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Physiological Adjustment of *Arthrocnemum macrostachyum* and *Nitraria retusa* to Saline Habitats in Sinai, Egypt

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Abstract: The physiological adaptive features pertaining two different halophytes, *Arthrocnemum macrostachyum* and *Nitraria retusa*, inhabiting North and South Sinai Peninsula were investigated during spring and summer. Site1 represents North Sinai (El-Arish region) and site2 representing South Sinai (Ras Sudr region). Site1 was characterized by higher rainfall and lower temperature compared to site2. The soils of both sites are characterized by higher electrical conductivity during summer and their water contents are higher in the lower than the upper horizon. Results showed that *A. macrostachyum* possessed higher succulence ratio in both seasons compared to *N. retusa*, while the opposite was true in cell-membrane electrical conductivity. In each species the cell-sap osmotic potential decreased during the summer which was appreciably lower in *A. macrostachyum*. In summer, *A. macrostachyum* showed higher levels of photosynthetic pigments (Chl. a, b and carotenoids) compared to those in spring while the opposite was found in case of *N. retusa*. In each species, the activities of catalase and peroxidase increased during summer, where *N. retusa* recorded higher activities of catalase than *A. macrostachyum* in both sites and for peroxidase in site1. Superoxide dismutase activity was higher in spring than summer in *N. retusa* in both sites but for *A. macrostachyum* in site1. Ascorbic acid content was significantly higher in both sites during spring in both species, while MDA has accumulated in summer in both sites for *N. retusa* and in *A. macrostachyum* in site1. *N. retusa* has experienced higher proline level in spring at site2. Each species possessed higher nitrogen content during summer in both sites. In each species and in each site, the DNA and RNA contents were significantly higher in spring. *A. macrostachyum* showed appreciably higher levels of all protein-bound amino acids in spring in site1 and the opposite was true in site2. In *N. retusa* a similar increase was recorded in the two sites in spring, except for glycine and methionine in site1 and alanine and valine in site2. The SDS-PAGE of the total proteins indicated that during spring and summer seasons *A. macrostachyum* was characterized by the presence of persistent bands having molecular weights (MW) ~23 and 15.79-16 kDa in site1 and ~15.6 kDa in site2 in both sites, while *N. retusa* displayed common bands having MW 22.30-22.17and 16.15-16.56 kDa in site1 and ~22 and 16.35 kDa in site2. High molecular weight protein bands appeared in site-2 during spring having MW 96.02 kDa and 103.04 kDa in *N. retusa* and *A. macrostachyum*, respectively. In summer, *A. macrostachyum* showed another bands having MW ranging between 24 and 62 kDa while *N. retusa* showed five proteins bands having MW ranging between 24 and 70 kDa.

Key words: Amino acids; antioxidant enzymes; *Arthrocnemum macrostachyum*; *Nitraria retusa*; DNA, photosynthesis; protein pattern, RNA; saline habitat; Sinai Peninsula

INTRODUCTION

Halophytes are species of the natural flora which form a group of ecologically, physiologically and biochemically specialized plants capable of functioning and reproducing normally in saline soils. This property is attributed to some of their specific physiological and biochemical features such as decreased osmotic potential of the cell sap and specific ion-transportation systems ensuring a relatively low content of ions in the cytoplasm (Shamsudinov, 1997). Depending on the regulating mechanism, these halophytes are classified into

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either succulent or excretive. The osmotic adjustment in plants subjected to salt stress can be achieved by the accumulation of compatible osmolytes which have low molecular weights such as sugars, organic acids, polyols, and nitrogen-containing compounds (Ashraf and Harris, 2004). The dramatic increase in the accumulation of certain basic proteins is noteworthy, since they may serve as a more stable form in the presence of high concentration of Na^+ in the cytoplasm (Singh et al., 1987). Yen et al. (1997) reported that the SDS-PAGE of proteins revealed that the accumulation of five polypeptides was enhanced by the addition of 200 mM NaCl to culture media of *Mesembryanthemum crystallinum* which may be considered as a general cellular adaptive mechanism to abiotic stresses in this halophyte.

The main objective of the present work was to define some major responses of two halophytic species *Arthrocnemum macrostachyum* (Moric.) K. Koch. (succulent) and *Nitraria retusa* (Forssk.) Asch. (excretive) to saline habitats in North and South Sinai and to demonstrate the adaptive mechanism of salt tolerance in each species.

MATERIALS AND METHODS

The Study Sites:

Two sites with different levels of salinity and aridity were selected in Sinai Peninsula. Site 1 (31˚ 05' 33" N, 33˚ 37' 13" E) is an inland coastal salt marsh near El-Arish, N. Sinai, whereas site 2 (29˚ 37' 30" N, 32˚ 41' 28" E) is the deltaic portion of Wadi Sudr on the eastern shore of the Gulf of Suez, S. Sinai.

