Effect of Na_2SO_4 on the growth, water relations, proline, total soluble sugars and ion content of *Atriplex halimus* subsp. *schweinfurthii* through *in vitro* culture

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Abstract

Atriplex halimus subsp. schweinfurthii is a perennial halophyte, which is widely distributed in the Algerian salt steppes. This study reports the effect of sodium sulphate (Na_2SO_4) on the growth, tissue water content, proline, total soluble sugars and ion content of the species under *in vitro* conditions. Optimal growth was observed at 50mM Na_2SO_4 and declined with a further increase in salinity. Water potential (Ψ_w) of plants became more negative with an increase in salinity. Ca^{2+}, Mg^{2+}, and K^+ concentration of plants decreased with increasing salinity, while Na^+ and SO_4^{2-} increased. Both proline and total soluble sugars content of shoots and roots were highest at 250 mM Na_2SO_4. This study has demonstrated that salt tolerance in *A. halimus* subsp. *schweinfurthii* is achieved by appropriate osmotic adjustment involving accumulation of inorganic and organic solutes. At high salinities, growth reduction probably occurs as a result of high concentrations of Na^+ and SO_4^{2-} and their interference with other ions such as K^+. This variety can be used locally as a fodder for livestock and could be useful in sand dune stabilization.

Keywords: *Atriplex halimus subsp. schweinfurthii*, *In vitro* culture, Proline, Total soluble sugars, Ion content, Water potential (Ψ_w), Halophyte, Steppe.

Introduction

Draining salinised soils or irrigating with high quality water from remote sources are extremely costly. Therefore, selecting plants tolerant to salinity is an alternative strategy for a sustainable agriculture in those marginal lands (Drevon et al. 2001). Much of the research quantifying the salt tolerance of plant species has been based on experiments in which NaCl is the predominant salt. There has been comparatively little research examining plant responses to situations where Na_2SO_4 dominates. However, Na_2SO_4 is present at higher concentrations than NaCl in the soils and groundwater in many areas of the world including parts of Algerian steppes (Halitim 1988).

In vitro culture technology is one of the most worldwide methods of biotechnology, allows efficient and rapid clonal propagation of many economically important crops, especially for species that have long reproductive cycles (Margara 1982).

Atriplex sp. (saltbushes) are dominant in many arid and semi-arid regions of the world, particularly in habitats that combine relatively high soil salinity with aridity (Le Houérou 2000). Saltbushes have been used as a resource for domestic livestock, and for rehabilitation of degraded lands. The utilization of halophytic plants in pasture and fodder production in saline soils is the only economic solution presently available (Khan & Duke 2001).

Salinity may decrease biomass production because it causes a lowering of plant water potentials, specific ion toxicities, or ionic imbalances (Munns 2002). Plants protect themselves from salt toxicity by minimizing Na^+ uptake and transport to the shoot

(Tester & Davenport 2003). Osmotic adjustment under saline condition may be achieved by ion uptake, synthesis of osmotica or both (Parida & Das 2005).

Atriplex halimus subsp. schweinfurthii (Chenopodiaceae) is one of the most abundant perennial halophytes found in Algerian salt steppes in association with *Salsola vermiculata* and *Suaeda fruticosa* (Ortiz-Dorda et al. 2005), is highly tolerant to NaCl (Nedjimi et al. 2005, Bajji et al. 1998). However, its tolerance to Na_2SO_4 has not been published. This study was conducted to determine the effects of sodium sulphate on growth, water relations and solutes accumulation of Atriplex halimus subsp. schweinfurthii under *in vitro* conditions.

Materials and methods

The seeds of *A. halimus subsp. schweinfurthii* were collected from the area of *El Mesrane*, province of Djelfa (Algeria), more precisely in the zone of the chott Zahrez (3°03'E longitude, 34°36'N latitude and 830m elevation). After removal of the fruiting bracts, seeds were surface sterilised for 30 s in ethanol 97%, followed by treatment in formaldehyde 0.8% for 40 min, calcium hypochlorite 5% for 20 min and rinsed three times with sterile deionised water (Bajji et al. 1998). The seeds were placed to germinate into Petri dishes on two layers sterilized filter paper. The filter paper was moistened every 24 hours with 5 ml of sterile deionised water.

