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Physiological and Biochemical Changes of Two Halophytes, *Nitraria retusa* (Forssk.) and *Atriplex halimus* (L.) Under Increasing Salinity

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Abstract: The effect of NaCl stress on the growth, nutriments contents and antioxidative activities were investigated in two xero-halophytes *Nitraria retusa* and *Atriplex halimus*. Plants were grown in 0-800 mM NaCl for 120 days under glasshouse conditions. Both xero-halophytic species showed positive plant growth for low levels of salinity. Increasing concentrations of salinity from 400-800 mM NaCl induced decrease in plant growth and chlorophyll content in the two species, especially in *A. halimus*. In addition both species were able to accumulate a large quantity of Na^+ and to maintain a higher leaf water content which was probably associated with a greater capacity for osmotic adjustment whereas the contents of K^+, Ca^{2+} and Mg^{2+} decreased significantly at diverse degree, resulting in an increase in the Na^+/K^+ ratio when NaCl concentrations increased. At higher salinity, it appears that salt excretion was involved in the strategies for salt tolerance in both xero-halophytic species, especially in *N. retusa*. A significant variability to the response to oxidative stress induced by salinity was found between both species. The relative better salt tolerance of *N. retusa* compared to *A. halimus* plants may be related to their higher polyphenols and carotenoids accumulation and antioxidant activity, associated with lower Malondialdehyde (MDA) content and Hydrogen peroxide (H_2O_2) concentration. In *A. halimus*, the secondary metabolites contribution in the restriction of oxidative damages caused by salt stress was disputed. As a whole, the data suggest that both *N. retusa* and *A. halimus* salt stressed plants might represent potential sources of polyphenols for economical use. But there is distinction in the implication of non-enzymatic antioxidants to limit oxidative damage.

Key words: Plant growth, nutriments contents, antioxidant compounds, salt stress, *Nitraria retusa*, *Atriplex halimus*

INTRODUCTION

Soil salinity is the main abiotic factor limiting plant growth and productivity around the world. This problem is more severe in arid and semi-arid regions (Munns, 2002). Salinity influences plants through osmotic effects, ion specific effects and oxidative stress (Pitman and Lauchli, 2002; Munns and Tester, 2008; Patel *et al*., 2009). All these factors cause adverse effects on plant growth and development at physiological and biochemical levels (Munns, 2002; Munns *et al*., 2006) and at the molecular level (Tester and Devenport, 2003). Osmotic effects are due to salt-induced decrease in the soil water potential. Salinity results in a reduction of K^+ and Ca^{2+} content and an increased level of Na^+ and Cl^- which forms its ionic effects. Indeed, high levels of Na^+ inhibit K^+ absorption which results in a Na^+/K^+ antagonism (Tester and Davenport, 2003). High concentrations of Na^+ disturb intracellular ion homeostasis which leads to membrane dysfunction, attenuation of metabolic activity and secondary effects that cause growth inhibition thereby leading to cell death (Ashraf, 2004). The ability of plant cells to maintain sodium concentrations low in the cytosol is a vital process associated with the ability of plants to grow under high salt regimes (Ashraf and Harris, 2004; Munns and Tester, 2008). Mechanisms of salt tolerance are of two main types: those minimizing the entry of salts into the plant and those minimizing the concentration of salt in the cytoplasm (Munns, 2002). Popp (1995) showed that tolerance mechanisms of halophytes include combinations of salt exclusion (from root and leaf), excretion (salt glands, bladder hairs and re-translocation), succulence, transport and compartmentalization and compatible solutes. Clearly, tolerance in the form of osmotic adjustment plays an important role in halophytes residing in saline environments (Flowers and Colmer, 2008).

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In addition to its known components of osmotic stress and ion toxicity, salt stress is also manifested as an oxidative stress by inducing an imbalance in cell compartment in the production of ROS and antioxidant defense (Mittler, 2002; Ksouri *et al*., 2007; Parvaiz and Satyawati, 2008). ROS includes superoxide (O_2^-), hydroxyl radicals (OH^-), hydrogen peroxide (H_2O_2) and singlet oxygen ($^1O_2$). These ROS are highly reactive and can alter the normal cellular metabolism through oxidative damage to proteins and nucleic acids as well as causing peroxidation of membrane lipids (Moller *et al*., 2007). On the other hand, Malondialdehyde (MDA), a product of lipid peroxidation has been considered an indicator of salt-induced oxidative damage and tends to show greater accumulation under salt stress (Sudhakar *et al*., 2001; Meloni *et al*., 2003). In order to prevent oxidative damages, plants have evolved a complex antioxidant system which includes both enzymatic and non-enzymatic (low-molecular mass antioxidants) components differentially found in cell compartments (Mittler, 2002; Jithesh *et al*., 2006; Hamed *et al*., 2007), responsible for maintaining the levels of ROS under tight control.

Antioxidant compounds (non-enzymatic antioxidants) such as phenolic compounds ascorbic acid, glutathione and carotenoids are employed by plants to eliminate ROS. Plant phenolic compounds are secondary metabolites, their beneficial effects are related to their antioxidant activity (Heim *et al*., 2002), particularly their ability to scavenge free radicals, to donate hydrogen atoms or electrons or to chelate metal cations (Sakihama *et al*., 2002; Balasundram *et al*., 2006). Additionally to their role as antioxidant, these compounds exhibit a wide range of medicinal properties such as anti-allergic, antiinflammatory, anti-thrombotic, cardio-protective and vasodilatory effects (Balasundram *et al*., 2006). In plants, polyphenol synthesis and accumulation is generally stimulated in response to as salinity suggesting that the presence of those metabolites is related to increased salt tolerance of plants (Navarro *et al*., 2006; Noreen and Ashraf, 2009; Kiarostami *et al*., 2010).

In Tunisia, a considerable diversity of halophytic species of multiple interests including therapeutic practices and economical applications as new sources of natural antioxidants in dietary food. *Nitraria retusa* is one of the leading shrubs in steppes, deserts and saline soils belonging to the family *Nitrariaceae*. It is an important sand controller, its leaves and twigs are occasionally grazed by sheep, goats and camels (Heneidy, 1996). The *Chenopodiaceae Atriplex halimus* is a Mediterranean xero-halophyte saltbush species, highly resistant to drought (Le Houerou, 2000) and salinity (Bajji *et al*., 1998). Atriplex plays an important role as a forage crop for both wild and domestic animals in arid regions. To date, little is known about the antioxidant system response to salt stress in the two species.

In this study, we have examined the effects of salinity on plant growth, water relation, ions accumulation and secondary metabolites in these two fodder species with increasing salinities levels. Therefore, the main objective is to evaluate the difference in the response of *N. retusa* and *A. halimus* plants to different NaCl salinity levels and to identify the degree of tolerance they develop to confront salt stress, in order to better understand their differences on salt stress tolerance.

MATERIALS AND METHODS

Plant growth conditions: *A. halimus* seeds were collected from Sabkha of Kalbia (10°59'.631°E and 35°49'. 33°2N) (Tunisia), sterilized for 20 sec in 3% (w/v) calcium hypochlorite and rinsed three times with deionised water. Seeds were sowed to germinate in plastic containers filled with a mixture of marketed peat and sterile sand (1:1, v/v) and irrigated with deionised water. For *N. retusa* plants were propagated by grafting from a source plant growing wild in the salt region of Sabkha of Kalbia. Polyethylene bags filled with sandy soil were employed as plant-growing containers in the plant propagation phase. Plants were initially grown in half-strength Hoagland solution to supply the macro- and micro-nutrients. When seedlings were around 7 cm in height (2-3 months old), they were placed in plastic pots (5 L) filled with mixture of peat and perlit (2:1, v/v). Irrigation was with one-half strength Hoagland solution and with distilled water on alternated days for acclimatization during 15 days. The experiments were conducted in a greenhouse under controlled conditions with the following regimes: temperature; min/max 17/35°C; relative humidity; min/max 30/70% and photoperiod (14/10 h day/night).