Plant and Soils Samples:

Three samples of *Arthrocnemum macrostachyum* and *Nitraria retusa* were collected from each site in the spring (March) and summer (August) of 2003. Wherever a plant sample was collected a soil sample was taken at two successive depths (0-20 cm and 20-40 cm of the rhizosphere). All measurements were recorded from the shoot of *A. macrostachyum* (with rudimentary leaves) and the leaves of *N. retusa*.

Plant and Soil Analyses:

Physical Parameters:

Succulence ratio was calculated as the initial fresh weight/the dry weight. Osmotic potential of cell sap was measured using Osmometer (030, Gonotec, Berlin, Germany) and the results were expressed as MPa. Electrical conductivity (EC) of cell membrane (as a measure of membrane leakage) was measured by EC-meter and expressed as $\mu S\ cm^{-1}$. Soil water content was calculated from the difference between fresh and dry weights and the electrical conductivity was calculated in $\mu S\ cm^{-1}$ according to Jackson (1962).

Chemical Analysis of Plant Samples:

The photosynthetic pigments (chlorophyll a, chlorophyll b and carotenoids) were determined quantitatively as described by Metzner *et al.* (1965). Soluble proteins were extracted according to Beauchamp and Fridovich (1971) and used in the assay of catalase (CAT; EC 1.11.1.6), peroxidase (POX; EC 1.11.1.7) and superoxide dismutase (SOD; 1.15.1.1) according to Kato and Shimizu (1987). Enzyme activities were expressed in µM of the substrate converted $min.^{-1}\ g^{-1}$ fresh weight. Ascorbic acid concentration was determined according to Rai (2001) and expressed in mg $100ml^{-1}$. Malondialdehyde (MDA; a peroxidation product of the unsaturated fatty acid linolenic acid, 18:3) was estimated as $\mu M\ g^{-1}$ fresh weight using the method of Heath and Packer (1968).

Total nitrogen content was determined using the modified micro-Kjeldahl method and results were expressed as mg $100g^{-1}$ dry weight. Free proline was determined according to the method of Bates *et al.* (1972). Nucleic acids were determined according to Guinn and Oklahoma (1966) and the values of DNA and RNA were expressed in $\mu g\ g^{-1}$ fresh weight. Individual amino acid content of protein-bound amino acids was determined according to the method of Anderson *et al.* (1977) using the Beckman HPLC (System gold; programmable solvent Model 126).The protein pattern as revealed by SDS-PAGE was carried out as described by Laemmli(1970). Scanning of the bands was analyzed by the Gel Documentation System which determines molecular mass (MM) in kDa of each polypeptide band in relation to a standard marker (M) using Gel Proanalyzer version 3 Media Cybernetics Imaging Exports software (Gel Doc. 2001 BioRad System).

All data were treated statistically by applying the two-way ANOVA test using the SPSS version 12 programme for Windows.

RESULTS AND DISCUSSION

Climatic and Soil Conditions of Sites:

Climatic records of sites 1 and 2 during March (spring) and August (summer) 2003 are presented in Table 1. In site 2 (at Wadi Sudr) the air was consistently warmer and much drier than site 1 (at El-Arish). However, soil water content was much higher in site 2 than in site 1 (Table 2). Rainfall was scarce during spring in site 1 and non-existent during summer as well as during both seasons in site 2 indicating the extreme aridity prevailing in the deserts of Egypt. Ground water was the major source of water supply to plants in the two sites during both seasons. Soil water content increased with depth. Electrical conductivity (EC) of the soil solution, as an indicator of its mineral content, was significantly higher in site 2 than in site 1 during both seasons (Table 2). It increased with depth in site 2 and from spring to summer in both sites.

Table 1: Meteorological records of two sites in Sinai Peninsula for March and August 2003, representing the spring and summer seasons, respectively. Data are provided by the Meteorological stations at Sheikh Zuwayed (N. Sinai) and Ras Sudr (S. Sinai) for sites 1 and 2, respectively.

	Site 1(El-Arish, N. Sinai)		Site 2 (Wadi Sudr, S. Sinai)	
	March	August	March	August
Average temperature (°C)	14.2	26.4	15.7	29.4
Relative humidity (%)	81.4	92.4	57.4	60.8
Rainfall (mm)	41.2	00.0	00.0	00.0

