Mechanisms of salt tolerance in the halophytes *Atriplex nummularia* Lind. and *Atriplex leucoclada* Boiss.

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To my father in spirit whom I always remember, and my dear mother for her love and to my wife Walaa who helped me to finish this work and finally to my son Ziad that I wish him a good future.

SUMMARY

The aim of this study was to compare the morphological, physiological and biochemical responses of *Atriplex nummularia* and *A. leucoclada* at various NaCl salinities. The plants were grown under artificial conditions in a gravel/hydroponic quick check system with 0, 25, 50, 100 or 150% seawater salinity. Increasing NaCl salinity stimulated the plant growth of both *Atriplex* species, with a maximum at 50% SWS, corroborating the halophilic nature of these species. The salinity threshold of both *Atriplex* species was slightly above 50% SWS, while the C_{50} was at 140 and 114% SWS for *A. nummularia* and *A. leucoclada* respectively. High salinities inhibited the growth of both *Atriplex* species, with more adverse effect on *A. leucoclada*. The growth reduction could not be explained by water deficit because both *Atriplex* species were able to reduce their shoot water potential as a consequence of a decreased osmotic potential (mainly by Na^+ and Cl^-- accumulation) in all plant organs. Several mechanisms were involved in both species to reduce the excessive ion accumulation (toxicity): 1) The old leaves were shedded and used for the dispose of Na^+ and Cl^-; 2) the bladder hairs protected the actively photosynthesizing tissues by the accumulation of toxic ions; 3) ion dilution was achieved as a result of increasing leaf succulence; 4) the WUE of photosynthesis was increased. Elevating salinity led in both *Atriplex* species also to a drastic decrease of the K^+, Ca^{2+} and Mg^{2+} concentrations and to symptoms of ion deficiency. In both cases (either ion deficiency or ion toxicity) salt resistance was apparently related to the ability to compartmentalize the harmful ions and to maintain adequate concentrations of the essential ions, especially, in the cytoplasm of the actively metabolic tissues. In this context, *A. nummularia* presented higher selective ion uptake and transport capacities at the root level in comparison to *A. leucoclada*. In both species, high dark respiration rates (DR) were observed for plants grown at the optimal salinity level, reflecting the high energy requirements for the transport and the sequestration of ions and biosynthesis of compatible solutes such as carbohydrates and proline. High NaCl concentrations significantly inhibited the net CO_2 assimilation rate, the transpiration rate, the stomatal resistance, and the internal CO_2 concentration. The reduced photosynthesis may be attributed to the salt-induced reduction in chlorophyll contents especially Chl(b) and the (ultrastructural) changes in the chloroplasts. As a common reaction, high NaCl salinity led to a decrease of the occurrence of RubisCo large subunit in the leaves of both *Atriplex* species. However, PPDK, ALdP, MDH, HMT, and SAMS were all up-regulated in *A. nummularia*, but all down-regulated in *A. leucoclada* in response to high NaCl salinity. These results can be interpreted as a higher responsiveness of *A. nummularia* to balance the C_4-photosynthesis. Reduced growth seems to be due to ion toxicity and ion imbalance in both species. The lower selectivity of ion uptake and transport capacities, its high energy demand and its lower responsiveness to balance the photosynthesis are the major reasons for lower salt resistance of *A. leucoclada*.

Keywords: Salt tolerance mechanisms, *Atriplex nummularia*, *Atriplex leucoclada*.

Zusammenfassung

Das Ziel dieser Studie war der Vergleich und die Untersuchung der Effizienz der Salzresistenzmechanismen von *Atriplex nummularia* und *A*. *leucoclada,* mit einem Schwerpunkt auf strukturelle, physiologische und molekulare Anpassungen Die Pflanzen wurden unter Gewächshausbedingungen in einen Schnelltestsystem (QCS) mit 0, 25, 50, 100 und 150% Meerwassersalinität (SWS) bewässert. NaCl stimulierte das Pflanzenwachstum beider *Atriplex* Arten bis zu einem Wachstumsoptimum von 50% SWS. Es gab jedoch einen signifikanten Unterschied bei der C_{50}. Sie betrug 140% für *A. nummularia* und nur 114% SWS für *A. leucoclada.* Außerdem hemmte eine Erhöhung der Salinität auf über 50% SWS besonders das Wachstum von *A. leucoclada*. *A. nummularia* war somit eindeutig die salzresistentere der beiden untersuchten Arten. Es gab keinen Hinweis, dass ein Wasserdefizit die o.g. Wachstumsabnahme bei hoher Salinität bewirkte, da das Sprosswasserpotential und die osmotischen Potentiale in allen Pflanzenorganen ausreichend und vorwiegend durch Na^+ und Cl^- Akkumulation abnahmen. Letzteres wurde als Hinweis für potentielle Ionentoxizität gewertet. Verschiedene Mechanismen waren in beiden Arten vorhanden um die NaCl-Toxizität zu reduzieren: 1) Die adulten Blätter wurden zur Beseitigung von exzessiv akkumuliertem NaCl abgeworfen; 2) die Blasenhaaren akkumulierten NaCl zum Schutz der photosynthetisch aktiven Gewebe; 3) toxische NaCl-Konzentrationen wurde durch eine gesteigerte Blattsukkulenz vermieden; 4) die WUE der Photosynthese wurde erhöht. Als ein Beleg für Ionen-Mangel wurde die salzbedingt deutliche Abnahme der K^+, Ca^{2+} und Mg^{2+} Konzentrationen in allen Organen beider Arten interpretiert. Sowohl bei Ionen-Mangel als auch bei Ionen-Toxizität ist die Salzresistenz mit der Fähigkeit verbunden, intrazellular die schädlichen Ionen vom Zytoplasma fern zu halten (kompartimentieren) aber gleichzeitig ausreichend hohe Konzentrationen der essentiellen Ionen in diesem stoffwechselaktiven Kompartiment zur Verfügung zu stellen. In diesem Zusammenhang zeigte *A. nummularia* im Vergleich mit *A. leucoclada* eine signifikant höhere Ionenselektivität bei der Aufnahme und dem Transport. Der dadurch aber auch durch die Synthese von stoffwechselverträglichen Substanzen (wie zum Beispiel Kohlenhydrate und Prolin) erhöhte Energiebedarf wurde in beiden Arten durch eine erhöhte Dunkelatmung (DR) abgedeckt. Salinität führte außerdem zum Stomataverschluss (hoher stomatärer Widerstand) und damit verbunden niedrigen Transpirationsraten. Die salzbedingt verminderte Photosyn-these könnte zurückzuführen sein auf niedrige C_i-Konzentrationen und Chlorophyll-gehalte sowie Schädigungen der Chloroplasten (Aufblähen der Thylakoidstapel) und einer möglichen Hemmung des Membrantransports, die mit der Anhäufung von großen Stärke-Körnern einher ging. 150% SWS bewirkte außerdem eine Abnahme der RubisCo- (große Untereinheit) in den Blättern beider *Atriplex* Arten. Auf dem C_4-Weg der Photosynthese erhöhte allerdings nur *A. nummularia* die Expression von PPDK, ALdP, MDH, HMT und SAMS während *A. leucoclada* deutliche Abnahmen dieser Proteine aufwies. Dieses Ergebnis kann als Beleg für eine resistenzerhöhende Regulation der Photosynthese bei *A. nummularia* gewertet werden. Die niedrige Selektivität bei der Ionenaufnahme und dem Transport, der hohe Energiebedarf und die fehlende Regulation im C_4-Weg der Photosynthese sind die Hauptgründe für die vergleichsweise niedrige Salzresistenz von *A. leucoclada*.

Schlüsselwörter: Salztoleranzmechanismen, *Atriplex nummularia*, *Atriplex leucoclada*.

LIST OF ABBREVIATION

ΔT	difference between leaf and air temperature
2D	two-dimensional
3-PGA	3-phosphoglyceric acid
A	net photosynthesis
AAS	atomic absorption spectrometry
ab	abaxial epidermis
ad	adaxial epidermis
ALADIM	advanced Laser Desorption Ionization Mass Analyzer
ALdP	plastidic fructose-bisphosphate aldolase
APS	ammonium peroxy disulfate
Arg	arginine
Asp	aspargine
ATP	adenosine 5, triphosphate
AW	ash weight [g]
bc	bladder cell
bh	bladder hair
BSA	bovine serum albumin
bs	bundle sheath
C_{an}	atmospheric CO_2 concentration [ppm]
ch	chloroplast
CHAPS	[(3-cholamidopropyl)-dimethylammonio]-propanesulfonate
Chl	chlorophyll
C_i	intercellular CO_2 concentration [ppm]
cr	calcium oxalate crystal
cw	cell wall
DHAP	dihydroxyacetone phosphate
DM	dry matter [g]
DR	dark respiration [$mol*m^{-2}*s^{-1}$]
DTT	dithiothreitol
DW	dry weight [g]
E	transpiration rate [$mol*m^{-2}*s^{-1}$]
EDTA	ethylenediamine tetraacetic acid
EDXA	Energy dispersive X-ray microanalysis
ep	epidermal cell
er	endoplasmic reticulum
FBP	fructose1,6-bisphosphate
FMOC-Cl	9-fluoroenylmethyl chloroformate
FW	fresh weight [g]
g	golgi body
GAP	D-glyceraldehyde-3-phosphate
GluA	Glutamic acid
gr	grana
HMT	5-methyltetrahydropteroyltriglutamate-homocysteine transmethylase
HPLC	high pressure liquid chromatography
IEF	isoelectric focusing
IPG	immobilised pH gradient
kDa	kiloDalton

La	adult leaf
Lc	light compensation point [µmol*m^{-2}*s^{-1}]
Lj	juvenile leaf
LMA	leaf mass to area ratio [mg*cm^{-2}]
Ls	light saturation point [µmol*m^{-2}*s^{-1}]
m	mitochondrion
MALDI-TOF	Matrix-Assisited Laser Desorption Ionisation Time-Of-Flight mass spectrometry
MDH	malate dehydrogenase
me	mesophyll cell
Met	methionine
MPa	mega pascal
MW	molecular weight
n	nucleus
OAA	oxaloacetic acid
PAGE	polyacrylamide gel electrophoresis
pd	plasmodesmata
pI	isoelectric point
pl	plastoglobuli
PPDK	pyruvate orthophosphate dikinase
PPFD	photosynthetic photon flux density [µmol*m^{-2}*s^{-1}]
pro	proline
PSII	photosystem II
QCS	Quick Check System
R	root
Rs	stomatal resistance [s*cm^{-1}]
RubisCO	ribulose bisphosphate carboxylase/oxygenase
s	starch
Sa	adult stem
$SA_{K:Na}$	selective absorption capacity of K^+ over Na^+
SAL	surface area per leaf [cm^2]
SAM	s-adenosyl-L-methionine
SAMS	s-adenosyl-L-methionine synthase
SDS	sodium dodecylsulfate
SD	standard deviation
SEM	scanning electron microscope
Sj	juvenile stem
$ST_{K:Na}$	selective transport capacity of K^+ over Na^+
st	stomata
SWS	seawater salinity
t	tracheary element
TEM	transmission electron microscope
TEMED	N,N,N',N'-tetramethylene diamine
th	thylakoid
TotAA	total amino acid
TSC	total soluble carbohydrate
TSP	total soluble protein
ue	upper epidermis
va	vacuole

vb	vascular bundle
ve	vesicle
WUE	water use efficiency of photosynthesis [µmol*m^{-2}*s^{-1}CO2 / mol*m^{-2}*s^{-1} water]
xp	xylem parenchyma
Φ_c	photosynthetic efficiency [µmol CO_2*μmol^{-1} Quantum]
ψ_s	water potential [MPa]

CONTENTS

1 INTRODUCTION

Approximately half of the world's land surface is perennial desert or dry lands, characterized by a climate with insufficient rainfall to meet the sustainable agricultural production (UNFPA, http://www.unfpa.org/seed/unso/pub-htm/dryland-population.pdf). These areas are mostly inhabited by developing countries with dense population (about 16% of the world's population). The high rate of population growth in these regions associated with the poor economic performance, water shortages, possible global climate changes, mismanagement, improper irrigation systems, deterioration of the vegetative cover, degradation of soil fertility and structure are leading to the desertification (FAO, 2005).

The spread of desertification threatens agricultural productivity worldwide by removing arable land from crop production. According to the UNCCD (http://www.unccd.com), more than 1 billion people in 110 countries are at risk of being displaced as a consequence of desertification. The widespread of desertification is usually related to the water shortage, the most pressing problem for mankind, most notably in the arid and semi-arid regions (Shay, 1990; Hamdy, 2002). Although two thirds of the earth is covered by water surfaces, only 2.5% is fresh water, while the majority (97.5%) is unusable saline water, containing about 3 - 5% salt, mostly sodium chloride (Hamdy, 2002). The usable portion of the freshwater resources for drinking, industry, and agriculture is less than 1% of all freshwater, and 0.01% of all water on the earth (Ghassemi *et al*., 1995; Hamdy, 2002). These dry areas can only be more productive by irrigation (Flowers, 2004). Unfortunately, high probability of salinization throws an immediate question over the sustainability of using irrigation to increase food production (Ghassemi *et al*., 1995).

Salinity is one of the oldest and serious agricultural problems, especially in countries where irrigation is an essential aid to agriculture (Flowers and Yeo, 1995; Munns, 2002; Rengasamy, 2002). It affects about 7 – 10 % of the world's total area, mostly located in arid and semi-arid regions (Szabolcs, 1994; Glenn *et al*., 1998; FAO, 2005). Additionally, the extension of irrigated agriculture and the intensive use of water resources combined with high evaporation rates in the arid and semi-arid regions increase the concentration of water soluble salts close to the soil surface.

Inevitably, this leads to an acceleration of secondary salinization that usually results in losses of once productive agricultural land (Choukr-Allah, 1996; Lieth and Mochtschenko, 2002; Lambers, 2003; Munns, 2005). At present, about one third of the world's cultivated lands are salt-affected due to unsustainable irrigation practices and about 1.6 million ha/year of irrigated lands go out of production due to salinization (Flowers, 1999; Glenn *et al*., 1999; Tanji, 2002; FAO, 2005).

Shortage of the precious natural resources (i.e. land and water) combined with an increasing demand for food as the population increase seriously limits the economic development and threatens the mankind existence worldwide, but particularly in arid and semi arid-regions. The situation in Egypt is an example for the critical overload. The agricultural production in Egypt is not increasing proportionally to population growth because of limited natural resources. Geographically speaking, Egypt is located between 27 – 30 °N latitude with a total area of one million square kilometres. Most of the population is concentrated in only a small portion (3.5 – 4% of the total area). The major parts of the country (96%) have a very low annual precipitation, ranging between 0 mm in the desert to 200 mm in the northern coastal region. Because of the insufficient rain, the agricultural production of the country is almost entirely dependent on the Nile River (55.5 BCM per year). As in many countries of the arid region, the competition for fresh water increases. Thus water of better quality is used primarily for drinking, whereas water of lower quality such as brackish or even saline water is often used for irrigation. Unfortunately, the salinity of such water resources typically exceeds the limit tolerated by our conventional crops.

The primary value of increasing salt tolerance of crops will be sustainability of irrigation (Tester and Davenport, 2003; Flowers, 2004). Given the amount by which food production will have to be increased, it seems reasonable to predict that changing of salt tolerance of crops will be an important aspect of plant breeding in the future. An alternative approach is domestication of naturally occurring halophytes for a sustainable crop production since they have already the requisite level of salt tolerance (Boer and Gliddon, 1998; Lieth *et al*., 1999). The study of halophytes with potential to become economically important is the aim of this investigation. About 2500 halophytic species are known throughout the world (Lieth *et al*., 1999; Koyro, 2006). They constitute the basis for the selection of plant

material that combined economic utility with the ability to grow, produce and reproduce under high salinity conditions (Aronson, 1989; Lieth *et al*., 1999). The optimum and sustainable utilization of halophytes as cash crops has the potential of making an important contribution to the food and feed production in many arid and semi-arid regions (Choukr-Allah, 1993; Lieth and Al Masoom, 1993; Glenn *et al*., 1999; Lieth *et al*., 1999; Koyro and Huchzermeyer, 1999a; Ahmad and Malik 2002; Barrett-Lennard, 2002; Zhao *et al*., 2002; Koyro, 2003b; Liu *et al*., 2006). It also permits the use of saline water, thereby reduces some of the demand for high quality water. However, application of halophytes is complex and its sustainable use needs great care, in considering agronomic, water management and economic factors without ignoring long term effects of this practice on the soil properties and on crop yield (Hamdy, 2002). In many developing countries, utilization of halophytes as cash crops has entered the realm of economic feasibility (Boer and Gliddon, 1998; Lieth and Mochtschenko, 2002). A number of halophytes are used as food crops such as many *Atriplex* species, *Aster tripolium*, *Salicornia europea*, *Avicennia marina*, *Avicennia germinans*, *Kosteletzkya virginica* and *Zizania aquatica* (O'Leary 1984; Lieth and Mochtschenko, 2002). Several halophytic species could provide good fodder for livestock and wildlife such as *Atriplex nummularia*, *Atriplex leucoclada*, *Suaeda fruticosa*, *Spartina alternifora*, *Sporobus virginicus*, *Leptochloa fusca* (Güth, 2001; Lieth and Mochtschenko, 2002). Wood from certain mangroves such as *Avicennia marina, Rhizophora mangle* and *Laguncularia racemosa* as well as *Tamarix amnicola* have been used traditionally for house and ship building (Güth, 2001; Lieth and Mochtschenko, 2002). Several halophytes such as *Salsola kali* contain chemicals of interest for medical and pharmaceutical purposes (Menzel and Lieth, 1999). In many arid and semi-arid regions halophytes like *Batis maritime*, *Mesembrianthemum crystalinum* and *Sesuvium portulacastrum* may help improve landscapes and roadsides. In addition, many halophytes can be used as ornamental plants such as *Aster tripolium* and *Limoneastrum monopetalum* (Lieth *et al*., 1999; Lal, 2001; Güth, 2001; Lieth and Mochtschenko, 2002). *Suaeda salsa* and *Batis maritima* can also be applied in saline land reclamation (phyto-remediation) (Zhao, 1991; Lieth *et al*., 1999). Use of halophytes has also substantial environmental benefits, since several halophyte species have the potential for coastline protection,

dune stabilisation or CO_2 sequestration to reduce the atmospheric CO_2 pollution (Güth, 2001; Lieth and Mochtschenko, 2002).

In this long list of useful plants, the family *Chenopodiaceae*, contains some particularly economically important halophytic generis such as *Atriplex*. As described so far, the genus *Atriplex* comprises about 200 species worldwide, mostly grown in arid and semiarid saline habitats. *Atriplex* species have been generally recognized for their high salt and drought tolerance. They evolved a number of adaptive mechanisms enabling them to survive and grow under saline and dry conditions (Watson *et al*. 1987; McKell, 1994; Glenn *et al*., 1997; Osman and Ghassaeli, 1997). The adaptability of these species to such stressful environments is a key for their utilization (Kelley *et al*., 1982). Owing to their favourable crude protein and acceptable nutritional contents, many *Atriplex* species have been introduced specifically for the purpose of increasing forage productivity and rehabilitating the marginal land, particularly in the arid and semi-arid regions (Kelly *et al*., 1982; McKell, 1994; Swingle *et al*., 1996). They are extremely important as forages for the livestock and wildlife on a year-round basis especially during the off-season periods (Goodall, 1982; Le Houérou, 1995). According to Nefzaoui (1997), more than one million ha in the Middle East have been cultivated with different *Atriplex* species for this purpose. In this respect, some *Atriplex* species (*A. lentiformis*, *A. barclayana*, *A. europea*) produce about 0.6 – 2.6 tones proteins per year and per ha, and thus under saline conditions (O'Leary *et al*., 1985). Additionally, *Atriplex* shrubs can be used as roadside plants (Stark, 1966), fuel (Nord and Countryman, 1972), in reclamation of salty soils (Goodin and Mozafar, 1972), and soil stabilisation (McKelly, 1974).

Among the salt tolerant *Atriplex* species, *A. nummularia* Lind. and *A. leucoclada* Bioss, have high potentials to become cash crops. *A. nummularia* has been long promoted as one of the most promising *Atriplex* species for extensive use as a forage crop on saline and marginal lands in several Mediterranean countries. As a plant with C_4 carbon fixation, it exhibits high water use efficiency (WUE) of photosynthesis (Osmond *et al*., 1980; Miyamoto and Mueller, 1994) and high salinity and drought tolerances (Sharma, 1982). There are several relevant information about its high fodder quality such as high biomass production, high protein content (approximately 15%), high digestible matter content, low fibre and moderate ash

content, within the range of conventional forage sources (National Academy of Sciences, 1971; Kelley *et al*., 1982; Glenn *et al*., 1997).

A*. leucoclada* is a native species, used as forage in the saline and marginal area of North Egypt. However, less data are available on *A. leucoclada* in comparison to *A. nummularia*.

Despite the ecological importance of *A. nummularia* and *A. leucoclada* and their potential use in the salt-affected area, knowledge about the levels of salt tolerance and their responses to salt stress (mechanism of salt tolerance), particularly about *A. leuococlada*, has not been sufficiently assessed. Understanding the morphological, physiological and biochemical mechanisms underlying the salt tolerance could provide important information needed for the sustainable utilisation of both *Atriplex* species and provide basic information about the effect of salinity on the growth and development of these plants (Lieth and Mochtschenko, 2002; Koyro and Huchzermeyer, 2004). Hence one aim of the current study is to assess the potential of sustainable utilization for *A. nummularia* and *A. leucoclada* as cash crops, and to compare the performance of these closely related *Atriplex* species at various NaCl salinities using the quick check system (QCS, Koyro and Huchzermeyer, 1999a). This system seems ideal to get detailed and precise information about the threshold of salinity tolerance and the individual mechanisms for salt tolerance.

It seems essential to select methods (including some general and specific physiological or biochemical parameters) closely related to the four major constrains of plant growth under saline conditions i.e. water relations, CO_2 gas-exchange, ion toxicity and nutrient imbalance constrains. One of these parameters is the biomass production. The plant species differ greatly in their growth responses to salt stress. While the glycophytes show generally a dramatic growth inhibition under saline condition, halophytes can tolerate and grow in a substrate rich in NaCl (Flowers *et al*., 1986). Many reports revealed that some halophytic species do not only tolerate high salinity levels but reach their optimal growth at moderate salinities (Ungar, 1991; Koyro *et al*., 2006; Liu *et al*., 2006). Growth stimulation at moderate salinity (100 - 200 mol*m^{-3} NaCl) has been reported for several *Atriplex* species such as *A. infata* F. Muell (Ashby and Beadle, 1957), *A. nummularia* (Uchiyama, 1987; Dunn and Neales, 1993), *A. hastata* (Black, 1965; Dunn and Neales, 1993), *A. halimus*

(Bajji *et al*., 1998) and *A. griffithii* (Khan *et al*., 2000). If the salinity increases above a threshold level, the growth of many halophytic species is markedly reduced (Greenway, 1968; Uchiyama, 1987; Ungar, 1996; Khan *et al*., 2000; Lu *et al*., 2002; Kefu *et al*., 2003; Koyro *et al*., 2006).

The primary deleterious effect of raising water salinity on plant growth is due to the disturbance of water homeostasis. Plants that grow in saline habitats face the problem of having low water potential in the substrate (Marschner, 1995). Low soil water potential interferes with the plant ability to take up water from the medium and, hence, causes growth reduction, along with a range of physiological and biochemical changes similar to those caused by water deficit (Sohan *et al*., 1999; Romero-Aranda *et al*., 2001; Koyro, 2000; Flowers, 2004; Munns, 2005). At low soil water potential, salt-tolerant plants adjust osmotically (accumulate solutes) and maintain a potential fort the influx of water. Several authors observed that water and osmotic potentials of many halophytic species decrease with the increase of substrate salinity (Khan *et al*., 2000; Romero-Aranda *et al*., 2001; Lu *et al*., 2002; Gulzar *et al*., 2003a, b and 2005).

One of the primary physiological targets of the induced water deficit (see above) is the photosynthetic capacity of plants (Lawlor and Cornic, 2002). Low soil water potential can result in reduction of cell turgor pressure that provides the expansive forces necessary for cell wall extension (Frensch and Hsiao, 1994; Marschner, 1995; Koyro, 1997). This can lead to an inhibition of plant growth, reduction of leaf area and promotion of leaf senescence and abscission. As a result, the total leaf area decreases, leading to reduced photosynthetic capacity and biomass production. Moreover, water deficit induces a rapid closure of stomata to avoid further water loss via transpiration (Cornic, 1994; Tester and Davenport, 2003). As a consequence, the apparent photosynthesis rate declines due to restricted availability of CO_2 for carboxylation reactions in the leaves (stomatal limitation of the photosynthesis) (Brugnoli and Björkman, 1992; Huchzermeyer and Koyro, 2005). Salt induced changes in leaf anatomy, such as increased thickness of cell walls and decreased intercellular spaces between mesophyll cells can also restrict CO_2 diffusion toward the chloroplasts (Evans and Caemmerer, 1996; Lauteri *et al*., 1997; Delfine *et al*., 1998). Limitation of CO_2 supply to Rubisco is a major contribute to reduction in photosynthesis in many C_3 carbon fixating plants.

However, in C_4 plants there have been few attempts to quantify stomatal and non stomatal limitations to photosynthesis under stress conditions. In latter one, CO_2 is fixed via phosphoenolpyruvate carboxylase in mesophyll cells into C_4 acids, which are transported to bundle sheath cells where they serve as donors of CO_2 to the C_3 cycle via C_4 acid decarboxylases. By integrating the two CO_2 assimilation pathways consecutively in the spatially cooperative mesophyll and bundle sheath cells, C_4 plants can achieve high photosynthetic efficiency, especially under conditions that cripple C_3 plants (Hatch, 1997; Furbank *et al*., 2000). A major advantage of C_4 plants over C_3 plants is not only higher photosynthetic efficiency, but more effective capacity to coordinate the rate of photosynthesis and transpiration. This may be significant in considering the ecological conditions under which many C_4 plants such as *Atriplex* are grown (high salinity and high light intensity) (Furbank and Taylor, 1995; Hatch, 1997). In many halophytic species, stomatal limitation of photosynthetic capacity reduces the transpiration rate and leads to higher water use efficiency in some halophytes (Naidoo *et al*., 1995; Robinson *et al*., 1997; Koyro and Huchzermeyer, 2004). There is, however, a long-standing controversy as to whether salt stress mainly limits photosynthesis through stomatal closure or metabolic impairments (non stomatal limitations) (Dionisio-Sese and Tobita, 2000; Lovelock and Ball, 2002). Non-stomatal limitation of photosynthesis has been attributed to an inhibited coupling factor activity (Tezara *et al*., 1999), reduced carboxylation efficiency (Wise *et al*., 1990; Jia and Gray, 2004), reduced amount or activity of crucial photosynthetic enzymes such as Rubisco (Parry *et al*., 2002), reduced RuBp regeneration (Giménez *et al*., 1992; Gunasekera and Berkowitz, 1993), and reduction of the contents of photosynthesis pigments (Seemann and Critchley, 1985; Abdullah and Ahmed, 1990; Hamada and El-Enany, 1994; Hajar *et al*., 1996; Koyro, 2006). Apparent photosynthesis was significantly suppressed in high salt stressed *A. prostrata* (Wang *et al*., 1997), *A. nummularia* (Uchiyama, 1987; Dunn and Neales, 1993), *A. centralasiatica* (Qiu *et al*., 2003). In most cases, growth reduction was usually accompanied by a decreased photosynthetic capacity (Dunn and Neales, 1993; Lu *et al*., 2003 a, b; Koyro and Huchzermeyer, 2004). The reduction of CO_2 supply to the carboxylation sites in the leaves as a result of stomatal and/or non-stomatal limitations increases the potential for photoinhibition and photooxidation (Lawlor, 2002; Lovelock and Ball 2002). These processes are

closely associated with an excess of excitation energy in PSII and over reduction of the photosynthetic electron transport (ET) chain (Osmond and Grace, 1995). If no protective mechanisms are employed, reactive oxygen species (ROS, such as superoxide, hydrogen peroxide, hydroxyl radicals and singlet oxygen) accumulate in the plants. ROS accumulation can damage many important molecules such as proteins, lipids and nucleic acids (Asada, 1999; Ben Amor *et al*., 2005), and leads to inactivation or damage of PSII (Hopkins, 1999; Parida, 2004).

Ion toxicity is one major problem for plants growing on saline soils. Although Na^+ is important in many C_4 species for the conversion of pyruvate to phosphoenolpyruvate and for pyruvate translocation across membranes (Ohnishi *et al*., 1990; Murata *et al*., 1992), excessive Na^+ may account for specific ion toxicity (Levitt, 1980). Symptoms of Na^+ toxicity such as chlorosis and necrosis at the leaf tips and margins, followed by leaf death are common in many plant species (Zhu, 2001; Munns, 2002). This leads apparently to a reduction of the leaf lifetime, and hence reduces the photosynthetic capacity and plant production (Munns, 1993, 2002). Further, Na^+-induced biochemical and ultra-structural changes in the chloroplasts may negatively impact photosynthesis (Fedina *et al*. 1994; Koyro, 2002; Lovelock and Ball 2002; Fiadalgo *et al*., 2004). Accumulation of Na^+ in the leaf apoplast may additionally result in an osmotic damage (Öertli, 1968; Flowers *et al*., 1991). Further, high cytoplasmic Na^+ concentration interferes with the K^+ binding sites, and hence inhibits a wide range of important metabolic processes that crucially depend on K^+ (Blaha *et al*., 2000; Munns, 2005). Although the toxic effects of Na^+ have been frequently reported, those of chloride were relatively ignored. Cl^- toxicity may be primarily due to the osmotic effect of high Cl^- in the cell walls (Öertli 1968; Marschner, 1995), or Cl^- accumulation in the cytosol, where it can affect protein synthesis and enzyme activity (Flowers *et al*. 1977; Gibson *et al*. 1984). Cl^- may also interfere with anionic sites involved in binding of RNA and anionic metabolites such as bicarbonate, carboxylates and sugar phosphates (Wyn Jones and Pollard, 1983; Serrano, 1996; Xu *et al*., 2000).

Plant growth inhibition can be also seen due to the effect of both Na^+ and Cl^- to limit the uptake of the essential nutrients required for growth (nutrient imbalance). High Na^+ concentration in the rooting medium is known to influence uptake, transport and utilization of major cations such as K^+, Ca^{2+} and Mg^{2+} and affects ion

homeostasis within the plant (Marschner, 1995; Hasegawa *et al*., 2000; Silberbush and Ben-Asher, 2001; Wyn Jones and Gorham, 2002; Tester and Davenport, 2003; Liu *et al*., 2006). This effect was observed in many NaCl stressed plants such as *A. amnicola* (Aslam *et al*.,1986), *A. nummularia* (Uchiyama, 1987; Ramos *et al*., 2004), *A.barclayana* (Nerd and Pasternak, 1992), *A. griffithii* (Khan *et al*., 2000), and *A. hortensis* (Wilson *et al*., 2000).

K^+ is a major macronutrient essential for many cell processes. The physiological roles of K^+ in the plants have been frequently reviewed (Marschner, 1995; Shabala *et al*. 2003). K^+ is involved in enzyme activation, turgor formation, regulation of stomatal movement and maintenance of osmotic homeostasis. Counterbalancing the large excess of negative charge, K^+ is also equilibrated with Na^+ to provide a correct environment for protein synthesis in condition of hyperionic stress. K^+ uptake at the root/soil boundary is achieved by highly K^+ selective pathways, whereas Na^+, at least in part, appears to move through less selective systems (Tester and Davenport, 2003). Because of the similarity between Na^+ and K^+ in their hydrated ionic radii (Amtmann *et al*., 2001; Tester and Davenport, 2003; Munns, 2005), Na^+ competes with K^+ at the sites of entry. Thus the K^+ uptake decreases, resulting in ion toxicity, ion imbalance and growth reduction (Nakamura *et al*., 1990; Marschner, 1995; Shabala *et al*., 2003).

Also, NaCl salinity severely affects Ca^{2+} uptake and transport, so that the shoots of stressed plants frequently show symptoms of Ca^{2+} deficiency (Cramer *et al*., 1987; Ehret *et al*., 1990; Francois *et al*., 1991). The physiological functions of Ca^{2+} have been comprehensively reviewed by Marschner (1995), and Cramer (2002). Briefly, it acts as a structural component of the apoplastic macromolecules. This function is related to its capacity for coordination by which it provides stable but reversible intermolecular linkages, predominantly in the cell walls and at the plasma membrane. Ca^{2+} has been also recognized as a transducer of hormonal and environmental signals to the responsive elements of cell metabolism (Lynch *et al*., 1989 and Bennet and Breen, 1991). The displacement of Ca^{2+} ions by Na^+ at the plasma membrane surface could induce the depolarization of the plasma membrane, inducing leakage of cytosolic K^+ from the cell (Shabala and Newmann, 2000; Cramer, 2002). Adequate Ca^{2+} contents in the salt-treated root tissues are thought to be a prerequisite for the maintenance of a high K^+/Na^+ selectivity

(Hajibagheri *et al*., 1987; Cramer, 2002). Therefore, the ratio Ca^{2+}/Na^{+} in plants appears as the more reliable indicator for salt stress (or tolerance) than the Na^{+} concentration alone (Ben Hayyim *et al*., 1987; Cramer *et al*., 1986).

The uptake of a further macronutrient, Mg^{2+} depends also on the Na^{+} competition. Mg^{2+} deficiency can influence many metabolic processes including enzymatic reactions such as RubisCo, Fructose-1, 6-Bisphosphatase, ATPases and most kinases (Marschner, 1995). Additionally, Mg^{2+} plays a key role in the chlorophyll and protein synthesis (Wyn Jones and Pollard, 1983). Mg^{2+} deficiency in plants often results in ultrastructural changes, especially in the chloroplast (Puech and Mehne-Jakobs, 1997). Hence, Mg^{2+} deficiency would have damaging effects on photosynthetic activity of plants (Sun and Payn, 1999; Fischer and Bremer, 1993; Koyro, 2000).

High external Cl^{-} concentration can also restrict the absorption of NO_3^{-}, PO_4^{3-} and SO_4^{2-} (Termaat and Munns, 1986; Marschner, 1995; Fisarakis *et al*., 2001). There is not much information available about the effect of high Cl^{-} on the uptake and contents of these anions in *Atriplex* species.

Salt tolerance is brought about by a range of interconnected physiological, morphological and biochemical processes that are controlled by specific gene expression (Marschner, 1995; Rengasamy *et al*., 2003, Tester and Davenport, 2003; Munns, 2005; Koyro, 2006). Basically, two main strategies are involved in salinity tolerance of plants: salt inclusion and salt exclusion. Both strategies are not mutually exclusive and may work in parallel in salt-tolerant species (Marschner, 1995; Munns, 2002).

Salt-including plants can use high salt (Na^{+} and Cl^{-}) concentrations as a cheap osmoticum to create and maintain water potential gradient and turgor necessary for water uptake and expansive growth (Koyro and Huchzermeyer, 1999b; Romero-Aranda *et al*., 2001; Lu *et al*., 2002; Tester and Davenport, 2003; Gulzar *et al*., 2005; Ottow *et al*., 2005). Many *Atriplex* species are known to rely on this mechanism and accumulate large amounts of Na^{+} and Cl^{-}, mainly in the shoots, like *A. barclayana* (Nerd and Pasternak, 1992), *A. nummularia* (Uchiyama, 1987, Ramos *et al*., 2004), *A. griffithii* (Khan *et al*., 2000) and *A. hortensis* (Wilson *et al*., 2000). Although salt inclusion mechanism facilitates osmotic adjustment and turgor maintenance, it can lead to ion toxicity and nutritional imbalance in the cytoplasm

(Koyro and Huchzermeyer, 1999b; Blumwald *et al*., 2000). Thus, adaptation by salt inclusion requires either salt tolerance, or avoidance mechanisms (Marschner, 1995). Tolerance of high salt accumulation can be achieved by various mechanisms. In some salt-including plants, the old or adult leaves act as ion sinks and accumulate large quantities of ions (Flowers and Yeo, 1992; Munns, 1993, 2002). This may restrict ion deposition into meristematic and actively growing and photosynthesizing leaf cells. Some species (natrophilic species) can replace K^+ by Na^+ not only in its function as osmoticum but also, to lesser extent, in some metabolic processes (Marschner, 1995; Mäser *et al*., 2002). The compartmentation of excessively accumulated Na^+ and Cl^- provides an efficient mechanism to avert the deleterious effects of these ions in the cytosol and help to maintain homeostasis of other essential ions like K^+, Ca^{2+} and Mg^{2+} necessary for the metabolic activities (Flowers and Yeo, 1992; Marschner, 1995; Serrano, 1996; Bressan *et al*., 1998; Koyro and Huchzermeyer, 1999b; Blumwald *et al*., 2000; Hasegawa *et al*. 2000; Munns, 2005). Approaches which have been used, such as X-ray microanalysis (Milis *et al*. 1985; Leigh and Story, 1993; Koyro *et al*., 1997, 2006; Flowers and Hajibagheri, 2001) generally support the hypothesis that maintenance of low cytosolic Na^+ concentration is important in salinity tolerance.

As a matter of fact, both Na^+ sequestration into the vacuole and Na^+ extrusion in the apoplast are active processes, since Na^+ has to be transported against its electrochemical potential (Blumwald *et al*., 2000; Tester and Davenport, 2003). Many membrane proteins are known to be involved in ion sequestration, compartmentation and redistribution. One important protein is the Na^+/H^+ antiporter, which mediated Na^+ extrusion at the plasmalemma or sequestration at the tonoplast (Wang *et al*., 2001; Morsomme and Boutry, 2000; Wyn Jones and Gorham, 2002). This antiporter is powered by the operation of the H^+-ATPase (Sussman, 1994). An increase in H^+-ATPase activities was observed in salt stressed *A. nummularia* roots and leaves (Braun *et al*., 1988; Niu *et al*., 1996). Increasing leaf succulence is another important adaptive feature that contributes to the regulation of internal ion concentrations through a dilution (Flowers *et al*., 1986; Koyro and Huchzermeyer, 1999b; Koyro, 2002; Debez *et al*., 2006). High substrate salinity was found to increase the leaf succulence of *A. hastata* (Black, 1958), *A. nummularia* (Ashby and

Beadle, 1957; Greenway, 1968), *A. patula* (Longstreth and Nobel, 1979), *A. amnicola* (Aslam *et al*., 1986), and *A. halimus* (Debez *et al*., 2003).

Atriplex species show a wide range of adaptation from the morphological to the physiological adaptations that include the ability to remove salt through bladder hairs. It is a common feature of *Atriplex* leaves and important for salt removing from the leaf tissues and hence prevent dangerous accumulation of toxic salt in the photosynthetic tissues (Black, 1954; Osmond *et al*., 1969; Mozafar and Goodin, 1970, Kelley *et al*., 1982; Schirmer and Breckle, 1982; Waisel 1991). In *A. halimus*, Mozafar (1970) and Mozafar and Goodin (1970) found that ion concentrations (Na^+ and Cl^-) of the leaf tissues remained almost constant, while those of the bladder hairs increased with increasing salt treatments and reached concentrations of almost saturated NaCl solutions. Also, ion contents of the bladder hairs were correlated with the salt concentrations in the leaves of *A. nummularia* (Uchiyama, 1987).

Salt exclusion is the second important adaptive feature beside salt inclusion. It is involved in the regulation of internal salt load (Marschner, 1995; Munns, 2002). The mechanisms conferring salt exclusion in plants have been reported by several authors (Greenway and Munns, 1980; Koyro and Huchzermeyer, 1999b; Storey and Walker, 1999; Koyro *et al*., 2006). These mechanisms include: prevention of ion entry into the root symplasm and subsequent unloading into the xylem, selective uptake of K^+ over Na^+ by root cells, preferential loading of K^+ rather than Na^+ by the stelar cells and removal of salt from the xylem in the upper part of the roots, the stem, petiole or leaf sheaths (Marschner, 1995; Storey and Walker, 1999; Munns, 2002; Tester and Davenport, 2003). Additionally, some salt-excluders get rid of salt by excreting it back into the environment by intensive re-translocation from the shoot to the root (Bhatti and Wieneke, 1984; Munns, 2002). The exclusion of salt from the phloem ensures that salt is not transported to the growing tissues of the shoot (Munns *et al*., 1988; Tester and Davenport, 2003). Salt exclusion from the leaves (excretion) through salt glands is an important mechanism, which helps to maintain a steady salt balance in the leaves over long periods of the vegetative cycle (Flowers *et al*., 1986; Ball, 1988). Whereas salt exclusion minimizes ion toxicity, it may accelerate water deficit that may reduce the plant growth under high saline conditions (Gorham *et al*., 1985; Koyro and Huchzermeyer, 1999 b).

As well excluders and includers need the accumulation of compatible solutes in the cytoplasm and organelles to counteract the increased osmolality of apoplast or the cell vacuoles (Hasegawa *et al*. 2000; Rontein *et al*., 2002; Aziz and Khan, 2003; Tester and Davenport, 2003; Ashraf and Harris, 2004). These solutes are non-toxic at high concentrations, have low weight, and are highly soluble. They protect plant cells from salt stress by turgor maintenance, detoxification of reactive oxygen species (ROS), and by stabilisation of quaternary structure of proteins (Yancey *et al*., 1982; Bohnert and Jensen, 1996). Major categories of organic osmotically active solutes are known to accumulate in plants under salt stress including simple and complex sugars, and sugar alcohols (Popp and Smirnoff, 1995; Bohnert and Jensen, 1996; Bajji *et al*., 1998; Murakeozy *et al*., 2003). Others include nitrogen-containing compounds such as amino acids, quaternary amino acid derivatives (proline, glycine betaine), and tertiary amines and sulfonium compounds (Nuccio *et al*., 1999; Mansour, 2000). A positive correlation between the substrate salinity and accumulation of proline and glycine betaine was reported in *A. spongiosa* and *Suaeda monica* (Storey and Wyn Jones, 1979), *A. gmelini* (Matoh *et al*., 1987), *A. semibaccata* and *A. halimus* (Koheil *et al*., 1992), *A. braclayana* (Nerd and Pasternak, 1992), *A. griffithii* (Khan *et al*., 1998), and *A. halimus* (Bajji *et al*., 1998).

As has been stated by Koyro and Huchzermeyer (2004), a prerequisite for the sustainable utilisation of the halophytes is the precise knowledge about their salinity tolerance and the various mechanisms enabling them to grow at saline habitats. Hence, a major motivation of the present study was to determine the levels of salt tolerance of *A. nummularia* and *A. leucoclada* and to compare the morphological, physiological and biochemical responses (mechanisms) of both species to various water salinities. Salt tolerance is a complex trait and often implicates a strong reliance between various mechanisms of which several were above-mentioned. Therefore, the influence of various NaCl salinity levels on several essential parameters related mainly to the four major constraints for plant growth on saline substrates as mentioned above were studied in both *Atriplex* species. It was planned to get an overview about the general mechanisms by measuring the following parameters: plant growth (fresh, dry, and ash weights, leaf number, leaf area, leaf mass to area ratio, and shoot/root ratio), water relations (water content,

water and osmotic potentials), gas-exchange (net photosynthesis, photosynthetic efficiency, dark respiration, intercellular CO_2 concentration, stomatal resistance, water use efficiency, and light saturation and light compensation points), and composition of minerals and compatible solutes in different parts of both plants. Such general information would give an impression about the salinity tolerance level and the different mechanisms underlying the adaptation to high NaCl-salinity. In addition, further special investigations (proteomics, EDXA, and structural as well as ultrastructural investigations) are expected to complete the view about the individual salt tolerance mechanisms of these species. Considering that salt tolerance is multigenic and implicates the regulation of several genes with determinant role in salt adaptation, one specific aim of this study was to quantify changes in the leaf proteome of both *Atriplex* species in response to salt stress, and to eventually identify candidate salt responsive proteins. The response of *Atriplex* species to salinity is widely reported, but ion composition and sequestration in the bladder hairs are often disregarded. Therefore, the elemental composition and distribution of different leaf cells such as bladder, epidermal, guard cells was determined. Further, light as well as scanning and transmission electron microscopical studies were conducted to identify salt-induced structural and ultrastructural changes in the leaves of both *Atriplex* species and their ecological advantages. All these scientific informations (general and specific) were correlated and used to determine and compare the level of salinity tolerance of the both *Atriplex* species. The comparison of these closely related *Atriplex* species is expected to give more and better information about the small differences or factors contributing to the high salt tolerance of these species and to open the vision on their future integration in programs of amelioration of crops.

2 MATERIAL AND METHODS

The present work was carried out at the “Institut für Botanik”, Gottfried Wilhelm Leibniz University, Hannover and the “Institut für Pflanzenökologie”, Justus-Liebig-University, Giessen, Germany. It aimed at investigating the physiological and structural mechanisms of the halophytes *A. nummularia* and *A. leucoclada* for surviving high NaCl salinity (up to 750 mol*m^{-3} NaCl). The experiments were conducted in the greenhouse with three successive cultures for each species during the period from October 2002 until October 2004.

2.1 Cultivation and growth conditions

Seeds of *A. nummularia* and *A. leucoclada* were obtained from the Desert Research Institute, Cairo, Egypt. They were washed with running tap water for 24 - 48 h to remove the excess of salts, and other germination inhibitors. The seeds were then sown in plastic flats containing a soil mixture of ED-73 soil (Hawita, Lauterbach, Germany) and vermiculite, 1:1 (v/v), and kept on a bench in the greenhouse at 25 ± 2 ºC daytime and 15 ± 2 ºC night time temperatures for a photoperiod of 16 h. After the emergence of the first two true leaves (two weeks after the germination), the young seedlings were transplanted into a multi-pot tray containing a soil mixture of F-E type T soil (Hawita, Lauterbach, Germany) and compost (1: 1, v/v). Five weeks later, fifty plants of uniform size were selected, and transferred into a gravel/hydroponic culture named Quick Check System (QCS) (Koyro and Huchzermeyer, 1999a) (Fig. 1). The plants were cultivated individually in black plastic pots (25 cm diameter). The free surface of the culture substrate was covered with a black plastic foil in order to prevent the plants from being spattered with the nutrient solutions, and to inhibit the growth of algae on the surface. The plants were irrigated with a basic nutrient solution modified after Epstein (1972) using a drip irrigation system. The composition of the nutrient solution is shown in Table (1). The plants were grown under photoperiodic conditions (16 h light/8 h dark) in the greenhouse. Temperatures were 25 ± 2 ºC during the day and 15 ± 2 ºC during the night. The relative humidity ranged from 50 to 70%, and the light intensity was in the range of 250 µE*m^{-2}*s^{-1} at the plant level.

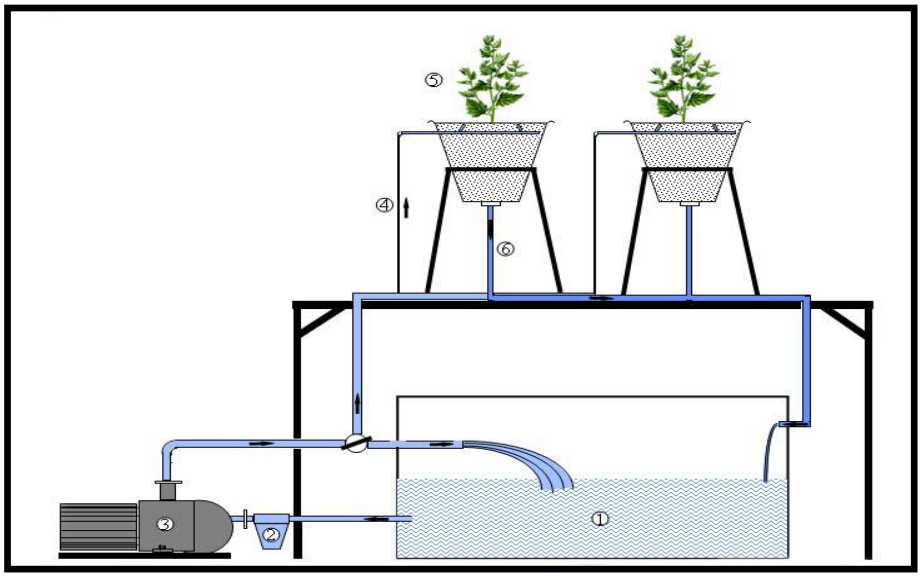

Fig. 1: Scheme of the *Atriplex* quick check system (QCS). (1) nutrient solution; (2) filter; (3) pump; (4) irrigation tube; (5) *Atriplex* plant and (6) draining tube.

Table 1: Composition of the basic nutrient solution (modified by Epstein, 1972), which used to irrigate *Atriplex* plants in the QCS.

Salts	**Concentration**
	(mol*m^{-3})
KNO_3	1.0
$Ca(NO_3)_2$	1.0
$NH_4H_2PO_4$	1.0
$(NH_4)_2HPO_4$	1.0
$MgSO_4$	1.0
	(µmol*m^{-3})
Fe-EDTA	20.0
H_3BO_3	25.0
KCl	50.0
$MnSO_4$	2.0
$ZnSO_4$	2.0
$CuSO_4$	0.5
H_2MoO_4	0.5

The addition of NaCl to the basic nutrient solution started after a period of another 2 weeks by raising the NaCl concentration in the nutrient solution in steps of 100 mol*m^{-3} NaCl each day until the final concentrations were achieved. The highest salinity treatment was reached after eight days, and there were altogether five salinity treatments (eight replicate pots for each treatment): control, 125, 250, 500 and 750 mol*m^{-3} NaCl (equivalent to 0, 25, 50, 100 and 150% SWS). The plants were watered daily with the nutrient solution every 4 h for 30 min starting at midnight. The nutrient solutions were changed every 2 - 3 weeks to avoid nutrient depletion. The experiment was performed for a total period of 11 - 12 weeks. During this period, the development of the cultures was photographed weekly with a digital camera.

2.2 Harvest procedure

The plants were harvested 11-12 weeks after the initiation of NaCl treatment (three replicates were harvested from each treatment). The plants were separated into roots (R), adult leaves (La), juvenile leaves (Lj), adult stems (Sa) and juvenile stems (Sj). The root segments were washed for 1 - 2 min with ice-cold 0.2 mol*m^{-3} $CaSO_4$ solution, and then for 1 - 2 min in distilled water in order to remove the excess of the nutrient solution and salts in the root free spaces. They were then blotted carefully with tissue paper to remove the surface water. The fresh weight of the roots, the adult and juvenile leaves and the adult and juvenile stems was directly determined. The adult and juvenile leaf numbers per plant, leaf area per leaf (SAL), leaf mass to area ratio (LMA) (defined as fresh weight per surface area) and the ratio of shoot /root fresh weight were captured.

