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Effect of Different levels of Salt Stress and Salicylic Acid on Morphological Characteristics of four Mass Native Basils (*Ocimum basilcum*)

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Abstract

In this study, four cultivars of basil (*Ocimum basilcum*) in four salinity stress levels (0, 50, 100 and 150 mM) and tow salicylic acid levels (0 and 0.5 mM) evaluated in a Completely Randomized Design (CRD) base factorial design with three replicates in pots. Results showed significant differences within salinity treatments in all 4 cultivars studied. Highest values measured for traits in cultivars were observed in Shandabad Tabriz and Isfahan cultivars. Also lowest values measured for plant height, fresh and dry weight of stem, stem diameter and internod length were observed in Isfahan cultivar. But salinity effect on these cultivars increased along with severity, linearly. Leaf area was most affected and decreased to 239.16 cm^2 (150 mM) from 784.15 cm^2 in 50 mM. Salicylic acid was positively affected all traits except internodes length and root fresh weight/shoot fresh weight. Generally Sabzevar and Shiraz cultivars showed sensitivity to salinity stress, especially in higher levels. This study should help understand some morphological responses of four native cultivars of *Ocimum basilcum* to salinity stress in Iran.

***Keywords*: abiotic stress, SA, native cultivars, Plant response, growth factors**

Introduction

Vegetables are sources of minerals and vitamins for human diet. With increasing population there an increasing demands of vegetables throughout the world urging a great necessity for increasing the production of vegetables. But the productivity of vegetable is affected due to various abiotic stress factors such as drought, salinity and low nutrient conditions. Soil salinity is becoming a serious problem throughout the world. A large area of the cultivable land is being converted into saline soils due to indiscriminate use chloride and sulphate containing fertilizers to build up of these ions in the soil profile (Maiti et al., 2004)**.** Very little information is available on research on salinity in vegetables (Shanono and grieve, 1999). Salinity affected the quality and productivity of vegetables (Sharma et al., 2001). It is reported that at certain concentrations, saline water might be utilized to irrigate vegetable crops (Kowaski and Pailada, 1995).

Salinization affects many irrigated areas mainly due to the use of brackish water. Worldwide, more than 45 million hectares of irrigated land have been damaged by salt, and 1.5 million hectares are taken out of production each year as a result of high salinity levels in the soil (R Munns and Tester, 2008). High salinity affects plants in several ways: water stress, ion toxicity, nutritional disorders, oxidative stress, alteration of metabolic processes, membrane disorganization, reduction of cell division and expansion, genotoxicity (Zhu, 2007). Together, these effects reduce plant growth, development and survival.

Some plant species are clearly more flexible than others in their requirements for survival in salty environments. Native plants in each country often have superior properties and higher resistance in different fields. However, in most cases the characteristic of these plants have not been well studied and is not known precisely (Sanchez-Blanco et al., 1998). Different adaptive mechanisms may be involved in gradual acclimation to salinity in contrast to adjustment to a sudden shock. The sensitivity to salinity of a given species may change during ontogeny. Salinity tolerances may increase or decrease depending on the plant species and/or environmental factors. For some species, salt sensitivity may be greatest at germination, whereas for other species, sensitivity may increase during reproduction (Marschner, 1986). Plants have evolved several mechanisms to acclimatize to salinity. It is possible to distinguish three types of plant response or tolerance: a) the tolerance to osmotic stress, b) the Na+ exclusion from leaf blades and c) tissue tolerance (R Munns and Tester, 2008).

The different results were dedicated from the effect of salinity stress on the quantitative and qualitative parameters. For instance, the salinity stress significantly affected stem length, root length, shoot wet weight, root wet weight and shoot dry weight, root dry weight, internodes length, stolon length, biomass, Essential oil yield and Essential oil percent. An increase in the salinity lead to reduce in length of stem and root, fresh weight of stem and root, dry weight of stem and root, internodes length, total biomass and essential oil percent and yield (Khorasaninejad et al., 2010). Ashraf and Orooj (2006) reported that salinity treatment lead to reduction of growth and plant developments. Root and shoot length, are most important parameter for salinity stress.