Five days after the germination, the seedlings having between 10 and 15 mm height were transferred into test tubes (one per tube) containing 20 ml of Murashige & Skoog (1962) medium supplemented with vitamins of Morel & Wetmore (1951), 0.1mM Fe-EDTA, 20 g/l sucrose (source of carbon), and 8 g l^{-1} of Agar (Bactoagar-Difco), and autoclaving for 20 minutes at 120°C and 150 kPa. pH was adjusted to 5.8 before adding KOH (Margara, 1982). The fresh medium was amended with sodium sulphate Na_2SO_4 (0, 50, 100, 150, 200 and 250 mM). The seedlings allowed growing for 30 d in a culture chamber whose temperature and photoperiod were controlled. The temperature was regulated at 25°C ± 1°C, under an illumination of 170 µmol $s^{-1}m^{-2}$ provided by a series of fluorescent lamps. The photoperiod was 16 h of light, and 8 h of darkness. The relative humidity was about 70%.

Ten plants of each treatment were randomly selected. To eliminate accumulation from salt on the surface, the plants were washed meticulously with distilled water. Fresh and dry weight of the plant shoots and roots were measured after 30 d of culture. Dry mass (shoots and roots) were determined after drying for 48 h in a forced-draft oven at 60°C. Shoot or root water content was calculated as (FW-DW), where FW and DW represent the fresh and dry weights, respectively. Shoot water potential (Ψ_w) was measured using a plant moisture stress instrument (PMS Instrument Co.) according to Sholander et al. (1965).

Sodium (Na^+) and potassium (K^+) concentrations were determined by the use of flame emission photometer (JENWAY PFP7 model) and calcium (Ca^{2+}) and magnesium (Mg^{2+}) by atomic absorption spectrophotometer (Perkin Elmer Analyst 300) following nitric- perchloric acid digestion. Sulfate ion content was measured with a DX-100 ion chromatograph. Proline and total soluble sugars were determined according to Bates et al. (1973) and Dubois et al. (1956) respectively.

The results of growth, ion contents, water potential and proline were subjected to one way ANOVA test to determine if significant differences were present among means. The Newman-Keuls test was carried out to determine if significant (P < 0.05) differences occurred among individual treatments.

Results

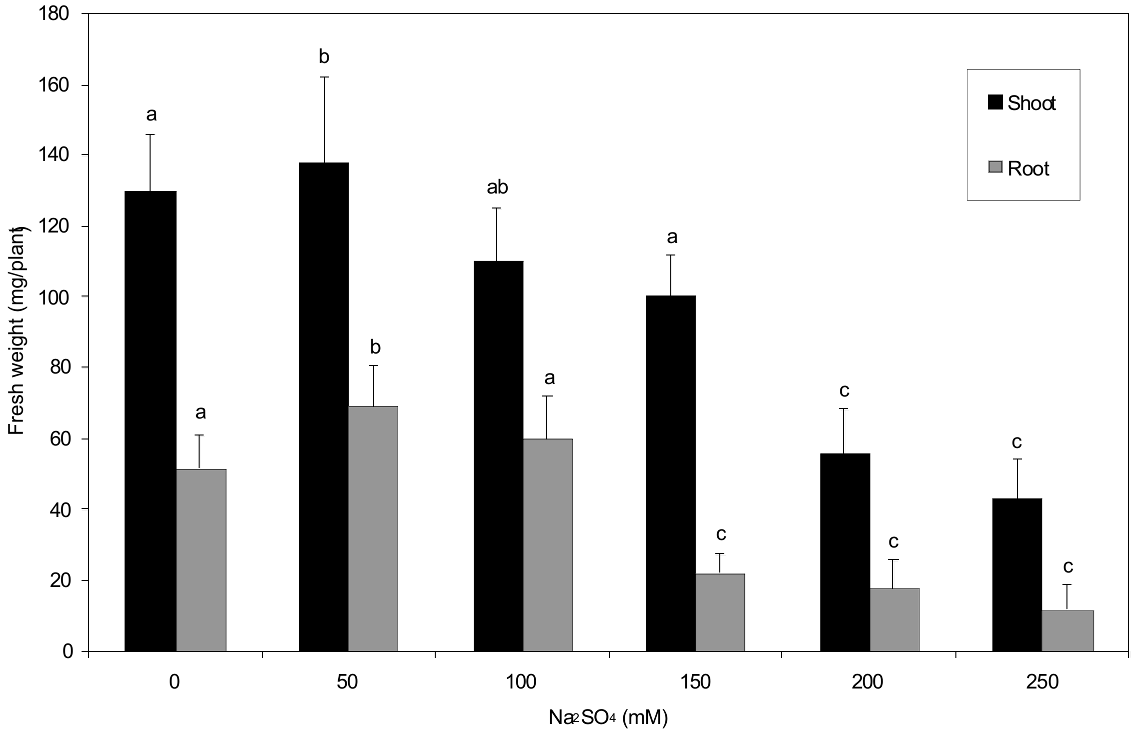
A one-way ANOVA indicated both shoot fresh weight (F=26.38, P<0.0001) and root fresh weight (F=12.62, P<0.0001) of A. halimus subsp. schweinfurthii plants were affected by salinity (Fig. 1). Optimal growth of shoot and root fresh weights were recorded at 50 mM Na_2SO_4 and declined with a further increase in salinity (Fig. 1).