Representative specimens of about 200 – 300 mg from each plant organ (R, Sa, Sj, La and Lj) were taken, and stored at -80 ºC for the quantitative chemical analysis. In order to determine ion contents of the bladder hairs separately, which covered all shoot parts and those of the leaf tissues, the adaxial and abaxial surfaces of the adult and juvenile leaves were rinsed in 25 ml distilled water (bladder hair fractions). These fractions were stored at 4 ºC for the determination of the ion contents. To obtain the dry and ash weights of the different plant organs, specimens of about 300 – 500 mg of these washed plant materials (R, Sa, Sj, La and Lj) were dried for 48 h at 105 ºC, and then weighed, and ashed in a muffle

furnace at 550 ºC for 12 h. The water content of all plant organs was determined as percentage of fresh weight.

2.3 Water relations

2.3.1 Determination of water potential

The leaf water potential of both *A. nummularia* and *A. leucoclada* was psychrometrically measured on the abaxial surfaces of the intact leaves with a Dew Point Microvoltmeter (Wescor Type HR330, WESCOR INC, USA). Hence the determination of water potential psychrometrically depends on the transpiration rate at the leaf surface through the stomata, it was difficult to obtain a real water potential values according to this method because of the presence of about 1 - 3 dense layers of bladder hairs which cover all leaf surfaces especially the young ones. Thus, the water potentials of the young shoots were determined by using the pressure pump (Scholander *et at*., 1965). The measurements were taken 4 and 8 weeks after the salt addition in the early morning between 08:00 and 09:00 o'clock, three replicates for each plant and three plants for each treatment.

2.3.2 Determination of osmotic potential

In order to determine the real osmotic potential of the plant organ tissues, about 200 - 300 mg of the washed plant materials (R, Sa, Sj, La and Lj) (three replicates each treatment) were placed in Eppendorf tubes, and heated in a water bath 80 ºC for 10 minutes. The samples were then crushed to extrude the tissue sap. A small hole was drilled in the bottom of each Eppendorf tube. The microtube was encased in a second intact empty Eppendorf tube, and centrifuged at 13000 X g for 10 minutes. The tissular sap was moved through the hole of the upper Eppendorf tube into the empty one. It was then collected for the measurements of the osmotic potential using an osmometer (Osmomat 030, Genotec GMBH, Berlin). A 300 mOsmol NaCl solution was used as a standard, and the calibration was checked after every ten readings. The readings were then converted to pressure units by using the salinity conversion table (Koyro, 2003a).

2.4 Chlorophyll contents and CO_2 gas exchange

2.4.1 Determination of chlorophyll and carotenoid contents

At harvest time, the pigment concentrations (Chl (a), (b) and carotenoids) were spectrophotometrically determined in the adult and juvenile leaves of both *Atriplex* species according to Lichtenthaler and Wellburn (1983).

2.4.2 CO_2-gas exchange and photosynthesis analysis

A closed photosynthesis measurement system LI-COR 6200 (LI-COR, Lincoln, NE, USA) was used to estimate the net CO_2 assimilation rate of *A. nummularia* and *A. leucoclada* under different water salinity and light intensity levels. The photosynthesis measurements were taken 4 and 8 weeks after the initiation of salt treatment.

CO_2- gas exchange of the second or third uppermost fully expanded leaves was measured at different levels of PPFD (0, 400, 800, 1200, 1600 and 2000 $\mu mol*m^{-2}*s^{-1}$) using a 200 W halogen light source. CO_2- gas exchange measurements were started after a pre-illumination of about 5 minutes for each light intensity level. The values of net photosynthesis and light intensities were then blotted to obtain the light-photosynthesis response curve for each species at each water salinity level. The photosynthetic efficiency (Φ_c), light saturation point (Ls) and the light compensation point (Lc) were estimated using SigmaPlot software according to Spilatro (1998). Net photosynthesis (A) ($\mu mol*m^{-2}*s^{-1}$), transpiration rate (E) ($mmol*m^{-2}*s^{-1}$), intercellular (C_i) and atmospheric (C_{an}) CO_2 concentrations (in ppm), stomatal resistance (R_s) ($s\ cm^{-1}$), and leaf and air temperature were determined (three different leaves from each plant and three plants from each treatment). Additionally, the ratio C_i/C_{an}, water use efficiency (WUE) as A/E, and the difference between leaf and air temperature (ΔT) were calculated. All measurements were taken at light intensity of 2000 $\mu mol*m^{-2}*s^{-1}$ (suggested to be the light saturation point for photosynthesis in both *Atriplex* species under the greenhouse conditions). All measurements were taken at atmospheric CO_2 concentration of 419.27 ± 29.85 ppm, 30.84 ± 0.67 ºC air temperature, and 35.7 ± 2.87% relative humidity. The photosynthesis measurements were achieved between 09:00 and 15:00 o'clock.

2.5 Determination of mineral elements

2.5.1 Determination of cation contents

Approximately 0.1 g of pulverized dried plant material from all organs (R, Sa, Sj, La and Lj) was ashed in a muffle furnace at 550 ºC for 12 h. Then the ashes of these organs were extracted with HNO_3 (32%) according to Steubing and Fangmeier (1992). The extractions and the bladder hair fractions (washing solution) were then diluted and the Na^+, K^+, Ca^{2+} and Mg^{2+} were measured in these extractions using an atomic absorption spectrophotometer (Perkin Elmer model PE 2100). The selective absorption ($SA_{K:\ Na}$) and the selective transport ($ST_{K:\ Na}$) capacity for K^+ over Na^+ was calculated according to the following equations:

$SA_{K:\ Na}$= (available Na^+/K^+ in the soil)/(Na^+/K^+ in the root) (Pitman, 1960).

$ST_{K:\ Na}$= (Na^+/K^+ in part A)/(Na^+/K^+ in part B) (Wang and Zhu, 1994).

2.5.2 Determination of anion contents

The concentrations of Cl^-, NO_3^-, SO_4^{2-}, PO_4^{-3}, malate, and oxalate in the press saps of the different plant organs (R, Sa, Sj, La and Lj) as well as in the bladder hair fractions were determined by using an ion chromatographic system (Meteohm AG, Herisau, Switzerland). This system consisted of ion chromatograph 690 equipped with a conductivity meter (detector), IC pump 697 and an autosampler. The press sap as well as the bladder hair fractions were diluted, and filtered through cellulose acetate filters of 0.45 µm pore size. The separation process was carried out using an IC anion column (Hamilito PRP-X100, 250 mm) based on a polystyrene-divinylbenzene copolymer. P-Hydroxybenzoid acid/Benzoat solution (2.5 mol*m^{-3} p- Hydroxybenzoid acid, 1 mol*m^{-3} Sodiumbenzoat, 2.5 % methanol, pH 8.5, 300 µS/cm conductivity) was used as an eluent. The analytical conditions were as follows: flow rate 2 ml/min, maximum pressure 34 MPa, injection volume 100 µl.

2.6 CNS analysis

About 2 – 3 g of the R, La and Lj fresh materials from each *Atriplex* species were harvested, directly freeze-dried, and ground into a fine powder. Carbon (C), nitrogen (N), and sulphate (S) contents of these organs were determined using a

Vario MAX CNS analyser (Elementar Analyse system, GmbH, Germany). L-Glutamic acid (C and N), and Sulphanylamid (S) were used as standards.

2.7 Determination of free amino acid (FAA) contents

2.7.1 Amino acids extraction

Initially, all chemicals used in the extraction of the amino acids were of HPLC grade, and purchased from Merck (Darmstadt, Germany). The amino acids were extracted according to Högy (2002). Samples of about 0.1 g of the freeze-dried plant materials (R, La, Lj) were mixed in centrifugation tubes with 8.8 ml 4% 5-sulfosalicylic acid dihydrate containing 0.0284 $mol*m^{-3}$ β-2-thienyl-DL-alanine, and homogenized with an ultra homogenizer for 40 sec. The samples were kept on ice for about 1 h, and then 1.2 ml of NaOH 2 M was added to each sample. After that the samples were centrifuged at 20000 X g at 4 ºC for 10 min, and the supernatants were collected, and filtered through 0.45 µm cellulose-acetate filter. The extractions were then diluted (1:10 v/v) with 1M borate buffer, pH 8.5, and stored at -20 ºC for further analysis.

2.7.2 Reversed-phase high performance liquid chromatography (RP-HPLC)

The extractions were derivatized with 9-fluoroenylmethyl chloroformate (FMOC-Cl) according to Einarsson *et al*. (1983). Free amino acids were determined by using a reversed-phase high performance liquid chromatograph (Varian, USA) equipped with a Varian autosampler model 410 and a Varian 210 pump. The amino acids were separated using an analytical ODS column (Aminotag 80 TM, 150 x 4.6 mm) protected by a PR-8 guard column (10 x 3.2 mm). The column temperature was set to 33 °C. The solvent system consisted of 0.015 M sodium citrate dihydrate plus 0.01 M tetramethylammonium chloride, pH 3.84 (A), and acetonitrile (B). The flow rate was set to 1.4 ml/min, and the injection volume was 10 µl. The derivatives were analysed using a HPLC fluorescence detector (Shimadzu RF-535) with a wavelength of 260 nm as excitation and 310 nm as emission wavelength

2.8 Determination of total soluble carbohydrate (TSC) contents

The total soluble carbohydrate (TSC) contents in the press sap of all plant organs (R, Sa, Sj, La and Lj) of the studied *Atriplex* species were assayed photometrically according to Kleber *et al*. (1987) and Volk (1996).

2.9 Determination of total soluble protein (TSP) contents

The total soluble protein (TSP) contents in the press sap of all plant organs (R, Sa, Sj, La and Lj) of both *Atriplex* species were determined photometerically according to Bradford (1976).

2.10 Two-dimensional polyacrylamide gel electrophoresis (2D-PAGE)

All chemicals used in the protein extraction, and gel electrophoresis were of the highest obtainable grade. At harvest time, the third and fourth uppermost fully expanded leaves of three randomly selected *A. nummularia* and *A. leucoclada* plants were collected from the control, and high salt treatment (750 mol*m^{-3} NaCl). The leaves were ground immediately to a fine powder in liquid nitrogen, and stored at -80 ºC until use. The proteins were extracted according to Zörb *et al*. (2004), and the protein content of these extractions (supernatants) was determined by the Bradford assay (1976).

The first dimension electrophoresis (Isoelectric focusing) was carried out according to Westermeier and Naven (2002), and Zörb *et al*. (2004). Isoelectric focusing was performed with the IPGphor system (Amersham Pharmacia Biosciences, UK) using Immobiline Dry gel strips (11 cm length) with linear pH gradients 3 – 10 (Amersham Pharmacia Biosciences, UK). For each dry strip gel, a sample of 200 µl protein extraction plus lysis solution buffer (8 M urea, 4% CHAPS, 40 mol*m^{-3} Tris base, and a trace of bromophenol blue) (protein concentration of 150 µg) was prepared. The IEF was started under the following conditions: 10 h rehydration; then 100 V for 2h; 500 V for 1 h; 1000 V for 1 h; and finally 8000 V for 5 h at 20 ºC and 50 µA for each strip. The focused IPG strips were then equilibrated with 5 ml equilibration buffer (A) [50 mol*m^{-3} Tris-Cl pH 8.8, 6 M urea, 30% (v/v) glycerol (87%), 2% (w/v) SDS, 1% (w/v) DTT, and a trace of bromophenol blue] at room temperature for 15 min. The alkylation of the reduced

room temperature for 15 min. The alkylation of the reduced proteins was conducted in 5 ml of the same equilibration buffer for 15 min using 2.5% (w/v) iodoacetamide instead of DTT. The strips were then rinsed with SDS electrophoresis running buffer (5 mol*m^{-3} Tris, 38.4 mol*m^{-3} glycine, 0.1% SDS) for a few seconds.

The second dimension was performed according to Schägger and von Jagow (1987), and Zörb *et al*. (2004). Each IPG strip was placed on top of a vertical in-house made 12.5% polyacrylamide-SDS gel (20x18x0.1 cm). Molecular weight standards (Rainbow, Amersham Pharmacia Biotech, UK) in the range of 14 – 220 KDa were used. About 5 µl 1:10 diluted protein marker was positioned on the acidic side of each gel strip. The IPG strip and the marker were then sealed with 1% w/v agarose containing traces of bromophenol blue. The second dimension was run at 4 ºC using 15 mA per gel for 15 min followed by 45 mA per gel for about 3 – 4 h. The gels were then fixed in a solution of 50% Ethanol and 10% glacial acetic acid for 1 h. The electrophoretically separated proteins were visualized by immersing the gels in Coomassie Brilliant Blue (50% Ethanol, 10% glacial acetic acid, and 0.05% Coomassie Brilliant Blue R 150) overnight, and then destained with glacial acetic acid 10% for 4 h.

The Coomassie stained gels were scanned, and the protein spots of the scanned images were counted using Delta 2D Software (DECODON GmbH, Germany). To compare the protein profiles of the control with the salt treated leaves, the master gel (control) was warped with that of the salt treated ones using the Delta 2D programme after setting specific vector points. Then dual channel images of the 2D gel were obtained. The optical density of each spot in the dual channel images was determined as percentage of the total polypeptide-associated optical density using the quantitation tool in the Delta 2D software. The experimental pI and MW of the differentially regulated protein spots were used for the identification using the public databases SWISS-PROT, TrEMBL and NCBInr (http://www.expasy.com).

2.11 Mass spectrometry

2.11.1 In-gel tryptic digestion and preparation of samples

The selected protein spots were manually excised from the Coomassie stained 2D-gels, and submitted for peptide mass fingerprinting. They underwent in-gel digestion with trypsin according to the published methods of Shevchenko *et al*. (1996). The excised spots were destained with 75 µl of (30% ethanol, and 70% glacial acetic acid). The gel pieces were dehydrated in 30 µl 100% acetonitrile (CH_3CN) for approximately 10 min, and then dried in a SpeedVac (Fa. Thermo Electron, Dreieich). Each sample was incubated in 150 µl of (10 mol*m^{-3} DTT plus 0.1 M NH_4HCO_3) at 55 ºC for 1 h, and was then derivatized with 150 µl (55 mol*m^{-3} iodoacetamide and 100 mol*m^{-3} NH_4HCO_3) at room temperature in the dark for 45 min. Subsequently, each sample was washed successively with 30 µl 0.1 M NH_4HCO_3 for 30 min, 30 µl 100% acetonitrile for 10 min, 30 µl 0.1 M NH_4HCO_3 for 10 min, and finally dehydrated with 30 µl 100% acetonitrile for 10 min. Afterward, the samples were completely dried in the SpeedVac. The gel pieces were then digested with trypsin (trypsin sequence grade, Roche Diagnostics, Penzberg, Germany). For each sample, 30 µL trypsin solution 12.5 ng/µL in 50 mol*m^{-3} NH_4HCO_3 was added, and the digestion was performed at 37°C overnight. The resulting peptides were obtained by successive extraction of the digested gel pieces. Firstly, the samples were centrifuged for 2 min, and the peptide-containing supernatants were collected (S1) in a new microtube. The gel pieces were extracted again with 30 µl of Ethanol 50% plus 0.1% trifluoroacetic acid (1:1v/v) for 10 min, centrifuged for 2 min. Then the supernatants were collected, and pooled together with those of S1. Finally, the gel pieces were extracted with 30 µl solution of [acetonitrile 100% and NH_4HCO_3 20 mol*m^{-3} in water] (1:1 v/v) for 10 min and centrifuged for 2 min to obtain the S2.

2.11.2 Mass Spectrometric Analysis and protein identification

Mass analysis of the proteolytic digestions was carried out at the “Institut für Anorganische und Analytische Chemie”, Justus-Liebig-University, Giessen. The analysis was achieved using a home-built MALDI-TOF mass spectrometer (ALADIM II) (Advanced Laser Desorption Ionization Mass Analyzer). The peptide extractions were mixed with the matrix solution [10 mg/ml 2,5-hydroxybenzoic acid

in 0.1% (v/v) trifluoroacetic acid, 30% (v/v) acetonitril]. The mixtures were applied to sample targets using micro-pipettes, and were then dried by a stream of warm air. The ionization was performed with a pulsed nitrogen laser (LSI- NSD, wavelength 337 nm, 3 ns pulse duration, 200 µJ per laser pulse). The mass spectra were recorded by summing up the responses of at least 100 individual laser shots. The typical mass resolving power was between 5000 and 7000. Peptide mass standards (substance p, Mellitin, Insulin) were used to calibrate the mass spectrometer. MALDI spectra were acquired by the ULISSES software package (version. 8. 2, copyright Bernhard Spengler, 1985 - 2000). Peak lists of the tryptic peptide masses were searched against the public protein databases of Mascot (http://www.matrixscience.com), ProFound (http://www.proteometrics.com) and Aldente (http://www.expasy.org/cgi-bin/aldente/form.cgi).

2.12 Structural investigations

2.12.1 Light and Transmission Electron Microscopy

Representative specimens of the shoot tips (including immature leaves) and juvenile fully expanded leaves of untreated control and NaCl treated (750 mol*m^{-3}) plants were collected from both *A. nummularia* and *A. leucoclada* at the harvest time. Several small segments (about 1 mm^2) of these organs were pre-fixed in an ice-cold fixative solution. The fixative consisted of 1% (v/v) formaldehyde and 2% (v/v) glutaraldehyde in 50 mol*m^{-3} pipes-buffer (pH 6.8). Since the osmosis of the cell is still active during the aldehyde fixation, sucrose was used as an additive in the fixative to form a weak hypotonic solution in accordance with the osmotic potential of the leaves to minimize the osmotic shock that might be able to disturb the cellular and the ultra cellular structures. The plant materials were degassed, and further fixed at 4 ºC for 4 h. The specimens were then washed three times (15 minutes each) in osmotically adjusted pipes-buffer (pH 6.8), and post-fixed for 4 hours with 1% (w/v) osmium tetroxide solution in the same buffer in a fume hood (Harlay and Fergusen, 1990). After having been washed 4 - 5 times with double distilled water, the specimens were dehydrated in graded series of acetone (5, 15, 30, 50, 70, 80, 90, 95 and 100%). The samples were embedded in Spurr's resin (1969) and polymerised at 70 ºC for 12 h.

The embedded materials were sectioned with a glass knife for the light microscopy investigations using an ultra microtome (Ultracut E, Reichert Jung). The semi-thin (2 µm) cross sections were stained with methylene blue (in 2% Ethanol) on a heating plate for 1 min. The leaf cross sections were examined with an Olympus AX 70 light microscope, and photographed with an Olympus C-35A D-2 digital camera on the same microscope. The measurements and the image analysis were done using an image analysis programme (Soft Imaging System) on a PC.

In order to study the ultrastructural changes in the leaves after NaCl treatment, thin sections (5–10 nm) were cut with a glass knife on an ultra microtome (Ultracut E, Reichert Jung). The sections were mounted on 200–300 mesh copper grids. They were then stained with 2% uranyl acetate (saturated solution in 60% Ethanol) for 8 min, and after that with lead citrate (2% in 0.2 M NaOH) (Reynolds, 1963) for 5 min. The sections were examined, and photographed using a LEO-912-AB-OMEGA (Zeis-Leica) Transmission Electron Microscope.

2.12.2 Scanning Electron Microscopy

For a closer examination of the leaf surfaces, scanning electron microscope investigations were carried out. Specimens of adult and juvenile leaves of the two studied *Atriplex* species were fixed in glutaraldhyde 2.5% in 50 mol*m^{-3} pipes-buffer (pH 6.8) at 4 ºC for 4 h. After washing them 3 - 4 times with the same buffer, the specimens were dehydrated with ascending concentrations of alcohol (5, 15, 30, 50, 70, 80, 90, 95 and 100%). They were then dried after the critical point method (Hall *et al*., 1978), and mounted onto aluminium stubs, and sputtered with a thin gold layer. The samples were examined, and photographed using a Philips XL-20 Scanning Electron with an accelerating voltage of 5 - 15 kV, and a working distance of 11 – 13 mm.

2.13 Energy dispersive X-ray microanalysis

Small fresh segments of the adult and juvenile leaves from the control and high salt treated *A. nummularia* and *A. leucoclada* plants were vacuum dried for about 10 minutes. Subsequently a thin gold layer was sputtered on the surfaces of the specimens using Hummer 6.2. Sputter coater. After the transfer of the specimens into a Philips XL-20 Scanning Electron Microscope (SEM), Energy disper-

sive X-ray-microanalyses (EDXA) were performed on the upper (adaxial) and lower (abaxial) leaf epidermal cells, the stomatal guard cells, and the bladder cells according to Koyro (1997). The analysis was conducted with a KEVEX-Si/Li-detector with Beryllium window (500 V). Typical analytical conditions were as follows: working distance, 11-13 mm; magnification 500 X; analysis area 10 µm; beam (sample) current, 150 µA; accelerating voltage, 15 kV; and counting time of 100 seconds. For each sample, three replicates were measured. The calculation and correction was made using EDAX DX-4 eDXi system version 3.02. Only the K_α values of each element were taken, and the ZAF correction procedure (where Z is the correction due to the atomic number of the matrix, A is the photoelectric absorption factor of x-rays in the specimen, and F is the fluorescence correction factor) was applied to convert the characteristic peak intensities into weight and atomic fractions.

2.14 Statistical analysis

The results of this study are presented as the means of nine replicates (three cultures each species, and three replicates each treatment) ± standard deviations (SD). All data sets were analysed with one-way-ANOVA using the SPSS for Windows statistical data analysis package (SPSS Inc., 2002, release 11, Chicago, IL, USA) in order to determine if significant differences were found among means. The LSD test was employed to determine if significant ($P< 0.05$) differences occurred between individual treatments and organs. In some cases, and in order to meet all assumptions for ANOVA, a Log^{10} transformation was performed when the original data were not distributed normally.

3 RESULTS

3.1 Effect of salinity on the growth and development

3.1.1 Visual observations

Comparative growth responses of *A. nummularia* and *A. leucoclada* plants to varying water salinity levels (0, 125, 250, 500 and 750 mol*m^{-3} NaCl) (13 weeks treatment) are illustrated in Figures 2 a and b respectively (see also appendix, Fig. A1). Variations between the different salinity treatments in both species were evident four to five weeks after the commencement of salinity treatment. As shown in Fig. 2a, the absence of NaCl in the nutrient solution led to a distinct reduction in the growth (shoots and roots) of *A. nummularia* control plants. The plants were thin with a small number of branches and leaves. Increasing NaCl salinity generally stimulated the growth of plants relative to the controls. The optimal plant growth and development was reached at low and moderate salinities (125–250 mol*m^{-3} NaCl). The growth stimulation at low and moderate salinities was accompanied by increases in leaf numbers, area and succulence, plant height, branching, shoot and root weight and length. The leaves of these plants appeared light green to yellowish green (Fig. 3b). *A. nummularia* plants showed a healthy habitus and a conspicuous growth even under high water salinity (750 mol*m^{-3} NaCl), where their growth (shoot and root) was still higher as in the controls. At this salinity, the plants appeared intensely blue-green with succulent leaves (Fig 3c).

A. leucoclada control plants grew normally with some signs of Na^+ deficiency (chlorosis) in their leaves especially the adults (Fig. 2b and 4a). Increasing water salinity up to 500 mol*m^{-3} NaCl slightly (but not significantly at $P < 0.05$) enhanced the plant growth with a maximum at 250 mol*m^{-3} NaCl. High NaCl concentrations adversely affected the growth (shoot and root) of *A. leucoclada* plants, with more severe effect in comparison to *A. nummularia* plants. The plants were dwarf with shallow root system and few branches and leaf numbers. The leaves were also very small, thick and light green in color with visible Na^+ toxicity symptoms i.e. burning of the leaf tips and margins (Fig. 4c).

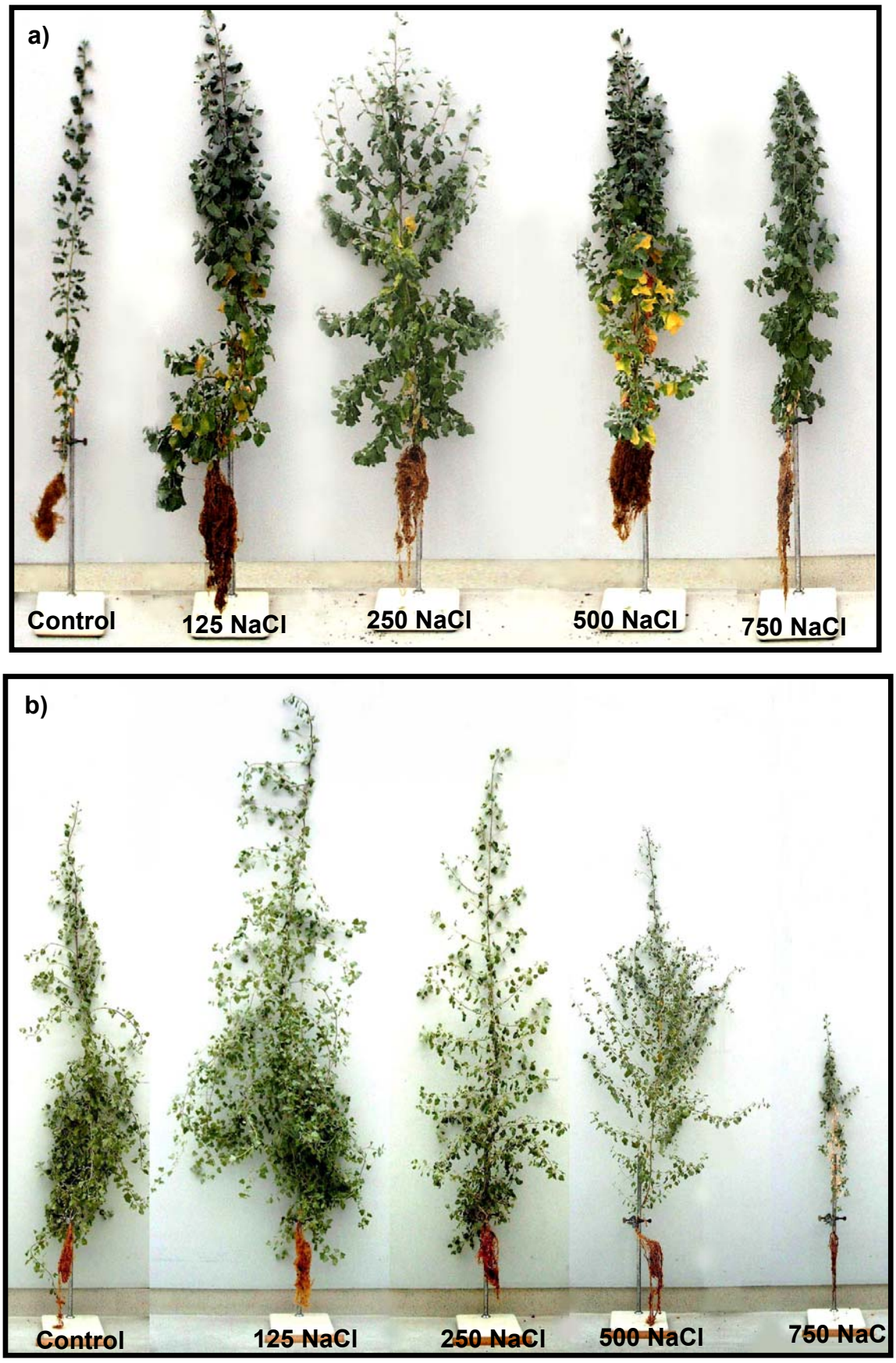

Fig. 2: Effect of different water salinity levels on the growth and development of a) *A. nummularia* and b) *A. leucoclada* plants after13 weeks of salinity treatments.

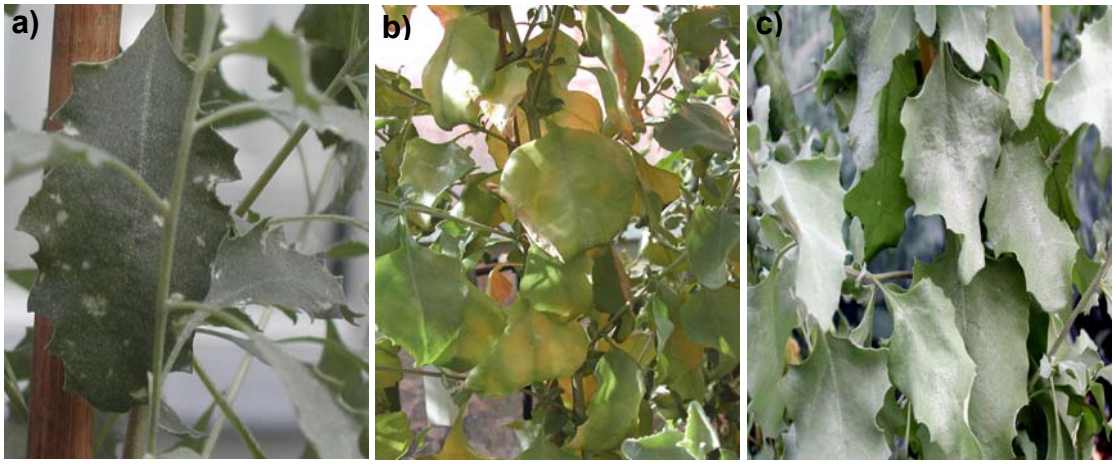

Fig. 3: The appearance of *A. nummularia* leaves as affected by different salinities; a) under control conditions; b) 125 and c) 750 mol*m^{-3} NaCl.

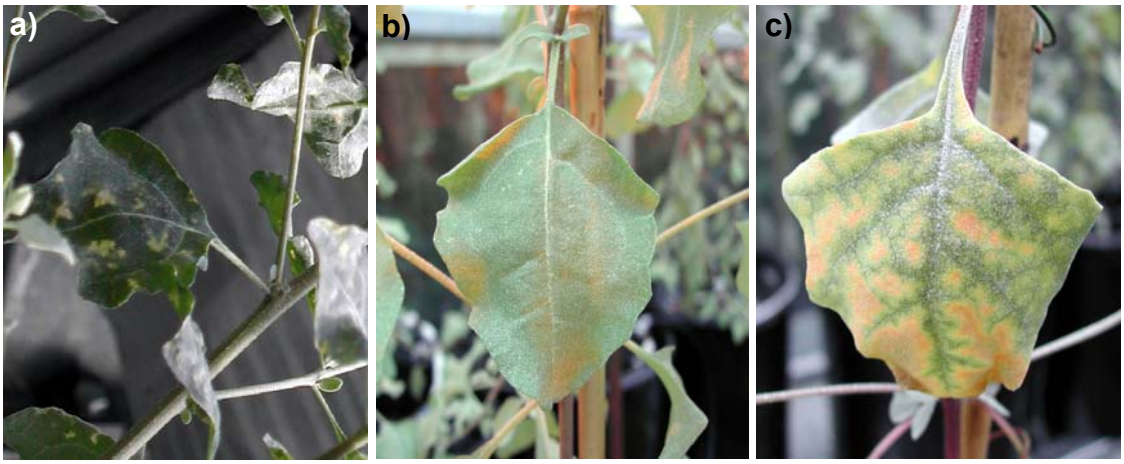

Fig. 4: The appearance of *A. leucoclada* leaves as affected by different salinities; a) under control conditions; b) 125 and c) 750 mol*m^{-3} NaCl.

3.1.2 Growth parameters

3.1.2.1 Fresh weight and biomass production

The effects of different water salinity levels on the growth (expressed as fresh weight in grams) and development of different *A. nummularia* plant organs (R, Sa, Sj, La and Lj) are shown in Fig. (5a). The general tendency was that increasing water salinity induced a progressive increase in the growth of the different plant organs with optimal growth at low to moderate salinity (125 – 250 mol*m^{-3} NaCl). Significant (P< 0.05) increases of about 550% and 111% in the plant fresh weight (PFW) were observed at moderate and high salinity, respectively, relative to the control plants (see appendix, Table A1). This increase in plant fresh weight was mainly caused by increase in the shoot fresh weight (ShFW) rather than in the root fresh weight (RFW) especially at the full strength salinity. Additionally, increasing salinity markedly increased the leaves fresh weight (LFW) than the stem fresh weight (SFW) even at high salinity level. Consequently, the shoot/root fresh weight ratio increased significantly with raising NaCl salinity level, with maximum values at moderate salinity (see appendix, Table 1). As shown in Fig. (5a), salinity threshold of *A. nummularia* plants (initial significant reduction in the maximum expected yield, Shannon and Grieve, 1999) was reached at 50% SWS and the C_{50} value at 140% SWS.

The mean plant fresh weight of *A. leucoclada* controls was higher than that of *A. nummularia* control plants, reached 261.9 ± 50.5 g. As can be seen in Fig. (5b), *A. leucoclada* plants did not show any significant (P< 0.05) increase in all plant organs fresh weight up to 500 mol*m^{-3} NaCl salinity with one exception: low to moderate salinity led to a significant increase in the leaves fresh weight (see appendix, Table A2). High salinity treatments dramatically reduced the fresh weight of all plant organs (see appendix, Table A2). No significant increases were observed in the shoot/root fresh weight ratio with raising water salinity (see appendix, Table A2). Fig. (5b) reveals that the salinity threshold of *A. leucoclada* was reached at 50% and the C_{50}-value at 114% SWS.

3.1.2.2 Leaf number, area and leaf mass to area (LMA) ratio

The average leaf number per plant of *A. nummularia* controls was about 25.2 ± 4.2 and 328.9 ± 100.4 for the adult and juvenile leaves, respectively. As shown in

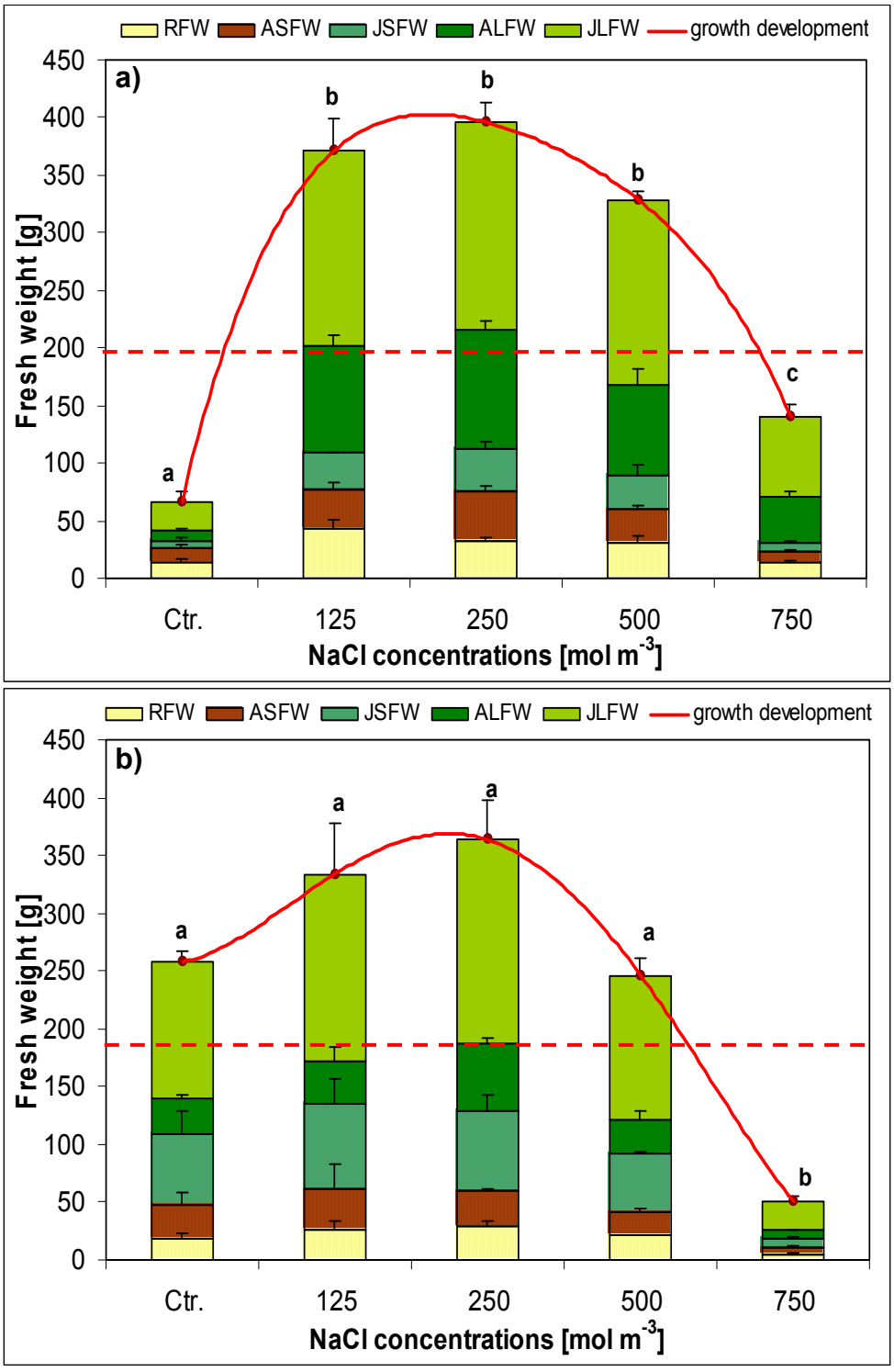

Fig. 5: Development and growth responses of the different organs (expressed as fresh weights) of a) *A. nummularia* and b) *A. leucoclada* plants at different NaCl salinities. The dotted red lines mark the C_{50} values. RFW, root; ASFW, adult stems; JSFW, juvenile stems; ALFW, adult leaves; JLFW, juvenile leaves. Each column represents the mean values of nine replicates and the bars represent standard errors. Columns with the same letter are not significantly different at $P < 0.05$, LSD test.

Table (2a), NaCl salinity led to a transient increase in the adult and juvenile leaf numbers with maximum increase at moderate salinity (250 mol*m^{-3} NaCl). *A. leucoclada* control plants had significant (P< 0.05) more adult and juvenile leaves (83.8 ± 1.4 and 1297.0 ± 125.8 respectively) per plant than *A. nummularia.* The leaf number per plant increased slightly with increasing water salinity up to 250 mol*m^{-3} NaCl. Unlike *A. nummularia*, high salinity level led to significant reductions in leaf number per plant in *A. leucoclada* and caused about 46 and 61% reductions in the adult and juvenile leaf number pre plant respectively relative to controls (Table 2b).

The surface area per leaf (SAL) of the adult leaves was significantly (P< 0.05) higher than that of the juvenile ones in both *Atriplex* species and at all salinity treatments (Table 2a, b). In *A. nummularia*, increasing water salinity transiently increased the SAL of both adult and juvenile leaves. Highest SAL was recorded at low (for adult leaves) and moderate (for juvenile leaves) water salinity. Further increase in the water salinity lowered the (SAL) of both adult and juvenile leaves with more adverse effect on the juvenile leaves. In *A. leucoclada*, SAL of both adult and juvenile leaves was comparatively lower than *A. nummulara* for all salinity treatments. It declined gradually for both adult and juvenile leaves as the salinity increased in the external nutrient solution (Table 2b).

The leaf mass to area (LMA) ratio of the adult leaves was more or less equal to that of juvenile ones in both *Atriplex* species under control conditions (Table 2a, b). In both *Atriplex* species and for both adult and juvenile leaves, LMA ratio increased significantly as the salinity level increased, the effect was more obvious in the adult leaves, The juvenile leaves of *A. nummularia*, however, showed significant (P< 0.05) higher LMA ratio than those of *A. leucoclada* at the whole range of salinities (Table 2a, b).

3.1.2.3 Dry weight

Under control conditions, *A. nummularia* plants had comparatively higher dry weight in % fresh weight (DW in % FW) for all organs than *A. leucoclada* except in the case of the root (Fig. 6 a and b). On average over all the plant organs, the DW in % FW ranged from 16.6 ± 2.4 to 36.6 ± 2.3 % in *A. nummularia* and from 9.2 ± 1.8 to 34.6 ± 1.9 % in *A. leucoclada*. In *A. nummularia*, a general trend of transient decrease in DW in % FW of all plant organs was observed as the salinity rose, with

Table 2a: Influence of different water salinity levels on the leaf number per plant, surface area per leaf (SAL) and leaf mass to area ratio (LMA) of *A. nummularia*. La, adult leaves; Lj, juvenile leaves.

Treatments	Leaf number*plant^{-1}(n)		SAL (cm^2)		LMA (mg*cm^{-2})	
	La	Lj	La	Lj	La	Lj
Control	25.22^a ± 4.22	328.89^a ± 100.41	54.04^a ± 8.19	29.00^a ± 9.67	30.68^a ± 3.55	30.19^a ± 3.11
125 NaCl	117.33^b ± 13.65	1153.11^b ± 150.84	139.85^b ± 18.65	44.16^b ± 9.43	40.04^b ± 2.03	37.22^b ± 5.50
250 NaCl	127.00^b ± 16.25	1338.11^b ± 277.04	81.20^c ± 19.08	44.21^b ± 9.33	46.06^{bc} ± 2.58	41.89^b ± 3.13
500 NaCl	103.33^b ± 7.57	1101.56^b ± 211.08	76.06^{ac} ± 13.85	34.67^{ab} ± 9.75	48.98^{bc} ± 5.46	42.66^b ± 5.62
750 NaCl	54.67^c ± 12.98	455.56^a ± 94.68	61.88^{ac} ± 10.45	31.43^a ± 8.43	56.74^c ± 5.20	42.05^b ± 4.87

Each value represents the mean of nine replicates. Means within a column followed by the same letter are not significantly different at $P< 0.05$ as determined by LSD test.

Table 2b: Influence of different water salinity levels on the leaf number per plant, surface area per leaf (SAL) and leaf mass to area ratio (LMA) of *A. leucoclada*. La, adult leaves; Lj, juvenile leaves.

Treatments	Leaf number*plant^{-1}(n)		SAL (cm^2)		LMA (mg*cm^{-2})	
	La	Lj	La	Lj	La	Lj
Control	83.83^a ± 1.44	1297.00^a ± 125.80	64.75^a ± 3.93	22.18^a ± 9.47	32.86^a ± 4.45	27.30^a ± 1.48
125 NaCl	93.78^a ± 26.59	2044.56^b ± 438.64	61.42^{ab} ± 6.40	21.53^a ± 5.78	46.49^b ± 3.23	28.86^a ±1.96
250 NaCl	109.78^a ± 5.85	2071.11^b ± 353.22	51.39^b ± 8.83	17.01^{ab} ± 6.21	51.74^{bc} ± 2.25	32.68^b ±3.01
500 NaCl	84.78^a ± 4.30	1560.89^{ac} ± 134.40	35.58^c ± 6.04	14.18^{ab} ± 3.98	51.40^{bc} ± 2.98	32.92^b ± 3.06
750 NaCl	45.89^b ± 6.11	512.67^c ± 55.34	27.12^c ± 5.16	8.39^b ± 4.23	53.99^c ± 3.19	35.23^b ± 1.06

Each value represents the mean of nine replicates. Means within a column followed by the same letter are not significantly different at $P < 0.05$ as determined by LSD test.

maximum reductions at low and moderate salinities (Fig. 6a). High salinity treatment did not significantly affect the DW in % FW of the adult and juvenile stems but caused reductions of about 35, 20, and 7% in the DW in % FW of La, Lj, and R relative to controls.

The same pattern of a transient decrease in the DW in % FW of all plant organs was observed for *A. leucoclada* (Fig. 6b). Low and moderate salinities did not significantly affect the DW in % FW of the adult and juvenile leaves and roots but significantly lowered that of adult and juvenile stems relative to the controls. Unlike *A. nummularia*, high NaCl concentration significantly increased the DW in % FW of the different *A. leucoclada* plant organs (except for the adult stem which was relatively less affected) and caused about 40, 30, 60 and 7% increases in La, Lj, Sj and R relative to the controls.

3.1.2.4 Dry matter

Fig. 7 (a and b) show that the dry matter content in % dry weight (DM in % DW) of all *A. nummularia* plant organs was significantly higher than those of *A. leucoclada* under control conditions (except for the adult stems). On average over all different plant organs, the DM in % DW ranged between 84.4 and 96.3% in *A. nummularia* and between 73.8 and 96.6% in *A. leucoclada*, with highest values in adult stems of both species. Increasing salinity level caused remarkably decreases in the DM in % DW in all plant organs of both *Atriplex* species relative to controls, with more adverse effect on *A. leucoclada*, especially at the highest salinity level (Fig. 7 a and b). At this salinity level, *A. nummularia* showed reductions of about 25, 10, 6, 6 and 4% in the DM in % DW of La, Lj, Sa, Sj and R respectively, while these reductions were 20, 15, 8, 14, 18% in *A. leucoclada*.

3.1.2.5 Ash weight

Ash contents (AW in % DW) of the different plant organs of *A. nummularia* were lower as in *A. leucoclada* at all salinity treatments (Fig. 8 a and b). In both *Atriplex* species, the adult leaves had generally the highest AW in % DW being 16.3 ± 2.7% and 26.2 ± 2.5% in *A. nummularia* and *A. leucoclada* respectively while the adult stems showed the lowest (about 3% for both species). There was a correlation between NaCl concentration in the nutrient solution and AW in % DW of the different

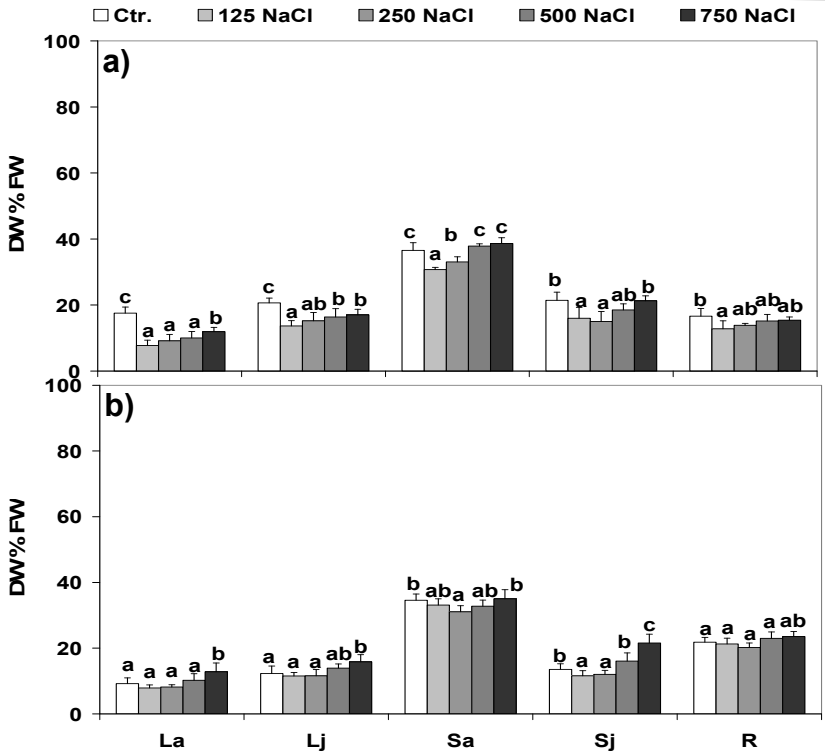

Fig. 6: Plant organs dry weight in % fresh weight (DW in %FW) of a) *A. nummularia* and b) *A. leucoclada* at different water salinities. La, adult leaves; Lj, juvenile leaves; Sa, adult stems; Sj, juvenile stems; R, roots. Each column represents the mean values of nine replicates and the bars represent standard errors. Columns with the same letter are not significantly different at $P< 0.05$, LSD test.

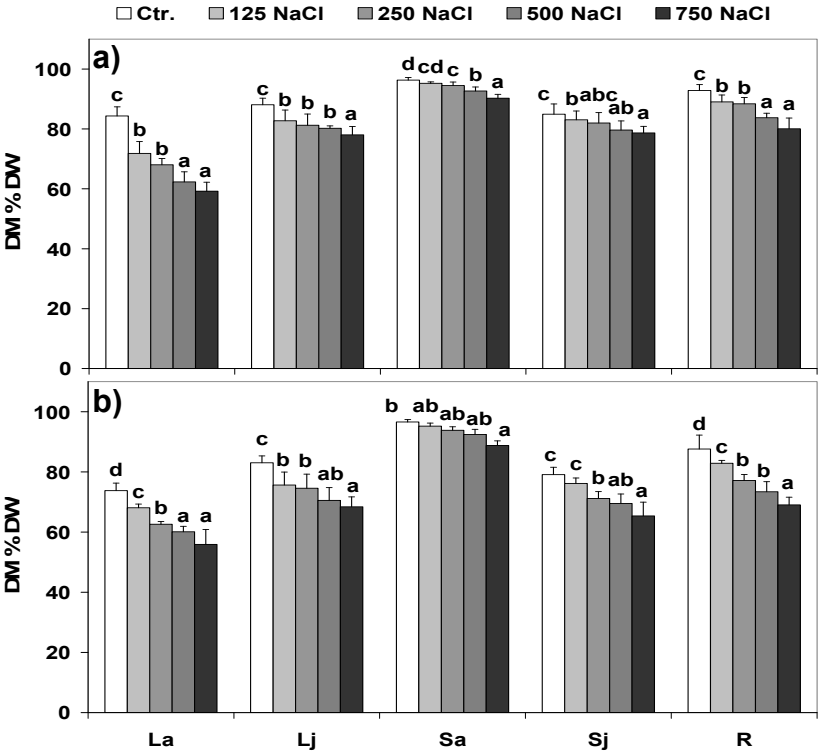

Fig. 7: Dry matter content in % dry weight (DM in % DW) of the different plant organs of a) *A. nummularia* and b) *A. leucoclada* as affected by different water salinities. La, adult leaves; Lj, juvenile leaves; Sa, adult stems; Sj, juvenile stems; R, roots. Each column represents the mean values of nine replicates and the bars represent standard errors. Columns with the same letter are not significantly different at $P< 0.05$, LSD test.

plant organs of both *Atriplex* species (Fig. 8 a and b). At high water salinity level, the AW in % DW of the adult leaves was highest, being 38.8 ± 1.9 and 41.9 ± 2.8% in *A. nummularia* and *A. leucoclada* respectively, whereas that of the adult stem was lowest, being 9.8 ± 1.3 and 11.2 ± 1.6% in *A. nummularia* and *A. leucoclada* respectively.

3.2 Effect of salinity on the water relations

3.2.1 Water contents

On average over the different organs, water content (H_2O in % FW) of *A. nummularia* control plants ranged between 63.4 ± 2.3 (adult stem) and 83.4 ± 2.4 (root). Transient increases in the water contents of all plant organs were observed as the NaCl concentration in the external nutrient solution increased (Fig. 9a). The maximum water contents, ranged between 69.3% (adult stems) and 92.3% (adult leaves) were reached at low to moderate salinities. Further increase in salinity level reduced the water contents of all plant organs (relative to the plants grown at moderate salinity) (Fig. 9a). High salinity treatment slightly increased the water contents of the adult and juvenile leaves, and did not significantly affect that of adult and juvenile stems relative to controls. The root water content was relatively less affected by increasing water salinity level (Fig. 9a).

