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HAMID MOHAMMADI[1, 2*], JAVID KARDAN[1]

[1] Faculty of Agriculture, Azarbaijan Shahid Madani University, Tabriz-Iran
[2] The Halophyte Biotechnology Research Center, Azarbaijan Shahid Madani University, Tabriz-Iran. Correspondence to: Hamid Mohammadi,
E-mail: hm34476@yahoo.com; hmohammadi@azaruniv.edu

Morphological and physiological responses of some halophytes to salinity stress

ABSTRACT

A pot experiment was conducted to examine whether the morphological and physiological characteristics of some halophytes may be affected by salt stress. For this purpose, a factorial experiment based on randomized complete block design was carried out with three replications. The treatments were some halophytes (*Salicornia europaea*, *Atriplex leucoclada,* and *Kochia scoparia*) and salinity stress levels [Electrical conductivity 0 (Hoagland's solution), Hoagland's solution consisting of 100, 200, 300 and 500 mM NaCl]. Among the halophytes tested, *Salicornia europaea* had significantly higher shoot and root of dry matters compared to the other halophytes in all salt treatments. Salinity stress resulted in an increase in photosynthetic pigments up to 200 mM and thereafter, it decreased in all of the studied plants. Photosynthetic pigments, ranked in a descending order, were high in *Kochia scoparia*, *Salicornia europaea,* and *Atriplex leucoclada*. In addition, salinity stress led to an enhancement in malondialdehyde (MDA) and H_2O_2. The tolerance of *Salicornia europaea* under high salinity stress was associated with low MDA and H_2O_2 contents as well as high contents of photosynthetic pigments. The shoot and root Na^+ increased considerably by augmenting the salinity levels in all halophytic plants; however, there was a significant difference among halophytes at higher salinity levels. The shoot K^+ decreased by increasing the salinity levels, but K^+ partitioning pattern varied among the halophytes. Under saline conditions, the shoot and root Na^+/K^+ ratio of all halophytes grew. The highest and the lowest of Na^+ were observed in *Salicornia europaea* and *Kochia scoparia*, respectively. Thus, the Na^+/K^+ ratio could be considered as an indicator of salt evaluation. Nitrogen, protein content, dry matter digestibility (DMD), and metabolizable energy (ME) were high in *Salicornia europaea* plants in comparison to other plants at 200–500 mM salinity levels; in contrast, acid detergent fiber (ADF) and netural detergent fiber

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(NDF) were low. According to the results of this study, the tolerance of halophytes towards NaCl is possibly due to the differences in damage from reactive oxygen species (ROS), especially H_2O_2, and toxicity to metabolism Na^+.

Keywords: salinity stress, halophytes, morphological parameters, physiological parameters

INTRODUCTION

Salt tolerance is very complex in the majority of plant species, because salt stress is known to induce tissue dehydration, ion toxicity, nutritional imbalance, or a combination of these effects (21). Approximately one billion hectares of lands in the whole world are saline, constituting a serious threat to farmers (13). Increased soil salinity is one of the natural detrimental factors that have a negative effect on plant growth and development (12). Plants can be divided into two broad groups on the basis of their response to high concentrations of salts. Halophytes are native to saline soils and complete their life cycles in that environment. Glycophytes or nonhalophytes, are not able to resist salts to the same degree as the halophytes do (37). With an increasing amount of arable land undergoing salinization (36) accompanied by increasing food demands from the growing human population, the need to develop salt-tolerant crops and to identify the degree of salinity tolerance within crops is becoming more important. It has been reported that plant growth, metabolism and nutrient uptake are adversely affected under saline conditions (32).

Generally, two types of mechanism of salt tolerance have been identified in higher plants (21). In the first mechanism, the growing medium salinity induces specific ion effects on plants, and the plants, in turn, respond by excluding toxic ions such as Na^+ and Cl^- from the leaves in different ways. In the second mechanism, the ions absorbed by cells are accumulated in the vacuoles. However, the patterns of ion accumulation have been successfully used in discriminating between salt-tolerant and salt-sensitive plants (21).