The *Ocimum* genus, belonging to the *Lamiaceae* family, includes herbs and shrubs distributed in tropical and subtropical regions of Asia, Africa and the Americas. The most important species of *Ocimum* genus is *O. basilicum* L.; this species, usually named common basil or sweet basil, is considered economically useful because of their basic natural characteristics as essential oil producers (Lawrence, 1993). Sweet basil is a popular culinary herb used in food and oral care products (Machale *et al.,* 1997). The essential oil of the plant is also used as perfumery. Also, basil is well known as a plant of a folk medicinal used as carminative, galactogogue, stomachic and antispasmodic tonic and vermifugem, also, basil tea taken hot is good for treating nausea, flatulance and dysentery (Sajjadi, 2006). I Available literature data indicates that there is a great deal of diversity in growth characteristics and the composition of essential oil of the genus *Ocimum*. Such observations have been attributed to the abundant cross-pollination that occurs within this genus resulting in considerable degrees of variation in the genotypes (Lawrence, 1988).

It is well known that vegetable crops are grown under high input situation salinity is not a great problem but occasionally farmers irrigate water their crops with brackish-saline water which may affect seedling growth. Therefore, salinity tolerant genotypes could do better under such conditions. Due to an increase in the salinity of irrigation waters and importance of basil (as vegetable and medicinal plant) aim of the present work was to study the morphological adaptation of four native cultivars with salicylic acid under different salinity conditions. Sensitiveness and resistance to salt stress was tested in order to know the response of this cultivars to different salt concentrations in soil solution.

Material and methods

Design and location experiment

The research done in the form of a factorial experiment based on completely randomized design with four salinity levels of sodium chloride (0, 50, 100 and 150 mM), two levels of SA (0, 0.1 mM) and four local accessions of basil, Purple Shandabad Tabriz basil, green Shiraz Basil, green Isfahan Basil and green Basil in the years 2012-2013, with 3 replications in Horticultural research Station, Ferdowsi University of Mashhad, Iran.

Plant material

Seeds were prepared from research Center of respective provinces. Seeds directly were planted in pots with a diameter of 30 cm (span pot) and four plants were kept in each pot. Medium consisting of soil, sand and manure in the ratio 3:1:1, respectively.

Salt solutions and SA treatment

The 6-8 leaf stage plants were prepared for NaCl treatments with different concentrations: 0, 50, 100 and 150 mM. Salicylic acid applied 4 days prior to salinity stress as foliar spray (0 (distilled water) and 0.5) mM.

Characteristics Measuring

To measure the characteristics of the samples were harvested at full bloom and the factors evaluated included: root and stem length (plant height), stem diameter, number of branches and leaves, leaf area, internode length, dry and fresh weight of stem, leaf and root, root length/stem length, root fresh weight/shoot fresh weight and root dry weight/shoot dry weight.

Leaf area samples were determined using a Li-3100 area meter (LI, Lincoln, Nebraska, USA). Root and shoot length was measured after picking up plants at the end of experiment period using a ruler. Roots, leaves and stems of examined plants were weighted after picking up from experimental plots. They were then died (70 ^{0}C for 48 h), and dry weight were recorded.

In this study the ratio of root to shoot were assessed in both weight and length (plant height). Stem diameter and internode distance was measured using a caliper. Number of leaves and branches of plants were counted.

Data analysis

Results of analysis of variance tests were performed with SPSS software and the graph drawing was done with Excel software. The mean yield using Duncan's multiple range test 5% and 1% were performed with SPSS software.

Results and Discussion

Basil cultivars

Analysis of variance showed significant effects of salinity within different growth characteristics of examined basil cultivars, SA and salinity stress ($p < 0.01$ and $p < 0.05$) (Table 1a, b). The cultivar effect was statistically significant in all traits ($p \leq 0.001$ and $p < 0.05$) (Table 1a, b).