A. leucoclada controls had comparatively higher water content than *A. nummularia*, ranging from 65.4% (adult stem) to 90.8% (adult leaves). Low to moderate salinities resulted in slight increases in the water contents of all *A. leucoclada* plant organs (Fig. 9b). Unlike *A. nummularia,* high water salinity level significantly reduced the water contents of adult and juvenile leaves and juvenile stems but did not affect those of adult stems and root (Fig. 9b).

3.2.2 Water potential

Water potentials of the control plants (ψ_s) (determined in the young shoots using the Scholander pressure pump) were -0.17 ± 0.04 and -0.17 ± 0.06 MPa in *A. nummularia* and *A. leucoclada* respectively. Fig. 10 (a and b) showed that both plants lowered the (ψ_s) of their juvenile shoots gradually and significantly as the salinity in the external solution increased. At the highest salinity treatment, the

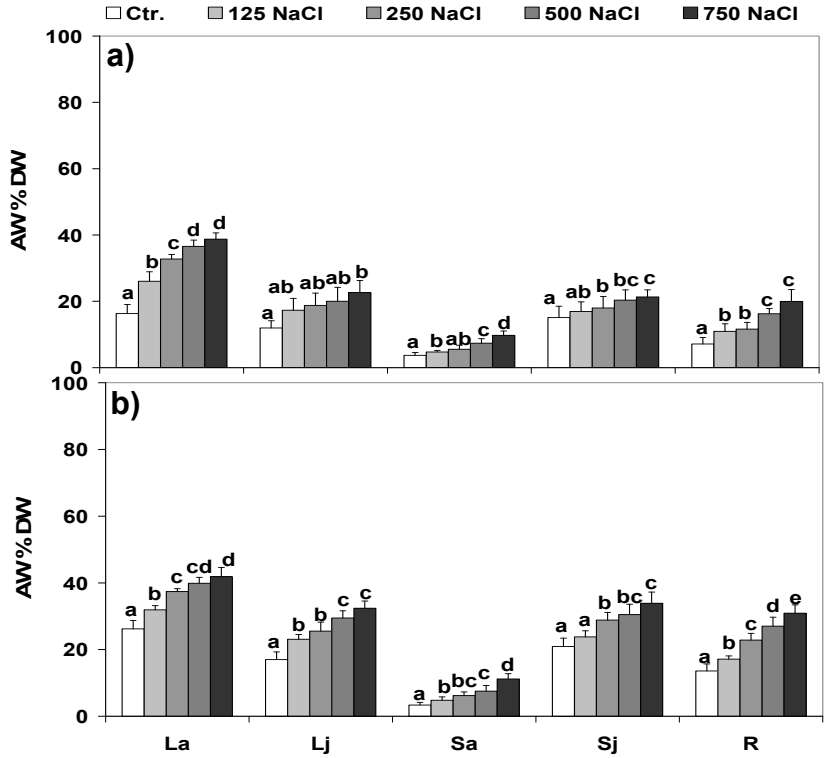

Fig. 8: Ash weight in % dry weight (AW in % DW) of the different plant organs of a) *A. nummularia* and b) *A. leucoclada* as affected by different water salinities. La, adult leaves; Lj, juvenile leaves; Sa, adult stems; Sj, juvenile stems; R, roots. Each column represents the mean values of nine replicates and the bars represent standard errors. Columns with the same letter are not significantly different at $P< 0.05$, LSD test.

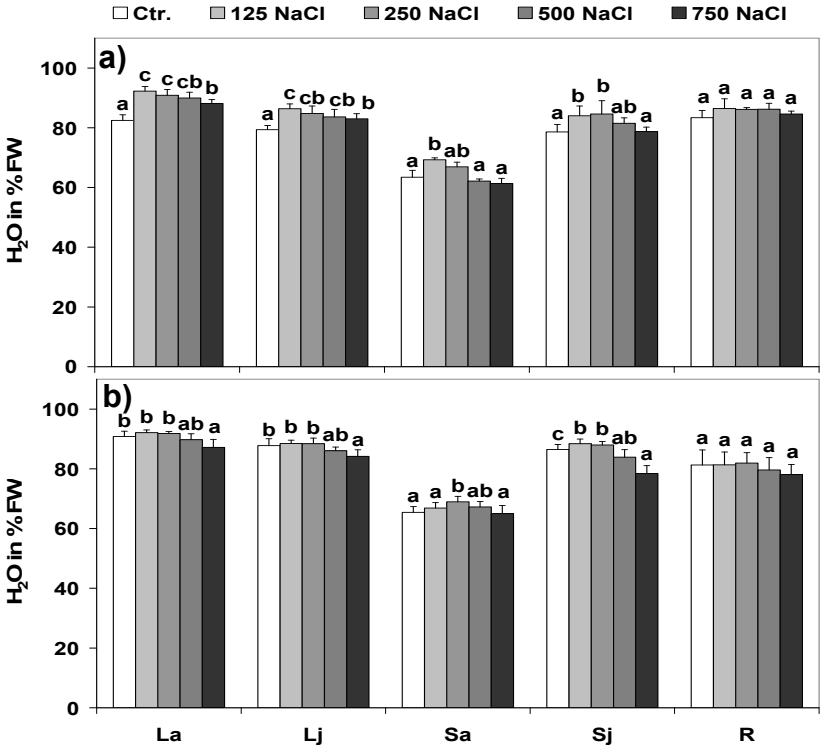

Fig. 9: Effect of various NaCl salinity levels on the water contents (H_2O in % FW) of the different plant organs of a), *A. nummularia* and b), *A. leucoclada*. La, adult leaves; Lj, juvenile leaves; Sa, adult stems; Sj, juvenile stems; R, roots. Each column represents the mean values of nine replicates and the bars represent standard errors. Columns with the same letter are not significantly different at $P< 0.05$, LSD test.

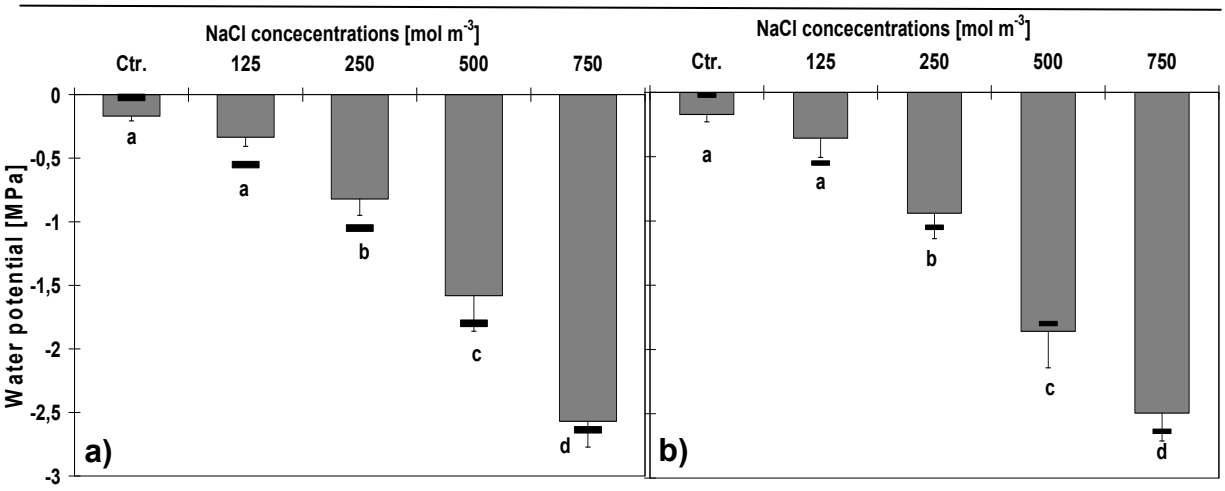

Fig. 10: Water potentials of the juvenile shoots of *A. nummularia* (a) and *A. leucoclada* (b) under elevated NaCl concentrations. The black lines show the substrate water potentials. Each column represents the mean values of nine replicates and the bars represent standard errors. Columns with the same letter are not significantly different at $P< 0.05$, LSD test.

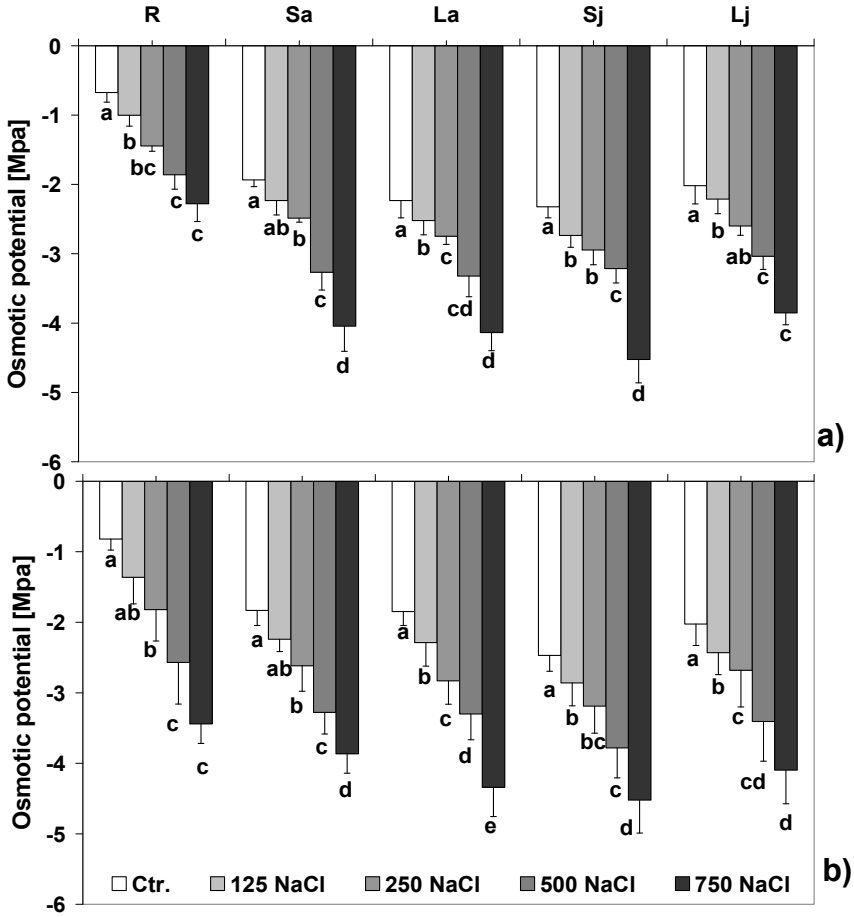

Fig. 11: Osmotic potential (in press sap) of the different organs of *A. nummularia* a) and *A. leucoclada* b) at different water salinity levels. La, adult leaves; Lj, juvenile leaves; Sa, adult stems; Sj, juvenile stems; R, roots. Each column represents the mean values of nine replicates and the bars represent standard errors. Columns with the same letter are not significantly different at $P< 0.05$, LSD test.

juvenile shoots of *A. nummularia* exhibited slightly lower water potential (-2.6 ± 0.4 MPa) than did those of *A. leucoclada* (-2.3 ± 0.3 MPa). It was observed that the values of the shoot water potentials obtained using Scholander pressure pump were slightly higher than the substrate water potential at all salinity treatments (Fig. 10 a and b).

3.2.3 Osmotic potentials

The osmotic potential of the press sap of *A. nummularia* control plants ranged between -0.7 and -2.3 MPa on average over the different plant organs. The highest osmotic potential was recorded in the root tissues and the lowest (more negative values) were in the juvenile stem ones. The osmotic potentials of all plant organs clearly decreased and became more negative as the water salinity increased (Fig. 11a). It reached from -2.3 ± 0.3 MPa (R) to -4.5 ± 0.3 MPa (Lj) at high salinity treatment.

The osmotic potentials of the different organs of *A. leucoclada* were more or less similar to those of *A. nummularia*, ranging from -0.8 MPa (R) to -2.5 MPa (Sj). As in *A. nummularia*, the osmotic potentials of all *A. leucoclada* plant organs became more negative as the water salinity increased (Fig. 11b). At the full strength salinity, *A. leucoclada* plants performed lower (more negatively) osmotic potentials than *A. nummularia*, being -3.4 ± 0.3 MPa (R) to -4.5 ± 0.5 MPa (Sj).

3.3 Effect of salinity on the pigment contents and the CO_2-gas exchange

3.3.1 Chlorophyll a, b and carotenoid contents

In general, chlorophyll (a), chlorophyll (b) and carotenoid contents (based on the leaf area) of the juvenile leaves were higher than those of the adult leaves in both *Atriplex* species and at all levels of salinity. Additionally, Chl(a) was the dominant pigment in adult and juvenile leaves of both *Atriplex* species. In *A. nummularia* control plants, the mean Chl(a) concentration was 61.9 and 72.1 µg* cm^{-2} in the adult and juvenile leaves, respectively. The Chl a/Chl b ratio ranged between 2.2 and 2.8 in the adult and juvenile leaves, respectively. As shown in Fig.

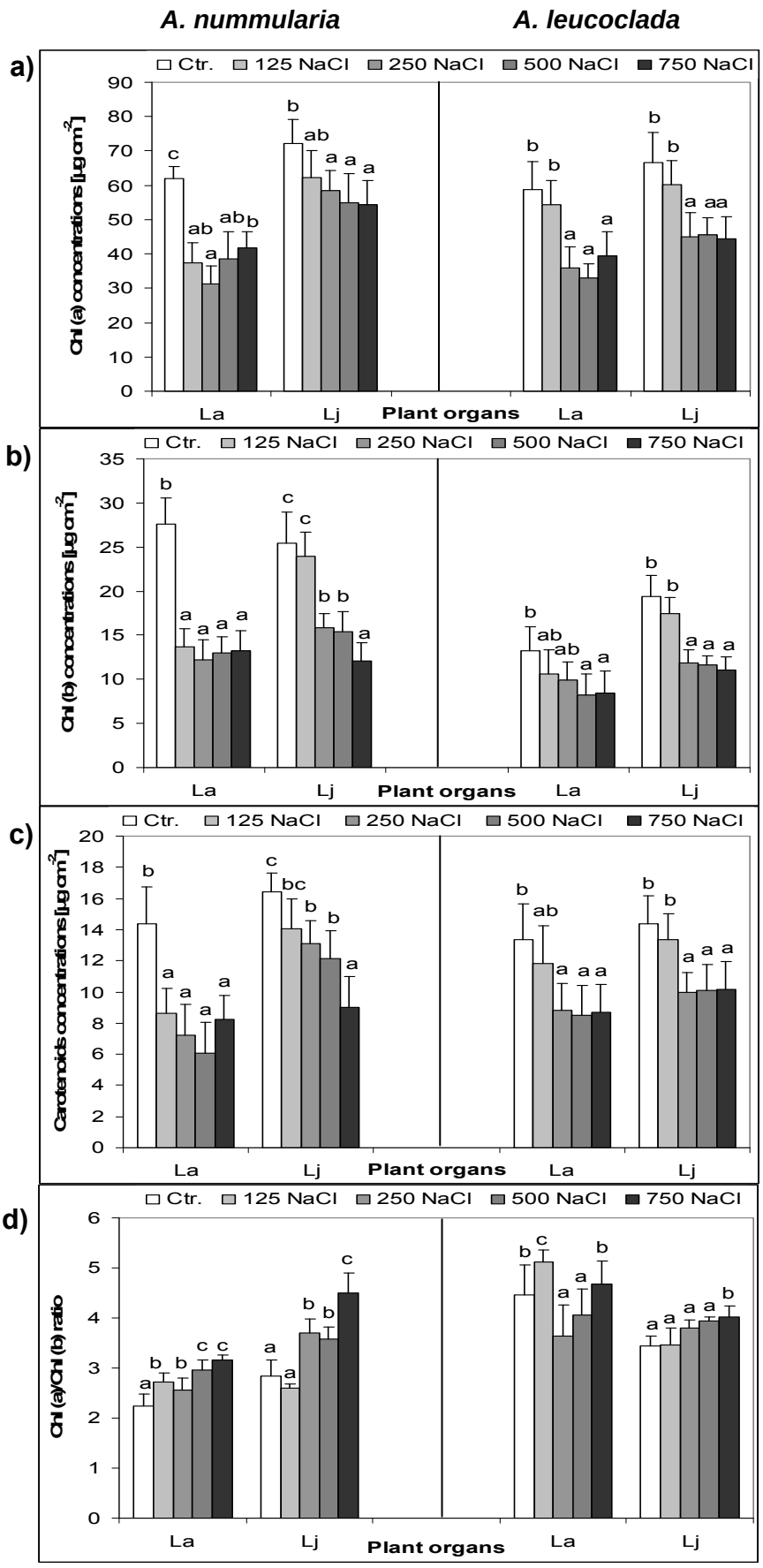

Fig. 12: Influence of NaCl salinity on the Chl (a) (a), Chl (b) (b), carotenoids concentrations (c) and Chl (a)/Chl (b) ratios (d) of the leaves of *A. nummularia* (left) and *A. leucoclada* (right). Each column represents the mean values of nine replicates and the bars represent standard errors. Columns with the same letter are not significantly different at $P < 0.05$, LSD test.

12 (a - c), the pigment contents of the adult leaves were transiently reduced as the water salinity level increased. Moderate salinity caused reduction of about 50% in the Chl(a), Chl(b) and carotenoid contents relative to the controls. Further increase in the water salinity slightly increased the pigment contents of the adult leaves. However, these increases were significant only for Chl(a). As for the juvenile leaves Chl(a), Chl(b) and carotenoid contents were reduced gradually with increasing water salinity to reach minimum values at the highest NaCl treatment (Fig. 12 a - c). Accordingly the Chl a/Chl b ratio of the adult leaves increased with increasing salinity.

The adult and juvenile leaves of *A. leucoclada* control plants had lower Chl(a), Chl(b) and carotenoid concentrations than those of *A. nummularia*. The Chl(a) concentrations ranged between 58.7 and 66.5 $\mu g*cm^{-2}$ for the adult and juvenile leaves of *A. leucoclada* control plants respectively. Under this condition, the Chl a/Chl b ratio was much higher (4.45 and 3.43 for the adult and juvenile leaves respectively) compared to those of *A. nummularia*. Similarly, increasing water salinity decreased the contents of all the three pigments in both adult and juvenile leaves with respect to controls (Fig. 12 a - c). This effect was more severe on Chl(b) and consequently the Chl a/b ratio increased with increasing NaCl salinity. Unlike *A. nummularia*, no significant ($P < 0.05$) differences in the Chl(a), Chl(b) and carotenoid contents of both adult and juvenile leaves were observed at salinities above 250 $mol*m^{-3}$ NaCl (Fig. 12 a - c).

3.3.2 CO_2-gas exchange

Raising water salinity induced profound changes in the CO_2-gas exchange parameters of both *Atriplex* species. Average net photosynthesis rate (A) of *A. nummularia* control plants was 18.3 ± 0.4 $\mu mol*m^{-2}*s^{-1}$. It significantly and steadily declined as the salinity increased, reached only about 25% of the control at the highest salinity treatment (Table 3a). Furthermore, the calculated photosynthetic efficiency (Φ_c) ($\mu mol\ CO_2*\mu mol^{-1}$ Quantum) declined steadily with increasing water salinity level, being lowest at the highest salinity treatment (Table 3b). In tendency, the light saturation point (Ls) increased from 953.33 $\mu mol*m^{-2}*s^{-1}$ under control conditions to 2647.36 $\mu mol*m^{-2}*s^{-1}$ at the highest water salinity level (Table 3b and Fig.13a). Consequently the light compensation point (Lc) gradually increased with

Table 3a: Effect of elevated NaCl salinity on the net photosynthesis rate (A), transpiration rate (E), water use efficiency (WUE), Stomatal resistance (Rs), ratio of the internal to the external CO_2 concentration (Ci/Can), and difference between the leaf and atmosphere temperature (ΔT) of *A. nummularia*. All of these values are at the light saturation point of photosynthesis.

Treatments	A [µmol*m^{-2}*s^{-1}]	E [mol*m^{-2}*s^{-1}]	WUE A/E	Rs [s*cm^{-1}]	Ci/C$_{an}$	ΔT °C
Control	18.25^{a} ± 0.38	5.61^{a} ± 0.14	3.26^{a} ± 0.15	2.08^{a} ± 0.34	0.50^{a} ± 0.04	1,64^{a} ± 0,14
125 NaCl	13.95^{b} ±1.23	2.98^{b} ± 0.24	4.69^{b} ± 0.09	4.04^{b} ± 0.65	0.35^{b} ± 0.04	2,27^{ab} ± 0,48
250 NaCl	12.65^{b} ± 0.74	2.74^{b} ± 0.31	4.70^{b} ± 0.31	5.62^{b} ±1.01	0.31^{b} ± 0.03	3,15^{b} ± 0,29
500 NaCl	8.94^{c} ± 0.46	1.72^{c} ± 0.19	5.24^{b} ± 0.34	8.03^{c} ± 0.42	0.26^{b} ± 0.02	3,40^{b} ± 0,05
750 NaCl	5.01^{d} ± 0.51	0.75^{d} ± 0.10	6.75^{c} ± 0.86	15.95^{d} ± 0.68	0.22^{c} ± 0.04	4,19^{c} ± 0,08

Means within a column followed by the same letter are not significantly different at $P< 0.05$ as determined by LSD test. Each mean represents nine replicates.

Table 3b: Calculated photosynthetic efficiency (Φ_c), dark respiration (DR), light compensation point (Lc) and light saturation point (Ls) of *A. nummularia* plants grown under various NaCl salinities. The calculation was done using SigmaPlot software.

Treatments	Φ_c [µmol CO_2*µmol^{-1} Quantum]	DR [mol*m^{-2}*s^{-1}]	Lc [µmol*m^{-2}*s^{-1}]	Ls [µmol*m^{-2}*s^{-1}]
Control	0.044	-1.48	32.29	953.33
125 NaCl	0.025	-1.47	56.41	1591.46
250 NaCl	0.026	-1.73	61.14	1500.26
500 NaCl	0.017	-1.16	63.09	1417.55
750 NaCl	0.009	-0.84	88.94	2647.37

Table 4a: Effect of elevated water salinity on the net photosynthesis rate (A), transpiration rate (E), water use efficiency (WUE), Stomatal resistance (Rs), ratio of the internal to the external CO_2 concentration (Ci/Can), and difference between the leaf and atmosphere temperature (ΔT) of *A. leucoclada*. All of these values are at the light saturation point of photosynthesis.

Trearments	A [µmol*m^{-2}*s^{-1}]	E [mol*m^{-2}*s^{-1}]	WUE A/E	Rs [s*cm^{-1}]	Ci/C$_{an}$	ΔT °C
Control	16,06^{a} ± 0,83	3,77^{a} ± 0,19	4,26^{a} ± 0,02	3.18^{a} ± 0.19	0.40^{a} ± 0.02	1,96^{a} ± 0,13
125 NaCl	14,16^{b} ± 0,40	2,97^{b} ± 0,25	4,79^{ab} ± 0,32	4.18^{b} ± 0.31	0.35^{a} ± 0.02	2,41^{a} ± 0,37
250 NaCl	10,73^{c} ± 0,78	1,91^{c} ± 0,11	5,63^{bc} ± 0,08	8.04^{c} ± 0.62	0.26^{b} ± 0.00	3,64^{b} ± 0,39
500 NaCl	8,15^{d} ± 0,74	1,37^{d} ± 0,17	5,98^{c} ± 0,28	11.67^{d} ± 0.28	0.23^{bc} ± 0.03	4,28^{bc} ± 0,36
750 NaCl	4,96^{e} ± 0,18	0,84^{e} ± 0,12	6,07^{c} ± 0,74	15.55^{e} ± 0.25	0.17^{c} ± 0.04	4,92^{c} ± 0,75

Means within a column followed by the same letter are not significantly different at *P*< 0.05 as determined by LSD test. Each mean represents nine replicates.

Table 4b: Calculated photosynthetic efficiency (Φ_c), dark respiration (DR), light compensation point (Lc) and light saturation point (Ls) of *A. leucoclada* plants grown under various NaCl salinities. The calculation was done using SigmaPlot software.

Treatments	Φ_c [µmol CO_2*µmol^{-1} Quantum]	DR [mol*m^{-2}*s^{-1}]	Lc [µmol*m^{-2}*s^{-1}]	Ls [µmol*m^{-2}*s^{-1}]
Control	0.030	-0.95	31.31	1385.77
125 NaCl	0.027	-1.01	35.97	1315.18
250 NaCl	0.026	-1.25	45.87	1142.34
500 NaCl	0.022	-0.76	33.77	1185.06
750 NaCl	0.009	-0.52	53.20	1824.42

increasing NaCl concentration in the nutrient solution, reached maximum at the highest water salinity (Table 3b). Increasing water salinity gradually and significantly increased the stomatal resistance. At the highest salinity level, there was approximately 8-fold increase in the stomatal resistance relative to the control plants (Table 3a). This increase in the stomatal resistance was correlated with a strong reduction in the transpiration rate (E), which reached minimum level at the highest salinity treatment (Table 3a). This close coordination between the transpiration and net photosynthesis rates under elevated salinity led to a significant increase in the water use efficiency (WUE) with increasing water salinity. Compared to control, WUE was increased by about 100% in plants grown at the highest salt concentration (Table 3b). Salinity distinctly reduced the ratio of the internal to external CO_2 concentration (C_i/C_{an}) from 0.5 ± 0.04 under control conditions to 0.2 ± 0.03 at 750 mol*m^{-3} NaCl treatment. A significant increase in the ΔT (difference between leaf and air temperature) was observed from 1.6 ± 0.1 °C under control conditions to 4.2 ± 0.1 °C at the highest water salinity treatment. The dark respiration (DR) increased transiently with elevated water salinity, being maximum at 250 mol*m^{-3} NaCl (Table 3b).

The control plants of *A. leucoclada* showed comparatively lower net photosynthetic rates (16.1 ± 0.8 µmol*m^{-2}*s^{-1}) than those of *A. nummularia* (Table 4a). The net photosynthetic rates was reduced gradually and significantly ($P< 0.05$) with increasing substrate salinity and reached similar level to that of *A. nummularia* at the highest salinity treatment (Table 4a). The calculated photosynthetic efficiency (Φ_c) of *A. leucoclada* controls was distinctly lower than that of *A. nummularia*. It declined gradually as the substrate salinity increased and reached a comparable level to that of *A. nummularia* at the highest salinity level (Table 4b). Light response curves (Fig. 13b) indicated that the photosynthesis was saturated at higher light intensity (1385.77 µmol*m^{-2}*s^{-1}). Salinity led to an increase (in tendency) of the light saturation point (Ls) which was highest (but lower than that of *A. nummularia*) at 750 mol*m^{-3} NaCl treatment (Fig. 13b and Table 4b). This was associated with increases in the light compensation point. The transpiration rates (E) of *A. leucoclada* controls were clearly lower than those of *A. nummularia* controls. It was significantly reduced as the salinity rose and reached minimum at 750 mol*m^{-3} NaCl treatment (Table 4a). The reduction of the transpiration rates were accompanied by a

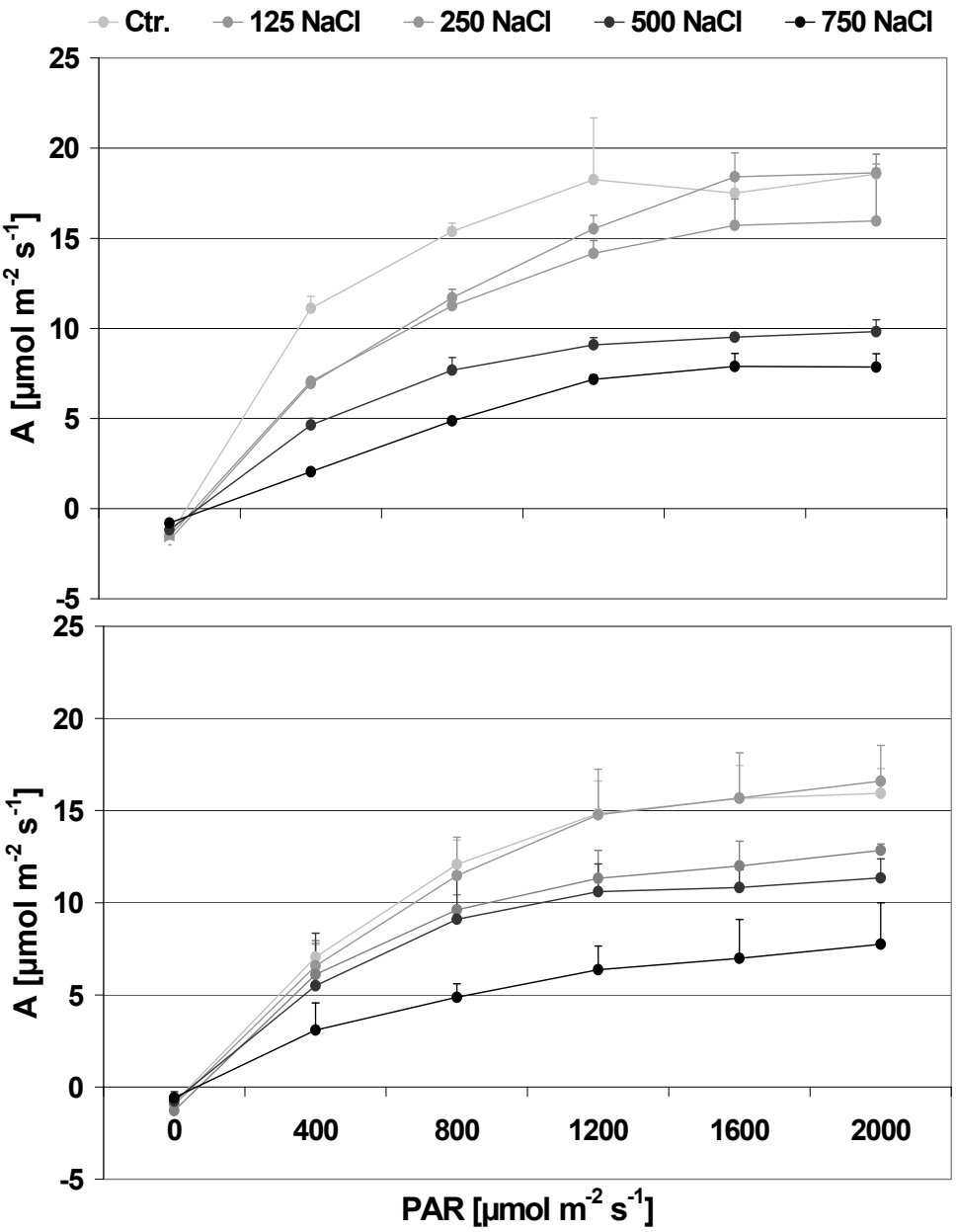

Fig. 13: Light response curves of *A. nummularia* a) and *A. leucoclada* b) at various NaCl salinities. A, Net photosynthesis rate [µmol*m^{-2}*s^{-1}]; PAR, photosynthetically active radiation [µmol*m^{-2}*s^{-1}].

significant increase in the stomatal resistance (5-fold increase) at 750 mol*m^{-3} NaCl salinity. As can bee seen in Table (4a), the WUE of *A. leucoclada* controls was higher than that of *A. nummularia* controls. Raising water salinity enhanced the WUE of *A. leucoclada* and the highest salinity treatment induced about 45% increase in the WUE relative to the control. The C_i/C_{an} ratio dropped from 0.4 ± 0.02 for control plants to 0.2 ± 0.04 for plants grown at 750 mol*m^{-3} NaCl. ΔT increased significantly (much more than in *A. nummularia*) from 1.9 ± 0.13 °C under control conditions to 4.9 ± 0.8 °C under the highest NaCl salinity (Table 4a). With respect to DR, it was markedly lower than that of *A. nummularia* under control conditions. Elevating NaCl salinity up to 250 mol*m^{-3} NaCl increased the DR, and then it declined to the same levels observed in *A. nummularia* plants at 750 mol*m^{-3} NaCl (Table 4b).

3.4 Effect of salinity on the mineral ion contents

Ion contents of both *Atriplex* species were clearly affected by elevated NaCl salinity. Since *Atriplex* species develop bladder hairs on all their plant parts especially the leaves, ion composition of these bladder hairs and the plant tissues were determined separately.

3.4.1 Cation composition

3.4.1.1 Na^+ contents

At all NaCl salinity treatments and in both *Atriplex* species Na^+ concentrations in the roots were lower than in the shoots. Tissue Na^+ concentrations of *A. nummularia* control plants ranged from 30.9 ± 3.7 mmol*kg^{-1} fw (R) to 147.0 ± 12.9 mmol*kg^{-1} fw (La). Analysis of variance revealed that Na^+ contents of all organs increased progressively and significantly ($P < 0.05$) as the external NaCl concentrations increase (Fig. 14a). High salinity level resulted in 6 – 20 fold increases in the Na^+ concentration relative to controls depending on the plant organs. At the highest salinity treatment, the roots tended to maintain low Na^+ concentrations (301.5 ± 8.5 mmol*Kg^{-1} fw), while the shoot parts contained a comparable Na^+ levels to those of the nutrient solutions (slightly lower in the adult stems) (Fig. 14a). The adult leaves, however, accumulate high Na^+ concentration

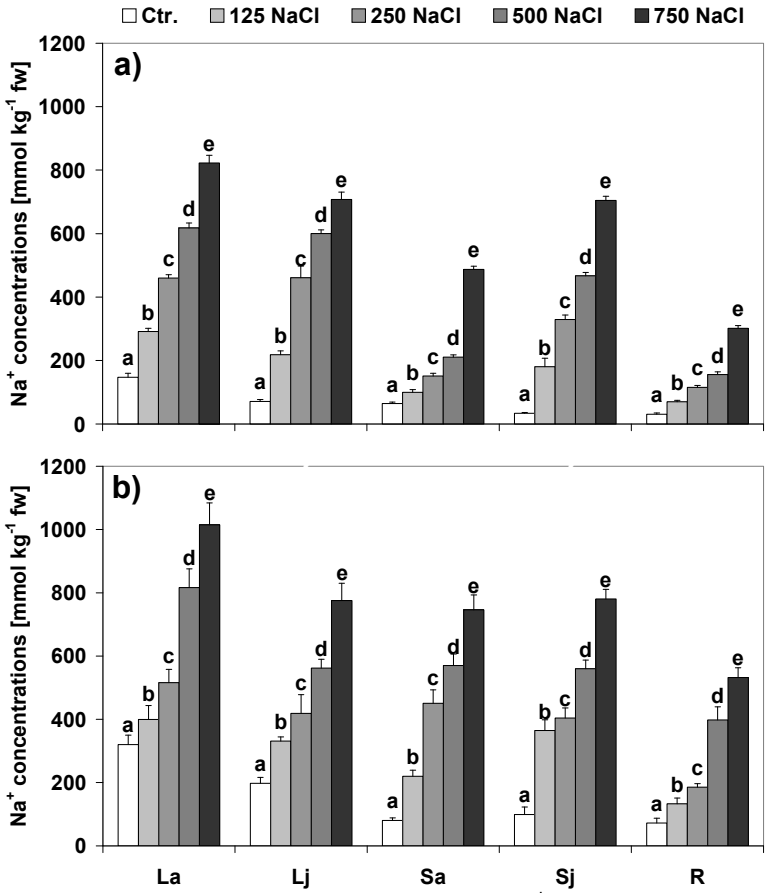

Fig. 14: Effect of increasing NaCl salinity on the Na^+ concentrations (per Kg fresh weight) of the different organs of a) *A. nummularia* and b) *A. leucoclada.* La, adult leaves; Lj, juvenile leaves; Sa, adult stems; Sj, juvenile stems; R, roots. Each column represents the mean values of nine replicates and the bars represent standard errors. Columns with the same letter are not significantly different at $P< 0.05$, LSD test.

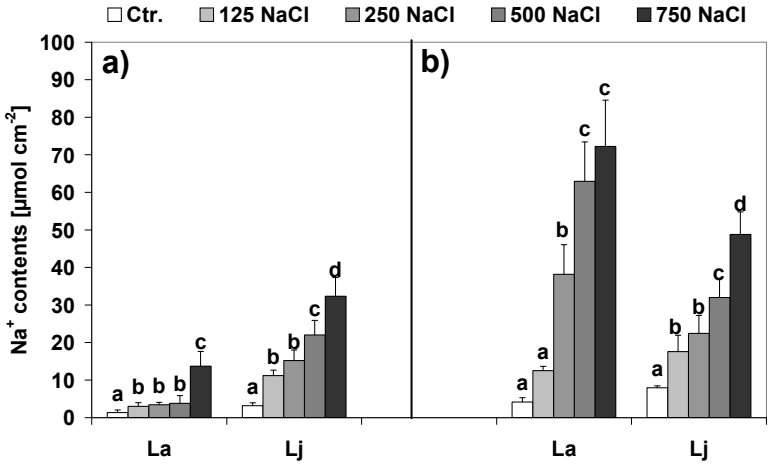

Fig. 15: Na^+ excretion of the bladder hairs (µmol*cm-2) of the adult and juvenile leaves a) *A. nummularia* and b) *A. leucoclada* at varying NaCl salinity levels. La, adult leaves; Lj, juvenile leaves. Each column represents the mean values of nine replicates and the bars represent standard errors. Columns with the same letter are not significantly different at $P< 0.05$, LSD test.

(822.3 ± 24.3 mmol*kg^{-1} fw) in comparison to the other organs and the nutrient solution (Fig. 14a). The leaves of *A. nummularia* control plants secreted considerable amounts of Na^+, being 1.3 ± 0.7 and 3.2 ± 0.8 µmol*cm^{-2} for the adult and juvenile leaves respectively. The Na^+ excretion significantly (P< 0.05) and gradually increased as the NaCl concentration in the nutrient solution increased (Fig. 15a). Expressed on the leaf fresh weight, Na^+ excretion of the bladder hairs increased with increasing water salinity and exceeded slightly that of the corresponding leaf tissues and that of the nutrient solution at the high salinity treatment only for the juvenile leaves bladder hairs (data not shown).

In general, Na^+ contents of the different organs of *A. leucoclada* plants were higher than those of *A. nummularia* at all salinity levels (Fig. 14b). Na^+ was the predominant cation in all *A. leucoclada* plant organs. It ranged from 72.6 ± 14.7 (R) to 320.4 ± 29.4 mmol*kg^{-1} fw (La) under control conditions. As expected, Na^+ ion contents of the different plant organs increased incrementally with NaCl salinity (Fig. 14b). The roots had distinctly lower Na^+ concentrations (532.4 ± 30.6 mmol*kg^{-1} fw) than the shoots. Similar to *A. nummularia*, the adult leaves accumulated much higher Na^+ concentration (1015.1 ± 68.5 mmol*kg^{-1} fw) compared to the other organs (Fig. 14b).

Under control conditions, Na^+ excretion of the bladder hairs were 4.2 ± 1.1 and 8.0 ± 0.5 µmol*cm^{-2} for the adult and juvenile leaves respectively (Fig. 15b). Significant (P < 0.05) increases were observed in the bladder hairs Na^+ excretion as the water salinity rose. The adult leaves bladder hairs accumulated significantly higher Na^+ than did the juvenile leaf bladders at the highest salinity level. About 17 and 6 folds increases were observed in Na^+ excretion of the adult and juvenile leaves bladder hairs, respectively, relative to controls. On leaf fresh weight base, Na^+ excretion of the bladder hairs of the adult and juvenile leaves were 1.5 and 2 fold, respectively, as high as in the corresponding leaf tissues. In contrast to *A. nummularia*, age of the leaves had no influence on the Na^+-excretion in *A. leucoclada*.

3.4.1.2 K^+ contents

K^+ was the dominant cation in *A. nummularia* plant tissues under control conditions. It reached 77.9 – 226.8 mmol*kg^{-1} fw on average over all the plant

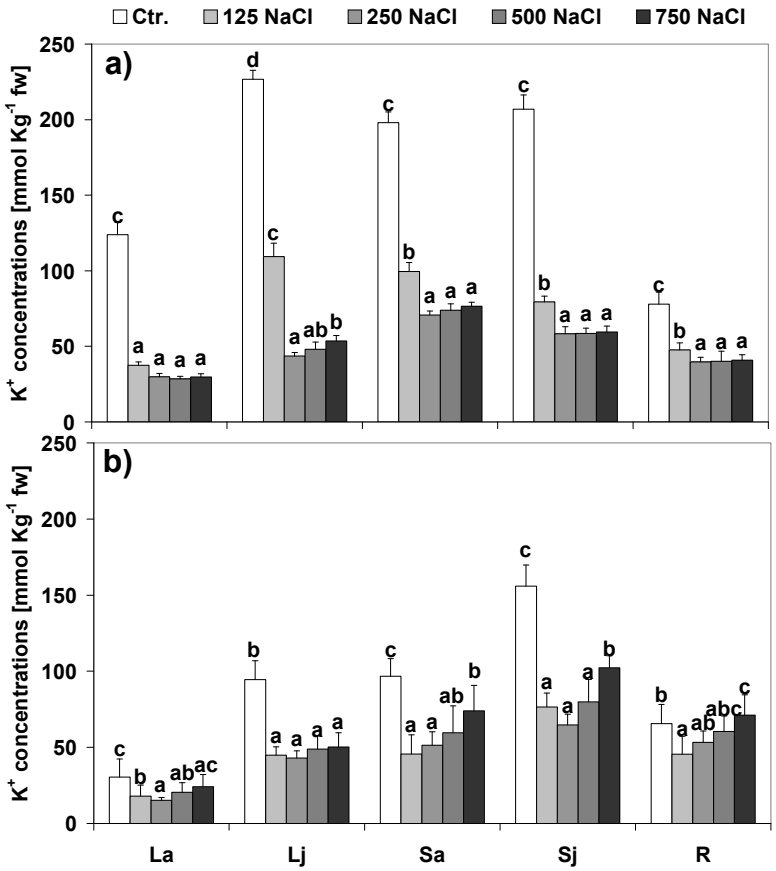

Fig. 16: Effect of increasing NaCl salinity on the K^+ concentrations (per Kg fresh weight) of different organs of a) *A. nummularia* and b) *A. leucoclada.* La, adult leaves; Lj, juvenile leaves; Sa, adult stems; Sj, juvenile stems; R, roots. Each column represents the mean values of nine replicates and the bars represent standard errors. Columns with the same letter are not significantly different at $P< 0.05$, LSD test.

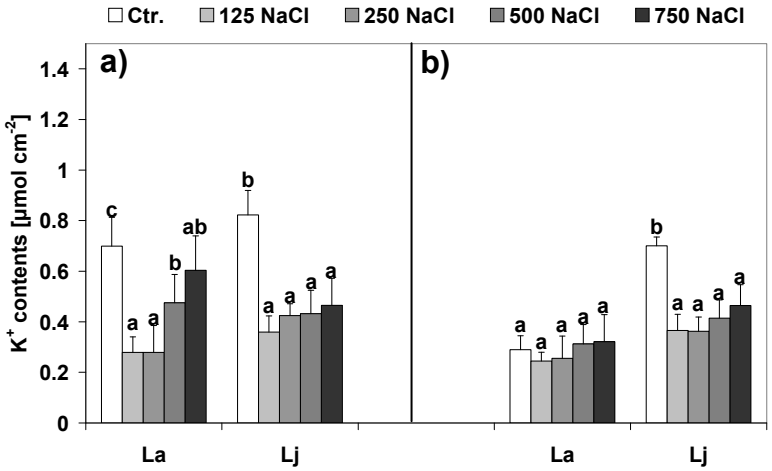

Fig. 17: K^+ excretion (leaf area basis) of the bladder hairs of the adult and juvenile leaves of a) *A. nummularia* and b) *A. leucoclada* at varying NaCl salinity levels. La, adult leaves; Lj, juvenile leaves. Each column represents the mean values of nine replicates and the bars represent standard errors. Columns with the same letter are not significantly different at $P< 0.05$, LSD test.

organs. The lowest K^+ concentrations were found in the roots while the highest K^+ concentrations were in the juvenile leaves (Fig. 16a). K^+ concentrations decreased transiently in all plant organs (particularly in the shoots) with increasing NaCl salinity, and reached a minimum at 250 mol*m^{-3} NaCl. Thereafter, K^+ concentrations of all plant organs slightly increased. However, this effect was significant only for the juvenile leaves (Fig. 16a).

On the basis of leaf area, K^+ excretion of the leaf bladder hairs were much lower than those of the corresponding tissues. It was highest for the hairs of both adult and juvenile leaves, resulting in 0.7 ± 0.1 and 0.8 ± 0.1 µmol*cm^{-2}, respectively, under control conditions. K^+ excretion of the bladder hairs decreased transiently with increasing NaCl salinity with minimum concentrations at the low salinity level (Fig. 17a). Further increase in NaCl salinity led to an increase of K^+ excretion of the adult leaves bladder hairs, while it did not significantly affect that of the juvenile leaves bladder hairs (Fig. 17a).

Tissues K^+ concentrations of *A. leucoclada* were lower than those of *A. nummularia* at control conditions. They ranged between 30.5 ± 11.8 mmol*kg^{-1} fw (La) and 156.0 ± 13.8 mmol*kg^{-1} fw (Lj). As shown in Fig. (16b), the raising of the external NaCl salinity transiently declined the K^+ contents of all plant organs, with more adverse effect on the shoots. Moderate salinity caused the highest reductions in K^+ concentrations (about 50 – 60 % in the shoot parts and 20 % in roots). Thereafter, increasing water salinity slightly but significantly enhanced K^+ contents of all plant organs, except that of the juvenile leaves (Fig. 16b). Fig. 17b shows that NaCl salinity transiently reduced the K^+ excretion of the bladder hairs of both adult and juvenile leaves.

3.4.1.3 Na^+/K^+ ratios

NaCl salinity gradually and significantly ($P < 0.05$) increased the Na^+/K^+ ratio of all *A. nummularia* plant organs (Table 5). At the highest NaCl salinity level, the roots and adult stems exhibited the lowest Na^+/K^+ ratios (7.4 and 6.4 respectively), whereas the adult leaves had the highest Na^+/K^+ ratio (27.8). Na^+/K^+ ratios in the bladder hairs of both adult and juvenile leaves were always higher than under

Table 5: Na^+/K^+ ratios of the different plant organs and bladder hairs of A. nummularia and A. leucoclada under varying NaCl salinity levels. La, adult leaves; Lj, juvenile leaves; Sa, adult stems; Sj, juvenile stems; R, roots; Bh.La, bladder hairs of the adult leaves; Bh.Lj, bladder hairs of the juvenile leaves.

	A. nummularia							*A. leucoclada*						
Treatments	**La**	**Lj**	**Sa**	**Sj**	**R**	**Bh. La**	**Bh. Lj**	**La**	**Lj**	**Sa**	**Sj**	**R**	**Bh. La**	**Bh. Lj**
Ctr.	1.19^{a} ± 0.09	0.31^{a} ± 0.02	0.32^{a} ± 0.02	0.16^{a} ± 0.02	0.40^{a} ± 0.06	1.72^{a} ± 0.61	4.03^{a} ± 1.17	10.58^{a} ± 1.46	2.16^{a} ± 0.21	0.85^{a} ± 0.09	0.73^{a} ± 0.10	1.02^{a} ± 0.24	15.87^{a} ± 2.37	11.04^{a} ± 1.04
125 NaCl	7.76^{b} ± 0.75	2.03^{b} ± 0.11	0.99^{b} ± 0.08	2.23^{b} ± 0.30	1.45^{b} ± 0.16	11.26^{b} ± 4.46	30.33^{b} ± 5.50	26.01^{b} ± 10.62	7.50^{b} ± 1.06	5.23^{b} ± 1.68	4.79^{b} ± 0.42	3.23^{b} ± 1.35	48.77^{b} ± 7.65	41.39^{b} ± 10.52
250 NaCl	15.10^{c} ± 1.03	10.34^{c} ± 0.75	2.15^{c} ± 0.19	5.61^{c} ± 0.42	2.91^{c} ± 0.37	13.40^{b} ± 8.10	32.39^{c} ± 7.08	34.57^{c} ± 4.94	9.87^{c} ± 1.88	9.01^{c} ± 1.68	6.30^{c} ± 0.82	3.52^{b} ± 0.39	179.79^{c} ± 86.74	67.20^{bc} ± 15.84
500 NaCl	21.90^{d} ± 1.12	12.50^{d} ± 1.19	2.91^{d} ± 0.21	7.94^{d} ± 0.48	3.94^{d} ± 0.45	10.82^{b} ± 5.52	55.30^{cd} ± 15.53	43.18^{d} ± 12.53	11.85^{d} ± 2.18	10.32^{d} ± 2.97	7.18^{d} ± 1.11	6.68^{c} ± 0.85	199.85^{c} ± 40.83	76.39^{c} ± 4.22
750 NaCl	27.83^{e} ± 2.80	13.42^{d} ± 0.90	6.40^{e} ± 0.28	11.95^{e} ± 0.72	7.42^{e} ± 0.71	23.97^{c} ± 9.25	69.38^{d} ± 14.88	45.30^{d} ± 13.31	15.79^{e} ± 2.37	10.45^{d} ± 1.95	7.66^{d} ± 0.60	7.73^{c} ± 1.61	280.92^{d} ± 29.45	106.15^{d} ± 19.73

Means within a column followed by the same letter are not significantly different at $P< 0.05$ as determined by LSD test. Each mean represents nine replicates.

Table 6: $SA_{K:\ Na}$ of the different plant organs and bladder hairs of A. nummularia and A. leucoclada under varying NaCl salinity levels. La, adult leaves; Lj, juvenile leaves; Sa, adult stems; Sj, juvenile stems; R, roots; Bh.La, bladder hairs of the adult leaves; Bh.Lj, bladder hairs of the juvenile leaves.