At the end of the acclimatization phase, seedlings of *N. retusa* and *A. halimus* were divided into 5 groups for treatments (10 plants treatment^{-1}): 1) 0 mM NaCl (control); 2) 100 mM NaCl; 3) 200 mM NaCl; 4) 400 mM NaCl and 5) 800 mM NaCl. These treatments were watered with 200 mL of salt solutions every 2 days to avoid excessive accumulation of salt due to loss of water during evaporation. At the initiation of the experiment, salinity concentrations were gradually increased by 100 mM NaCl in both halophytic species at 2nd day interval to reach maximum salinity levels. Salt solutions were completely replaced once a week to maintain salinity levels in the pots. The experiment was performed for a total period of 120 days.

Growth activity and water content: The plant material was 1st cleaned with distilled water. After the water on the plant was absorbed by tissue paper, Fresh Mass (FM) was measured. The Dry Mass (DM) was measured after the fresh material was dried at 70°C for 48 h. Relative Growth Rate (RGR) was calculated as follows:

$$RGR = [\ln(DM_2/DM_1)]/(t_2-t_1)$$

Where:

DM = The total dry mass
t = The time
1, 2 = The beginning and the end of a salinization period, respectively

The Water Content of the Leaves (LWC) was determined as follows:

$$LWC = [(FM - DM)/FM] \times 100$$

Photosynthetic pigment estimation: About 1 g of fresh leaves tissue was used for each extraction. Tissue was homogenized in liquid nitrogen and total pigments extracted in 80% acetone.

The absorbance of the extracts was measured on a spectrophotometer (Hitachi U-2001, Japan) at three wavelengths (470, 645 and 663 nm) and chlorophylls (a, b) and carotenoids concentrations were calculated according to Arnon (1949).

Ion analysis: Dried samples (15 mg from four independent plants per treatment) were ground into a fine powder for wet digestion and dry ashing. The ash was dissolved with concentrated nitric acid and then set to a volume of 20 mL with distilled water. Cations such as Na^+ and K^+ were determined with a flame photometer (Model 410, Corning, Halstead, UK). Ca^{2+} and Mg^{2+} concentrations were measured (in press sap) after dilution with deionised water with an atomic absorption spectrophotometer.

Hydrogen peroxide (H_2O_2) estimation: The H_2O_2 content was determined as described by Velikova. Fresh leaf tissue (0.5 g) was homo-genized with 5 mL of 0.1% (w/v) Trichloroacetic Acid (TCA) inapre-chilled pestle and mortar. This homogenate was then centrifuged at 12,000 g for 15 min. Total 0.5 mL of the supernatant 0.5 mL of potassium phosphate buffer (pH 7.0) and 1 mL of potassium iodide were added.

The mixture was vortexed and its absorbance was read at 390 nm using a UV-visibles spectrophotometer (UV-2500, Shimadzu Corp., Kyoto, Japan) and the H_2O_2 concentration was calculated according to the standard curve.

Lipid peroxidation: Lipid peroxidation was estimated by determining the Malonyldialdehyde (MDA) content in the leaves according to the method of Cakmak and Horst (1991). Fresh leaf samples (0.5 g) were ground in 5 mL of 0.1% (w/v) Trichloroacetic Acid (TCA) at 4°C. The homogenate was centrifuged at 12,000 Hg for 5 min. Total 1 mL aliquot of the supernatant was mixed to 4 mL of 0.5% (w/v) Thiobarbituric Acid (TBA) prepared in 20% (w/v) TCA and incubated at 90°C for 30 min. Thereafter, the reaction was stopped in ice bath. Centrifugation of the samples was performed at 10,000 Hg for 5 min and absorbance of the supernatant was measured at 532 nm on a spectrophotometer (UV-2500, Shimadzu Corp., Kyoto, Japan). After subtracting the non-specific absorbance at 600 nm, the malondialdehyde content was calculated using its absorption coefficient (ε) and expressed as nmol malondialdehyde g^{-1} fresh mass following the formula:

$$MDA\ (nmol\ g^{-1}\ FM) = \left[(A532 - A600) \times V \times 1000)/\varepsilon\right] \times W$$

Where:

ε = The specific extinction coefficient (= 155 mM cm^{-1})
V = The volume of crushing medium
W = The fresh weight of leaf
A600 = The absorbance at 600 nm wavelength
A532 = The absorbance at 532 nm wavelength

Extraction and analysis of phenolic compounds: The extraction procedure was determined using the method described by Waterman and Mole (1994) with some modifications. Briefly, lyophilised leaf samples (5 g) were extracted twice with 100 mL of 70% methanol thereafter with 100 mL of 70% acetone at the temperature of 4°C. Then extraction was renewed with absolute methanol. The extracts were filtered, mixed and concentrated at 240 mbar pressure in a roto-evaporator (Heidolph Elektro GmbH and Co., WB 2000, Kelheim, Germany) at 40°C. After the elimination of organic solvents, the total aqueous excerpt was centrifuged at 15,000 Hg for 15 min then washed by the dichloromethane to remove the chlorophylls and the lipids traces. The gotten total aqueous phase was evaporated until dryness and the residual was taken in the absolute methanol to constitute a total phase containing the set of polyphenols. Phenolic content was assayed using the Folin-Ciocalteu reagent following Singleton's method slightly modified (Dewanto *et al*., 2002). An aliquot (0.125 mL) of appropriately diluted sample extract was added to 0.5 mL of distilled water and 0.125 mL of the Folin-Ciocalteu reagent. The mixture was shaken and allowed to stand for 6 min before adding 1.25 mL of 7%

Na_2CO_3 solution. The solution was then diluted with deionized water to a final volume of 3 mL and mixed thoroughly. After incubation for 90 min at 23°C in dark, the absorbance was measured at 765 nm by using a UV-Vis spectrophotometer (UV-2500, Shimadzu Corp., Kyoto, Japan). Total phenolic content of leaves (three replicates per treatment) was expressed as mg gallic acid equivalents per gram of dry weight (mg GAE/g DM) through the calibration curve with gallic acid. The calibration curve range was 0-400 mg mL^{-1}. Total flavonoid content was measured by a colorimetric assay developed by Dewanto *et al.* (2002). An aliquot of suitable diluted samples or standard solution of (þ)-catechin was added to a $NaNO_2$ and mixed for 6 min before adding 0.15 mL of a freshly prepared $AlCl_3$ (10 g/100 mL). After 5 min, 0.5 mL of 1 mol L^{-1} NaOH solution was added. The final volume was adjusted to 2.5 mL with distilled water and thoroughly mixed. Absorbance of the mixture was determined at 510 nm against the same mixture without the sample as a blank. Total flavonoids of plant part were expressed as mg (þ)-catechin/g DM (mg CE/g DM) through the calibration curve of (þ)-catechin.

Free radical-scavenging activity of extracts: Free radical-scavenging activity of leaf polyphenolic extracts using DPPH assay was determined as described by Hatano *et al.* (1988). The dried plant extract was diluted in pure methanol at different concentrations (10, 20, 100 and 200 mg mL^{-1}) then 1 mL of each diluted plant extract was added to 0.25 mL of a 0.2 mmol L^{-1} DPPH. methanolic solution. The mixture of different extract concentration and DPPH were placed in the dark at room temperature for 30 min. The absorbance of the resulting solution was then measured at 517 nm against methanol as blank using a UV/Vis spectrophotometer and corresponded to the ability of extracts to reduce the stable radical DPPH to the yellow-colored diphenyl picrylhydrazine. The radical-scavenging activities (%) of the tested samples were evaluated by comparison with a control which contained 2 mL of DPPH solution and 1 mL of methanol. The antioxidant activity of each sample was calculated following the formula:

$$\text{Antioxidant activity (\%)} = [(Ac - As)/Ac] \times 100$$

Where:
Ac = The absorbance of the control
As = The absorbance of the tested sample after 30 min

Statistical analysis: A one-way ANOVA was achieved to compare the mean values using the SPSS statistical package ($p<0.05$). In case of significant differences, Duncan post-hoc tests were performed to compare the means.