Table 1. Mean square analysis of studied traits in this experiment

SV	df	LA (cm^2)	Lnu	Bnu	Ph (cm)	SFW (gr)	LFW (gr)	SDW (gr)	LDW (gr)
SA	1	327850**	6944**	36.2**	404**	281**	497.0**	34.0**	9.5**
S	3	2237089**	35013**	97.6**	1679**	984**	433.9**	119**	22.4**
C	3	246253**	14299**	18.9**	925**	207**	143.3**	15.7**	4.26**
SA*S	3	30071.3*	332*	4.93*	7.29*	5.29*	13.35*	1.79**	0.24^{ns}
SA*C	3	6977.7^{ns}	296*	0.68^{ns}	19.0**	10.4**	2.29^{ns}	1.18*	0.27*
S*C	9	71413.3**	651**	6.7**	37.0**	57.5**	29.6**	8.03**	0.40**
SA*C*S	9	5325.9^{ns}	130^{ns}	0.75^{ns}	6.649*	9.85**	4.79^{ns}	0.69^{ns}	0.09^{ns}
Error	64	9632.9	94.58	1.5	2.57	1.8	3.81	0.412	0.095

(a)

SV	df	SD (mm)	InL (mm)	RL (cm)	RFW (gr)	RDW (gr)	RL / Ph	RFW / SFW	RDW / SDW
SA	1	0.14**	0.02^{ns}	294**	13**	6.09**	0.012^{ns}	0.012**	0.008*
S	3	3.13**	364.13**	1218.3**	169**	14.1**	0.095**	0.013**	0.175**
C	3	1.3**	1178.9**	160.15**	9.66**	3.04**	0.047**	0.001*	0.007**
SA*S	3	0.01^{ns}	1.03^{ns}	7.00^{ns}	0.10^{ns}	0.05^{ns}	0.012*	0.001**	0.004^{ns}
SA*C	3	0.01^{ns}	18.71*	15.00*	1.18**	0.183*	0.004^{ns}	0.001**	0.002^{ns}
S*C	9	0.14**	30.97**	46.75**	0.44**	0.98**	0.016**	0.003**	0.008**
SA*S*C	9	0.01^{ns}	8.88^{ns}	2.07^{ns}	0.06^{ns}	0.07^{ns}	0.002^{ns}	0.000^{ns}	0.003*
Error	64	0.044	6.56	4.12	0.139	0.057	0.004	0.00	0.002

(b)

SA: Salicylic acid, S: Salinity, C: Cultivar, SA*S: Salicylic acid * Salinity, SA*C: Salicylic acid * Cultivar, S*C: Salinity * cultivar, SA*C*S: Salicylic acid * Cultivar * Salinity, E: Error, LA: Leaf Area, Lnu: Leaf number, Bnu: Branch number, Ph: Plant height, SFW: Stem fresh weight, LFW: Leaf fresh weight, SDW: Stem dry weight, LDW: Leaf dry weight, SD: Stem diameter, InL: Inter-nod length, RL: Root length, RFW: Root fresh weight, RDW: Root dry weight, RL/Ph: Root length/plant height, RFW/SFW: Root fresh weight/Shoot fresh weight, RDW/SDW: Root dry weight/Shoot dry weight. no: not significant, *, ** indicate significance at $P < 0.05$, 0.01, respectively

The cultivar effect was statistically significant in all characteristics. Growth characteristics of four basil cultivar studied are shown in Table 2a,b. According to these results, there are noticeable differences among measured traits in them. Highest values are mainly recorded in Shandabad Tabriz and Isfahan cultivars (Table 2a,b). Sabzevar and Shiraz cultivars the next scores, respectively. For grow basil in these salinity conditions at a glance, it seems that between 4 basil cultivar studied, Shandabad Tabriz cultivar be suggested the first candidate, followed by Isfahan cultivar as alternatives. Unlike the Shandabad Tabriz and Isfahan cultivars, two other cultivars are not suitable for irrigation with saline waters (salinity levels higher than 50mM). The salt tolerance can be different at different growth stages of the plant. Therefore in some species, salt sensitivity may be greatest at germination, whereas for other species, sensitivity may increase during reproduction (Marschner, 1986).