	A. nummularia							*A. leucoclada*						
Treatments	**Ns→R**	**R→Sa**	**Sa→La**	**Sa→Sj**	**Sj→Lj**	**La→Bh**	**Lj→Bh**	**Ns→R**	**R→Sa**	**Sa→La**	**Sa→Sj**	**Sj→Lj**	**La→Bh**	**Lj→Bh**
Ctr.	0.10^{a} ± 0.02	1.23^{ab} ± 0.18	0.27^{a} ± 0.03	2.01^{a} ± 0.20	0.52^{a} ± 0.08	0.73^{a} ± 0.27	0.08^{a} ± 0.02	0.04^{a} ± 0.01	1.21^{a} ± 0.31	0.08^{a} ± 0.01	1.17^{a} ± 0.12	0.33^{a} ± 0.04	0.73^{a} ± 0.24	0.19^{a} ± 0.01
125 NaCl	80.83^{b} ± 7.49	1.48^{c} ± 0.20	0.13^{c} ± 0.02	0.45^{b} ± 0.04	1.10^{b} ± 0.16	0.74^{a} ± 0.22	0.08^{a} ± 0.02	41.87^{b} ± 13.72	0.63^{bc} ± 0.20	0.26^{b} ± 0.16	1.09^{a} ± 0.33	0.65^{b} ± 0.12	0.71^{ab} ± 0.27	0.20^{a} ± 0.03
250 NaCl	86.98^{bc} ± 7.14	1.36^{bc} ± 0.18	0.14^{c} ± 0.02	0.38^{c} ± 0.04	0.54^{a} ± 0.05	1.35^{a} ± 0.46	0.32^{b} ± 0.10	68.38^{c} ± 7.82	0.40^{c} ± 0.07	0.26^{b} ± 0.05	1.43^{b} ± 0.20	0.65^{b} ± 0.11	0.23^{bc} ± 0.07	0.18^{a} ± 0.08
500 NaCl	112.69^{d} ± 2.89	1.36^{bc} ± 0.18	0.13^{c} ± 0.01	0.37^{c} ± 0.05	0.64^{a} ± 0.05	2.44^{b} ± 1.00	0.24^{c} ± 0.06	72.28^{c} ± 9.14	0.71^{b} ± 0.29	0.24^{b} ± 0.04	1.41^{b} ± 0.22	0.61^{b} ± 0.06	0.20^{c} ± 0.03	0.15^{a} ± 0.01
750 NaCl	97.06^{c} ± 9.24	1.16^{a} ± 0.11	0.23^{b} ± 0.03	0.54^{d} ± 0.04	0.89^{c} ± 0.05	1.40^{a} ± 0.61	0.20^{c} ± 0.07	95.73^{d} ± 18.34	0.79^{b} ± 0.31	0.25^{b} ± 0.10	1.36^{b} ± 0.19	0.49^{c} ± 0.06	0.18^{c} ± 0.05	0.16^{a} ± 0.03

Means within a column followed by the same letter are not significantly different at $P< 0.05$ as determined by LSD test. Each mean represents nine replicates.

control conditions. The bladder hairs of juvenile leaves had higher Na^+/K^+ ratio than those of the adult leaves at all salinity levels. The Na^+/K^+ ratio increased significantly with increasing water salinity, and reached a maximum (24.0 and 74.2 for the adult and juvenile leaves bladder hairs respectively) at the highest salinity treatment (Table 5). Na^+/K^+ ratio of all plant organs as well as of the bladder hairs of *A. leucoclada* was much higher than that of *A. nummularia* at all salinity treatments. The same trend of increasing Na^+/K^+ ratio of all plant organs and bladder hairs was observed with elevating NaCl salinity (Table 5). At the highest NaCl salinity, the adult leaves had the highest Na^+/K^+ ratio (45.3), while the juvenile stems and roots had the lowest Na^+/K^+ ratio (7.7 and 7.7 respectively).

3.4.1.4 K^+/Na^+ selectivity

Selective absorption capacity of K^+ over Na^+ ($SA_{K: Na}$) (estimated according to the equation of Pitman, 1960) was low (0.10 ± 0.02) in *A. nummularia* control plants. Increasing NaCl concentrations in the substrate led to a steep rise of the $SA_{K: Na}$ up to more than 110 fold at the seawater salinity (Table 6). The control plants of *A. leucoclada* presented very low $SA_{K: Na}$ values (0.04 ± 0.08). $SA_{K: Na}$ increased as the salinity rose, being 95-fold higher than the controls at the highest salinity treatment. $SA_{K: Na}$ values were generally lower than those of *A. nummularia* at the whole range of salinity treatments.

Table (6) shows that the selective transport capacity of K^+ over Na^+ ($ST_{K: Na}$) (calculated according to the equation of Wang and Zhu, 1994) was highest under control conditions for all organs of both *Atriplex* species. $ST_{K: Na}$ was generally less than 1 in all organs of both *Atriplex* species and at the whole range of salinities (Table 6) with two exceptions; $ST_{K: Na}$ from R to the Sa in *A. nummularia* and $ST_{K: Na}$ from Sa to Sj in *A. leucoclada* which were always higher than one at all salinity levels.

3.4.1.5 Ca^{2+} contents

Tissues Ca^{2+} concentrations of *A. nummularia* were highest at control condition, ranging from 22.0 $mmol*kg^{-1}$ fw (R) to 53.7 $mmol*kg^{-1}$ fw (La). Ca^{2+} concentrations of all plant organs decreased as the NaCl salinity increased up to 250 $mol*m^{-3}$ NaCl (Fig. 18a). Thereafter, increasing water salinity did not

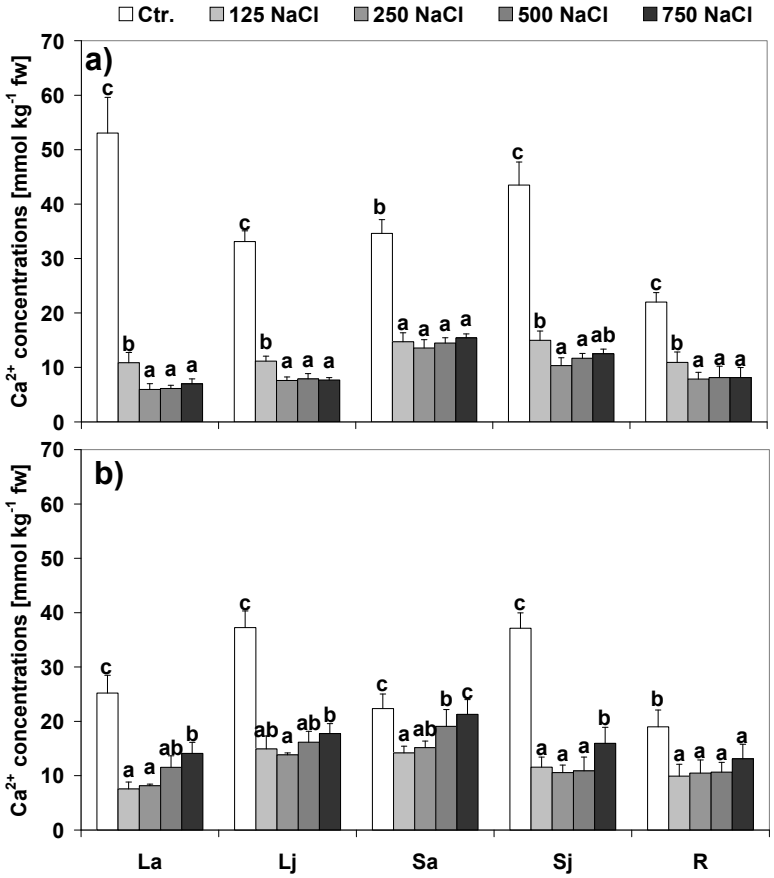

Fig. 18: Effect of increasing NaCl salinity on the different organs Ca^{2+} concentrations (fresh weight basis) of a) *A. nummularia* and b) *A. leucoclada.* La, adult leaves; Lj, juvenile leaves; Sa, adult stems; Sj, juvenile stems; R, roots. Each column represents the mean values of nine replicates and the bars represent standard errors. Columns with the same letter are not significantly different at $P< 0.05$, LSD test.

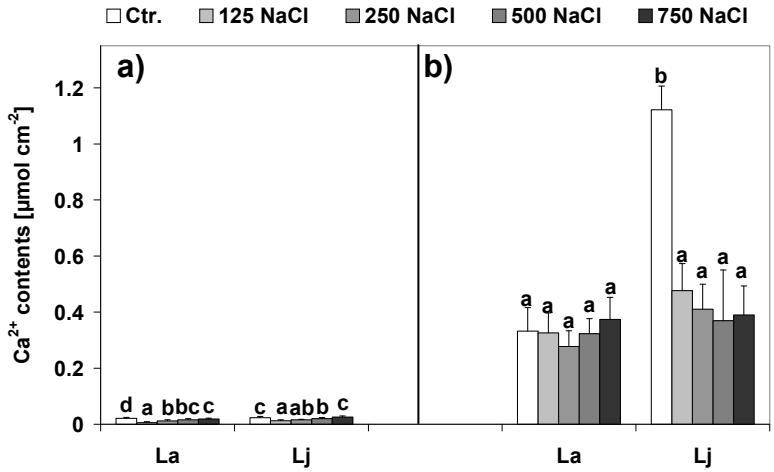

Fig. 19: Ca^{2+} excretion (leaf area basis) of the bladder hairs of the adult and juvenile leaves of a) *A. nummularia* and b) *A. leucoclada* at varying NaCl salinity levels. La, adult leaves; Lj, juvenile leaves. Each column represents the mean values of nine replicates and the bars represent standard errors. Columns with the same letter are not significantly different at $P< 0.05$, LSD test.

significantly impact the Ca^{+2} concentrations of all organs except the juvenile stems, which showed a small but significant ($P < 0.05$) increase at the highest salinity treatment. Small amounts of Ca^{2+} (about 0.02 µmol*cm^{-2}) were detected inside the bladder hairs of both adult and juvenile leaves under control conditions. Increasing water salinity led to a transient decrease of the Ca^{2+} excretion, with lowest Ca^{2+} concentrations occurred at a salinity of 125 mol*m^{-3} NaCl (Fig.19a).

Control plants of *A. leucoclada* had lower Ca^{2+} concentrations (19.0 – 37.3 mmol*kg^{-1} fw) on average over the plant organs than *A. nummularia*. Ca^{2+} concentrations transiently decreased with increasing external NaCl concentrations (Fig. 18b). Further increase in the water salinity significantly increased the Ca^{2+} contents of all organs with the exception of the roots. At high salinity treatment, Ca^{2+} was still lower than in the controls. Fig. 19b reveals that the bladder hairs of both adult and juvenile leaves of *A. leucoclada* contained much higher Ca^{2+} concentrations than those of *A. nummularia* at the whole range of salinities. Additionally, the juvenile leaves excreted comparatively higher Ca^{2+} than adult ones at all salinity levels. Increasing NaCl salinity lowered significantly the Ca^{2+} excretion of the bladder hairs of the juvenile leaves, while it did not significantly affect those of the adult leaves bladder hairs (Fig. 19b).

3.4.1.6 Mg^{2+} contents

Mg^{2+} concentrations of *A. nummularia* ranged from 25.8 mmol*kg^{-1} fw (R) to 38.3 mmol*kg^{-1} fw (Lj and Sj). Moderate NaCl salinity decreased the Mg^{+2} contents of all plant organs (Fig. 20a). The same effect was observed for Mg^{2+} excretion of the bladder hairs of both the adult and juvenile leaves. It dropped significantly as the salinity rose to 125 mol*m^{-3} NaCl and then remained constant at higher salinities (Fig. 21a).

Mg^{2+} concentrations of *A. leucoclada* control plants were higher than those of *A. nummularia* controls in all plant organs with exception of the adult stems. On average over all the different plant organs, Mg^{2+} concentrations of *A. leucoclada* ranged between 25.8 mmol*kg^{-1} fw (Sa) and 61.9 mmol*kg^{-1} fw (Lj). Mg^{2+} concentrations of all plant organs declined transiently in response to elevated water salinity (Fig. 20b). At high salinity treatment, the plants maintained consistently higher Mg^{2+} concentrations than *A. nummularia* in all organs. With regard to the

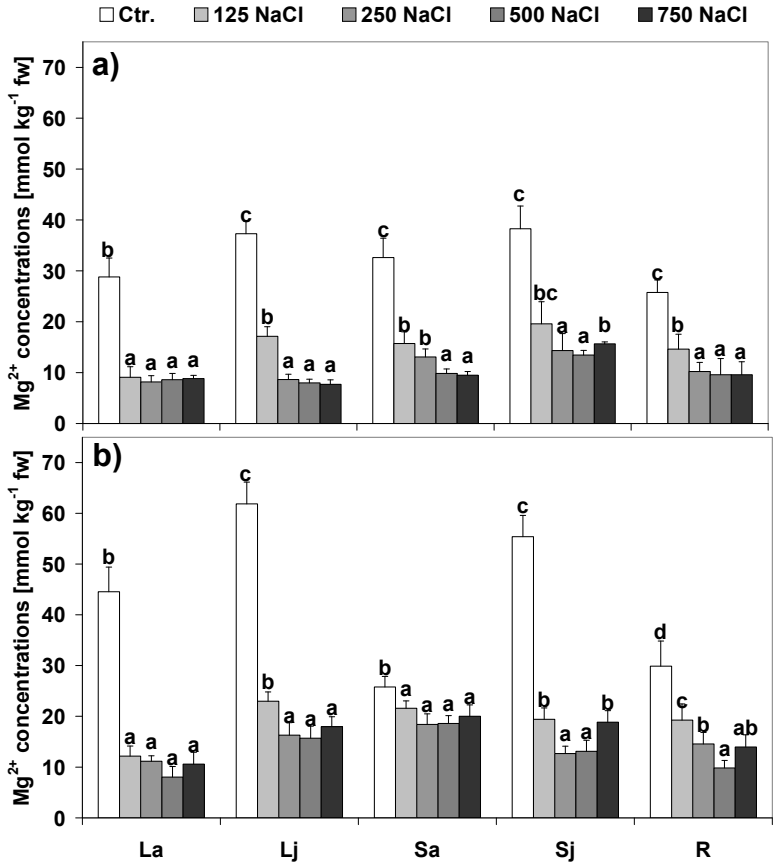

Fig. 20: Effect of increasing NaCl salinity on the different organs Mg^{2+} concentrations (fresh weight basis) of a) *A. nummularia* and b) *A. leucoclada.* La, adult leaves; Lj, juvenile leaves; Sa, adult stems; Sj, juvenile stems; R, roots. Each column represents the mean values of nine replicates and the bars represent standard errors. Columns with the same letter are not significantly different at $P< 0.05$, LSD test.

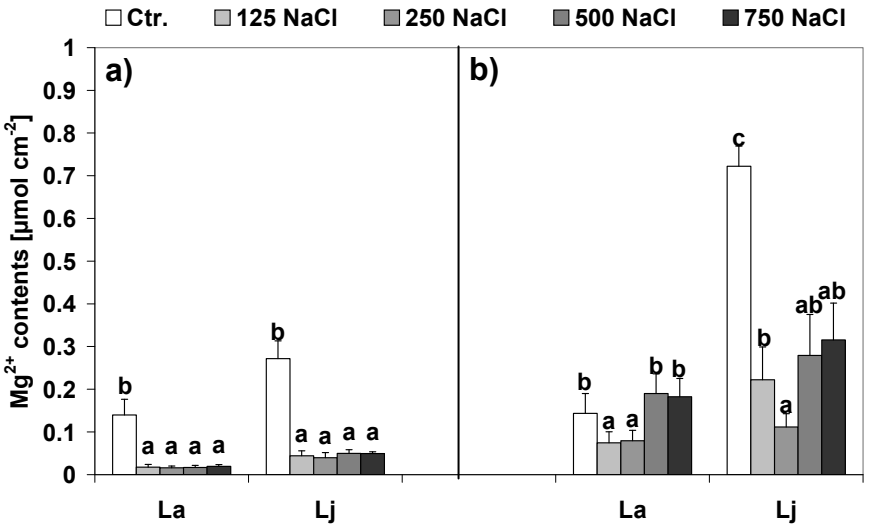

Fig. 21: Mg^{2+} excretion (leaf area basis) of the bladder hairs of the adult and juvenile leaves of a) *A. nummularia* and b) *A. leucoclada* at varying NaCl salinity levels. La, adult leaves; Lj, juvenile leaves. Each column represents the mean values of nine replicates and the bars represent standard errors. Columns with the same letter are not significantly different at $P< 0.05$, LSD test.

bladder hairs, they accumulated higher Mg^{2+} content than *A. nummularia*, particular, in the juvenile leaves. A minimum Mg^{2+} content inside the bladder hairs of both adult and juvenile leaves was observed at the moderate salinity (Fig. 21b).

3.4.2 Anion composition

3.4.2.1 Inorganic anions

3.4.2.1.1 Chloride contents

Cl^- was generally the dominant anion in the plant tissues of both *Atriplex* species at all salinity levels. In control plants of *A. nummularia*, the Cl^- concentrations of all organs were distinctly higher than in the nutrient solution. They ranged between 31.2 (Lj) and 201.5 (Sj) mmol*l^{-1} press sap. Cl^- concentrations of all plant organs increased significantly ($P < 0.05$) in parallel to the NaCl concentration in the culture solution (Fig. 22a). The pattern of Cl^- accumulation was similar to that of Na^+ i.e. it was accumulated preferentially in the shoot parts. However, Cl^- concentrations were comparatively lower than Na^+ concentrations in all plant organs especially at the highest salinity level. Even the bladder hairs accumulated much lower Cl^- than Na^+ at all salinity levels. Regardless of salinity treatment, Cl^- concentrations in the juvenile leaves bladder hairs were distinctly higher compared to those of the adult ones (Fig. 23a). At control conditions, low Cl^- excretion was measured, ranging from 0.3 ± 0.1 to 0.6 ± 0.1 µmol*cm^{-2} for the bladder hairs of the adult and juvenile leaves respectively. Increasing NaCl salinity led to a steep rise of the Cl^- excretion in the juvenile leaves bladder hairs, while that of the adult leaf bladder hairs did not significantly change up to a salinity of 750 mol*m^{-3} NaCl (Fig. 23a).

Tissue Cl^- concentrations of *A. leucoclada* control plants were in the range between 42.4 (R) and 297.23 (Sj) mmol*l^{-1} press sap. The Cl^- concentration in the tissue of all plant organs was correlated with the NaCl concentration applied to the nutrient solution. They consistently increased as the water salinity rose reaching a maximum at the highest water salinity level (Fig. 22b). At this level Cl^- increasing was proportionally higher in the roots than in all other organs. As can be seen in Fig. 23b, the bladder hairs of *A. leucoclada* contained comparatively higher Cl^- contents than those of the *A. nummularia* at all salinity levels. As in *A. nummularia*, the bladder hairs of *A. leucoclada* accumulate lower Cl^- rather than Na^+ and the bladder

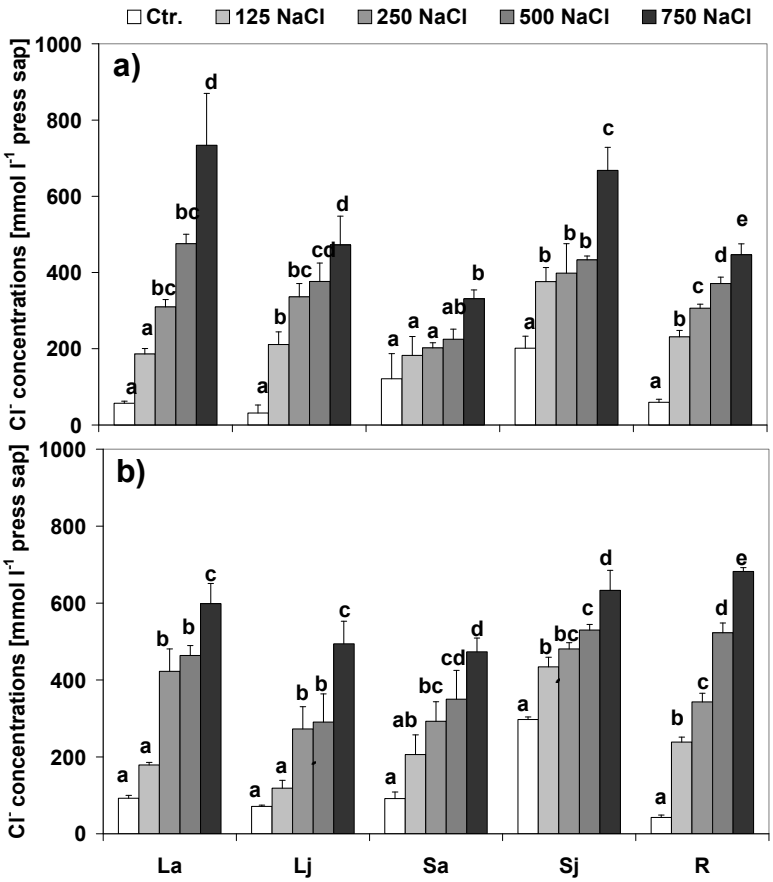

Fig. 22: Effect of increasing NaCl salinity on Cl^- concentrations of the different organs of a) *A. nummularia* and b) *A. leucoclada.* La, adult leaves; Lj, juvenile leaves; Sa, adult stems; Sj, juvenile stems; R, roots. Each column represents the mean values of nine replicates and the bars represent standard errors. Columns with the same letter are not significantly different at $P< 0.05$. LSD test.

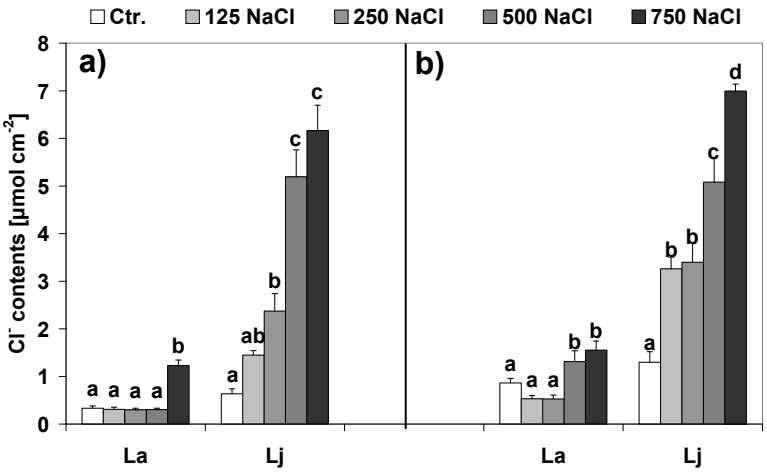

Fig. 23: Cl^- excretion (leaf area basis) of the bladder hairs of the adult and juvenile leaves of a) *A. nummularia* and b) *A. leucoclada* at varying NaCl salinity levels. La, adult leaves; Lj, juvenile leaves. Each column represents the mean values of nine replicates and the bars represent standard errors. Columns with the same letter are not significantly different at $P< 0.05$. LSD test.

hairs of the juvenile leaves contained generally higher Cl^- contents compared to those of the adult ones. Cl^- contents inside the bladder hairs of the juvenile leaves increased gradually and significantly as the salinity rose, while those of the adult leaves bladder hairs increased only significantly at 500 and 750 mol*m^{-3} NaCl (Fig.23b).

3.4.2.1.2 Nitrate contents

Tissue NO_3^- concentrations of *A. nummularia* control plants varied from 8.1 to 63.4 mmol*l^{-1} press sap on average over all the plant organs. Salinity transiently and significantly reduced the nitrate contents of all plants organs (Fig. 24a). The maximum reduction in nitrate contents occurred at the low and moderate salinities and was severe in the juvenile organs (Lj and Sj) and roots (Fig. 24a). Thereafter, nitrate contents of all plant organs increased significantly with increasing water salinity. At 750 mol*m^{-3} NaCl salinity, nitrate concentrations in the adult leaves and roots exceeded the control levels while those of juvenile leaves and adult and juvenile stems did not significantly differ relative to their controls (Fig. 24a). NO_3^- contents of the bladder hairs were much lower as in the corresponding leaf tissues. Salinity declined transiently nitrate excretion of the bladder hairs of the adult and juvenile leaves, with a minimum excretion at 250 and 500 mol*m^{-3} NaCl for juvenile and adult leaves, respectively (Fig. 25a).

Untreated *A. leucoclada* control plants had markedly higher NO_3^- concentrations (on average over all organs 13.2 –8.0 mmol*l^{-1} press sap) than those of *A. nummularia*. Increased salinity led to a decrease of nitrate concentrations of all plants organs, with minimum concentrations being 4.5 – 23.6 mmol*l^{-1} press sap at the highest salinity treatment (Fig. 24b). The bladder hairs contained small quantities of NO_3^- (0.01 and 0.14 µmol*cm^{-2}) in adult and juvenile bladder hairs, respectively. As shown in Fig. (25b), salinity reduced significantly the nitrate excretion of the juvenile leaves while it did not significantly affect the adult ones.

3.4.2.1.3 Phosphate contents

A. nummularia control plants contained high tissue phosphate concentration, ranging between 31.0 (R) to 167.8 (Sa) mmol*l^{-1} press sap. Phosphate

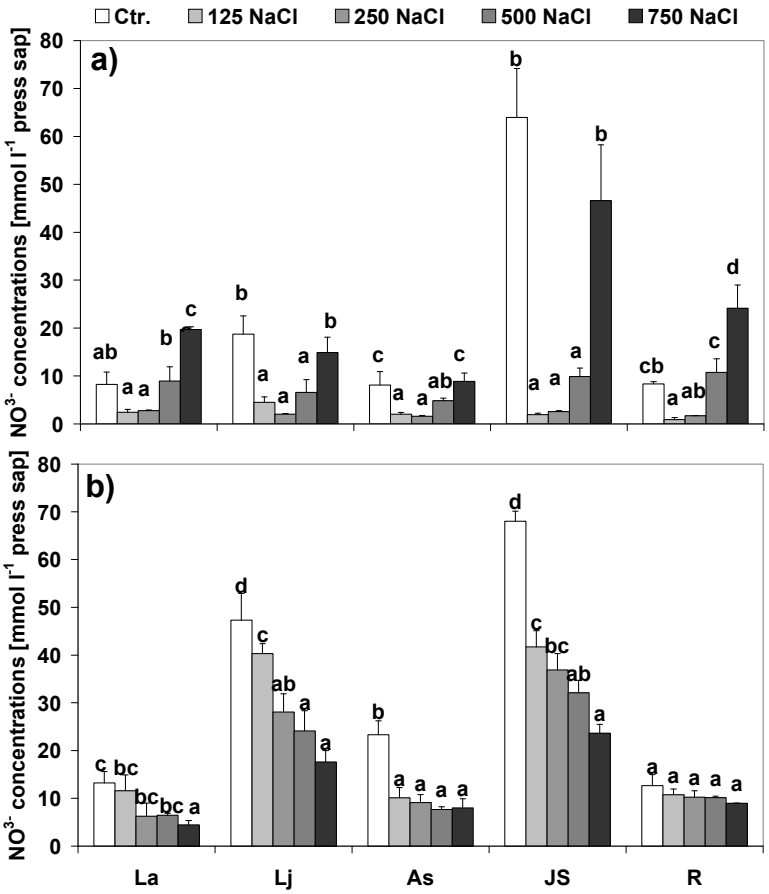

Fig. 24: Effect of increasing NaCl salinity on NO^{3-} concentrations of the different organs of a) *A. nummularia* and b) *A. leucoclada.* La, adult leaves; Lj, juvenile leaves; Sa, adult stems; Sj, juvenile stems; R, roots. Each column represents the mean values of nine replicates and the bars represent standard errors. Columns with the same letter are not significantly different at $P< 0.05$, LSD test.

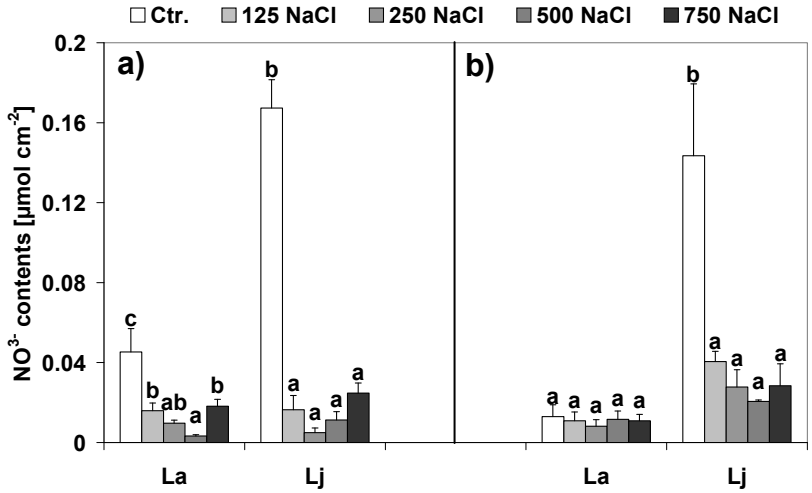

Fig. 25: NO^{3-} excretion (leaf area basis) of the bladder hairs of the adult and juvenile leaves of a) *A. nummularia* and b) *A. leucoclada* at varying NaCl salinity levels. La, adult leaves; Lj, juvenile leaves. Each column represents the mean values of nine replicates and the bars represent standard errors. Columns with the same letter are not significantly different at $P< 0.05$, LSD test.

concentration in all plant organs tended to decrease as the water salinity increased (Fig. 26a). These declines were transiently in the adult leaves, juvenile stems and roots and gradually in the juvenile leaves and adult stems. Analysis of variance between the means showed that the effect of NaCl salinity on the tissues phosphate concentrations was not statistically significant.

On average over all the plant organs, *A. leucoclada* had lower phosphate concentrations (of about 29.7 – 92.6 mmol*l^{-1} press sap) than *A. nummularia* and a decline occurred with increasing NaCl salinity (Fig. 26b). These decreases were transient only for the adult and juvenile leaves. Statistically, there were no differences in the phosphate concentrations of the adult leaves and juvenile stems at the whole range of salinity treatments. At high salinity level, phosphate was accumulated in the leaves, reaching nearly the control levels but it decreased by 36, 27 and 46% in the adult stems, juvenile stems and roots respectively (Fig. 26b).

3.4.2.1.4 Sulphate contents

Sulphate concentrations in the tissues of *A. nummularia* control plants ranged between 11.1 (Sj) and 137.3 mmol*l^{-1} press sap (La). Fig. 27a shows that salinity of 125 mol*m^{-3} NaCl led to a decline of the sulphate concentration of all organs with the exception of the adult leaves where the concentrations were slightly but not significantly higher. For all organs, no significant changes in the sulphate concentrations were found at salinities higher than 125 mol*m^{-3} NaCl.

Sulphate concentrations were slightly lower in *A. leucoclada* than in *A. nummularia* for all plant organs. They ranged from 6.5 (Sa and R) to 14.9 (Lj) mmol*l^{-1} press sap. The sulphate concentrations in all plant organs declined as the salinity increased with the exception of the juvenile leaves where they increased slightly (Fig. 27b). These effects, however, were not statistically significant for the juvenile leaves, adult stems and roots.

3.4.2.2 Organic anions

3.4.2.2.1 Oxalate contents

The oxalate concentrations of *A. nummularia* control plants ranged between 8.5 (La) and 47.0 (Lj) mmol*l^{-1} press sap. Oxalate concentrations decreased transiently as the salinity rose in all plant organs with the exception of the adult

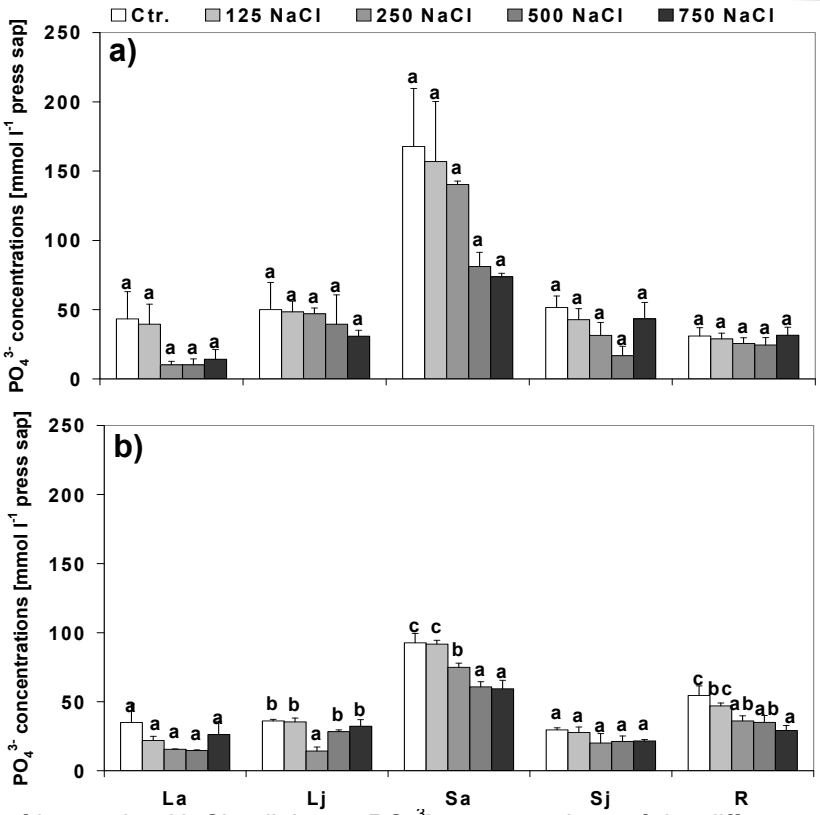

Fig. 26: Effect of increasing NaCl salinity on PO_4^{3-} concentrations of the different organs of a) *A. nummularia* and b) *A. leucoclada.* La, adult leaves; Lj, juvenile leaves; Sa, adult stems; Sj, juvenile stems; R, roots. Each column represents the mean values of three replicates and the bars represent standard errors. Columns with the same letter are not significantly different at $P < 0.05$, LSD test.

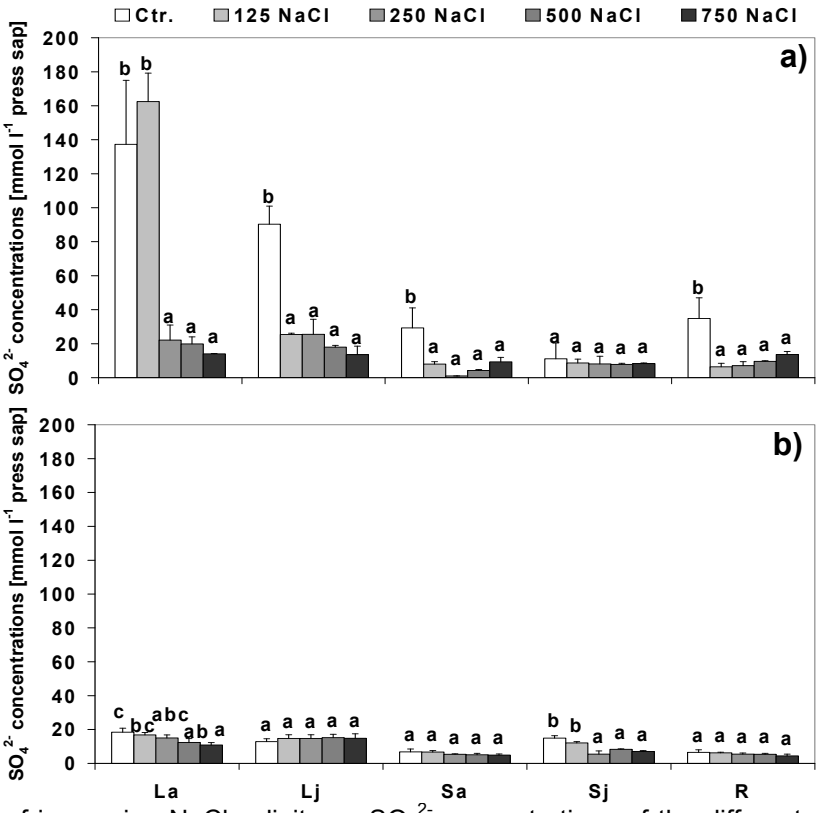

Fig. 27: Effect of increasing NaCl salinity on SO_4^{2-} concentrations of the different organs of a) *A. nummularia* and b) *A. leucoclada.* La, adult leaves; Lj, juvenile leaves; Sa, adult stems; Sj, juvenile stems; R, roots. Each column represents the mean values of three replicates and the bars represent standard errors. Columns with the same letter are not significantly different at $P < 0.05$, LSD test.

leaves in which the oxalate gradually increased (Fig. 28a). Oxalate contents of all organs exceeded the control levels at the highest salinity treatment. Root oxalate contents remained approximately constant at the whole range of salinity treatments (Fig. 28a).

Fig. (28b) reveals that oxalate concentrations of *A. leucoclada* plants were in the mean lower (8.2 – 20.5 mmol*l^{-1} press sap) than those of *A. nummularia* under control conditions. Their concentrations increased with elevating salinity. However, this effect was statistically significant only for the adult and juvenile leaves.

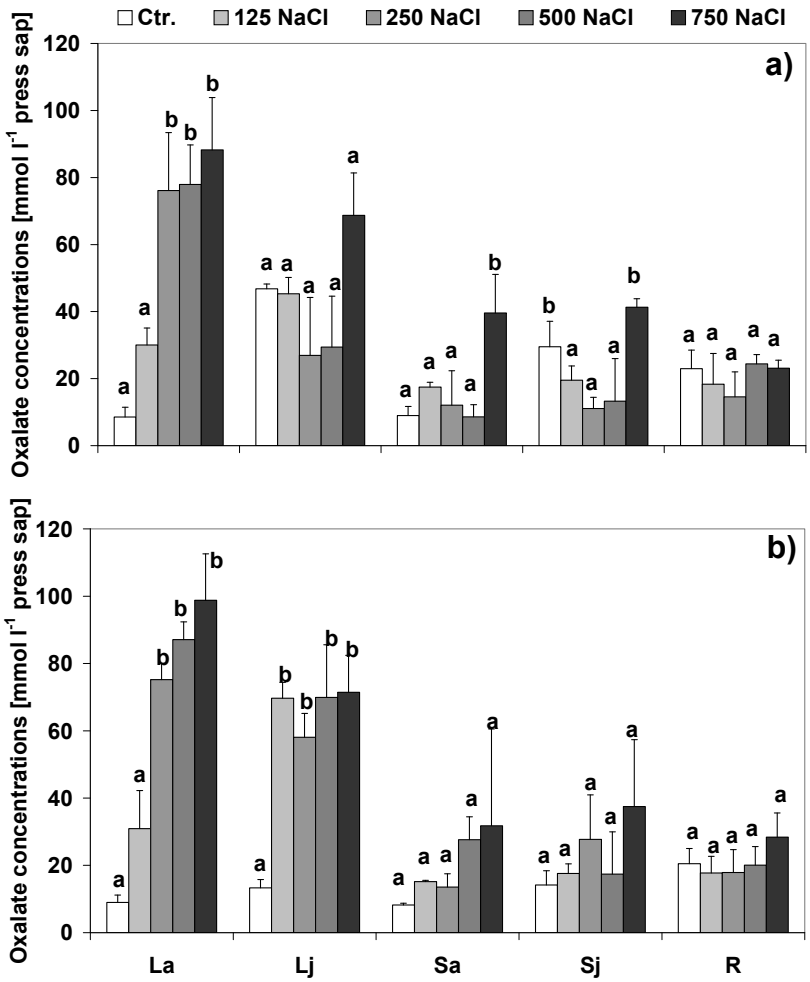

Fig. 28: Oxalate concentrations of the different organs of a) *A. nummularia* and b) *A. leucoclada* at varying NaCl salinity levels. La, adult leaves; Lj, juvenile leaves; Sa, adult stems; Sj, juvenile stems; R, roots. Each column represents the mean values of nine replicates and the bars represent standard errors. Columns with the same letter are not significantly different at $P< 0.05$, LSD test.

3.4.2.2.2 Malate contents

Malate content of the different organs of both *Atriplex* species were also measured, but its concentrations were always below the detection level. Therefore, it was not possible to get convincing results.

3.5 Effect of salinity on carbon, nitrogen and sulphur contents

3.5.1 Carbon contents

Comparable carbon contents (in % DW) were found in the adult and juvenile leaves and roots of *A. nummularia* plants under control condition, being 41.2 ± 0.7, 41.0 ± 1.1 and 40.2 ± 2.7% respectively. Carbon contents of these organs declined as the external NaCl concentration increased (Fig. 29a). Raising water salinity from 0 to 125 mol*m^{-3} NaCl reduced significantly (P < 0.05) the carbon content in the adult leaves and roots. Further increases in salinity did not significantly affect the adult leaves carbon content, but slightly and significantly increased that of the roots (Fig. 29a). As for the juvenile leaves, %C reduced gradually with increasing water salinity, with maximum and significant reduction at the highest water salinity level (Fig. 29a). At this level, %C of the different plant organs was lower than the controls measured 33.9 ± 3.1, 33.1 ± 3.1 and 35.2 ± 3.5 for the adult leaves, juvenile leaves and roots respectively.

With the exception of the roots, % C of *A. leucoclada* was significantly (P < 0.05) lower than that of *A. nummularia* under control conditions. It was 37.5 ± 2.3, 35.5 ± 2.2 and 38.2 ± 4.0% for the adult leaves, juvenile leaves and the roots of *A. leucoclada* controls respectively. As shown in Fig. 29b, salinity gradually reduced the carbon contents of the adult and juvenile leaves, with less adverse effect on the juvenile leaves, while it did not significantly (P < 0.05) impact those of the roots. At the highest water salinity level, the roots exhibited the highest carbon content (38.2 ± 6.1%), while the adult and juvenile leaves showed lower levels (25.6 ± 3.2 and 27.1 ± 1.1% respectively).

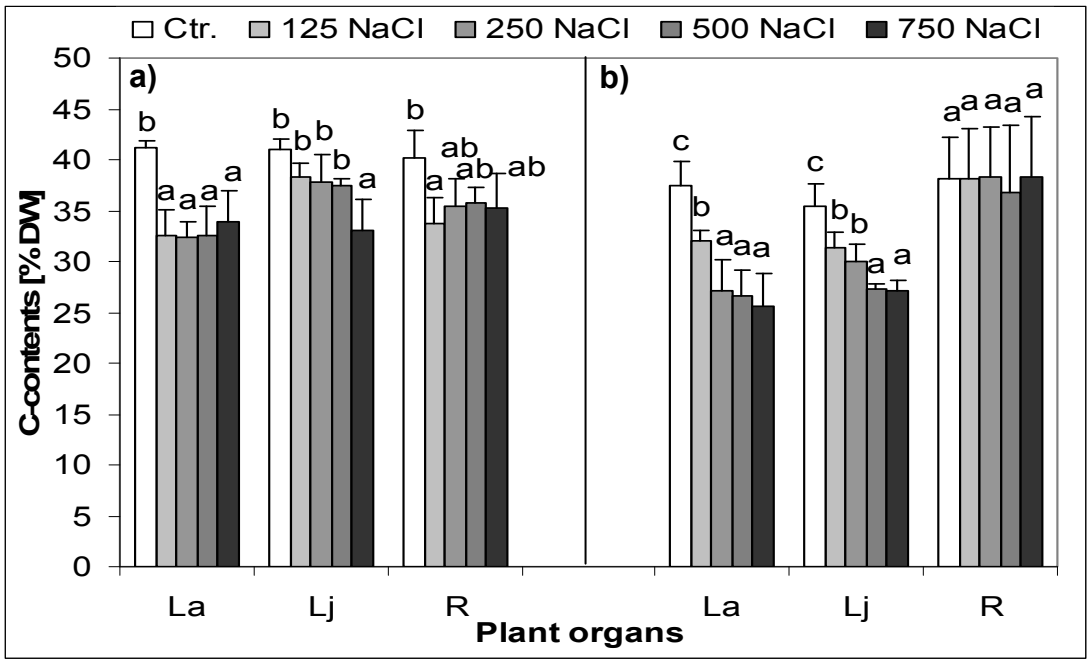

Fig. 29: Effect of various NaCl-salinity on the carbon contents (% dry weight) of the adult leaves (La), juvenile leaves (Lj) and roots (R) of a) *A. nummularia* and b) *A. leucoclada.* Each column represents the mean values of nine replicates and the bars represent standard errors. Columns with the same letter are not significantly different at $P< 0.05$, LSD test.

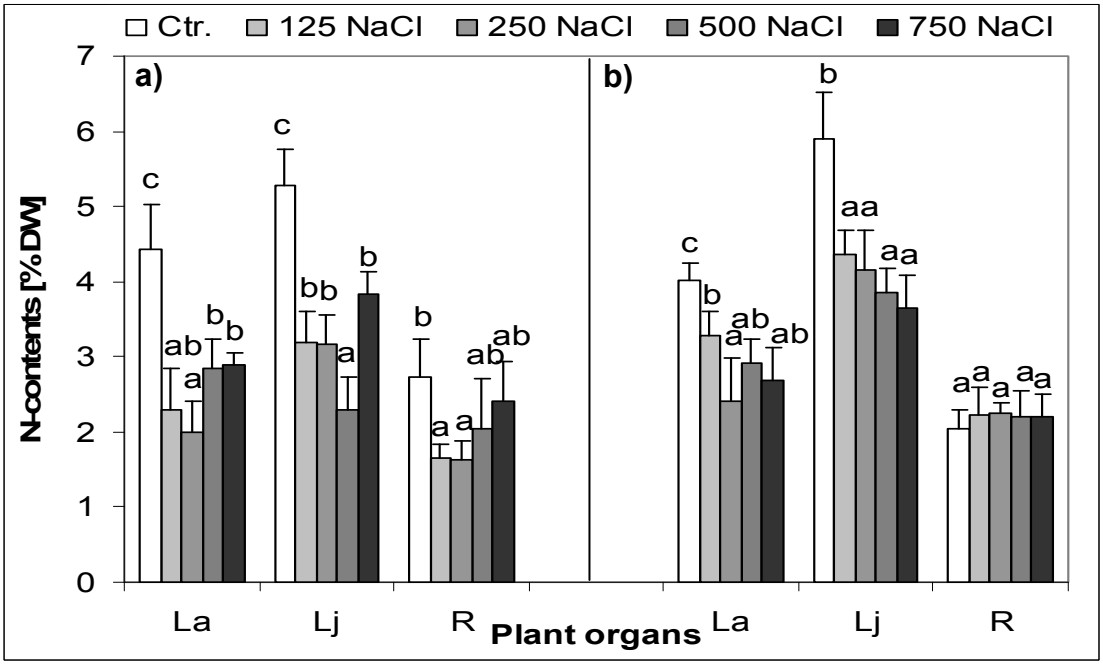

Fig. 30: Effect of various NaCl-salinity on the nitrogen contents (% dry weight) of the adult leaves (La), juvenile leaves (Lj) and roots (R) of a) *A. nummularia* and b) *A. leucoclada.* Each column represents the mean values of nine replicates and the bars represent standard errors. Columns with the same letter are not significantly different at $P< 0.05$, LSD test.

3.5.2 Nitrogen content

%N of *A. nummularia* control plants averaged 4.4 ± 0.6, 5.3 ± 0.5 and 2.8 ± 0.5% in the adult, juvenile leaves and roots respectively. It decreased transiently with increasing water salinity (Fig. 30a). The highest significant ($P < 0.05$) reduction in the %N occurred at the moderate salinity for the adult leaves and roots and at salinity of 500 mol*m^{-3} NaCl for the juvenile leaves. High salinity treatment significantly ($P < 0.05$) reduced the %N, with more severe effect on the adult leaves (Fig. 30a). It caused about 35, 28, and 13% reduction in the adult leaves, juvenile leaves and roots respectively relative to controls.

A. leucoclada control plants had relatively lower %N than *A. nummularia* (except for the juvenile leaves). The mean %N was 4.0 ± 0.2, 5.9 ± 0.6 and 2.0 ± 0.3% in the La, Lj, and R respectively. In tendency, %N of the La and Lj was reduced, while that of the roots did not significantly change as the substrate salinity increased (Fig. 30b). There were reductions of about 34 and 38% in the nitrogen content of the La and Lj respectively at the highest salinity treatment.

3.5.3 Sulphur content

In *A. nummularia*, the juvenile leaves contained higher sulphur contents (0.8 ± 0.1% (dry weight basis) than the adult leaves (0.4 ± 0.1%) and the roots (0.3 ± 0.1%) under control conditions. NaCl salinity transiently increased the %S of the adult leaves, gradually and significantly ($P < 0.05$) reduced %S of the juvenile leaves, but did not significantly affect that of the roots (Fig. 31a). High salinity level reduced the %S to about 24, 45, and 3% in the adult leaves, juvenile leaves and roots respectively.

Concerning *A. leucoclada*, %S was lower in both juvenile leaves and roots and higher in adult leaves than in *A. nummularia* under control conditions. The mean %S of *A. leucoclada* controls was 0.8 ± 0.1, 0.6 ± 0.1, 0.3 ± 0.1% for the juvenile leaves, adult leaves and roots respectively. Similar to *A. nummularia*, raising water salinity increased transiently the %S of the adult leaves, gradually decreased that of the juvenile leaves and did not significantly affect that of roots (Fig. 31b). Reductions of about 8, 60 and 23% in the %S of adult leaves, juvenile leaves and roots, respectively, occurred at the highest water salinity treatment.

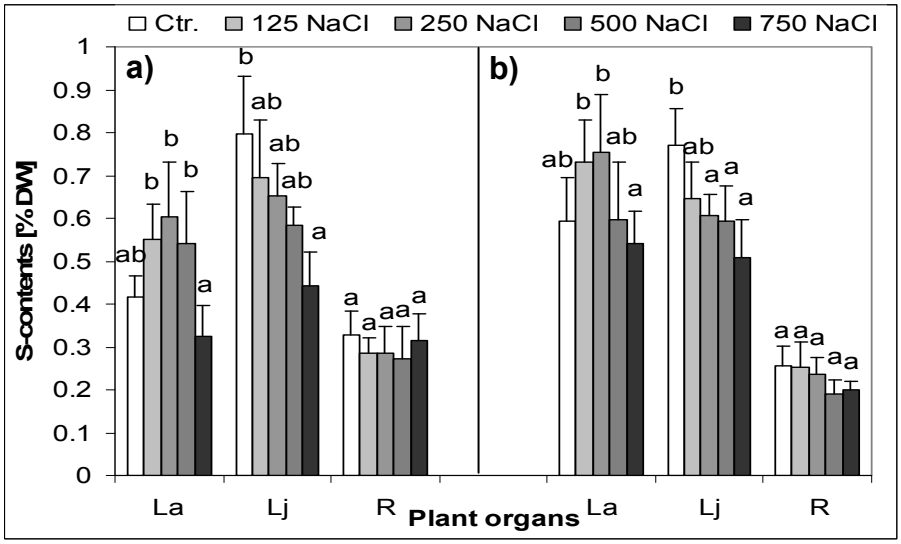

Fig. 31: Effect of various NaCl-salinity on the sulphur contents (% dry weight) of the adult leaves (La), juvenile leaves (Lj) and roots (R) of a) *A. nummularia* and b) *A. leucoclada.* Each column represents the mean values of nine replicates and the bars represent standard errors. Columns with the same letter are not significantly different at $P < 0.05$, LSD test.