Salinity also affected significantly both shoot dry weight (F=8.75, P<0.0001) and root dry weight (F=6.56, P<0.05) (Fig.1). Shoot and root dry weight were promoted at 50 mM Na_2SO_4 and declined at salinities above 150 mM Na_2SO_4 (Fig. 1).

Salinity significantly affected tissue water content (succulence) of A. halimus subsp. schweinfurthii shoots (F=26.18, P<0.0001) and roots (F=15.22, P<0.001) on a unit water per plant basis. Tissue water (mg $plant^{-1}$) increased slightly at low salinities, but declined at higher salinities (Fig. 2).

A one-way ANOVA of the water status of A. halimus subsp. schweinfurthii revealed that salinity significantly affected the water potential (R^2=0.99, P<0.0001) of plant. Water potential of A. halimus subsp. schweinfurthii plants became increasingly negative with an increase in media salinity (Fig. 3).

A one-way ANOVA of the ion content of A. halimus subsp. schweinfurthii revealed that salinity significantly affected Ca^{2+} (F=4.69, P<0.01), Mg^{2+}



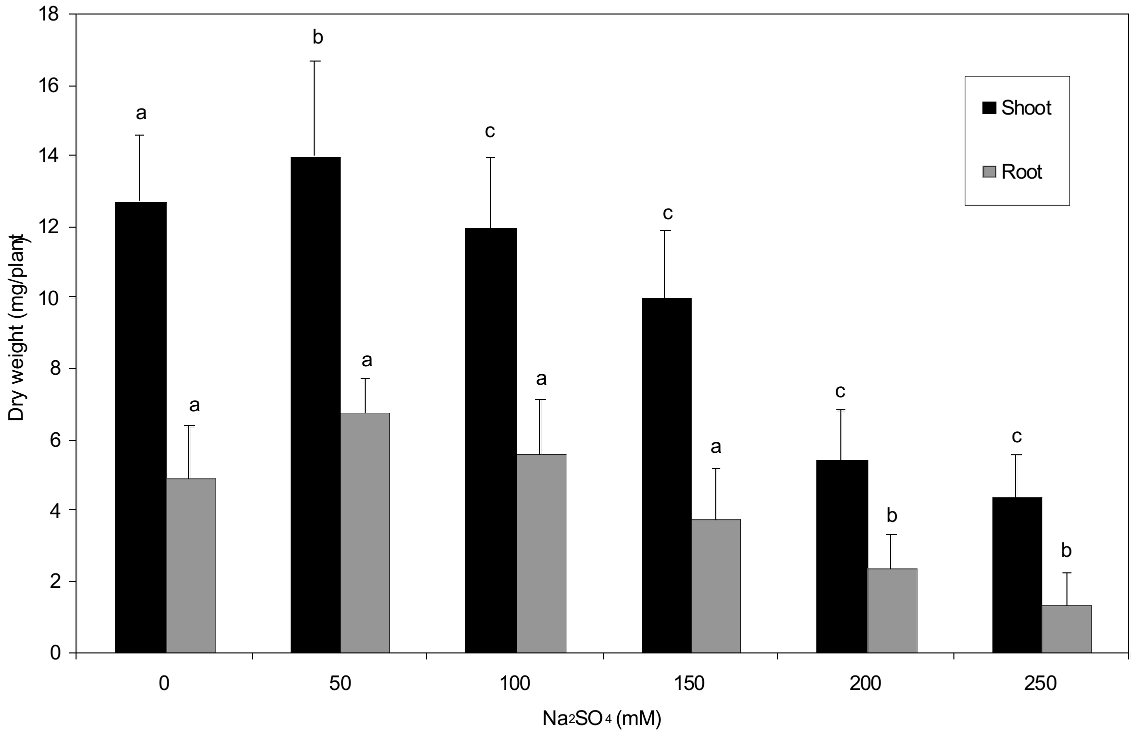


Figure 1. Effect of Na_2SO_4 (0, 50, 100, 150, 200 and 250 mM) on the fresh and dry weight in *Atriplex halimus* subsp. *schweinfurthii* vitroplants. Bars represent standard error. Different letters above bars represent a significant difference (P <0.05) between treatments.