RESULTS

Plant growth and leaf water content: Sodium chloride treatments modified growth of both species in a concentration-dependent indicated by a several parameters such as shoot dry weight, root dry weight and Relative Growth Rate (RGR). In the two halophytic species, salt stress induced modifications in Dry Mass (DM) were more pronounced in the shoots than in the roots of the plants (Fig. 1a). The dry mass gain of plants was stimulated up to 200 mM NaCl and then reduced significantly in the highest NaCl treatment. In addition, the relative growth rate decreased significantly when the salinity level increased 200 mM in Nitraria and Atriplex

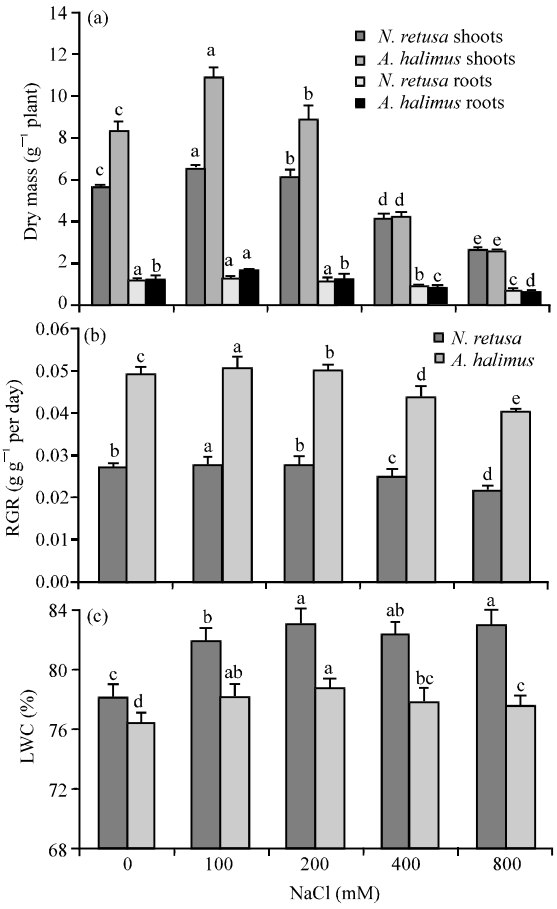

Fig. 1: a) Shoots and roots dry mass (g^{-1} plant); b) Relative Growth Rate (RGR, g g^{-1} day^{-1}) and c) Leaf Water Content (LWC, %) in *N. retusa* and *A. halimus* plants treated with various salt concentrations. Bars followed by the same letter do not differ statistically at $p<0.05$ (Duncan's multiple range test). Averages of four repetitions are presented with bars indicating SE

(Fig. 1b). Thus, moderate salinity appeared to be optimal for the growth of these two halophytic species. For example, shoots dry mass at 100 mM NaCl was about 15.5-29.7% higher for *N. retusa* and *A. halimus* plants, respectively. As compared with the control, shoot dry mass decreased at 800 mM NaCl by 51.5 and 69% in *N. retusa* and *A. halimus*, respectively. The increase of salt concentration induced promotion of the relative water content in the both species, especially in *N. retusa*. This parameter was maximal in 200-800 mM NaCl in *N. retusa* and at 100-200 mM NaCl in *A. halimus* (Fig. 1c).

Nutriments contents: Both species accumulated Na^+ with increasing NaCl concentration and the highest levels was observed in their shoots. Indeed at 200 mM NaCl salt stress treatment, Na^+ content of shoots and roots was (8.9; 3.9) and (3.8; 3.2) times than that in the control in *N. retusa* and *A. halimus*, respectively (Table 1). At 800 mM NaCl level, the values of *N. retusa* shoots Na^+ content reached 10.6 times than that in control and 5.4 times than that in control in *A. halimus*. In contrast, K^+ content of shoots decreased with increasing salt supply in the two plant species. At 200 mM NaCl level, the percentage inhibition of shoots K^+ content as compared with control non-treated plants was 37.1% in *A. halimus* and 30.4% in *N. retusa* (Table 1).

The rooted potassium content decreased down to a minimum values in *N. retusa* and *A. halimus* plants grown in the presence of 200 mM NaCl which represents a 31.5 and 42.8% of reduction as compared with the control, non-treated plants. For higher salinities levels (400-800 mM) roots K^+ content was insensitive in *N. retusa* or decreased slightly in *A. halimus*. The data shows that the Na^+/K^+ ratio increases progressively with increasing salt concentration. In shoots grown at 200 mM NaCl treatment, this ratio was 12.8 times greater in *N. retusa* and only 6.1 times greater as compared with the control for *A. halimus*. The Ca^{2+} content was significantly higher in shoots grown at control non-saline condition than shoots grown in different salinities levels (Table 1). Ca^{2+} roots content was lowest in *N. retusa* roots grown in the range of 200-800 mM NaCl and in *A. halimus* roots grown at 200 and 800 mM, significantly higher in *N. retusa* roots grown at 100 mM and in *A. halimus* roots grown at 400 mM and highest in roots grown at 0 mM. Mg^{2+} shoots content of *A. halimus* decreased slightly as NaCl concentrations increased. While in *N. retusa* there were no significant differences in the Mg^{2+} shoots content between shoots grown at control non-saline condition and these grown at 800 mM (Table 1). Mg^{2+} roots content was lowest in *N. retusa* roots grown at 800 mM NaCl and in *A. halimus* roots grown at 100 mM NaCl. The highest values were observed at 100 mM an 800 mM NaCl in *N. retusa* and *A. halimus* roots, respectively. A negative correlation between shoots dry mass and its sodium contents was observed in both species ($r = -0.57$, $F = 3.4$, $p = 0.291$ in *N. retusa*; $r = -0.79$, $F = 8.1$, $p = 0.071$ in *A. halimus*; Fig. 2a and b).

Table 1: Effect of NaCl treatments on the accumulation of sodium, potassium, calcium, magnesium and Na/K^+ ratio in shoot and root tissues of *N. retusa* and *A. halimus*

	NaCl (mM)				
Elements	0	100	200	400	800
Shoots of *N. retusa*					
Na (μmol g^{-1} DM)	573±52.00^e	3087±214^d	5104±86^c	7574±172^a	6089±613^b
K (μmol g^{-1} DM)	1104±83.0^a	791±31.00^b	768±54.0^b	834±33.0^b	687±25.0^c
Ca (μmol g^{-1} DM)	623±35.00^a	449±23.00^b	361±11.0^c	319±10.0^d	282±7.00^e
Mg (μmol g^{-1} DM)	355±16.00^a	330±15.00^b	307±12.0^c	314±13.0^c	359±6.00^a
Na/K	0.52±0.09^d	3.90±0.27^c	6.66±0.44^b	9.08±0.56^a	8.86±0.85^a
Roots of *N. retusa*					
Na (μmol g^{-1} DM)	597±53.00^e	1961±68.0^d	2377±136^c	2815±128^b	3376±227^a
K (μmol g^{-1} DM)	1164±59.0^a	875±78.00^b	797±48.0^c	895±19.0^b	738±26.0^c
Ca (μmol g^{-1} DM)	673±42.00^a	441.00±24^b	362±7.00^c	357±8.00^c	369±5.00^c
Mg (μmol g^{-1} DM)	530±19.00^c	639.00±18^a	567±14.0^b	516±30.0^c	460±22.0^d
Na/K	0.51±0.07^d	2.25±0.19^c	2.99±0.32^b	3.14±0.17^b	4.57±0.35^a
Shoots of *A. halimus*					
Na (μmol g^{-1} DM)	1058±57.0^d	2527±78^c	4059±230^b	5578±232^a	5734±191^a
K (μmol g^{-1} DM)	1484±51.0^a	1041±74^b	933±32.00^c	712±33.0^d	592±28.0^e
Ca (μmol g^{-1} DM)	484±27.00^a	380±11.0^b	361±10.00^c	297±29.0^d	237±15.0^e
Mg (μmol g^{-1} DM)	405±15.00^a	284±7.00^b	236±12.00^c	190±16.0^d	137±11.0^e
Na/K	0.71±0.14^e	2.43±0.23^d	4.35±0.26^c	7.84±0.61^b	9.70±0.71^a
Roots of *A. halimus*					
Na (μmol g^{-1} DM)	259±11.0^d	954±31.0^b	835±60.00^c	1545±700^a	1472±49^a
K (μmol g^{-1} DM)	605±44.0^a	395±24.0^b	346±17.00^b	315±6.00^c	263±11.0^d
Ca (μmol g^{-1} DM)	371±7.00^a	354±14.0^{ab}	294±12.00^c	350±8.00^b	312±17.0^c
Mg (μmol g^{-1} DM)	251±6.00^{bc}	173±9.00^d	264±6.000^b	236±15.0^c	281±10.0^a
Na/K	0.42±0.05^d	2.42±0.23^c	2.41±0.21^c	4.90±0.28^b	5.58±0.16^a

