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Influence of Different Sodium Chloride Concentrations on Selected Physiological Responses of Salvinia

SAFAA AL-HAMDANI[1]

ABSTRACT

In this study we examined selected physiological responses of salvinia (*Salvinia minima*) to NaCl concentrations of 0.0, 2.5, 3.0, 3.5, and 4.0 g/L. The plants were grown for seven days under a controlled environment of 25 C ± 1 C, 120 µmol $m^{-2}s^{-1}$ photon flux density, and 14 h photoperiod. Salvinia growth (dry weight) significantly declined at NaCl concentrations of 3.0, 3.5, and 4.0 g/L. Fresh weight was similarly reduced by the different NaCl concentrations. The reduction in growth coincided with a decline in CO_2 assimilation, which showed negative value at 4.0 g/L. Chlorophyll *a* and chlorophyll *b* concentrations were significantly enhanced at NaCl concentration of 2.5, 3.0, and 3.5 g/L. However, no significant differences in chlorophyll *a* and chlorophyll *b* concentrations were observed at 4.0 g/L NaCl. The different concentrations of NaCl resulted in similar increases in anthocyanin concentration. A decrease in water potential was associated with increase in salt concentrations in the growth media. The decrease in water potential was shown to follow the increase in Na^+ accumulate, and soluble sugar seemed to have a negligible effect on water potential.

Key words: aquatic plant, Na uptake, salt, salvinia, sodium chloride.

INTRODUCTION

Salt contamination of many terrestrial habitats has increased, due in part to using poor water quality for irrigation and lowering of the water table (Neumann 1997). In areas of low precipitation where water leaching is low and the water table is near the surface, a high rate of evapotranspiration is often associated with gradual buildup of salt on the soil surface (Grunwald et al. 1995). Following a heavy rain event, excess salt is rapidly washed into surrounding freshwater systems causing elevation in salt concentration. In the southeastern United States, especially coastal areas, massive freshwater wetlands are slowly being lost (McKee and Mendelssohn 1989). One of the major causes for this loss is saltwater intrusion (Morgan 1977). For example, as the Mississippi River Delta subsides, sea level is slowly rising, causing intrusion of seawater into normally freshwater marshes and rivers (Chabreck and Linscombe 1982). In addition, the wide and deep canals of waterways used by barges and other large shipping vessels often allow rapid movement of brackish water into freshwater systems. This problem is not restricted to United States ecosystems. In Australia, freshwater ecosystems increasingly are threatened by salinity due to rising saline groundwater, causing gradual changes in the physical environment (Nielson et al. 2003). The extent of salinity damage depends on both the rate of salinization and the actual increase in salt levels (Morgan 1977). Most of the aquatic habitats undergo little ecological stresses at salinities below 1 mg/L (Nielsen et al. 2003). However, salinity was reported to influence factors such as habitat modification and loss of food resources, which may influence changes in the overall structure of the ecosystem (Blinn and Bailey 2001). Salt concentrations exceeding 1 g/L were found to reduce plant growth and both sexual and asexual reproduction in Myriphyllum *crispatum* (James and Hart 1993).

The genus *Salvinia*, from the family Salviniaceae, is comprised of 1 genus and 12 known species (Nauman 1993). *Salvinia minima* is a small, free-floating freshwater fern found in tropical and temperate regions (DeBusk and Reddy 1987). Salvinia is a non-native invasive aquatic fern, accidentally introduced to the United States (Schmitz et al. 1991). Salvinia demonstrated the ability to withstand aluminum (Al) concentrations of 20 mg/L Al through manipulation of the media pH from 3.9 to near 7 within 24 h (Gardner and Al-Hamdani 1997). In addition, Salvinia was shown to survive chromium (Cr) concentrations of 2.0 mg/L Cr (VI) and double its population in 9 days (Nichols et al. 2000). Hoffman et al. 2004, reported that salvinia showed the ability to survive lead (Pb) concentrations of 20 to 40 µM Pb(II) and 200 µM of $AsO_4$3-. The high uptake of Pb and arsenic (As) was associated with reduction in salvinia growth and alteration in frond appearance. Under optimal conditions, populations of salvinia can double in size in approximately 3.5 days (Gaudet 1973), a relatively high growth rate that makes it a possible candidate for NaCl remediation. The objectives of this study were to evaluate the response of salvinia growth, photosynthetic rate, photosynthetic pigments, soluble sugar accumulation, and osmotic potential to selected concentrations of NaCl (0.0, 2.5, 3.0, 3.5, and 4.0 g/L), concentrations that represent the wide range of possibilities found in nature. In addition, the uptake of Na^+ by salvinia grown at the different concentrations was determined.