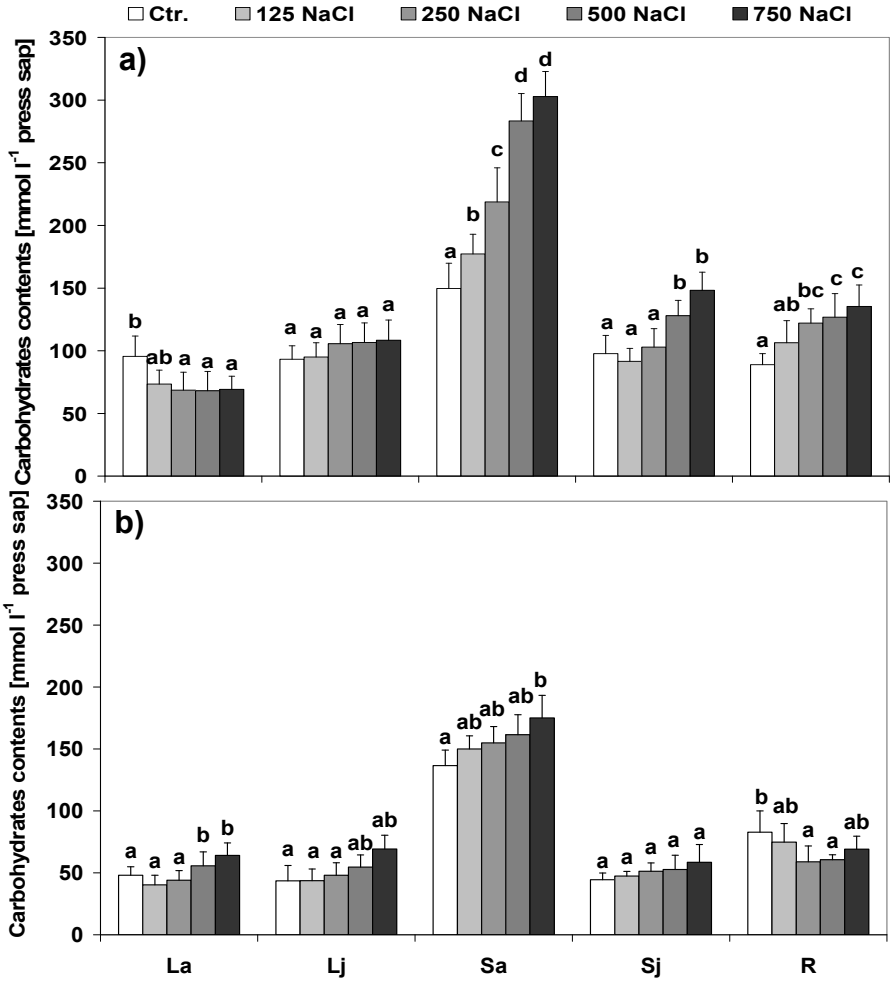

Fig. 32: Total soluble carbohydrate contents of the different plant organs of a) *A. nummularia* and b) *A. leucoclada* as affected by elevated NaCl salinity. La, adult leaves; Lj, juvenile leaves; Sa, adult stems; Sj, juvenile stems; R, roots. Each column represents the mean values of nine replicates and the bars represent standard errors. Columns with the same letter are not significantly different at $P < 0.05$, LSD test.

3.6 Effect of salinity on the total soluble carbohydrates content

In *A. nummularia* control plants, the total soluble carbohydrate content (TSC) on average over all plant organs ranged between 88.9 (R) and 149.7 (Sa) mmol*l^{-1} press sap. With increasing NaCl salinity, the TSC contents of all plant organs (except the adult leaves) notably increased and exceeded clearly the initial values at the highest salinity treatment (Fig. 32a). This effect was more pronounced in the adult stems where an increase of 100% in the TSC occurred at 750 mol*m^{-3} NaCl. Comparison of the means among the different treatments showed that the increase in TSC content with increasing salinity was not significant in the juvenile leaves. As for the adult leaves, TSC concentration significantly decreased with elevated salinity up to 250 mol*m^{-3} NaCl. Further increase in water salinity did not significantly affect the TSC content (Fig. 32a).

The TSC content of the different *A. leucoclada* plant organs was considerably lower than that of the *A. nummularia* at the whole range of salinity treatments. It ranged between 43.56 (Lj) and 136.61 (Sa) mmol*l^{-1} press sap under control conditions. Salinity treatments increased gradually the TSC contents of the Sa, Sj and Lj to exceed that of the controls at the highest salinity treatment, whereas it transiently reduced the TSC contents of the La and R (Fig. 32b). At the high salinity treatment, TSC content of both organs increased significantly and exceeded that of control only in the adult leaves.

3.7 Effect of salinity on the amino acids (AA) content and composition

3.7.1 Total Amino acid (TotAA) content

In control plants of *A. nummularia*, the total amino acid (TotAA) content of the juvenile leaves was highest (588.86 µmol*g^{-1} DW) followed by the adult leaves (460.26 µmol*g^{-1} DW) and the roots (209.60 µmol*g^{-1} DW). Increasing NaCl salinity decreased transiently the TotAA content of both adult and juvenile leaves, with lowest TotAA concentration at salinity of 125 mol*m^{-3} NaCl (Table 7). At this salinity level, approximately 20 and 49% reductions in the TotAA contents were noted for the adult and juvenile leaves respectively. TotAA content increased with a further increase in the NaCl salinity and reached a maximum (503.58 and 852.66 µmol*g^{-1}

DW in the adult and juvenile leaves respectively) at the highest salinity treatment. As for the roots, TotAA contents significantly ($P< 0.05$) and continuously increased with the rise of salinization level, reached maximum (411.77 µmol*g^{-1} DW) at the highest salinity treatment (Table 7).

Data presented in Table (7) reveal that the TotAA contents of the adult and juvenile leaves of *A. leucoclada* were nearly similar (408.97 and 438.19 µmol*g^{-1} DW) and relatively lower than those in *A. nummularia* under control condition. The TotAA contents of the leaves decreased transiently as the external salinity rose. The maximum reduction (about 16%) was observed at 125 and 250 mol*m^{-3} NaCl treatments for the juvenile and adult leaves respectively. High salinity treatment increased the TotAA contents of the leaves, and caused about 34 and 79% increases in the TotAA of adult and juvenile leaves respectively (Table 7). As shown in Table (10), the roots of *A. leucoclada* control plants presented a slightly higher TotAA content (264.77 µmol*g^{-1} DW) compared to those of *A. nummularia* controls. The TotAA contents of the roots of *A. leucoclada* increased transiently (but not significantly) as the substrate salinity rose (Table 7).

Table 7: Contents (µmol*g-1 DW) of total amino acids (TotAA) in the adult leaves (La), juvenile leaves (Lj) and roots (R) of *A. nummularia* and *A. leucoclada* at various NaCl salinities.

Treatments	*A. nummularia*			*A. leucoclada*		
	La	Lj	R	La	Lj	R
Ctr.	460.27 **a** ±110.38	588.86 **b** ± 98.64	209.60 **a** ± 39.49	408.97 **ab** ± 37.38	438.19 **a** ± 28.04	264.77 **a** ± 23.71
125 NaCl	363.66 **a** ± 45.36	295.43 **a** ± 23.88	298.27 **ab** ± 2.57	376.54 **ab** ± 83.49	366.53 **a** ± 84.00	375.01 **a** ± 106.93
250 NaCl	443.34 **a** ± 97.46	407.36 **ab** ± 117.51	322.30 **ab** ± 97.45	340.02 **a** ± 22.98	382.15 **a** ± 85.00	398.55 **a** ± 54.16
500 NaCl	466.04 **a** ± 68.24	516.84 **ab** ± 50.23	460.06 **b** ± 67.84	376.74 **ab** ± 13.35	427.02 **a** ± 61.35	346.27 **a** ± 171.14
750 NaCl	503.58 **a** ± 112.45	852.66 **c** ± 76.75	411.77 **b** ± 59.54	548.56 **b** ± 55.10	787.06 **b** ± 127.02	331.95 **a** ± 18.24

Means within a column followed by the same letter are not significantly different at $P< 0.05$ as determined by LSD test. Each mean represents three replicates.

3.7.2 Amino acids composition

The analysis of the amino acids revealed that Arginine (Arg), Aspargaine (Asp), Glutamic acid (GluA) and Proline (Pro) are the most abundant amino acids in the different organs of both *Atriplex* species. The levels of all the other amino acid were relatively low and seem to be not much affected by salinity treatment. Thus we decided to concentrate only on the changes of Arg, Asp, GlutA and Pro in the La, Lj and R of both *Atriplex* species in response to various NaCl treatments.

Arg, the least abundant amino acid in *A. nummularia* control plants, accounted for only 3, 3 and 5% of the TotAA of the adult leaves, juvenile leaves and roots respectively. As shown in Fig. 33, it transiently decreased in these organs as the NaCl salinity raised, reaching a minimum concentration at the low and moderate salinities. High salinity treatment induced about 8 and 10 folds increases in the Arg concentrations of the adult and juvenile leaves respectively, while it resulted in approximately 25% reductions in the roots Arg concentrations. *In A. leucoclada* control plants, Arg concentrations were distinctly lower in the adult leaves and slightly higher in the juvenile leaves and roots compared to those of *A. nummularia* (Fig. 33). It accounted for about 1.2, 8 and 8% of the TotAA in the adult leaves, juvenile leaves and roots. Arg concentrations reduced transiently in the adult and juvenile leaves, while it did not significantly change in the roots with increasing salinity (Fig. 33). There were about 4 and 0.4 folds increases in its concentration in the adult and juvenile leaves respectively under high salinity conditions. By contrast, the roots displayed about 0.9 fold reduction in the Arg concentration at this salinity level.

Low concentrations of Asp were also detected in *A. nummularia* control plants. They contributed to about 4.5, 5 and 4.7% of the TotAA of the adult leaves and juvenile leaves and roots respectively. Fig. 33 shows that raising external NaCl salinity level did not significantly affect Asp concentration in the adult leaves, but transiently decreased that of the juvenile ones. As for the plant roots, Asp contents increased initially at low NaCl concentrations, then remained unchanged up to 250 mol*m^{-3} NaCl salinity then increased significantly ($P < 0.05$) at the highest salinity treatment. High NaCl treatment caused about 40% reduction in the Asp content of both adult and juvenile leaves and about 60% increase in the root. Relatively low Asp content were also detected in *A. leucoclada* control plants, accounting for about

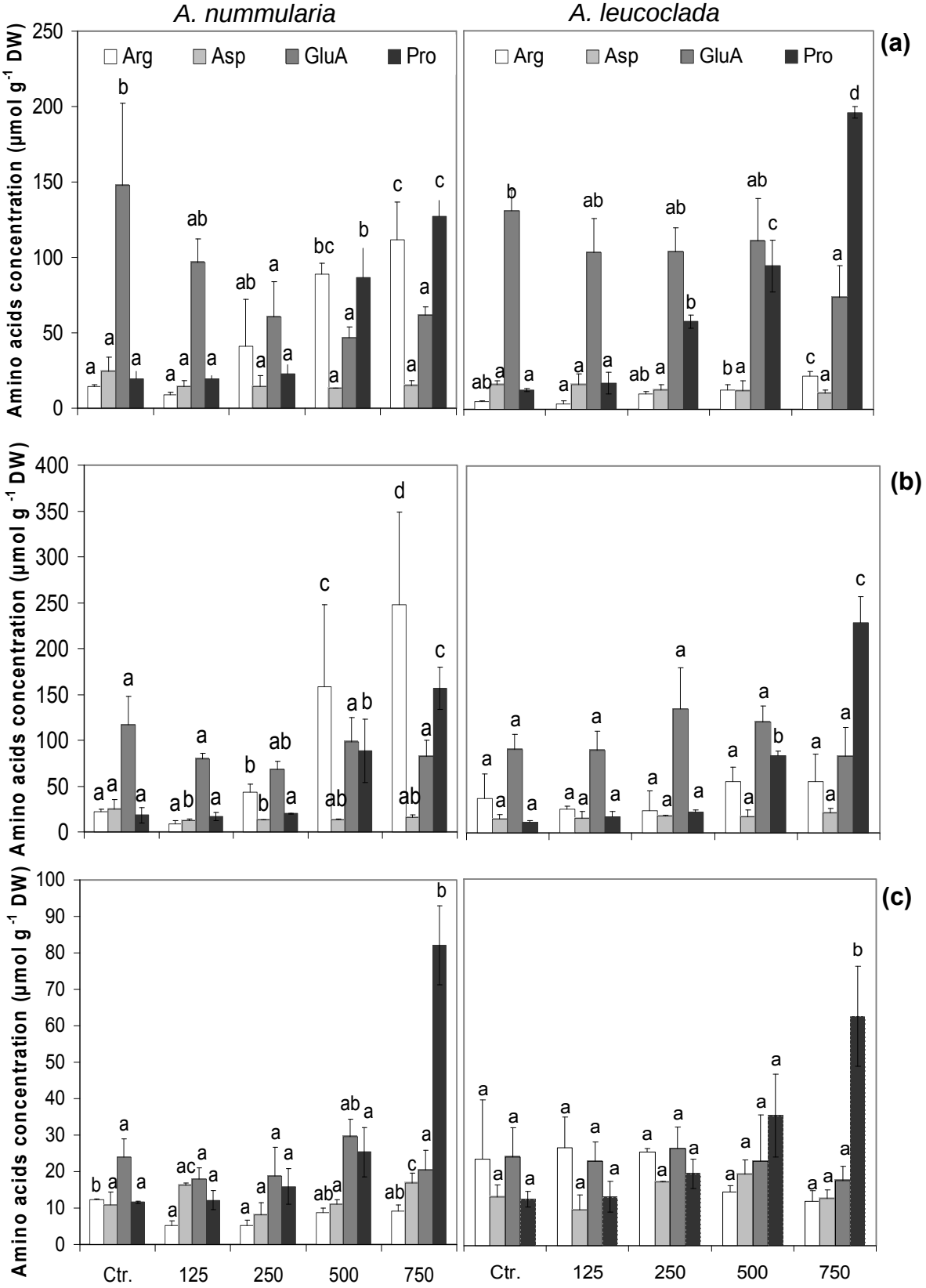

Fig. 33: Arg, Asp, GluA and Pro concentrations (µmol*g^{-1} DW) in the the adult leaves (a), juvenile leaves (b) and roots (C) of *A. nummularia* and *A. leucoclada* at different NaCl salinities. La, adult leaves; Lj, juvenile leaves; Sa, adult stems; Sj, juvenile stems; R, roots. Each column represents the mean values of nine replicates and the bars represent standard errors. Columns with the same letter are not significantly different at $P< 0.05$. LSD test.

4, 3 and 5% of the TotAA of the adult leaves, juvenile leaves and roots respectively. Unlike *A. nummularia*, NaCl treatments had no significant impact on the Asp contents of the different organs of *A. leucoclada* (Fig. 33).

As shown in Fig. 33, GluA was the most abundant amino acids accounted for about 31, 19 and 11% of the TotAA in the adult leaves, juvenile leaves and roots of *A. nummularia* respectively. GluA concentrations decreased in these organs with the rise of salinization level. However, this effect was significant only for the adult leaves. In *A. leucoclada*, GluA concentrations contributed to about 31, 20 and 9% of the TotAA in the adult leaves, juvenile leaves and roots respectively. In tendency, GluA concentration was reduced with increasing NaCl salinity. This effect which was significant only for the adult leaves (Fig. 33).

Low proline (Pro) concentrations were also found in *A. nummularia* plants grown under control conditions. Proline concentration was accounted for about 4, 3 and 5% of the TotAA in the adult leaves, juvenile leaves and roots respectively. Its concentrations in the different plant organs increased slightly (not significantly) with increasing NaCl salinity up to 250 mol*m^{-3} NaCl (Fig. 33. Higher salinity, however, induced progressive increases in the Pro contents, particularly in the juvenile leaves. There were about 6, 8 and 7 fold increases in the Pro levels in the adult leaves, juvenile leaves and roots respectively at the high salinity level. It was the most abundant amino acid accounted for 25 – 40% of the total amino acids in the different plant organs at this salinity. Pro concentration accounted for about 3, 2.5 and 4.5% of the TotAA of the adult leaves, juvenile leaves and roots of *A. leucoclada* control plants. As in *A. nummularia*, Pro concentrations increased slightly at low salinities, and significantly much more pronounced at the high salinity treatments (Fig. 33). The leaves of *A. lecuoclada* accumulated clearly much higher Pro concentrations compared to those of A*. nummularia* at the highest water salinity, while the reverse was observed for the roots. At high salinity level, approximately 15, 20 and 5 fold increases were found in the Pro contents of the adult leaves, juvenile and roots of *A. leucoclada* plants (Fig. 33).

3.8 Osmotic balance

It is well known that plant survival under saline conditions depends on the ability to balance the osmotic burden by decreasing the tissue osmotic potential.

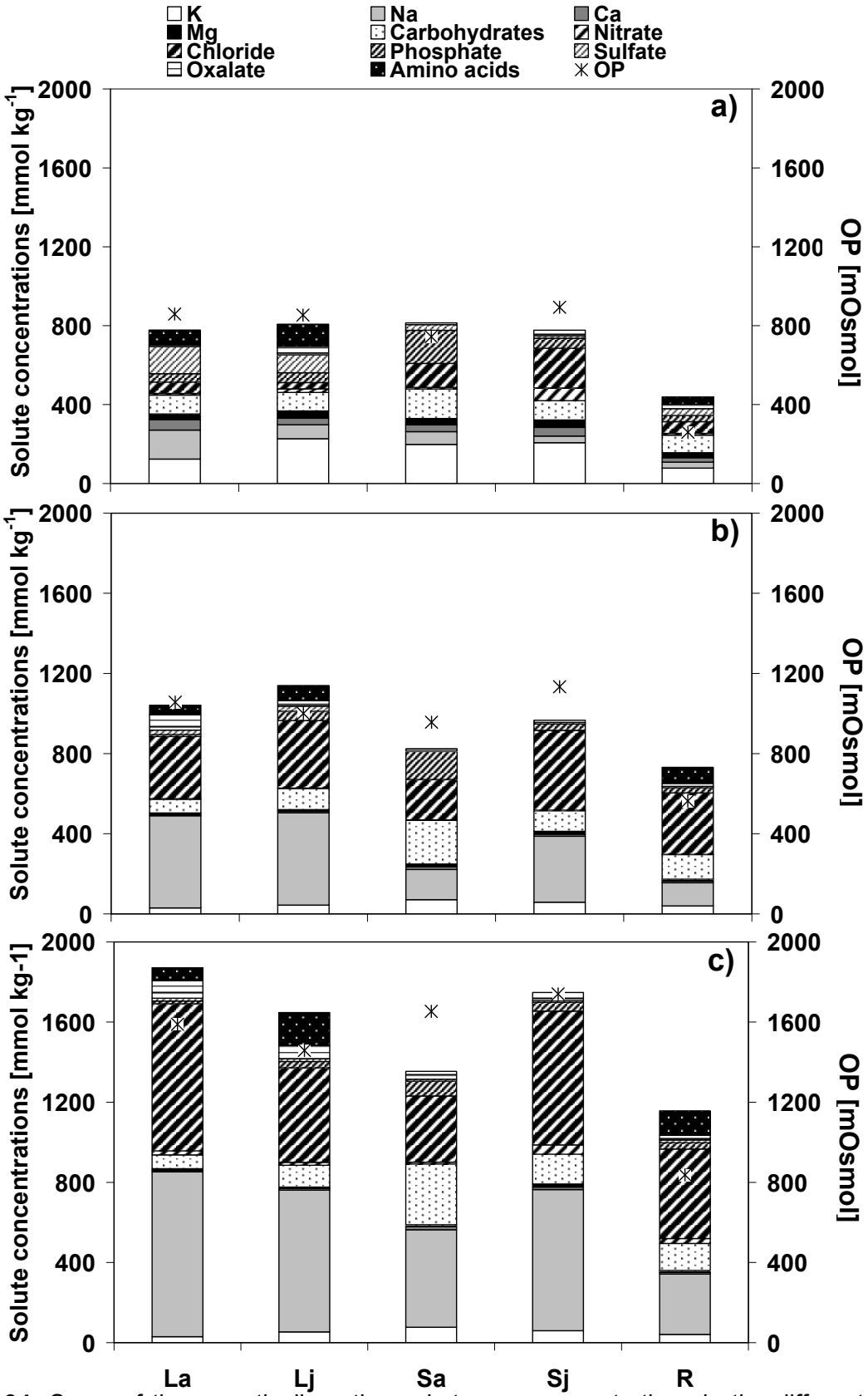

Fig. 34: Sums of the osmotically active substances concentrations in the different organs of *A. nummularia* plants at control (a), 250 mol*m^{-3} NaCl (b) and 750 mol*m^{-3} NaCl (c) salinities. The asterix marks the corresponding osmotic potential of the press saps.

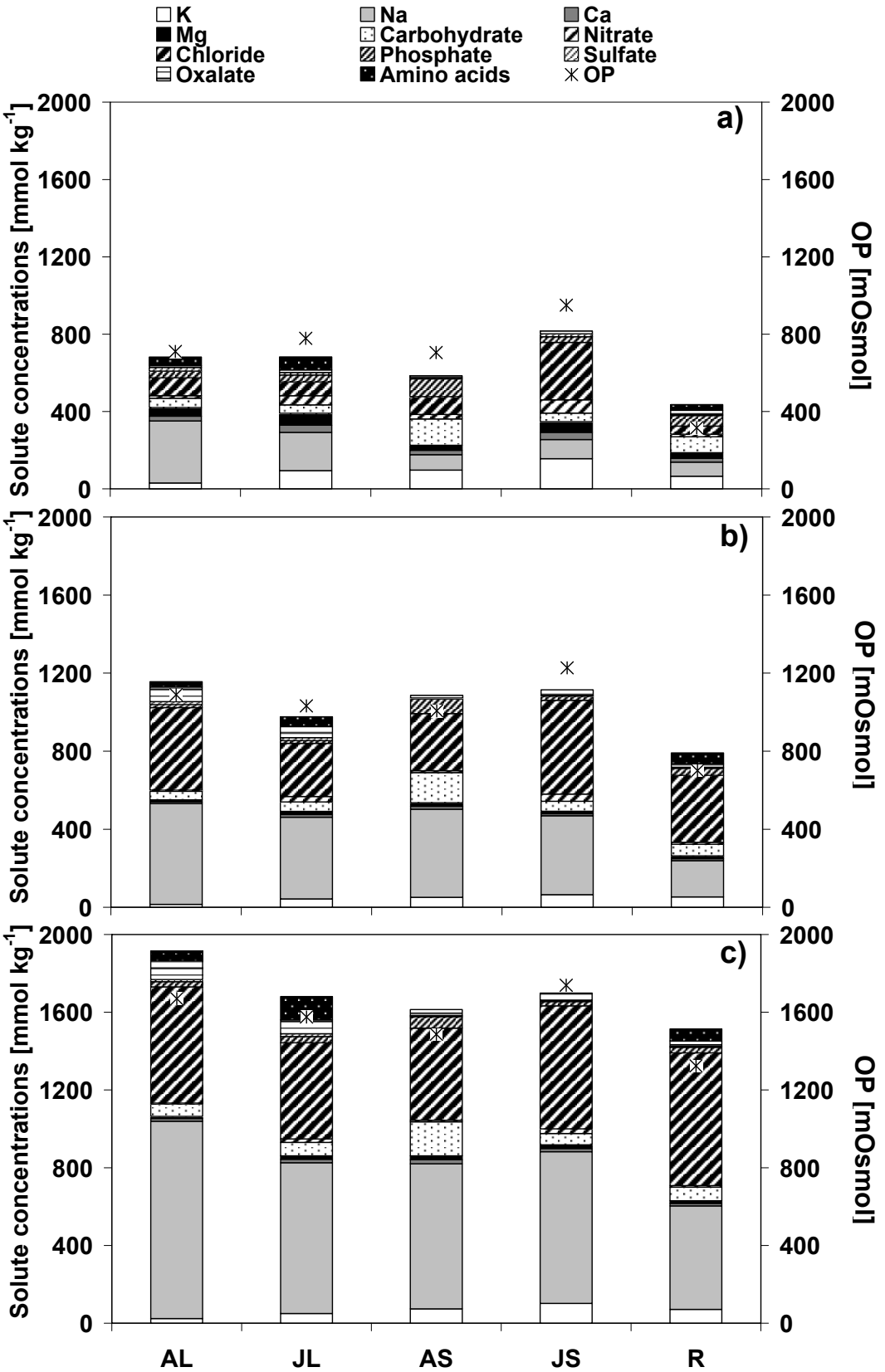

Fig. 35: Sums of the osmotically active substances concentrations in the different organs of *A. leucoclada* plants at control (a), 250 mol*m^{-3} NaCl (b) and 750 mol*m^{-3} NaCl (c) salinities. The asterix marks the corresponding osmotic potential of the press saps.

This balance of osmotic potential will be reached by increasing the concentrations of osmotically active substances within the plant tissues in response to elevated water salinity. As can be seen in Fig. 34 and 35, the sums of all osmotically active solute concentrations increased parallel to the external salinity in all organs of both *Atriplex* species. From quantitive point of view, the sum of solute concentrations were sufficient to explain more than 95% of the osmotic potentials of all organs of both *Atriplex* species at all salinity levels. The accumulation of Na^+ and Cl^- ions in the different plant organs seem to play the main role in the osmotic adjustment in all plant organs especially at high salinity treatment

3.9 Effect of salinity on the total soluble protein content

The mean total soluble protein (TSP) content of *A. nummularia* controls ranged between 0.2 (R) and 0.9 (Lj) $mg*l^{-1}$ press sap (Fig. 36a). Elevating substrate salinity transiently decreased the TSP concentration of adult and juvenile leaves and juvenile stems (Fig. 36a). High salinity treatment significantly ($P < 0.05$) enhanced the TSP content of juvenile leaves to reach higher level than the control only in the juvenile stems. As shown in Fig. 36a, no significant differences in the adult leaves TSP content were observed at salinities above 125 $mol*m^{-3}$ NaCl. In contrary, the soluble protein content of the adult stems and the roots increased gradually as the water salinity rose. However, this effect was only statistically significant in the roots (Fig. 36a).

A. leucoclada plants exhibited generally lower TSP than *A. nummulatria* plants at all salinity treatments except in the roots. TSP on average over the different organs of *A. leucoclada* control plants ranged from 0.3 (La) to 4.3 (Sa and Sj) $mg*l^{-1}$ press sap. Increasing salinity levels transiently reduced the TSP of all plant organs, with maximum reductions at low and moderate salinities (Fig. 36b) . High salinity treatments increased the TSP contents which exceeded the control level in all organs except for the juvenile leaves. However, comparison of means among treatments showed that the changes in TSP content of juvenile stems were not statistically significant.

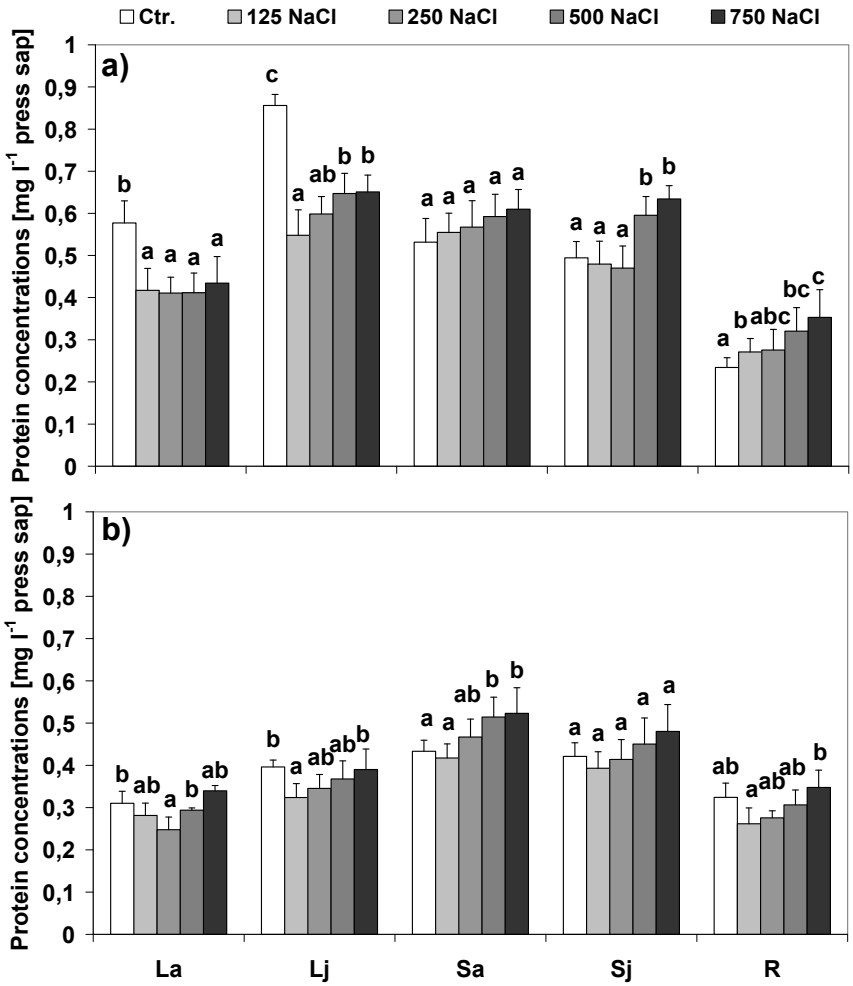

Fig. 36: Total soluble protein content (TSP) of the different plant organs of a) *A. nummularia* and b) *A. leucoclada* as affected by elevated NaCl salinity. La, adult leaves; Lj, juvenile leaves; Sa, adult stems; Sj, juvenile stems; R, roots. Each column represents the mean values of nine replicates and the bars represent standard errors. Columns with the same letter are not significantly different at $P< 0.05$, LSD test.

3.10 Proteomics

3.10.1 2D-gel electrophoresis and protein expression patterns

Figures 37 a and 38a show representative 2D-gel electrophoresis of the fully expanded leaves of *A. nummularia* and *A. leucoclada* respectively. Using the Delta 2D software (DECODON GmbH, Germany), about 200 - 250 protein spots could be reproducibly detected on the Comassie-stained gels of both *Atriplex* species (control condition). Most of these proteins occurred in the pH range of 5 – 8. The protein expression patterns of salt treated leaves of *A. nummularia* and *A. leucoclada* (Fig. 37b and 38b respectively) were compared with those of the corresponding controls. A general repression effect of high salinity treatment on the protein expression of the leaves was noted in both *Atriplex* species. The number of down regulated proteins and the degree of repression were higher in *A. leucoclada* than *A. nummularia*. Approximately 55 and 85% of the protein spots in *A. nummularia* and *A. leucoclada*, respectively, were differentially regulated under high salinity condition. In *A. nummularia*, about 5% of protein spots were up regulated, 38% down regulated, and 13% disappeared at high salinity concentration. In the case of *A. leucoclada*, there were about 0% up regulation, 46% down regulation and 39% disappear in the protein spots. Moreover, no new protein spot was detected in both *Atriplex* species under high salinity condition compared to control.

Due to the high number of differentially expressed proteins, we decided to concentrate only on those with percentage volume higher than 0.1. On these criteria, about 70 and 79 protein spots were selected for *A. nummularia* and *A. leucoclada* leaves, respectively. The numbers on the gels in Figures 37a and 38a refer to them. Of these proteins, 48 spots were down regulated, 12 spots were up regulated and 10 spots disappeared in *A. nummularia* at high NaCl concentration. In *A. leucoclada*, 60 spots were down regulated and 19 spots disappeared at high salinity level. The ratio of repression or induction (percentage spot volume of control/percentage spot volume of 750 $mol*m^{-3}$ NaCl) of these proteins was calculated. About 33 protein spots that were regulated by a factor of at least two folds (repression or induction) when compared to the corresponding control were selected for further characterization. The experimental pI and MW were used firstly for the identification of these proteins using the public databases SWISS-PROT, TrEMBL and NCBInr (http://www.expasy.com). Searching using only

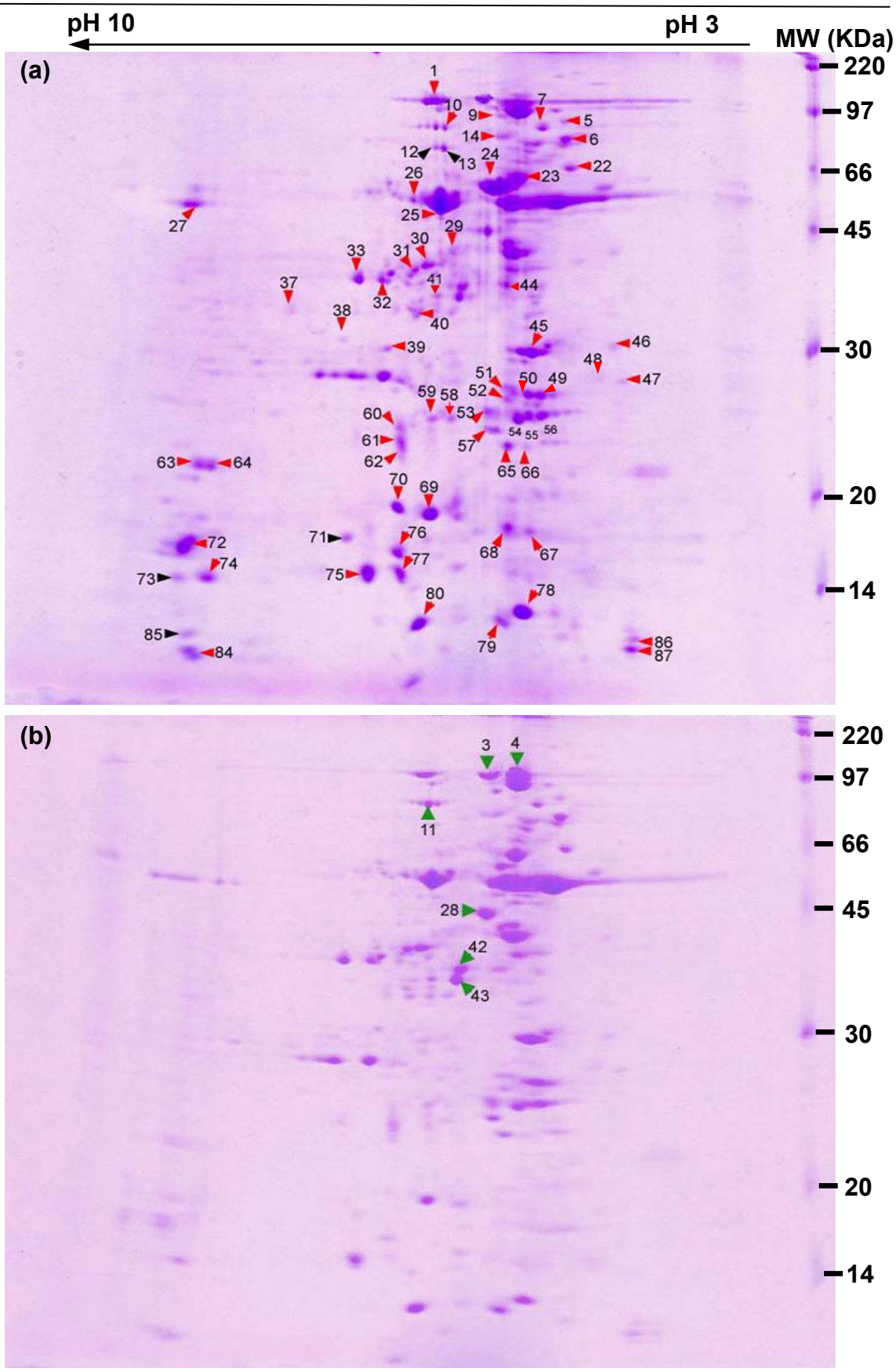

Fig. 37: Representative 2D-gel electrophoresis of the leaf proteins from untreated (a) and 750 mol*m^{-3} NaCl treated (b) *A. nummularia* plants. The numbers on the gels refer to the proteins which are differentially regulated under salinity condition and have percentage volume higher than 0.1% as quatified with Delta 2D software. The red arrows refer to the down-regulated proteins, the green arrows refer to the up-regulated proteins and the black ones refer to those proteins, which disappear under salinity treatment.

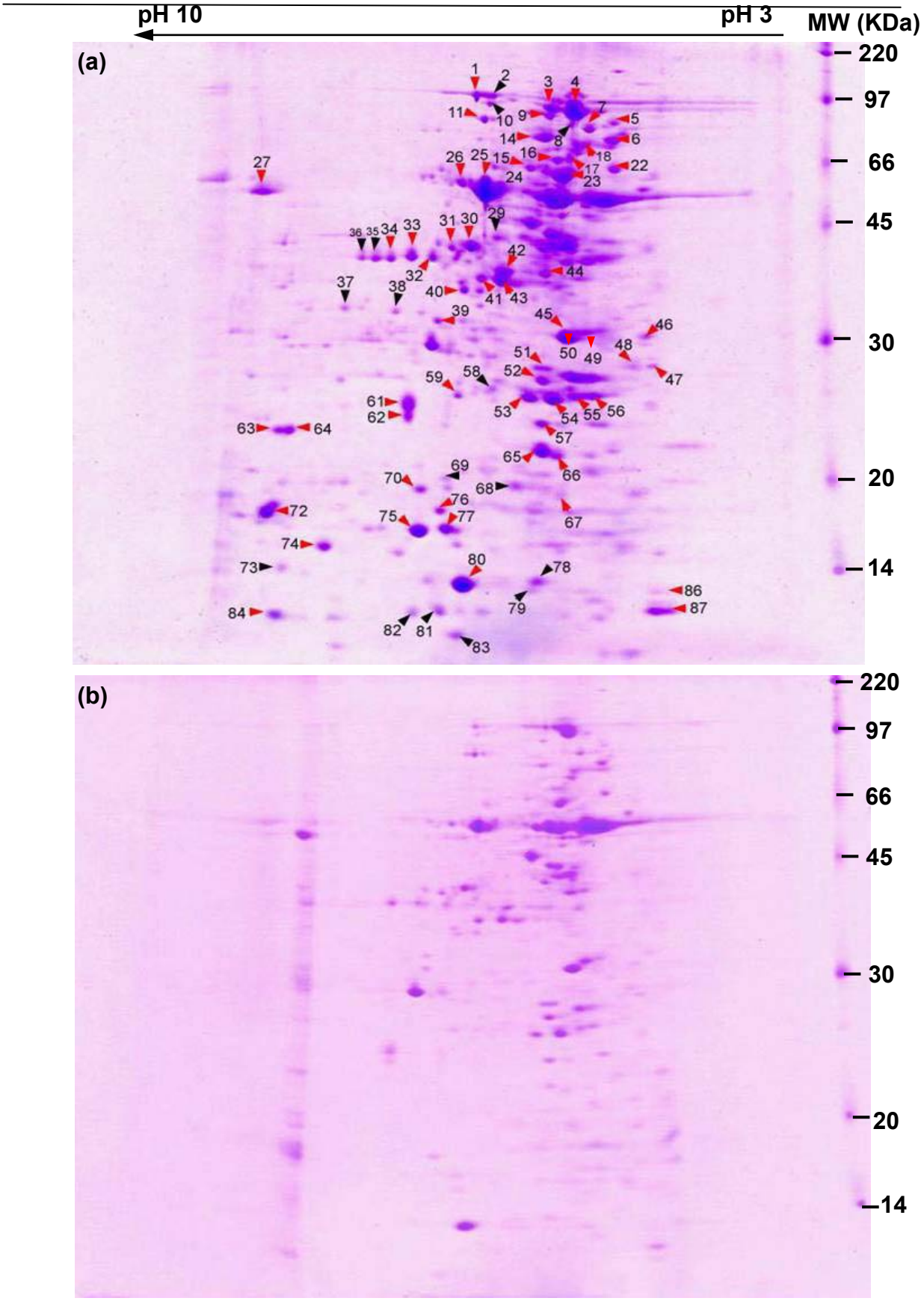

Fig. 38: Representative 2D-gel electrophoresis of the leaf proteins from untreated (a) and 750 mol*m^{-3} NaCl treated (b) *A. leucoclada* plants. The numbers on the gels refer to the proteins which are differentially regulated under salinity condition and have percentage volume higher than 0.1% as quatified with Delta 2D software. The red arrows refer to the down-regulated proteins, the green arrows refer to the up-regulated proteins and the black ones refer to those proteins, which disappear under salinity treatment.

the experimental pI and MW did not give valuable information about these proteins and a long list of probable protein names was obtained for each protein spot (see appendix, Table A4).

3.10.2 MALDI-TOF mass spectrometry and protein identification

Since searching the databases failed to give valuable information about these proteins, some of them were analyzed using Matrix Assisted Laser Desorption/Ionization - Time Of Fight - Mass Spectrometry (MALDI-TOFF-MS). Because of the high costs of this method, only 11 protein spots that showed very conspicuous expression profiles were chosen: Spots 1, 25, 61, 75, 76 and 77 (which showed more than 2-fold down regulation under high salinity treatment in both *Atriplex* species), and spots 4, 11, 28, 42 and 43 (which were down regulated in *A. leucoclada* but up regulated in *A. nummularia* under high NaCl salt stress). Figs. A2 and A3 (see appendix) show representative mass spectra (peptide mass fingerprint) of the analyzed protein spots. The peptide mass fingerprint (PMF) of each protein spot was then searched against the public protein databases Mascot (http://www.matrixscience.com), ProFound (http://www.proteometrics.com) and Aldente (http://www.expasy.org/cgi-bin/aldente/form.cgi) for the identification. Only six protein spots (4, 11, 25, 28, 42 and 43) were successfully identified with high scoring (probability) whereas five proteins (1, 61, 75, 76 and 77) were not identified using these databases. All proteins in Table (8) are the first protein candidates in the search result lists. The identified proteins can be classified into two groups.

I- Proteins involved in photosynthesis and carbohydrate metabolism – This group includes four protein spots (spot No. 4, 25, 42 and 43), with a relation to the C_4 photosynthesis pathway.

Spot No. 4 was identified as Pyruvate orthophosphate dikinase (PPDK) (EC 2.7.9.1) similar to the PPDK from *Mesembryanthemum crystallinum* and *Zea mays*. As is shown in Figs. 37 and 38, the PPDK was up regulated and down regulated in the leaves of *A. nummularia* and *A. leucoclada* respectively at high salinity treatment.

Spot No. 25 was identified (with high confidence) as ribulose1,5-bisphosphate carboxylase-oxygenase large subunit (EC 4.1.1.39). It was markedly down

Table 8: Identification of some salt responsive proteins of the leaves of *A. nummularia* and *A. leucoclada* using MALDI-TOF-MS. All protein names in the table are the first protein candidate in the search result of the used data bases. The induction or repression factor was calculated as the ratio of percentage spot volume of the control to percentage spot volume of the high salt treatment.

Spot	Protein name	Theoretical/experimental		Induction/repression factor	
		MW (KDa)	p*I*	*A. nummularia*	*A. leucoclada*
1	• Putative disease resistance protein	115/97	6.4/6.5	2.42	2.77
	• Phosphoenolpyruvate carboxylase.	109/97	6.0/6.5		
4	• Pyruvate orthophosphate dikinase.	95/95	5.1/5.5	0.89	2.04
11	• 5-methyltetrahydropteroyltriglutamate-homocysteine transmethylase.	85.06/80	5.9/6.5	0.26	3.17
25	• Ribulose 1, 5-bisphosphate carboxylase-oxygenase large subunit.	50.04/52	6.1/6.5	2.55	2.99
28	• S-adenosyl-L-methionine synthase	43.6/45	5.6/5.8	0.500	2.06
42	• plastidic fructose-bisphosphate aldolase	42.8/37	6.9/6.5	0.49	5.71
43	• Cytoplasmic malate dehydrogenase.	36/37	6.0/6.5	0.40	4.91
61	• P protein-like	22/25	5.6/7	2.55	8.54
	• Isopentenyl-diphosphate delta–isomerase	27/25	5.3/7		
	• Hypothetical protein.	29/25	5.9/7		
75	• Hypothetical protein	19/18	10.8/7.5	1.93	n.d.
	• Putative mitochondrial malate dehydrogen.	22/18	7.6/7.5		
	• Disease resistance-like protein.	18/18	8.5/7.5		
	• Phytochrome B	21/18	7.2/7.5		
76	• 17.9 kDa class II heat shock protein	18/20	7.7/7.2	10.10	n.d.
77	• Hypothetical protein	19/18	6.1/7.2	23.12	n.d.
	• Pin II-type proteinase inhibitor 12 (framnet)	24/18	6.3/7.2		
	• S-receptor kinase related protein (fragment)	18/18	6.4/7.2		
	• Chlorophyll a/b-binding protein	28/18	6.4/7.2		

Mw: molecular weight; p*I*: isoelectric point; n.d.: not determind (spot protein was not detect in the gels of high salinity treatment)

regulated under high NaCl salinity treatment in comparison to the corresponding control in both *Atriplex* species (Figs. 37 and 38).

Spot No. 42: A plastidic fructose-bisphosphate aldolase (ALdP) (EC 4.1.2.13), similar to that of *Nicotiana paniculata* was detected on this spot. The abundance of ALdP increased in the salt treated leaves of *A. nummularia* while it decreased in those of *A. leucoclada* (Figs. 37 and 38).

Spot No. 43 was identified as malate dehydrogenase (MDH) (EC 1.1.1.37), similar to that of *Zea mays* and *Mesembryanthemum crystallinum.* The abundance of this protein increased significantly in the leaves of *A. nummularia* whereas it decreased markedly in those of *A. leucoclada* at high salinity treatment (Figs. 37 and 38).

II- Proteins implicated in nitrogen metabolism and amino acid biosynthesis – This group includes spots No 11 and 28.

Spot No. 11 was identified as 5-methyltetrahydropteroyltriglutamate-homocysteine transmethylase (HMT) (EC 2.1.1) homologous to that of *Mesembrynthemum crystalinum*. High salinity treatment induced a significant increase in abundance of 5-methyltetrahydropteroyltriglutamate-homocysteine transmethylase in the leaves of *A. nummularia* and decrease in those of *A. leucoclada* (Figs. 37 and 38).

Spot No. 28 was identified as S-adenosyl-L-methionine synthase (SAMS) (EC 2.5.1.6). The enzyme was distinctly up regulated in the leaves of *A. nummularia* while it was slightly down regulated in those of *A. leucoclada* at saline condition (Figs. 37 and 38).

As for the other four protein spots more than one probable candidate protein were obtained for each spots (Table 8). This may be because of the corresponding *Atriplex* proteins were not included in the data bases or because of their low mass spectrum qualities or due to the mixture of numerous protein spots localized in the same pI or MW range. These proteins were just measured once by MALDI-TOFF-MAS and with additional measurements they could be identified.

3.11 Energy dispersive X-ray microanalysis (EDXA)

Ion contents (Na, Cl, K, P, S, Mg and Ca) and their distribution within the bladder cells, epidermal and guard cells of the adaxial and abaxial epidermal layers were determined for the adult and juvenile leaves of both *Atriplex* species. Results of EDXA analysis revealed that there were no major differences between the adaxial and abaxial epidermal layers. Thus, Table A3 (appendix) contains only the data of the adaxial epidermal cells. *In A. nummularia* controls, the bladder cells contained generally high amounts of Na and Cl in comparison to the epidermal cells (Fig. 39). The high accumulation of Na and Cl in the bladder cells was associated with relatively low K, Mg, P, Ca contents. The guard cells, on the other hand, were characterized by slightly high P and lower Na contents in comparison to the epidermal cells (Fig. 39). As shown in Fig. 39, S forms a relatively constant proportion in all measured cells under control conditions. Salinity significantly increases Na and Cl and trims Mg, P, S, K and Ca contents of the epidermal cells, guard cells and bladder cells (Fig. 39). These results are in agreement with those of atomic absorption spectrophotometerical ones. In some cases, numerous crystals were observed on the leaf surface of the plants grown at high salinity level after the collapse of the bladder hairs (Fig. 39). EDXA analysis of these crystals revealed that they consisted mainly of Na and Cl.

The same pattern of ion content and distribution was more or less noticed in *A. leucoclada* plants under control conditions (Fig. 40). However, conspicuous high Mg and low Cl contents were observed in all measured cell types of *A. leucoclada* under control conditions (compared to *A. nummularia*). The same trend of increased Na and Cl and decreased Mg, P, S, K and Ca contents were observed in all measured cell types (i.e. epidermal cells, guard cells and bladder cells) of *A. leucoclada* leaves at high saline conditions (Fig. 40).

At this salinity treatment (750 mol*m^{-3} NaCl), lower Na contents were detected in the leaf epidermal cells and in the guard cells of *A. leucoclada* as in *A. nummularia* (Fig. 40). By contrast, P contents in these parts were clearly higher in *A. leucoclada*. Additionally, the guard cells of *A. leucoclada* juvenile leaves showed high Cl contents at saline conditions (Fig. 40c).