The data are the means of four replications ±SE. Values in each row with the same letter are not significantly different ($p = 0.05$) as described by Duncan's test

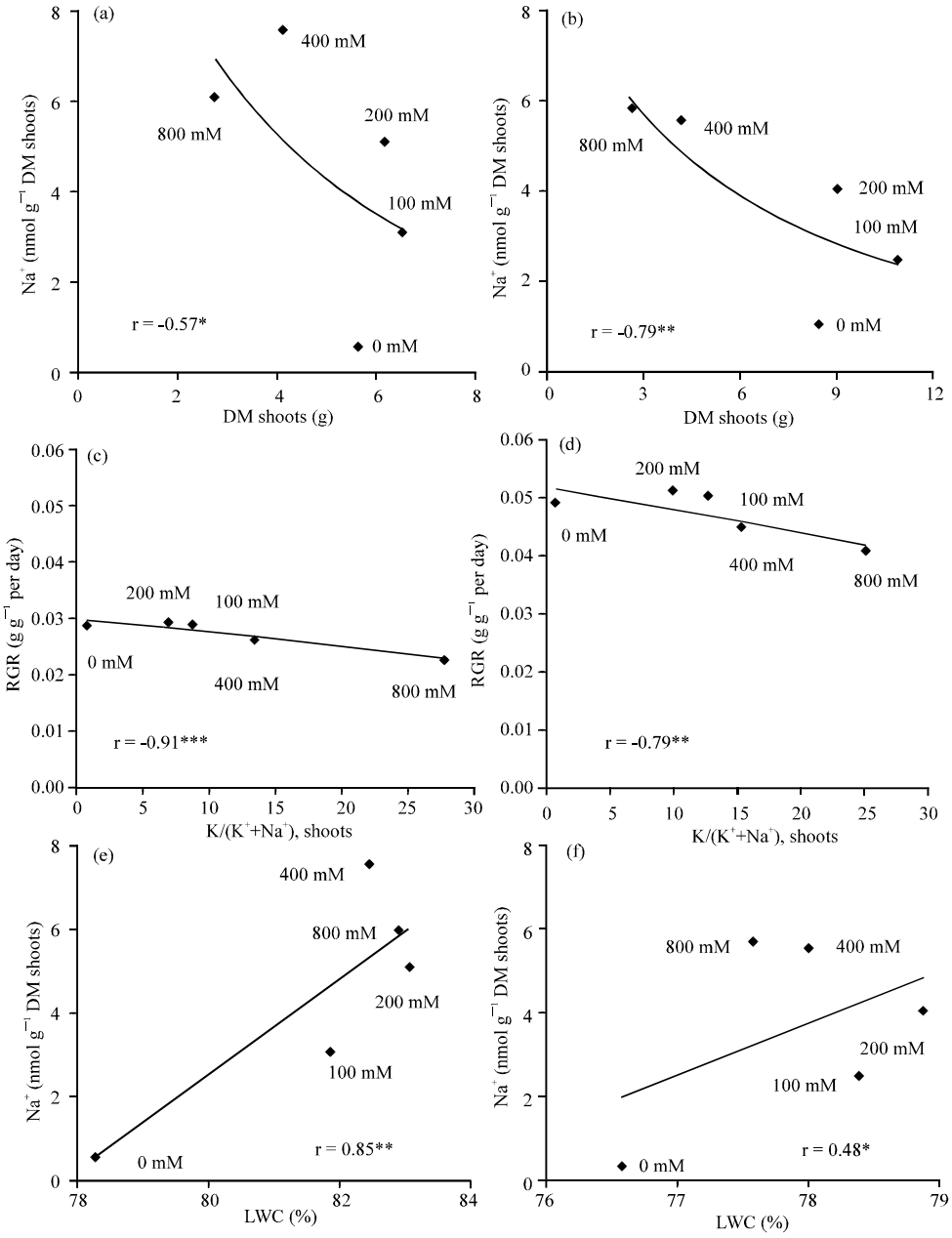

Fig. 2: Correlations between the shoots dry mass and its sodium contents; a and b) between the shoots RGR and ionic selectivity K^+ (K^++Na^+); c and d) and between foliar relative water content and its sodium content; e and f) at both species cultivated under salt stress (a, c and e: *N. retusa*; b, d and f: *A. halimus*). An average of 4 repetitions and confidence interval was calculated at the threshold of 95%

In addition, a highly negative relationship was observed between RGR and ionic selectivity $K^+/(K^+ + Na^+)$ ($r = -0.91$, $F = 27.9$, $p<0.0001$ in *N. retusa*; $r = -0.79$, $F = 8.1$, $p = 0.057$ in *A. halimus*; Fig. 2c and d). On the other hand, a significant positive correlation was observed between the shoots sodium contents and their relative water contents ($r = 0.85$, $F = 5.6$, $p = 0.071$ in *N. retusa*; $r = 0.48$, $F = 1.2$, $p = 0.411$ in *A. halimus*; Fig. 2e and f).

Pigment composition: Carotenoid contents of xero-halophytic species was unaffected by salinity. In addition, chlorophyll contents of *N. retusa* and *A. halimus* did not differ significantly by the presence of NaCl until 200 and 400 mM levels, respectively. Although at 800 mM NaCl, chlorophyll a and b was 5.6-24% and 7.1-27.7% lower than in the unsalinized control plants for *N. retusa* and *A. halimus*, respectively (Table 2).

Oxidative stress evaluation: Results presented in Table 3 showed that in both species, lipid peroxidation (MDA content) changed with increasing salinity. As compared with control, the leaf MDA content showed significantly increase only at higher salinities (400-800 mM NaCl). At moderate salinity, the MDA content was unaffected in the leaves of *A. halimus* moreover, this parameter decreased significantly and reached 74.7% compared with control plants at 100 mM NaCl in *N. retusa*. On the other hand the MDA accumulation was greater in *A. halimus* than in *N. retusa* thus at 800 mM NaCl level, lipid peroxidation increased by 33.7 and 92% in *N. retusa* and *A. halimus*, respectively (in relation to control). For the H_2O_2 generation, it has been noted that in both species, this parameter as well as the MDA accumulation was correlated with growth inhibition; it increased in all treatments which reduced growth and it was always significantly higher in *A. halimus* as compared to that in *N. retusa* (Table 3). In *N. retusa*, the H_2O_2 content decreased significantly between 9 and 12.2% at 100-200 mM NaCl and increased significantly at 400 and 800 mM NaCl to 125 and 177% of the control, respectively. In *A. halimus*, the H_2O_2 content decreased slightly by 2.3% at 100 mM NaCl.

Table 2: Effects of NaCl treatments (0, 100, 200, 400 and 800 mM) on the concentration of Hydrogen peroxide (H_2O_2) and Malondialdehyde (MDA) in leaves of *N. retusa* and *A. halimus*

Species	NaCl (mM)	H_2O_2 (µmol g^{-1} FM)	MDA (nmol g^{-1} FM)
N. retusa	0	12.3±0.8^{e}	6.5±0.5^{e}
	100	11.2±0.7^{d}	4.9±0.4^{d}
	200	10.8±0.8^{d}	5.8±0.5^{e}
	400	15.4±1.1^{b}	7.6±0.6^{b}
	800	21.8±1.5^{a}	8.7±0.6^{a}
A. halimus	0	14.2±1.2^{cd}	7.2±0.5^{e}
	100	13.8±1.1^{d}	6.9±0.6^{e}
	200	15.1±1.1^{c}	7.3±0.6^{e}
	400	19.2±1.4^{b}	10.7±0.8^{b}
	800	34.2±2.3^{a}	13.8±1.0^{a}

Data are means values ±SE of four measurements. Values in each column with the same letter are not significantly different (p = 0.05) as described by Duncan's test

At higher salinities, the H_2O_2 generation was significantly higher in *A. halimus* than that *N. retusa*. In comparison with the control plants, this parameter increased to 135 and 241% under 400 and 800 mM NaCl, respectively (Table 3). There was a positive linear correlation ($r = 0.87$, $F = 54.2$, $p<0.0001$ in *N. retusa*; $r = 0.94$, $F = 145.3$, $p<0.0001$ in *A. halimus*; Fig. 3a) between the MDA accumulation and H_2O_2 content in both species. On the other hand, a highly negative relationship was observed between MDA accumulation and biomass production ($r = -0.89$, $F = 67.9$, $p<0.0001$ in *N. retusa*; $r = -0.93$, $F = 123.1$, $p<0.0001$ in *A. halimus*; Fig. 3b).

