

THE EFFECT OF SALINITY ON THE GROWTH OF THE HALOPHYTE *ATRIPLEX HORTENSIS* (CHENOPODIACEAE)

S. SAI KACHOUT¹ – A. BEN MANSOURA² – K. JAFFEL¹ – J.C. LECLERC³ – M.N. REJEB² – Z.
OUERGHI¹

¹*Laboratoire de physiologie végétale, faculté des sciences de Tunis, campus universitaire,
Tunisie*

²*Institut National de Recherche en Génie Rural, Eaux et Forêts (INRGREF), Tunisie*³*Equipe
d'Ecophysiologie Appliquée Faculté de Sciences et Techniques Saint Etienne, France*

**Corresponding author
e-mail: salmasey@yahoo.fr*

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Abstract. The effect of salt stress on the growth of the *Atriplex hortensis* was determined by growing plants in soils receiving a one-time irrigation of nutrient solution containing low, medium, and high levels of NaCl. *Atriplex* plants were grown in sand culture under controlled greenhouse conditions. The experiment was compared to two varieties of *A. hortensis*. Growth and yield were progressively declined by increasing NaCl concentrations. *Atriplex hortensis*, an annual halophyte, exhibits significant reduction in height and biomass under saline stress conditions. The photosynthetic activity decreases when plants are grown under saline conditions leading to reduced growth and productivity. Low levels of salinity (5 g/l NaCl) did not cause substantial inhibition of growth but increasing concentrations of salt induced a progressive decline in length and weight of the plants. Salt stress induced a significant decrease in leaf area, but it had no significant effect on leaf water content. The results of present study indicate no differences in salinity tolerance in both *Atriplex* plants. Another possible conclusion is that improved tolerance to salt stress may be accomplished by decline in growth and photosynthetic activity. Based on these findings the tolerant *Atriplex* can be grown in moderately NaCl-contaminated soils.

Keywords: *Atriplex*; salt stress; biomass production; chlorophyll concentration; water content; stomatal conductance.

Introduction

Salinity is one of the most important environmental factors limiting crop production of marginal agricultural soils in many parts of the world. Salinity effects on plants include ion toxicity, osmotic stress, mineral deficiencies, physiological and biochemical perturbations, and combinations of these stresses [31, 67, 68, 74, 102]. Salt ion toxicity has numerous deleterious effects on plants such as denaturing cytosolic enzymes [68]. Many investigations on quantification of salt tolerance of plant species have been based on experiments in which NaCl was the predominant salt [18, 42, 44]. Salt stress affects many aspects of plant metabolism and, as a result, growth and yields are reduced. Excess salt in the soil solution may adversely affect plant growth either through osmotic inhibition of water uptake by roots or specific ion effects. High concentrations of salts have detrimental effects on plant growth [23, 63] and excessive concentrations kill growing plants [20]. Halophytes are distinguished from glycophytes by their tolerance of saline conditions. The use of halophytic plants in pasture and fodder production on saline soils is the only economically feasible solution available [40]. Halophytes are plants that grow naturally in saline environments, such as salt marshes, salt spans and salt deserts [36]. Halophytic species differ widely in the extent to which they

accumulate ions and their overall degree of salt tolerance [25, 26]. *Atriplex* species (saltbushes) are dominant in many arid and semi-arid regions of the world, particularly in habitats that combine relatively high soil salinity with aridity [60, 75, 77]. Several species belonging to the genus *Atriplex* are well adapted to harsh environmental conditions and therefore constitute a useful material for the identification of physiological mechanisms and genes involved in abiotic stress resistance [14, 90, 100]. *Atriplex* is a halophyte saltbush species highly resistant to drought [50], salinity [7], and heavy-metal stress [55]. *Atriplex* spp. are among a group of halophytes that complete their life cycle at high salinity levels and have the ability to accumulate high concentrations of micronutrients much greater than the required minimum [82, 101]. *Atriplex* spp. has increased biomass production with salt increments in the growth medium ranging from 5 to 10 g l⁻¹ NaCl [41, 72]. A similar promotion of growth has also been reported for other halophytic species [9, 18].

It is suggested that *Atriplex* spp. may be more suitable for revegetating very saline soils and also be a good source of productive feed [33, 76].

