Environmental Research Journal 6 (1): 1-13, 2012
ISSN: 1994-5396

Effect of Salt Stress on Growth, Water Relations, Solute Composition and Photosynthetic Capacity of the Xero-Halophyte *Nitraria retusa* (L.)

[1]Faycal Boughalleb, [2]Hichem Hajlaoui and [1]Mounir Denden
[1]Laboratoire de Physiologie Vegetale, Institut Superieur Agronomique de Chott Meriem (ISA-CM), BP 47, 4042 Chott-Meriem (Sousse), Tunisia
[2]Institut National de Recherche en Genie Rural, Eaux et Forets (INRGREF), United' Experimentation Agricole, Oued Souhail, BP 20, 8000 Nabeul, Tunisia

Abstract: *Nitraria retusa* is common fodder shrub. The increasing interest in the utilisation of such shrubs in saline medium of North Africa requires evaluating the salinity effects on growth, water and solutes relationship, photosynthesis parameters in order to investigate salt-resistance mechanisms. Plants were grown in 0-800 mM NaCl under controlled conditions and harvested in three periods (after 60, 120 and 240 days). During the first harvest, the growth of *N. retusa* was promoted up to 400 mM NaCl, only to 200 mM NaCl in the two last harvests. Salt stress caused a marked decrease in osmotic potential, a significant accumulation of Na^+ and Cl^- and a concomitant decrease in K^+ and Ca^{2+} contents while magnesium, nitrogen and phosphorus contents were not greatly affected. Plants are able to maintain a higher leaf water content which was probably associated with a greater capacity for osmotic adjustment. The organic osmotica that can be involved in osmotic adjustment was proline, soluble sugar and at least degree glycinebetaine. Moderate salinity had a stimulating effect on growth rate, net CO_2 assimilation (P_n), transpiration (E) and stomatal conductance (g_s). At higher salinities levels, these physiological parameters decreased significantly. There was no significant changes on the chlorophyll fluorescence for *N. retusa* stressed plants. Carotenoid content was highest at 800 mM. For the chlorophyll content, it was unaffected up to 400 mM and then decreased slightly at 800 mM. Mesophyll of *N. retusa* leaves were thinner in salt-stressed plants while epidermis thickness was unaffected by salinity and the stomatal density decreased significantly with higher salt treatments. The results suggest that *N. retusa* show high tolerance to high salinity. The tolerance to salinity appears to be achieved through two mechanisms compartimentation of ions at moderate salinity and salt excretion at very higher salinity.

Key words: *Nitraria retusa*, solute and water relations, osmotic adjustment, leaf anatomical changes, chlorophyll fluorescence, gas exchange

INTRODUCTION

Soil salinity is one of the most important environmental factors limiting plant growth and productivity around the world (Munns, 2002). Salinity may decrease biomass production because it causes a lowering of plant water potentials, increasing specific ion toxicities or ionic imbalances (Munns and Tester, 2008). NaCl toxicity enhances the sodium content and consequently affects the absorption of other mineral elements (Greenway and Munns, 1980). Indeed, high levels of Na^+ inhibit Ca^{2+} and K^+ absorption which results in a Na^+/K^+ and Na^+/Ca^{2+} antagonism (Tester and Davenport, 2003). Metabolic toxicity of Na^+ is largely due to its ability to compete with K^+ for binding site essential for cellular function (Aziz and Khan, 2001).

Salt tolerance mechanisms of halophytes include combinations of salt exclusion (from root and leaf), excretion (salt glands, bladder hairs and re-translocation), succulence, compartmentalization and compatible solutes (Popp, 1995). On the other hand Debez *et al.* (2004) reported that sodium exclusion method of salt tolerance appears less efficient than sodium accumulation particularly in the succulent xerophytes. A large number of plant species accumulate glycinebetaine and proline in response to salinity stress and their accumulation may play a role in combating salinity stress (Ashraf and Harris, 2004). Organic solutes are accumulated in the cytosol to balance the solute potential of the vacuole which is dominated by ions (Greenway and Munns, 1980). Whereas data do not always indicate a positive

Corresponding Author: Faycal Boughalleb, Laboratoire de Physiologie Vegetale, Institut Superieur Agronomique de Chott Meriem (ISA-CM), BP 47, 4042 Chott-Meriem (Sousse), Tunisia

correlation between the osmolyte accumulation and the adaptation to stress (Ashraf and Harris, 2004; Martinez *et al*., 2005; Ashraf and Foolad, 2007).

The reduction in plant growth observed in many species subjected to excess salinity is often associated with a decrease in their photosynthetic ability (Brugnoli and Bjorkman, 1992). Salt may affect growth indirectly by decreasing the rate of photosynthesis. Stomatal (closure of stomata) and non-stomatal (including damage to photosynthetic apparatus) factors may be involved in reduction of photosynthesis rate (Bethke and Drew, 1992; Dionisio-Sese and Tobita, 2000; Kao *et al*., 2003). Different studies have reported a relationship between non-stomatal effects and the presence of high leaf Na^+ and Cl^- concentrations (Bethke and Drew, 1992; James *et al*., 2002).

Measurement of chlorophyll fluorescence has been used to evaluate the integrity of photosystem II upon exposure to stress (Shabala, 2002). Various studies have shown that salinity stress can predispose plants to photoinhibition and photodamage of PSII and finally inhibit its activity (Everard *et al*., 1994; Jimenez *et al*., 1997; Netondo *et al*., 2004) whereas other studies have shown that salt stress had no significant effect on PSII (Delfine *et al*., 1998; Lu *et al*., 2003; Morant-Manceau *et al*., 2004).

Because of the increasing land surfaces affected by salinity, understanding how some potential halophytic crop plants cope with salinity is becoming a major topic. The aim is to engineer crops that will be able to grow on such soils. For this study researchers selected *N. retusa* (L.), 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). To now the responses of *N. retusa* to salinity are of particular importance as they are among the most important species that can be used both as a fixative soil and as fodders in dry lands of the centre and south of Tunisia. There was defined the growth-related parameters, solute and water relations and correlated them with chlorophyll fluorescence, photosynthetic pigments composition and gas exchange parameters. In order to evaluate the response of *N. retusa* plants to different NaCl salinity levels and to identify and understand salt resistance mechanisms induced to confront saline stress.

MATERIALS AND METHODS

Plant growth conditions: *Nitraria retusa* plants were propagated by grafting from a source plant growing wild in the salt region of Sabkha Kalbia (Tunisia). 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 micronutrients. When seedlings were around 7 cm in height (3 months old), plants 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%; photoperiod (14/10 h day/night). At the end of the acclimatization phase, *N. retusa* seedlings were divided into five groups for treatments: 0 mM NaCl (control); 100, 200, 400 and 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 at 2 days 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 240 days.

Growth activity, leaf parameters and water relations: All the growth parameters were measured for three harvests (60, 120 and 240 days after the highest salt concentration was reached). Plants from each treatment were sampled to determine leaf number and leaf height. The Leaf surface Area (LA) was measured by using leaf area meter DT-Scan (Delta-Scan Version 2.03, Delta-T Devices, Ltd., England). For the biomass determination, the plant material was first 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), Net Assimilation Rate (NAR) were calculated as follows:

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

$$NAR = [(DM_2-DM_1)/(LA_2-LA_1)]/[\ln (LA_2/LA_1)/(t_2-t_1)]$$

Where:

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

Leaf succulence was assessed using both Leaf succulence (L_S) and Leaf thickness (L_T) ratios as:

L_S = Leaf FM/Leaf surface area (LA)

L_T = Leaf Water Content (LWC)/ Specific Leaf Area (SLA)

with SLA being the specific leaf area SLA ($m^2\ g^{-1}$) = Leaf surface Area (LA)/Leaf DM ; Water content of leaves were determined as LWC = (FM-DM)/DM. The osmotic potential (Ψ_s) of the sap was measured using a vapour pressure osmometer (model 5500, Wescor, CA).