Leaf phenolic compounds: The behaviour of two species to total polyphenols variation under salt stress was different. Thus, *A. halimus* maintained a fairly steady level of polyphenols whereas *N. retusa* indicated a significant raise over salt stress. In *N. retusa* leaves, the amount of total polyphenols remained virtually independent of salt concentration in the 0-100 mM range and increased slightly at 200 mM NaCl (Table 2) however, higher salinities were accompanied by large increases in total polyphenols, representing 1.7 and 2.4 fold the control, respectively at 400 and 800 mM NaCl. In *A. halimus*, the highest level of total polyphenols content (136% of the control) observed at 100 mM was associated with the higher growth stimulation in this species. However, the amount of total polyphenols remained unchanged but slightly higher than that in control in the 200-800 mM range. In salt treated plants of the two species, the variation of total flavonoids concentration was similar to

Table 3: Effects of NaCl concentrations on photosynthetic pigments (chlorophyll a and b and carotenoids), total phenols, total flavonoids, antioxidant activity percentage of polyphenolic compounds and the correlation between total phenols accumulation and the antioxidant activity in leaves of *N. retusa* and *A. halimus*

Species	NaCl (mM)	Chl a (mg g^{-1} FM)	Chl b (mg g^{-1} FM)	Carotenoids (mg g^{-1} FM)	Total phenols (mg GAE g^{-1} DM)	Total flavonoids (mg CE g^{-1} DM)	Antioxidant activity (%)
N. retusa	0	200.4±3.1^{b}	85.2±1.7^{a}	24.1±0.7^{c}	56.5±3.7^{d}	35.5±2.3^{cd}	11.0±0.7^{d}
	100	205.8±2.6^{a}	83.8±1.6^{a}	26.0±0.7^{b}	58.5±3.5^{cd}	37.0±2.5c	12.5±0.9^{d}
	200	202.4±2.4^{ab}	81.2±1.4^{a}	24.2±0.8^{c}	61.2±3.3^{c}	33.6±2.7^{d}	14.7±1.3^{c}
	400	199.1±5.9^{b}	79.6±2.3^{a}	25.9±0.9^{b}	94.5±5.9^{b}	52.7±4.1^{b}	30.5±2.2^{b}
	800	189.1±6.5^{c}	79.1±3.1^{a}	27.1±0.6^{a}	135.7±8.6^{a}	84.0±6.4^{a}	49.5±3.5^{a}
A. halimus	0	181.8±2.8^{a}	104.4±2.3^{ab}	19.5±0.7^{a}	15.2±1.7^{e}	7.2±0.9^{d}	7.5±0.5^{d}
	100	177.2±3.5^{b}	107.6±4.1^{a}	20.8±1.9^{a}	20.7±1.9^{a}	9.5±0.8^{a}	8.7±0.7^{d}
	200	174.8±2.4^{bc}	102.1±4.0^{b}	20.7±0.6^{a}	16.9±1.3^{b}	8.7±0.9^{b}	11.7±0.9^{c}
	400	172.4±2.4^{c}	99.4±3.8^{c}	20.1±0.9^{a}	16.1±1.4^{b}	7.4±0.6^{d}	17.8±1.5^{b}
	800	138.1±4.9^{d}	75.4±2.5^{d}	19.6±0.8^{a}	16.4±1.2^{b}	7.5±0.6^{cd}	24.7±1.8^{a}
Regression coefficient (r)							
N. retusa		-	-	-	0.99***	-	-
A. halimus		-	-	-	-0.28*	-	-

Data are means values±SE of four measurements. Values in each column with the same letter are not significantly different (p = 0.05) as described by Duncan's test; *p<0.05, **p<0.01 and ***p<0.001

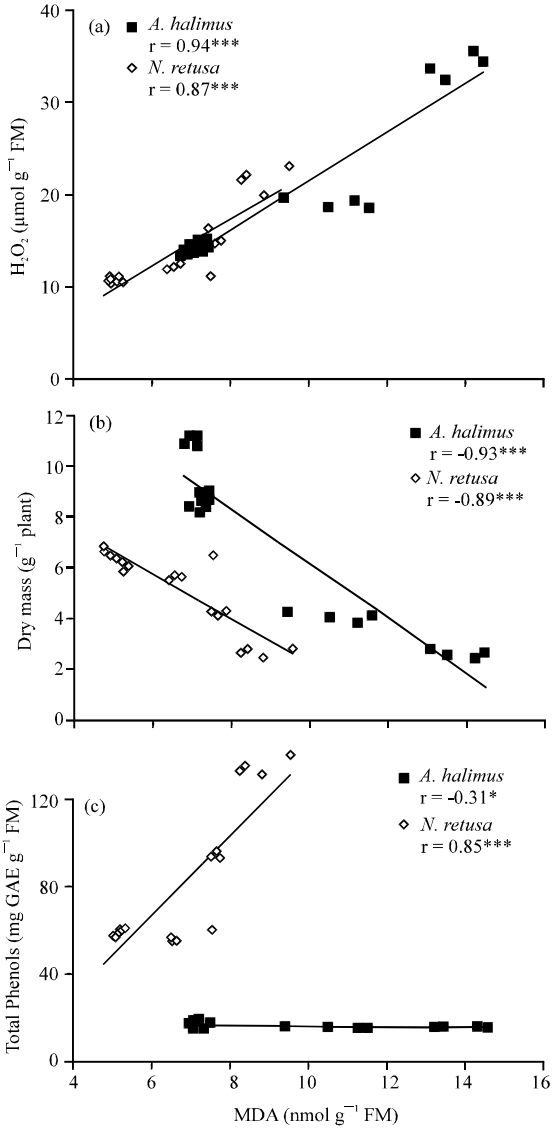

Fig. 3: Relationships between hydrogen peroxide (H_2O_2) contents; a) shoots biomass accumulation; b) and total phenols accumulation; c) with Malondialdehyde (MDA) amount at *N. retusa* and *A. halimus* species cultivated under salt stress. An average of 4 repetitions and confidence interval was calculated at the threshold of 95%

polyphenols content. Results reported in Table 2 showed that the flavonoids content increased significantly, especially at higher salinities (400-800 mM NaCl) in *N. retusa* leaves, reaching 236% of control at 800 mM. In *A. halimus* leaves, the flavonoids content was significantly increased at 100-200 mM NaCl and remained unchanged by salt stress in the 400-800 mM range. A positive correlation between phenols accumulation and lipid peroxidation (MDA content) was observed in *N. retusa* (r = 0.85, F = 46.4, p<0.0001; Fig. 3c) while this correlation was significantly negative in *A. halimus* (r = -0.31, F 1.9, p = 0.175; Fig. 3c).

Free radical-scavenging activity: The free radical-scavenging activity expressed in percentage of antioxidant activity using DPPH assay was evaluated in the two halophytes species under salinity treatments. The results (Table 2) showed that in both species, the percentage of antioxidant activity of leaf extracts remained statistically constant during the treatment of 100 mM NaCl while it increased significantly at with higher salinities (200-800 mM NaCl). This accretion of scavenging activity was much more pronounced in *N. retusa* compared to *A. halimus*, especially at higher salt stress treatments (400-800 mM NaCl). For example at 800 mM, the free radical-scavenging activity of polyphenolic compounds extracted from the leaves was 3.6 and 4.8 fold of the control in *A. halimus* and *N. retusa*, respectively. A highly positive relationship was observed between phenols accumulation and antioxidant activity in *N. retusa* plants (r = 0.99, F = 2325.5, p<0.0001; Table 2) whereas this relationship was significantly negative in *A. halimus* (r = -0.28, F = 1.5, p = 0.236; Table 2).