These plants could be promising, since *Atriplex* species have special bladders in the leaves that act as salt sinks for the removal of the excess of salt [47]. In the arid zones and other dry lands, halophytic plants often dominate because of their tolerance to drought and salinity [24]. *Atriplex* spp. are among the most salt-tolerant higher plants. They have adapted to salinity by tolerating salts internally and/or by excreting salt [41, 62]. However, the presence of high salt levels does not seem to be required for optimal growth. It is reported that soil salinity suppresses shoot growth more than the root growth [57, 81]. [23] reported that salinity causes reduction in leaf area as well as in rate of photosynthesis, which together result in reduced crop growth and yield. Also, high concentration of salt tends to slow down or stop root elongation [45] and causes reduction in root production [23].

In general, low salinity levels do not appear to have a deleterious effect on the growth of *Atriplex* spp. and may actually stimulate growth [3, 15, 59, 104]. However, high salinity levels may cause a reduction in total growth of *Atriplex* spp., especially in leaf biomass [6, 28, 66, 80, 85, 95, 97].

In this research, growth parameters such as dry mass, leaf area, plant height and root elongation of *Atriplex* plant were determined in order to get a general view of the effects of salinity on the overall growth of *A. hortensis*. Chlorophyll concentration, water content and stomatal conductance of plants were measured in order to understand how salinity affects the physiology of plants. Among Chenopodiaceae the genus *Atriplex* is the most studied, probably because *Atriplex* species are used for rehabilitation of saline soils.

Materials and Methods

Plants

Seeds of *Atriplex hortensis* were taken from a botanic garden: Denmark House, Pymoor, Ely, Cambridgeshire (CN seeds). Plants were grown in a greenhouse at 28/20 °C (day/night) under a photoperiod of 16 h.

Atriplex seeds were planted in pots of 14 cm diameter and 25 cm depth; each pot contained 3,5 kg soil. The soil characteristics were as follows: sandy loam in texture, sand 52.3%; silt 10.5%; clay 12%; pH 8.75 and organic matter 1.5%. Ten seeds per pot

and six replicates were used for each treatment. Irrigation was applied on a two days basis to achieve soil water field capacity level. Treatments were as follows:

Control and Salt Treatment:

a- control (0 NaCl) b- 5 g/l NaCl

c- 10 g/l NaCl d- 15 g/l NaCl

Growth parameters of Atriplex

The plant height (cm), root system length (cm) and leaf area (cm²) were measured (after 3 months). Plants were washed with distilled water and separated into shoots and roots. The dry weight (dw) was obtained after oven drying the plants at 60 °C for 48 hours. The dry weights of the root and shoot systems were also determined.

The effect of salinity on physiology parameters was studied in terms of water content, chlorophyll concentration and stomatal conductance.

Plant pigments

Chlorophyll a and b were estimated spectrophotometrically [64], after acetone extraction of the pigments from fresh leaves. Chlorophyll concentration was determined with four replicate plants. A leaf sample of 0.1 g was ground and extracted with 5 mL of 80% (v/v) acetone in the dark. The slurry was filtered and absorbancies were determined at 645 and 663 nm.

Statistical Analysis

The pot experiment was set up in randomized complete block design replicated five times. ANOVA (SAS version 10.0) was employed for statistical analysis of data. Statistical significance was defined as $P < 0.05$

Results

Effect of salinity on leaf growth

Increasing NaCl concentrations in the irrigation solution significantly decreased total leaf area of both *Atriplex* varieties (Fig. 1). The leaf area of both varieties was nearly equal concentration in green and red, respectively. At 15 g/l NaCl total leaf area was reduced by 13% (red) and 47% (green) compared with the controls.

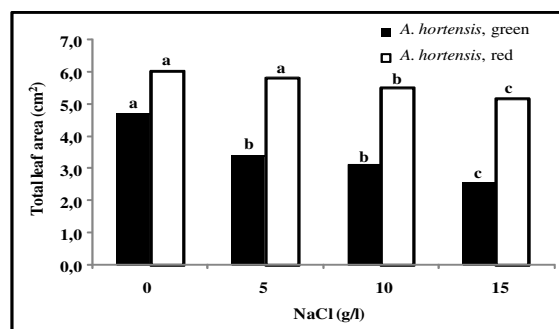


Figure 1. The effect of salinity on total leaf area in the two *Atriplex* varieties green and red.