Anatomical study: At the end of the experiment, small pieces of leaf tissue (approx. 5×5 mm, ten matures leaves from the median parts of ten plants per treatment) were excised from the midportion of laminate leaves. Cut tissues were fixed in freshly prepared FAA (Formaldehyde: glacial acetic acid: 70% ethanol 5:5:90 by volume) overnight at room temperature. After washing with a 0.1 M phosphate buffer (pH 7.4), they were dehydrated by passage through a tertiary butyl alcohol series (15-100%) and embedded with warm (56-58°C) paraffin. The resulting blocks were then cut in 10 μm sections with rotary microtome and stained with 0.5% (w/v) toluidine blue O in 0.1% (w/v) borax. Observations were performed under a light microscope (Leitz, Germany) and the measurements of various cells and tissues were taken with an ocular micrometer and exact values were calculated with a factor derived by comparing ocular with stage micrometers. The stomatal density (number per unit leaf area) was measured in the same day of collection in the abaxial surface of ten mature leaves per treatment.

Photosynthetic pigments: 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-2000, Krefeld, Germany) at three wavelengths (470, 645 and 663 nm) and chlorophyll (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. For chloride determination, shoot and root samples of 100 mg were ground and extracted in 10 mL of distilled water by heating at 80°C for 3 h. Chloride concentration in the extracts was performed using a digital chloridometer (Haake-Buchler Instrument, Inc., Saddlebrook, NJ-USA). The mineral phosphorus was carried out by using the colorimetric method of Fleury and Leclerc at 436 nm. Nitrogen content was determined by the Kjeldhal method adapted to the colorimetric method at 660 nm. After mineralization of 1 g of sample by sulfuric acid in the presence of a catalyser (potassium sulphate oxide and mercury) and dilution, the rate of nitrogen was given by the Technicon autoanalyser II system.

Glycinebetaine, proline and total soluble sugars: Organic solutes content were determined in leaves of four plants per treatment. Dry plant material (25 mg) was extracted with 80% ethanol at 80°C. The solution was filtered and concentration of total soluble sugars was determined by the anthrone colorimetric method. Glycinebetaine was extracted by stirring finely ground-dried samples with demineralised water at 100°C for 1 h. Glycinebetaine content was determined spectrophotometrically after reaction with KI-I2 at 365 nm (Grieve and Grattan, 1983). Proline was also determined spectrophotometrically following the ninhydrin method described by Bates *et al.* (1973). Approximately 300 mg of dry tissue was homogenized in 10 mL of 3% aqueous sulphosalicylic acid and filtered. To 2 mL of the filtrate, 2 mL of acid ninhydrin were added followed by the addition of 2 mL of glacial acetic acid and boiling for 60 min. The mixture was extracted with toluene and the free proline was quantified spectrophotometrically at 520 nm from the organic phase using toluene as a blank.

Analysis of photosynthetic gas exchange: Net CO_2 assimilation rate (P_n), stomatal conductance (g_s), intercellular CO_2 concentration (C_i) and transpiration (E) of the seedlings were measured on leaves of five plants per treatment at 120 days after salt stress using a Portable Photosynthesis system (Li-6200, Lincoln, NE-USA). Measurements were made between 8:00 and 9:00 h to avoid photoinhibitory damage potentially resulting from high light stress at midday. Temperature, relative humidity and light intensity during measurement of gas exchange were 23-26°C, 80% of the ambient humidity and 1000 mmoL/m^2/sec, respectively. The CO_2 concentration in the chamber was 360 mmoL moL^{-1}. Leaf Water Use Efficiency (WUE) was estimated as the quotient of the photosynthetic rate over the transpiration rate (P_n/E).

Measurements of chlorophyll fluorescence: Photosystem 2 (PSII) photochemistry was measured on dark and light-adapted leaves using a portable fluorometer (PAM-2000, Walz, Germany). By using fluorescence parameters determined in both light and dark-adapted leaves, calculations were made of:

- The maximal potential PSII efficiency, Fv/Fm = (Fm-F0)/Fm
- The photochemical quenching coefficient, q_P = (F'm-Fs)/(F'm-F'0)
- Efficiency of excitation energy capture by open PSII reaction centers, F'v/F'm = (F'm-F'0)/F'm
- Quantum yield of PSII electron transport, Φ_{PSII} = (F'm-Fs)/F'm
- The nonphotochemical quenching, NPQ = (Fm/F'm)-1 (Demmig-Adams and Adams, 1996)

The minimal Fluorescence level (F0) and the maximal Fluorescence level (Fm) presented the fluorescence signal from a dark-adapted leaf when all reaction centers are open using a low intensity pulsed measuring light source and during a pulse of saturating light when all reaction centers are closed, respectively. F'0 is the minimal fluorescence in the light-adapted state, obtained by turning off the light temporarily to drain the electrons from PSII, F'm is the maximal value when all reaction centers are closed after a pulse of saturating light and Fs is the fluorescence at steady-state photosynthesis.

Statistical analysis: The data were subjected to Analysis of Variance (ANOVA) and comparisons between the mean values of treatments were made by the Duncan post hoc tests ($p<0.05$). Statistical analyses were performed using the SPSS statistical package (SPSS 13).

RESULTS

Plant growth: A two-way ANOVA showed a significant individual effect of salinity and time of harvest and their interaction in all growth parameters of *N. retusa* (Table 1). Shoot dry mass, Relative Growth Rate (RGR) and Leaf Area (LA) were significantly enhanced at 100 and 200 mM NaCl in the first harvest (ca. 107-110, 103 and 108-109.5% of the control, respectively) and only at 100 mM in the two last harvests (Table 1). Moderate salinity appeared thus to be optimal for the growth of *N. retusa*. In the first harvest (60 days), root dry mass did not show a significant effect of salinity. In addition, shoot DM, plant height, leaf number, RGR and LA were not inhibited up to 400 mM NaCl but only up to 200 mM in the others harvests. At higher salt levels, all these parameters decreased significantly.

Osmotic potential and leaf water relations: In the two first harvests there is shown that *N. retusa* leaf water content values were higher in the presence than in the absence NaCl levels (Table 1). Whereas leaf water content significantly decreased at higher salt levels (400-800 mM) in the third harvest (240 days). Consequently, leaf succulence ratio (L_S) increased in average by 18.2 and 12.9% at 800 mM NaCl in the two first harvests, respectively but was reduced by 27% in the third harvest. Leaf thickness (L_T) increased only at higher salinities treatments in the three harvests. At 800 mM there was observed an increase in L_T by approximately, 16.7, 44.9 and 71.5% in the first, second and third harvest, respectively (Table 1). The osmotic potential of salt treated plants decreased with increasing NaCl concentration (Table 1), the ANOVA showed a significant individual effect of time of harvest and his interaction with salinity in osmotic potential of *N. retusa*.

Table 1: Effects of NaCl concentrations on growth, Leaf Area (LA, cm^2), Net Assimilation Rate (NAR, $g\ m^{-2}$ per day), Leaf succulence (L_S, $mg\ cm^2$), Leaf thickness (L_T, µm), Water Content (LWC, $mL\ g^{-1}$ DM) and osmotic potential (Ψ_s,-MPa) of *N. retusa* plants after 60, 120 and 240 days of treatments