Salinity stress causes extensive crop losses in many parts of the world due to the lack of salt tolerance in major field crops. Enhancing tolerance to salinity in crops will be an important goal of plant breeders in future to ensure food supply for the growing world population (12). A wide range of variation in the level of salt tolerance found in halophytes clearly demonstrates the genetic basis of salt tolerance. Although it is widely recognized that the genetic and physiological basis of salt tolerance in plants is inherently complex owing to the involvement of multigene controlled traits or mechanisms, the lack of a thorough understanding of these mechanisms and their contribution toward salt tolerance is a major limitation to developing salt-tolerant plants (2).

An improved osmotic adjustment is a major factor in growth stimulation of halophytes by high Na supply. Growth responses of halophytes to Na under saline conditions reflect the need for an osmoticum during osmotic adjustment to salinity stress. Many halophytes osmotically compensate for high external osmotic potential by accumulating Na salts, often NaCl from the environment. Growth stimulation by Na is particularly apparent in the Chenopodiaceae and among nonchenopods (23).

Iran, like other developing countries, is situated in the arid and semi-arid areas and is faced with a series of problems, including limited natural resources, poor water quality, soil affected with salinity, and food shortages. Thus, extensive research, particularly into the management of soils affected by salinity, must be performed in order to solve these problems. In the cultivation of halophytes, it appears that management practices on soils are ideal, especially when there is insufficient good-quality water. Halophyte has been highly regarded by researchers in many countries (3, 4). A number of plant species have been selected for their production or potential supply when they are irrigated

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with saline water and seawater (16, 18). Some halophyte species have been domesticated as forage plants (5, 26). Shoots of *Salicornia europaea bigellovi*, *Sesuvium portulacastrum*, *Chenopodium album*, *Portulaca oleracea*, and *Suaeda maritima* are utilized for vegetables, salads and pickles in various parts of the country (10). The experiment was aimed at investigating the number of the reactions of halophytic plants in soils affected by salinity as a result of using poor-quality water in order to overcome desertification and utilization of soil and too salty water.

MATERIALS AND METHODS

PLANT MATERIALS AND TREATMENTS

The seeds of studied halophytes (*Salicornia europaea*, *Atriplex leucoclada* and *Kochia scoparia*) were obtained from seed and plant Agricultural Research Institute Karaj, Iran. All seed samples were surface sterilized with 10% sodium hypochlorite solution for 5 min and washed three times with distilled water.

In a pot experiment, halophytes were exposed to NaCl salinity, using a complete blocks randomized design with factorial arrangement and each treatment was replicated 3 times. Plants were grown in pots (with 25 cm diameter) containing perlit. Ten seeds were sown in each pot. After germination the seedlings were thinned to three of uniform size per pot. Supplementary light was provided in the greenhouse for 16 h per day. The daytime and nighttime temperatures of the greenhouse were 24.5 and 14.8^{0}C, respectively. Irrigation was made using 6 saline solutions (control, 100, 200, 300 and 500 mM) in a ratio of 1:1 of NaCl/$CaCl_2$ prepared in half-strength Hoagland solution. The NaCl concentrations in Hoagland's solution (25) were used to raise the plants following sowing. The salt treatments were begun following sowing.

All measurements were made at vegetative stage after 42 days of salt treatments. Plants were separated into shoots and roots and washed with distillated deionised water and weighed after being shade-dried. Some samples were frozen in liquid nitrogen for 2 min, then stored at -70^{0}C for all measurements such as plastid pigments, MDA and H_2O_2 contents.

DETERMINATION OF NA AND K IONS

Ion Na and K measurements were taken from the 2 N chloride acid extract of the samples that had been burned at 600 ºC for 4 h, using a flame photometer (PF5 Carl Ziess Germany model) (31).

DETERMINATION OF H_2O_2 CONTENT

Hydrogen peroxide content in leaves were determined according to Velikova et al. (2000). Flag leaf tissues (0.07 g) were homogenized in an ice bath with 5 ml of 0.1% (w/v) trichloroacetic acid (TCA). The homogenate was centrifuged at 12,000g for 15 min and 0.5 ml of the supernatant was added to 0.5 ml of 10 mm potassium phosphate buffer (pH 7.0) and 1 ml of 1 m KI. The absorbance of the supernatant was measured at 390 nm (38).