DISCUSSION

Halophytes show growth stimulation at NaCl concentrations that are inhibitory to the growth of non-halophytes (Osmond *et al*., 1980). In agreement with this idea, both *N. retusa* and *A. halimus* responded to low salinities by increased biomass production and relative growth rate. But higher salinities levels (400-800 mM NaCl) were inhibitory to plant growth, especially in *A. halimus*. The stimulation of plant growth at moderate salinity is consistent with previous studies on salt requiring halophytes (Moghaieb *et al*., 2004; Silveira *et al*., 2009). The depressive action of salt on growth appeared by a significant reduction of the aerial organ growth activity suggesting that the shoot was more sensitive to salinity than the root. The decline in plant growth due to high salinity is associated with a number of physiological and biochemical processes governing plant growth.

Deleterious effects of salinity are thought to result from osmotic effects, ion toxicities and nutrient deficiencies (Munns, 1993, 2002; Munns *et al*., 2006). In this research, the negative relationship between the aerial biomass production of *A. halimus* (r = - 0.79**, Fig. 2b) and Na^+ content suggest that the growth decrease was due to the ionic toxicity. However, the relative low

correlation detected in *N. retusa* (r = - 0.57*, Fig. 2a) seems to be the consequence of the decrease in Na^+ content at high salinities. The two species mainly accumulated inorganic ions, especially Na^+ by salt treatment. Ion distributional patterns in halophytic plants were typical for those of salt tolerant species where the major portion of the absorbed ions is translocated to the shoots (Munns, 1993). In contrast, increasing the external NaCl concentration was accompanied by a concomitant decrease in K^+ content and increase Na^+/K^+ ratio. A high concentration of Na^+ can interfere with K^+ uptake, resulting in deficiency and stunted growth (Meloni *et al*., 2008). Thus, the interaction between relative K^+ and Na^+ concentration has been considered a key factor in determining salt tolerance in plants. The Ca^{2+} contents were reduced in both studied species grown at high salinity while Mg^{2+} levels were less affected by salt stress, especially among *N. retusa* indicating a restriction of the uptake of these nutrients at different degree. As with K^+, reduced tissue concentrations of Mg^{2+} and Ca^{2+} may be due to interference with their uptake by Na^+ or possibly their activity in the external solution was reduced due to changes in ionic strength, ion-pair formation and precipitation (Hu and Schmidhalter, 2005). These results are consistent with those reported in other halophytes (Pagter *et al*., 2009; Patel *et al*., 2009).

On the other hand, the high correlation between the RGR and the ionic selectivity showed that *N. retusa* and *A. halimus* shoots (r = -0.91*** and r = -0.79**, respectively; Fig. 2c and d) remained highly selective for K^+ ions. This reflects absorption and a selective accumulation of K^+ compared to Na^+. The selectivity towards cations (K^+, Ca^{2+} and Mg^{2+}) was affected at higher salinities. The failure of the selective barrage led to a selective invasion of tissues by Na^+. The results (Fig. 1c) showed that the accumulation of Na^+ in photosynthetic organs was associated with an improvement of water content for all levels of salinity in the both species suggesting that the decrease in plants growth under high salinity can not be explained by reduction in cell turgor. The ability of these species to keep leaf LWC at a level above the control, despite high external salinity makes them less vulnerable to the osmotic and ionic stress caused by salinity (Silveira *et al*., 2009). The positive relationship between water and Na^+ contents (r = 0.85** in *N. retusa* and r = 0.48* in *A. halimus*, Fig. 2e and f) suggests that *N. retusa* and *A. halimus* plants are able to accumulate Na^+ in their leaves and to maintain turgor and osmotic adjustment. It appeared that salinity tolerance was maintained in these species essentially by using Na^+ as an osmoticum and compartmentalizing it within leaf cells away from the cytosol, perhaps by Na^+/H^+ antiport at the vacuolar membranes (Zhu, 2003; Apse and Blumwald, 2007). At high salinities, the response of both species especially *N. retusa* was quite different and the external concentration of 400 mM appeared to be critical. A reduction in Na^+ concentration indicating that the mechanism adopted by the plant to survive the salt stress was not the same in the presence of either low or high NaCl doses. It appears that this mechanism of salt regulation in *N. retusa* was capable to secreting excess salts from leaves. This result was in accordance with the findings of El-Bana (2002) that consider *N. retusa* and *Tamarix nilotica* as excretive frutiscents. In addition, a considerable part of the Na^+ absorbed may be accumulated in trichomes present at high densities on the leaf surface (Lefevre, 2007), avoiding toxic build-up of Na^+ in the apoplastic tissues of the leaves.

The excessive accumulation of Reactive Oxygen Species (ROS) in plants is one of the major damage induced bay salinity. Multiple antioxidant systems and substrates are believed to overcome the effects of salinity-induced oxidative stress by scavenging the ROS and thus protect cells from this damage (Jahnke and White, 2003; Athar *et al*., 2008). The modulation in the levels of secondary metabolites (Chlorophylls, carotenoids, flavonoids and phenolics, etc.) are of greater importance in the prevention of stress induced oxidative damage (Gould *et al*., 2000; Sgherri *et al*., 2004). In the experiments, chlorophyll a and b contents remained unaffected up to 200 and 400 mM NaCl in *A. halimus* and *N. retusa*, respectively and then decreased at higher salinity levels (Table 3).

Similar results were reported for leaf chlorophyll content of *Artimisia anethifolia* (Lu *et al*., 2003) and *Hordeum vulgare* (Khosravinejad *et al*., 2008). Decrease in chlorophylls level under salt stress may be due to reduction in pigment biosynthesis or enzymatic chlorophyll degradation (Yang *et al*., 2009). The carotenoid content was unaffected bay salinity in *A. halimus* leaves however there was significant increase in this pigment at higher salinities in *N. retusa* leaves. Carotenoids can protect photosynthetic system against reactive oxygen species generate under salt stress (Parida and Das, 2005; Parvaiz and Satyawati, 2008). The maintenance of carotenoids in *A. halimus* and its promotion in *N. retusa* may be due to their protective role against reactive oxygen species. The product of lipid peroxidation (content of MDA) and the generation of hydrogen peroxide have been considered as indicators of oxidative damage (Meloni *et al*., 2003). In the both species, the leaf H_2O_2 and MDA accumulation was increased significantly only at higher NaCl concentration (400-800 mM NaCl). This accumulation was greater in

A. halimus than in *N. retusa* (Table 2). The high positive correlation between H_2O_2 generation and MDA amount (r = 0.87*** in *N. retusa*; r = 0.94*** in *A. halimus*, Fig. 3a) confirmed the hypothesis that H_2O_2 brings about lipid peroxidation leading to membrane damages (Hichem *et al*., 2009). Furthermore, a negative correlation was observed between shoot biomass production and leaf MDA contents (r = -0.89*** in *N. retusa*; r = -0.93*** in *A. halimus*, Fig. 3b) in both species under salt stress indicating that low lipid peroxidation resulted in increased biomass production which may be attributed to the highly tolerance of these species to moderate salinities and their effective detoxification mechanisms. However, the increase of MDA and H_2O_2 contents coupled with reduced plant growth at higher salinities indicating that membrane stability had been destroyed and lipid peroxidation had occurred. The increase in lipid peroxidation may be due to the incapability of antioxidants to scavenger reactive oxygen species results from salt stress. Similar results were observed on other halophyte such as *Cakile maritima* and *Limonium bicolor* (Amor *et al*., 2005; Li, 2008).

The phenolic compounds produced through the phenyl propanoid pathway has been considered pertinent in oxidative stress tolerance (Moyer *et al*., 2002; Sgherri *et al*., 2004). Their accumulation can be changed by salt stress but this is critically dependent on the salt sensitivity of plants (Kim *et al*., 2008). In the present study, the species differed in their polyphenols accumulation induced by salinity. Thus, the content of these secondary compounds (phenols and flavonoids) was significantly increased with salt stress in *N. retusa* leaves which is similar to that in red pepper and *Cakile maritima* reported by Navarro *et al*. (2006) and Ksouri *et al*. (2007).