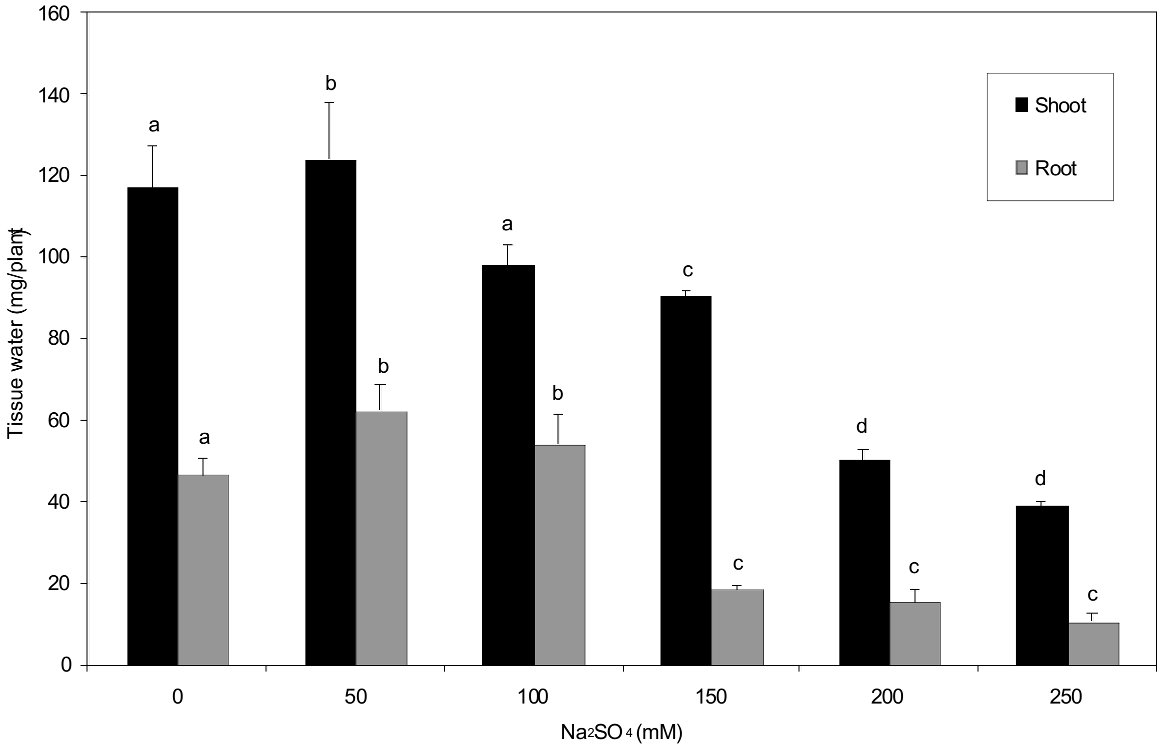


Figure 2. Effect of Na_2SO_4 (0, 50, 100, 150, 200 and 250 mM) on tissue water content in *Atriplex halimus* subsp. *schweinfurthii* vitro-plants. Bars represent standard error. Different letters above bars represent a significant difference (P <0.05) between treatments.