DETERMINATION OF THE MDA CONTENT

For the measurements of lipid peroxidation in leaves, the thiobarbituric acid (TBA) test, which determines MDA as an end product of lipid peroxidation (24), was used. An aliquot (0.07 g) of flag leaves was homogenized in 5 ml of 0.1% (w/v) TCA solution. The homogenate was centrifuged at 12,000 *g* for 15 min and 0.5 ml of the supernatant was added to 1 ml of 0.5% (w/v) TBA in 20% TCA. The mixture was incubated in boiling water for 30 min, and the reaction was stopped

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by placing the reaction tubes in an ice bath. Then the samples were centrifuged at 10,000 *g* for 5 min, and the absorbance of the supernatant was measured at 532 nm, subtracting the value for non-specific absorption at 600 nm. The amount of MDA-TBA complex (red pigment) was calculated from the extinction coefficient 155 mM^{-1} cm^{-1}.

PIGMENTS DETERMINATION

Chlorophyll (Chl) and carotenoids (Car) were estimated by extracting the leaf material in 80% acetone. Absorbances were recorded at 663, 645 and 470 nm (29). Photosynthetic pigment contents were calculated from the equations as described by Lichtenthaler & Wellburn (29).

FORAGE QUALITY

Crude protein (CP %) of the shade-dried samples was determined using the Kjeldahl technique (1). Acid detergent Fiber (ADF) and Neutral Detergent Fiber (NDF) were determined according to AOAC (1980) method. Dry matter digestibility (DMD) (34) was estimated by the formula DMD % = 83.58- 0.824 ADF % + 2.626 N % suggested by Oddy et al. (34). Metabolizable energy (ME) was predicted with the equation ME = 0.17 DMD % – 2 suggested by A.O.A.C. (1).

STATISTICAL ANALYSIS

The data were analyzed by SAS statistical package and the mean comparisons were made following Duncan's Multiple Range Test at P = 0.05 by MSTATC (version 2.10, Inc, Michigan state university).

RESULTS AND DISCUSSION

GROWTH PARAMETERS

Analysis of variance (ANOVA) indicated that the shoot and the root dry matter (DM) were significantly ($P<0.05$) altered under the treatments employed (Table 1). However, the maximum shoot and root DM were observed in plants exposed to 100 and 200 mM of NaCl, respectively (Table 2). By increasing the salinity stress (200–500 mM), the shoot and the root DM decreased in the plants studied, but this reduction was much less in *Salicornia europaea*. In other words, the exposure to 400 and 500 mM NaCl severely decreased the root and the shoot DM, respectively, except for *Salicornia europaea* which could grow at 500 mM, and all *Atriplex leucoclada* plants died under 500 mM salt stress. The results demonstrated that the growth of the plants studied was stimulated by increasing salt concentrations, and a significant difference was observed between the three plants genus. Similarly, it was reported that the effect of salinity on growth varies among halophytes (14), and dry mass is stimulated under salinity stress (20). Our findings also confirmed that the overall growth and development of halophytes plants decreased as the salt concentrations increased, which, as previously mentioned, could be due to the reduction of water potential that is responsible for plant devel-

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opment. Such growth stimulation at moderate salinity in halophytes may be attributed to the improvement in shoot osmotic status as a result of increased ion uptake (33). Reduced growth at high salinities is probably associated with the reduced turgor and the high energy cost of massive salt secretion and osmoregulation.

PHOTOSYNTHETIC PIGMENTS

Data analysis showed that there were significant differences in the plastid pigments (chlorophyll a, chlorophyll b, total chlorophyll and carotenoids) under salinity stress, type of halophytes and their interactions (Table 1). The effectiveness of the process of salt concentrations in various halophytes was different in each halophyte. The highest content of chlorophyll (chlorophyll a, b, and total chlorophyll) was observed in *Salicornia europaea* with 100 mM NaCl. In all of the three halophytes, the content of chlorophyll decreased by increasing salinity stress (Table 2). It appears that reduced photosynthesis and the subsequent decreased growth under stress conditions, generally result from the reduction in chlorophyll content. The main reason for the decline in chlorophyll content, especially under severe stress conditions, may be the loss of the activity of enzymes involved in chlorophyll synthesis (ALA-Hydrogenase) (35). In our present study, the plants exposed to lower concentrations of salinity (100 mM) had an improvement in their photosynthetic pigment contents when compared to the other concentrations applied (Table 2). Furthermore, the lowest content of carotenoid was observed in 500 mM salinity stress. In contrast, the carotenoid content in 100 mM salinity stress was more than that in the control, which was probably due to the antioxidant system induced by low concentrations of salt. This observation agrees well with the findings reported by Ashraf et al. (2009) who stated that carotenoid has ROS scavenging capability under salt stress (6).

