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Effect of salt stress on nutrient concentration, photosynthetic pigments, proline and foliar morphology of *Salvinia auriculata* Aubl.

Efeito do estresse salino sobre as concentrações de nutrientes, pigmentos fotossintéticos, prolina e na morfologia foliar de *Salvinia auriculata* Aubl.

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Abstract: Aim: This study aimed to investigate the effects of NaCI and Na_2So_4 salts on Ca, CI, K, Mg N, P, S and Na content as well as on the content of photosynthetic pigments (chlorophyll-*a*, chlorophyll-*b* and carotenoids), proline content and on the foliar morphology of *Salvinia auriculata* Aubl. **Methods:** The plants were collected in Jacu lagoon, located in the North of Rio de Janeiro State, and after a five-day-acclimation period, experiments were performed in the greenhouse with 0, 100, 200 mM concentrations of NaCI and Na_2SO_4 salts and the usual techniques for light and electron microscopy. **Results:** After seven days of experiment, a decrease in the content of Ca^{2+}, K^+, Mg^{2+}, P, N ions as well as in the content of photosynthetic pigments (chlorophyll-*a,b* and carotenoids) in *Salvinia auriculata* under saline treatments was observed. The proline content showed an upward tendency as compared to the control. Under transmission electron microscopy, it was observed that, on the foliar limb, there was a membrane system disorder, mainly of chloroplasts, with higher presence of starch grains of plant cells subjected to salinity. Under scanning electron microscope, the integrity of trichomes and foliar limb cells of *Salvinia auriculata* subjected to the control treatment as well as the changes caused by salinization on the surface of cells were observed. **Conclusion:** As for all the salinization effects evaluated, it was noticed that the increase in Na_2SO_4 salt concentration resulted in higher morphological and nutritional alterations in the floating aquatic macrophyte, *Salvinia auriculata.*

Keywords: floating aquatic macrophyte, salinization, cells, microscopy.

Resumo: Objetivo: Este estudo teve como objetivo investigar os efeitos dos sais NaCl e Na_2SO_4 sobre o conteúdo de Ca, Cl, K, Mg, N, P, S, Na; teor de pigmentos fotossintéticos (clorofila-*a*, clorofilab-*b* e carotenóides); teor de prolina e a morfologia das folhas de *Salvinia auriculata* Aubl.**; Métodos:** As plantas foram coletadas na lagoa do Jacu localizada no Norte do Estado do Rio de Janeiro, e após um período de cinco dias de aclimatação foram realizados experimentos em casa de vegetação com as concentrações 0, 100, 200 mM dos sais NaCl e Na_2SO_4 e utilizadas as técnicas usuais para microscopia óptica e eletrônica; **Resultados:** Após sete dias de experimento foi verificado a diminuição do conteúdo dos íons Ca^{2+}, K^+ , Mg^{2+}, P, N e do teor de pigmentos fotossintetizantes (clorofila-*a*,*b* e carotenóides) em *Salvinia auriculata* submetidas aos tratamentos salinos. O teor de prolina mostrou uma tendência ao incremento quando comparado ao controle. Sob microscopia eletrônica de transmissão, no limbo foliar foi observado uma desorganização do sistema de membranas, principalmente dos cloroplastos com maior presença de grãos de amido das células das plantas submetidas à salinidade. Sob microscópio eletrônico de varredura foi observada a integridade dos tricomas e das células do limbo foliar de *Salvinia auriculata* submetida ao tratamento controle e mudanças na superfície das células provocadas pela salinização**; Conclusões:** Para todos os efeitos da salinização avaliados, foi notado que o incremento na concentração do sal Na_2SO_4 resultou em maiores alterações morfológicas e nutricionais à macrófita aquática flutuante *Salvinia auriculata*.

Palavras-chave: macrófita aquática flutuante, salinização, células, microscopia.

1. Introduction

High concentrations of soluble salts occur in terrestrial environments or in aquatic environments, which may happen naturally or anthropogenically (Larcher, 1995). The naturally occurring salinization is recognized as primary and the anthropogenic form as secondary (Williams, 1999).

The primary salinization is a natural process which occurs in regions where there is water deficit, in other words, low rainfall and high evaporation potential, which leads to a progressive increase in the concentration of salts released by weathering or by sea spray that may reach the water bodies as consequence of storms and winds (Suzuki et al., 1998; 2002; Roache et al., 2006). Unlike primary salinization, secondary salinization results from human activities (Neumann, 1997). The secondary salinization of water bodies may occur through irrigation of agricultural crops which may leach the accumulated salt in irrigated soils into river and lake waters downstream (Rengasamy, 2006) and, in the specific case of coastal lagoons, through the process of opening sand fences between the sea and the coastal lagoons (Suzuki et al.,1998; 2002).

In the Northern region of Rio de Janeiro state, the intrusion processes of saline or brackish water in coastal aquatic ecosystems may cause indirect process of salinization of other water bodies that do not present direct contact with the sea, through exchange of water by groundwater. Groundwater in this region is sub-outcropping (Dantas et al., 2000), which causes intense exchange between shallow aquatic ecosystems and groundwater supply. Allied to this feature, the negative water balance observed in this region (Chagas and Suzuki, 2005) may worsen the salinization process in these ecosystems, which causes alterations in the biotic communities that grow there.

Ecological variations in the aquatic macrophyte communities related to salinization are fully documented, which involves alterations in abundance, vegetation distribution in aquatic ecosystems (Kipriyanova et al., 2007; Watson and Byrne, 2009), variations in growth, reproduction and survival of macrophytes (Warwick et al., 1997Muschal, 2006), and usually reduces species richness (Greenberg et al., 2006; Sharpe and Baldwin, 2009).