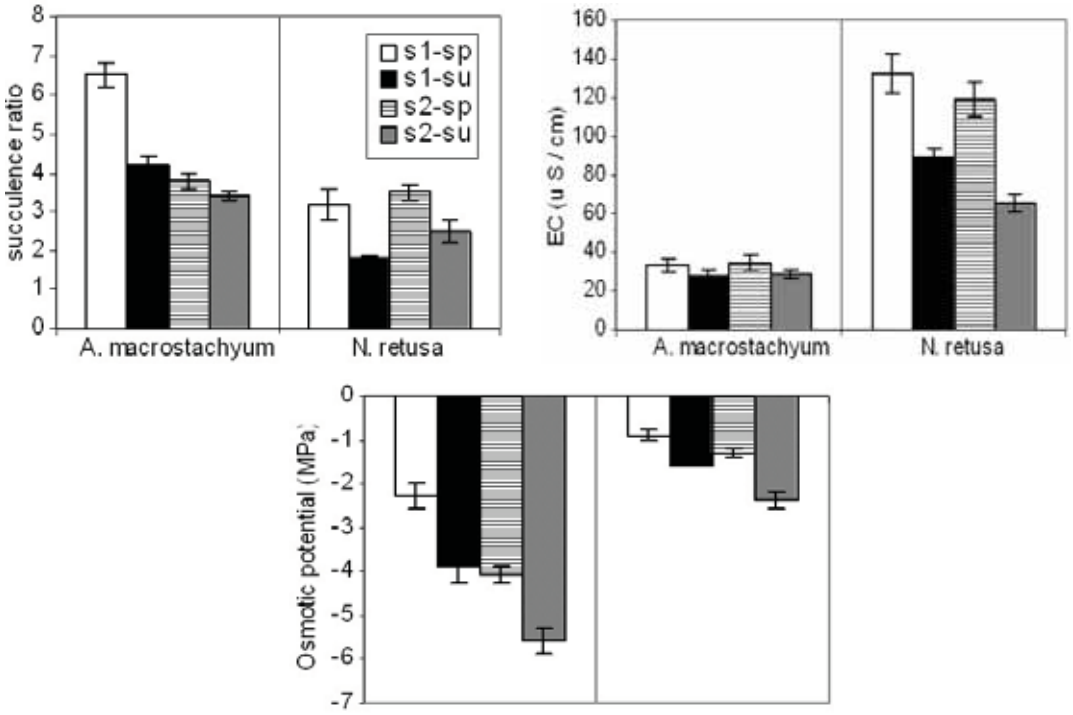
Table 2: Electrical conductivity (EC) and water content (%) at two depths of the soil of two sites in Sinai Peninsula during the spring and summer seasons. Values represent means ± SD, n = 3. F values: ** = highly significant at $p \leq 0.01$; * = significant at $p \leq 0.05$.

	Site 1 (El-Arish, N. Sinai)		Site 2 (Wadi Sudr, S. Sinai)	
	Depth (cm)		Depth (cm)	
Measurements	0-20	20-40	0-20	20-40
	Spring (March 2003)			
EC (μ S cm^{-1})	11.1±0.2	11.1±0.6	28.2±1.4	125.8±3.4
water content (%)	60.1±1.2	69.9±0.5	84.2±1.8	97.7±2.3
	Summer (August 2003)			
EC (μ S cm^{-1})	64.7±0.5	48.3±1.3	139.3±5.2	225.2±6.2
water content (%)	40.2±1.3	49.9±0.8	51.1±2.5	65.5±1.5

Plant Variables:

In each site, succulence ratios (SR) were significantly higher during spring than summer for both *A. macrostachyum* and *N. retusa* (Fig. 1). The maximum succulence ratio was recorded in site 1 for *A. macrostachyum*. For each species, electrical conductivity (EC) of cell membrane and osmotic potential (OP) of cell sap were consistently higher during spring than summer, although the change in EC was not significant in *A. macrostachyum*. EC and OP were significantly higher in *N. retusa* than *A. macrostachyum*. The latter decreased significantly during summer for each species in both sites (Fig. 1). The content of each of chl-a, chl-b and carotenoids increased significantly in *A. macrostachyum* during summer than spring, while the opposite was true in *N. retusa* (Fig. 2).

In each site, the activities of CAT and POX increased significantly in the two species during summer than spring (Fig 3). SOD activity decreased significantly during summer in *A. macrostachyum* in site 1 and in *N. retusa* in site 2. The only significant increase in SOD activity during summer was recorded in *A. macrostachyum* in site 1. Ascorbic acid content increased significantly during spring in each species in the two sites. Malondialdehyde (MDA) accumulated during summer in both species in site 1 and in *N. retusa* only in site 2. However, the total nitrogen (TN) content increased significantly in the two species during summer in each site, free proline, DNA and RNA contents followed an opposite trend (Fig. 4). During summer each protein-bound amino acid decreased in *A. macrostachyum* in site 1 but increased in site 2, except methionine (Fig. 5). A similar decrease in each amino acid was recorded in *N. retusa* during summer in the two sites, except for glycine and methionine in site 1 and alanine and valine in site 2.



	A. macrostachyum			*N. retusa*		
	Succulence	EC	OP	Succulence	EC	OP
seasons	**	*	**	**	**	**
sites	**	ns	**	*	*	**
interaction	**	ns	ns	ns	ns	*

Fig. 1: Changes in succulence ratio, electrical conductivity (EC) and osmotic potential (OP) of *Arthrocnemum macrostachyum* and *Nitraria retusa* growing in two saline sites (s1 at El-Arish, N. Sinai; s2 at Wadi Sudr, S. Sinai), between the spring (sp) and summer (su) seasons of 2003. Value of each bar represents mean ± SD, n = 3. F values: ** = highly significant at $p \leq 0.01$; * = significant at $p \leq 0.05$.