MALONDIALDEHYDE (MDA) AND HYDROGEN PEROXIDE (H_2O_2)

There was a noticeable difference between halophyte types in MDA and H_2O_2 contents under salinity stress (Table 1). By increasing the salt concentrations, MDA and H_2O_2 levels increased in all three halophytes (Table 2). The minimum and the maximum contents of MDA were observed in the plants exposed to salt concentrations at 100 mM in *Salicornia europaea* and 500 mM in *Kochia scoparia*, respectively (Table 2). A trend similar to MDA changes was found for H_2O_2 accumulation under applied treatments. The level of H_2O_2 in *Salicornia europaea* plants exposed to 100 mM was lower than that of other plants; therefore, lipid peroxidation was less pronounced in such plants (Table 2). As can be seen, MDA is produced through lipid peroxidation and salt stress by inducing oxidative stress and production of ROS, leading to the oxidation of proteins and lipids, and

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Table 1. Analysis of variance (ANOVA) for studied traits in halophytic plants under salinity stress

Traits	Mean squares for source of variation				
	Block	Factor a (Salinity stress)	Factor b (Halophytes)	Interaction a×b	Error
Root dry weight	0.0003 ^{ns}	0.0863**	0.8430**	0.0161**	0.0005
Shoot dry weight	0.0421 ^{ns}	0.6947**	0.0375**	0.0510**	0.0041
Chlorophyll *a*	0.0022 ^{ns}	0.05**	0.017**	0.004**	0.0018
Chlorophyll *b*	0.0007 ^{ns}	0.026**	0.025**	0.003**	0.0017
Total chlorophyll	0.0035 ^{ns}	0.14**	0.077**	0.01**	0.0044
Carotenoid	0.22 ^{ns}	4.24**	7.10**	0.27**	0.23
Shoot MDA contents	0.07 ^{ns}	15.62**	0.48**	8.30**	0.505
Shoot H_2O_2 contents	0.12 ^{ns}	16.98**	7.53**	11.07**	0.23
Root Na^+	3.83 ^{ns}	282.11**	442.17**	180.43**	1.24
Shoot Na^+	92.28 ^{ns}	5220.99**	8406.76**	3311.00**	50.76
Root K^+	3.54 ^{ns}	62.01**	36.52**	8.52**	0.55
Shoot K^+	3.60 ^{ns}	473.04**	304.30**	70.59*	4.18
Root Na^+/K^+ ratio	1.51 ^{ns}	26.30**	33.18**	16.50**	0.50
Shoot Na^+/K^+ ratio	6 ^{ns}	99.41**	240.09**	78.92**	1.57
N%	0.0005 ^{ns}	0.86**	0.16**	0.034**	0.0007
CP%	0.02 ^{ns}	33.82**	6.42**	13.30**	0.029
ADF	0.17 ^{ns}	83.90**	152.59**	195.39**	0.14
NDF	0.23 ^{ns}	305.02**	564.73**	555.63**	0.06
DMD	0.11 ^{ns}	1138.40**	1163.29**	744.67**	0.10
ME	0.0033 ^{ns}	23.56**	29.36**	14.03**	0.002

*, ** Significantly different at the 5 and 1% probability level, respectively, ^{ns} not significant.

subsequent destruction of membrane structure (17). Moreover, it is likely that the accumulation of H_2O_2 is caused by the lack of superoxide dismutase activity and its isozymes under salt stress. However, H_2O_2 can improve the tolerance of plants towards salt stress, because it is an active oxygen species, which is widely generated in many biological systems and mediates various physiological and biochemical processes in plants (28).