	Periode (days) NaCl (mM)														
	60					120					240				
Parameters	0	100	200	400	800	0	100	200	400	800	0	100	200	400	800
Shoot DM ($g\ plant^{-1}$)	1.50^{b}	1.60^{a}	1.60^{a}	1.50^{b}	1.40^{c}	5.60^{c}	6.50^{a}	6.10^{b}	4.10^{d}	2.70^{e}	15.30^{c}	18.40^{a}	16.20^{b}	8.10^{d}	2.80^{e}
Root DM ($g\ plant^{-1}$)	0.50^{a}	0.50^{a}	0.50^{a}	0.50^{a}	0.50^{a}	1.10^{a}	1.20^{a}	1.10^{a}	0.90^{b}	0.70^{c}	2.80^{b}	3.00^{a}	3.00^{a}	1.30^{c}	0.70^{d}
Shoot/Root ratio	2.80^{b}	2.80^{b}	3.20^{a}	2.80^{b}	2.60^{b}	4.80^{ab}	5.20^{a}	5.50^{a}	4.30^{b}	3.50^{c}	5.30^{b}	6.00^{a}	5.40^{b}	6.40^{a}	3.70^{c}
Plant height (cm)	14.10^{a}	15.20^{a}	14.40^{a}	13.70^{a}	11.40^{b}	45.00^{b}	57.60^{a}	53.90^{a}	34.80^{c}	16.30^{d}	71.80^{b}	77.10^{a}	65.20^{c}	38.40^{d}	16.90^{e}
Leaf number	147.00^{a}	146.00^{a}	148.00^{a}	146.00^{a}	136.00^{b}	393.00^{b}	432.00^{a}	425.00^{a}	395.00^{b}	257.00^{c}	717.00^{b}	759.00^{a}	703.00^{b}	625.00^{c}	350.00^{d}
RGR10^{-2} ($g/g/day$)	3.48^{b}	3.60^{a}	3.59^{a}	3.48^{b}	3.41^{c}	2.85^{b}	2.95^{a}	2.89^{b}	2.59^{c}	2.20^{d}	1.78^{b}	1.85^{a}	1.80^{b}	1.51^{c}	1.11^{d}
Leaf area (cm^2)	73.00^{b}	78.00^{a}	80.00^{a}	74.00^{b}	63.00^{c}	313.00^{b}	$362^{a}.00$	360.00^{a}	267.00^{c}	151.00^{d}	639.00^{c}	772.00^{a}	697.00^{b}	341.00^{d}	158.00^{e}
NAR ($g\ m^{-2}\ day^{-1}$)	8.60^{b}	8.80^{ba}	8.70^{ba}	8.50^{b}	9.00^{a}	10.50^{ab}	10.80^{a}	10.10^{b}	8.80^{c}	9.20^{c}	8.10^{a}	8.20^{a}	8.00^{a}	6.90^{b}	4.60^{c}
L_S ($mg\ cm^{-2}$)	49.40^{c}	51.90^{b}	52.90^{b}	53.60^{b}	58.40^{a}	51.80^{d}	61.80^{b}	57.60^{c}	65.80^{a}	58.50^{c}	51.00^{bc}	54.90^{ab}	56.30^{a}	47.10^{c}	37.20^{d}
L_T (µm)	681.00^{c}	697.00^{cb}	721.00^{b}	789.00^{a}	795.00^{a}	729.00^{c}	838.00^{b}	852.00^{b}	1009.00^{a}	1057.00^{a}	738.00^{b}	784.00^{b}	809.00^{b}	1184.00^{a}	1266.00^{a}
LWC ($mL\ g^{-1}$ DM)	7.10^{d}	7.80^{c}	8.70^{b}	9.70^{a}	8.90^{b}	10.10^{d}	11.60^{c}	12.40^{b}	13.00^{a}	10.50^{d}	10.20^{c}	10.80^{b}	11.50^{a}	9.80^{c}	8.70^{d}
Ψs (-MPa)	1.50^{a}	1.90^{d}	2.20^{c}	3.50^{b}	4.80^{a}	2.10^{a}	2.30^{d}	2.50^{c}	3.30^{b}	5.00^{a}	1.80^{a}	3.20^{d}	3.80^{c}	4.90^{b}	6.10^{a}

Plants were cultivated on nutrient solution supplied with 0, 100, 200, 400 or 800 mM NaCl during 2, 4 and 8 months. Data are means of five measurements. Values within each treatment period marked with at least one same letter not significantly different ($p<0.05$) as described by Duncan's test

Table 2: Anatomical variables of leaves from *N. retusa* plants treated with different salinity levels (0, 100, 200, 400 and 800 mM NaCl): Upper Epidermis (UE), Lower Epidermis (LE), Mesophyll thickness (M_T), Leaf thickness (L_T) and stomatal density

	NaCl (mM)				
Parameters	0	100	200	400	800
UE (μm)	26.1±1.1^a	26.2±1.2^a	26.3±0.9^a	26.5±1.30^a	26.4±1.2^a
M_T (μm)	685.2±5.8^b	731.3±7.4^b	755.2±7.1^b	1129.2±6.80^a	1211.2±9.1^a
LE (μm)	26.5±1.2^b	26.9±1.1^b	27.3±1.3^{ab}	27.6±1.40^{ab}	28.5±2.1^a
L_T (μm)	738.1±8.6^b	784.3±7.9^b	809.6±6.8^b	1184.6±12.7^a	1266.1±14.2^a
Stomatal density (number mm^{-2})	122.0±5.0^a	124.0±7.0^a	121.0±6.0^a	118.0± 4.0^b	105.0±4.0^c

Plants were harvested after 8 months of salt treatments. Data are means of ten measurements. Values in each line with the same letter are not significantly different ($p<0.05$) as described by Duncan's test

Leaf anatomy: A comparison of control plants with saline treated plants showed that the epidermis thickness of *N. retusa* was not altered significantly (Table 2). However, salt stress induced a significant increase of the mesophyll thickness as well as of the entire lamina thickness and the highest values were measured in the range of 400-800 mM NaCl (Table 2). Stomatal density decreased significantly ($p<0.05$) at salinity levels exceeding 200 mM NaCl in *N. retusa* leaves.

Solutes accumulation: The concentration of ions in *N. retusa* plant varied among salinity treatments. The one-way ANOVA analysis indicated a significant relationship ($p<0.0001$) between salinity level and content of Na^+, Cl^-, K^+, Ca^{2+}, Mg^{2+}, N and P within the plants. As shown in the Fig. 1a, Na^+ and Cl^- concentration of shoots ($F = 321.6$, $F = 307.3$, respectively; $p<0.0001$) and roots ($F = 233.5$, $F = 413$, respectively; $p<0.0001$) significantly increased as the salinity increased whereas researchers observed a significant ($p<0.05$) reduction in shoot Na^+ contents at 800 mM NaCl as compared to the values measured in 400 mM treatments. In addition the shoot Cl^- content between salinity levels 400 and 800 mM NaCl did not differ significantly ($p<0.05$). Potassium content decreased with increasing salt supply in both organs ($F_{shoot} = 39.9$, $F_{root} = 40.3$; $p<0.0001$) (Fig. 1b). At 100 mM NaCl level, the percentage inhibition of shoots and roots K^+ content as compared with control non-treated plants was in the range of 28.3 and 24.8%, respectively. There was no significantly difference among the shoots K^+ content from 100 up to 400 mMNaCl ($p<0.05$) among roots K^+ content from 100-400 mM and from 200-800 mM. The Ca^{2+} content of shoots ($F = 174.4$, $p<0.0001$) and roots ($F = 139.4$, $p<0.0001$) decreased with salinity treatments (Fig. 1b). Compared with control non-stressed plants, *N. retusa* grown under 800 mM NaCl conditions had only 45.2% of the Ca^{2+} in the shoots. Higher salinities have not supplementary effect on roots Ca^{2+} concentration as shown by the non-significant difference among roots from 200 mM up to 800 mM NaCl. The Mg^{2+} content in the shoots ($F = 11.1$, $p<0.0001$) and roots ($F = 36.8$, $p<0.0001$) changed significantly in response to different salinity

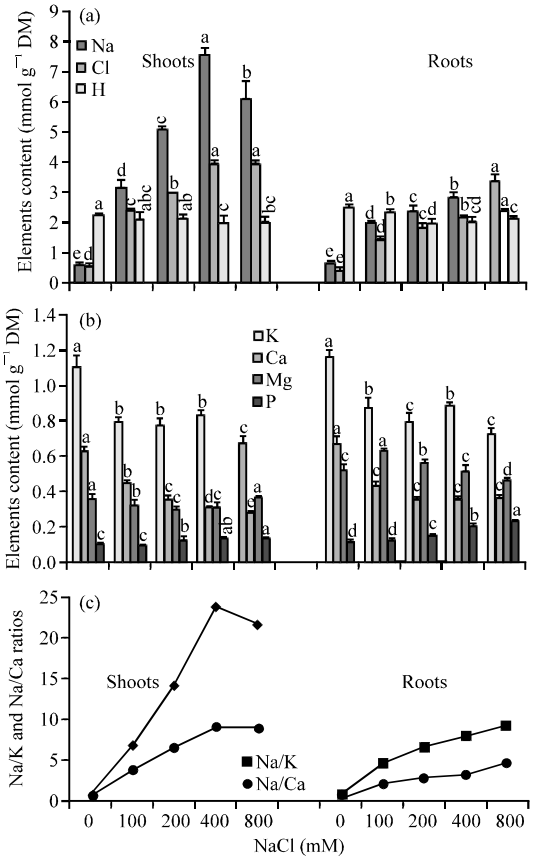

Fig. 1: Effect of NaCl treatments on the accumulation of sodium, chloride and nitrogen (a), potassium, calcium, magnesium and phosphorus (b), Na/K and Na/Ca ratios (c) in shoot and root tissues of *N. retusa* plants salt-treated for 120 days. Bars followed by the same letter do not differ statistically at $p<0.05$ (Duncan's test). Averages of four repetitions are presented with bars indicating SE