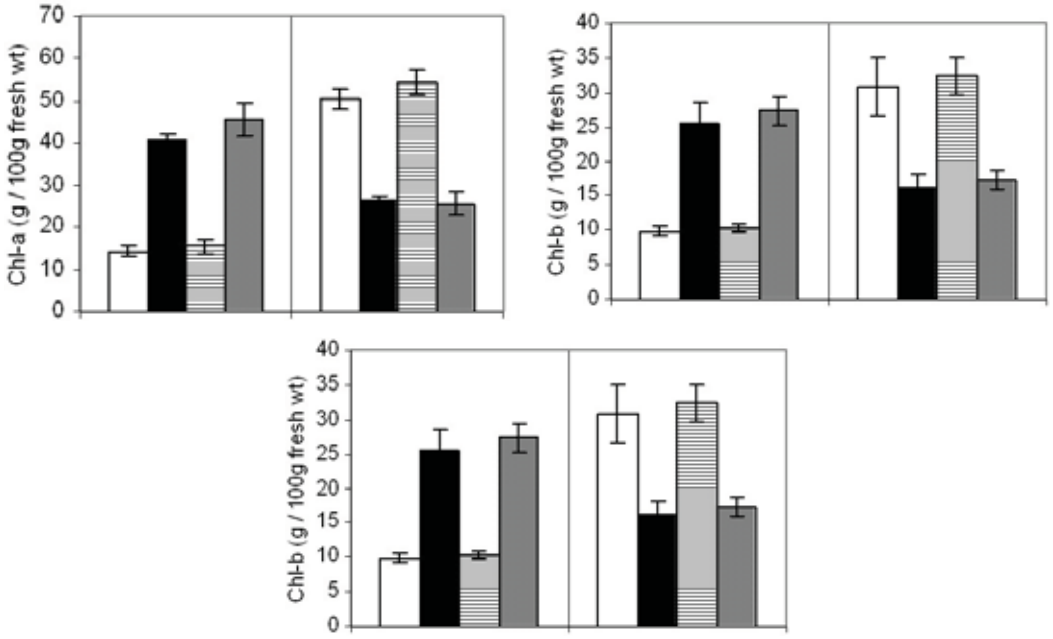
The seasonal changes in protein pattern (Table 3) can be distinguished into three groups: (a) proteins synthesized in summer, (b) proteins degraded in summer, and (c) proteins persistent from spring to summer. These three groups which are represented in Table 3 might be summarized as follows:

A. macrostachyum:

Group a:
Site 1: four protein bands (MM 61.66, 42.47, 26.93, 24.75 kDa)
Site 2: three protein bands (MM 62.44, 42.47, 22.86 kDa)

Group b:
Site 1: four protein bands (MM 61.11, 41.74, 24.56, 15.79 kDa)
Site 2: five protein bands (MM 103.04, 61.11, 41.05, 30.86, 24.10 kDa)

Group c:
Site 1: four protein bands (MM 23.04, 23.23, 15.79, 16 kDa)
Site 2: two protein bands (MM 15.67-15.56 kDa)



	A. macrostachyum			*N. retusa*		
	Chl-a	Chl-b	carotenoids	Chl-a	chl-b	carotenoids
seasons	**	**	**	**	**	**
sites	*	ns	*	ns	ns	ns
interaction	ns	ns	ns	ns	ns	ns

Fig. 2: Changes in chlorophyll a (chl-a), chlorophyll b (chl-b) and carotenoid contents of *Arthrocnemum macrostachyum* and *Nitraria retusa* growing in two saline sites (s1 at El-Arish, N. Sinai; s2 at Wadi Sudr, S. Sinai) between the spring (sp) and summer (su) seasons of 2003. Value of each bar represents mean ± SD, n = 3. F values: ** = highly significant at $p \leq 0.01$; * = significant at $p \leq 0.05$.

N. Retusa:

Group a:
Site 1: three proteins (MM 70.10, 43.90, 24.71 kDa)
Site 2: three proteins (MM 42.47, 32.31, 24.33, kDa)
Group b:
Site 1: two proteins (MM 42.47, 24.17 kDa)
Site 2: five proteins (MM 96.02, 61.26, 42.82, 31.52, 24.53 kDa)

Group c:
Site 1: four proteins (MM 22.3 and 22.47, 16.15, 16.56 kDa)
Site 2: three proteins (MM 22.14, 21.98, 16.35 kDa).

Although *A. macrostachyum* and *N. retusa* inhabited the same sites, they seem to possess different mechanisms of adjustment to salinity and heat stresses. The greater capacity of *A. macrostachyum* for osmotic adjustment by the synthesis of more acidic solutes compared to *N. retusa* as reported by Austin (1993) was represented by the higher SR and lower EC and OP compared to *N. retusa* which agreed with the results of Serag (1999) and Subbarao *et al. (*2000).