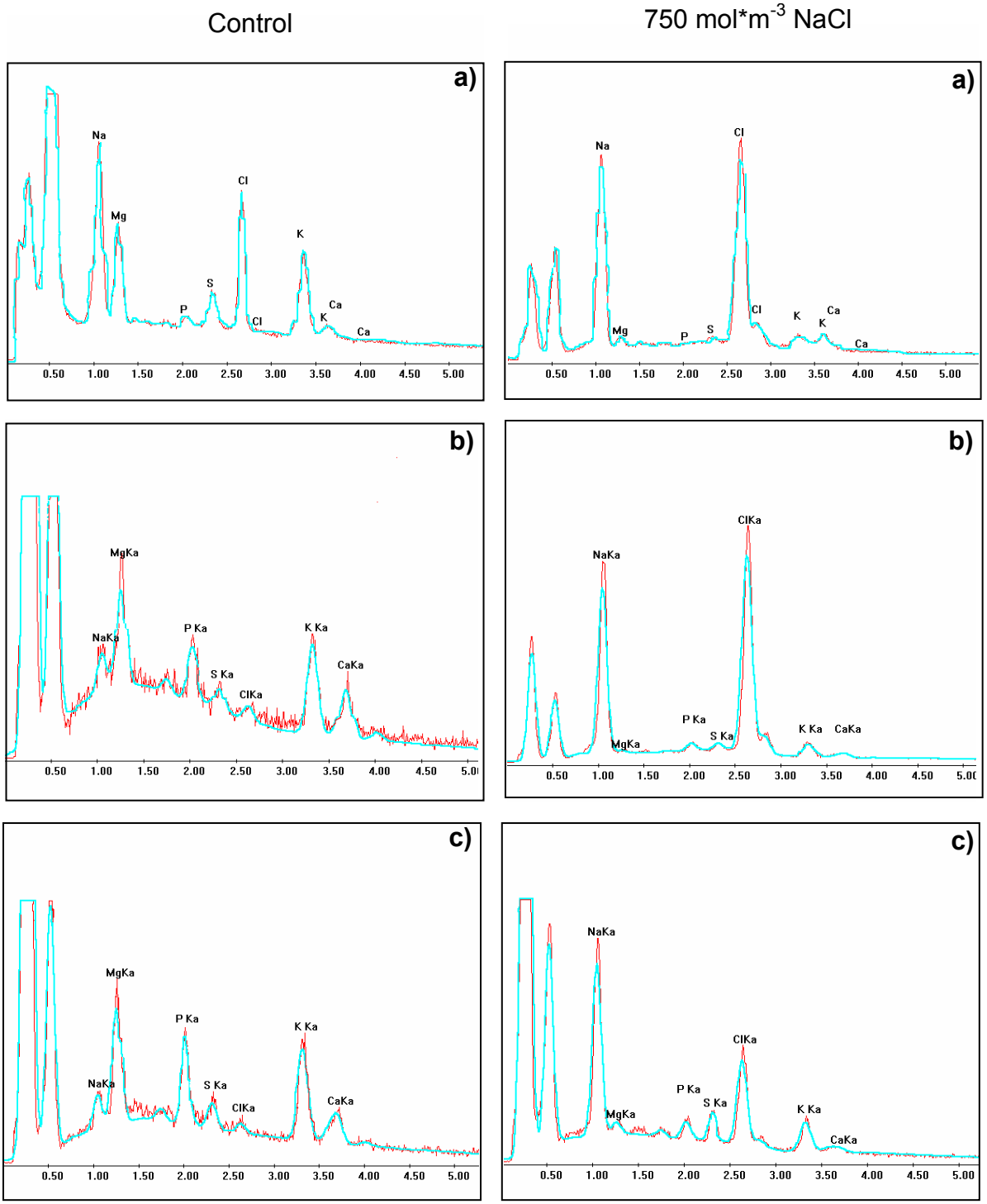

Fig. 39: Representative energy-dispersive x-ray microanalytical spectra of the bladder cells (a), epidermal cells (b) and guard cells (c) of the juvenile leaves (adaxial epidermis) of *A. nummularia* controls (left) and salt treated plants (right).

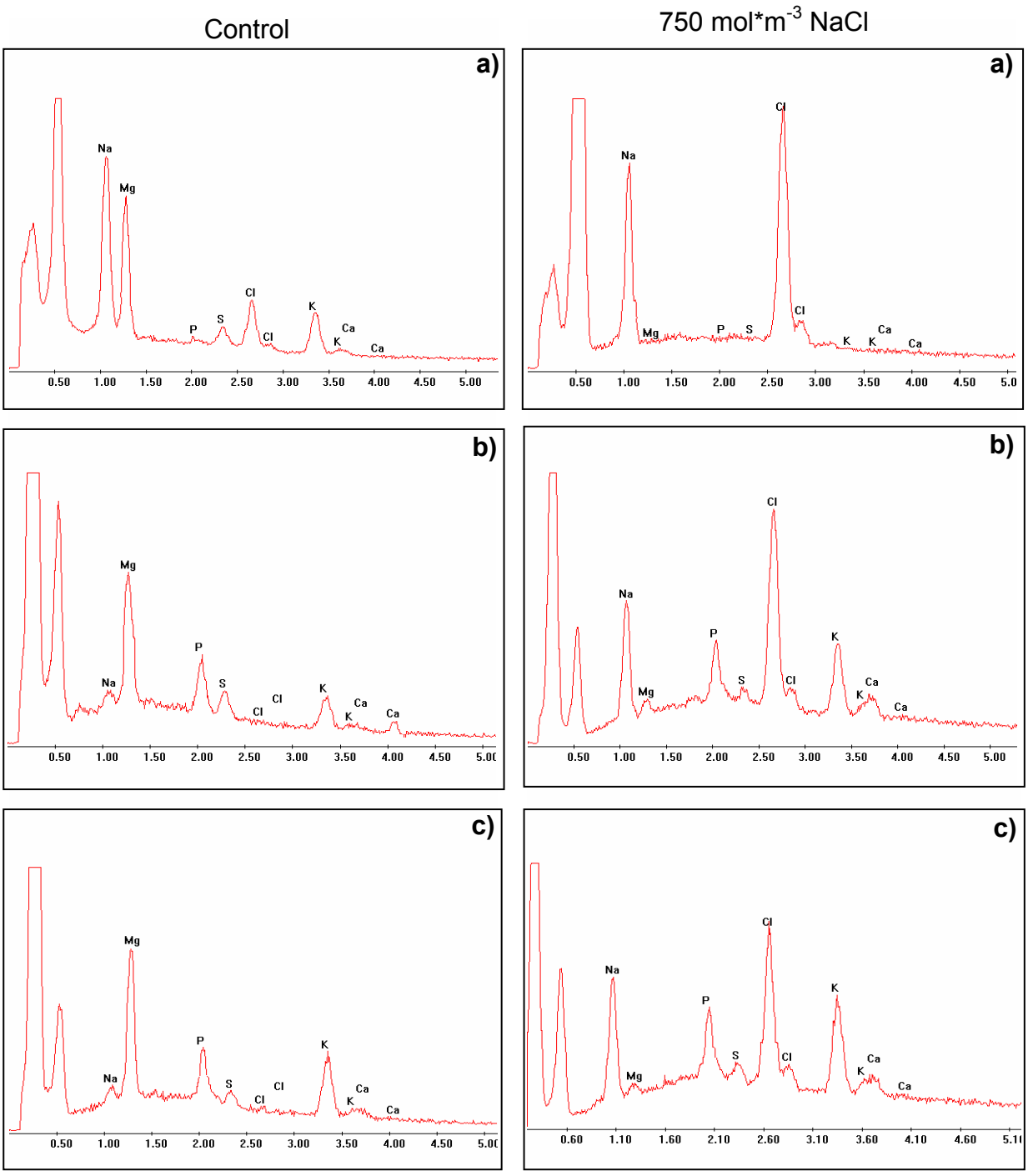

Fig. 40: Representative energy-dispersive x-ray microanalytical spectra of the bladder cells (a), epidermal cells (b) and guard cells (c) of the juvenile leaves (adaxial epidermis) of *A. leucoclada* controls (left) and salt treated plants (right).

3.12 Effect of salinity on the leaf structure

3.12.1 Light and Scanning Electron microscopy

Light microscopical investigations revealed that the leaves of both *Atriplex* species had similar anatomical structure. They have a dorsiventral anatomy, with adaxial (upper) and abaxial (lower) epidermal layers enclosing in between a mesophyll with numerous vascular bundles (Fig. 44a and 45b). The epidermal cells of both adaxial and abaxial sides have thick outer walls covered with thin smooth cuticle layers. Stomata of the anomocytic type are scattered on both the adaxial and abaxial surfaces with an irregular distribution. Accordingly, the leaf is amphistomatal. The stomatal density has been estimated as 32.4 ± 6.2 and 34.1 ± 4.6 stomata*mm^{-2} in *A. nummularia* and *A. leucoclada* respectively. The stomatal aperture of *A. nummularia* was 17.1 ± 5.7 µm in length and 3.4 ± 0.2 µm in width while in *A. leucoclada* it was 13.5 ± 5.1 µm and 2.9 ± 0.3 µm in length and width respectively. The leaves of both *Atriplex* species are abundantly covered with 1 – 3 layers of special epidermal trichomes, the so-called bladder hairs or vasiculated hairs (Fig. 41 and 42). These bladder hairs give the leaves (in particular the juvenile ones) their greyish appearance. The bladder hair consists of a thin stalk (of 1 – 3 cells) bearing a large bladder cells with 140 – 200 µm in diameter. The stalk cells are characterized by a dense plasmatic content, while the bladder cell has a giant central water containing vacuole.

The formation of such bladder hairs begins in the early stages of the leaf development. Study of transverse sections of the very young leaves revealed that the bladder hairs originate as embossments from the dermatogen. The first periclinal division leads to the bladder initial, while the stalk cell was formed by subsequent divisions or in some cases it develops directly to the large bladder cell. The new developing bladder hairs press against the older mature ones which begin to collapse after a stretching of the stalk cells. It was generally observed that the adult leaves of both *Atriplex* species have much less bladder hairs per unit leaf as the juvenile ones.

The hypodermis beneath the upper and lower epidermis composed of 1 – 2 layers of large, isodiametric, colourless, thin walled parenchymatus cells (Fig. 44a and 45a). The diameters of these cells ranged between 51.3 ± 12.5 and 75 ± 18.3 µm in *A. nummularia* and *A. leucoclada* respectively. Large intercellular spaces

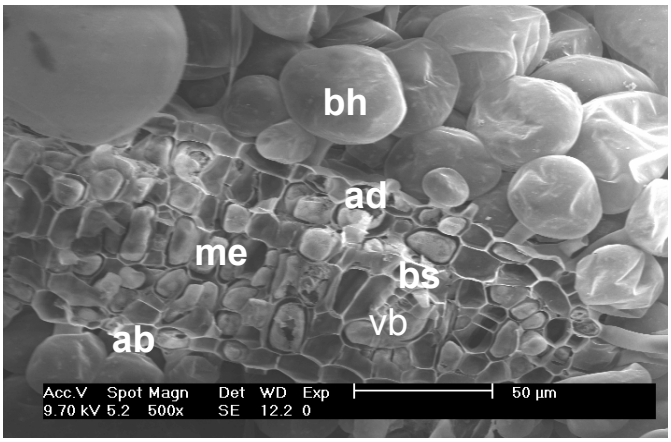

Fig. 41: Representative SEM photograph of a vacuum hydrated and transversely fractured surface leaf of *A. nummularia* control plant. ab, abaxial epidermis; ad, adaxial epidermis; bh, bladder hairs; bs, bundle sheath; me, mesophyll; vb, vascular bundle.

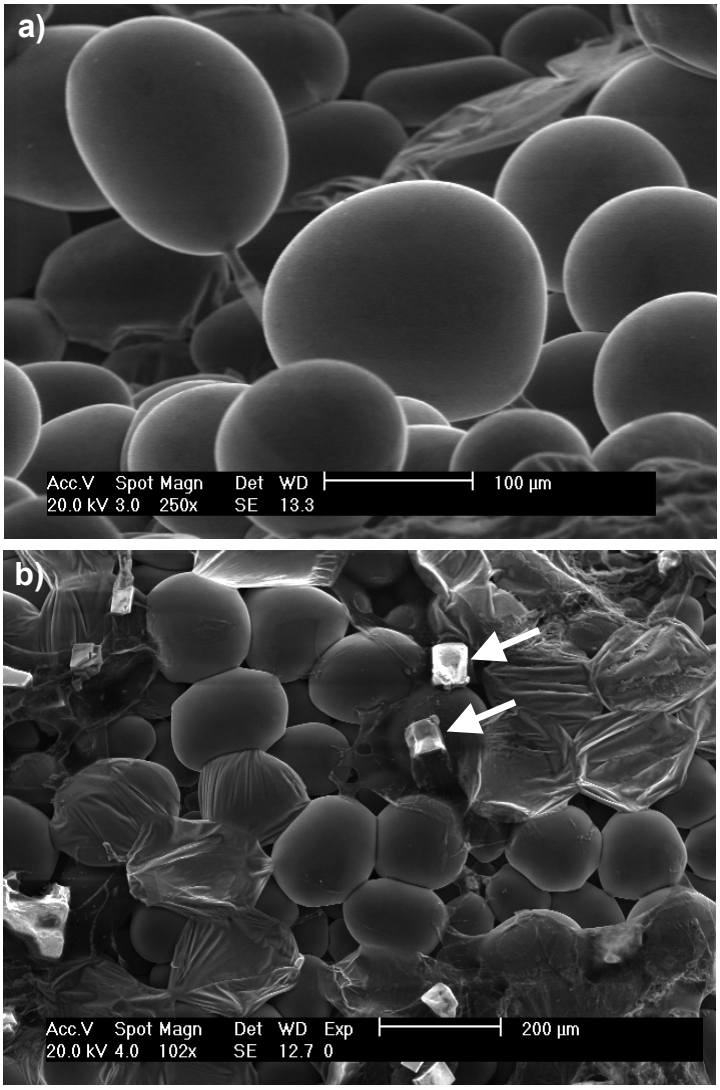

Fig. 42: Representative SEM photographs of the juvenile leaf surface of *A. leucoclada* (a) at control condition and (b) at high salinity level (750 mol*m^{-3} NaCl). Note the various stages of bladder hairs development and degeneration. Typical NaCl crystals (arrows) can be seen on the leaf surface at high salinity treatment (b).

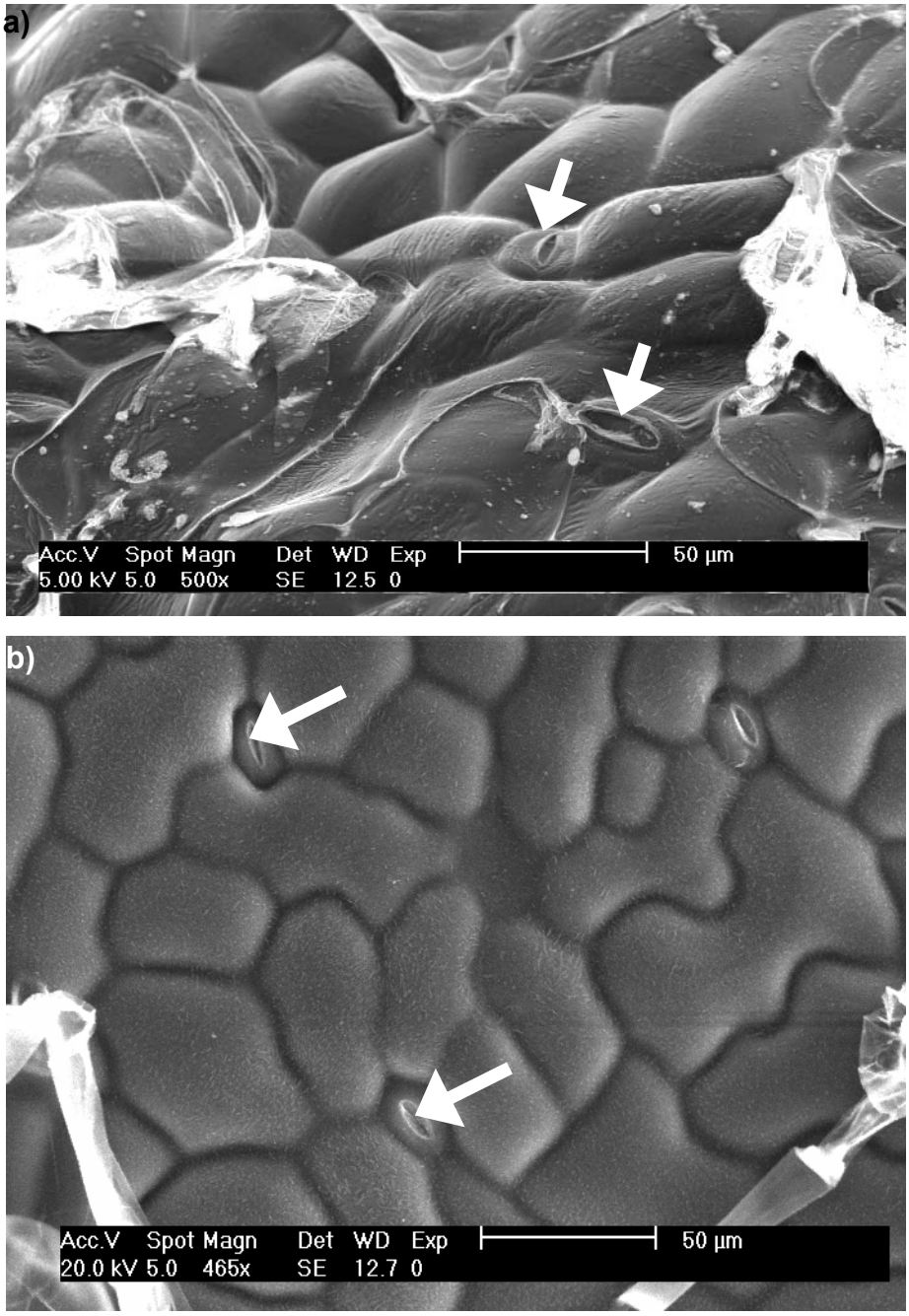

Fig. 43: Representative scanning electron micrographs of *A. nummularia* adult leaf surface at control conditions (a) and at 750 mol*m^{-3} NaCl (b). Note the open stomata in (a) and the closed stomata in (b).

occur between the hypodermal cells especially underneath the stomata (substomatal chamber) (Fig. 45a, 46a and 47a). Numerous large crystals of calcium oxalate (druses) were observed in the hypodermal cells particularly in the juvenile leaves of both *Atriplex* species (Fig. 44a). *Atriplex* leaves show the typical C_4 anatomy i.e., the mesophyll is well developed into mesophyll layer and bundle sheath layer, both are arranged concentric around the vascular strands. The mesophyll is formed out of one layer of thin walled, short columner cells. They were 24.1 ± 5.9 µm and 13.2 ± 2.5 µm for the anticlinal and periclinal dimensions respectively in *A. nummularia* while those of *A. leucocalda* were bigger and reached 39 ± 4.1 µm and 22.4 ± 6.0 µm. The mesophyll cells have large vacuoles and a few small organelles embedded in the cytoplasm. Generally, the mesophyll cells are arranged radially around the bundle sheath. The bundle sheath consists of isodiametric chlorenchymatous cells which are radially arranged around the vascular bundles. Their diameters were 64.0 ± 11.5 µm in *A. nummularia* and 47.3 ± 16.1 µm in *A. leucoclada*. These cells have thick walls (especially the outer tangential ones), small vacuoles, and they are densely packed with large starch containing-chloroplasts. The cells of the bundle sheath are very small on the abaxial side. The lateral vascular bundles show the overall feature of an open lateral veins. The tracheary elements had diameters of 13.9 ± 3.2 µm and 12.7 ± 2.6 µm in *A. nummularia* and *A. leucoclada* respectively.

Salinity did not affect the basic anatomical features of the leaves in both *Atriplex* species. In tendency, salinity slightly reduced the epidermal cell size of the adaxial and abaxial epidermises of both *Atriplex* species. The bladder hairs density seems to be less affected with salinity treatment rather aging. Stomatal density was slightly reduced to 29.5 ± 4.1 and 30.4 ± 7.2 stomata*mm^{-2} in *A. nummularia* and *A. leucoclada* respectively under saline conditions. Most stomata on the adaxial and abaxial sides of the NaCl-treated adult leaves were closed and their guard cells have distinctly thicker walls (Fig. 43b) and (A4, appendix). The stomatal aperture measured 11.7 ± 3.8 µm in length and 1.3 ± 0.3 µm in width for *A. nummularia* whereas it averaged 12.1 ± 4.1 µm in length and 1.4 ± 0.6 µm for width in *A. leucoclada*.

The most obvious salt-induced changes concern the leaf thickness in both *Atriplex* species. Exposure to high salinity treatment significantly increased the leaf

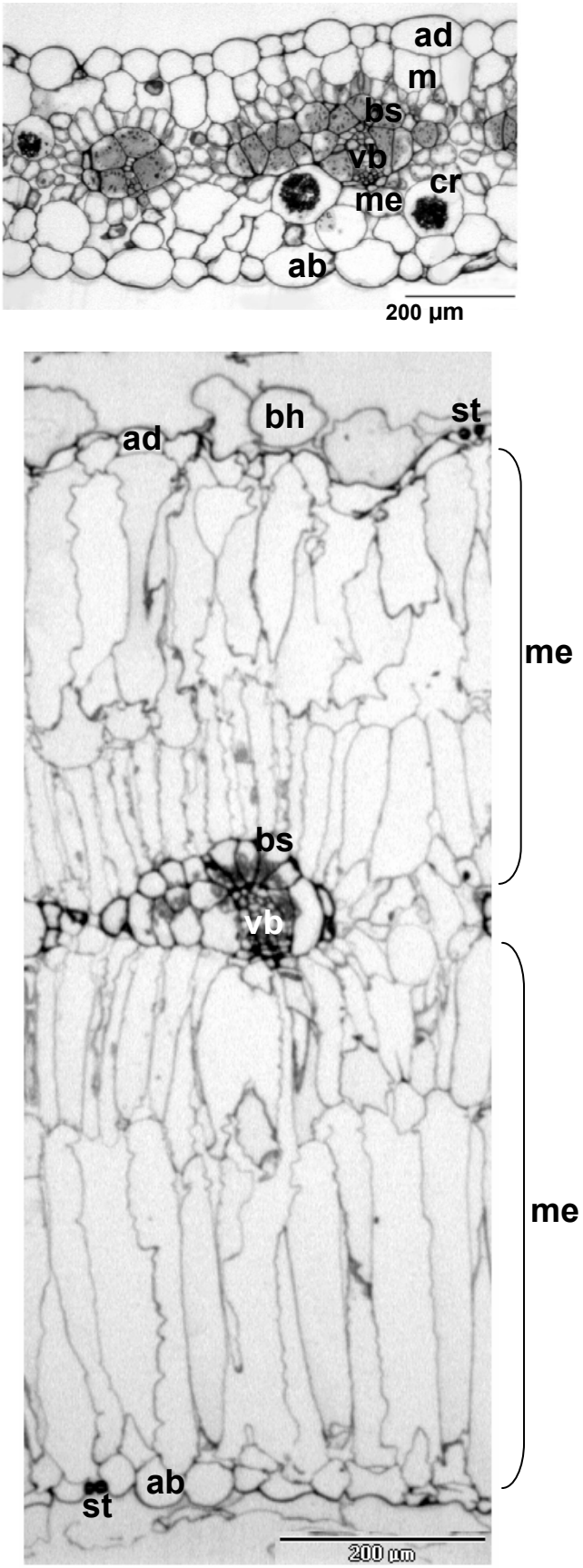

Fig. 44: Transverse sections of the fully expanded mature leaves of *A. nummularia* a) at control condition and b) at 750 mol*m^{-3} NaCl salinity. ab, abaxial epidermis; ad, adaxial epidermis; bh, bladder hairs; bs, bundle sheath; cr, calcium oxalate crystal; me, mesophyll; st, stomata; vb, vascular bundle.

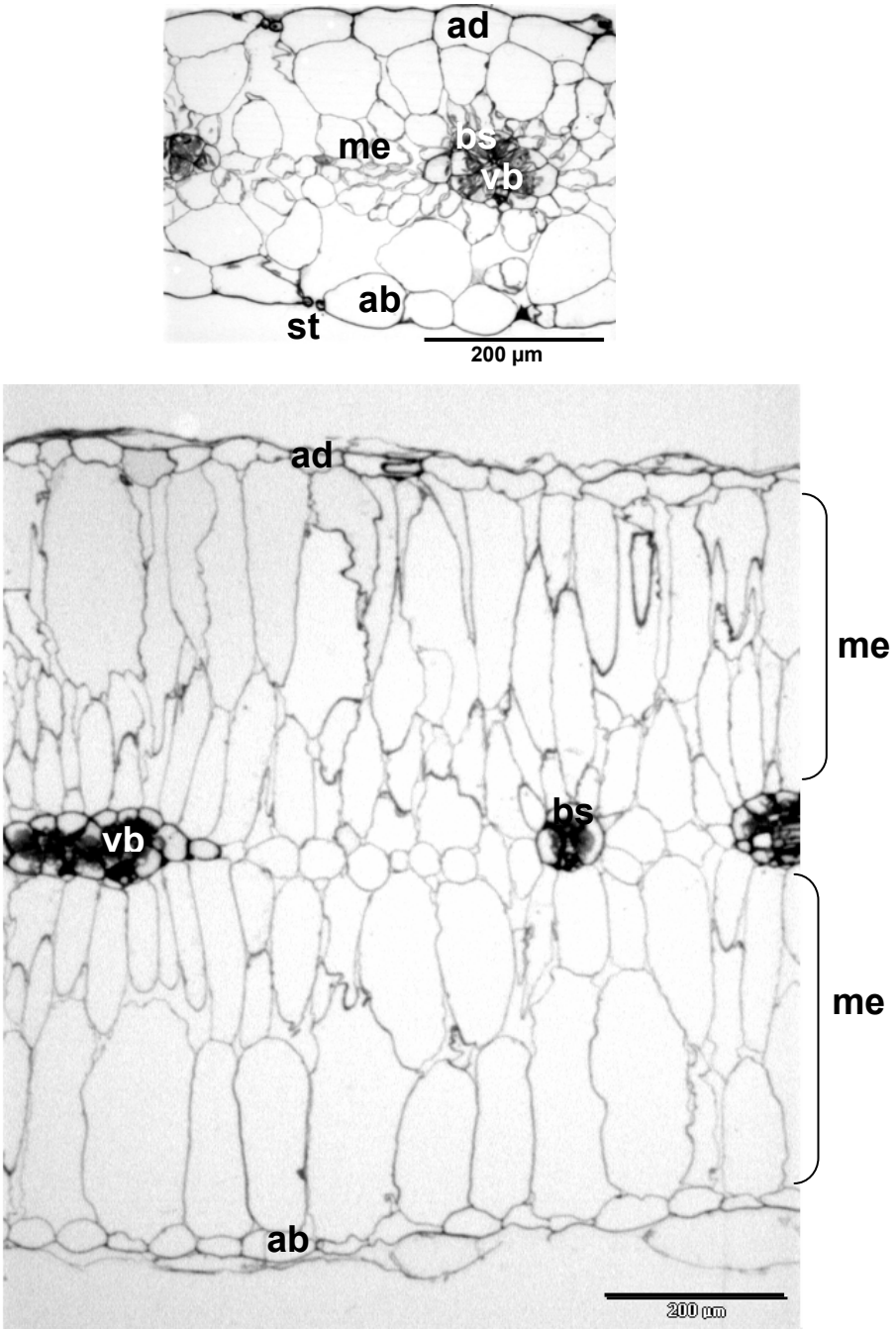

Fig. 45: Transverse sections of the fully expanded mature leaves of *A. leucoclada* a) at control and b) at 750 mol*m-3 NaCl salinity. ab, abaxial epidermis; ad, adaxial epidermis; bh, bladder hairs; bs, bundle sheath; me, mesophyll; st, stomata; vb, vascular bundle.

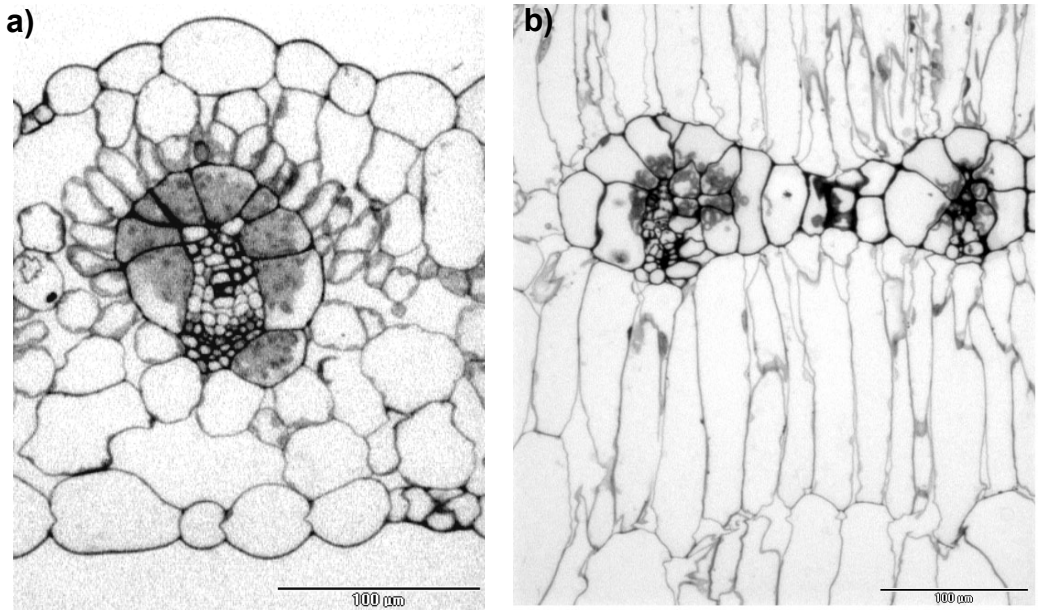

Fig. 46: The structure of the mesophyll and bundle sheath of a) untreated and b) salt treated *A. nummularia* leaves.

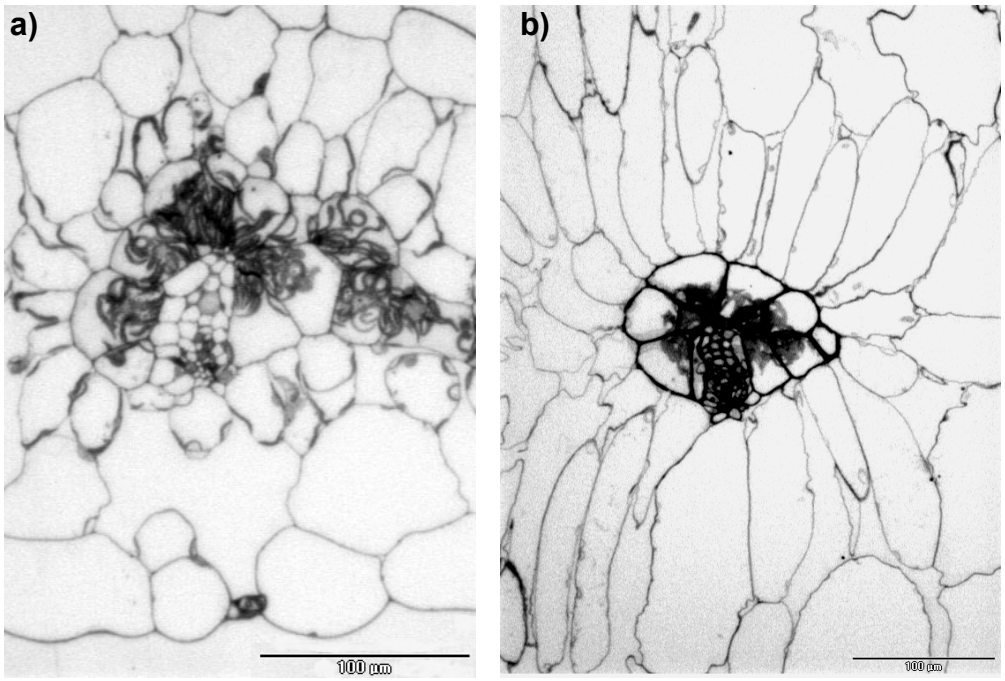

Fig. 47: The structure of the mesophyll and bundle sheath of a) untreated and b) salt treated *A. leucoclada* leaves.

thickness by about 3.5 – 4 and 2.5 – 3 folds in *A. nummularia* and *A. leucoclada* respectively relative to the corresponding controls (Fig. 44b and 45b). This increase is mainly due to the extension of the hypodermal and mesophyll cells particularly in the anticlinal direction. Additionally, this leads to reduction in the intercellular spaces between the mesophyll cells by about 90 and 82% in *A. nummularia* and *A. leucoclada* respectively (compared to controls, Fig. 46 and 47). Furthermore, salinity resulted into a distinct decrease in calcium oxalate crystals within the mesophyll cells (Fig. 44b and 45b). In contrast to the hypodermal and mesophyll layers, no significant salt-induced changes were found in the size of bundle sheath cells. The tracheary element diameter was slightly affected by salinity. Their diameters declined in response to salinity, ranging 9.2 ± 3.1 µm and 8.9 ± 2.8 µm in *A. nummularia* and *A. leucoclada* respectively.

3.12.2 Ultrastructure investigations

The ultrastructure of the different leaf cells of *A. nummularia* and *A. leucoclada* is relatively similar. The epidermal cells are characterized by large central vacuoles, some chloroplasts, ER, mitochondria and prominent nucleus in a peripheral cytoplasm layer (Figs. 48a and b). Of central interest was the ultrastructure of the bladder hairs, particularly, the stalk cell. As can be seen in Figs. 48c and 49a, the stalk cell of the bladder hair contains a large nucleus, several mitochondria, endoplasmic reticulum, chloroplasts and a few small vacuoles impeded into a dense cytoplasm. The nucleus of the stalk cell is especially larger in relation to cell size, filling up the central portion of the cell (Fig. 49a). On contrary, the bladder cell has an extremely large vacuole with a prominent nucleus imbedded in the peripheral cytoplasm. Numerous plasmodesmata were observed between the epidermal and stalk cells and between the stalk and the bladder cells (Fig. 49b). It was obvoius that the bladder hairs did not show any significant responses to salinity treatments with exception of increasing vesicles observed in the bladder cells especially in *A. leucoclada* (Fig. 55a).

As described before, the major leaf veins of both *Atriplex* species are surrounded by the bundle sheath cells which having conspicuous morphological differences to the surrounding mesophyll cells. The bundle sheath cells have a dense cytoplasmic content (chloroplasts, mitochondria, ER, etc.) (Figs. 50). The

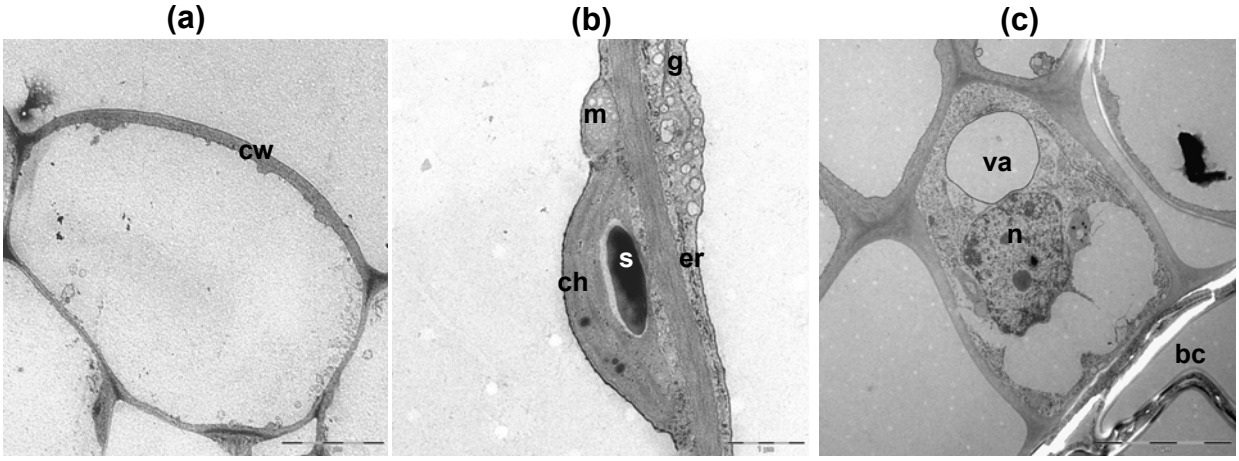

Fig. 48: Different portions of the adaxial epidermis of control leaf of *A. leucoclada*. (a) epidermal cell with its outer thick cell wall. Note the small vesicles adjacent to the radial cell wall (arrows). (B) portion of two adjacent epidermal cells showing the some cytoplasmic organelles. (c) stalk cell of a bladder hair. bc bladder cell; ch, chloroplasts; cw; cell wall; er, endoplasmic reticulum; g, golgi bodies; m, mitochondria; n, nucleus; s, strach and va, vacuoles.

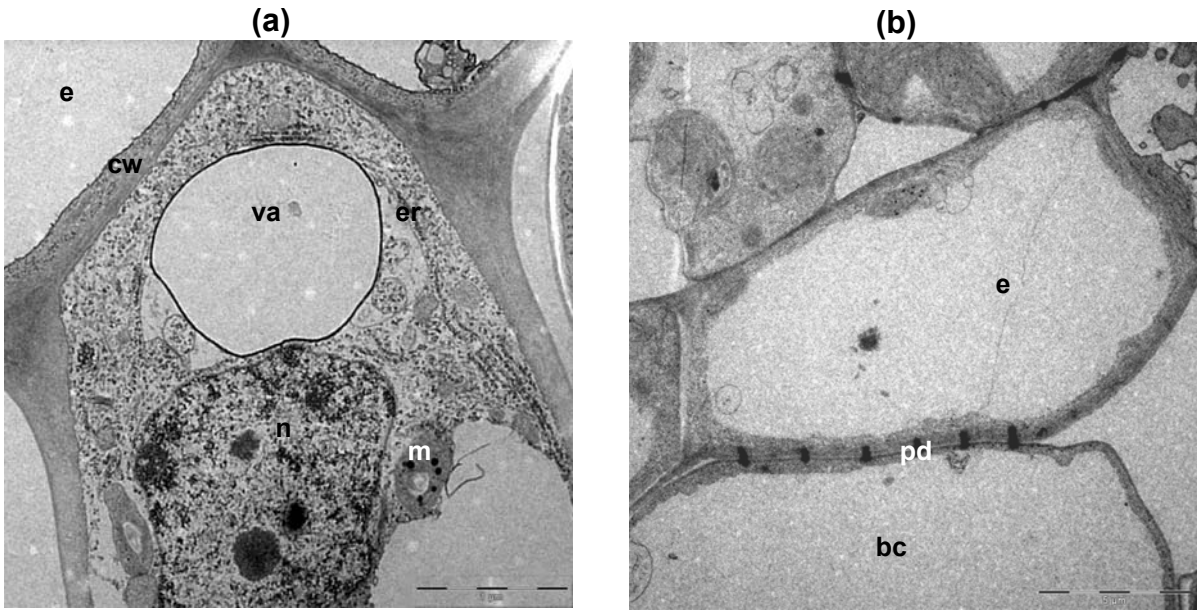

Fig. 49: Stalk cell ultrastructure (A) and portion of the bladder cell with an adjacent cell of the adaxial epidermis fro untreated control leaf of *A. leucoclada* (B). bc, bladder cell; ch, chloroplasts; cw; cell wall; e, epidermal cell; er, endoplasmic reteculum; m, mitochondria; n, nucleus; pd, plasmodesmata; va, vacuoles

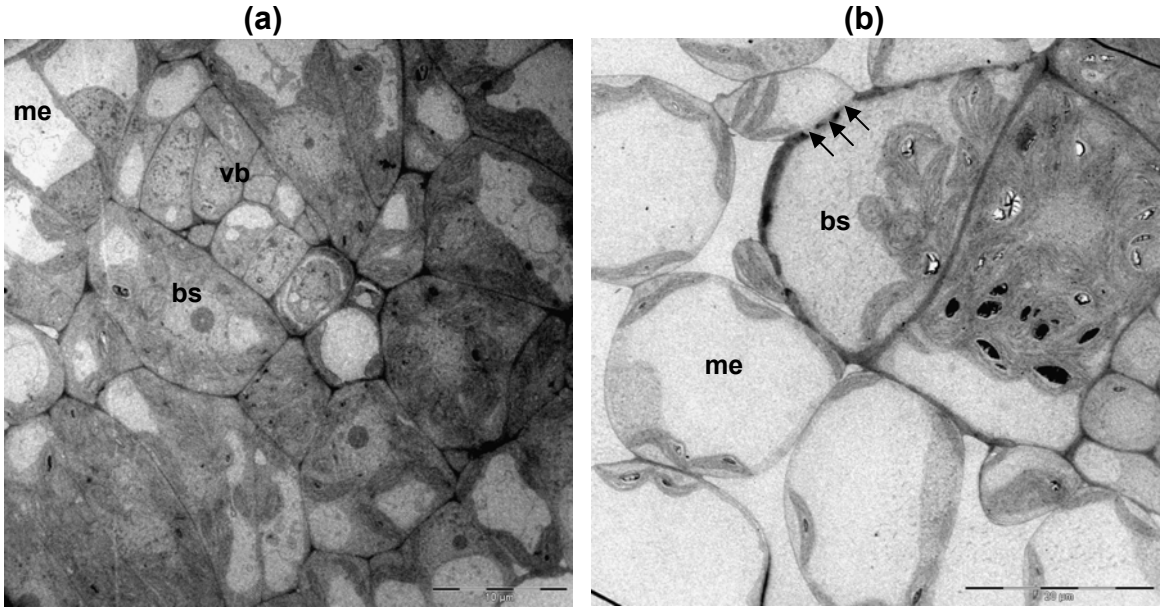

Fig. 50: Bundle sheath cells surrounding a vascular bundle with the adjacent mesopyll cells from control leaf of *A. leucoclada*. Note the much more dense cytoplasmic contents in the bundles sheath cell as in the mesophyll cells (a, b), the thick walls of the bundle sheath cells with several plasmodesmata (arrows) in (b). bs, bundle sheath cell; me, mesophyll cell and vb, vascular bundle.

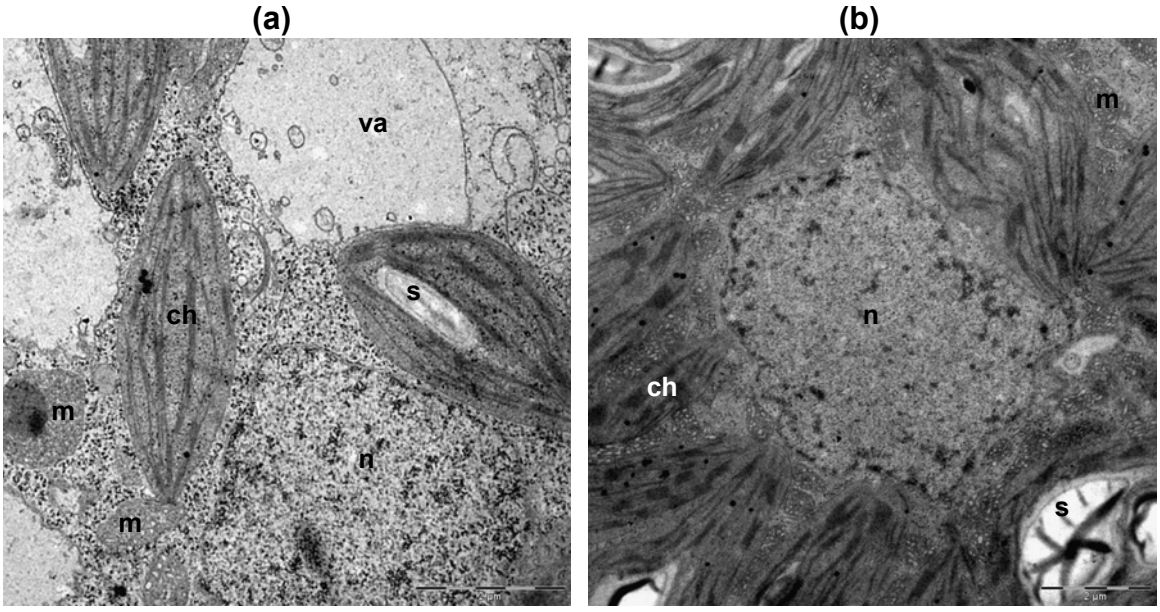

Fig. 51: Part of a mesophyll cell (a) and bundles sheath cell (b) from a control leaf of *A. nummularia*. Note the large chloroplasts with normal grana and large starch grains and the high number of mitochondria in the bundle sheath cell (b). ch, chloroplast; m, mitochondria; n, nucleus; s, strach and va, vacuole.

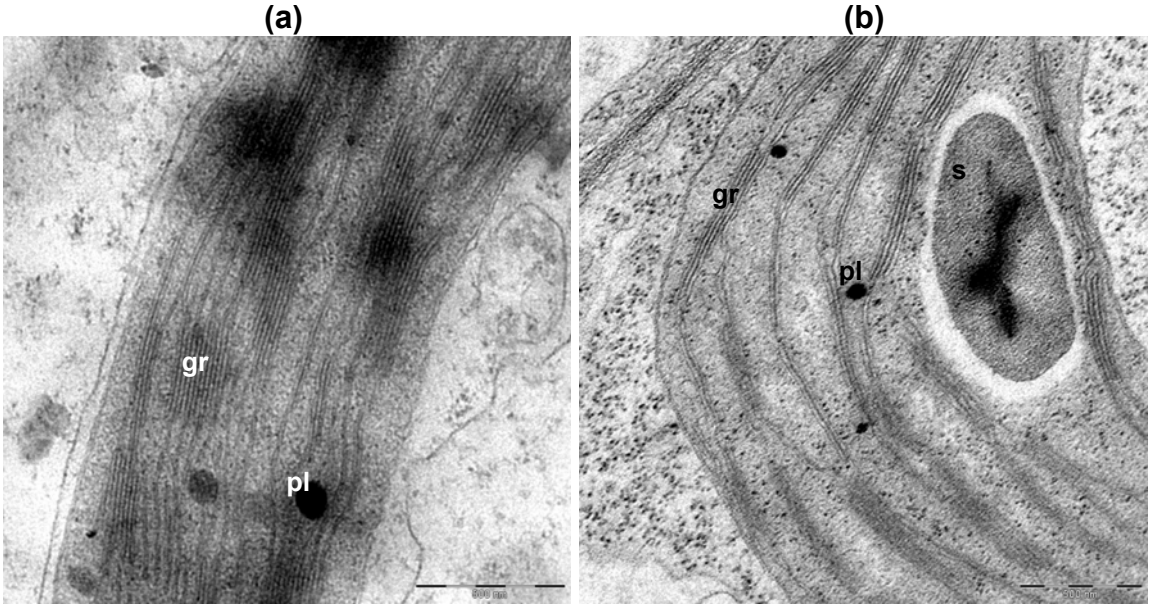

Fig. 52: Part of the bundle sheath cell (a) and mesophyll cell (b) of a control leaf of *A. nummularia*. gr, grana; pl, plastoglobuli and s, strach.

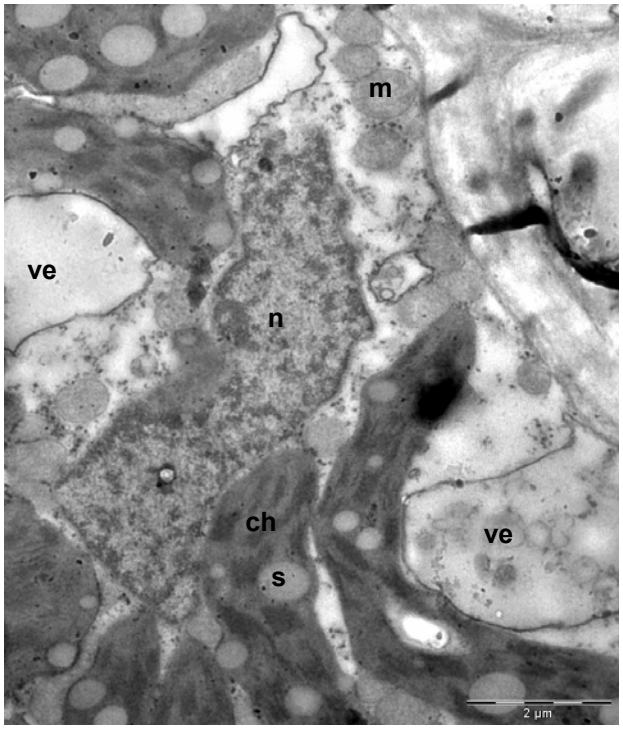

Fig. 53: Part of a bundle sheath cell of a salt treated leaf of *A. nummularia*. ch, chloroplasts; m, mitochondria; n, nucleus; s. strach and ve, vesicles.

chloroplasts of the bundle sheath cells are long and relatively thick and have more grana and the stroma lamellae are shorter compared with those of the mesophyll cells (51a, b). There was much more starch accumulated in the bundle sheath chloroplasts as in the mesophyll ones (Figs. 52a and b). The bundle sheath cells are also characterized by a higher number of relatively big mitochondria in comparison to the mesophyll cells (Fig. 51b). Additionally, they have large cell nuclei located in the centre while that of the mesophyll cells are situated peripherally and are again somewhat smaller than the ones of the bundle sheath. The bundle sheath cells are characterized by a relative thick cell wall in comparison to the mesophyll cells (Fig. 50b). Several plasomodesmata were observed between mesophyll and bundle sheath cells in the leaves of control and salt treated leaves in both *Atriplex* species (Fig. 50b).

In general, salinity induced swelling of several organelles in both the mesophyll and bundles sheath cells like chloroplasts, golgi bodies, mitochondria, and cell nuclei (Fig. 53). These salt-induced changes were more obvious in the bundle sheath cells than the mesophyll cells. The numbers of chloroplasts decreased slightly and the overall shape of them was changed as a result of membrane swelling from elliptical to an elongate, cup or horseshoe shape in the NaCl treated leaves (Fig. 53 and 54a and b). Further, the thylakoid membranes were de-stacked and the granal and lamellar spacing increased. Several starch grains were accumulated in the chloroplasts of salt treated leaves (Fig. 54a). In some cases, the bundle sheath cells of high salt treated leaves have slightly large, somewhat misshaped cell nuclei when compare with those of the controls (Fig. 53). Salinity did not impact the ultrastructure of the mitochondria in both the bundle sheath and mesophyll cells. However, there were higher numbers of larger mitochondria in the bundle sheath cells of salt treated leaves compared to the control (Fig. 53, 45a). It was observed also that salinity led to increase the vesicle numbers in the bundle sheath and mesophyll cells as well as in the epidermal and bladder cells (Fig. 55 a and b).

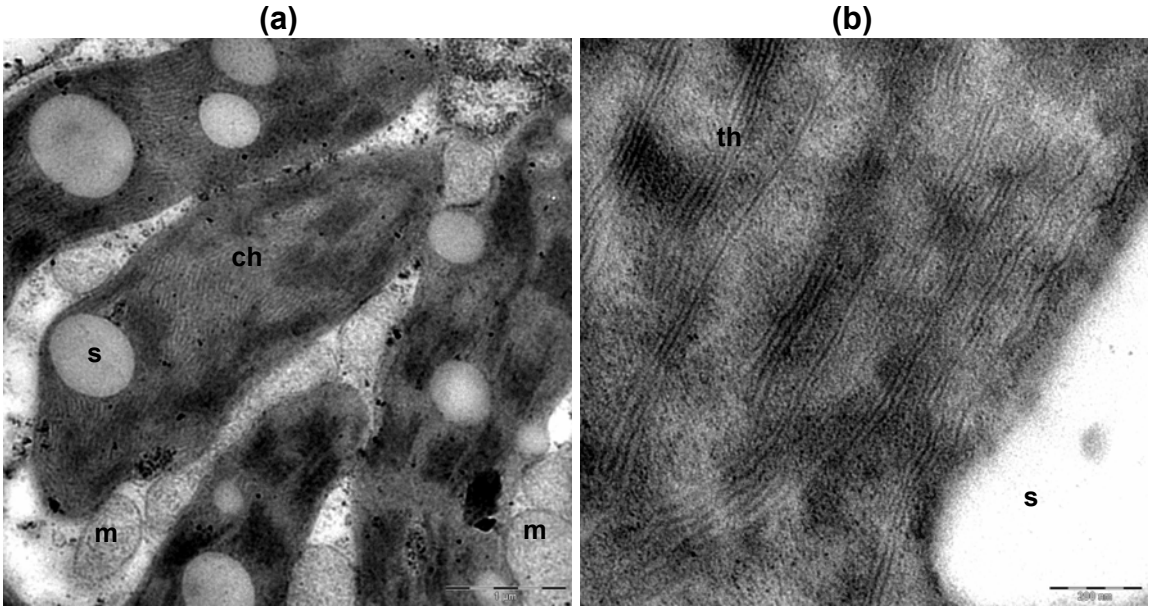

Fig. 54: Part of a bundle sheath cell of a salt treated leaf of *A. nummularia*. Note the presence of many mitochondria of a large size, the large starch grains accumulated in the chloroplasts, the de-stalking of the chloroplasts (a) and the disrupted thylakoid membranes in (b).
ch, chloroplasts; m, mitochondria; s, strach and th, thylakoid membrane.