treatments. In the shoots, plants grown in the range from 100-400 mM NaCl exhibited significantly lower Mg^{2+} content. However, the root Mg^{2+} content under some salinity treatments (100 and 200 mM NaCl) was higher

than under non-salinized condition, only plants grown at 800 mM NaCl presented lower Mg^{2+} content. The nitrate concentration in roots and shoots was not largely influenced by moderate NaCl levels. At higher salinities levels (400-800 mM NaCl), nitrate concentration in *N. retusa* shoots was reduced by about 9.3 and 6.8% compared with the control, respectively (Fig. 1a). Phosphate content was not affected with increasing salt supply in both organs in contrast researchers observed a increased values in some salinities treatments (Fig. 1b). The data shows (Fig. 1c) that the Na^+/K^+ ratio increases progressively with increasing salt concentration. Also a similar tendency was observed for Na^+/Ca^{2+} in all plant parts. For organic compounds, the study (Fig. 2a) showed that proline concentration was 1.6 fold higher at 100 mM and 3.3 fold higher at 800 mM NaCl than that in control while it was maintained at constant value in the range 100-200 mM NaCl. The Glycinebetaine (GB) content per unit dry weight increased consistently with increasing external salt. Soluble Sugar (SS) content decreased in *N. retusa* with moderate salt levels (100-200 mM NaCl) but increased with higher salinities.

Pigment composition and gas exchange: Chlorophyll contents of *N. retusa* was unaffected by the presence of NaCl until 400 mM NaCl levels. Although, at 800 mM NaCl, total chlorophyll content was 6% lower than in the insalinized control (Table 3). Carotenoid content was promoted by salinity and the highest values were measured at 800 mM NaCl. Moderate NaCl-salinity (up to 200 mM NaCl) had a stimulating effect on the photosynthesis parameters of *N. retusa* ($p<0.05$) with only minor changes of the intercellular CO_2 concentration. At higher salinities a distinct decrease of the stomatal conductance (g_s) and the intercellular CO_2 concentration (C_i) and consistent reductions of the net photosynthesis (P_n) and the transpiration rate (E). There was a good linear correlation (Fig. 3a) ($r^2 = 0.97$, $F = 959.9$, $p<0.0001$) between photosynthesis and stomatal conductance. The intercellular CO_2 concentration (C_i) was slightly correlated with P_n (Fig. 3b) ($r^2 = 0.35$, $F = 9.7$, $p = 0.006$). A highly positive relationship was observed between P_n and DM (Fig. 3d) ($r^2 = 0.93$, $F = 286.7$, $p<0.0001$) whereas a insignificant negative linear relationship between P_n and sodium content (Fig. 4) ($r^2 = 0.14$, $F = 3$, $p = 0.1$).

Salinity effect on PSII photochemistry: Researchers first investigated the changes in PSII photochemistry in the dark-adapted leaves. Table 2 shows that there was no significant difference in the maximal quantum yield of PS II (Fv/Fm) between control and salt-treated *N. retusa*

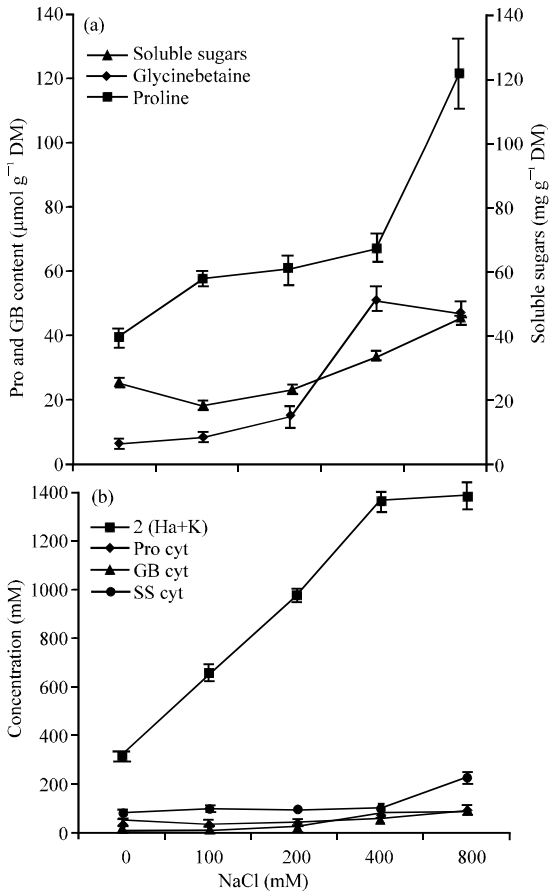

Fig. 2: (a) Effect of NaCl concentrations on proline, glycinebetaine and soluble sugar contents, (b) comparison between the global ionic concentration [2 (Na+K), mM] and cytoplasmic concentration of proline (Pro cyt), glycinebetaine (GB cyt) and soluble sugars (SS cyt) of *N. retusa* shoots after 120 days of treatments. Bars followed by the same letter do not differ statistically at $p<0.05$ (Duncan's test). Averages of four repetitions are presented with bars indicating SE

plants measured at predawn. For the PSII photochemistry in the light-adapted leaves, in both control and salt stressed plants at midday, durant the experiment there was no changes in the efficiency of excitation energy capture by open PSII reaction centers (F'v/F'm) in stressed plants as compared to control plants. In the ranges of salinity (0-400 mM NaCl) there was no changes in the actual PSII efficiency (Φ_{PSII}) in salt-treated plants as compared to control plants. At higher salinity, this parameter decreased significantly as compared to control plants. Table 3 shows that salt stress had no effects on the photochemical quenching coefficient (q_P). Table 3

Table 3: Effects of NaCl concentrations on photosynthetic pigment contents, net CO_2 assimilation rate (Pn), stomatal conductance (gs), transpiration (E), internal CO_2 concentration (Ci), Water-Use Efficiency (WUE), maximum efficiency of PSII photochemistry (Fv/Fm), efficiency of excitation capture by open PSII reaction centres (F'v/F'm), actual PSII efficiency (Φ_{PSII}), photochemical quenching coefficient (q_P) and Non-Photochemical Quenching coefficient (NPQ) in leaves of *N. retusa* after 120 days of treatments

	NaCl (mM)					ANOVA (F-values)
Parameters	0	100	200	400	800	Salinity (S)
Carotenoid (mg/g/FM)	24.1±0.700^{c}	26.0±0.700^{b}	24.2±0.800^{c}	25.9±0.900^{b}	27.1±0.600^{a}	15.5***
Chl a (mg/g/FM)	200.4±3.100^{b}	205.8±2.500^{a}	202.4±2.400^{ab}	199.1±5.800^{b}	189.1±6.400^{c}	13.3***
Chl b (mg/g/FM)	85.2±1.700^{a}	83.8±1.600^{ab}	81.2±1.400^{bc}	79.6±2.300^{c}	79.1±3.100^{c}	7.4**
Chl a+b (mg/g/FM)	285.6±3.900^{a}	289.6±3.600^{a}	283.6±4.200^{ab}	278.7±6.400^{b}	268.2±4.700^{c}	15.6***
P_n (µmoL m sec^{-1})	19.1±0.140^{b}	22.4±0.450^{a}	22.8±0.470^{a}	17.6±0.240^{c}	12.2±0.300^{d}	786.2***
g_s (mmoL m sec^{-1})	615.0±14.30^{b}	760.0±21.50^{a}	778.0±17.10^{a}	519.0±23.20^{c}	288.0±21.30^{d}	526.8***
E (mmoL m sec^{-1})	4.29±0.03^{b}	4.75±0.04^{a}	4.70±0.05^{a}	3.62±0.03^{c}	2.86±0.07^{d}	481.4***
C_i (µmoL moL^{-1})	324.0±3.700^{a}	281.0±3.100^{b}	253.0±3.400^{c}	245.0±4.300^{d}	206.0±3.500^{e}	419.2***
WUE (µmoL CO_2 mmoL^{-1} H_2O)	4.46±0.03^{b}	4.73±0.09^{a}	4.86±0.11^{a}	4.87±0.07^{a}	4.26±0.13^{c}	17.2***
Fv Fm^{-1}	0.84±0.02^{a}	0.83±0.03^{b}	0.85±0.03^{a}	0.85±0.02^{a}	0.84±0.02^{ab}	4.7**
F'v F'm^{-1}	0.44±0.02^{b}	0.45±0.02^{a}	0.46±0.02^{a}	0.45±0.01^{a}	0.44±0.01^{b}	8.8***
OFSII	0.26±0.01^{b}	0.27±0.01^{a}	0.27±0.01^{a}	0.25±0.01^{c}	0.21±0.01^{d}	329.0***
q_P	0.60±0.02^{c}	0.61±0.01^{b}	0.62±0.02^{a}	0.61±0.03^{ab}	0.60±0.01^{d}	39.8***
NPQ	1.23±0.12^{e}	1.28±0.15^{d}	1.30±0.17^{c}	1.57±0.18^{b}	1.87±0.22^{a}	1767.9***

