), results showed that, simple effect of gibberellin led to increase relative leaf water content, so that, its maximum amount was observed in treatment of gibberellin line and the minimum amount was obtained in the treatment without gibberellin. The results of mean comparison of double effect of salinity and ascorbate indicated that, ascorbate caused to increase relative water content in the same concentrations of stress application (Table 3). The amount of RWC was reported by 63% in the sample of *Arabidopsis* mutants which contained 30% ascorbate compared with its natural type while, in natural sample, it was reported by 76%. These results showed that, the presence of ascorbate was effective to adjust salinity effects (Huang et al., 2005).

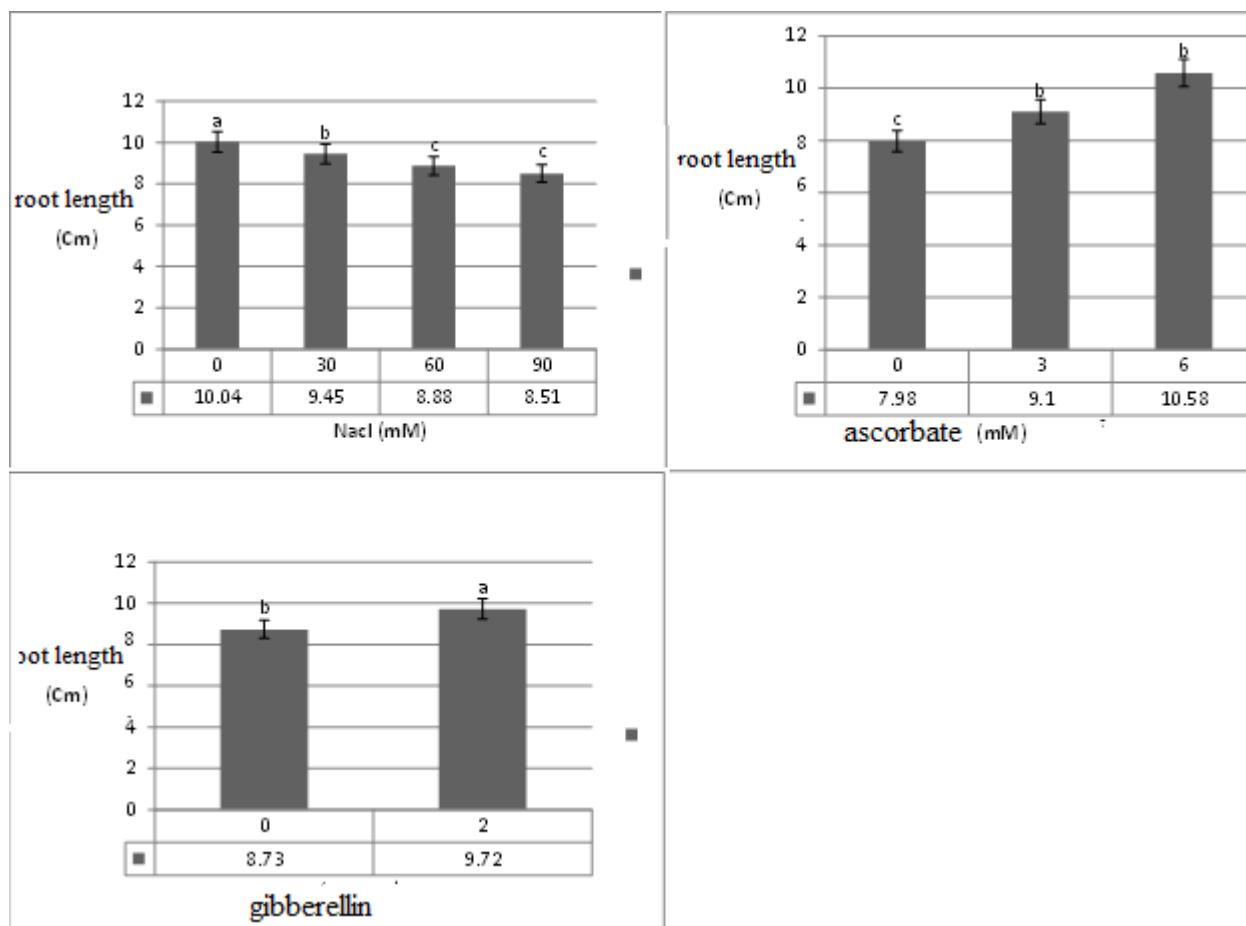


Figure 1: Simple effects of root length:

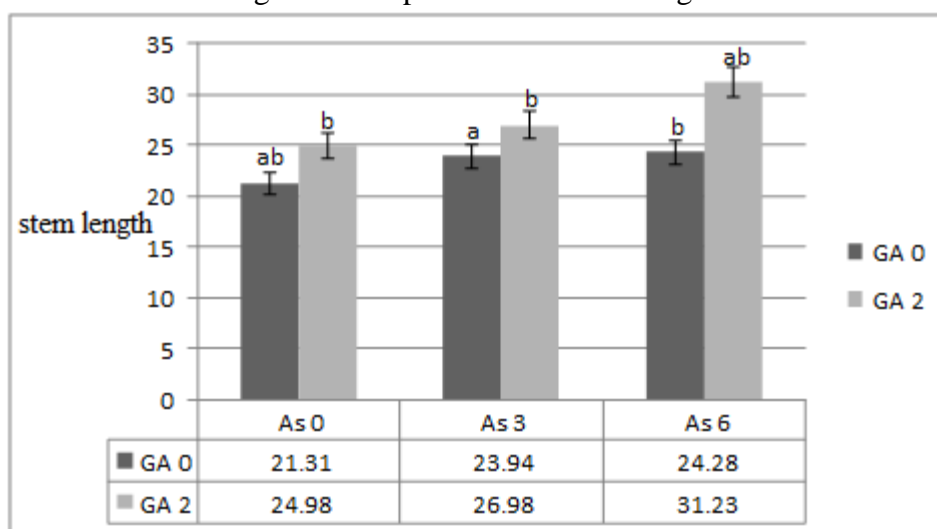


Figure 2: Double interaction of gibberellin and ascorbate on stem length:

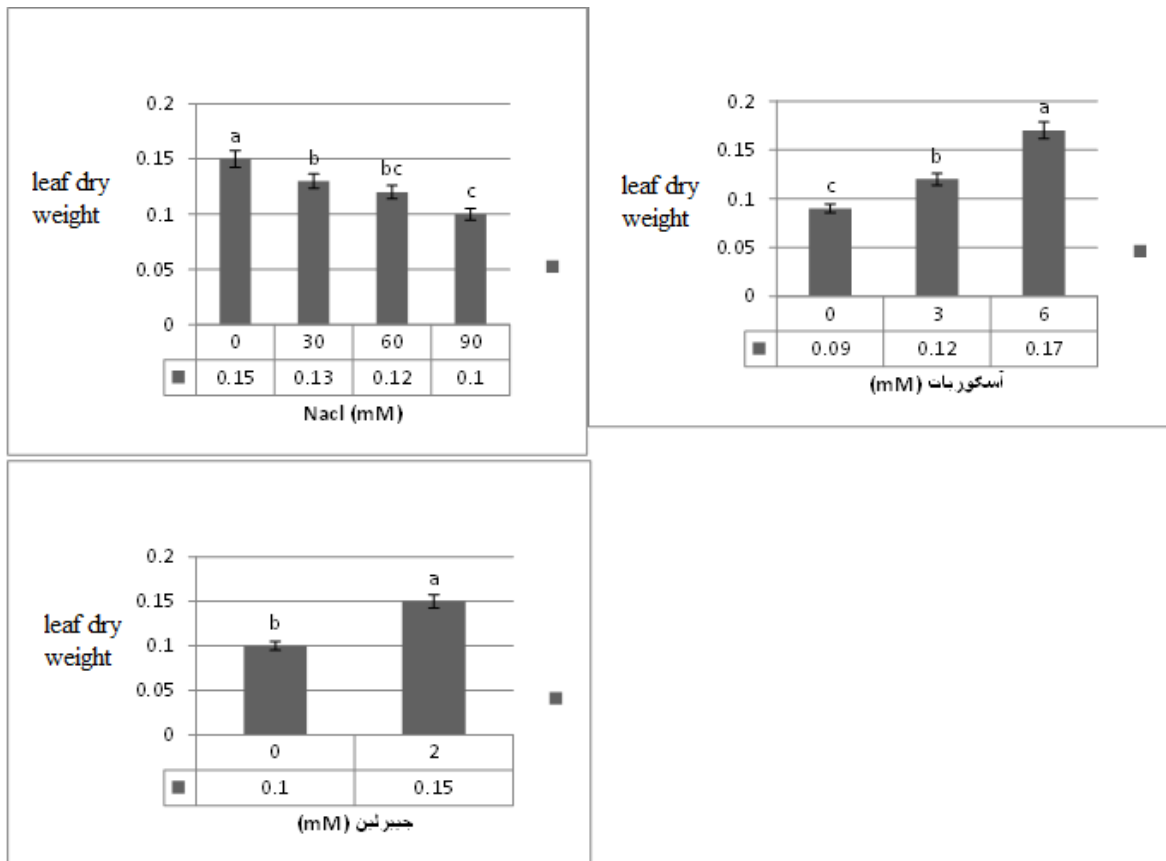


Figure 3: Simple effects of leaf dry weight

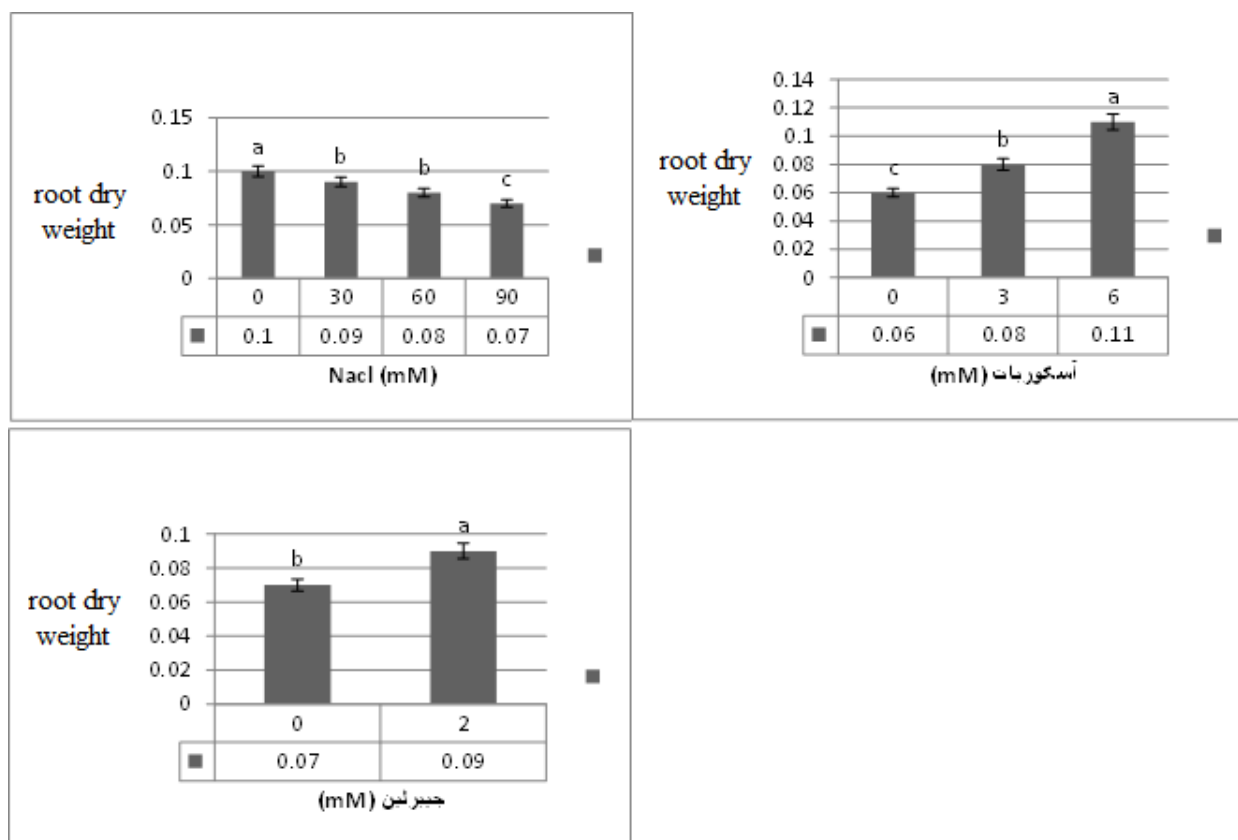


Figure 4: Simple effects of root dry weight

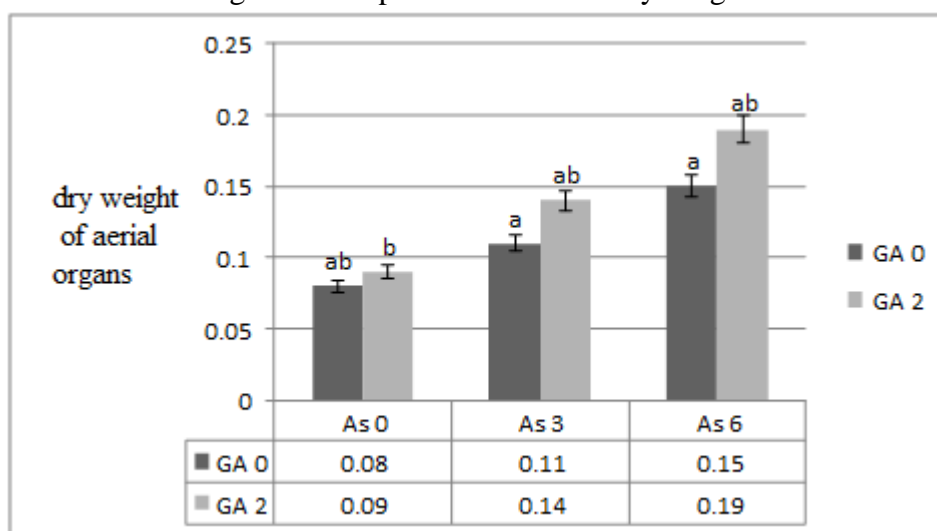


Figure 5: Double interaction of gibberellin and ascorbate on dry weight of aerial organs