Finally, according to this study, we can say that in the 6-8 leaf stage to flowering stage, Shandabad Tabriz and Isfahan cultivars compared with salicylic acid spray, is best option for cultivated in waters with salinity between 100 and 150 mM. Therefore we are needed more research on the response of different cultivars to salinity at different growth stages.

Table 2. Means comparison of investigated growth characteristics of four basil cultivar studied

a)

C	LA (cm)	Lnu	Bnu	Ph (cm)	SFW (gr)	LFW (gr)	SDW (gr)	LDW (gr)
Sh T	560.94[b]	106.58[b]	12.91[a]	48.29[c]	20.62[c]	24.28[b]	5.77[c]	3.25[b]
Sa	475.49[a]	83.58[a]	12.29[a]	36.87[b]	15.74[b]	22.02[a]	4.82[b]	2.59[a]
Is	690.65[c]	133.54[c]	14.37[b]	34.33[a]	14.29[a]	26.83[c]	3.80[a]	3.22[b]
Sh	476.99[a]	81.15[a]	12.87[a]	37.20[b]	14.45[a]	21.48[a]	4.61[b]	2.44[a]

(b)

C	SD (mm)	InL (mm)	RL (cm)	RFW (gr)	RDW (gr)	RL / Ph	RFW / SFW	RDW / SDW
Sh T	3.83[b]	49.78[d]	26.92[b]	8.37[c]	2.97[b]	0.55[a]	0.185[a]	0.360[a]
Sa	3.68[b]	37.16[b]	21.08[a]	7.17[a]	2.34[a]	0.57[a]	0.191[ab]	0.347[ab]
Is	3.31[a]	33.41[a]	22.5[a]	7.80[b]	2.26[a]	0.65[b]	0.186[a]	0.349[ab]
Sh	3.76[b]	39.57[c]	22.08[a]	6.98[a]	2.19[a]	0.58[a]	0.200[b]	0.321[a]

C: Cultivar, Sha: Shandabad Tabriz, Sab: Sabzevar, Isf: Isfahan, Shi: Shiraz, LA: Leaf Area, Lnu: Leaf number, Bnu: Branch number, Ph: Plant height, SFW: Stem fresh weight, LFW: Leaf fresh weight, SDW: Stem dry weight, LDW: Leaf dry weight, SD: Stem diameter, NL: Inter-nod length, RL: Root length, RFW: Root fresh weight, RDW: Root dry weight, RL/Ph: Root length/plant height, RFW/SFW: Root fresh weight/Shoot fresh weight, RDW/SDW: Root dry weight/Shoot dry weight. Figures with different letters in each column are significantly different at P<5%.

Salt stress

Leaf Area and leaf number

Salinity stress showed a significant effect leaf area and leaf number (p<0.01) (Table1a). In leaf area characteristic Highest and lowest values were observed in 50 mM and 150 mM salinity treatment, respectively (Table 2a). Also in leaf number characteristic highest and lowest values were observed in control and severest salinity stress (150 mM) (Table 2a). As happens in many plant cultivars, all four cultivars examined had a lower total biomass and leaf area at the end of the stress period in comparison to control.

However, each cultivars act differently in response to salinity stress. Our results agree with Vahdati *et al* (2012) on Clover plants which reduced leaf area from 184.147 to 40.21 cm^2 during salinity increase. Leaf area represent an important physiologic index in characterization of intensity to some metabolic process (growing, transpiration, photosynthesis, respiration, etc) (Sumalan and Dobrei, 2002). The immediate response of salt stress is reduction in the rate of leaf surface expansion leading to cessation of expansion as salt concentration increases (Wang and Nil, 2000). However in case of *Cakile maritime* leaf area was salinity dependent and significantly stimulated in moderate levels of stress (ksouri et al., 2007). As a morphological point of view reduction in the canopy area can be considered as an avoidance mechanism (Torrecillas *et al.,* 2003). Under saline conditions it is known that the properties or a decreased photosynthesis rate (Franco *et al*., 1997).