Different letters represent a significant difference ($P < 0.05$) between treatment

Effect of salinity on dry weight

Shoot dry weights at all stages of development were reduced progressively with increasing NaCl concentrations while reversibly.

Increasing concentration of salt in soil significantly retarded ($p < 0.05$) dry weight of stems and roots (Table. 1). Dry weight significantly decreased ($p < 0.05$) for shoots (leaves + stems), roots and total biomass of plants in response to increasing concentration of salt (Table. 1). Percentage relative weight of tissues of salinized plants compared to those of control plants were computed as (salinized tissue dry weight/control dry weight) x100. Dry weight values of tissues given in Tab. 1 were used for the calculation of percentage relative weight of tissues. Values of percentage relative weight varied from 88 to 52 % for shoots, from 80 to 39 % for roots and from 87 to 50 % for total biomass in response to increasing soil salinity from 5 to 15 g/l. In experiment, dry plant weight decreased dramatically with the increasing NaCl concentration. The greatest dry plant weight of *Atriplex* was obtained with the first treatment in all range of salinity treatments. The general tendency was that increasing concentrations of salt induced a progressive decline in the length of shoots and in the weight of roots, stems and leaves.

Table 1. Effect of salinity on root and shoot dry weights of *Atriplex hortensis*. Different letters represent a significant difference ($P < 0.05$) between treatments.

Treatment	Shoot		Root		Total weight	
	(g/plant)	(%)	(g/plant)	(%)	(g/plant)	(%)
<i>A hortensis</i> varieties green						
0 g NaCl/l	14,545 a	100	2,253 a	100	16,798 a	100
5 g NaCl/l	12,838 b	88	1,758 b	78	14,596 b	87
10 g NaCl/l	9,702 c	67	1,295 c	57	10,997 c	65
15 g NaCl/l	7,538 d	52	0,889 d	39	8,426 d	50
LDS _{0,05}	0,278	-	0,066	-	0,271	-
<i>A hortensis</i> varieties red						
0 g NaCl/l	14,355 a	100	2,091 a	100	16,447 a	100
5 g NaCl/l	12,538 b	87	1,680 b	80	14,218 b	86
10 g NaCl/l	10,139 c	71	1,301 c	62	11,440 c	70
15 g NaCl/l	7,460 d	52	0,936 d	45	8,396 d	51
LDS _{0,05}	0,247	-	0,054	-	0,247	-

Mean from five replicate pots are given and different letters in the same column are significantly different at the level of 0.05.

Effect of salinity on chlorophyll concentration

Salinity stress (5, 10 and 15 g/l NaCl) resulted in significantly progressive decline the photosynthetic pigments (chlorophyll a and b). Similarly, Chl b concentration decreased with 20% at NaCl concentrations of 5 g/l. Red variety had significantly more Chl a and Chl b than green variety at all NaCl concentrations. The strongest effect of salt occurred with two varieties between 10 and 15 g/l NaCl.

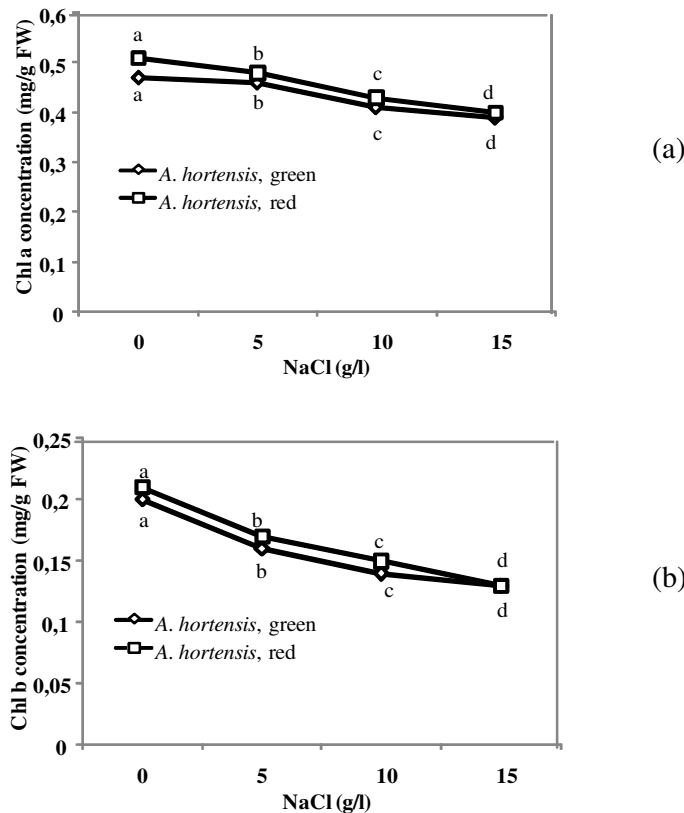


Figure 2. Effects of increasing NaCl concentration in the irrigation on chlorophyll a (a) and chlorophyll b (b) in the two *Atriplex* varieties green and red.