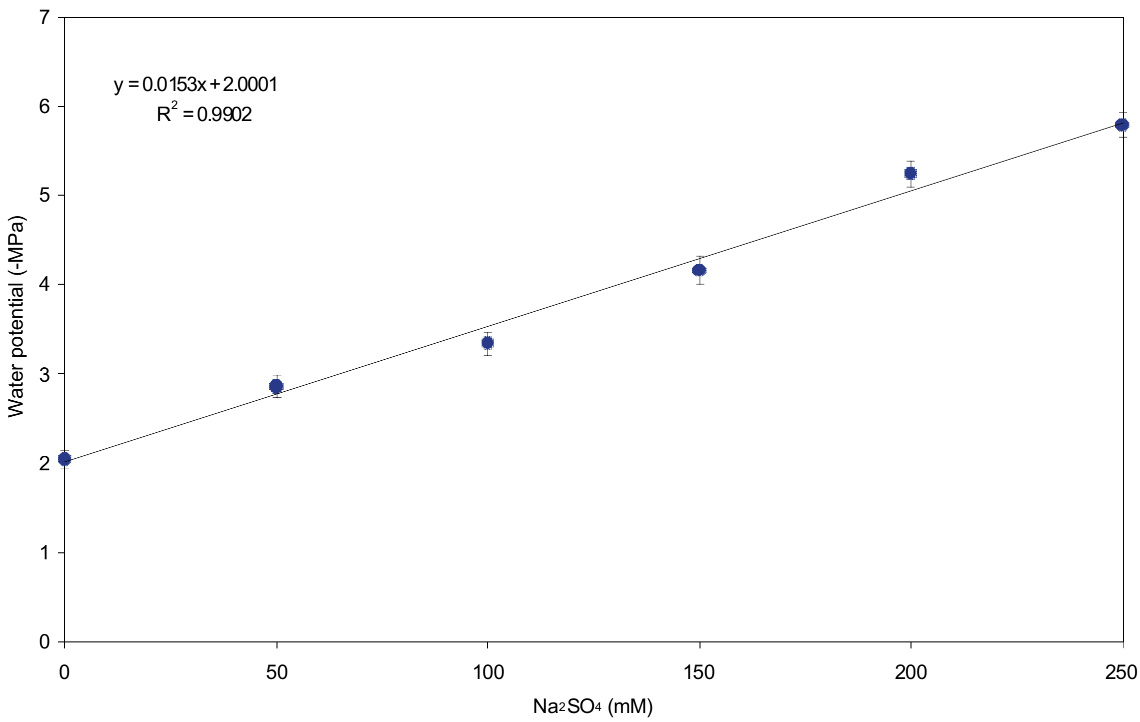


Figure 3. Effect of Na_2SO_4 (0, 50, 100, 150, 200 and 250 mM) on water potential in *Atriplex halimus* subsp. *schweinfurthii* vitro-plants. Bars represent standard error. The linear regression equation is shown.

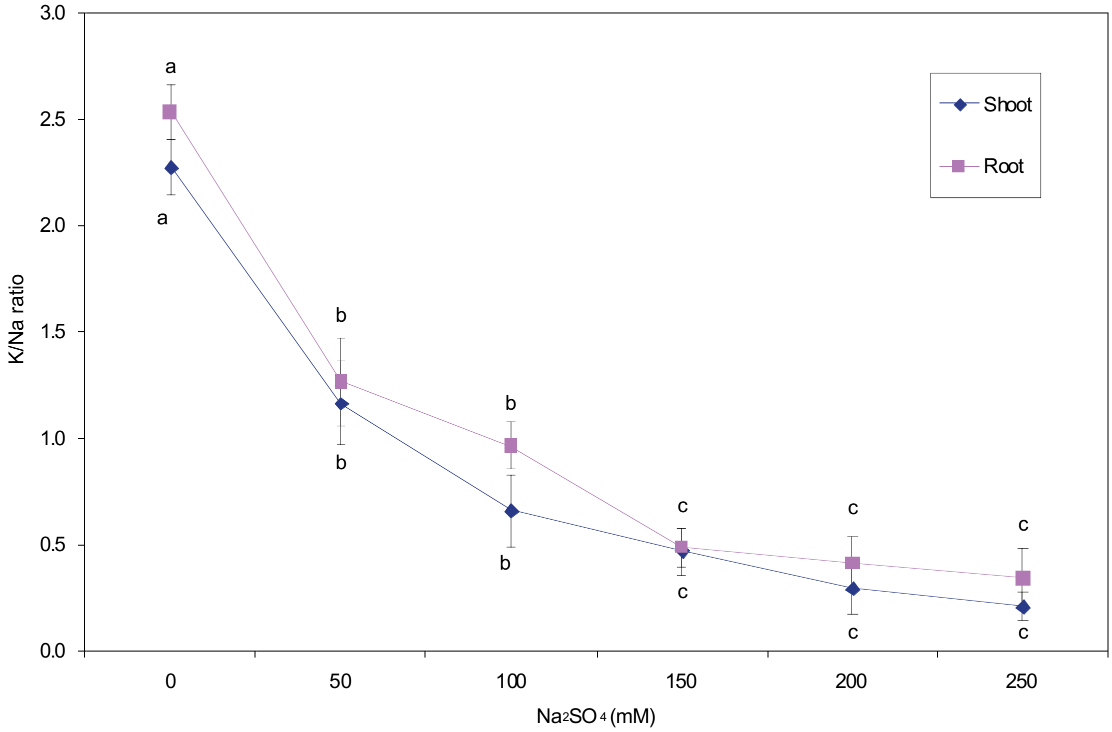


Figure 4. Effect of Na_2SO_4 (0, 50, 100, 150, 200 and 250 mM) on K/Na ratio in *Atriplex halimus* subsp. *schweinfurthii* vitro-plants. Points represent mean±standard error. Different letters above points represent a significant difference (P <0.05) between treatments.