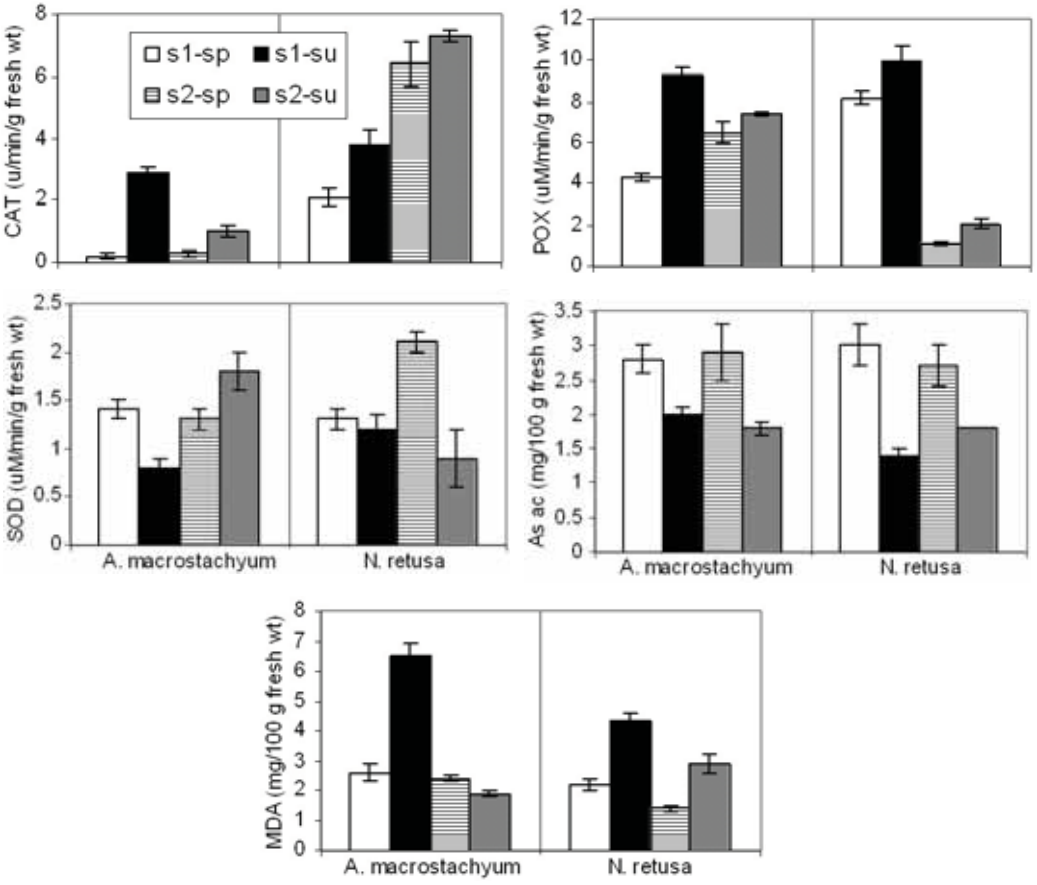
A. macrostachyum tended to retain higher contents of chl-a, chl-b and carotenoids during the summer, which might be considered as an adaptive mechanism to cope with the decreased air and soil

Table 3: Changes revealed by SDS-PAGE in protein patterns of *Arthrocnemum macrostachyum* and *Nitraria retusa* growing in two saline sites (site 1 at El-Arish, N. Sinai; site 2 at Wadi Sudr, S. Sinai) between the spring (March) and the summer (August) seasons of 2003. MM = molecular mass.

	A. macrostachyum				*N. retusa*			
Marker MM (KDa)	site 1		site 2		site 1		site 2	
	spring	summer	spring	summer	spring	summer	spring	summer
	-	-	103.04	-	-	-	-	-
97.40	-	-	-	-	-	-	96.02	-
	-	-	-	-	-	70.10	-	-
66.20	-	-	-	62.44	-	-	-	-
	-	61.66	-	-	-	-	-	-
	-	-	-	-	-	-	61.26	-
	61.11	-	61.11	-	-	-	-	-
45.00	-	-	-	-	-	43.90	-	-
	-	-	-	-	-	-	42.82	-
	-	42.47	-	42.47	42.47	-	-	42.47
	41.74	-	41.05	-	-	-	-	-
	-	-	-	-	-	-	-	32.31
31.00	-	-	-	-	-	-	31.52	-
	-	-	30.86	-	-	-	-	-
	-	-	-	-	27.37	27.17	-	-
	-	26.93	-	-	-	-	-	-
	-	24.75	-	-	-	24.71	-	-
	24.56	-	-	-	-	-	24.53	-
	-	-	-	-	-	-	-	24.33
	-	-	24.10	-	24.17	-	-	24.17
	23.04	23.23	-	-	-	-	-	-
	-	-	-	22.86	-	-	-	-
	-	-	-	-	22.30	22.47	22.14	21.98
21.50	-	-	-	-	16.15	16.56	16.35	16.35
	15.79	16.00	15.67	15.56	-	-	-	-
14.40	-	-	-	-	-	-	-	-

moisture (Ahmed, 2002). The remarkably higher carotenoid content might be regarded as one of the adaptive responses which lead to delaying senescence and maintaining the survival of stressed plants through protection against oxidative processes by reducing singlet oxygen formation via absorption of energy from the exited states of chlorophyll, as well as by directly quenching singlet oxygen (Halliwell and Gutteridge, 1985). In *N. retusa*, the decrease in the photosynthetic pigments during summer might be attributed to the inhibitory effect of NaCl on the biosynthesis of pigments, increasing their degradation, damage of chloroplast thylakoids and/or to the increased activity of the chlorophyll degrading enzyme chlorophyllase as was demonstrated by Mark *et al*. (1999) and Kasim and Hamada (2003). The decrease in chlorophyll content associated with the appearance of some protein bands lead De la Rosa-Ibrro and Maiti (1995) to suggest that nitrogen may have been shifted to the synthesis of protein instead of chlorophyll.