For *A. halimus*, leaf phenolics content was significantly increased at 100 mM NaCl and decreased at higher salinities suggesting that great tolerance of *A. halimus* to moderate NaCl treatment was concomitant with leaf enrichment in polyphenols. The antioxidant activity of phenolic compounds can play an important role in neutralizing ROS (Zheng and Wang, 2001). In the research, the total antioxidant capacity estimated by DPPH assay was enhanced in the both species by salinities superior to 100 mM NaCl. Similar results have been reported by Ksouri *et al*. (2007) for the antioxidant activity of polyphenolic extracts of salt treated *Cakile maritima* leaves. A highly significant positive correlation between antioxidant activity and phenolic compounds (r = 0.99***, Table 2) was observed in *N. retusa*. Similar results have been demonstrated by the other researchers (Duh, 1999; Kim *et al*., 2006; Naciye *et al*., 2008). *N. retusa* is a good source of phenolic antioxidant compounds. The flavonoids (isorhamnetin, isorhamnetin-3-O-glucoside, isorhamnetin-3-O-rutinoside and isorhamnetin-3-O-robinobioside) were reported as the major antioxidant active phenolic compounds in this species (Salem *et al*., 2011). An increase in total phenolic content and antioxidant activity of *N. retusa* under salinity can reduce oxidative stress. The significant negative correlation between total phenols accumulation and antioxidant activity observed in *A. halimus* (r = -0.28*, Table 2) suggest that total antioxidant capacity could be contributed by phenolic compounds and other unknown antioxidant compounds. The higher MDA and lower polyphenols contents in *A. halimus* leaves compared to *N. retusa*. Moreover, the higher capacity to scavenge hydroxyl radical observed in *N. retusa* in comparison with *A. halimus* suggest a relative imbalance between ROS generation and scavenging systems in *A. halimus* plants under higher salinities (400-800 mM NaCl). However, the promotion of biomass production observed at 100 mM NaCl favoured the production of the polyphenols. A similar propriety was observed in *Cakile maritima* (Ksouri *et al*., 2007).

Furthermore, this significant positive correlation between biomass production and leaf phenolic contents and the significant negative correlation between leaf MDA and phenolic contents in *A. halimus* under salt stress, lest to conclude that the high tolerance of *A. halimus* to moderate salinity in terms of high biomass production may be due to low production of MDA content. This reduction in MDA contents may be due to reduced production of ROS hence decreased oxidative stress.

CONCLUSION

As conclusion, the results indicate that both the halophytic studies species *N. retusa* and *A. halimus* showed increase in growth at moderate salinities. On the other hand, they showed differential responses in the accumulation of antioxidant compounds. *N. retusa*, responded to NaCl stress by enhancing phenolic compounds however, this secondary metabolites was increased remarkably only at moderate salinity in *A. halimus*. The results revealed that the ability to survive at higher salinities in *N. retusa* plants may due to the inclusion of great amount of Na^+ in relation to their advanced leaf succulence and to improved resistance to oxidative stress via increased the accumulation of phenolic compounds. It appeared that this spices was salt secreting at higher salinities. In *A. halimus*, salt tolerance may be due to its ability to use Na^+ in the shoots for

osmotic adjustment. In this species it appears that the non-enzymatic antioxidants such as phenolic compounds have not major role to prevent oxidative damages. The minor inhibition of the growth and increase of the hydrogen peroxide generation and lipid peroxidation at higher salinities (400-800 mM NaCl) in *N. retusa* suggest the better tolerance to salt stress of this species as compared to *A. halimus*.

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REFERENCES

Amor, N.B., K.B. Hamed, A. Debez, C. Grignon and C. Abdely, 2005. Physiological and antioxidant responses of the perennial halophyte *Crithmum maritimum* to salinity. Plant Sci., 168: 889-899.

Apse, M.P. and E. Blumwald, 2007. Na^+ transport in plants. FEBS Lett., 581: 2247-2254.

Arnon, D.T., 1949. Copper enzyme in isolated chloroplast polyphenoloxidase in Beta vulgaris. Plant Physiol., 24: 1-15.

Ashraf, M. and P.J.C. Harris, 2004. Potential biochemical indicators of salinity tolerance in plants. Plant Sci., 166: 3-16.

Ashraf, M., 2004. Some important physiological selection criteria for salt tolerance in plants. Flora, 199: 361-376.

Athar, H., A. Khan and M. Ashraf, 2008. Exogenously applied ascorbic acid alleviates salt-induced oxidative stress in wheat. Environ. Exp. Bot., 63: 224-231.

Bajji, M., J.M. Kinet and S. Lutt, 1998. Salt stress effects on roots and leaves of *Atriplex halimus* L. and their corresponding callus cultures. Plant Sci., 137: 131-142.

Balasundram, N., K. Sundram and S. Samman, 2006. Phenolic compounds in plants and agri-industrial by-products: Antioxidant activity, occurrence and potential uses. Food Chem., 99: 191-203.

Cakmak, I. and W.J. Horst, 1991. Effect of aluminium on lipid peroxidation, superoxide dismutase, catalase and peroxidase activities in root tip of soybean (*Glycine max*). Physiol. Plant., 83: 463-468.

Dewanto, V., X. Wu, K.K. Adom and R.H. Liu, 2002. Thermal processing enhances the nutritional value of tomatoes by increasing total antioxidant activity. J. Agric. Food Chem., 50: 3010-3014.

Duh, P.D., 1999. Antioxidant activity of water extract of four Harng Jyur (*Chrysanthemum morifolium* Ramat) varieties in soybean oil emulsion. Food Chem., 66: 471-476.

El-Bana, M.I., 2002. Floristic Composition of a Threatened Mediterranean Sabkhat of Sinai. In: Sabkha Ecosystems, Barth, H.J. and B. Boer (Eds.). Kluwer Academic Publishers, Cairo, Egypt, pp: 109-122.

Flowers, T.J. and T.D. Colmer, 2008. Salinity tolerance in halophytes. New Phytol., 179: 945-963.

Gould, K.S., K.R. Markham, R.H. Smith and J.J. Goris, 2000. Functional role of anthocyanins in the leaves of *Quintinia serrata* A. Gunn. J. Exp. Bot., 51: 1107-1115.

Hamed, K.B., A. Castagna, E. Salem, A. Ranieri and C. Abdelly, 2007. Sea fennel (*Crithmum maritimum* L.) under salinity conditions: A comparison of leaf and root antioxidant responses. Plant Growth Regul., 53: 185-194.

Hatano, T., H. Kagawa, T. Yasuhara and T. Okuda, 1988. Two new flavonoids and other constituents in licorice: Their relative astringency and radical scavenging effects. Chem. Pharm. Bull., 36: 2090-2097.

Heim, K.E., A.R. Tagliaferro and D.J. Bobilya, 2002. Flavonoid antioxidants: Chemistry, metabolism and structure-activity relationships. J. Nutr. Biochem., 13: 572-584.

Heneidy, S.Z., 1996. Palatability and nutritive value of some common plant species from the Aqaba Gulf area of Sinai, Egypt. J. Arid Environ., 34: 115-123.

Hichem, H., D. Mounir and E. Naceur, 2009. Differential responses of two maize (*Zea mays* L.) varieties to salt stress: Changes on polyphenols composition of foliage and oxidative damages. Ind. Crop Prod., 30: 144-151.

Hu, Y. and U. Schmidhalter, 2005. Drought and salinity: A comparison of their effects on mineral nutrition of plants. J. Plant Nutr. Soil Sci., 168: 541-549.

Jahnke, L.S. and A.L. White, 2003. Long-term hyposaline and hypersaline stresses produce antioxidant responses in the marine algae *Dunaliella tertiolecta*. J. Plant Physiol., 160: 1193-1202.

Jithesh, M.N., S.R. Prashanth, K.R. Sivaprakash and A.K. Parida, 2006. Antioxidative response mechanisms in halophytes: their role in stress defence. J. Genet., 85: 237-254.

Khosravinejad, F., R. Heydari and T. Farboodnia, 2008. Effects of salinity on photosynthetic pigments, respiration, and water content in two barley varieties. Pak. J. Biol. Sci., 11: 2438-2442.