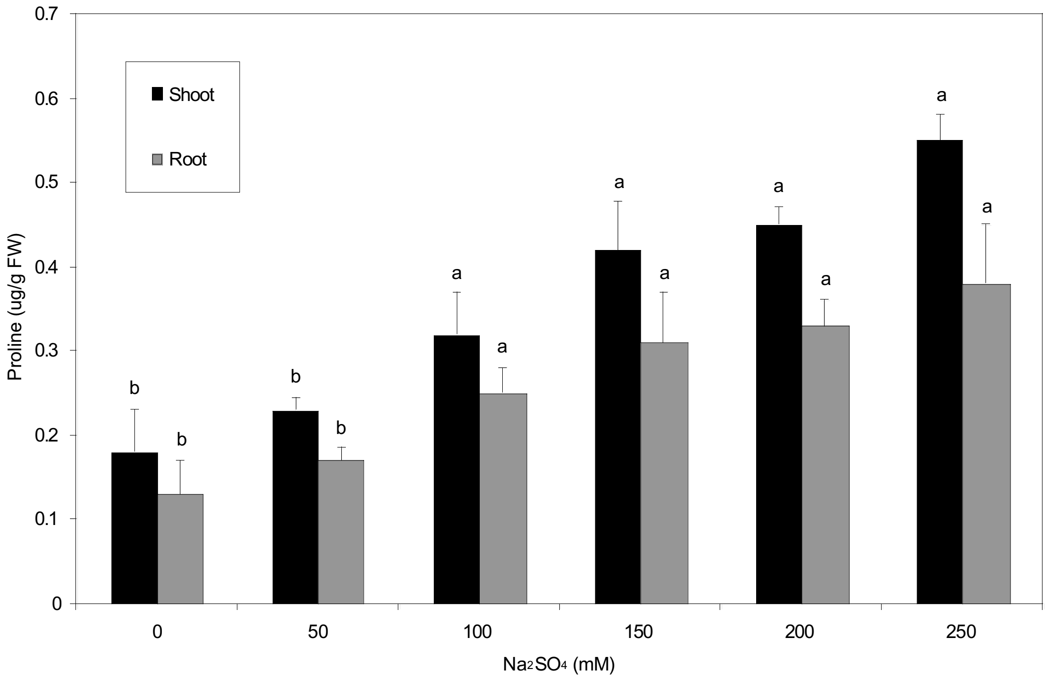


Figure 5. Effect of Na_2SO_4 (0, 50, 100, 150, 200 and 250 mM) on the proline content in *Atriplex halimus* subsp. *schweinfurthii* vitro-plants. Bars represent standard error. Different letters above bars represent a significant difference (P <0.05) between treatments.

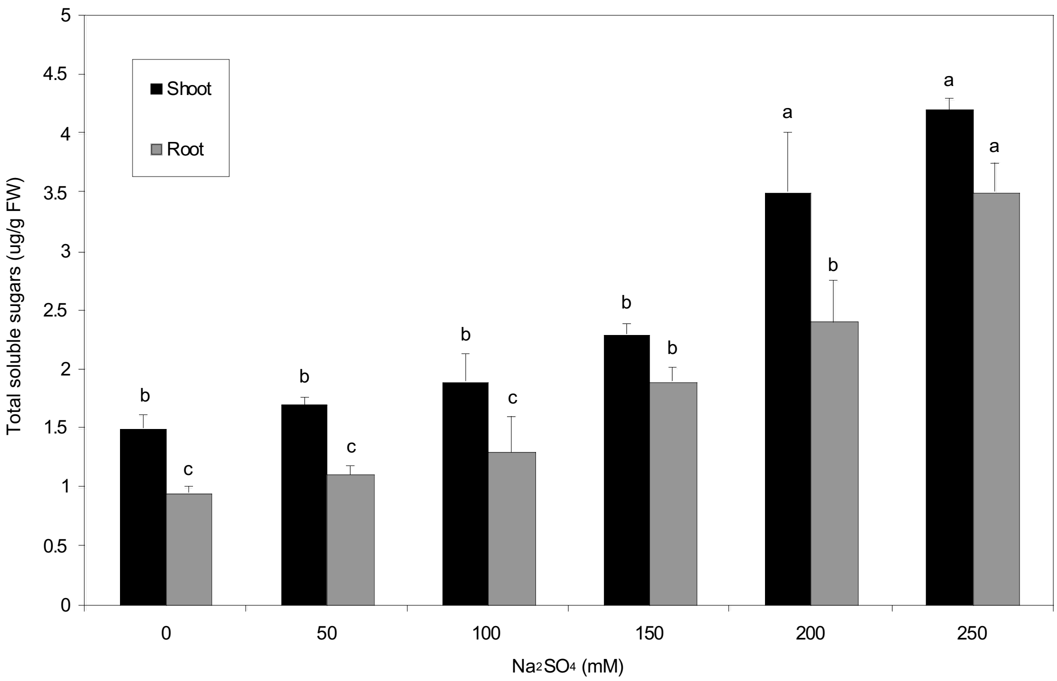


Figure 6. Effect of Na_2SO_4 (0, 50, 100, 150, 200 and 250 mM) on the total soluble sugars content in *Atriplex halimus* subsp. *schweinfurthii* vitro-plants. Bars represent standard error. Different letters above bars represent a significant difference (P <0.05) between treatments.