Data are means values±SE of four measurements. Values in each line with the same letter are not significantly different (p = 0.05); as described by to Duncan's Test. The probabilities are shown as *p<0.05, **p<0.01, ***p<0.001 and not significant (ns)

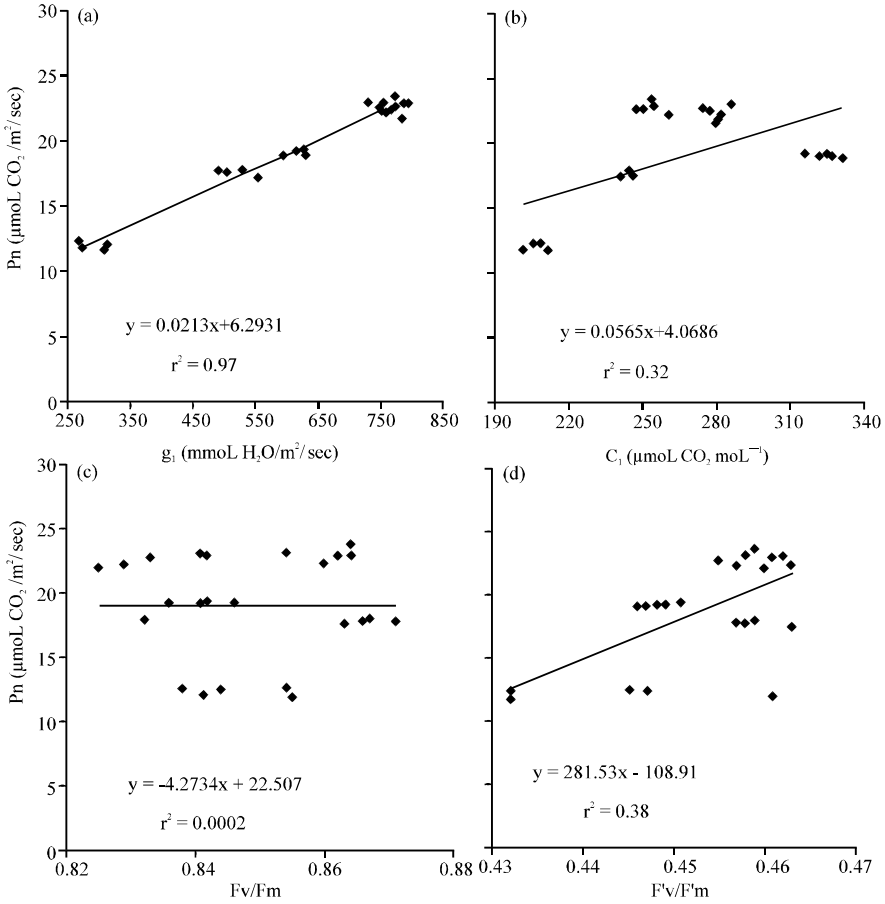

Fig. 3: (a) Relationships between net CO_2 assimilation rate (Pn) and stomatal conductance (gs), b) internal CO_2 concentration (Ci), c) dark-adapted fluorescence (Fv/Fm), d) and light-adapted fluorescence (F'v/F'm) in *N. retusa* species cultivated under salt stress for 120 days. An average of 5 repetitions and confidante interval was calculated at the threshold of 95%

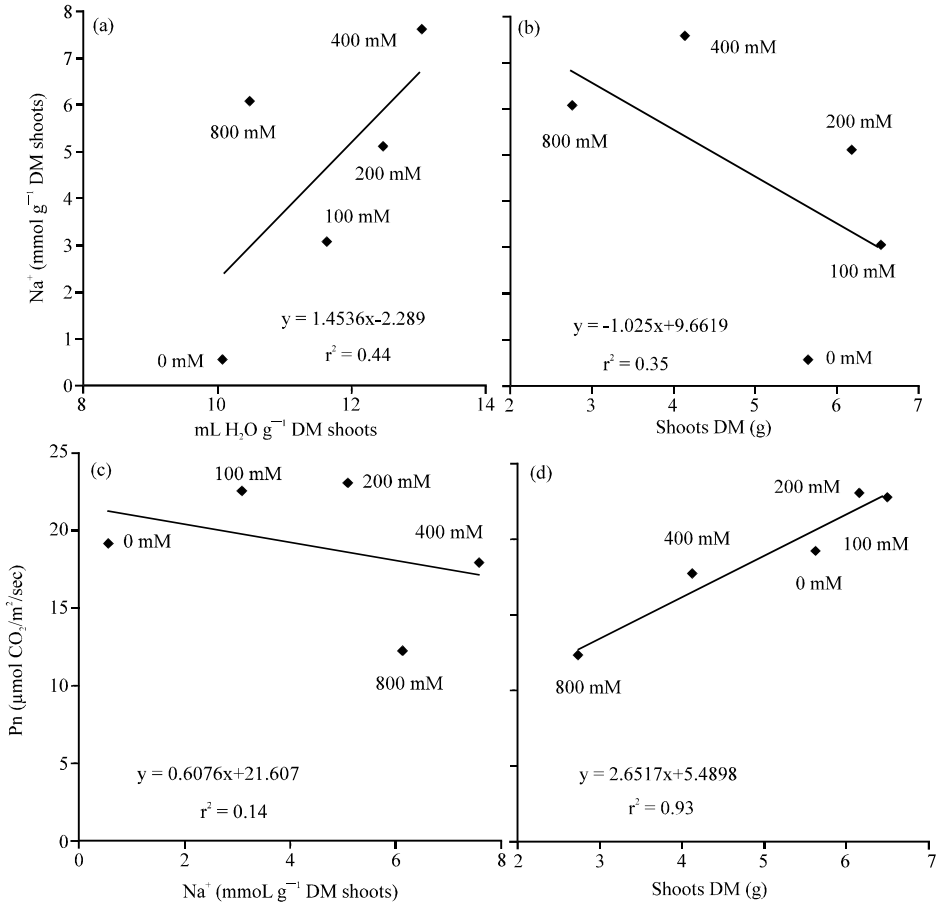

Fig. 4: (a) Correlations between the shoots sodium contents and shoots water content, b) shoots Dry Mass (DM), c) net CO_2 assimilation rate (Pn) and d) between net CO_2 assimilation rate (Pn) and the shoots Dry Mass (DM) in *N. retusa* plants cultivated under salt stress for 120 days. An average of 4 repetitions and confidante interval was calculated at the threshold of 95%

shows also that NaCl stress in the root medium increased the Non-Photochemical Quenching coefficient (NPQ) of the leaves. At the highest salinity level (800 mM), NPQ was 1.5 times greater as compared with control non-salinized plants. The maximal quantum yield of PS II (Fv/Fm) was not associated with the photosynthetic rate of *N. retusa* leaves (Fig. 4c) ($r^2 = 0.0002$). However, the efficiency of PSII (F'v/F'm) was more correlated with P_n (Fig. 4d) ($r^2 = 0.38$, $F = 14.4$, $p = 0.001$).

DISCUSSION

Data collected for dry weight, relative growth rate and leaf area indicated that *N. retusa* growth was stimulated by moderate salinity, in agreement with previous studies (Moghaieb *et al*., 2004; Silveira *et al*., 2009) on salt stressed halophytes. Indicating that *N. retusa* is a true halophyte because it remains viable and completes its life cycle at salinity exciding seawater values. However, long period of stress (240 days) combined with higher salinities levels (400-800 mM NaCl) were inhibitory to plant growth. The depressive action of salt on growth appeared by a significant reduction of the aerial organ growth activity (Table 1). Moreover, the results showed an decrease of Shoot-Root Ratio (RSR) under saline conditions (Table 1) which indicate a more pronounced salt effect on shoot dry weight than on root dry weight and suggest that shoot was more sensitive to salinity than the root (Tejera *et al*., 2004).