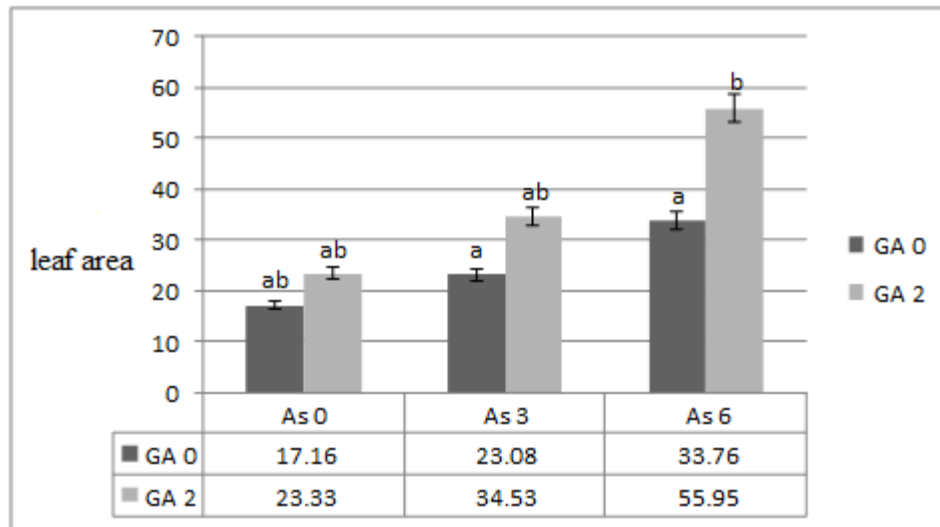


Figure 6: Double interaction of gibberellin and ascorbate on leaf area

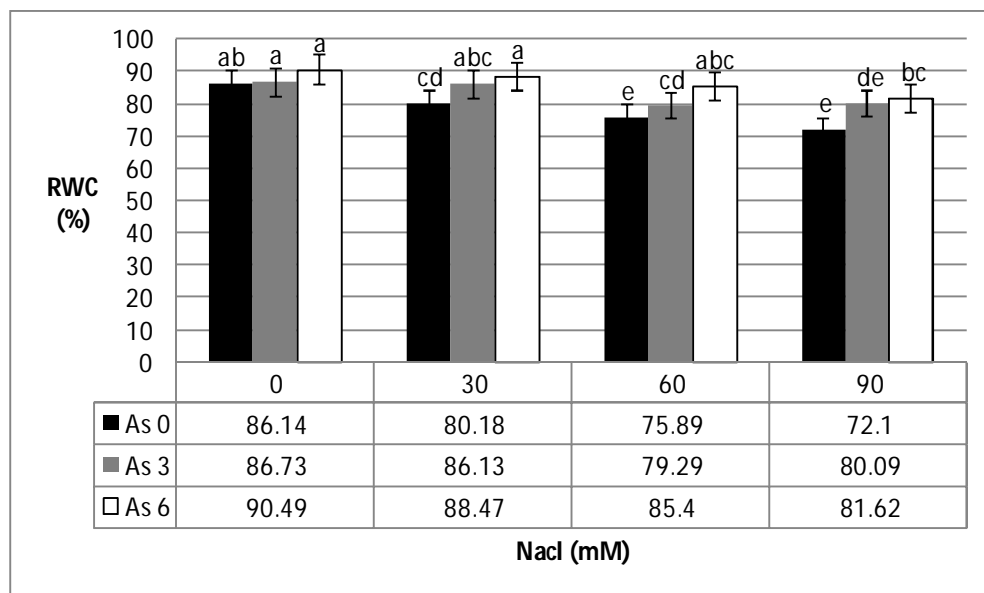


Figure 7: Interaction of salinity and ascorbate on relative water content (RWC)

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Table 1. Results of variance analysis of vegetative traits and relative leaf water content in application of different treatments of salinity, ascorbate and gibberellin

RWC	Leaf area	Leaf dry weight	Root dry weight	Dry weight of aerial organs	Stem length	Root length	Freedom degree	Variation sources
478/3 ^{**}	781/08 ^{**}	0/0065 ^{**}	0/012 ^{**}	0/008 ^{**}	79/68 ^{**}	10/73 ^{**}	3	salinity
293/23 ^{**}	4227/3 ^{**}	0/043 ^{**}	0/029 ^{**}	0/021 ^{**}	49/77 ^{**}	23/55 ^{**}	1	Gibberellin
504/27 ^{**}	4993/04 ^{**}	0/049 ^{**}	0/062 ^{**}	0/058 ^{**}	16/74 ^{**}	54/5 ^{**}	2	Ascorbate
12/25 ^{ns}	18/6 ^{ns}	0/0008 ^{ns}	0/00094 ^{ns}	0/00025 ^{ns}	6/17 ^{ns}	0/47 ^{ns}	3	gibberellin×salinity
27/05 [*]	144/35 ^{ns}	0/0016 ^{ns}	0/00011 ^{ns}	0/00057 ^{ns}	2/31 ^{ns}	0/35 ^{ns}	6	Ascorbate×salinity
23/07 ^{ns}	533/16 [*]	0/00019 ^{ns}	0/00082 ^{ns}	0/0012 [*]	35/25 ^{**}	0/18 ^{ns}	2	gibberellin×Ascorbate
10/56 ^{ns}	37/6 ^{ns}	0/00042 ^{ns}	0/00011 ^{ns}	0/00025 ^{ns}	6/07 ^{ns}	0/50 ^{ns}	6	gibberellin×salinity×Ascorbate
14/08	118/05	0/00074	0/00083	0/00053	5/65	0/68	72	Error
4/53	34/7	21/58	10/11	18/22	9/36	8/94		Variation coefficient (percent)