[1]Department of Biology, Jacksonville State University, 700 Pelham Road, Jacksonville, AL 36265-1602; e-mail: sah@jsu.edu; office: 256-782-5801, fax: 256-782-5587. Received for publication September 2, 2007 and in revised form May 4, 2008.

MATERIALS AND METHODS

Salvinia with a total of 25 fronds were placed into 60 250-ml Erlenmeyer flasks containing 125 ml of various NaCl concentrations (0.0, 2.5, 3.0, 3.5, and 4.0 g/L.) dissolved in 10% Hoagland's solution (Hoagland and Arnon 1938). Twelve flasks per treatment were used for each of NaCl concentration. The plants were grown for seven days under a controlled environment of 25C ± 1 C, 120 µmol $m^{-2}s^{-1}$ photon flux density and 14 h photoperiod. The plants of six samples (flasks) from each treatment were used to test for growth, chlorophyll, anthocyanins, photosynthetic rate, soluble sugar concentration, and water potential. The remaining six samples of each treatment were utilized for determining Na^+ uptake.

The fresh weight of each of the randomly selected samples from each treatment was recorded; the plant dry weight was determined after 48 h in an oven at 65 C. Approximately 0.1 g fresh weight of each sample was used for measuring chlorophyll *a*, *b*, and carotenoid concentration. The sample was placed into 5 ml of N, N-Dimethylformamide (DMF) solution. The samples were incubated in the dark for 36 h at 4 C. Chlorophyll *a* and *b* were determined spectrophotometrically at wavelengths of 647 and 664.5 nm (Inskeep and Bloom 1985). Anthocyanin was determined by homogenizing 0.1 g fresh plant sample in 5 ml methanol containing 1% HCl (v/v) for 2 min on ice. The homogenate was filtered and absorbance of the extract was determined spectrophotometrically following the method of Mancinelli (1990).

Carbon dioxide assimilation and internal CO_2 concentrations of six randomly selected samples from each treatment were measured four hours after the onset of the light period at day seven. The selected plants of each sample were enclosed in a flow-through plexiglass assimilation chamber (4.5 by 11.8 by 7.3 cm) of a Li-Cor 6200 photosynthesis system (Lincoln, NE) as described by McDermitt et al. (1989). Standard measurement conditions were 25 C, 120 µmol m^{-2} s^{-1} photon flux density and 45 to 50% relative humidity.

Leaf water potential of six samples of each treatment was measured as described by Al-Hamdani et al. (1990). Leaf discs (0.24 cm^2) were removed from each plant using a leaf cutter psychrometer. The disks were sealed in the psychrometer chamber, which was then allowed to equilibrate (temperature and vapor) in a 25 C water bath. Using a thermocouple psychrometer meter (Model 85, Logan, UT), the wet bulb depression was determined. Using the resulting readings, water potential was calculated for the individually calibrated psychrometers. The same number of samples of the same leaves was used for molality determination. Cell sap, 100 µL, was extracted from each of the selected leaves with a French Press, loaded on a paper disk, and measured with a vapor pressure osmometer (Model 5520, Wescor, Logan, UT).

Soluble sugar analysis was conducted following a procedure slightly modified from Chatterton et al. (1987). The dry samples were ground into a fine powder, and a 100 to 500 mg portion was placed in a sealed vial and used for the determination of soluble sugars as reported in detail by Wilson and Al-Hamdani (1997).