Salinity increase in aquatic ecosystems affects most plants and causes ionic and osmotic stresses (Owens, 2001), several biochemical and morphological alterations as well as nutrient imbalance (Muhammed et al., 1987; Jampeetong and Brix, 2009). At the beginning or during the period of plant exposure to salinity, fundamental factors to plant life such as growth, photosynthesis, protein synthesis, lipid metabolism, productivity (Parida and Das, 2005) and nutritional balance are affected (Grattan and Grieve, 1999).

Nutrients, in general, have several functions in plant structure, metabolism and osmoregulation of plant cells (Taiz and Zeiger, 2009). However, one of the most important salt stress effects on plants is induced by nutritional disorders, which result from salinity effect on availability, absorption and transport of nutrients within the plant (Munns and Tester, 2008). Nutrient deficiency as well as ion toxicity and osmotic stress are factors attributed to the deleterious effect of salinity on plant growth and productivity (Nublat et al., 2001).

Chlorophyll is the principal agent responsible for photosynthesis and, under adverse conditions, chlorophyll level is a good indicator of photosynthetic activity (XinWen et al., 2008). Thus, we quantified the content of photosynthetic pigments in order to infer the effect of salt concentrations on the photosynthetic activity of *S. auriculata.*

To achieve ion balance in the vacuoles, the cytoplasm accumulates compounds called compatible solutes or osmolytes which do not interfere with normal biochemical reactions (Hasegawa et al., 2000; Parida and Das, 2005). Compatible solutes are neutral molecules, non-toxic, that stabilize proteins and membranes and prevent denaturation at high salt concentrations (Yancey, 2005) and, even at low concentrations, compatible solutes avoid water loss, ion imbalance, reducing intracellular concentration of salts (Burg and Ferraris, 2008). The compatible solutes accumulated in the cytoplasm of plant cells under salt stress include proline, valine, isoleucine, aspartic acid, pinitol, betaine, glucose, fructose, saccharose, mannitol and inositol (Parida and Das, 2005). In the current study we evaluated the proline accumulation that presents advantages as compared to other compatible solutes because it has less metabolic reactions besides being a short-chain amino acid (Burg and Ferrari, 2008). Proline is an essential amino acid for primary metabolism as a component of proteins and it is mainly synthesized from glutamate (Szabados and Savouré, 2009). Proline accumulation plays adaptive roles in stress tolerance (Verbruggen and Hermans, 2008), storing carbon and nitrogen (Hare and Cress, 1997).

The analysis of anatomy and ultrastructure of leaf blade cells through scanning and transmission

electron microscopy is meant to be a useful tool for subcellular understanding of alterations caused by salt stress. The integrity of cell membranes and the enzymatic activities also tend to be weakened with salinity toxic effects (Zhu, 2003). All these alterations may result in reduction in productivity or death of plants (Parida and Das, 2005). There is no study about anatomy and ultrastructure of leaf blade cells through scanning and transmission electron microscopy on *S. auriculata* subjected to salt stress.

Toxicity in plants results mainly from high concentrations of ions Na^+, CI^-, (Chinnusamy and Zhu, 2003), although most studies on salinity effect on plants are associated with NaCI excess and few studies have focused on Na_2SO_4 performance in growth (Renault et al., 2001; Stoeva and Kaymakanova, 2008) and in physiology of plants (Pagter et al., 2009). Na^+ and CI^- are present in greater amounts in seawater (Makita and Harata, 2008); thus, aquatic environments close to the sea may present high concentrations of Na^+ and CI^-. High concentrations of SO_4^{2-} may be found in aquatic environment where there are ores and anthropogenic activities such as agriculture (Davies, 2007). Proximity to seawater also influences sulphate concentration in coastal aquatic environments (Esteves, 1998). According to Davies (2002), Na_2SO_4 is the best candidate for evaluating sulphate toxicity as it does not precipitate easily, which keeps relatively consistent exposure levels of sulphate for the test duration. However, just as a reminder, ion Na^+ associated with anion SO_4^{2-} also causes osmotic stress on organisms (Davies, 2002).

The floating aquatic macrophyte, *Salvinia auriculata* Aubl. (Salviniaceae), occupies several wetlands and lagoons with moderately saline waters and close to the Northern coast of Rio de Janeiro State. Thus, we investigated *S. auriculata* potential for survival and growth in high salinity water and demonstrated the characteristics of this plant to different concentrations of salts NaCI and Na_2SO_4.

2. Materials and Methods

Salvinia auriculata was collected in Jacu lagoon, Municipality of Campos dos Goytacazes, Rio de Janeiro. Jacu lagoon is considered to be a freshwater lagoon, with electrical conductivity around 500 uS. cm^{-1}. At the laboratory the plants were washed in running water to remove any organic or inorganic material attached. The plants were stored in plastic containers with Hoagland and Arnon (1950) solution culture modified for a five-day period for acclimatization. Later, the experiment was performed in the greenhouse with salt treatments (0, 100 and 200 mM): NaCI and Na_2SO_4 (Merck) added to 8 L of solution culture in different experimental units. After seven days of experiment, fresh samples of *S. auriculata* were collected for evaluation of leaf anatomy and ultrastructure, photosynthetic pigments and proline. The rest of the samples made up of leaves and roots were placed in paper bags and oven dried (60 °C) for 4 days. After this drying period, the samples were ground in a knife mill and aliquots were separated for the determination of Ca, CI^-, K^+, Mg^{2+}, N, P, S and Na.