Effect of salinity on growth

Plant height decreased with increasing salinity for the both varieties (Fig. 3a). Plant height decreased in the plants supplemented with NaCl and their rate of loss was proportional to the concentration of the NaCl. Stem height decreased rapidly at 100 cm in 15 g/l NaCl concentration for two varieties. The plant height of both varieties was nearly equal at all NaCl concentration, suggesting no difference in varietal response to NaCl.

Increasing concentration of salt in soil significantly retarded ($p < 0.05$) elongation of stems and roots (Fig. 3b). Root growth of two *Atriplex* varieties responded differently to increasing salinity. As salinity level increased from control (0 g/l) to (15 g/l), green variety exhibited a trend of increasing in root mass. In contrast, root elongation of red variety decreased linearly with increasing salinity.

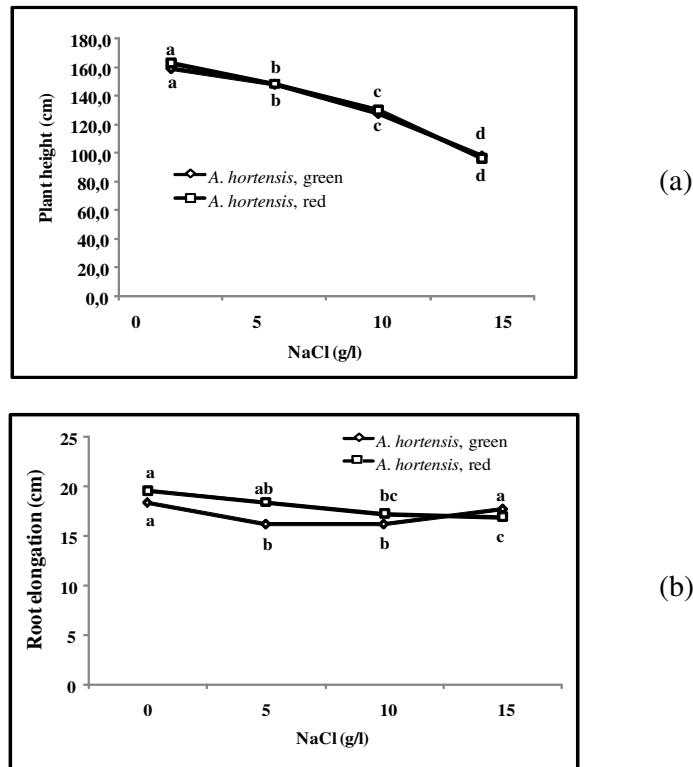


Figure 3. Effects of increasing NaCl concentration in the irrigation on plant height (a) and root elongation (b) in the two *Atriplex* varieties green and red

Effect of salinity on water content

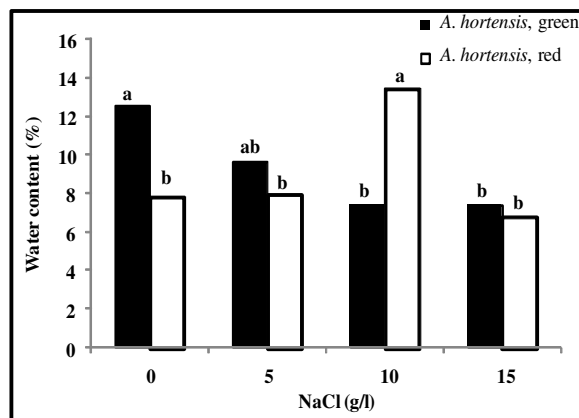


Figure 4. The effect of salinity on total leaf area in the two *Atriplex* varieties green and red. Different letters represent a significant difference ($P < 0.05$) between treatments

The water content of leaves of *A. hortensis* red variety was highest in plants grown at 10 g/l NaCl, followed by the leaves grown at 5 g/l and the lowest water content was in

leaves grown at 15 g/l NaCl (Fig. 4). Further increase in salinity caused a reduction in water content to a very low value at higher salinities.