Deleterious effects of salinity are thought to result from osmotic effects, ion toxicities and ionic imbalances (Munns and Tester, 2008; Patel *et al*., 2009). In addition, Na^+ may have a direct toxic effect such as when it interferes with the function of potassium as a cofactor in various reactions (Khan *et al*., 2000b). In the present study, *N. retusa* plant accumulates inorganic ions especially Na^+ and Cl^- by salt treatment to sustain the osmotic potential and maintain a flow of water into the plant. This accumulation was increased with increasing NaCl concentration and the shoots were the preferential

site for Na^+ accumulation (Fig. 1a). In contrast, increasing the external NaCl concentration was accompanied by a concomitant decrease in K^+ and Ca^{2+} contents. Mg^{2+} concentrations were less affected by salt stress. These results are consistent with those reported for *Salicornia rubra* (Khan *et al*., 2001) and *Plantago crassifolia* (Vicente *et al*., 2004). Nitrate content remained slightly affected by salt stress as shown by the fact that its concentration of shoots decreased only by <6.9% with 800 mM NaCl. Witch is in agreement with other researches indicating that salinity reduces N accumulation in plants (Garg *et al*., 1993).

In this regard, Munns (2002) show that 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 by which the species would be able to resist a greater ion concentration. The vast accumulation of Na^+ in this halophytic species with moderate salinity appeared that the second mechanism is responsible for the salinity tolerance in *N. retusa*. Demonstrating that salinity resistance of this species is not linked to their ability to restrict sodium accumulation in the aerial part. The results (Fig. 4a) showed that the accumulation of Na^+ in photosynthetic organs was associated with an improvement of water content for a all levels of salinity ($r^2 = 0.44$, $F = 14.5$, $p = 0.001$) and with increased leaf thickness and succulence (Table 1). Succulent plants are characterized by thick leaves, increased vacuole volume and mesophyll cell size (Mimura *et al*., 2003). Succulence enables the dilution of internal ion content. It is in agreement with the performance of other halophytes like *Suaeda fruticosa* (Khan *et al*., 2000a) and *Atriplex stocksii* (Khan *et al*., 2000b).

This result reflects probably an ability of the plants to use the dominant ions (Na^+) for the osmotic adjustment. To test the validity of this hypothesis, researchers compared the shoots water content, their global ionic concentration estimated by [2 ($Na^+ + K^+$)/water content] and the ionic concentration of the culture medium (Fig. 2b). This data shows in spite of the salinity levels, the global ionic concentration of shoots exceeds that of the nutrient solution. *N. retusa* plants are therefore able to maintain the hypertonia in relation to its culture medium. However, this hyper-adjustment which would be provided exclusively by K^+ in control non-treated plants and by Na^+ in plants subjected to salt is associated with an improvement in the hydration of tissues in plants grown at moderate salinity levels. These results suggest that under moderate salinity, the plant uses Na^+ (and very likely Cl^-) for osmotic adjustment. At higher salinities, response of *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 salt excretion was involved in salt regulation mechanisms of *N. retusa* plants. This result was in accordance with the findings of El-Bana (2002) that consider *N. retusa* and *Tamarix nilotica* as excretive frutiscents.

Many studies on halophytes and some tolerant glycophytes plants showed that a low foliar Na/K ratio is a salt tolerance index (Tester and Davenport, 2003; Ashraf and Orooj, 2006). In contrast Grieve *et al.* (2004) consider that K^+/Na^+ and K^+/Na^+ selectivity did not appear to be good indicators of salt tolerance. In the present study, Na^+/K^+ and Na^+/Ca^{2+} ratios increased with salinity in both plant parts (Fig. 1c). Furthermore, the relative weedy correlation between the RGR and the ionic selectivity [K/(K+Na)] seems to be the consequence of the strong Na^+ and K^+ competition. These results indicate that an ion transport mechanism exists for Na^+ and against K^+ and Ca^{2+} accumulation. This reflects absorption and a selective accumulation of K^+ compared to Na . The selectivity towards cations (K^+, Ca^{2+} and Mg^{2+}) is severely affected at higher salinities. The failure of the selective barrage led to a selective invasion of tissues by Na^+. The data of Fig. 3b show a weedy negative relationship ($r^2 = 0.35$, $F = 9.3$, $p = 0.009$) between the aerial biomass production and their Na^+ content. Suggest that ionic toxicity was slightly implied in the inhibition of growth. It may be due to progressively lower K^+ levels in the tissue. This phenomenon has been explained by the competitive interaction between Na^+ and K^+ and the inhibition of K^+ uptake by high Na^+ levels (Bernstein *et al*., 1995).

The accumulation of Na^+ and Cl^- should be balanced by stimulation of the synthesis of compatible compound in the cytoplasm (Khan *et al*., 1998). Sugars, proline and other organic solutes are considered to improve salt tolerance by contributing to osmotic balance and preserving enzyme activity in the presence of toxic ions (Greenway and Munns, 1980). The data (Fig. 2a) showed a progressive increase in proline with increasing salinity. Soluble sugar involved in alleviating salt stress in many halophytes (Shen and Chen, 2001; Song *et al*., 2006) in agreement with this view the data showed that soluble sugar increased as a result of salinity increase. For the glycinebetaine content, it was 17-120 fold lower than that in *Suaeda fruticosa* and *Atriplex griffithii* (Khan *et al*., 1998) indicating that *N. retusa* is not considered among the hyper-accumulator of GB. The significance of organic compounds accumulation, their role in osmoregulation and salt tolerance has been questioned. To see if these

organic compounds play a role in the osmotic adjustment between the cytoplasm and the vacuole, we compared the global ionic concentration [estimated by 2 (Na^++ K^+)/Water content] with that of the cytoplasm organic compounds contents (Fig. 2b). The latter is achieved by reducing organic compounds content to those in the water and on the assumption that the cytoplasm is 5% of the total cell volume (Fernandez-Ballester *et al*., 1998). Figure 2b shows a weedy contribution of GB and SS in maintaining osmotic balance between the vacuole and the cytoplasm while the role of proline was more important. These results are consistent with several other researches indicating that the osmotic adjustment is provided mainly by inorganic solutes in plants inclusive (Yang *et al*., 1990). Furthermore, Martinez *et al*. (2005) found that GB contributed only a few percent to osmotic adjustment even if a chloroplastic sequestration is assumed. Its importance in stress resistance may therefore be linked to its protective properties for membranes and numerous endocellular structures rather than to an osmotic function. Vicente *et al*. (2004) showed that proline does not seem to play an important role in the mechanism of salt tolerance in *Plantago crassifolia*.

In order to test the functionality of the photochemical apparatus, *N. retusa* plants was treated with high salinity (up to 800 mM NaCl). There was found that there were a little effect of salinity on the photochemistry of PSII. This is reflected by the fact that no significant changes were observed in their maximal efficiency of PSII photochemistry (Fv/Fm) and that the efficiency of PSII (F'v/F'm) was not affected by salinity in *N. retusa* plants for the duration of the experiment, suggesting that there was no damage to PSII (Table 3). There was a decline in Φ_{PSII} by approximately 15% between controls and 800 mM NaCl (Table 3). This effect appears mild and indicates that electron flow was not severely affected. There were also no changes in photochemical quenching coefficient (q_P) which indicate that the proportion of reaction centres remaining open were similar under control and saline conditions (James *et al*., 2002). In contrast, researches observed an increase in NPQ suggesting that salt treatment induced an enhancement in dissipation of damaging excess energy in order to protect the photosynthetic apparatus against photodamage (Schindler and Lichtenthaler, 1996; Qiu *et al*., 2003), in order to maintain an adequate balance between photosynthetic electron transport and carbon metabolism (Krause and Weis, 1991). In the experiments, chlorophyll a and b contents and total chlorophyll remained unaffected up to 400 mM in *N. retusa* and then decreased slightly at the highest salinities levels (800 mM) (Table 3). Similar results were reported for leaf chlorophyll content of *Artimisia anethifolia* (Lu *et al*., 2003). NaCl stress decreased chlorophyll content of the plant by increasing the activity of the cholorophyllase (Rao and Rao, 1981). The shown salt-induced increase of the carotenoid content in leaves of *N. retusa* (Table 3) could be function to protect the photosynthetic apparatus by dissipated the excess of energy (Lu *et al*., 2003).