Photosynthetic pigments (chlorophyll-*a,b* and carotenoids) were extracted with organic solvent dimethyl sulfoxide (Merk), and the samples were analyzed according to the methodology described in Wellburn (1994). Proline content was obtained according to Bates et al., (1973) method. Nutrient determination was performed by Atomic Absorption Spectrofotometry (AAS-ZEISS). Data obtained were analyzed by utilizing Kruskal-Wallis nonparametric statistical test and, later on, Dunn's test in order to compare the results between the treatments, using significance level of 0.05.

Sections of the middle third of the leaf-blade were fixed in a solution of glutaraldehyde 2.5%, formaldehyde 4% and sodium cacodylate buffer 0.05 M (pH 7.2) for 2 hours. The samples were post-fixed with 1.0%OsO_4 and dehydrated in an ascending series of acetone and embedded in Epoxy resin (Polybed) (Bozzola and Russel, 1992). Ultrathin sections (80 nm) cut by ultramicrotome REICHERT were placed on 300 mesh copper grids and, then, contrasted in solutions of uranyl acetate 5.0% and lead citrate. The material was observed and photographed using the transmission electron microscope ZEISS-TEM900 at an acceleration voltage of 80 KV.

As for the scanning electron microscopy, the samples were fixed and dehydrated, as described above, dried by the critical point method using CPD-030 BAL-TEC (Lienchtenstein), covered with 20 nm gold (SCD-050 BAL-TEC-Liechtenstein), and observed under microscope (DSM 962-ZEISS) at an acceleration voltage of 25 KV.

3. Results

The saline treatments negatively affected the nutrient content in *S. auriculata* (Table 1). The content of Ca^{2+}, K^+, Mg^{2+}, N and P decreased in *S. auriculata* in different saline treatments (100 mM

of NaCI, 200 mM of NaCI, 100 mM of Na_2SO_4 and 200 mM of Na_2SO_4). However, the nutritional content reduction mainly occurred in plants subjected to saline treatments with 200 mM of Na_2SO_4. The results in content of Ca^+, K^+, Mg^{2+}, N and P of plants subjected to control treatment (0) and of plants subjected to treatment with 200 mM of Na_2SO_4 differed significantly ($p < 0.05$).

CI^- content was higher in plants subjected to 100 and 200 mM NaCI treatments as compared to the ones subjected to control treatment as well as to 100 and 200 mM Na_2SO_4 treatments; the significant differences occurred between 100 and 200 mM NaCI treatments and the other treatments (0, 100 and 200 of Na_2SO_4).

S content was higher in plants subjected to 100 and 200 mM Na_2SO_4 treatments; significant differences occurred between 100 and 200 mM Na_2SO_4 treatments and the other treatments (control, 100 and 200 mM of NaCI). The ratio Na^+/K^+ tended to increase in saline treatments as compared to the control; however, the ratio Na^+/K^+ was higher in plants subjected to 200 mM Na_2SO_4 treatment, and significant difference occurred between the plants subjected to control treatment and the ones subjected to 200 mM Na_2So_4 treatment. N content differed significantly ($p < 0.05$) between the plants subjected to control treatment and the ones subjected to 200 mM Na_2SO_4 treatment. However, it was also observed that N content in *S. auriculata* had a tendency to decrease in plants subjected to 100 and 200 mM NaCI treatments as well as 100 mM Na_2SO_4 treatment.

P content tended to decrease in all saline treatments as compared to plants subjected to control treatment, but the significant difference ($p < 0.05$) occurred between the plants subjected to control and 200 mm Na_2SO_4 treatments.

The content of chlorophyll-*a*, chlorophyll-*b*, total chlorophyll (chlorophyll-*a*+*b*) and carotenoids in *S. auriculata* decreased when the plants were subjected to saline treatments (100 and 200 mM of NaCl; 100 and 200 mM ofNa_2SO_4) and compared to the ones subjected to control treatment; the significant difference ($p < 0.05$) was noticed between *S. auriculata* subjected to control and 200 mM Na_2SO_4 treatments (Table 2). The ratio chlorophyll-*a*/chlorophyll-*b* (Chlo-*a*/Chlo-*b*) tended to increase in saline treatments, although significant differences did not occur.

Proline content (Table 3) tended to increase in *S. auriculata* subjected to saline treatments, mainly 200 mM of NaCl and Na_2SO_4 treatment. However, the statistical test did not show significant differences between the treatments.

Table 1. Concentration of nutrients ($mg.g^{-1}$ DW) in the tissues of *Salvinia auriculata* subjected to salt stress induced by NaCl and Na_2SO_4. In parentheses are the minimum and maximum values. The letters refer to statistical difference between the control and all saline treatments.