Antioxidant defenses have been induced in *A. macrostachyum* in site 1 more efficiently compared to *N. retusa,* especially in summer. The increased activities of catalase and peroxidase and the decreased content of ascorbate can be considered as a defense machanism in each species during summer. The results suggest that the behavior of each antioxidant enzyme is related to the species and habitat conditions. However, superoxide dismutase (SOD) showed lower activity in summer in both sites for *N. retusa* and in site 1 for *A. macrostachyum* compared to spring, which can be correlated, to some extent, with ascorbate content. Such decrease in SOD activity can be due to the formation of H_2O_2 as a by-product which should be a potential damaging agent at higher salt concentration during summer. In contrast, malondialdehyde (MDA) which is a peroxidation product showed greater levels in summer in the two sites for *N. retusa* and in site 1 for *A. macrostachyum*, indicating the occurrence of lipid peroxidation, which can be correlated also with the behavior of SOD activity. With increased succulence in spring the elevated levels of ascorbate, as a non-enzymatic antioxidant, can play a crucial role in determining the tolerance of each halophyte to oxidative stress. It is possible that elevated antioxidant levels could be associated with the degree of salt tolerance in each studied halophyte under each habitat conditions in each season.

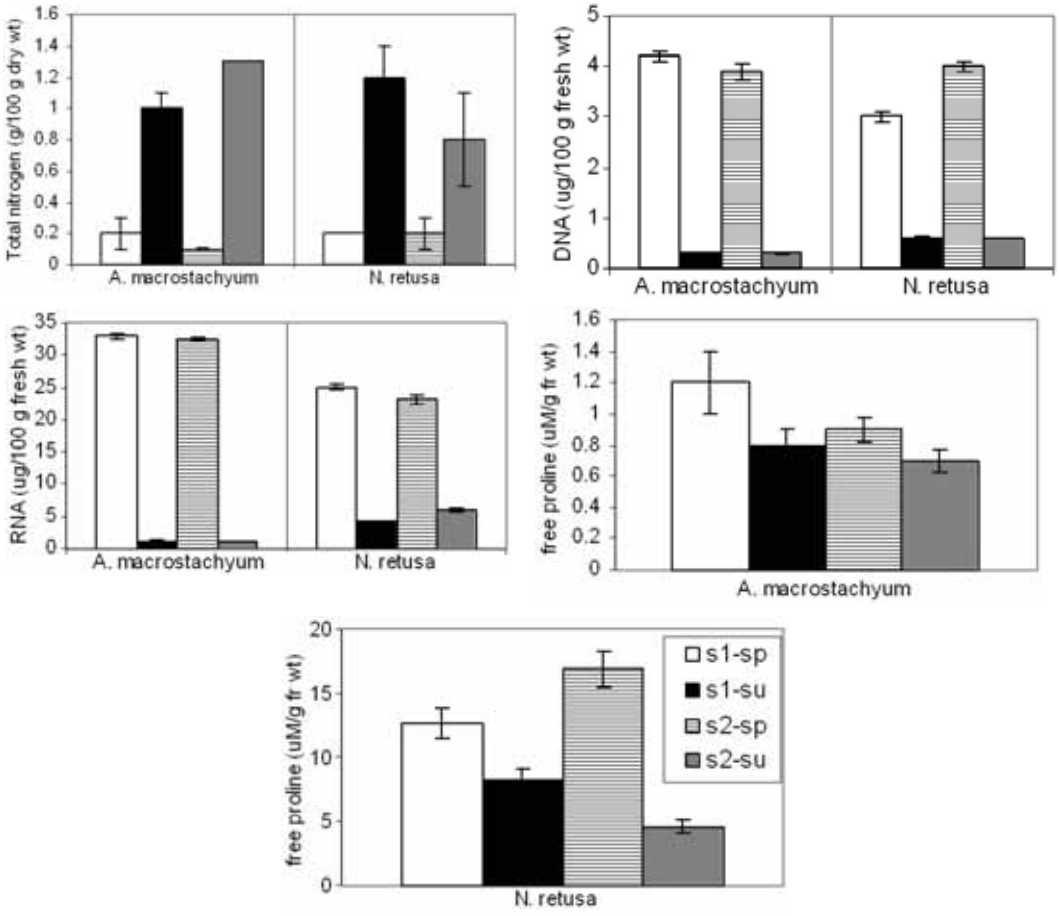


Factors	*A. macrostachyum*					*N. retusa*				
	CAT	POX	SOD	As ac	MDA	CAT	POX	SOD	As ac	MDA
seasons	*	**	ns	**	**	**	**	**	**	**
sites	*	ns	**	ns	**	**	**	*	ns	**
interaction	ns	**	**	ns	**	ns	ns	**	*	ns