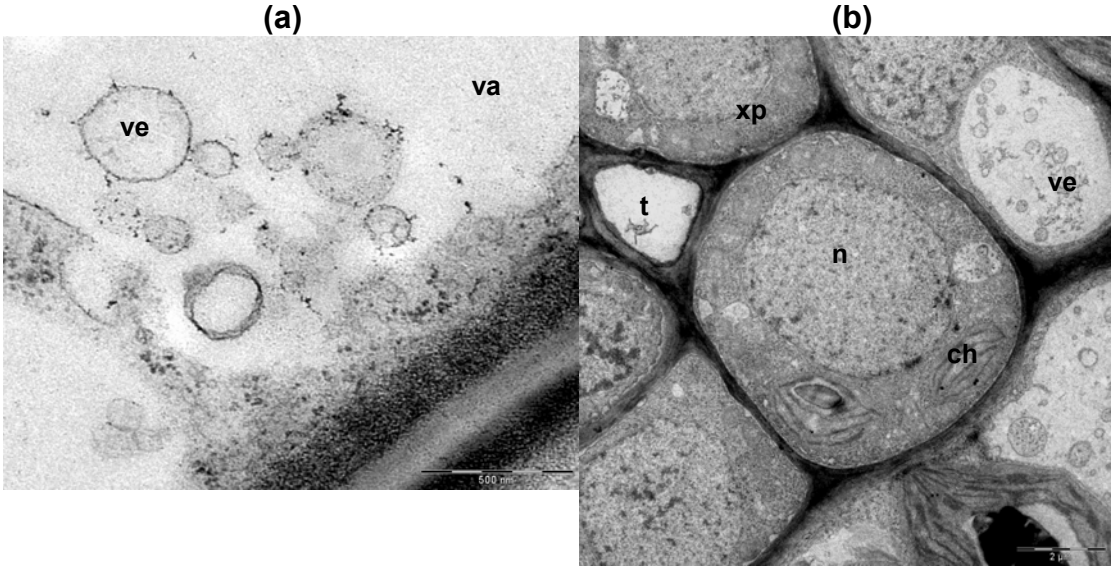

Fig. 55: Numerous vesicles in the bladder cells of salt treated leaf from *A. leucoclada* (a) and in the xylem parenchyma of salt treated leaves of *A. nummularia* (b).
ch, chloroplast; n, nucleus; t, tracheary element; va, vacuoles; ve, vesicles and xp, xylem parenchyma.

4 DISCUSSION

Considering the fresh weight as an indicator of plant growth capacity, it is obvious from the results obtained in the present study that the growth of both *Atriplex* species was significantly stimulated by moderate salinity (250 mol*m^{-3} NaCl) (Fig. 2a, b and 5a, b). Thus this salinity level could be considered as the optimal salinity. Growth stimulation under moderate salinities (100 - 200 mol*m^{-3} NaCl) has been reported previously for many *Atriplex* species such as *A. nummularia* (Uchiyama, 1987; Dunn and Neales, 1993; Ramos *et al*., 2004), *A. spongiosa* (Storey and WynJones, 1979), *A. undulata* (Smith and McComb, 1981), *A. hortensis* (Jeschke and Stelter, 1983), *A. barclayana* (Nerd and Pasternak, 1992), *A. halimus* (Bajji *et al*., 1998; Debez *et al*., 2003), *A. griffithii* (Khan *et al*., 2000), and *A. centralasiatica* (Qiu *et al.,* 2003). The increase in tissue water content seems to account for the increase in plant fresh weight in both *Atriplex* species at moderate salinity as has been suggested for other plant species (Nerd and Pasternak, 1992; Prado *et al*., 2000). This is supported by the trends of water content, which correlated with those of the plant fresh weight (Fig. 9a, b).

Although the presence of NaCl is rarely essential for the growth of halophytes (Flowers, *et al*., 1977), its absence in the nutrient solution markedly inhibited the growth of *A. nummularia*. Similar observation has been reported earlier by Brownell (1968) and Brownell and Crossland (1972) who found that *A. nummularia* plants grown in a sodium-free culture solution showed distinct symptoms of leaf chlorosis and their growth was reduced. Unlike *A. nummularia*, *A. leucoclada* showed distinctly better performance at control conditions. This might be due to the higher Na^+ accumulation observed in *A. leucoclada* controls compared to those of *A. nummularia* (Fig. 14a, b). The requirement of Na^+ for the conversion of pyruvate to phosphoenolpyruvate under light conditions (Murata *et al*., 1992), for the control of pyruvate translocation across membranes through a Na^+/H^+ symport (Ohnishi *et al*., 1990), and for the maintenance of chloroplast structural integrity (Brownell and Bielig, 1996), in addition to its osmotic role may explain its importance for the growth of both *Atriplex* species, particularly, *A. nummularia*. Our results suggest that *A. nummularia* could be considered as an obligate halophyte, and *A. leucoclada* as a facultative halophyte (Waisel, 1972). Supraoptimal levels of salinity inhibited the growth of both

Atriplex species in this study, with more adverse effect on *A. leucoclada*. It is worth noting that the fresh weight of high salt stressed *A. nummularia* plants was reduced, but still greater than their respective controls (111% increases). Similar observation has been reported previously by Ashby and Beadle (1957) for *A. nummularia* plants. While salinity threshold of both *Atriplex* species was similar (slightly above 50% SWS), the C_{50} was at salinity of 140 and 114% SWS in *A. nummularia* and *A. leucocalada* respectively. These results indicate that *A. nummularia* is comparatively more productive, in terms of maintenance of biomass production as *A. leucoclada* under saline condition. Reduced biomass as a response to high substrate salinity is quite common in halophytes (Greenway, 1968; Waisel, 1972; Priebe and Jäger, 1978; Richardson and McKell, 1980; Aslam *et al.*, 1986; Uchiyama, 1987; Ungar, 1996; Wang *et al*., 1997; Koyro *et al*., 2006; Liu *et al*., 2006). It is assumed to be an adaptive mechanism for the survival under saline condition because it allows the plant to rely on multiple resources to cope with salinity stress (Zhu, 2001).

Water homeostasis is indispensable for plant growth under saline condition. The initial effect of NaCl on plant growth is due to an osmotic effect, resulting from the low substrate water potential (Munns, 2002; Tester and Davenport, 2003; Ashraf and Harris, 2004). As a response, both *A. nummularia* and *A. leucoclada* lowered their shoot water potential as a consequence of decreased osmotic potential of all plant organs (Fig. 11 a and b). It was observed that the values of water potential obtained using the scholander apparatus were relatively higher than the expected values and than those of the external nutrient solutions. This may be attributed to a technical reason. The results show that both species were able to maintain a constant osmotic potential gradient between the leaf and root tissues and the external solution. This would explain the enhanced water content of both species especially at moderate salinities (Fig. 9a, b) and indicates that both *Atriplex* species had osmotically adjusted. Reduction of the tissue osmotic potential was associated with a substantial excessive accumulation of Na^+ and Cl^- and decreased K^+ concentration in all plant organs of both *Atriplex* species (Fig. 14a, b and 22a, b). This reveals that these species use the accumulation of Na^+ and Cl^- for osmotic adjustment. An increase of ion accumulation for the decrease of plant osmotic potential is a common trait in many halophytic species such as *A. nummularia* (Ashby and Beadle, 1957), *A. triangularis* (Karimi and Ungar, 1984), *A. semibacata* (Viliers *et*

al., 1996), *A*. *prostrata* (Karimi and Ungar, 1984; Wang *et al*., 1997), and *A*. *griffithii* (Khan *et al*., 2000). Salt inclusion mechanism is considered to be less energy and carbon demanding compared to the adjustment by organic solutes (Wyn Jones, 1981; Yeo, 1983; Raven, 1985; Munns, 2002; Koyro and Huchzermayer, 1999b).

Data presented in Fig (34 and 35) show that tissue Na^+ and Cl^- concentrations of all plant organs of both *Atriplex* species were sufficient to explain, from quantitive point of view, more than 90% of the tissue osmotic potentials. Moreover, *A. leucoclada* plants exhibited larger capacity for osmotic adjustment than *A. nummularia* as it is obvious from Fig (11 a and b). This may be referred to its high affinity for Na^+ accumulation even under control conditions, reflected in the higher ion (Na^+ and Cl^-) concentrations and higher AW in % DW values in comparison to *A. nummularia* (Fig. 8, 14 and 22). However, high Na^+ and Cl^- accumulation in excess of what is required for osmotic adjustment might lead to tissue dehydration and/or ion toxicity. Such conditions resulted ultimately in growth reduction, inhibition of new leaf initiation and the formation of small ones, some with symptoms of nutrient disorders as observed at high salinity especially in *A. leucoclada* (Fig. 2a, b).

Salt inclusion mechanism in dicotyledonous halophytes is generally associated with high capacity to compartmentalize the harmful ions (at the cellular, intracellular and interorgan levels) to maintain low Na^+ concentrations in the actively metabolic tissues. As evident from Fig. 14 and 22, the shoots of both *Atriplex* species were preferential sites for Na^+ and Cl^- accumulation, thereby the plants avoid ion accumulation in the root tissues. Such ion distribution between the root and shoot confirms that these species are salt includers (Flowers *et al*., 1977 and Flowers and Läuchli, 1983). In many halophytes, the adult leaves function as ion sinks to protect the actively growing and photosynthesizing tissues of the juvenile leaves (Yeo and Flowers, 1983; Jeschke, 1984; Pasternak, 1987; Cramer and Bowman, 1991; Koyro and Huchzermeyer, 1999b). This may be true for both *Atriplex* species since the adult leaves exhibited slightly higher Na^+ concentrations as the juvenile ones (Fig. 14 a and b). Salt tolerance of both *Atriplex* species is linked also to their capacity to remove or compartmentalize the excess of salts mainly Na^+ and Cl^- from the salt sensitive metabolic sites of the leaf into the bladder hairs on the leaf surface (Black, 1954; Osmond *et al*., 1969; Mozafar and Goodin, 1970; Waisel, 1972; Schirmer and Breckle, 1982; Waisel, 1991). Na^+ and Cl^- concentrations of the bladder hairs (leaf

area base) of both *Atriplex* species were correlated with NaCl concentrations in the growing medium. Similar observation was also reported for many other *Atriplex* species such as *A. vesicaria* (Osmond *et al*., 1969), *A. halimus* (Mozafar, 1970; Mozafar and Goodin, 1970), *A. confertifolia* (Breckle, 1974), and *A. nummularia* (Uchiyama, 1987). Comparing the salt contents of the bladder hairs and leaf tissues reveals that about 21% and 50% of the leaf Na^+ contents was excreted in the bladder hairs of the adult and juvenile leaves respectively in *A. nummularia*. In *A. leucoclada*, the bladders of the adult and juvenile leaves contained equal Na^+ concentrations (about 60% of that of the leaves). These observations indicate the significant role that the bladder hairs play in salt removing from the leaves and thus prevent dangerous accumulation of toxic salts in their tissues. This conclusion is further confirmed by the EDAX analysis which show that the bladder hairs on the leaves of both *Atriplex* species contain extremely high Na^+ and Cl^- concentrations relative to other leaf cell such as the epidermal and guard cells (Fig. 39 and 40). Generally, Na^+ and Cl^- contents (leaf area basis) of the bladder hairs of the juvenile leaves were clearly higher than those of the bladders of the adult ones. In agreement with Kelley *et al*. (1982), this may be due to the fact that the formation of bladder hairs in both *Atriplex* species starts in the early stages of the leaf development. This suggests that the basic role of the bladders is the protection of the young developing leaves from toxic salt levels (Schirmer and Breckle, 1982). The low density of bladder hairs on the adult leaves, particularly, in *A. nummularia* is a prove that ion dilution as a result of increasing leaf succulence is the main strategy to avoid the toxic effect of harmful ions. At the cellular level, ion compartmentation into cell vacuoles separate the harmful ions from cytosolic enzymes, resulting in adequate K^+/Na^+ ratio in the cytosol, important for the metabolic activity (Koyro and Huchzermeyer, 1999b; Blumwald *et al*., 2000; Hasegawa *et al*., 2000; Munns, 2002; Tester and Davenport, 2003). Additionally, it provides an osmotic driving force for the water uptake.

As mentioned above, ion sequestration is a cost-effective mechanism with respect to the amount of energy and resources spent. However, this mechanism by itself is an energy-consuming process (accumulation of Na^+ occurs against a concentration gradient) and usually accompanied by organic solutes synthesis (extra energy requirements) in the relatively small compartment cytoplasm (approximately 10 % of the cell volume) to balance the low osmotic potential in the vacuole (Rontein

et al., 2002). It is believed that Na^+ compartmentation is mediated by the action of Na^+/H^+ antiporters at the tonoplast and at the cell membranes. The proton gradient that drives the antiporter is generated by tonoplast H^+-ATPases and pyrophosphatases (Sussman, 1994). In *A. nummularia* leaf cells, Milis *et al*. (1985) and Hassidim *et al*. (1990) found that Na^+ fluxes across the tonoplast and the vacuolar Na^+ content increased as the external Na^+ concentration increased. Further, NaCl-dependent increase in Na^+/H^+ antiporter activity was correlated with an increase in plasma membrane H^+-ATPase activities (Braun *et al*., 1988). Also, several studies have shown that enhanced vacuolar H^+-ATPase activity is correlated with better growth in many halophytes like *Spartina townsendii*, *Populus euphratica*, *Salicornia bigelovii* and *Salsola salsa* under saline conditions (Koyro *et al*., 1993; Ma *et al*., 2002; Parks *et al*., 2002; Kefu *et al*., 2003). It would be therefore interesting to investigate the salt-induced changes of H^+-ATPase and ion distribution at the cellular level in the leaves and roots of both *Atriplex* species and to integrate the results to the ongoing comparison between them.

In the present study, increased leaf succulence of both *Atriplex* species was due to considerable enlargement of the hypodermis and palisade parenchyma (Fig. 44 b and 45 b). The big vacuoles of these enlarged cells would allow a better and an effective Na^+ compartmentation in these vacuoles and ensure adequate K^+/Na^+ ratio in the cytoplasm. Cell enlargement was accompanied with increased vacuolation in the mesophyll, epidermal and bladder cells of the salt treated leaves of both *Atriplex* (Fig. 55 a, b). Clearly, increasing vacuolation in response to salinity is an evidence for a high activity of salt compartmentation, which allows the leaf to translocate salts from the cytoplasm into the vacuoles or into the bladder hairs (Kurkova and Balnokin, 1994; Koyro, 2002; Mitsuya *et al*., 2002). In addition, the mesophyll cells, in particular, the bundle sheath cells of salt treated leaves of both *Atriplex* species had more mitochondria as compared to those of the controls (Fig. 53 and 54a). The mitochondria of the salt affected plants were also larger. Although, the mitochondrial respiration was not measured in this study, the salt induced increase in mitochondrial number and size gives reason for the assumption that an additional supply of energy is required for salt compartmentalisation and osmotic adaptation.

As Na^+ and Cl^- are compartmentalized in the cell vacuole, compatible solutes should be synthesized and accumulated in the metabolizing cell compartments to

counteract the increased osmolality of the vacuole (Hasegawa *et al*. 2000; Rontein *et al*., 2002; Tester and Davenport, 2003; Huchzermeyer and Koyro, 2005; Ashraf *et al*., 2006). An important group of compatible solutes which are investigated in *A. nummularia* and *A. leucoclada* are the carbohydrates. As shown in Fig. (32 a, b), elevating water salinity increased the content of total soluble carbohydrates (TSC) in most plant organs of both *Atriplex* species. Accumulation of TSC in plants has been frequently reported in response to salinity stress and is thought to have an important role in the osmotic adjustment of salt-tolerant plants (Ashraf, 1994; Ashraf and Tufail, 1995; Popp and Smirnoff, 1995; Bajji *et al*., 1998; Murakeozy *et al*., 2003). Several hypotheses have been mentioned to explain the accumulation of carbohydrates even with suppressed photosynthesis under salt stress. Carbohydrate accumulation in response to salinity is thought to result primary from the decreased export due to shortage of energy source (e.g. ATP) (Munns and Termaat, 1986) or ion deficiency (Marschner, 1995; Mehne-Jakobs, 1995). In both *Atriplex* species, carbohydrate accumulation might be also related to the disturbance of carbohydrate metabolism which regulated by various synthesizing and degrading enzymes that may be ion-specifically controlled (Rathert, 1982; Singh *et al*., 1996). Regardless of salinity treatment, *A. nummularia* accumulated proportionally higher TSC contents in all organs compared to *A. leucoclada*. This may explain at least in part the higher dry matter content (DM in % FW) of *A. nummularia* compared to *A. leucoclada* (Fig. 7 a, b). Higher TSC accumulation in *A. nummularia*, especially in the root, may help to maintain the water absorption and its influx and transport to the shoot.

Another group of compatible solutes are the amino acids, which reportedly accumulated under salt stress (Wyn Jones, 1981; Rabe, 1990; Ashraf, 1994; Mansour, 2000; Mirsa and Gupta, 2005). In this study, analysis of the amino acid composition reveals that increasing TotAA was mainly achieved by large fractions of proline (pro) (Fig. 33). Pro was found to accumulate in La, Lj, and R of both *Atriplex* species with raising salinity as has been previously observed in the leaves of *A. spongiosa* and *Suaeda monica* (Storey and Wyn Jones, 1979), *A. halimus* (Bajji *et al*., 1998), spinach (Di Martino and Fuggi, 2001), *Populus euphratica* (Watanabe *et al*., 2000) and sugar beet (Ghoulam *et al.*, 2002). It was observed that *A. leucoclada* (less salt-tolerant) accumulated much higher pro (4 – 18 fold increase relative to controls) compared to *A. nummularia* (more salt-tolerant) (5 – 7 fold increase relative

to controls) at the highest water salinity level. Similar correlation between the level of salt tolerance and pro accumulation was also found in barley and cotton plants under water stress (Hanson *et al*., 1977; Ferreira *et al*., 1979) and in tomato (Tal *et al.,* 1979), soybean (Moftah and Michel, 1987), rice (Lutts *et al*., 1999) under salt stress. Results of this study show that the overall concentration of pro is too low to be significant for osmotic adjustment (Fig. 34 and 35). This does not preclude the importance of pro because it is mainly synthesized and restricted in the cytoplasm, providing merely about 10% of cell volume. Thus its importance as a cytoplasmic osmolyte might be higher than is suggested here from contents on the basis of total dry weight. In addition to its role as an osmoticum, pro is presumed to be osmoprotectant involved in stabilizing cellular membranes, protecting proteins and enzymes or acting as a stress signal (Rudolph *et al*., 1986; Lone *et al*., 1987; Bandurska, 1993; Mansour, 1998; Gadallah, 1999; Hoekstra *et al*., 2001; Maggio *et al*., 2002; Vinocur and Altman, 2005). Also it may function as free-radical scavengers (Bohnert and Shen 1999; Diamant *et al*. 2001; Lin *et al*., 2002; Misra and Gupta, 2005) and as a nitrogen and carbon sources during the limited growth and photosynthesis under stress conditions (Tester and Davenport, 2003; Wang and Showalter, 2004; Mirsa and Gupta, 2005).

Results of the present study show that *A. nummularia* controls had higher affinity for K^+ uptake than those of *A. leucoclada*, leading to a significant participation in osmotic adjustment (Fig. 34 and 35). Nevertheless, NaCl treatment drastically decreased K^+ concentrations of all organs of both *Atriplex* species. Similar results have been observed previously, and interpreted as a result of competition between K^+ and Na^+ uptake in the roots (Hajibagheri *et al*., 1987; Alberico and Cramer, 1993; Hasegawa *et al*., 2000; Zhu, 2003) or due to the changes in the membrane integrity caused by the displacement of Ca^{2+} by Na^+ (Cramer *et al*. 1985; Marschner, 1995; Gupta *et al*., 2002; Tester and Davenport, 2003). Thus, the ratio Na^+/K^+ increased significantly with elevating water salinity in both *Atriplex* species as previously observed in *A. hortensis*, *A. prostrata*, *A. amnicola*, and *A. nummularia* (Jeschke and Stelter, 1983; Karimi and Ungar, 1984; Aslam *et al*., 1986; Uchiyama, 1987; Ramos *et al*., 2004). It has been assumed that Na^+ can replace K^+ to a certain degree in some cellular activities, especially in its osmotic functions in the vacuole, stomatal regulation and enzyme activation (Flowers and Läuchli, 1983; Mäser *et al*., 2002).

This might explain why the growth of both studied *Atriplex* species was stimulated at moderate salinities when K^+ concentrations declined. However, the severe reduction in K^+ concentration seems to be responsible for the general trend of inhibited protein synthesis in both *Atriplex* species as evident from the results of TSP (Fig. 36) in combination with the pattern of protein expression (2D-gel electrophoresis) (Fig. 37 and 38). Further, decreased K^+ concentration in the leaves might contribute to the low photosynthetic capacity presumably by disrupting the function of PSII as reported by Ball *et al*. (1987). Blumwald *et al*. (2000) and Lacerda *et al*. (2001) reported that salt tolerance is partially correlated with the ability to avoid the accumulation of Na^+ and/or to maintain adequate levels of K^+ in the shoots. As shown in Table 5, both *Atriplex* species were able to maintain low Na^+/K^+ ratio in the roots and the meristematic tissues (juvenile leaves and stems) where the metabolic demands are expected to be greatest and the sensitivity to Na^+ is highest. In general, *A. nummularia* had lower Na^+/K^+ ratios compared to *A. leucoclada* and apparently, due to this reason, *A. nummularia* is more salt-tolerant than *A. leucoclada* at high salinity. The substantial differences in Na^+ and K^+ accumulation between the two *Atriplex* species may attribute basically to the difference in the selective ion uptake and transport capacities at root level as reported by Wang *et al*. (2002). In this study, the calculated selective absorption of K^+ over Na^+ ($SA_{K:\ Na}$) which was very low in both *Atriplex* species under control conditions increased steadily with increasing water salinity. Interestingly, $SA_{K:\ Na}$ was higher in *A. nummularia* than *A. leucoclada* at the whole range of salinity treatments. Additionally, the selective transport capacity of K^+ over Na^+ ($ST_{K:\ Na}$) from R to Sh, from Sj to the Lj and from Sa to La was generally higher in *A. nummularia* compared to *A. leucoclada* at the whole range of salinities. Together, these observations strongly suggest that *A. leucoclada* transfers higher amounts of Na^+ to the shoots, and it is not capable to maintain an adequate K^+ concentration in its tissues under salt stress as does *A. nummularia*.

Increasing external NaCl concentration resulted also in a significant reduction in Ca^{2+} and Mg^{2+} contents of all organs of both *Atriplex* species (Fig. 18 a, b and 20 a, b). These effects were previously reported for *A. canescens* (Richardson and Mckell, 1980), *A. nummularia* (Uchiyama, 1987), *Beta vulgaris* ssp. *maritima* (Koyro and Huchzermeyer, 1999a), *A*. *griffithii* (Khan *et al*., 2000), *Salvadora persica* (Maggio *et al*., 2000) and many other halophyte species (Gul *et al*., 2000; Koyro,

2000; Ghoulam *et al*., 2002; Wyn Jones and Gorham, 2002; Ashraf *et al*., 2006; Liu *et al*., 2006). A high Na^+ concentration is thought to displace Ca^{2+} from the plasma membrane, causing a loss of integrity and leakage of cytosolic K^+ from cells, influencing K^+/Na^+ selectivity (Cramer *et al*., 1985; Epstein, 1998; Cramer, 2002; Tester and Davenport, 2003). Although Ca^{2+} contents were depressed in all organs of the salinized plants, there was not a continuous decline at salinities higher than 250 mol*m^{-3} NaCl. Rather, Ca^{2+} contents are maintained at nearly similar levels (in *A. nummularia*) or slightly increased (in *A. leucoclada*). This might be due to a dilution effect resulted from the severe growth inhibition at the high water salinity or due to an elevation of the cytosolic free Ca^{2+} caused by high salinity level as reported by Lynch *et al*. (1989) and Okazaki *et al*. (1996). The increase of Ca^{2+} concentration may also result from dissolving of calcium oxalate crystals observed in the mesophyll cell of the control leaves (Fig. 44a). These crystals disappeared completely in response to high water salinity treatment (Fig. 44b), suggesting that they may serve as Ca^{2+} as well as oxalate buffer when the uptake of Ca^{2+} is hindered at the high NaCl salinity level (Koyro *et al*., 1997; Koyro *et al*., 1999). It is probable that increasing Ca^{2+} contents at the high salt treatment is related to the function of Ca^{2+} as a secondary messenger (Knight and Knight, 2001). Thus, increase of Ca^{2+}, in particular, in all *A. leucocalda* organs at high salt treatment reflects the dependency of *A. leucoclada* plants on Ca^{2+} to alleviate Na^+ toxicity (Knight *et al*., 1997; Epstein, 1998; Sanders *et al*., 1999).

Mg^{2+} is essential for chlorophyll and protein synthesis and about 25% of the leaves Mg^{2+} is located in the chloroplasts (Marschner, 1995). It plays also an important role in the activation of some key enzyme in plants like RubisCo and ATP synthase (Marschner, 1995; Koyro, 2000) and carbohydrate synthesis (Greger and Linberg, 1987). Thus reduction of Mg^{2+} concentration observed in the leaves of both *Atriplex* species under salt stress may contribute to the reduction of protein synthesis, chlorophyll content and hence the declined photosynthesis rates. In agreement with Fischer and Bussler (1988) and Marschner (1995), reduced Mg^{2+} concentrations in the leaves might also have contributed to the carbohydrate accumulation observed in both *Atriplex* species under salt stress (Fig. 32 a, b).

NO_3^- concentration decreased sharply in all plant organs of both Atriplex species as a result of the competition with Cl^-. Many authors attributed the reduction

of NO_3^- uptake at high salinity to a direct competition between Cl^- and NO_3^- (Kafkafi *et al*., 1982; Cramer *et al*., 1985; Feigin *et al*., 1987; Bar *et al*., 1997; Zhu, 2002), while others referred this reduction to the declined water uptake under salt stress (Lea-Cox and Syvertsen, 1993). Reduction of NO_3^- uptake might be also attributed to the disturbance of sugar metabolism under salt stress. High NO_3^- assimilation rates are important for the synthesis of N-containing compounds (i.e. proline) that have crucial role in the osmotic adjustment (Jefferies, 1980; Ashraf, 1994; Mansour, 2000; Mirsa and Gupta, 2005). However, NO_3^- assimilation would occur on the expense of NO_3^-, leading also to reduce its concentration in the leaves (Fig. 24a, b). As known NO_3^- assimilation is a high-consuming energy process and may be occurred at expense of CO_2 assimilation. This may explain the reduction in the carbon content (% dry weight) observed in the leaves of both Atripex species at high salinity (Fig. 29 a, b). Unlike *A. leucoclada*, *A. nummularia* plants grown at salinities of 100 – 150% SWS tend to accumulate high NO_3^- concentration in their organs to level that exceed the controls in the adult leaves and roots. This may be related to the salt tolerance of *A. nummularia* as assumed by Kafkafi *et al*. (1982). However, low nitrate concentrations seem to be responsible for the reductions in the total N-content (% dry weight) as observed in both *Atriplex* species in response to salinity (Fig. 30a, b). Similar conclusions were reported previously (Feigin *et al*., 1991; Pessarakli, 1991; Al-Rawahy *et al*., 1992). When compared to *A. nummularia*, *A. leucoclada* plants grown at the highest water salinity exhibited generally lower total N-content. This might be attributed to the accumulation of free amino acids (mainly proline) and enhanced protein level in *A. nummularia* at high saline condition. It is tempting to speculate that the reduction in the N-content may affect the photosynthetic capacity, since about 50% of leaf N-content is located in the photosynthetic machinery (Evans, 1989; Pons and Westbeek, 2004). Additionally, reduction of leaf N-content as a result of high salinity might lower the net photosynthesis by decreasing the chlorophyll contents (Marschner, 1995; Ignatova *et al*., 2005); chloroplast size (Lawlor, 2002) and grana number (Laza *et al*., 1993).

Sulphate concentration, the least abundant anion in both *Atriplex* species, generally decreased with increasing water salinity. As has been suggested by Pérez-Pérez-Alfocea *et al*. (1993) and Santamaria *et al*. (1998), the reduction in tissue SO_4^{2-} can be explained by the antagonism between Cl^- and SO_4^{2-} ions. Sulphate

assimilation might be also responsible for the low SO_4^{2-} concentrations observed especially in the plant leaves of both *Atriplex* species. Assimilation of SO_4^{2-}, however, is necessary for the incorporation of sulphur into amino acids, protein, coenzymes and reduced sulfate compounds such glutathione (Rennenberg, 1989; Marschner, 1995). Glutathione plays a key role in detoxification of oxygen radicals and hydrogen peroxide which are expected to be increased under salinity stress (Noctor *et al*. 2002; Pfannschmidt 2003; Huchzermeyer and Koyro, 2005). Increased total S-content observed in the adult leaves at the optimal salinity levels (Fig. 31) might be due an increase in the assimilation of S and the biosynthesis of S-containing organic compound such as, GSH to mitigate the salt-induced oxidative stress. High salinity, however, reduced the S-contents in both *Atriplex* species. This is not surprisingly since the SO_4^{2-} contents were reduced in response to increasing water salinity. Low sulphur content may affect the synthesis of sulphur containing amino acids and hence LHC polypeptides (Marschner, 1995). This could explain the reduction of chlorophyll contents in the leaves (Burke *et al*., 1986; Dietz *et al*., 1989) and then the photosynthesis. Additionally, low sulphur content reduces the root hydraulic conductivity, stomatal aperture, net photosynthesis (Karmoker *et al*., 1991) and then the leaf area and the plant growth (Edelbauer, 1980; Burke *et al*., 1986).

Although Na^+ was reported to play crucial roles in the photosynthesis of C_4 plants, high Na^+ concentrations significantly inhibited the net photosynthesis (A) of both studied *Atriplex* species (Tables 3a and 4a). Interestingly, the leaves of both *Atriplex* species grown at this salinity continued to photosynthesize, although at a reduced rate, when as much as 30% of their dry weight was ash. There are many earlier reports, which show that plant photosynthetic capacity is inhibited by salt stress (Brugnoli and Björkman, 1992; Dunn and Neales, 1993; Alarcón *et al*., 1994; Ashraf, 1999; Bayuelo-Jimenez *et al*., 2003; Qiu *et al*., 2003; Koyro *et al*., 2006). Inhibition of photosynthesis in response to increasing salinity could be an important factor limiting the accumulation of dry matter and hence inhibits the growth of both *Atriplex* species. Figures (13 a, b) show that the required light intensity to saturate the photosynthesis of both *Atriplex* species decreased with increasing water salinity. This is likely a consequence of the lower chlorophyll concentrations per unit area. Commensurate with the reduction in photosynthetic ability, the CO_2 compensation

point (Lc) increased in response to water salinity (Tables 3b and 4b). Similar results were found by Everard *et al*. (1994), Lutts *et al*. (1996) and El-Shintinawy (2000).

Reduction of the net photosynthesis (A) coincided with progressive decrease in the leaf transpiration rates (E) (Tables 3a, 4a), leading to a conservation of water. Similar characteristics for water conservation have also been reported for many halophytic species under saline conditions (Ayala and O'Leary, 1995; Wang *et al*., 1997; Carrol *et al*., 2001; Liu and Stützel, 2002; Debez *et al*., 2006; Koyro *et al*., 2006). Lower transpiration rate also represents an adaptive mechanism to cope with high water salinities, since it could reduce salt loading into the leaves and hence prolong the leaf lifespan by maintaining salts at subtoxic levels (Everard *et al*., 1994; Volkmar *et al*., 1998; Koyro, 2006). It is worth noting that reduction in the transpiration rate was more severe in *A. nummularia* compared to *A. leucoclada* at the high water salinities, further suggesting that *A. nummularia* is better adapted to high salinities. Salt-induced reduction in the diameters of the tracheary element of the leaves might contribute to low water conductance and hence low water loss through transpiration (Belda and Ho 1993; Lovisolo and Shubert 1998). Decreased transpiration rate was also due to the partial closure of the stomata as can be seen in Fig. (43b). EDAX-microanalysis revealed that Na^+ contents of the stomatal guard cells increased in response to high water salinity. In agreement with Thiel and Blatt (1990), the replacement of K^+ by Na^+ as a result of excessive Na^+ accumulation in the guard cells might be responsible for the inhibition of stomatal opening and hence declined the transpiration rate at high salinity. Interestingly, the guard cells of the salt treated leaves of *A. leucoclada* maintained lower Na^+ but higher K^+ and P contents compared to *A. nummularia* (Fig. 39c and 40c). This indicates that the guard cells of *A. nummularia* can effectively utilize Na^+ instead of K^+ to achieve the regulation of turgor and hence control the transpiration rate compared to *A. leucoclada*, which needs adequate K^+ concentration for the turgor regulation. For both *Atriplex* species, salt-induced reduction in the transpiration rate was proportionally much higher than that of photosynthesis, leading to improve the water use efficiency (WUE). Such an increase in the WUE has been recorded for many halophytic species in response to salinity stress (De Jong, 1978; Osmond *et al*., 1980; Ayala and O'Leary, 1995; Naidoo *et al*., 1995). According to Naidoo and Mundree (1993) and Koyro (2000), increasing WUE is an important feature for the long-term survival of plants and would

be an advantage in saline environments. Interestingly, *A. nummularia* showed slightly higher WUE than *A. leucoclada* at the highest water salinity, further indicating that this species is highly adapted to grow under saline and arid conditions.

In both *Atriplex* species, salinity impacted the photosynthesis *a priori* by an enhanced stomatal closure which leads to substantial reduction of CO_2 diffusion to the carboxylation sites (Lauteri *et al*., 1997; Khan *et al*., 2000). This interpretation is supported by the linear proportionality of net photosynthesis (A), transpiration rate (E), the stomatal resistance (Rs) and the internal CO_2 concentration (Tables 3a and 4a). Similarly, a positive correlation between photosynthesis and stomatal conductance has been found in *Atriplex prostrata* (Björkman *et al*., 1972; Wang *et al*., 1997), *Avicennia marina* (Ball and Farquhar, 1984), cotton leaves (Brugnoli and Björkman, 1992); *Atriplex nummularia* and *Atriplex hastata* (Dunn and Neales, 1993) and *Atriplex centralasiatica* (Qiu *et al*., 2003). In agreement with Delfine *et al*. (1998), Tuffers *et al*. (2001), salt-induced changes observed in the leaf structure (i.e., increasing in the leaf thickness and succulence) of both *Atriplex* species (Fig. 44b and 45b) contribute also to the reduction of photosynthetic rate by decreasing CO_2 diffusion. In addition, the severe reduction of photosynthesis at high water salinities may attribute also to non stomatal limitations (Everard *et al*.,1994; Dionisio-Sese and Tobita, 2000; Sobrado, 2005).

Leaf chlorophyll content is another factor which can limit the net photosynthesis. As a general effect of increasing water salinity, Chl(a) and (b) contents (leaf area base) decreased significantly in both studied *Atriplex* species (Fig. 12 a, b). NaCl-induced decrease in chlorophyll content is widely reported (Karimi and Ungar, 1984; Viliers *et al*., 1996; Ashraf and Rehman, 1999; Delfine *et al*., 1999; Khan *et al*., 2000; Kaya *et al*., 2001). The disruption of the chloroplast membrane, instability of the pigment protein complex and enhanced chlorophyllase activity may contribute to the decrease in chlorophyll content at high salinities (Ashraf and Bhatti, 2000).

Low CO_2 assimilation rates of the salt treated plants means that the plants receive excess light energy, resulting into an increase in the ROS generation and hence induce an oxidative stress (Sicher, 1999). It is conceivable that salt stressed *Atriplex* plants developed some scavenging mechanisms in the light reaction system or they utilize the excessive energy for ion excretion or sequestration. The increase

in leaf succulence in addition to the reduction in chlorophyll content observed in both *Atriplex* species with increasing water salinity may lead to a reduction of the flow of electrons through the photosystem (reduction of apparent quantum efficiency) (Tables 3b and 4b). Additionally, the presence of 2 – 3 layers of bladder hairs, filled with salts on the leaf surfaces may form a strongly-light reflective mate on the leaves (Osmond *et al*., 1980; Sharma, 1982; Freitas and Breckle, 1992). This light-reflecting layer is thought to protect the photosystems from over reduction and photoinhibition under stress conditions (Osmond *et al*., 1980; Cornic, 1994; Mooney *et al*., 1997; Streb *et al*., 1997). Reduction in the total chlorophyll contents seems to be also an adaptive mechanism to cope with salt stress, since it may lead to decrease the over reduction of photosynthetic electron transport chain and hence the generation of ROS (Wang *et al*., 2003; Christian, 2005). Results of the current study showed that the reduction in Chl(b) was proportionally higher than that of Chl(a) and consequently the ratio Chl a/b was increased (Fig. 12 d). The reduction in Chl(b) content (mostly located in the LHC) could be interpreted as an adaptation in the LHC capacity. This can lead to reduce (optimize) the photosynthetic efficiency (Tables 3b and 4b) and hence reduces the oxidative stress (Moorthy and Kathiresan, 1999; Koyro, 2006).

Enhanced rates of leaf dark respiration (DR) in response to salinity might be also responsible for low net photosynthesis. Salt-induced increase in respiration is related to the high energy costs needed for salt-compartmentation and the biosynthesis of organic solutes (Tattini *et al*., 1997; Di Martino *et al*., 2003). This would presumably occur at the expense of net CO_2 assimilation and the result is growth reduction (Schwarz and Gale, 1981; Tottini *et al*., 1997). Our results showed that the DR rates in *A. nummularia* were higher than those of *A. leucoclada* at the whole range of salinity treatments (Tables 3b and 4b). This reflects the higher energy requirements for salt economy (transport and sequestration of ions) and biosynthesis of compatible solutes in *A. nummularia* plants even under control conditions. In both *Atriplex* species, the highest respiration rates were observed for plants grown at the optimal salinity level. This might be due to the fact that maintenance respiration of rapidly growing plants (at 250 mol*m^{-3} NaCl) is generally much higher than that of the more slowly growing plants (Koyro and Huchzermeyer, 1999b).

Suppression of photosynthetic activity is also related to the ion toxicity resulted from high Na^+ and Cl^- or due to nutrient deficiency in particular, K^+, Ca^{2+}, Mg^{2+} and

nitrogen. This impacts many aspects of the photosynthesis as discussed above. Impairment of the photosynthesis may also be attributed to the salt induced changes in the chloroplasts. De-stacking of the thylakoid membranes was the most obvious salt-induced change in the chloroplasts (Fig. 54a, b). This effect was also noted by Kelley *et al*. (1982), Hernández *et al*. (1995) and Parida *et al*. (2003), although other investigators did not observe de-stacking of the membranes (Bruns and Hecht-Buchholz 1990, Hernández *et al*. 1995, Mitsuya *et al.* 2000). As thylakoid stacking represents functional integrity of photosystems and optimal energy harvest and distribution, several investigators suggested that the photosynthetic membranes disarrangement occurred under salinity treatment disturbed the normal functioning of thylakoid membranes in energy capture and utilisation (Parida *et al*., 2003).

Large starch grains accumulated in the chloroplast of salt stressed leaves of both Atriplex species (Fig. 54a, b). A dramatic accumulation of starch in the chloroplasts was observed in some species such as *Atriplex hastat*a and *Suaeda maritim*a under saline conditions (Hajibagheri *et al*., 1985). The occurrence of starch grains in the chloroplast, in spite of decreased CO_2-assimilation rates, suggests that the export and utilization of carbohydrates under salt stress appears to be more reduced than photosynthetic CO_2-fixation (Jeannette *et al*. 2000; Pego *et al*. 2000). At the molecular level, reduction of the photosynthetic activity in both *Atriplex* at high salinity treatment was associated with a marked decreased in the abundance of RubisCo large subunits (protein spot No.25) and PPDK (protein spot No.4) as shown in the 2D- gel electrophoresis (Fig. 37b and 38b). RubisCo with its large subunit was the most abundant protein in the leaves of both *Atriplex* species under the control conditions. It was markedly down-regulated in response to high water salinity treatment in the leaves of both *Atriplex* species. This is matched with the disarrangement (de-stacking) of the thylakoid membranes observed at high salinity treatment. RubisCo is the first enzyme in the Calvin-Benson cycle of the photosynthetic fixation of CO_2 (Fig. 56). It catalyzes the reaction of ribulose 1, 5-bisphosphate and CO_2 to form 2 (3-PGA). Thus the rate of photosynthesis depends largely on the quantity and activity of Rubisco (Lorimer, 1981; Makino *et al*., 1983). This could explain, at least in part, the reduction of photosynthesis observed in both *Atriplex* species at high salinities. The synthesis and activity of RubisCo were reported to decrease under salt stress (Wyn Jones and Pollard, 1983; Nieva *et al*.,

1999; Rivelli *et al*., 2002). Pyruvate orthophosphate dikinase (PPDK) with the PEPC are crucial for the operation of the C_4 photosynthesis (Fig. 56). It catalyzes the reversible phosphorylation of pyruvate and hence plays an important role in the regeneration of phosphoenolpyruvate (PEP), the primary acceptor of CO_2 (Taiz and Zeiger, 1991; Kondo *et al*., 2000; Häusler *et al*., 2002). This reaction is critically controlled by light/dark-mediated regulation and is possibly a rate-limiting step in the C_4 photosynthesis (Edwards *et al*., 1985). Therefore, salt-induced depression of PPDK in *Atriplex* plants negatively impacted the C_4 acid cycle and consequently the net photosynthesis. This further confirms that stomatal factors were not solely responsible for the reduction in photosynthesis at high salinity levels.

Further, high salinity increased the abundance of plastidic fructose 1,6-bisphosphate aldolase (ALdP) (protein spot No. 42) in the leaves of *A. nummularia* while its abundance was decreased in *A. leucoclada* leaves (Fig. 37 and 38). Zörb *et al.*, (2004) found that Fructose 1,6-biphosphate aldolase was increased in salt-treated maize shoots. As shown in Fig. (56), ALdP is a key metabolic enzyme in the carbon reduction cycle (Calvin cycle). It catalyzes both the condensation of dihydroxyacetone phosphate (DHAP) and glyceraldehyde-3-phosphate (GAP) to form fructose 1,6-bisphosphate (an aldol condensation) and the reverse reaction, the cleavage of fructose 1,6-bisphosphate to the two triose phosphates (Anderson *et al*., 2005). ALdP is located in the chloroplast (starch synthesis) and the cytosol (sucrose biosynthesis) (Perham 1990, Marsh and Lebherz, 1992). Therefore it is extremely important to maintain a balance between export and regeneration in order that the Calvin cycle does not become depleted of intermediates (Fridlyand *et al.,* 1999; Raines *et al.*, 1999). The salt-induced up-regulation of ALdP in the leave of *A. nummularia* may explain the higher TSC content and the accumulation of starch in the chloroplasts (Fig. 54). On contrary, decreased expression of the ALdP in *A. leucoclada* may inhibit the photosynthesis and alter the starch metabolism in the leaves as a result of an accumulation of triose phosphates and a depletion of ribulose-1,5-bisphosphate and 3PGA as has been showed by Haake *et al.* (1998). This may explain the lower TSC contents and the severe growth inhibition observed in *A. leucoclada* under salt stress.

MDH (protein spot No. 43) has also been differentially regulated in the leaves of both *Atriplex* species. Whereas the abundance of this protein was significantly

increased in the leaves of *A. nummularia*, it was markedly decreased in those of *A. leucoclada* at high water salinity (Fig. 37 and 38). MDH is a key enzyme in the plant cell metabolism, particularly, in C_4 photosynthesis pathway as it generates the reducing power for the various biosynthesis processes (Kumar *et al*., 2000). This role is of great importance under stress conditions (Hare *et al*., 1998). MDH is existed in various isoforms, and located in different subcellular organelles such as cytoplasm, chloroplasts, and mitochondria (Fig. 56). The cytoplasmic MDH catalyzes the formation of malate from OAA, which inters in the mitochondria through the dicarboxylate transporter where the mitochondrial MDH catalyzes the conversion of malate into OAA (Gietl, 1992). Thus the mitochondrial and cytoplasmic forms function together to balance the reducing equivalents between the cytoplasm and mitochondria via an oxaloacetate-malate shuttle (Gielt, 1992; Musrati *et al*. 1998). Additionally, the chloroplastic MDH form is an essential component of the malate-pyruvate shuttle (Fig. 56), an important mechanism for a shuttle exchange of substrates and reducing equivalents across the cell membranes. In this respect, MDH catalyzes the reduction of oxaloacetate to malate in the mesophyll chloroplasts. Malate is then shuttled from the mesophyll to the bundle sheath cells where it is decarboxylated to pyruvate and CO_2. The CO_2 released in the bundle sheath cells through NADP specific malic enzyme is re-assimilated into carbohydrate by the reductive pentose phosphate pathway. Thus, malate in addition to acting as a carrier for CO_2 transport, also serves as a carrier of light-generated reducing equivalents from the mesophyll to the bundle sheath chloroplasts, where they are used for the reductive assimilation of CO_2 into carbohydrate (Fig. 56). The pyruvate (Pyr) generated by the decarboxylation is shuttled back to mesophyll cells to regenerate the primary CO_2 acceptor phosphoenolpyruvate (PEP) by pyruvate orthophosphate dikinase (PPDK) in the mesophyll chloroplasts (Edwards and Walker 1983; Orgen, 1984; Setoyama *et al*., 1988). NADP-MDH showed high activity in the mesophyll cells of C_4 plants, especially in the NADP-malic enzyme type (Taiz and Zeiger, 1991). In this study, salt-induced changes in the cytosolic MDH expression would affect the synthesis of OAA and hence interfere with the function and activity of TCA cycle (Salisbury and Ross, 1986). It may also affect the amino acid synthesis since OAA is the precursor for amino acids biosynthesis (Salisbury and Ross, 1986). In accordance with Kumar *et al*. (2000), increased MDH abundance under salt stress

appears to be an adaptive feature, leading to the maintaining of high activity of TCA cycle, optimum photosynthesis and maintaining high capacity for amino acids synthesis. This suggests that salt tolerance of *A. nummularia* may be correlated with increased MDH under high salinity level compared to *A. leucoclada.*

High salinity treatment induced a significant increase in the abundance of 5-methyltetrahydropteroyltriglutamate-homocysteine transmethylase (HMT) in the leaves of *A. nummularia* while it decreased its abundance in those of *A. leucoclada* (Fig. 37 and 38). This enzyme catalyzes the biosynthesis of methionine (Met) from homocysteine by transferring the methyl group from 5-methyltetrahydropteroyltri-L-glutamate to L-homocysteine in the presence of magnesium and phosphate ions (Fig. 57) (Guest *et al*., 1964). Hence, increased expression level of this protein should increase the methionine concentration in *A. nummularia* stressed leaves. Methionine has two major fates: incorporation into proteins or conversion into S-adenosyl-L-methionine (SAM). The majority of methionine is converted into SAM for the transmethylation reactions as reported by Zörb *et al*. (2004). This reaction is catalyzed by S-adenosyl-L-methionine synthase (SAMS) in the presence of ATP (Fig. 57). SAM is the major methyl-group-donor for several transmethylation reactions in the plant cell (Tabor and Tabor, 1984; Heby and Persson, 1990; Boerjan *et al*., 1994). It is a precursor for the biosynthesis of the phytohormon ethylene (Tiburcio *et al*., 1990, Kende, 1993) and polyamines (Heby and Persson, 1990; Moffatt and Weretilnyk, 2001). It is also required for the biosynthesis of phenylpropanoid compounds which have broad biological functions, including structural constituents of the cell wall (Higuchi, 1981; Lewis and Yamamoto, 1990; Campbell and Sederoff, 1996). In this study, SAMS was distinctly up-regulated in the leaves of *A. nummularia* while it was slightly down-regulated in those of *A. leucoclada* in response to salt stress (Fig. 37 and 38). It can be speculated that the induction of SAMS in *A. nummularia* may be related to increased Met content caused by the upregulation of 5-methyltetrahydropteroyltriglutamate-homocysteine transmethylase. This may ultimately lead to increase lignin and betaine contents in the stressed leaves of *A. nummularia*. Together, these observations foreshadow a link between the induction of these genes and the better performance of *A. nummualria* at high salinity stress. Similar conclusion was also reported for salt stressed tomato (Espartero *et al*., 1994; Sánchez *et al*., 2004),

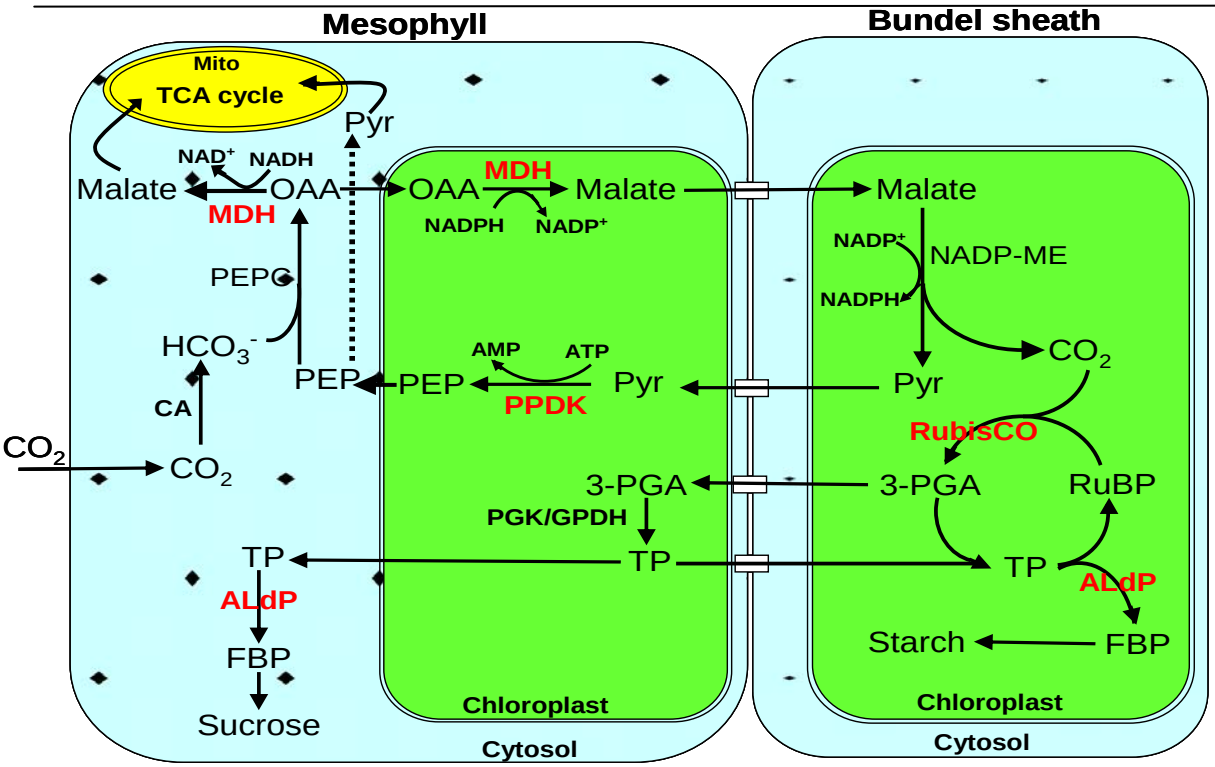

Fig. 56: Schematic representation showing the role of some C_4 photosynthesis-related enzymes (NADP-ME type). 3-PGA, 3-Phosphoglycerat; ALdP, fructose 1,6-bisphosphste aldolase; CA, carbonic anhydrase; FBP, fructose 1,6-bisphosphste; GPDH, Glucose-6-phosphat-Dehydrogenase; MDH, malate dehydrogenase; Mit, mitochondrion; OAA, oxaloacetate; PEP, Phosphoenolpyruvat; PEPC, phosphoenolpyruvate carboxylase; PGK, Phosphoglycerat-Kinase; PK, pyruvate kinase; PPDK, pyruvate orthophosphate dikinase; Pyr, Pyruvate; RuBP, Ribulose1-5-phosphat; TP, Triose phosphat; RubisCo, ribulose 1, 5-bisphosphate carboxylase-oxygenase.