	0	NaCl [mM]		Na_2SO_4 [mM]	
		100	200	100	200
Ca^{2+}	6.62^a	4.19^{ab}	3.79^{ab}	3.30^{ab}	2.73^b
	(6.53-6.81)	(6.53-6.81)	(3.78-3.86)	(3.21-3.39)	(2.71-2.79)
K^+	39.93^a	23.22^{ab}	18.58^{ab}	18.38^{ab}	9.92^b
	(39.86-40.10)	(23.21-23.40)	(18.49-18.78)	(18.02-18.93)	(9.87-10.16)
Na^+/K^+	0.06^b	1.17^{ab}	2.59^{ab}	2.37^{ab}	7.50^a
	(0.06-0.06)	(1.17-1.18)	(2.58-2.61)	(2.23-2.42)	(7.47-7.71)
Na^+	2.33^b	27.15^a	48.24^a	43.52^a	75.85^a
	(2.23-2.33)	(27.15-27.51)	(48.03-48.60)	(42.16-43.63)	(74.38-76.14)
Mg^{2+}	3.35^a	3.18^{ab}	3.07^{ab}	2.33^{ab}	1.30^b
	(3.23-3.36)	(3.18-3.23)	(3.06-3.08)	(2.33-2.34)	(1.28-1.32)
N	14.61^a	8.04^{ab}	4.98^{ab}	5.50^{ab}	3.85^b
	(14.61-14.75)	(6.14-9.99)	(4.83-4.98)	(5.41-5.56)	(3.75-3.95)
P	35.72^a	25.47^{ab}	19.48^{ab}	24.32^{ab}	18.26^b
	(35.23-37.35)	(25.29-26.65)	(18.82-19.66)	(21.84-24.71)	(17.47-18.99)
Cl^-	10.81^b	37.84^a	65.99^a	7.47^b	6.68^b
	(10.65-11.77)	(37.52-38.48)	(65.03-65.99)	(7.16-8.11)	(6.36-7.79)
S	7.72^b	5.7^b	5.52^b	73.19^a	101.87^a
	(7.64-7.85)	(5.73-5.94)	(5.51-5.60)	(71.60-73.57)	(101.62-103.46)

Table 2. Changes in chlorophyll-*a* (Chlo-*a*) and chlorophyll-*b* (Chl-*b*) concentrations, rates of chlorophyll-*a* and *b* (Chlo-*a*/Chlo-*b*), and carotenoids (Car) (mg.g^{-1} DW) in the aquatic fern *Salvinia auriculata* subjected to salt stress induced by NaCl and Na_2SO_4 salts. In parentheses are the minimum and maximum values. The small letters refer to statistical difference between the control and all saline treatments.

Sal	[mM]	Chl-*a*	Chl-*b*	Chl-*a*/Chl-*b*	Chl total	Car
	0	58.81^{a}	21.10^{a}	2.77^{a}	79.92^{a}	10.65^{a}
		(46.35-63.74)	(17.13-22.99)	(2.71-2.79)	(63-86.72)	(7.78-11.00)
NaCl	100	18.26^{ab}	7.06^{ab}	2.51^{a}	25.09^{ab}	4.12^{ab}
		(17.73-22.39)	(6.83-9.65)	(2.32-2.68)	(24.79-35.04)	(2.88-4.96)
	200	14.11^{ab}	4.85^{ab}	2.91^{a}	18.96^{ab}	4.03^{ab}
		(12.97-16.9)	(4.35-5.9)	(2.86-2.98)	(17.32-22.80)	(3.31-4.79)
Na_2SO_4	100	15.51^{ab}	5.32^{ab}	2.87^{a}	20.83^{ab}	3.16^{ab}
		(14.53-16.55)	(5.17-5.77)	(2.81-2.91)	(19.70-22.33)	(1.98-3.75)
	200	9.68^{b}	3.48^{b}	2.91^{a}	12.86^{b}	3.36^{b}
		(9.38-11.51)	(3.03-3.96)	(2.70-3.19)	(12.71-15.46)	(2.79-3.54)

Table 3. Changes in proline concentration (μmol.g^{-1} DW) in the aquatic fern *Salvinia auriculata* subjected to salt stress induced by NaCl and Na_2SO_4 salts. The letters refer to statistical difference between the control and all saline treatments.

Salt [mM]	Proline (mg.g^{-1} FW)
0	0.15^{a}
	(0.12-0.22)
100 NaCl	0.42^{a}
	(0.347-0.472)
200 NaCl	0.67^{a}
	(0.42-0.79)
100 Na_2SO_4	0.67^{a}
	(0.62-0.86)
200 Na_2SO_4	1.46^{a}
	(1.09-1.72)

Through the transmission electron microscopy images, it is possible to observe (Figure 1) that the chloroplast is denser in the leaf blade of plants subjected to control treatment (Figure 1a). In all the saline treatments, alterations in the chloroplast membranes were observed, and the chloroplast got a more rounded shape with starch grains inside. The highest disorder of chloroplasts occurred in 200 mM NaCl and 200 mM Na_2SO_4 saline treatments.

The lack of grana was visualized in *S. auriculata* cells under 100 mM Na_2SO_4 treatment. In cells of plants subjected to 200 mM NaCl and 200 mM Na_2SO_4 treatments, the total degradation of the cytoplasm was visualized.

Through leaf micromorphology it was possible to observe that the shape of the trichomes (Figure 2a and b) in *S. auriculata* subjected to control treatment was intact. In saline treatments (Figure 2c-f) progressive loss of turgidity of trichomes was observed as salinity increased. Alterations in *S. auriculata* trichomes were more observed in 200 mM Na_2SO_4 treatment.

In Figure 3, through scanning electron microscopy, it is possible to observe the integrity of trichomes (Figure 3a) and leaf blade cells (Figure 3b) of *S. auriculata* subjected to the control treatment. The leaf blade cells subjected to the control treatment (Figure 3b) present smoother surfaces when compared to those of plants subjected to saline treatments (Figure 3c-f). The most significant alteration in cell surface was observed in 200 mM Na_2SO_4 treatment (Figure 4e and f).