IONS ACCUMULATION

The root and the shoot Na^+ concentration increased considerably in all halophytes with the external salt concentration, but the response of halophyte types varied in this regard (Table 1). Increased salt concentration induced the accumulation of Na^+ in the root and the shoot of all halophytes; however, this increase was higher in *Atriplex leucoclada*, *Salicornia europaea* and *Kochia scoparia*, in a

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Table 2. Mean comparison of physiomorphological and biochemical traits in halophytic plants under salinity stress

Salinity stress (mM)	Halophytes	Root dry matter (gr)	Shoot dry matter (gr)	Chl a (mg gr^{-1}FW)	Chl b (mg gr-1 FW)	Total Chl (mg gr^{-1} FW)	Car (mg gr^{-1} FW)	H_2O_2 contents (µmol g^{-1}FW)	MDA contents (nmol/g FW)	Root Na^+	Shoot Na^+
0	*Salicornia*	0.653 a	0.916 a	0.209 cde	0.108 de	0.317 de	1.745 bcd	0.785 fg	0.495 de	1.383 ij	1.500 g
	Atriplex	0.118 fg	0.714 cd	0.217 bcde	0.116 cde	0.333 de	1.613 bcd	1.058 f	0.746 cde	2.160 hi	5.333 fg
	Kochia	0.125 fg	0.692 cd	0.268 abc	0.147 cde	0.415 bcd	2.832 a	0.776 fg	0.097 e	1.383 ij	0.613 g
100	*Salicornia*	0.663 a	0.801 bc	0.309 a	0.274 a	0.583 a	2.766 a	1.051 f	0.662 cde	7.600 f	36.900 d
	Atriplex	0.133 ef	0.794 bc	0.299 ab	0.185 bcd	0.484 abc	2.325 abc	2.679 d	1.503 bcd	14.583 cd	49.000 d
	Kochia	0.167 ef	0.729 cd	0.277 abc	0.202 abc	0.480 abc	3.124 a	2.288 de	1.745 bcd	2.300 hi	5.867 fg
200	*Salicornia*	0.557 b	0.637 de	0.168 def	0.202 abc	0.370 cd	1.471 cd	1.619ef	1.915 bc	10.200 e	47.800 d
	Atriplex	0.147 ef	0.716 cd	0.203 cde	0.124 cde	0.327 de	1.546 cd	3.094 d	2.798 b	23.567 b	111.000 a
	Kochia	0.189 de	0.880 ab	0.271 abc	0.234 ab	0.505 ab	3.135 a	3.934 c	2.471 b	3.600 gh	11.067 efg
300	*Salicornia*	0.495c	0.541 e	0.158 ef	0.142 cde	0.300 de	1.207 d	2.576 d	2.810 b	13.800 d	62.400 c
	Atriplex	0.071 gh	0.353 f	0.150 ef	0.081 e	0.231 e	1.124 d	6.021 a	5.594 a	31.033 a	112.317 a
	Kochia	0.118 fg	0.631 de	0.244 abcd	0.152 bcde	0.396 bcd	2.481 ab	4.067 c	2.512 b	4.467 g	15.333 ef
500	*Salicornia*	0.225 de	0.320 f	0.111 f	0.106 de	0.217 e	0.994 d	4.913 b	4.383 a	15.850 c	81.650 b
	Atriplex	0.000 i	0.000 g	0.000 g	0.000 f	0.000 f	0.000 e	0.000 g	0.000 e	0.000 j	0.000 g
	Kochia	0.020 hi	0.074 g	0.142 ef	0.153 bcde	0.296 de	1.626 bcd	6.746 a	5.150 a	5.550 g	20.200 e

*: Means followed by the same letter/s in each column are not significantly different based on Duncan's Multiple Range Test (n=3).

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descending order (Table 2). Organic compatibles, whose function is to balance the osmotic potential in the vacuole due to the accumulated Na^+ and Cl^- ions, are synthesized by the halophytes with differences in their carbon and nitrogen costs (15). In other words, *Atriplex leucoclada* plants could accumulate more Na^+ in the root and the shoot by increasing salinity stress (Table 2). When the amount of sodium increases in the root zone, this may lead to changes in cell osmotic pressure, and, plasmolysis besides the reduction in absorption of selective elements (19). Our results showed that although *Atriplex leucoclada* had lower biomass production, it accumulated more shoot Na^+ concentration and, hence, maintained considerably higher shoot Na^+/K^+ ratios as compared with the other halophytes (Table 2). The findings of this research corresponded with those reported by Khan et al. (2000) about *Atriplex* (27).