(F=12.52, P<0.0001), K^+ (F=18.45, P<0.0001), SO_4^{2-} (F=35.45, P<0.0001) and Na^+ (F=31.76, P<0.0001) content of plants. Sodium and sulfate content progressively increased in both shoots and roots with an increase in salinity, and this increase was greater in shoots compared to roots (Table1). The Ca^{2+}, Mg^{2+}, and K^+ content of plants decreased with an increase in salinity (Table 1). The K^+/Na^+ ratio was affected significantly (F=37.45, P<0.01) by Na_2SO_4 treatments, the higher the salinity, the lower is the ratio in both shoots and roots (Fig.4).

The concentrations of proline (F=337.45, P<0.0001) and total soluble sugars (F=11.35, P<0.0001) in roots and shoots were significantly affected by salinity. In shoots, proline and total soluble sugars concentration increased with salinity and peaked at 250 mM Na_2SO_4 (Figs. 5 and 6). Roots followed the same pattern but concentrations of proline and total soluble sugars were significantly lower than shoots (Fig. 5 and 6).

Discussion

Atriplex halimus subsp. schweinfurthii (Chenopodiaceae) is an Algerian perennial shrub. It is of interest because of its tolerance of environmental stresses and its use as a fodder shrub for livestock in low rainfall Mediterranean areas (Haddioui & Baaziz, 2001). It has been divided into two subspecies: *halimus* and *schweinfurthii (*Walker et al. 2005). Present study showed that *Atriplex halimus subsp.* schweinfurthii shoot and root biomass production were significantly stimulated at 50 mM Na_2SO_4, but were reduced at higher salinities. Some *Atriplex* species such as *A. nummularia, A. griffithii*, *and A. hortensis* are reported to grown at higher salinities, ranging to 100 to 200 mM NaCl (Ramos et al. 2004, Khan et al. 2000a, Wilson et al. 2000). Similar results have been reported for other halophytes which have optimal growth in the presence of salt (Ben Amor et al. 2005, Debez et al. 2004).

One of the basic mechanisms for survival under salt stress conditions relies on the compartmentalization of toxic ions in the vacuoles, which allows osmotic adjustment avoiding the inhibition of metabolic processes in the cytoplasm (Zhu 2001); succulence is an anatomical adaptation which, by increasing the vacuolar volume, permits the accumulation of larger amounts of water (and dissolved ions) in the shoots (Vicente et al. 2004). Exposure to high concentrations of NaCl increased succulence in shoots of *A. nummularia* (Ramos et al., 2004) and *A.* hortensis (Wilson et al. 2000). The present work showed that succulence in *A. halimus subsp. schweinfurthii* only increased slightly at low Na_2SO_4 treatment, but at higher salinities the succulence progressively decreased. This increase of succulence

Na_2SO_4 (mM)	Tissue ion concentration [µmol (g FW)$^{-1}$]				
	Na^+	K^+	Ca^{2+}	Mg^{2+}	SO_4^{2-}
Shoots					
0	126.5 51.8c	287.9 41.1a	141.4 21.7a	87.0 15.2a	135.3 38.4c
50	210.7 47.4c	245.3 34.5a	122.0 25.5b	77.3 17.8a	202.5 44.9b
100	322.1 55.2b	212.0 45.7a	128.7 27.6b	68.5 11.6b	288.1 50.2b
150	433.6 76.2a	202.5 31.9a	100.4 11.3c	51.8 15.6b	370.6 80.1b
200	492.7 96.8a	144.5 11.5b	89.9 30.1d	49.1 20.2b	445.5 98.8a
250	547.5 116.6a	112.7 22.4b	79.4 20.9d	60.7 16.1b	564.8 100.1a
Roots					
0	49.8 22.7c	126.2 34.5a	52.7 13.1a	37.5 4.6a	66.3 31.4c
50	88.5 32.1b	111.8 43.5a	46.6 20.2a	31.8 13.5b	77.5 44.2c
100	100.6 11.2b	96.8 22.4a	35.9 17.7b	30.7 7.6b	103.4 66.4b
150	151.2 38.1b	73.4 12.9b	36.2 13.6b	33.6 6.6b	145.5 45.9b
200	189.5 44.6b	77.9 11.6b	34.5 8.8b	29.0 5.3b	179.0 26.9b
250	217.1 33.9a	73.4 12.9b	34.3 7.9b	28.7 11.7b	283.3 35.2a

Mean values in rows for each parameter having the different letters are significantly different at $P < 0.05$ level by Newman-Keuls test.