4. Discussion

In the present study it was observed that induced salinity by both NaCI and Na_2SO_4 reduces Ca^{+} and Mg^{2+} content in *S. auriculata*. Rout and Shaw (2001), evaluating the consequences of induced salinization on Ca^{+} and Mg^{+} level, found out results that demonstrate the intracellular decrease of these ions in *Hydrilla verticillata*, *Najas gramenia* and *Najas indica*. In this study *S. auriculata* macrophytes subjected to Na_2SO_4 treatment were more affected, which corroborates what was observed by Renault et al. (2001), who studied the effects of induced salinity by NaCl and Na_2SO_4 on the concentration of Mg^{2+} ions in *Cornus stolonifera*. This event must have occurred because Ca^{+} and Mg^{2+} ions precipitate when combined with SO_4^{2-}, and the excess of Na^{+} and SO_4^{2-} reduces Mg^{2+} absorption (Esteves, 2009). Hu and Schmidhalter (2005) also highlight that, as in K^{+}, the decrease in Ca^{+} and Mg^{2+} absorption in plant tissues under salinity conditions may happen because of Na^{+}

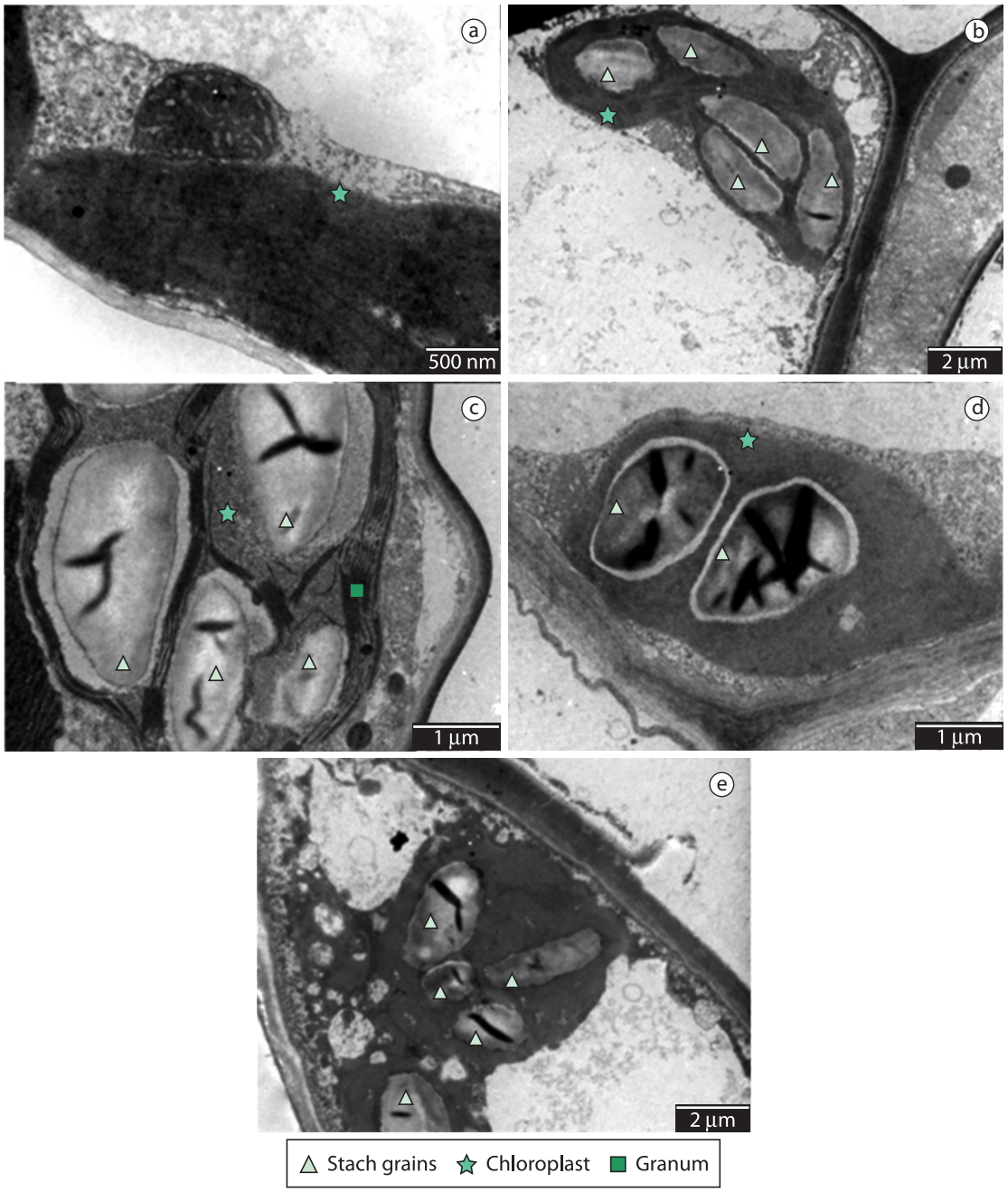

Figure 1. Transmission electron microscopy of the leaf of *Salvinia auriculata* showing stach grains, chloroplast, granum. a) Plant control, b) plant subjected to 100 mM NaCl, c) plant subjected to 200 mM NaCl, d) plant subjected to 100 mM Na_2SO_4, e) plant subjected to 200 mM Na_2SO_4.

interference or eventual ion-pair formation and subsequent precipitation. Other studies with plants subjected to salinization also show decrease in Mg^{2+} concentration (Niaz and Rasul, 1998; Rout and Shaw; 2001; Esteves and Suzuki, 2008; Japeetong and Brix, 2009), which belongs to the central structure of chlorophyll-*a* molecule and takes part in several enzymatic processes that involve phosphate transfer. Thus, the decrease in Mg^{2+} content may also have contributed to the decrease in photosynthetic pigment content.