Effect of salinity on stomatal conductance

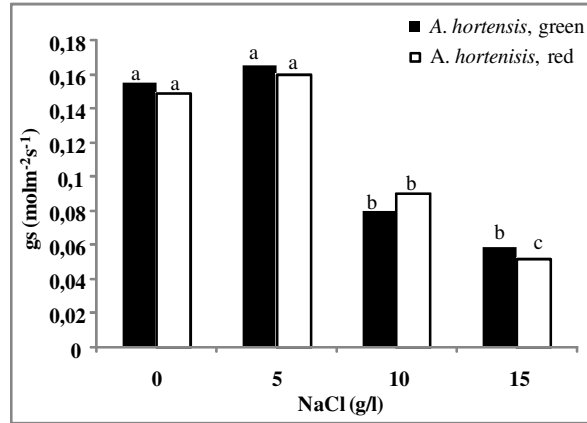


Figure 5. Effects of increasing NaCl concentration on stomatal conductance of the two *Atriplex hortensis* varieties green and red.

Stomatal conductance (Fig. 5) of both varieties decreased by about 50% with increasing external NaCl concentration. Mean stomatal conductance (gs) measures ranged from 0,16 molm⁻² s⁻¹ in low salinity treatments up to 0,06 molm⁻² s⁻¹ in high salinity treatments.

Discussion

Atriplex hortensis is highly tolerant of salinity. Halophytic *Atriplex* species show stimulation of growth at NaCl concentrations that are inhibitory to non-halophytes [77]. Saline soils and saline irrigations constitute a serious production problem for vegetable crops as saline conditions are known to suppress plant growth [87]. Increases in salinity lead to a reduction and/or delay in growth of both halophyte and glycophyte plants [37, 39, 96]. An important finding was that plants could survive at 15 g l⁻¹ salinity and this might be a suitable concentration to use to select highly salt-tolerant plants. Studies have shown that *Atriplex* spp., such as *A. nummularia*, *A. griffithii* and *A. hortensis*, could survive under highly saline conditions; with optimal growth occurring at 5 to 10 g l⁻¹ NaCl [41, 82, 101]. Other researchers have not observed growth stimulation by NaCl in *S. virginicus* [12, 70, 71].

Halophytes such as *Atriplex* spp. show a stimulation of growth at NaCl concentrations that are inhibitory to the growth of non-halophytes [77]. [3] showed that the growth of both *Atriplex inflata* F. Muell. and *A. nummularia* Lindl. was greater at 600 mm NaCl than in nutrient controls. *Atriplex* spp. vary in their degree of salt tolerance [80]. *Atriplex halimus* L. had the least decrease in dry mass production (40%) at 750 mm NaCl, whereas *A. calotheca* (Rafn.) Rafn. and Fries. (67%) and *A. nitens* Schkuhr (80%) had greater decreases but all three species were able to survive in this salt treatment [80]. [93] determined that *A. spongiosa* F. Muell. was able to grow in

medium containing over 600 mM NaCl, with dry mass production decreasing by 50% at 800 mM NaCl. Other chenopod halophytes, such as *Halosarcia pergranulata* [91], *Suaeda fruticosa* [41] and *Sarcocornia fruticosa* [84], have growth optima at moderate to high salinities.

In general, low salinity levels do not appear to have a deleterious effect on the growth of *Atriplex* spp. and may actually stimulate growth [3, 15, 59]. Many species of *Atriplex* are valued as livestock forage when herbage availability is low especially in arid environments and salt-affected area [34] because they have high content of crude protein, vitamins (A, C and D) and minerals such as chromium [61, 86].

In this study we show that the presence of medium-high concentrations of NaCl in the growth solution induced more deleterious effects on growth. These results fit with those previously reported in *Atriplex prostrata* [21]. Most species tested had their maximum biomass production in low salinity treatments. These results broadly match those obtained by [17, 25, 27, 29, 30, 38] who reported that low NaCl concentrations stimulate growth of some halophytic species, but an excess of salt decreases growth and biomass production. [56] reported that in most halophytic species growth decreases gradually with the increase of salt rate in the culture medium above a critical threshold specific to each species. Reduction of plant growth under saline conditions is a common phenomenon [4] but such reduction occurs differently in different plant organs. For example, in the present experiment, root dry weight was reduced more than shoot dry weight by salt stress. In contrast, [35] observed that salt stress inhibited the growth of shoot more than root in *Brassica* species.