Moderate salinity (up to 200 mM NaCl) was optimal for the photosynthetic capacity of *N. retusa* (maximal values gas exchange parameters) but higher salinities induced a significantly reduction in P_n, g_s and E. This response to NaCl is similar to that of *Salicornia rubra* and *Sarcocornia fruticosa* (Khan *et al*., 2001; Redondo-Gomez *et al*., 2006). While the intercellular CO_2 (C_i) was immediately decreased by salt stress. Stomatal closure could reduce internal CO_2 concentration and CO_2 assimilation rate (Dionisio-Sese and Tobita, 2000). Water use efficiency of *N. retusa* tends to increase at higher salinities, in spite of the decrease in P_n through a decrease in g_s. The decrease in both P_n and C_i with increasing salinity and the consequent decrease in g_s may indicate that stomatal closure were imposing a limitation on photosynthesis under saline stress. In addition, a highly significant correlation between net photosynthetic rate and stomatal conductance (Fig. 4a) ($r^2 = 0.97$) suggests stomatal conductance as one of the important causes limiting photosynthesis under salt stress. However, the decline in C_i was relatively weedy correlated with P_n (Fig. 4b) ($r^2 = 0.35$) which could be related to the presence of non-stomatal effects. In agreement with other studies reported that both stomatal and non-stomatal components are responsible for a decrease in photosynthetic ability (Everard *et al*., 1994; Lawlor, 2002). Regression analyses among net photosyntitic rate (P_n) and the maximal efficiency of PSII photochemistry (Fv/Fm) and the efficiency of PSII (F'v/F'm) on the other hand (Fig. 4c and d) shows no significant relationship between P_n and Fv/Fm and a weedy correlation between P_n and F'v/F'm ($r^2 = 0.38$), demonstrating that variations of these fluorescence parameters were very slightly associated with variations of photosynthesis. However, it is difficult to discriminate between a down-regulation of fluorescence parameters in dark or light adapted leaves (Fv/Fm, F'v/F'm) by stomatal closure or a regulation of these parameters by another factor.

It was observed that P_n showed a very lower negative correlation with leaf sodium content (Fig. 3c) ($r^2 = 0.14$) seems to be the consequence of the independence between either CO_2 assimilation rate or stomatal conductance and leaf Na^+ and/or Cl^- concentrations. Which indicate the greater capacity for intracellular ion compartmentation in *N. retusa*. Nevertheless, leaf sodium content of up to 200 mM NaCl was positively correlated with P_n. The nature of salt influence on photosynthesis depends partly on the ability of the stomata to use Na^+ I nstead of K^+ to regulate guard cell turgor

(Kerstiens *et al*., 2002). The promotion of photosynthesis rate up to 200 mM NaCl (Table 3) could be related to the replacement of K^+ by Na^+ in the unaltered guard cells of *N. retusa* (Eshel *et al*., 1974). Different studies have reported a relationship between non-stomatal effects and the presence of high leaf Na^+ and Cl^- concentrations (Bethke and Drew, 1992; James *et al*., 2002). The higher tolerance of PSII to photoinhibition indicates that the decrease in the CO_2 assimilation capacity in salt-adapted *N. retusa* leaves may not be due to the limitation of the photosynthetic electron transport.

On the other hand, the decline in CO_2 assimilation was associated with changes in anatomical properties of leaves including an increase in leaf thickness. The results showed that the presence of salt increased mesophyll thickness and reduced stomatal density (Table 2). This increase in mesophyll thickness provided the leaf with more intercellular spaces and thereby more mesophyll conductance. This accords well with the studies in olive (Bongi and Loreto, 1989), cotton (Brugnoli and Bjorkman, 1992) and *B. parviflora* (Parida *et al*., 2004). In addition, no increase in the density of mesophyll cells was observed in *Nitraria retusa* plants submitted to salt stress. The fact that decreases in CO_2 assimilation rate was in parallel with the decrease in both stomatal conductance and intercellular CO_2 concentration and probably a maintaining of the mesophyll conductance suggests that the decreased CO_2 assimilation rate at higher salinities levels in *N. retusa* leaves may be due mainly to the stomatal closure than non-stomatal factors (reduction of mesophyll conductance and action on the photochemistry of the leaves).

The highly positive relationship observed between DM and P_n (Fig. 3d) and the progressively reduction in the Net Assimilate Rate (NAR) (Table 1) showed that the reduction in plant growth with salinity could be the result of the decrease in photosynthetic ability. In accordance with the findings of Cramer *et al.* (1990) and Sanchez-Blanco *et al*. (2002) representing that photosynthesis could be the growth-limiting factor. The fact that growth and net photosynthesis rate varied in the same manner with increasing salinity suggest that the decrease in stomatal conductance and transpiration rate represent adaptive mechanisms to cope with excessive salt rather than only a negative consequence of it (Clark *et al*., 1999). This strategy tends to reduce the salt loading into the leaves and helps to increase the longevity by maintaining salts at subtoxic levels longer than it would occur if transpiration rates were not diminished (Everard *et al*., 1994).

CONCLUSION

The results shows that maximal growth was occurred at moderate salinities and that photosynthetic performance and biomass accumulation were reduced only at high NaCl concentrations beyond 400 mM NaCl and that PSII of *N. retusa* plants are tolerant to salinity. The decrease in photosynthetic rate may be due at least in part to the stomatal closure. The ability of *N. retusa* to cope with severe salt stress seem to be a result of the integration of different mechanisms, at moderate salinity there is inclusion of great amount of Na^+ and its sequestration in vacuoles in relation to the promotion of leaf succulence in conjunction with improved photosynthetic capacity but at higher salinities, *N. retusa* plants appears to be salt secreting. The compatible solute that can be involved in osmotic adjustment was mainly proline. Also there can indicate that tolerance of PSII to high salinity stress can be considered as an important strategy for *N. retusa* plants to grow in very high salinities levels.

REFERENCES

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

Ashraf, M. and A. Orooj, 2006. Salt stress effects on growth, ion accumulation and seed oil concentration in arid zone traditional medicinal plant ajwain (*Trachyspermum ammi* [L.] Sprague). J. Arid Environ., 64: 209-220.

Ashraf, M. and M.R. Foolad, 2007. Roles of glycinebetaine and proline in improving plant abiotic stress resistance. Environ. Exp. Bot., 59: 206-216.

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

Aziz, I. and M.A. Khan, 2001. Experimental assessment of salinity tolerance of Ceriops tagal seedlings and saplings from the Indus delta, Pakistan. Aquatic Bot., 70: 259-268.

Bates, L.S., R.P. Waldren and I.D. Teare, 1973. Rapid determination of free proline for water-stress studies. Plant Soil, 39: 205-207.

Bernstein, N., W.K. Silk and A. Lauchli, 1995. Growth and development of sorghum leaves under conditions of NaCl stress: Possible role of some mineral elements in growth inhibition. Planta, 196: 699-705.

Bethke, P.C. and M.C. Drew, 1992. Stomatal and non-stomatal components to inhibition of photosynthesis in leaves of *Capsicum annuum* during progressive exposure to NaCl salinity. Plant Physiol., 99: 219-226.

Bongi, G. and F. Loreto, 1989. Gas-exchange properties of salt-stressed olive (*Olea europea* L.) leaves. Plant Physiol., 90: 1408-1416.

Brugnoli, E. and O. Bjorkman, 1992. Growth of cotton under continuous salinity stress: Influence on allocation pattern, stomatal and non-stomatal components of photosynthesis and dissipation of excess light energy. Planta, 187: 335-345.

Clark, H., P.C.D. Newton and D.J. Barker, 1999. Physiological and morphological responses to elevated CO_2 and a soil moisture deficit of temperate pasture species growing in an established plant community. J. Exp. Bot., 50: 233-242.

Cramer, G.R., E. Epstein and A. Lauchli, 1990. Effects of sodium, potassium and calcium on salt-stressed barley. I. Growth analysis. Pysiol. Plantarum, 80: 83-88.

Debez, A., K.B. Hamed, C. Grignon and C. Abdelly, 2004. Salinity effects on germination, growth and seed production of the halophyte *Cakile maritima*. Plant Soil, 262: 179-189.

Delfine, S., A. Alvino, M. Zacchini and F. Lorets, 1998. Consequences of salt stress on conductance to CO_2 diffusion, rubisco characteristics and anatomy of spinach leaves. J. Plant Physiol., 25: 395-402.

Demmig-Adams, B. and W.W. III Adams, 1996. The role of xanthophyll cycle carotenoids in the protection of photosynthesis. Trends Plant Sci., 1: 21-26.