Fig. 3: Changes in activities of catalase (CAT), peroxidase (POX), superoxide dismutase (SOD), and the contents of ascorbic acid (As. ac.) and malondialdehyde (MDA) of *Arthrocnemum macrostachyum* and *Nitraria retusa* growing in two saline sites (s1 at El-Arish, N. Sinai; s2 at Wadi Sudr, S. Sinai) between the spring (sp) and summer (su) seasons of 2003. Value of each bar represents mean ± SD, n = 3. F values: ** = highly significant at $p \leq 0.01$; * = significant at $p \leq 0.05$.

The significant increase in the total nitrogen content in each species during summer which was in accordance with the results reported by Mosallam and Hassan (2001) may be related to enhanced activity of proteolytic enzymes with decreased synthesis of proteins (Dubey, 1994). However, higher levels of DNA during spring in each species can be due to active cell division. The decreased content of DNA in *A. macrostachyum* relative to *N. retusa* may be attributed to lower number of cells per unit weight (El-Shourbagy**,** *et al.***,** 1980). Abo Kassem *et al.* (2002) found that DNase activity decreased with different anion concentrations which showed a significant correlation with the reduction in protein associated with an increase in nucleic acid content in radish.

The higher level of free proline in *N. retusa* may be attributed to relatively higher total soluble salts in the soil as reported by Ahmed (2002) and it was found to be associated and correlated with protein–rich proline. The capacity of *N. retusa* for the synthesis of proline–rich protein, besides the



	A. macrostachyum				*N. retusa*			
	TN	proline	DNA	RNA	TN	proline	DNA	RNA
Seasons	**	**	**	**	**	**	**	**
Sites	*	*	ns	ns	ns	ns	**	ns
Interaction	**	ns	*	ns	ns	**	**	ns

Fig. 4: Changes in total nitrogen (TN), free proline, DNA and RNA contents of *Arthrocnemum macrostachyum* and *Nitraria retusa* growing in two saline habitats (s1 at El-Arish, N. Sinai; s2 at Wadi Sudr, S. Sinai), between the spring (sp) and summer (su) seasons of 2003. Value of each bar represents mean ± SD, n = 3. F values: ** = highly significant at $p \leq 0.01$; * = significant at $p \leq 0.05$.

accumulation of free proline compared to *A. macrostachyum* can be attributed to its role as an osmoprotectant for enzymes and membranes against stresses rather than as a compatible solute (Okuma *et al*., 2000).

The remarkably higher levels of the four bound-amino acids (glycine and methionine in site 1 and alanine and valine in site 2) in *N. retusa* during summer can be attributed to the stimulation of synthesis of adaptive protein types rich in these four amino acids. However, differences exist in the formation of adaptive protein types in each variety under each habitat. The synthesis of protein types rich in certain amino acids could be the key to survival for the species (El-Shourbagy *et al*., 1980). Although each species showed differences in the level of each of these four bound-amino acids in each season, the capacity was more remarkable for *N. retusa*. However, the decreased content of certain protein-bound amino acids in *A. macrostachyum* suggests losses of synthetic processes or