* and ** explain significant different at the levels of 5% and 1% respectively

Table 2. Mean comparison of salinity simple effect, gibberellin and ascorbate on vegetative traits

RWC (%)	Leaf area (cm ²)	Leaf weight (g)	dry	Root weight (g)	dry	Dry weight of aerial organs (g)	Stem length (cm)	Root length (cm)	
86/14 ^{ab}	27/65 ^d	0/11 ^{efgh}		0/08 ^{de}		0/10 ^{efg}	25/59 ^{cd}	8/47 ^{cd}	S _{0mM} +AS _{0mM}
86/73 ^a	31/25 ^b	0/13 ^{bcd}		0/09 ^{abc}		0/14 ^{bcde}	27/30 ^{bc}	10/09 ^c	S _{0mM} +AS _{3mM}
90/49 ^a	56/45 ^a	0/19 ^a		0/13 ^a		0/20 ^a	30/72 ^a	11/57 ^a	S _{0mM} +AS _{6mM}
80/18 ^{cd}	19/56 ^d	0/1 ^{igh}		0/06 ^{ef}		0/08 ^{igh}	23/43 ^d	8/29 ^{def}	S _{30mM} +AS _{0mM}
86/13 ^{abc}	29/41 ^{cd}	0/12 ^{bcde}		0/08 ^{bcde}		0/13 ^{cdef}	24/93 ^d	9/39 ^c	S _{30mM} +AS _{3mM}
88/47 ^a	49/03 ^{ab}	0/17 ^b		0/11 ^{ab}		0/17 ^b	27/57 ^b	10/67 ^{ab}	S _{30mM} +AS _{6mM}
75/89 ^e	18/47 ^d	0/08 ^{gh}		0/06 ^{fg}		0/08 ^{gh}	22/81 ^d	7/80 ^{ef}	S _{60mM} +AS _{0mM}
79/29 ^{cd}	38/04 ^d	0/11 ^{cdef}		0/08 ^{cde}		0/12 ^{defg}	25/62 ^d	8/66 ^{cd}	S _{60mM} +AS _{3mM}
85/4 ^{abc}	40/87 ^{ab}	0/16 ^{bc}		0/10 ^{abc}		0/15 ^{bc}	27/16 ^{ab}	10/18 ^{bc}	S _{60mM} +AS _{6mM}
72/10 ^e	15/32 ^d	0/07 ^h		0/05 ^g		0/06 ^h	20/76 ^e	7/36 ^f	S _{90mM} +AS _{0mM}
80/09 ^{de}	26/54 ^d	0/10 ^{defg}		0/06 ^{ef}		0/10 ^{hg}	24 ^d	8/26 ^{cde}	S _{90mM} +AS _{3mM}
81/62 ^{bc}	33/08 ^{bc}	0/15 ^{bcd}		0/09 ^{bcd}		0/14 ^{bcd}	25/58 ^{bc}	9/92 ^c	S _{90mM} +AS _{6mM}