According to the results, salt concentration, halophyte types and their interactions had significant effects on the content of root and shoot potassium (Table 1). Decreased accumulation of potassium in the root and the shoot was observed along with increased salt concentration in all halophytes. The maximum accumulation of potassium in the root and the shoot was related to *Atriplex leucoclada* without salt treatment (Table 2). Maintaining high levels of potassium is considered as the tolerance mechanism towards saline conditions (11), because potassium plays an important role in maintaining the water balance in plants and the continuation of the activity of enzymes (7). Under salt stress, sodium disrupts potassium uptake, thus reducing the accumulation of potassium.

FORAGE QUALITY PARAMETERS

The evaluated factors had significant impacts on nitrogen, protein content, DMD, and ME (Table 1). The highest nitrogen and protein contents were observed in *Atriplex leucoclada*, *Salicornia europaea* and *Kochia scoparia* plants exposed to salinity stress, respectively (Table 2). At 200–500 mM salinity stress levels, nitrogen, protein content, DMD, and ME were higher in *Salicornia europaea* plants in comparison to other plants (Table 2). The results indicated that high salinity induced a reduction in nitrogen uptake in *Atriplex leucoclada* and *Kochia scoparia* halophytes, so that the highest nitrogen in the above-mentioned plants was observed in low salinity levels (Table 2). The nitrogen element is an important nutrient whose uptake is disrupted by the presence of salt. Increasing the salt concentration in the root zone leads to losses of root hairs, thus negatively affecting nitrogen metabolism (9). Under salt stress, the reduction in protein content can be due to the degradation of proteins and the lack of their re-synthesis (22).

ADF and NDF were significantly ($P < 0.01$) affected by the experimental treatments (Table 1). The highest values of ADF and NDF (Table 2) were obtained in high salinity stress in all halophytes, but these values were lower in *Salicornia*

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europaea plants in comparison to other plants (Table 2). In other words, *Salicornia europaea* plants under high salinity have good forage quality and can be utilized for planting and sustainable development should be considered in saline areas. It has been demonstrated that among various common chemical determinations of plant materials; CP, DMD, and ME are mainly considered for evaluation of forage quality. In our study, crude protein content decreased with increasing salinity stress, whereas ADF and NDF of whole shoot increased under salinity stress. In other words, increasing of salinity stress caused a significant decrease in forage quality. These results may be due to a considerable change of lignin content. Also, more of tolerant plants to salt include high contents of non-protein nitrogen. For example, Benjamin et al. (8) reported that 42% of the nitrogen in *Atriplex barclayana* is non-protein nitrogen. This nitrogen will only be available for conversion to microbial protein in the rumen if a good supply of metabolisable energy is available or if added to a protein deficient feed (30).

CONCLUSIONS

The results of our study suggested that the growth of the halophytes studied is differently inhibited at high salinity. Reduced growth at high salinities is probably associated with insufficient osmoregulation and reduced turgor. The halophytes studied accumulate Na^+ in their root and shoot with different concentrations. Our findings demonstrated that *Salicornia europaea* plants could manage Na^+ in their root and shoot by increasing salinity stress, in contrast, *Atriplex leucoclada* plants could accumulate more Na^+ in their root and shoot at high salinity stress, and cell death may occur. The effect of salinity stress on growth and other parameters could result from the negative impact of salinity on photosynthesis pigments and imbalance nutrition that arise from toxicity to metabolism Na^+ and damage from reactive oxygen species (ROS). Furthermore, Nitrogen, protein content, DMD, and ME were high in *Salicornia europaea* plants in comparison to other plants at 200–500 mM salinity levels. Our results indicated that regardless of the reduction in growth parameters, *Salicornia europaea* is a valuable candidate crop to be employed under high salinity, where other traditional crops cannot grow or produce under high levels of salinity.

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