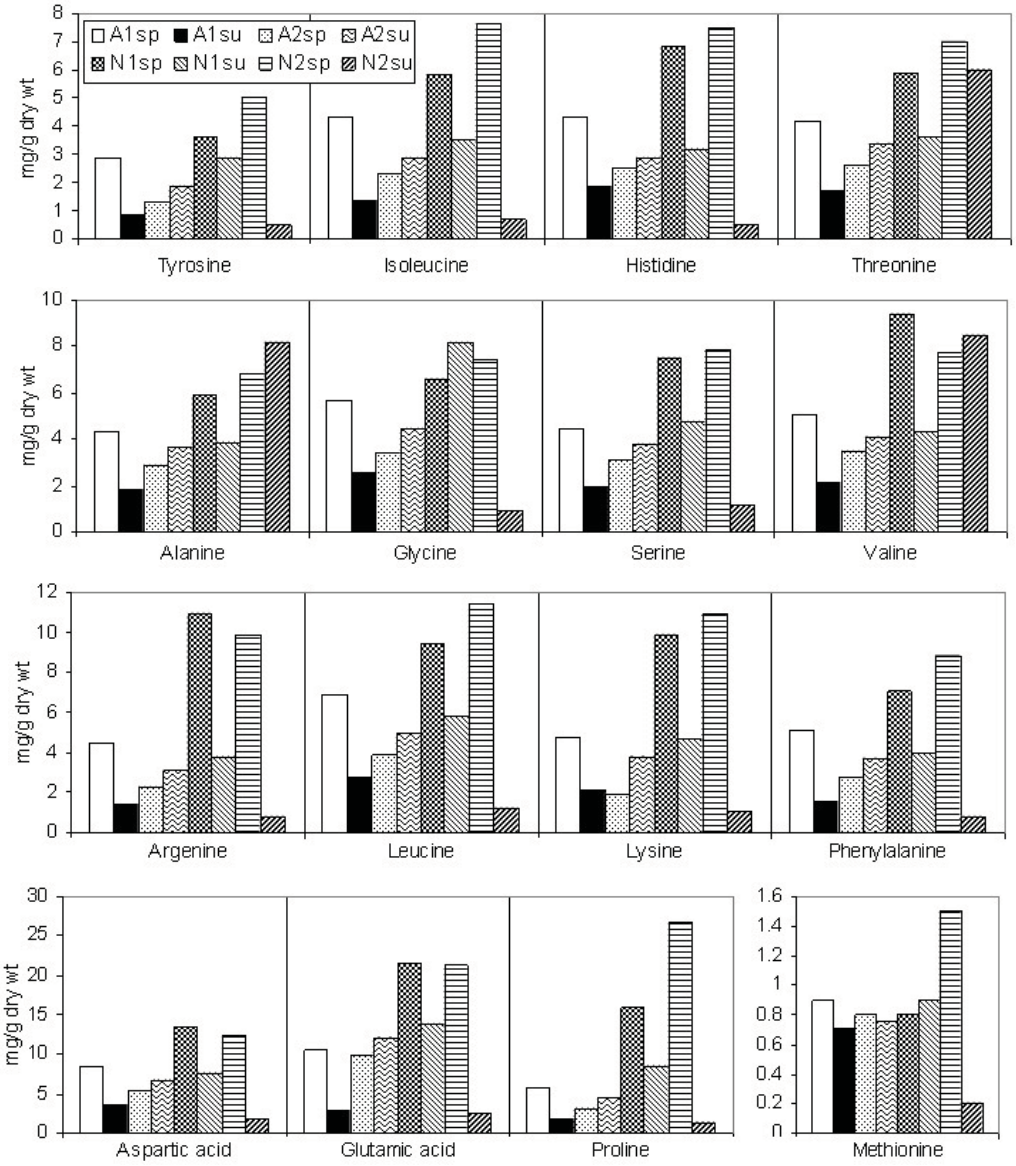


Fig. 5: Changes in 16 of the protein-bound amino acids of *Arthrocnemum macrostachyum* (A) and *Nitraria retusa* (N) growing in two saline habitats (1 at El-Arish, N. Sinai; 2 at Wadi Sudr, S. Sinai), between the spring (sp) and summer (su) seasons of 2003.

breakdown of various sub-cellular components (Levitt, 1972) compared to *N. retusa*. These results suggest that *N. retusa* having a superior capacity for stress tolerance relative to *A. macrostachyum* through the maintenance of synthetic machinery, since the preservation of certain protein-bound amino acids, even during summer, depends on nucleic acid metabolism.

Accumulation of specific protein can be considered a stable characteristic linked to increased survival or growth of species under stress conditions (La Rosa *et al*., 1989). In the present study there was a remarkable difference between *A. macrostachyum* and *N. retusa* regarding habitats. The proteins with molecular weight of 70 kD appeared only in *N. retusa* in summer, which being involved in lowering Na^+ influx, thus increasing tolerance to salt stress as reported by Schachtman *et al.* (1997). Pelham (1986) speculated that stress 70 kDa

proteins and homologues played a role in ATP-dependent protein folding and assembly, which received considerable support from studies in stressed and unstressed cells (Pelham,1990). It has been proposed that stress 70 kDa proteins and homologues infold processor proteins or maintain them in a form suitable for transport across membranes (Deshaies *et al*., 1988) or facilitate correct folding of proteins involved in protein transport processes (Beckman *et al*., 1990). However, each species was able to synthesize LMW proteins in the range of 23.04-23.23, in addition to 15.56-16 kDa in *A. macrostachyum* and 27.37, 27.17 and 16.15-16.56 kDa in *N. retusa* in both seasons. The HMW proteins with 103 and 96 kDa might be regarded as constitutive proteins which have been degraded during summer in *A. macrostachyum* and *N. retusa*, respectively.

A. macrostachyum and *N. retusa* were able to synthesize a greater proportion of proteins including those having MM of < 27.0 kDa, belonging to aquaporin (Arora *et al*, 2000) involved in the regulation of membrane water permeability (Maurel, 1997), associated with increased succulence. Accumulation of LMW protein bands such that of 26.9 kDa can be involved in the accumulation of proline during stress (Weigel *et al*, 1986). Nilson and Orcutt (2000) showed that osmotine (a type of responsive protein) may have a role in osmotic adjustment as an adaptive mechanism for salt stress either by stimulating rapid accumulation of proline and glycine betaine in the cytoplasm as a non-toxic osmoticum resulting in osmotic adjustment without perturbing metabolic function. Understanding the function of stress proteins in halophytes will require much additional work. Genetic approaches that could complement information gained from molecular and biochemical work must include the identification of specific mutant stress proteins.

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