Dionisio-Sese, M.L. and S. Tobita, 2000. Effects of salinity on sodium content and photosynthetic responses of rice seedlings differing in salt tolerance. J. Plant. Physiol., 157: 54-58.

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.

Eshel, A., Y. Waisel and A. Ramati, 1974. The Role of Sodium in Stomatal Movements of a Halophyte: A Study by X-Ray Microanalysis. In: German Society of Plant Nutrition, Wherman, J. (Ed.). University of Hannover, Germany.

Everard, J.D., R. Gucci, S.C. Kahn, J.A. Flore and W.H. Loescher, 1994. Gas exchange and carbon partitioning in the leaves of celery (*Apium graveolens* L.) at various levels of root zone salinity. Plant Physiol., 106: 281-292.

Fernandez-Ballester, G., V. Martinez, D. Ruiz and A. Cerda, 1998. Changes in inorganic solutes in citrus growing under saline stresses. J. Plant Nutr., 21: 2497-2514.

Garg, B.K., S.P. Vyas, S. Kathju, A.N. Lahiri, P.C. Mali and P.C. Sharma, 1993. Salinity–fertility interaction on growth, mineral composition and nitrogen metabolism of Indian mustard. J. Plant Nutr., 16: 1637-1650.

Greenway, H. and R. Munns, 1980. Mechanisms of salt tolerance in nonhalophytes. Ann. Rev. Plant Physiol., 31: 149-190.

Grieve, C.M. and S.R. Grattan, 1983. Rapid assay for the determination of water soluble quaternary ammonium compounds. Plant Soil, 70: 303-307.

Grieve, C.M., J.A. Poss, S.R. Grattan, D.L. Suarez, S.E. Benes and P.H. Robinson, 2004. Evaluation of salt-tolerant forages for sequential water reuses systems 2. Plant-ion relations. Agric. Water Manage., 70: 121-135.

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.

James, R.A., A.R. Rivelli, R. Munns and S. von Caemmerer, 2002. Factors affecting CO_2 assimilation, leaf injury and growth in salt-stressed durum wheat. Funct. Plant. Biol., 29: 1393-1403.

Jimenez, M.S., A.M. Gonzalez-Rodriguez, D. Morales, M.C. Cid, A.R. Socorro and M. Caballero, 1997. Evaluation of chlorophyll fluorescence as a tool for salt stress detection in roses. Photosynthetica, 33: 291-301.

Kao, W.Y., T.T. Tsai and C.N. Shih, 2003. Photosynthetic gas exchange and chlorophyll a fluorescence of three wild soybean species in response to NaCl treatments. Photosynthetica, 41: 415-419.

Kerstiens, G., W. Tych, M.F. Robinson and T.A. Mansfield, 2002. Sodium related partial stomatal closure and salt tolerance of *Aster tripolium*. New Phytol., 153: 509-515.

Khan, M.A., B. Gul and D.J. Weber, 2001. Effect of salinity on the growth and ion content of *Salicornia rubra*. Commun. Soil Sci. Plant Anal., 32: 2965-2977.

Khan, M.A., I.A. Ungar and A.M. Showalter, 2000b. Effects of salinity on growth, water relations and ion accumulation of the subtropical perennial halophyte *Atriplex griffthii* var. Stocksii. Ann. Bot., 85: 225-232.

Khan, M.A., I.A. Ungar and A.M. Showlter, 2000a. The effect of salinity on the growth, water status and ion content of a leaf succulent perennial halophyte, *Suaeda friticosa* L. Forssk. J. Arid Environ., 45: 73-84.

Khan, M.A., I.A. Ungar, A.M. Showalter and H.D. Dewald, 1998. NaCl-induced accumulation of glycinebetaine in four subtropical halophytes from Pakistan. Physiol. Plant., 102: 487-492.

Krause, G.H. and E. Weis, 1991. Chlorophyll fluorescence and photosynthesis: The basics. Ann. Rev. Plant Physiol. Plant Mol. Biol., 42: 313-349.

Lawlor, D.W., 2002. Limitation to photosynthesis in water stressed leaves: stomata versus metabolism and the role of ATP. Ann. Bot., 89: 871-885.

Lu, C., N. Qiu and Q. Lu, 2003. Photoinhibition and the xanthophylls cycle are not enhanced in the salt-acclimated halophyte *Artemisia anethifolia*. Physiol. Plant., 118: 532-537.

Martinez, J.P., J.M. Kinet, M. Bajji and S. Lutts, 2005. NaCl alleviates polyethylene glycol-induced water stress in the halophyte species *Atriplex halimus* L. J. Exp. Bot., 56: 2421-2431.

Mimura, T., M. Kura-Hotta, T. Tsujimura, M. Ohnishi and M. Miura *et al*., 2003. Rapid increase of vacuolar volume in response to salt stress. Planta, 216: 397-402.

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.

Morant-Manceau, A., E. Pradier and G. Tremblin, 2004. Osmotic adjustment, gas exchanges and chlorophyll fluorescence of a hexaploid triticale and its parental species under salt stress. J. Plant Physiol., 161: 25-33.

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

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

Netondo, G.W., J.C. Onyango and E. Beck, 2004. Sorghum and salinity: II. Gas exchange and chlorophyll fluorescence of sorghum under salt stress. Crop Sci., 44: 806-811.

Parida, A.K., A.B. Das and B. Mittra, 2004. Effects of salt on growth, ion accumulation, photosynthesis and leaf anatomy of the mangrove, *Bruguiera parviflora*. Trees Struct. Funct., 18: 167-174.

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.

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.

Qiu, N., Q. Lu and C. Lu, 2003. Photosynthesis, photosystem II efficiency and the xanthophyll cycle in the salt-adapted halophyte *Atriplex centralasiatica*. New Phytol., 159: 479-486.

Rao, G.G. and G.R. Rao, 1981. Pigment composition and chlorophyllase activity in pigeon pea (*Cajanus indicus* spreng) an gingelly (*Sesamum indicum* L.) under NaCl salinity. Indian J. Exp. Biol., 19: 768-770.

Redondo-Gomez, S., C. Wharmby, J.M. Castillo, E. Mateos-Naranjo and C. Luque *et al*., 2006. Growth and photosynthetic responses to salinity in an extreme halophyte, *Sarcocornia fruticosa*. Physiol. Plant., 128: 116-124.

Sanchez-Blanco, M.J., P. Rodriguez, M.A. Morales, M.F. Ortuno and A. Torrecillas, 2002. Comparative growth and water relations of *Cistus albidus* and *Cistus monspeliensis* plants during water deficit conditions and recovery. Plant Sci., 162: 107-113.

Schindler, C. and H.K. Lichtenthaler, 1996. Photosynthetic CO_2 assimilation, chlorophyll fluorescence and zeaxanthin accumulation in field-grown maple trees in the course of a sunny and a cloudy day. J. Plant Physiol., 148: 399-412.

Shabala, S.I., 2002. Screening Plants for Environmental Fitness: Chlorophyll Fluorescence as a Holy Grail for Plant Breeders. In: Advances in Plant Physiology, Hemantaranjan, A. (Ed.). Scientific Publishers, Jodhpur, pp: 287–340.

Shen, Y.G. and S.Y. Chen, 2001. Molecular mechanism of plant response to salt stress. Hereditas, 23: 365-369.

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.

Song, J., G. Feng, C.Y. Tian and F.S. Zhang, 2006. Osmotic adjustment traits of *Suaeda physophora*, *Haloxylon ammodendron* and *Haloxylon persicum* in field or controlled conditions. Plant Sci., 171: 113-119.

Tejera, N.A., R. Campos, J. Sanjuan and C. Lluch, 2004. Nitrogenase and antioxidant enzyme activities in *Phaseolus vulgaris* nodules formed by *Rhizobium tropici* isogenic strains with varying tolerance to salt stress. J. Plant Physiol., 161: 329-338.

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

Vicente, O., M. Boscaiu, M.A. Naranjo, E. Estrelles, J.M. Belles and P. Soriano, 2004. Responses to salt stress in the halophyte: Plantago crassifolia. J. Arid Environ., 58: 463-481.

Yang, Y.W., R.D. Newton and P.R. Miller, 1990. Salinity tolerance in sorghum 1. whole plant response to sodium chloride in *S. bicolor* and *S. halepense*. Crop Sci., 30: 775-780.