Ion balance, especially Na^+/K^+ balance, is essential for plant tolerance to salinity (Apse and Blumwald, 2007). In this study K^+ content in *S. auriculata* decreased with the saline treatments, and the ratio Na^+/K^+ increased. The increase in ratio Na^+/K^+ was also observed in the study of *S. natans* subjected to salinity with 50, 100 and 150 mM

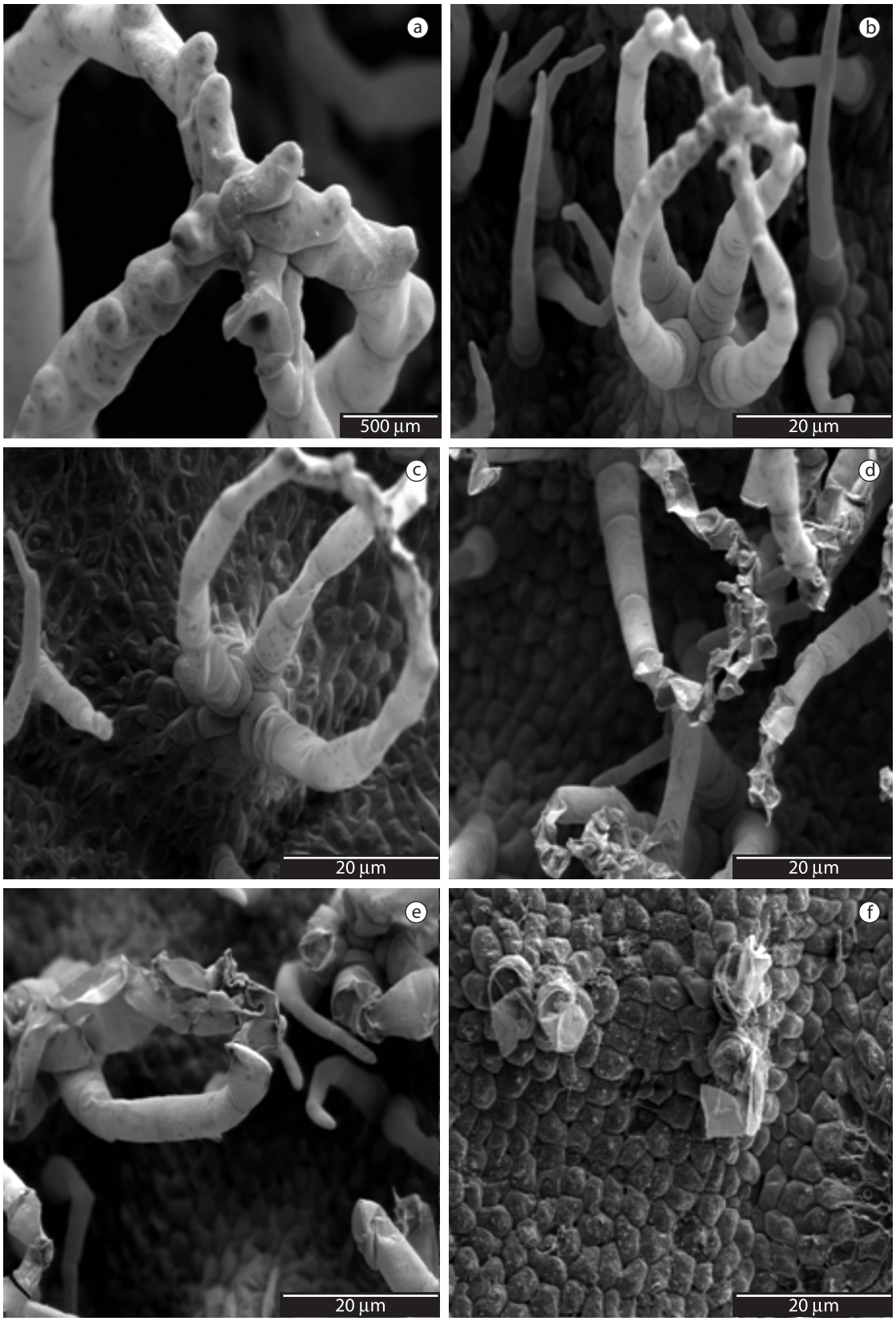

Figure 2. Scanning electron microscopy of the adaxial leaf surface of *Salvinia auriculata*. a) and b) Plant control, c) plant subjected to 100 mM NaCl, d) plant subjected to 200 mM NaCl, e) plant subjected to 100 mM Na_2SO_4, f) plant subjected to 200 mM Na_2SO_4.