Kiarostami, K., R. Mohseni and A. Saboora, 2010. Biochemical changes of *Rosmarinus officinalis* under salt stress. J. Stress Physiol. Biochem., 6: 114-122.

Kim, H.J., F. Chen, X. Wang and J.H. Choi, 2006. Effect of methyl jasmonate on phenolics, isothiocyanate and metabolic enzymes in radish sprout (*Raphanus sativus* L.). J. Agric. Food Chem., 54: 7263-7269.

Kim, H.J., J.M. Fonseca, J.H. Choi, C. Kubota and D.Y. Kwon, 2008. Salt in irrigation water affects the nutritional and visual properties of romaine lettuce (*Lactuca sativa* L.). J. Agric. Food Chem., 56: 3772-3776.

Ksouri, R., W. Megdiche, A. Debez, H. Falleh, C. Grignon and C. Abdelly, 2007. Salinity effects on polyphenol content and antioxidant activities in leaves of the halophyte *Cakile maritime*. Plant Physiol. Biochem., 45: 244-249.

Le Houerou, H.N., 2000. Utilization of fodder trees and shrubs in the arid and semiarid zones of West Asia and North Africa. Arid Soil Res. Rehabil., 14: 101-135.

Lefevre, I., 2007. Investigation of three Mediterranean plant species suspected to accumulate and tolerate high cadmium and zinc levels. Morphological, physiological and biochemical characterization under controlled conditions. Ph.D. Thesis, Catholic University of Louvain.

Li, Y., 2008. Kinetics of the antioxidant response to salinity in the halophyte *Limonium bicolor*. Plant Soil Environ., 54: 493-497.

Lu, C., G. Jiang, B. Wang and T. Kuang, 2003. Photosystem II photochemistry and photosynthetic pigment composition in salt-adapted halophyte *Artimisia anethifolia* grown under outdoor conditions. J. Plant Physiol., 160: 403-408.

Meloni, D.A., M.A. Oliva, C.A. Martinez and J. Cambraia, 2003. Photosynthesis and activity of superoxide dismutase, peroxidase and glutathione reductase in cotton under salt stress. Environ. Exp. Bot., 49: 69-76.

Meloni, D.A., M.R. Gulotta and C.A. Martinez, 2008. Salinity tolerance in *Schinopsis quebracho* colorado: Seed germination, growth, ion relations and metabolic responses. J. Arid Environ., 72: 1785-1792.

Mittler, R., 2002. Oxidative stress, antioxidants and stress tolerance. Trends Plant Sci., 7: 405-410.

Moghaieb, R.E.A., H. Saneoka and K. Fujita, 2004. Effect of salinity on osmotic adjustment, glycinebetaine accumulation and the betaine aldehyde dehydrogenase gene expression in two halophytic plants, *Salicornia europaea* and *Suaeda maritima*. Plant Sci., 166: 1345-1349.

Moller, I.M., P.E. Jensen and A. Hansson, 2007. Oxidative modifications to cellular components in plants. Annu. Rev. Plant Biol., 58: 459-481.

Moyer, R.A., K.E. Hummer, C.E. Finn, B. Frei and R.E. Wrolstad, 2002. Anthocyanins, phenolics and antioxidant capacity in diverse small fruits: Vaccinium, rubus and ribes. J. Agric. Food Chem., 50: 519-525.

Munns, R., 1993. Physiological process limiting plant growth in saline soils: Some dogmas and hypothesis. Plant Cell Environ., 16: 15-24.

Munns, R., 2002. Comparative physiology of salt and water stress. Plant Cell Environ., 25: 239-250.

Munns, R., R.A. James and A. Lauchli, 2006. Approaches to increasing the salt tolerance of wheat and other cereals. J. Exp. Bot., 57: 1025-1043.

Munns, R. and M. Tester, 2008. Mechanisms of salinity tolerance. Annu. Rev. Plant Biol., 59: 651-681.

Naciye, E., A. Guler and A. Erol, 2008. Antioxidant activity of rosemary extract, blackseed essential oil, carnosic acid, rosamarinic acid and sesamol. Food Chem., 110: 76-82.

Navarro, J.M., P. Flores, C. Garrido and V. Martinez, 2006. Changes in the contents of antioxidant compounds in pepper fruits at different ripening stages, as affected by salinity. Food Chem., 96: 66-73.

Noreen, Z. and M. Ashraf, 2009. Assessment of variation in antioxidative defense system in salt-treated pea (*Pisum sativum*) cultivars and its putative use as salinity tolerance markers. J. Plant Physiol., 66: 1764-1774.

Osmond, C.B., O. Bjorkman and D.J. Anderson. 1980. Physiological Processes in Plant Ecology. Springer-Verlag, Berlin.

Pagter, M., C. Bragato, M. Malagoli and H. Brix, 2009. Osmotic and ionic effects of NaCl and Na_2SO_4 salinity on *Phragmites australis*. Aquat. Bot., 90: 43-51.

Parida, A.K. and A.B. Das, 2005. Salt tolerance and salinity effects on plants: A review. Ecotoxicol. Environ. Saf., 60: 324-349.

Parvaiz, A. and S. Satyawati, 2008. Salt stress and phyto-biochemical responses of plants-a review. Plant Soil Environ., 54: 89-99.

Patel, A.D., H. Bhensdadia and A.N. Pandey, 2009. Effect of salinisation of soil on growth, water status and general nutrient accumulation in seedlings of *Delonix regia* (Fabaceae). Acta Ecol. Sinica, 29: 109-115.

Pitman, M.G. and A. Lauchli, 2002. Global Impact of Salinity and Agricultural Ecosystems. In: Salinity Environment-Plants Molecules, Lauchli, A. and V. Luttge (Eds.). Kluwer Academic Press, The Netherlands, pp: 3-20.

Popp, M., 1995. Salt Resistance in Herbaceous Halophytes and Mangroves. In: Progress in Botany, Behnke, H.D., U. Luttge, K. Esser, J.W. Kadereit and M. Runge (Eds.). Springer-Verlag, Berlin, pp: 416-429.

Sakihama, Y.M., F. Cohen, S.C. Grace and H. Yamasaki, 2002. Plant phenolic antioxidant and prooxidant activities: Phenolics-induced oxidative damage mediated by metals in plants. Toxicology, 177: 67-80.

Salem, J.H., I. Chevalot, C. Harscoat-Schiavo, C. Paris, M. Fick and C. Humeau, 2011. Biological activities of flavonoids from *Nitraria retusa* (Forssk.) Asch. and their acylated derivatives. Food Chem., 124: 486-494.

Sgherri, C., B. Stevanovic and F. Navari-Izzo, 2004. Role of phenolics in the antioxidative status of the resurrection plant *Ramonda serbica* during dehydration and rehydration. Physiol. Plant, 122: 478-485.

Silveira, J.A.G., S.A.M. Araujo, J.P.M.S. Lima and R.A. Viegas, 2009. Roots and leaves display contrasting osmotic adjustment mechanisms in response to NaCl-salinity in *Atriplex nummularia*. Environ. Exp. Bot., 66: 1-8.

Sudhakar, C., A. Lakshmi and S. Giridarakumar, 2001. Changes in the antioxidant enzyme efficacy in two high yielding genotypes of mulberry (*Morus alba* L.) under NaCl salinity. Plant Sci., 161: 613-619.

Tester, M. and R. Davenport, 2003. Na^+ tolerance and Na^+ transport in higher plants. Ann. Bot., 91: 503-527.

Waterman, P.G. and S. Mole, 1994. Extraction and Chemical Quantification. In: Analysis of Phenolic Plant Metabolites, Waterman, P.G. and S. Mole (Eds.). Blackwell Scientific Publications, Oxford, UK., ISBN-13: 9780632029693, pp: 80-84.

Yang, F., X. Xiao, S. Zhang and C. Li, 2009. Salt stress responses in *Populous cathagana* Rehder. Plant Sci., 176: 669-677.

Zheng, W. and S.Y. Wang, 2001. Antioxidant activity and phenolic compounds in selected herbs. J. Agric. Food Chem., 49: 5165-5170.

Zhu, J.K., 2003. Regulation of Ion homeostasis under salt stress. Curr. Opin. Plant Biol., 6: 441-445.