To determine sodium tissue content, six sample aliquots (0.1 to 0.7 g) of dried root and shoot from each treatment were refluxed for 15 min in 10 ml 6N HNO_3. Five ml of concentrated HNO_3 was added to the sample, and the reflux was continued until approximately 5 ml of solution was left. After the solution had cooled, 2 ml of water and 5 ml of 30% H_2O_2 were added. The samples were heated slowly, and H_2O_2 was added to the solution, 1 ml at a time, until effervescence ceased. The samples were allowed to cool, and HCl was added in a ratio of 1:2 (v/v). After all the plant tissues were digested, the solution was then brought up to 75 ml with distilled H_2O. The sodium extracted by the acid was determined with a Buck Model 210 VGP atomic absorption spectrophotometer at 357.9 nm.

This experiment was repeated three times and statistically analyzed as a randomized complete block design (Steel and Torrie 1980). This design ensured that observed differences in plant performances were largely due to treatments rather than variation among the four blocks (experiments conducted at different times). Mean separations for the treatments with significant F values (P = 0.05) of ANOVA analysis were based on the least significant difference (LSD) test (Steel and Torrie 1980).

RESULT AND DISCUSSIONS

Salvinia growth, as represented by plant dry weight, was similarly influenced by the presence of NaCl up to 3.0 g/L, in comparison to the control (Table 1). However, reduction in salvinia growth was associated with treatments of higher salt concentrations (3.5 and 4.0 g/L). On the other hand, salvinia fresh weight showed similar reduction with the introduction of the different NaCl concentrations to the growth media (Table 1). The 4.0 g/L rate resulted in a 40% reduction in fresh weight compared to the untreated controls. Increase in salt concentration was reported to inhibit the expansion of meristematic tissues, which is ultimately responsible for the growth of the plant (Neumann 1997). In general, aquatic plants have the ability to tolerate salt concentrations below 1 g/L (James and Hart 1993). The presence of NaCl in the growth media was shown to induce oxidative stress in plants (Bartosz 1997, Holmberg and Bülow 1998). Rout and Shaw (2001) reported a positive correlation between the plant salt tolerance and increase in antioxidative enzymes, particularly catalase and superoxide dismutase. The combined possible effect of oxidative stress and reduction in meristematic activities might contribute to the reduction in salvinia growth in the present of the salt. The decline in the salvinia growth in the presence of NaCl coincided with the reduction in photosynthetic rate (Table 1). Treatments receiving 2.5 and

Table 1. The effects of different NaCl concentrations on salvinia growth and gas exchange.

NaCl (g/L)	Dry weight (mg)	Fresh weight (mg)	Photosynthetic rate (µmol CO_2 $m^{-2}s^{-1}$)	Internal CO_2 (µL L^{-1})
0.0	45.00 a	716.50 a	1.25 a	340.09 a
2.5	43.20 ab	458.83 b	0.98 b	338.02 b
3.0	43.37 ab	444.00 b	0.75 b	356.20 ab
3.5	42.01 b	435.67 b	0.35 c	354.78 ab
4.0	34.00 c	430.00 b	-0.12 d	366.73 b

Mean within the column followed by the same letter are not significant at the 0.05 percent level of probability. Gas exchange measurement was at (25 C), 120 µmol m^2s^{-1} and ambient air pressure of 1 kPa.

3.0 mg/l NaCl showed similar reduction in photosynthetic rate in comparison to the control (Table 1). Further declines in photosynthetic rate were obtained at 3.5 and 4.0 g/L NaCl. A critical point of plant stress was reached when negative photosynthetic rate was obtained at 4.0 g/L NaCl, a concentration that might be considered higher than the uppermost level of NaCl concentration at which salvinia can survive. Continuous exposure to this concentration of NaCl would likely result in death through carbohydrates depletion.