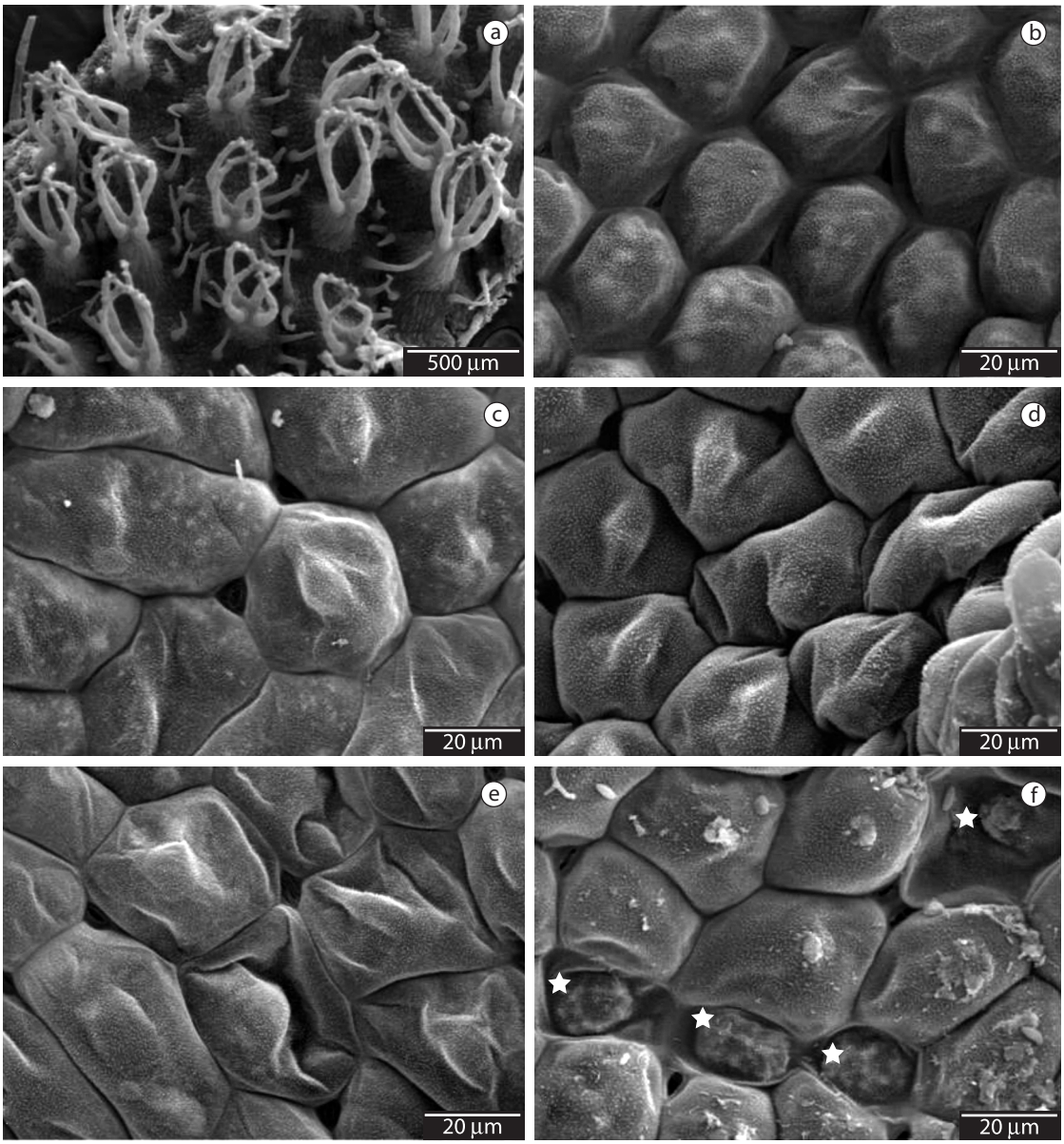

Figure 3. Scanning electron microscopy of adaxial leaf surface of *Salvinia auriculata*. a) and b) Plant control, c) plant subjected to 100 mM NaCl, d) plant subjected to 200 mM NaCl, e) plant subjected to 100 mM Na_2SO_4, f) plant subjected to 200 mM Na_2SO_4 (Stars – highly altered cells).

NaCI treatments. Jampeentong and Brix (2009) report, in this same study, that *S. natans* needs mechanisms to keep cellular homeostasis, and in high Na^+ concentration in the solution it caused K^+ deficiency. So, *S. auriculata* as well as *S. natans* need mechanisms that could keep cellular homeostasis.

Potassium is an essential nutrient for plant growth and development (Taiz and Zeiger, 2009), and it has several functions such as activation of essential enzymatic reactions, as the reactions that occur in pyruvate formation, it contributes to osmotic pressure in the vacuole and, therefore, cell turgor, which provides structural rigidity to non-lignified cells (Maathuis and Amtmann, 1999). In addition, K^+ deficiency reduces photosynthetic activity, chlorophyll content and Carbon fixation (Zhao et al., 2001).

In the scientific literature there is controversy about salinity effect on P absorption (Navarro et al., 2001). In saline areas P is easily available for plants, and Nitrogen is the principal limiting nutrient (Esteves and Suzuki, 2008). However, P absorption was inhibited in *Cornus stolonifera* Michx both in NaCl and in Na_2SO_4 treatments (Renault et al., 2001).

P and N content in *S. auriculata* decreased with saline treatments. It probably happened because the presence of Cl^- as well as SO_4^{-2} reduces P absorption

by wheat plants and sunflowers (Sen and Bal, 2009). The same is likely to occur in *S. auriculata*, which justifies our results that show a decrease in P concentration.

According to Ashraf and Sultana (2000), salinization interferes in absorption and in different stages of N metabolism. Salinity is considered to be one of the major factors responsible for N low availability in the environment (Debouba et al., 2006), as it inhibits the biological N fixation through reduction in nodulation (Elsheikh and Wood, 1990). The study on salinity effect on *Triglochin procerum* macrophyte revealed a decrease in the percentage of N (Roache et al., 2006). Similar result was also found in the study of *Eichhornia crassipes* subjected to salinity (Casabianca et al., 1995).Thus, salinity increment results in a decrease in the nutritional quality of plants due to N content reduction.