Branch number and nod length

Results from branch number and nod length showed that a significant difference between salinity treatments (p<0.01) (Table 1). Highest and lowest amounts measured for this trait were similarly observed in control and severest salinity stress (Table3a, b). Razmjoo et al., (2008) and Abdel-Rahman et al., (2011) reported that increased salinity reduction in the number of branches per plant. Decreasing inter-node length with increasing water salinity and water stress led to the reduction in stem height and other physiological and morphological characteristics of plant. Such decreases in plant height with increasing salinity are typical effects of the accumulation of toxic ions in cells, which adversely affect cell division and expansion (Munns, 1993). Our results agree with Alizadeh *et al*., (2011) on rice plants which reduced inter-node length during salinity increase.

Root and shoot length

Effect of salinity stress was highly significant in this trait (p<0.01) (Table 1). At the end of the experimental period, salinity had led to a significant decrease in root and shoot length. Highest and lowest amounts measured for this trait were similarly observed in control and severest salinity stress (Table3a, b).

Salinity stress results in a clear stunting of plants (Takemura et al., 2000). The root and shoot lengths are the important traits for salt stress because roots are in direct contact with the soil and absorb its water for shoot supply. For this reason, root and shoot lengths provide an important clue to the response of plants to salt stress (Jamil and Rha, 2004). Vahdati *et al*., (2012) stated that an increase in irrigation water salinity reduces root and shoot lengths. Also plant height was significantly inhibited by salinity and treated plants reached 70% of the height of control plants (Navaro *et al*., 2008) which agrees with the results of this study.

Root, shoot and leaf fresh and dry weight

According to UNOVA in Table 1, applying salinity stress significantly affected shoot, leaf and root fresh and dry weights of different organs in examined plants were significantly affected by salinity ($p<0.01$) (Table 1a, b). 22.41 (in 50 mM salinity stress), 27.27 (in 50 mM salinity stress) and 10.62 (in control) grams, respectively shoot, leaf and root fresh weights were decreased to 8.42, 18.27 and 4.58 grams by 266, 149 and 232%, respectively. Also dry weight of the different organs decreased with increasing salinity (Table 3a, b). Salt stress results in a considerable decrease in the fresh and dry weights of leaves, stems and roots (AliDinar et al., 1999; Chartzoulakis and klapaki, 2000).

Stem diameter

Stem diameter also decreased significantly with increasing salinity. Highest and lowest amounts measured for this trait was observed in control and severest salinity stress (Table 3a).

Root to shoot ratio

In root length/ shoot length and fresh weight root/ fresh weight shoot ratio highest amount measured was observed in control and there was no significant difference between 50, 100 and 150 levels salinity stress (Table 3b). Also in dry weight root/ dry weight shoot ratio highest and lowest (0.446 and 0.237) was observed in 150 mM and 50 mM salinity stress, respectively. Many studies have been reported that root dry weight/shoot dry weight, increases during exposure to stress (El-Tayeb, 2005). With increasing salinity, the plants will allocate more nutrients to the roots to grow more and more able to absorb water.