Table 1. Ionic content in shoots and roots of *Atriplex halimus subsp. schweinfurthii* grown in different Na_2SO_4 concentrations. Values represent means ± standard error

is proposed to be caused by an accumulation of ions (Munns 2002). Measurements of plant water status indicated that *A. halimus subsp. schweinfurthii* plants adjusted their water potential to more negative levels as salinity increased.

In halophytes, water relations and the ability to adjust osmotically have been seen as important determinants of the growth response (Ben Amor et al., 2005; Benlloch-Gonzalez et al. 2005, Kurkova et al. 2002, Maggio et al. 2000). It would appear that the growth response at moderate salinities may be largely the consequence of an increased uptake of solutes that are required to induce cell expansion, since this maintains the pressure potential in plant tissues. At high salinities, growth reduction might either be caused by a reduced ability to adjust osmotically as a result of saturation of the solute uptake system, or because of excessive demand on the energy requirements of such systems (Khan et al. 2000b). Other factors, such as nutrient deficiencies, may also play an important role (Munns 2005).

Atriplex halimus subsp. schweinfurthii accumulated a large amount of Na^+ and SO_4^{2-} ions in shoots and roots and lower amount of K^+, Mg^{2+}, and Ca^{2+}. Generally, ion accumulation was greater in shoots than in roots. Khan et al., 2005 reported that Na^+ and Cl^- concentrations increased with an increase in salinity, while Ca^{2+}, Mg^{2+}, and K^+ decreased. Ramos et al. (2004) reported that *A. nummilaria* stored a large amount of Na^+ and Cl^- in its organs compared with other cations. The accumulation of sodium and the parallel decrease of potassium levels in the shoot appear to be one of the general characteristics of halophytes, in agreement with the notion that Na^+ can compete with K^+ for the same binding sites, and will therefore interfere with potassium transport into the cell by using its physiological transport systems (Vicente et al. 2004). The K^+/Na^+ ratios in shoots and roots declined at 50 mM Na_2SO_4 from the control value and remained lower than the control at salinities from 150 to 250 mM Na_2SO_4 (Fig. 4) A low K^+/Na^+ ratio in salt treatments has been reported previously in other halophytes (Khan et al. 2005, Debez et al. 2004, Short & Colmer 1999). Generally the K^+/Na^+ ratio in shoots was lower than in roots indicating that the absorbed sodium is predominantly stored in roots.

Osmotic adjustment, which is necessary for growth in a saline environment, may be accomplished by accumulation of inorganic and organic solutes. Inorganic ions are believed to be sequestered in the vacuoles, while organic solutes are assumed to be compartmentalised in the cytoplasm to balance the low osmotic potential in the vacuole.

Halophytes are able to compartmentalize toxic ions in the vacuole under salt stress conditions. This response requires the accumulation in the cytoplasm of non-toxic osmolytes to maintain the osmotic balance (Parida & Das 2005). Accumulation of organic solutes such as sugars and amino acids in the cytoplasm plays an important role in osmotic adjustment in plants (Ashraf & Harris 2004, Watanabe et al. 2000). Proline is probably the most widely distributed compatible solute involved in the response to osmotic stress (Parida & Das 2005, Claussen 2005). In our study, high proline content was observed in *A. halimus subsp. schweinfurthii* grown under Na_2SO_4 stress (Fig. 5). Accumulation of sugars might make a greater contribution to osmotic adjustment than proline. Prado et al. (2000) reported that accumulation of soluble sugars in *Chinopodium quinoa* might lower the shoot osmotic potential. At high stress intensities of Na_2SO_4, *A. halimus subsp. schweinfurthii* plants had greatly increased amounts of total soluble sugars (Fig. 6). The two-fold increase in Na^+ and SO_4^{2-} in shoots and roots was accompanied by similar increases in proline and totals soluble sugars, suggesting that salinity stress induced proline and sugars accumulations.

The mechanism of salt tolerance in *A. halimus subsp. schweinfurthii* could involve striking a delicate balance between ion accumulation, osmotic adjustment, production of osmotica (proline and total soluble sugars), maintenance of water potential, and growth. At higher salinities, a significant reduction in growth occurs because of the plant's inability to adjust osmotically, and specific ion toxicities may have caused a significant reduction in growth. *A. halimus subsp. schweinfurthii* is a salt accumulating halophyte; it could be used in increasing forage production in salt affected soils.

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