As an expected result, S was higher in *S. auriculata* subjected to Na_2SO_4 saline treatment. SO_4^{2-} is the main form of S absorbed by plants (Salisbury and Ross, 1992). However, to take part in organic molecules as amino acids and proteins, or to be stored in the vacuoles, SO_4^{2-} is reduced to sulfites and sulfides by enzymatic actions (Nikiforova et al., 2006; Balieiro et al., 2007). Part of SO_4^{2-} excess is stored in the vacuole and the other part, by enzymatic actions, is reduced to sulfites and subsequently to sulfides that, after reacting with enzyme O-acetylserine, will form the amino acid cystein (Nikiforova et al., 2006) from which the amino acid methionine will be formed (Taiz and Zeiger, 2009). As S content did not increment in plants under NaCI treatment, it is possible to suppose that sulfate, in this treatment, supplied the development demand of *S. auriculata*.

The content of chlorophyll-*a* and *b*, total chlorophyll (*a*+*b*) and carotenoids decreased with saline treatments (Table 2). Khan (2003) found out that saline stress slows down the production of photosynthetic pigments. Sharma e Hall (1991) highlighted that saline stress induces degradation of β-carotene, which causes a decrease in the content of carotenoids that are integrated constituents of thylakoid membranes and act in absorption and light transfer to chlorophyll; besides, they protect chlorophyll from photooxidation (Thaiz and Zeiger, 2009; Lima et al., 2004). Thus, degradation in carotenoid synthesis may imply degradation of chlorophylls (Lima et al., 2004). In the present study, degradation of chloroplasts, where photosynthesis occurs, was evidenced in the images of transmission electron microscopy (Figure 3). This degradation of the photosynthetic apparatus may have contributed to the reduction in the content of photosynthetic pigments. In studies performed with *Salvinia molesta* subjected to 50, 100 and 200 mM of NaCl (Upadhyay e Panda, 2005), the decrease in the content of carotenoids and chlorophyll was also observed. Due to this event, the capability of *S. auriculata* to colonize saline environment is probably reduced as well.

In the present study we noticed that proline content tended to increase with saline treatments, especially 200 mM of Na_2SO_4 (Table 3), although the results have not differed significantly ($p < 0.005$). In the study with *S. natans* subjected to salinity with 50, 100 and 150 mM of NaCl (according to Jampeetong and Brix, 2009), proline content showed an increase of about three times in plants subjected to 150 mM treatment when compared to the control. However, the authors highlighted that proline accumulation was very small in *S. natans*, which indicates that *S. natans* has limited capability to synthesize proline as a compatible compound that could increase tolerance or mitigate salt stress effects on this species. The fact that the results of proline content in *S. auriculata* subjected to salinity did not present significant differences may be linked to the limitation of *Salvinia* species in producing proline under salt stress situation.

In the study performed by Pagter et al. (2009) on *Phragmites australis* subjected to solution culture containing NaCl and Na_2SO_4 separately, the highest proline accumulation in leaves was found when compared to the control treatment, but the highest proline accumulation occurred in leaves of plants subjected to NaCl treatment.

From images of *S. auriculata* cell structure, it was noticed that the main alteration found in plants subjected to different saline treatments occurred in the chloroplasts (Figure 1). Ali et al. (2004) highlight that chloroplast integrity is linked with membrane stability which hardly ever remains intact in high saline concentrations. The increase in spaces between the thylakoids, grana disorganization and the highest presence of starch grains were also found in studies on rice, *Oryza sativa* L. (Marcondes and Garcia, 2009), and on pea (*Pisum sativum* L.) (Hernández et al., 1995) subjected to saline stress.

According to Ali et al., 2004, excessive salinization modifies the metabolic activity of cell wall, which causes deposition of several materials that limit the elasticity of cell wall and reduces turgor pressure. This fact may be related to the observation

made on epidermal cell surfaces of *S. auriculata* subjected to saline treatments: they become irregular as compared to the control (Figure 2). A sudden increase in salinity causes water loss in leaf cells and alteration in cell sizes, with reduction of area and depth (Munns and Tester, 2008).

In conclusion, we observed that *S. auriculata* is sensitive to high saline concentrations. Although we did not observe significant differences between the treatments for some evaluations, we could notice more severe morphological and biochemical changes in *S. auriculata* subjected to Na_2SO_4 than in *S. auriculata* subjected to NaCl through the results and visualization of the plants during the experiment. Besides that, it was possible to observe that the plants subjected to Na_2SO_4 saline treatments had probably irreversible damage as it is shown in the microscopy images (Figure 2 and 3). Despite the importance of S in structural and metabolic functions of plants, some intermediate routes that form sulfite, for instance, are toxic. So, the reactions that occur after SO_4^{2-} absorption aiming to synthesize organic compounds containing S are heavily regulated by the plants (Davidian and Kopriva, 2010). Sulfite may bring inactivation of S-S bonds (Hänsch and Mendel, 2005), causing inactivation of proteins and, consequently, reduction in plant growth or even cell death (Lang et al., 2007). Probably, high Na_2SO_4 concentrations may have affected the regulation of SO_4^{2-} assimilation in *S. auriculata,* and the largest amount of Na^+ associated with anion SO_4^{2-} has also worsen Na_2SO_4 negative effects on *S. auriculata*.

The results of this project indicate that *S. auriculata*, which widely develops in moderately saline environments in northern of Rio de Janeiro (possibly oligohaline that present salinity up to 5 g salt.L^{-1}), suffers morphological and physiological alterations that make them unable to develop and proliferate in mesohaline (salinity between 5 and 18 g salt.L^{-1}) and in euryhaline (salinity above 18 g salt.L^{-1}) environments.

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