Table 3. Means comparison Effect of different salinity levels on growth characteristics

a)

Salt	LA (cm^2)	Lnu	Bnu	Ph (cm)	SFW (gr)	LFW (gr)	SDW (gr)	LDW (gr)
0	784.15^c	137.46^d	14.87^c	47.66^d	20.46^c	26.83^c	6.37^c	3.85^d
50	840.31^c	128.7^c	14.41^c	44.46^c	22.41^d	27.27^c	6.84^c	3.54^c
100	340.45^b	81.08^b	12.75^b	35.04^b	13.82^b	22.23^b	3.64^b	2.25^b
150	239.16^a	57.6^a	10.42^a	29.54^a	8.42^a	18.27^a	2.17^a	1.87^a

b)

Salt (mM)	SD (mm)	InL (mm)	RL (cm)	RFW (gr)	RDW (gr)	RL / Ph	RFW/ SFW	RDW/ SDW
0	4.05^c	44.11^d	32.5^d	10.62^d	3.52^d	0.68^b	0.226^b	0.343^b
50	3.74^b	41.65^c	24.67^c	8.77^c	2.40^c	0.56^a	0.178^a	0.237^a
100	3.64^b	39.17^b	19.00^b	6.35^b	2.07^b	0.54^a	0.178^a	0.352^b
150	3.17^a	34.98^a	16.42^a	4.58^a	1.76^a	0.56^a	0.181^a	0.446^c

LA: Leaf Area, Lnu: Leaf number, Bnu: Branch number, Ph: Plant height, SFW: Stem fresh weight, LFW: Leaf fresh weight, SDW: Stem dry weight, LDW: Leaf dry weight, SD: Stem diameter, InL: Inter-nod length, RL: Root length, RFW: Root fresh weight, RDW: Root dry weight, RL/Ph: Root length/plant height, RFW/SFW: Root fresh weight/Shoot fresh weight, RDW/SDW: Root dry weight/Shoot dry weight. Figures with different letters in each column are significantly different at P<5%.

SA

The SA effect was statistically significant in all characteristics except inter-nod length ($p\leq0.05$) (Table 1a,b). Salicylic acid was positively affected all traits except internodes length and root fresh weight/shoot fresh weight (Table 4a, b). UNOVA results indicated that there was significant difference the interaction between SA and salinity in leaf area, leaf number, branch number, leaf fresh weight ($p<0.05$) and stem dry weight, root fresh weight/ shoot fresh weight ($p<0.01$). But no significant results was observed in other traits (Table 1a,b). Salicylic acid plays an important role in the defense response to stresses (salts, water, etc) in many plant species (Senaratna et al., 2000). Exogenously applications of salicylic acid helped to increase plant growth significantly in saline conditions (Stevens et al., 2006). Khodary (2004) has reported that SA increased the fresh and dry weight of shoots and roots of stressed maize plants, which is consistent with our results in brassica plants. Also Abd EL-Lateef Gharib (2006) reported that SA increased plant height, number of (branches, nodes and leaves) per plant, leaf area and fresh and dry weight of herbs in basil and marjoram under no-salinity.

Table 4. Means comparison of investigated growth characteristics under condition 0 and 0.5 mM

(a)

SA (mM)	LA (cm^2)	Lnu	Bnu	Ph (cm)	SFW (gr)	LFW (gr)	SDW (gr)	LDW (gr)
0	492.58	92.70	12.50	37.125	14.68	21.37	4.16	2.57
0.5	609.46	109.72	13.73	41.23	17.88	25.93	5.35	3.19

(b)

SA (mM)	SD (mm)	InL (mm)	RL (cm)	RFW (gr)	RDW (gr)	RL/ Ph	RFW/ SFW	RDW/ SDW
0	3.61	39.99	21.89	7.22	2.189	0.570	0.202	0.304
0.5	3.69	39.19	24.89	7.95	2.693	0.602	0.180	0.336

LA: Leaf Area, Lnu: Leaf number, Bnu: Branch number, Ph: Plant height, SFW: Stem fresh weight, LFW: Leaf fresh weight, SDW: Stem dry weight, LDW: Leaf dry weight, SD: Stem diameter, InL: Inter-nod length, RL: Root length, RFW: Root fresh weight, RDW: Root dry weight, RL/Ph: Root length/plant height, RFW/SFW: Root fresh weight/Shoot fresh weight, RDW/SDW: Root dry weight/Shoot dry weight.

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