Table 3. Mean comparison of salinity simple effect, gibberellin and ascorbate on vegetative traits

RWC (%)	Leaf area (cm ²)	Leaf weight (g)	dry	Root weight (g)	dry	Dry weight of aerial organs (g)	Stem length (cm)	Root length (cm)	
									salinity
87/79 ^a	38/45 ^a	0/15 ^a		0/1 ^a		0/15 ^a	27/87 ^a	10/04 ^a	0 mM
84/92 ^b	32/67 ^{ab}	0/13 ^b		0/09 ^b		0/13 ^b	25/31 ^b	9/45 ^b	30 mM
80/19 ^c	29/13 ^{bc}	0/12 ^{bc}		0/08 ^b		0/12 ^b	25/2 ^b	8/88 ^c	60 mM
77/94 ^d	24/98 ^c	0/10 ^c		0/07 ^c		0/1 ^c	23/45 ^c	8/51 ^c	90 mM
									Gibberellin
80/96 ^b	24/67 ^b	0/10 ^b		0/07 ^b		0/11 ^b	32/18 ^a	8/73 ^b	0 mM
84/45 ^a	37/94 ^a	0/15 ^a		0/09 ^a		0/14 ^a	27/73 ^b	9/72 ^a	2 mM
									Ascorbate
78/58 ^c	20/25 ^c	0/09 ^c		0/06 ^c		0/08 ^c	23/15 ^c	7/98 ^c	0 mM
83/06 ^b	28/81 ^b	0/12 ^b		0/08 ^b		0/12 ^b	25/46 ^b	9/1 ^b	3 mM
86/49 ^a	44/86 ^a	0/17 ^a		0/11 ^a		0/17 ^a	27/76 ^b	10/58 ^b	6 mM

Table 4. Mean comparison of salinity double effect

RWC (%)	Leaf area (cm ²)	Leaf weight (g)	dry weight (g)	Root weight (g)	dry weight of aerial organs (g)	Dry weight of aerial organs (g)	Stem length (cm)	Root length (cm)	
76/08 ^a	17/16 ^{ab}	0/07 ^{ab}	0/06 ^{ab}	0/08 ^{ab}	21/31 ^{ab}	7/56 ^{ab}	GA _{0mM} +AS _{0m} M		
82/23 ^a	23/08 ^a	0/1 ^a	0/07 ^a	0/11 ^a	23/94 ^a	8/6 ^a	GA _{0mM} +AS _{3m} M		
84/58 ^a	33/76 ^a	0/15 ^a	0/10 ^a	0/15 ^a	24/28 ^{ab}	10/01 ^a	GA _{0mM} +AS _{6m} M		
81/08 ^b	23/33 ^{ab}	0/11 ^b	0/07 ^b	0/09 ^a	24/98 ^b	8/4 ^b	GA _{2mM} +AS _{0m} M		
83/88 ^b	34/53 ^{ab}	0/14 ^b	0/09 ^b	0/14 ^{ab}	26/98 ^b	9/6 ^b	GA _{2mM} +AS _{3m} M		
88/41 ^b	55/95 ^b	0/19 ^{ab}	0/13 ^{ab}	0/19 ^{ab}	31/23 ^{ab}	11/15 ^{ab}	GA _{2mM} +AS _{6m} M		