Succulence is anatomical adaptation, which, by increasing the vacuolar volume, permits the accumulation of large amounts of water and dissolved ions in both shoots and roots [68]. Exposure to salinity concentrations has been shown to increase the tissue water content of halophytes [42, 51]. In halophytes, water content and the ability to make osmotic adjustments have been seen as important determinants of growth response [9, 10, 46, 58]. In the present study salinity stress resulted in decreased chlorophyll content. Saline stress led to leaf chlorosis, which ultimately resulted in significant photoinhibition and photodestruction of chlorophyll pigments. Similar results have been reported for other legumes [1, 92, 94].

The effect of salt stress on *Atriplex* leaf growth and shoot development is similar to its effect on sorghum [11] and lettuce [49]. Measurements of chlorophyll fluorescence provide quantitative information about photosynthesis through noninvasive means [98]. Salinity responses of chlorophyll a fluorescence have been studied in barley (*Hordeum vulgare* L.), [8, 48], rice (*Oryza sativa* L.), [54] and sorghum [53, 88, 89]. An increase in chlorophyll content has been thought to be due to the accumulation of NaCl in the chloroplast [43]. [65] also concluded that salt stress induced an increase in the chlorophyll content, which could be due to an increase in the number of chloroplasts in stressed leaves. The decreased in chlorophyll content under salinity stress is a commonly reported phenomenon and in various studies, because of its adverse effects on membrane stability [2, 5]. [73] found that the chlorophyll content reduction of leaves started to occur in plants grown at 100 mM NaCl and higher concentrations. These reductions could be attributed to the effect of salinity that causes inhibition of synthesis of chlorophyll or accelerating its degradation [83].

In general, salinity can reduce plant growth or damage the plants through: (i) osmotic effect (causing water deficit), (ii) toxic effects of ions and (iii) imbalance of the uptake

of essential nutrients. These modes of action may operate on the cellular as well as on higher organizational levels and influence all the aspects of plant metabolism [23, 45].

All these results agree with those previously reported for *A. griffithii* where the growth of plants cultivated in the absence or in the presence of 90 mM Na⁺ was similar even after 90 days [41]. Previous studies in *Atriplex amnicola* also indicated that this plant increased growth after additions of NaCl to the growth medium up to 25-50 mM but then growth declined as salt concentration was increased [6].

Results for reduction of shoot growth and leaf area development of *A. hortensis* with increasing salt concentration are in conformity with finding of [16], who reported that growth in Kenaf (*Hibiscus cannabinus*) under moderate salt stress was affected primarily through a reduction in elongation of stem and leaf area development.

There were very clear effects of NaCl on stomatal conductance in the present experiment, across the whole range of salinity similar to results published for *S. pectinata* by [32]. In other species, salinity decreases assimilation through reductions in leaf area [69, 79] and stomatal conductance [13, 78]. Exposure of halophytes to increasing salinity may result in partial closure of the stomata, in order to limit both transpiration and the transport of salts to the leaves [99]. Salinity is known to inhibit photosynthesis in a number of plant species [19, 22, 52, 103].

Conclusion

In summary our results show that *A. hortensis* is a highly salt tolerant annual halophyte, this plant has the ability to complete its life cycle under very high saline media. Their growth may be stimulated by the presence of salts in the growth medium. In conclusion, results presented here show that salinity reduces leaf growth and stomatal conductance variables. The remarkable reduction of total plant leaf area is likely to affect whole plant photosynthesis, contributing to the low biomass production. Chlorophyll fluorescence appears to be a useful indicator of salt stress at high NaCl concentrations. Finally, in this study, salinity stress results in a clear stunting of plant growth, which results in a considerable decrease in the dry weights of shoots and roots. Increasing salinity is accompanied also by significant reductions in stomatal conductance, plant height and root length. Increased research on the selection of halophytic species which have an economic utilization may enable the rehabilitation and revegetation of salt-affected lands given that the appropriate soil and irrigation management is applied.

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