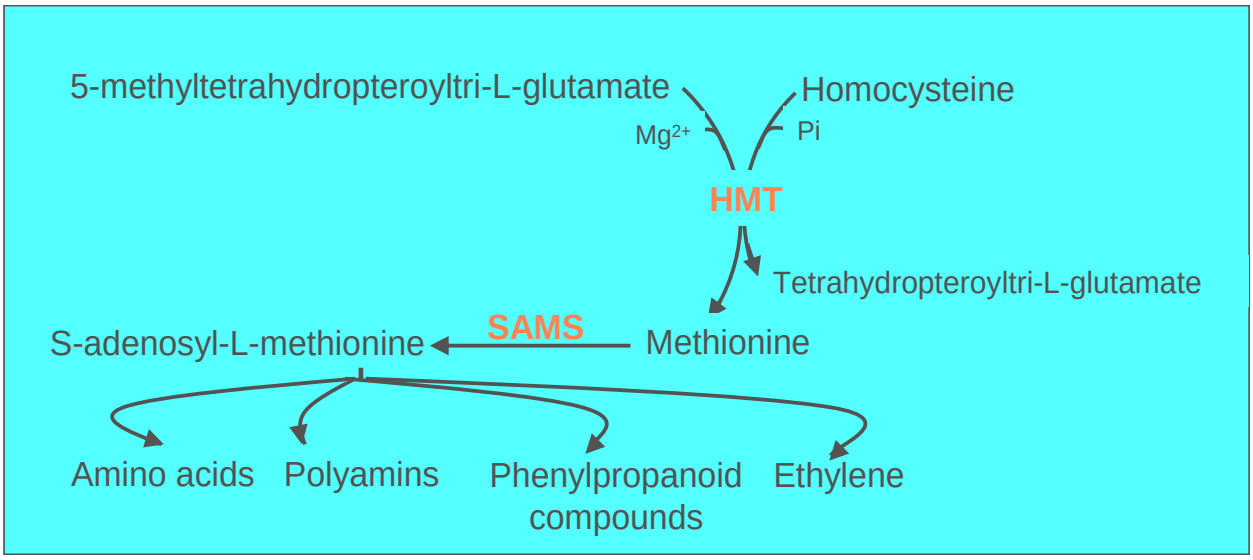

Fig. 57: Schematic representation showing the role of 5-methyltetrahydropteroyltriglutamate-homocysteine transmethylase (HMT) and S-adenosyl-L-methionine synthase (SAMS) in the plant N-metabolism.

maize roots (Zörb *et al*., 2004), and *A. nummularia* leaves (Tabuchi *et al*., 2005).

In conclusion the tolerance to NaCl was clearly different between the two *Atriplex* species. Whereas *A. nummularia* could grow normally (even better as the respective control) at salinity up to 150% SWS, the growth of *A. leucoclada* is severely inhibited at this salinity level. Neither reduced water uptake nor declined photosynthetic capacity appeared to be major reason for the reduced growth in both species. Rather, reduced growth seems to be due to ion toxicity and disturbed mineral nutrition, especially in *A. leucoclada*. The selective uptake and transport capacities, ion compartmentation and the regulation of salt loads to prevent build-up of toxic concentrations seem to be the main features which enable both *Atriplex* species to grow at high NaCl salinity.

Our results suggest that both species can be grown productively under saline condition up to 50% SWS. It has been observed however, that although animals may maintain live weight while grazing *Atriplex* species, they invariably loose condition (Casson *et al*., 1996). This may attribute to the increase in water intake (Atiq-Ur-Rehman *et al*., 1994) to counter the high amount of salts accumulated in *Atriplex* species (Wilson, 1996). As reported by Hopkins and Nicholson (1999) and Aganga *et al.* (2003), *Atriplex* species can be an effective fodder component in mixed diets for livestock. The advantages would be that adverse effects due to the high ion content of the plant tissues could be minimized, that animal performance and economic returns may be higher than direct grazing of the plant shoots. Finally, it should be mentioned that this study is the first step for developing these *Atriplex* species as cash crops (particularly *A. leucoclada*). And further field investigations are recommended to observe the performance of these species, since several responses underlying salt tolerance may be overlooked when operating outside the field context.

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DECLARATION

I, Sayed Abd El-monim Sayed Hussin, hereby declare that this thesis fort he degree of Ph.D. in Biology at the University of Hannover, Germany, is may own work and has never been submitted at any other University.

Hannover, November, 2006

Sayed Abd El-monim Sayed Hussin

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Fig A1: Gravel/hydroponic culture (quick check system) (QCS) of (a) *A. nummularia* and (b) *A. leucoclada.* The water salinity increased in the arrows directions.

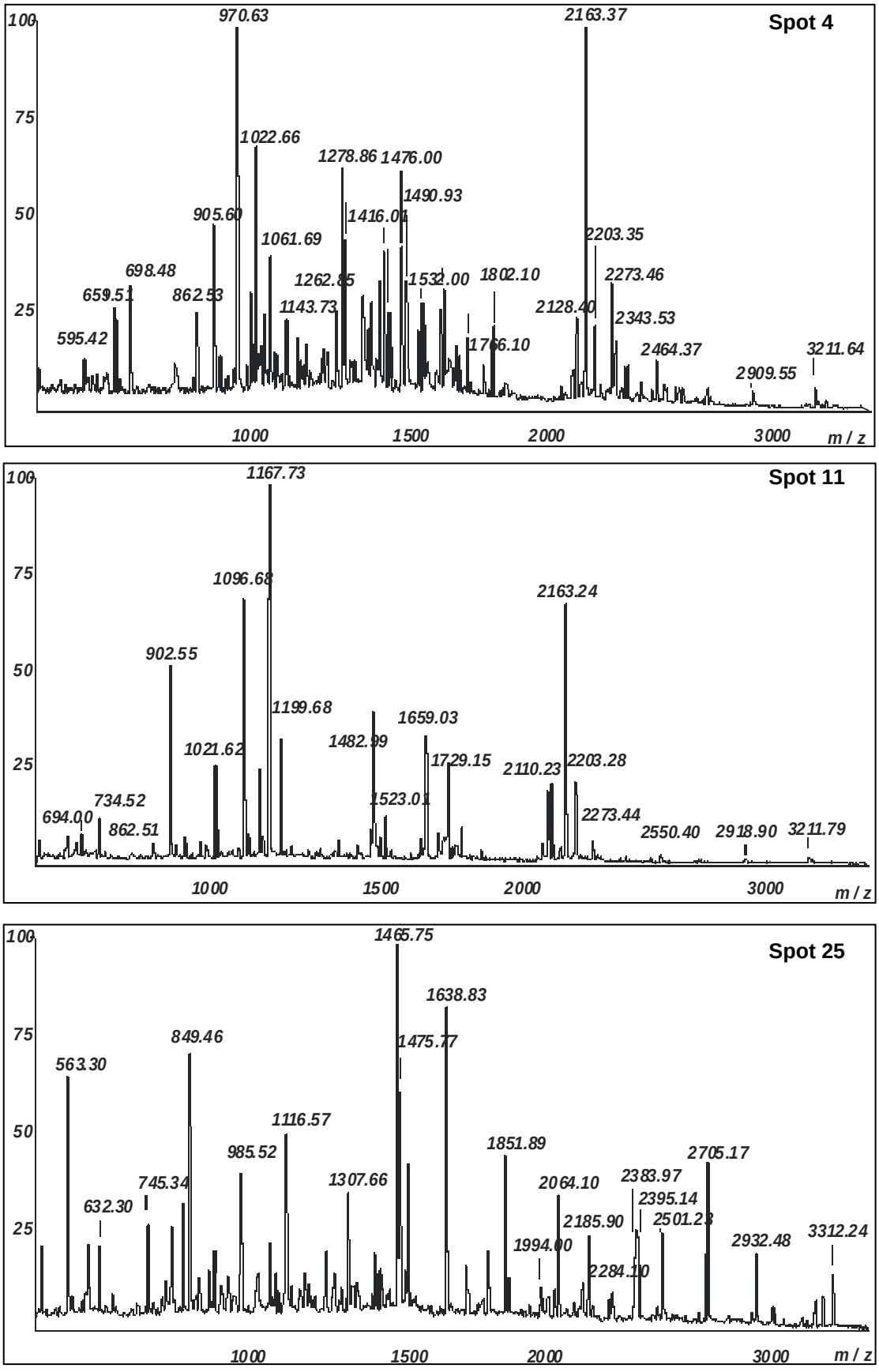

Fig. A2: Representative delayed extraction MALDI-TOF mass spectra of protein spots 4, 11 and 25, following in-gel trypsin digestion. The spots were excised from 2DE-gels of *A. nummularia* leaves.

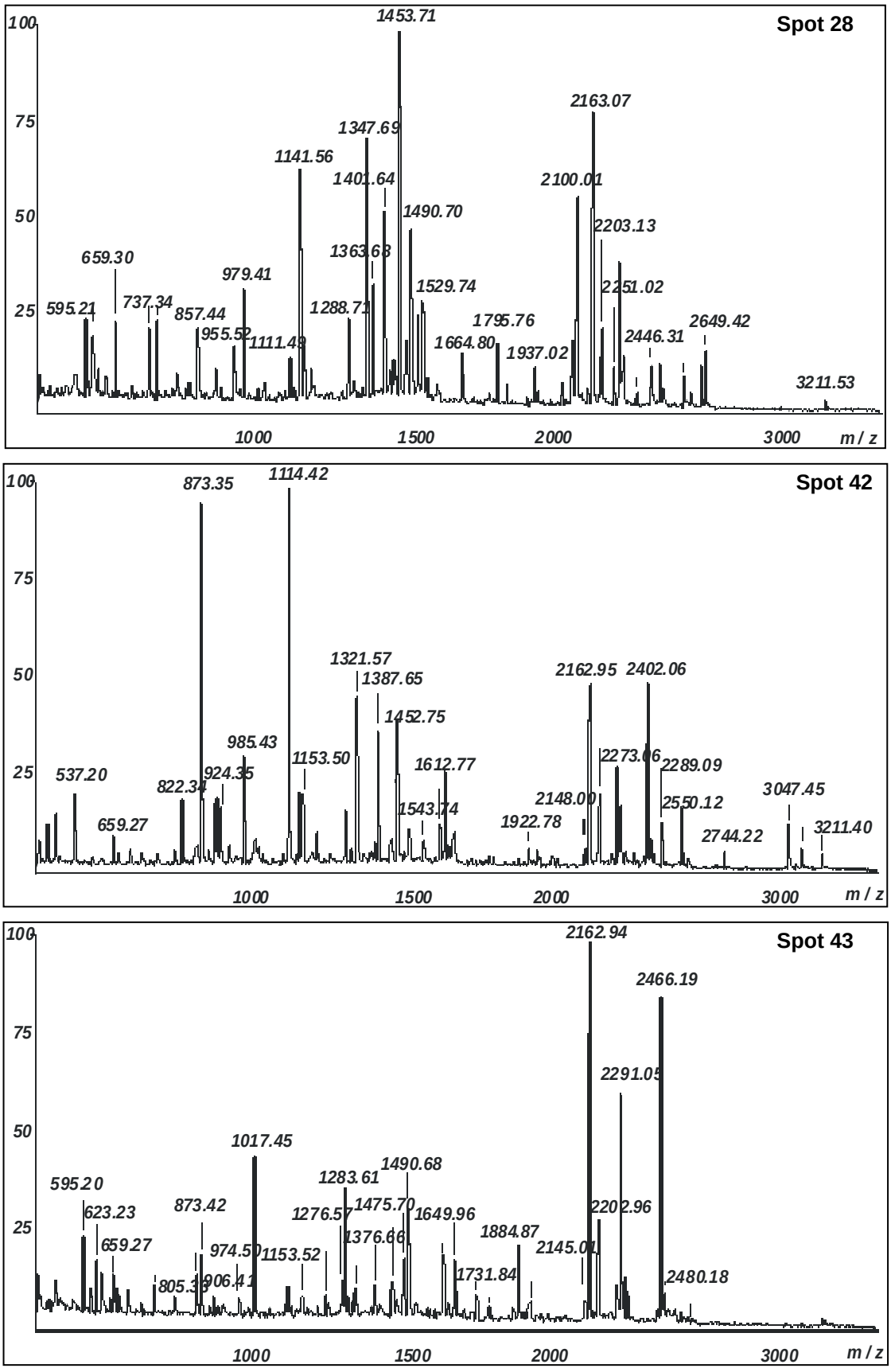

Fig. A3: Representative delayed extraction MALDI-TOF mass spectra of protein spots 28, 42 and 43, following in-gel trypsin digestion. The spots were excised from 2DE-gels of *A. nummularia* leaves.

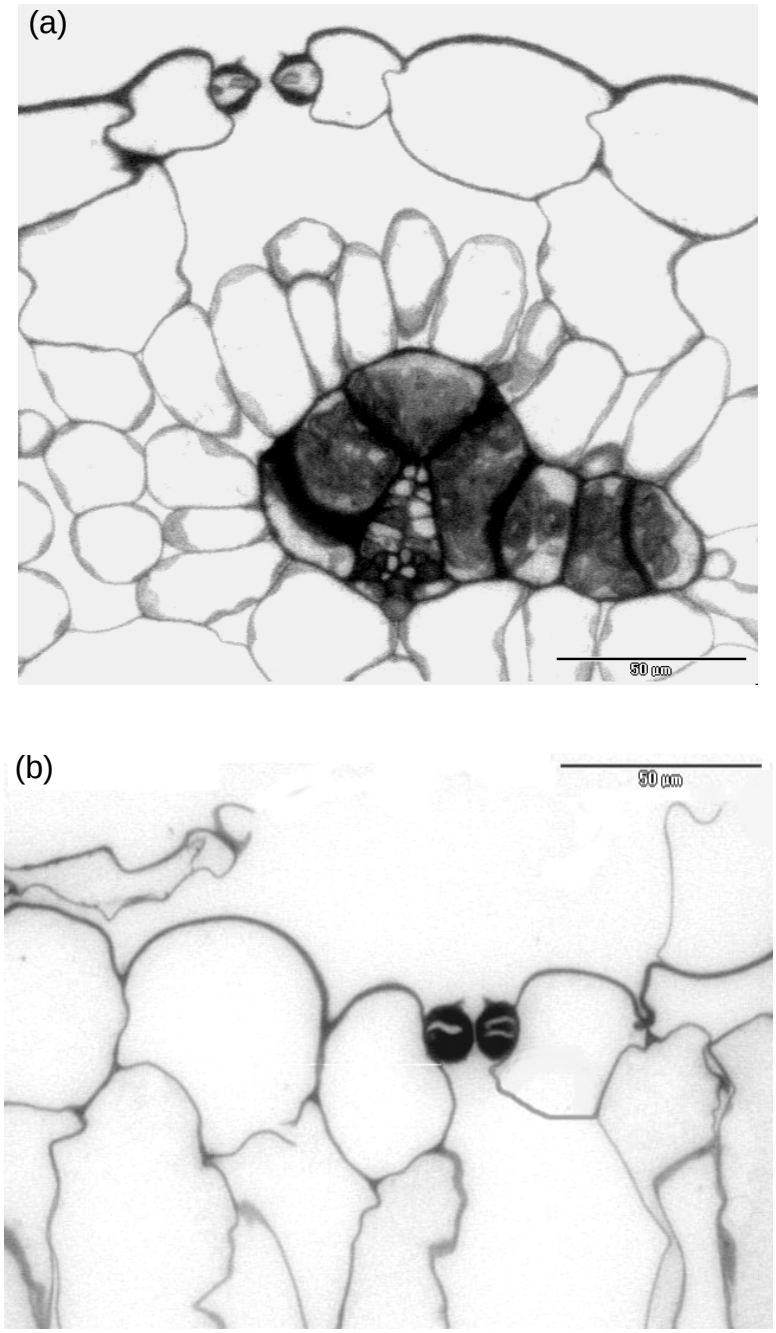

Fig. A4: An enlarged view of the upper epidermis of *A. nummularia* leaves a) untreated and b) treated with 750 mol*m-3 NaCl. Note the thick outer walls in both (a) and (b) and the very thick walls of the guard cells and the small aperture of the stomata under high salinity treatment.

Table A1: Effect of different water salinity levels on the leaf number per plant and the growth (expressed as fresh weight in g) of the different organs of *A. nummularia*. ALN, adult leaf number per plant; JLN, juvenile leaf number per plant; LN, leaf number per plant; ALFW, adult leaf fresh weight; JLFW, juvenile leaf fresh weight; LFW, leaf fresh weight; ASFW; adult stem fresh weight; JSFW, juvenile fresh weight; SFW, stem fresh weight; ShFW, shoot fresh weight; RFW, root fresh weight and PFW, plant fresh weight.

Tearments	ALN	JLN	LN	ALFW	JLFW	LFW	ASFW	JSFW	SFW	ShFW	RFW	PFW	Sh/R ratio
Ctr.	25.2^{a} ± 4.2	328.9^{a} ± 100.4	354.1^{a} ± 104.3	9.6^{a} ± 0.9	24.1^{a} ± 9.2	33.8^{a} ± 9.9	13.7^{a} ± 2.8	5.8^{a} ± 3.2	19.5^{a} ± 5.9	53.2^{a} ± 15.8	13.2^{a} ± 4.4	66.4^{a} ± 20.2	4.1^{a} ± 0.2
125 mM	117.3^{b} ± 13.7	1153.1^{b} ± 150.8	1270.4^{b} ± 164.4	93.1^{bc} ± 8.7	168.5^{b} ± 28.3	261.6^{b} ± 35.6	34.7^{b} ± 5.9	31.8^{b} ± 0.3	66.5^{bc} ± 6.1	328.0^{b} ± 41.7	44.2^{b} ± 10.9	372.2^{b} ± 51.9	7.6^{b} ± 1.1
250 mM	127.0^{b} ± 16.3	1338.1^{b} ± 277.0	1465.1^{b} ± 280.2	102.5^{b} ± 8.8	180.9^{b} ± 17.7	283.3^{b} ± 25.3	42.5^{c} ± 5.4	37.2^{b} ± 5.7	79.7^{c} ± 6.7	363.0^{b} ± 19.5	34.3^{b} ± 4.3	397.4^{b} ± 21.4	10.7^{c} ± 1.2
500 mM	103.3^{b} ± 7.6	1101.6^{b} ± 211.1	1204.9^{b} ± 207.2	79.0^{bc} ± 13.6	160.7^{b} ± 7.3	239.7^{b} ± 18.6	28.1^{b} ± 4.4	29.6^{b} ± 8.9	57.7^{b} ± 10.0	297.4^{bc} ± 28.2	30.3^{ab} ± 6.8	327.7^{b} ± 34.7	10.0^{bc} ± 1.6
750 mM	54.7^{c} ± 12.9	455.6^{c} ± 94.7	510.2^{a} ± 107.3	39.9^{d} ± 4.9	69.9^{c} ± 10.4	109.8^{c} ± 13.9	9.5^{a} ± 2.1	7.8^{a} ± 2.4	17.3^{a} ± 4.6	127.1^{c} ± 18.0	13.2^{a} ± 2.7	140.3^{c} ± 20.7	9.7^{bc} ± 0.7

Each value represents the mean of nine replicates. Means within a column followed by the same letter are not significantly different at $P< 0.05$ as determined by LSD test.

Table A2: Effect of different water salinity levels on the leaf number per plant and the growth (expressed as fresh weight in g) of the different organs of *A. leucoclada*. ALN, adult leaf number per plant; JLN, juvenile leaf number per plant; LN, leaf number per plant; ALFW, adult leaf fresh weight; JLFW, juvenile leaf fresh weight; LFW, leaf fresh weight; ASFW; adult stem fresh weight; JSFW, juvenile fresh weight; SFW, stem fresh weight; ShFW, shoot fresh weight; RFW, root fresh weight and PFW, plant fresh weight.

Tearments	ALN	JLN	LN	ALFW	JLFW	LFW	ASFW	JSFW	SFW	ShFW	RFW	PFW	Sh/R ratio
Ctr.	83.8^{a} ± 1.4	1297.0^{a} 125.8	1380.8^{a} ±126.5	30.5^{a} ± 3.4	117.9^{a} ± 9.1	148.4^{a} ± 12.5	28.6^{a} ± 11.5	61.9^{a} ± 19.6	90.6^{a} ± 30.5	238.9^{a} ± 41.5	22.9^{a} ± 9.3	261.9^{a} ± 50.5	11.2^{a} ± 3.0
125 mM	93.8^{a} ± 26.6	2044.6^{b} ± 438.6	2138.3^{b} ± 452.9	35.6^{a} ± 12.7	162.4^{a} ± 44.5	198.1^{ab} ± 53.3	34.7^{a} ± 2.5	74.8^{a} ± 21.3	109.5^{a} ± 42.8	307.6^{a} ± 96.0	24.9^{a} ± 4.5	332.5^{a} ± 100.5	12.1^{a} ± 1.6
250 mM	109.8^{a} ± 5.9	2071.1^{b} ± 353.2	2180.9^{b} ± 355.1	57.6^{b} ± 4.6	176.1^{a} ± 33.9	233.7^{b} ± 33.3	30.5^{a} ± 1.6	69.4^{a} ± 12.7	99.9^{a} ± 13.1	333.6^{a} ± 46.5	28.3^{a} ± 5.3	361.9^{a} ± 50.0	11.9^{a} ± 1.8
500 mM	84.8^{a} ± 4.3	1560.9^{ac} ± 134.4	1645.7^{ab} ± 130.4	29.4^{a} ± 7.5	125.4^{a} ± 14.8	154.8^{a} ± 7.9	20.9^{b} ± 2.9	49.5^{a} ± 2.9	70.4^{ab} ± 5.4	225.2^{a} ± 12.9	19.4^{ab} ± 3.5	244.5^{a} ± 14.7	11.9^{a} ± 2.2
750mM	45.9^{b} ± 6.1	512.7^{c} ± 55.3	558.6^{c} ± 50.0	8.3^{c} ± 0.6	23.7^{b} ± 5.1	32.0^{c} ± 5.3	5.7^{c} ± 1.2	6.9^{b} ± 1.5	12.6^{b} ± 2.6	44.7^{b} ± 7.41	5.3^{b} ± 1.15	49.9^{b} ± 8.4	8.5^{a} ± 0.9

Each value represents the mean of nine replicates. Means within a column followed by the same letter are not significantly different at $P < 0.05$ as determined by LSD test.

Table A3: Element contents (expressed as weight percent) of the adaxial bladder cells, epidermal and guard cells of the juvenile leaves of *A. nummularia* and *A. leucoclada* at control and high salinity (750 mol*m^{-3} NaCl) condition.

Species	Element	Bladder hairs		Epidermal cells		Guard cells	
		Control	750 NaCl	Control	750 NaCl	Control	750 NaCl
A. nummularia	**Na**	31.99 ± 0.00	45.54 ± 0.00	12.79 ± 0.20	40.10 ± 4.57	7.27 ± 1.12	40.28 ± 4.71
	Mg	13.95 ± 0.00	1.05 ± 0.00	21.17 ± 2.33	1.04 ± 0.25	21.57 ± 4.61	1.99 ± 0.14
	P	3.86 ± 0.00	0.51 ± 0.00	22.48 ± 4.19	1.34 ± 0.33	24.89 ± 5.23	3.43 ± 0.96
	S	6.67 ± 0.00	1.45 ± 0.00	4.55 ± 0.30	1.69 ± 0.21	6.32 ± 0.64	6.91 ± 0.45
	Cl	24.82 ± 0.00	49.91 ± 0.00	3.46 ± 0.27	48.42 ± 2.83	2.53 ± 0.18	27.09 ± 3.76
	K	14.41 ± 0.00	0.78 ± 0.00	20.77 ± 1.99	4.08 ± 1.38	23.03 ± 3.59	16.37 ± 2.89
	Ca	2.31 ± 0.00	0.96 ± 0.00	14.78 ± 2.08	3.02 ± 0.19	14.38 ± 2.42	3.82 ± 0.54
A. leucoclada	**Na**	32.62 ± 8.00	38.05 ± 3.32	6.66 ± 2.01	27.89 ± 5.61	7.54 ± 0.00	27.37 ± 0.00
	Mg	28.85 ± 4.48	0.95 ± 0.78	35.06 ± 7.97	2.87 ± 1.14	32.96 ± 0.00	3.77 ± 0.00
	P	5.81 ± 2.91	0.57 ± 0.26	23.51 ± 2.29	10.41 ± 3.71	25.26 ± 0.00	12.65 ± 0.00
	S	7.93 ± 2.92	1.58 ± 0.82	11.20 ± 2.62	3.67 ± 0.86	7.36 ± 0.00	3.40 ± 0.00
	Cl	11.45 ± 2.55	57.54 ± 1.26	0.76 ± 1.31	38.25 ± 3.63	0.30 ± 0.00	32.42 ± 0.00
	K	11.90 ± 2.74	0.65 ± 0.59	18.63 ± 3.28	14.55 ± 3.27	22.50 ± 0.00	17.41 ± 0.00
	Ca	1.13 ± 0.40	0.65 ± 0.57	4.77 ± 0.27	2.36 ± 0.57	4.10 ± 0.00	2.99 ± 0.00

Each value represents the mean of three replicates. Means within a column followed by the same letter are not significantly different at $P < 0.05$ as determined by LSD test.

Table A4: Probable candidate names of salt responsive proteins which regulated by a factor of at least two folds in gels of both *A. nummularia* and *A. leucoclada* leaves. Protein spots identification using the pI and MW was conducted by quiring the SWISS-PROT, TrEMBL and NCBInr data bases.

Spot No.	Experimental pI/MW	Probable candidate names obtained from Swiss- Prot, TrEMBL and NCBInr database
1	6.5 ± 0.1 / 97.0 ± 5.3	• **Phosphoenolpyruvate carboxylase.** pI: 6.04, MW: 109.48 • **Potassium channel.** pI: 6.31, MW: 99.21. • **Lipoxygenase.** pI: 6.34, MW: 97.45. • **Nitrate reductase.** pI: 6.33 – 6.61, MW: 98.81 - 101.42. • **Putative disease resistance protein.** pI: 6.26 - 6.60, MW: 101.44 – 94. 51. • **Plasma membrane ATPase.** pI: 6.25 – 6.54 , MW: 104.09 – 105.52. • **Sucrose synthase isoform.** pI: 6.40, MW: 91.65.
3	6.2 ± 0.2 / 97 ± 7.0	• **Alpha-xylosidase precursor.** pI: 6.32, MW: 99.64. • **Sucrose synthase.** pI: 5.98 - 6.28, MW: 92.24 – 93.20. • **Potassium channel.** pI: 6.27, MW: 93899.23. • **Disease resistance protein.** pI: 6.14 - 6.26, MW: 97.26 – 101.44. • **Nitrate reductase.** pI: 6.04 – 6.44, MW: 98.81 – 101.77. • **Lipoxygenase.** pI: 6.01 - 6.31, MW: 96.63 - 100.08.
4	5.5 ± 0.2 / 95.0 ± 7.1	• **Phosphoenolpyruvate carboxylase.** pI: 5.57 - 5.94, MW: 108.37 – 109.99. • **Probable disease resistance protein.** pI: 5.87 - 6.14, MW: 95.79 – 102.64. • **Calcium-transporting ATPase.** pI: 5.76, MW: 109.06. • **Glycine dehydrogenase.** pI: 5.64 – 5.94, MW: 105.54 – 105.56. • **Probable potassium transporter.** pI: 5.51, MW: 95.45. • **Heat shock protein 101.** pI: 5.81 – 5.90, MW: 100.9 – 101.29. • **Lipoxygenase.** pI: 6.01 – 6.16, MW: 96.64 - 97.18. • **ATPase, plasma membrane-type.** pI: 5.53 - 5.82, MW: 104.13 – 105.01. • **Sucrose synthase.** pI: 5.78 - 5.94, MW: 92.00 – 93.01.
11	6.5 ± 0.2 / 80.0 ± 3.2	• **Chloride channel protein.** pI: 6.81, MW: 85.41. • **Ethylene receptor**. pI: 6.92, MW: 82.64. • **Sodium/hydrogen exchanger.** pI: 6.58, MW: 83.47. • **Photosystem I P700.** pI: 6.74, MW: 83.04. • **5-methyltetrahydropteroyltriglutamate-homocysteine transmethylase.**pI: 5.9 – 6.1, MW: 85.05 – 85.22. • **Methionine synthase.** pI: 6.0 – 6.2, MW: 85.3 – 88.0.
12	6.5 ± 0.3 / 80.0 ± 2.5	• **Chloride channel protein.** pI: 6.28, MW: 83.54. • **Ethylene receptor 2.** pI: 6.53 - 6.61, MW: 81.70 – 82.89. • **Sodium/hydrogen exchanger.** pI: 6.58, MW: 83.47. • **Phenylalanine ammonia-lyase.** pI: 6.26 – 6.32, MW: 77.78 – 78.99. • **Photosystem I P700.** pI: 6.46 – 6.74, MW: 81.74 – 83.33.
13	6.5 ± 0.1 / 78.0 ± 2.2	• **Photosystem I P700.** pI: 6.46 - 6.74, MW: 81.74 – 83.33. • **Phenylalanine ammonia-lyase.** pI: 6.26 – 6.32, MW: 77.78 – 78.99. • **Sodium/hydrogen exchanger 8.** pI: 6.58, MW: 83.47. • **Chloride channel protein.** pI: 6.28, MW: 83.54.

24	5.7 ± 0.3 / 56.0 ± 4.1	**Calcium-dependent protein kinase.** pI: 5.93 - 6.13, MW: 58.08 – 60.06. • **Light-independent protochlorophyllide reductase subunit B.** pI: 5.95 - 6.42, MW: 58.22 – 58.34. • **Phytoene dehydrogenase.** pI: 5.77 – 6.22, MW: 52.19 – 53.69. • **Photosystem II P680.** pI: 5.97 – 6.16, MW: 55.99 – 56.33. • **Ribulose bisphosphate carboxylase large chain.** pI: 6,27 - 6.68, MW: 52.17 – 53.16. • **Sucrose-binding protein.** pI: 6.08, MW: 57.22. • **Tyrosine decarboxylase.** pI: 5.89 – 6.20, MW: 54.42 – 59.52.
25	6.5 ± 0.2 / 52.0 ± 2.6	• **Cytochrome P450.** pI: 6.52, MW: 54.44. • **Glutathione reductase.** pI: 6.31 – 6.56, MW: 53.14 - 53.87. • **Putative glycosyltransferase.** pI: 6.52 – 6.57, MW: 50.07 – 53.09. • **Photosystem II 44 kDa reaction center protein.** pI: 6.34 - 6.71, MW: 50.04 – 51.87. • **Ribulose bisphosphate carboxylase large chain precursor.** pI: 6.27 - 6.68, MW: 52.17 – 53.16. • **Threonine dehydratase biosynthetic.** pI: 6.27, MW: 54.79. • **Probable vacuolar ATP synthase subunit H.** pI: 6.58, MW: 50.28.
28	5.8 ± 0.3 / 45.0 ± 2.7	• **Chalcone synthase.** pI: 5.75 – 6.24, MW: 42.01 – 43.74. • **S-adenosylmethionine synthetase.** pI: 5.67 - 6.02, MW: 42.55 – 42.65. • **Alcohol dehydrogenase 1.** pI: 5.91 – 6.20, MW: 40.01 – 41.57. • **ATP synthase a chain.** pI: 6.19, MW: 42.99. • **Aspartate aminotransferase.** pI: 6.10, MW: 44.36. • **Choline monooxygenase**. pI: 5.81, MW: 42.63. • **Glutamate dehydrogenase**. pI: 6.07, MW: 44.67. **Chaperone protein.** pI: 5.76, MW: 46.44.
39	7.3 ± 0.2 / 33.0 ± 2.0	• **Peroxidase 1 precursor.** pI: 7.17, MW: 33.29. • **Cell division control protein.** pI: 7.13, MW: 34.00. • **Ferredoxin--NADP reductase, chloroplast precursor.** pI: 7.07, MW: 35.20.
42	6.5 ± 0.1 / 37.0 ± 3.5	• **Alcohol dehydrogenase class III,** (S-(hydroxymethyl)glutathione dehydrogenase). pI: 6.78, MW: 40.82. • **Fructose-bisphosphate aldolase**. pI: 6.96, MW: 38.86. • **Chalcone synthase.** pI: 6.72, MW: 42.71. • **Gibberellin oxidase,** (Gibberellin C-20 oxidase). pI: 6.90, MW: 43.44.
43	6.5 ± 0.1 / 37.0 ± 3.5	• **Malate dehydrogenase**. pI: 6.33 – 6.39, MW: 33.41 – 35.68 • **Glyceraldehyde-3-phosphate dehydrogenase**. pI: 6.2 – 6.68, MW: 36.096 – 36.67. • **Ribose-phosphate pyrophosphokinase**. pI: 6.31, MW: 36.15. • **Aldose reductase**. pI: 6.51, MW: 35. 81 • **Ferredoxin-NADP reductase**. pI: 6.54, MW: 34.78

44	6.1 ± 0.2 / 39.0 ± 2.6	• **Alcohol dehydrogenase 1** (EC 1.1.1.1). pI: 6.15 - 6.29, MW: 40.48 - 40.88. • **S-adenosyl-L- methionine:norcoclaurine 6-O-methyltransferase.** pI: 6.27, MW: 38.69. • **Fructose-bisphosphate aldolase.** pI: 5.96 - 6.21, MW: 38.45 - 38.45. • **Arginase.** pI: 6.11, MW: 37.34. • **Glutamine synthetase.** pI: 5.94 - 6.21, MW: 39.20 – 39.57. • **Calcium-activated outward-rectifying potassium channel 1.** pI: 6.25, MW: 40.72. • **DNA-directed RNA polymerase alpha chain.** pI: 5.91 - 6.33, MW: 38.30 – 39.15. • **Thioredoxin reductase.** pI: 6.26, MW: 40.63. • **Vacuolar ATP synthase subunit C.** pI: 6.06, MW: 39.98.
56	5.9 ± 0.1 / 28.0 ± 3.0	• **L-ascorbate peroxidase, cytosolic**. pI: 5.72, MW: 27.43. • **Chloroplast envelope membrane protein.** pI: 5.71 - 6.07, MW: 26.75 – 27.64. • **Hydroxyacylglutathione hydrolase.** pI: 5.93 - 6.14, MW: 28.16 - 28.97. • **Probable aquaporin.** pI: 6.03, MW: 28.74. • **Proteasome subunit alpha type 3.** pI: 5.75 - 6.11, MW: 27.23 – 27.29. • **Ribonuclease 2 precursor**. pI: 5.82, MW: 27.23. • **Plastid-specific 30S ribosomal protein.** pI: 5.89, MW: 26.80. • **Stress-related protein.** pI: 5.86, MW: 27.54. • **Vesicle-associated membrane protein.** pI: 6.00, MW: 27.45.
58	7.0 ± 0.1 / 27.0 ± 3.1	• **Probable glutathione S-transferase.** pI: 6.77, MW: 25.66. • **Adenylate kinase.** pI: 6.91, MW: 26.93. • **NAD(P)H-quinone oxidoreductase chain K.** pI: 6.90, MW: 27.83. • **Proteasome subunit alpha type 7-A.** pI: 6.86, MW: 27.33. • **Vacuolar ATP synthase subunit E.** pI: 7.13, MW: 26.34. • **Putative cytochrome c biosynthesis ccmC-like mitochondrial protein.** pI: 6.89, MW: 26.08.
59	7.2 ± 0.2 / 27.0 ± 2.3	• **Embryonic abundant protein.** pI: 7.14, MW: 27.63. • **Vacuolar ATP synthase subunit E.** pI: 7.13, MW: 26.34. • **Adenylate kinase.** pI: 6.91, MW: 26.93. • **Alpha-expansin 23 precursor.** pI: 6.96, MW: 27.28. • **Aquaporin TIP3.1.** pI: 7.20, MW: 28.30.
61	7.0 ± 0.1 / 25.0 ± 2.0	• **ATP synthase delta chain.** pI: 6.88, MW: 22.29. • **Adenylate kinase.** pI: 6.91, MW: 26.93 – 27.33. • **NADH-ubiquinone oxidoreductase 27 kDa subunit.** pI: 6.92, MW: 23.17. • **Ubiquinol-cytochrome c reductase iron-sulfur.** pI: 6.93, MW: 23.20. • **Vacuolar ATP synthase subunit E.** pI: 7.13, MW: 26.34. • **Germin-like protein.** pI: 7.08, MW: 21.75.
64	9.2 ± 0.2 / 23.0 ± 2.0	• **Putative alpha-expansin.** pI: 9.26 - 9.75, MW: 24.25 – 25.65. • **NADH-ubiquinone oxidoreductase.** pI: 9.56, MW: 23.68 – 27.68. • **Proline-rich protein precursor.** pI: 9.58, MW: 22.23. • **Mitochondrial Ribosomal protein.** pI: 9.48 - 9.69, MW: 20.62 - 22.01. • **Chloroplast 30S ribosomal protein S2.** pI: 9.70, MW: 21.91 – 27.23.

65	6.3 ± 0.3 / 25.0 ± 3.0	• **Probable shikimate kinase.** pI: 6.38, MW: 24.84. • **Carbonic anhydrase.** pI: 6.06 – 6.19, MW: 23.27 – 25.57. • **Chloroplast envelope membrane protein.** pI: 6.07 – 6.44, MW: 26.94 – 27.40. • **Dehydrin DHN2.** pI: 6.35, MW: 24.49. • **Glutathione S-transferase.** pI: 6.06 - 6.34, MW: 23.35 – 24.63. • **NADH-ubiquinone oxidoreductase 27 kDa subunit.** pI: 6.44, MW: 23.15. • **Osmotin-like protein.** pI: 6.39, MW: 25.18. • **Proteasome subunit alpha type.** pI: 6.17, MW: 27.44. • **Peroxiredoxin.** pI: 6.13, MW: 24.08. • **Superoxide dismutase [Mn], mitochondrial precursor.** pI: 6.24, MW: 22.69. • **Vacuolar ATP synthase subunit E.** pI: 6.50, MW: 27.16.
67	5.5 ± 0.2 / 20 ± 2.8	• **ATP synthase B chain.** pI: 5.33 – 5.41, MW: 20.11 – 20.88. • **Auxin-induced protein 22D.** pI: 5.52, MW: 21.69. • **Chlorophyll a-b binding protein.** pI: 5.59, MW: 23.93. • **Dehydration-responsive element-binding protein.** pI: 5.49, MW: 20.64. • **Ferritin-1.** pI: 5.26 - 5.56, MW: 23.11 - 23.76. • **Germin-like protein.** pI: 5.26 – 5.65, MW: 21.55 – 22.17. • **Glutathione S-transferase.** pI: 5.45 – 5.65, MW: 23.69 – 24.11. • **Nodulin.** pI: 5.45 - 5.73, MW: 20.24 – 21.55. • **Proteasome subunit beta type.** pI: 5.31 – 5.61, MW: 22.75 – 23.86. • **Superoxide dismutase [Mn].** pI: 5.58, MW: 22.56. • **GTP-binding protein.** pI: 5.60, MW: 23.311.
68	5.3 ± 0.2 / 22.5 ± 3.2	• **Germin-like protein.** pI: 5.26 – 5.65, MW: 21.55 – 22.17. • **Glutathione S-transferase.** pI: 5.45 – 5.65, MW: 23.69 – 24.11. • **Nodulin.** pI: 5.45 - 5.73, MW: 20.24 – 21.55. • **Oxygen-evolving enhancer protein.** pI: 5.52 - 5.95, MW: 20.02 – 21.72. • **Superoxide dismutase [Mn].** pI: 5.46 - 5.89, MW: 22.35 – 23.00. • **GTP-binding protein.** pI: 5.60, MW: 23.311.
69	6.5 ± 0.1 / 20 ± 2.0	• **ATP synthase subunit I**. pI: 6.35, MW: 21.26. • **Germin-like protein.** pI: 6.46, MW: 21.46. • **NAD(P)H-quinone oxidoreductase subunit I**. pI: 6.42, MW: 21.16.
71	7.8 ± 0.1 / 20 ± 2.0	• **Oxalate oxidase.** pI: 7.80, MW: 21.20. • **Germin-like protein.** pI: 7.75 – 7.95, MW: 21.02 - 21.18.
72	9.2 ± 0.2 / 22.0 ± 2.5	• **Putative 3-methyladenine DNA glycosylase**. pI: 9.12, MW: 23.15. • **ATP synthase B chain.** pI: 9.13 - 9.48, MW: 20.06 – 20.98. • **Cytochrome b6.** pI: 9.10 - 9.14, MW: 24.12 - 24.16. • **Phospholipid hydroperoxide glutathione peroxidase.** pI: 9.05, MW: 19.30. • **Photosystem I assembly protein.** pI: 9.25 – 9.44, MW: 20.6 – 22.95.

74	9.5 ± 0.3 / 18.0 ± 3.0	• **Photosystem I reaction center.** pI: 9.05 - 9.38, MW: 17.27 - 17.85. • **Water stress-inducible protein.** pI: 9.19, MW: 17.32. • **Chaperone protein.** pI: 9.44, MW: 20.65. • **Disease resistance response protein.** pI: 9.06, MW: 20. 371 • **Germin-like protein.** pI: 9.04 – 9.30, MW: 19.56 – 21.40. • **Phospholipid hydroperoxide glutathione peroxidase.** pI: 9.01 - 9.05, MW: 19.30 – 19.43. • **Probable glutathione peroxidase.** pI: 9.28, MW: 19.33. • **Multiple stress-responsive zinc-finger protein ISAP1.** pI: 9.14, MW: 17.63. • **Mitochondrial 22 kDa protein.** pI: 9.44, MW: 21.65. • **Ribonuclease.** 9.00 - 9.27, MW: 22.64 - 23.51.
75	7.5 ± 0.2 / 18.0 ± 0.1	• **Ribulose bisphosphate carboxylase small subunit 1.** pI: 7.60, MW: 16.25.
76	7.2 ± 0.4 / 20.0 ± 3.5	• **Dehydrin.** pI: 7.10, MW: 18.46. • **Germin-like protein.** pI: 6.7 - 7.08, MW: 19.99 - 21.74. • **18.3 kDa class I heat shock protein (HSP 18.3).** pI: 6.76, MW: 18.27.
77	7.2 ± 0.3 / 18.0 ± 1.0	• **Dehydrin.** pI: 7.10, MW: 18.46. • **Nucleoside diphosphate kinase III.** pI: 7.07, MW: 17.12. • **NAD(P)H-quinone oxidoreductase subunit I.** pI: 7.51, MW: 20.09.
78	5.7 ± 0.2 / 16.0 ± 1.0	• **ATP synthase epsilon chain.** pI: 5.83 – 6.1, MW: 14.46 – 15.15. • **Superoxide dismutase.** pI: 5.75 – 5.93, MW: 14.94 – 15.37. • **Serine carboxypeptidase.** pI: 6.03, MW: 16199.23.
83	7.2 ± 0.1 / 10.0 ± 2.0	• **Probable nonspecific lipid-transfer protein.** pI: 7.03, MW: 10.92. • **Hypothetical mitochondrial protein.** pI: 6.96, MW: 12.12. • **Cytochrome c oxidase polypeptide.** pI: 7.14, MW: 7.04. • **2S sulfur-rich seed storage protein.** pI: 7.03, MW: 8.60.
84	9.4 ± 0.1 / 15.0 ± 2.5	• **Ubiquinol-cytochrome c reductase complex 14 kDa protein.** pI: 9.30, MW: 14470.76. • **Hypothetical mitochondrial protein.** pI: 9.12 – 9.41, MW: 12.00 – 16.45. • **Probable cytochrome c.** pI: 9.04 - 9.37, MW: 12.05 - 12.39. • **Hypothetical mitochondrial protein.** pI: 9.12 – 9.41, MW: 12.00 – 16.45. • **NAD(P)H-quinone oxidoreductase chain 4L.** pI: 9.03 – 9.43, MW: 11.27 – 11.33.
86	5.3± 0.2 / 15.0 ± 2.0	• **Thioredoxin H-type.** pI: 5.37, MW: 13.58. • **Superoxide dismutase [Cu-Zn].** pI: 5.16 – 5.44, MW: 14.97 – 15.70. • **Salt stress-induced protein.** pI: 5.19, MW: 15.06. • **Ribulose bisphosphate carboxylase small chain.** pI: 5.12 - 5.50, MW: 14.11 - 14.66. • **L-asparaginase precursor.** pI: 5.06 - 5.27, MW: 13.45 - 13.60. • **ATP synthase epsilon chain.** pI: 5.13 - 5.44, MW: 14.47 – 14.66.

87	5.3± 0.2 / 14.0 ± 1.6	• **L-asparaginase precursor.** pI: 5.06 - 5.27, MW: 13.45 - 13.60. • **ATP synthase epsilon chain.** pI: 5.13 - 5.44, MW: 14.47 – 14.66. • **Ribulose bisphosphate carboxylase small chain.** pI: 5.12 - 5.50, MW: 14.11 - 14.66. • **Thioredoxin H-type 1.** pI: 5.12 - 5.37, MW: 13.39 – 13.58. • **Plastid-specific 30S ribosomal protein 3.** pI: 5.08, MW: 13.79. • **50S ribosomal protein.** pI: 5.30, MW: 14.35.

Personal record

Personal Information

- **Family Name:** Hussin
- **First Name:** Sayed
- **Place and date of birth:** Cairo, 25 Oktober, 1972
- **Nationality:** Egyptian
- **Marital status:** Married
- **Position:** Assistant Lecturer in Department of Agricultural Botany, Faculty of Agriculture, Ain Shams University, Cairo, Egypt.

Education background

- **1978 - 1987:** Ground School in Cairo, Egypt.
- **1987-1990:** Secondary School, Cairo, Egypt .
- **1990-1994:** B. Sc. degree in Agriculture Sciences with major in Horticulture, final grade „very good“, Faculty of Agriculture, Ain Shams University, Cairo, Egypt.
- **1995 - 2000:** M. Sc. degree in Agricultural Botany, Faculty of Agriculture, Ain Shams University, Cairo, Egypt.
- **6/2002 - 9/2002:** German Language course at the Goethe Institut, Mannheim, Germany.
- **10/2002 – 10/2006:** Studies at the Institut für Botanik, Hannover University, Germany fort he award of Doctoral degree with the support of a DAAD fellowship.

Employment hisory

- **10/1995 - 5/2000:** Demonstrator in the Department of Agricultural Botany, Faculty of Agriculture, Ain Shams University, Cairo, Egypt.
- **6/2000 - 6/2002:** Assistant Lecturer in the Department of Agricultural Botany, Faculty of Agriculture, Ain Shams University, Cairo, Egypt.

List of publications

Published:

- **Koyro, H.-W.; Geissler, N.; Hussin, S; Huchzermeyer, B. (2006)** Mechanisms of cash crop halophytes to maintain yield and reclaim soils in arid areas. In: M. A. Khan, and D. J. Weber (Eds.), Task for Vegetation Science 40. Ecophysiology of High Salinity Tolerant Plants. Springer Publ., Pp. 345 - 366.

In Press:

- **Koyro, H.-W.; Geissler, N.; Hussin, S; Huchzermeyer, B. (2007)** Survival at extreme locations: Life strategies of halophytes - The long way from increasing salinisation of cultivated agricultural lands across molecular aspects of salt tolerance to the use of cash crop halophytes. In: C. Abdelly (Ed.) Biosaline Agriculture. Springer Publ. (in press).

In preparation:

- **S. Hussin, N. Geissler, S. Eisa, S. Habib and H.-W. Koyro (2007)** The potential of cash crop halophytes (*Atriplex nummularia* Lindl. and *Atriplex leucoclada* Bioss. to maintain yield and reclaim saline soils in arid areas. (Prep.)

- **S. Hussin, S. Habib, and H.-W. Koyro (2007)** Strategies of the halophyte *Atriplex leucoclada* Bioss. to survive at saline habitats. (Prep.)

- **H.-W. Koyro, N. Geissler, S. Hussin, S. Eisa, and S. Habib (2007)** Ecophysiological mechanisms of cash crop halopytes to maintain yields and reclaim saline soils in arid areas. (Prep.)

- **S. Eisa, S. Hussin, and H.-W. Koyro.** Physiological responses of *Chenopodium quinoa* under sea-water irrigation. (Prep.)