Internal CO_2 concentration was similar to the control at all the salt concentrations except that of 4.0 g/L, which was significantly higher from the control and the treatment receiving 2.5 g/l (Table.1). This might be considered as an indication that reduction in CO_2 assimilation was clearly independent from the internal CO_2. Ziska et al. (1990) reported a reduction in photosynthesis in Japanese plum *(Prunus salicina*) due to increasing salinity levels, attributed to an increase in leaf chloride levels. Furthermore, a decline in the activity of ribulose 1,5-bisphosphate carboxylase and pool size of triosephosphate, ribulose 1,5-bisphosphate (Rubp), and phosphoglycerate was observed in response to increasing salinity levels. A rise in leaf dark respiration was also detected in association with elevated leaf chloride concentration, which might further contribute to a decline in net CO_2 assimilation and plant growth. The light reaction mechanism of photosynthesis of Naupaka (*Scaevola sericea*) was also found to be hindered by increasing salinity stress (Goldstein et al. 1996). Specifically, irreversible inactivation of photosystem I and photosystem II resulted in osmotic and ionic effects of NaCl (Allakhverdiev et al. 2000). The negative value of CO_2 assimilation obtained at 4.0 g/l NaCl (Table 1) could indicate that respiration rate exceeded photosynthesis; therefore, 4.0 g/l NaCl can be considered as the detrimental concentration for salvinia growth.

Chlorophyll *a* and chlorophyll *b* pigments concentration were enhanced in the presence of NaCl in the growth media (Table 2). However, no significant difference was observed between the NaCl concentrations except to those plants grown at 4.0 g/L, which were similar to the control. Increased chlorophyll concentration in stressed plants can be interpreted as an index of tolerance to NaCl; however, it should not be the only consideration. Similar to this finding, Krishnaraj et al. (1993) reported that the presence of the salt resulted in an increase in chlorophyll *a* and *b* in wheat (*Triticum aestivum*). However, Al-Hamdani (2004) reported that kudzu (*Pueraria lobata)* maintained similar concentrations of chlorophyll *a* and *b* at NaCl concentrations of 0, 25, and 50 mM.

The different concentrations of sodium chloride were induced similar to increases in anthocyanin concentrations and were significantly higher than that of the control plants (Table 2). The increase in anthocyanin concentration occurred in response to the presence of salt in the growth media and might be considered a detoxification factor. This conclusion was also supported by Doong et al. (1993), who concluded that anthocyanins are produced by most aquatic plants in response to stress factors, such as high light intensity, high temperature, or nutritional limitations. Anthocyanin concentration was also found to be induced in azolla (*Azolla caroliniana*) by Al stress (Ayala-Silva and Al-Hamdani 1997) and by Cr (VI) (Wilson and Al-Hamdani 1997).

Dissolved solute, expressed as molality, significantly increased in response to increased NaCl (Table 3). The highest increase in molality was shown at salt concentration 3.0 g/L, which was significantly higher than those plants growing at 2.5 g/L. The significant increase in molality, number of moles of solute/weight of solvent in kg, was shown to coincide with a decrease in water potential values (Table 3). The capacity to alter tissue water potentials to a lower level than that of the soil is one of the common responses of plants to salinity stress to sustain the water uptake (Wang et al. 1997). Osmoregulation involves synthesis and accumulation of various organic and inorganic solutes to reduce water potential and maintain high turgor necessary for plant growth (Cram 1976, Mass and Nieman 1978). An increase in the soluble sugars, such as glucose and mannitol, in response to elevated salt content was found to play an active role in osmotic adaptation to salinity (Tattini et al. 1996). A highly negative leaf osmotic potential was shown in pigeon pea as result of increase NaCl concentration (Ashraf 1994). Allakhverdiev et al. (2000) reported that NaCl induced osmotic effects resulting in decreasing cytosol water content and increasing the intercellular salt concentration. In addition, the increase in intercellular salt concentration was caused by an influx of Na^+ ion through potassium/sodium channels. In this study, soluble sugar accumulation in salvinia grown at 2.5 g/L NaCl was significantly higher than the other treatments, which revealed similar soluble sugar accumulation (Table 3). The increase in soluble sugar can be interpreted as part of the plant adaptation process in reducing water potential and maintaining the high turgor pressure necessary for the plant to grow (Cram 1976, Mass and Nieman 1978).

Exposure of salvinia to higher concentrations of NaCl significantly increased the uptake of Na^+ (Table 4). This increase in salt uptake was significantly higher in plants

Table 2. The effects of different NaCl concentrations on chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*) and anthocyanin concentrations in salvinia.

NaCl (g/L)	Chl *a* (mg g^{-1} fr. wt)	Chl *b* (mg g^{-1} fr. wt)	Anthocyanin (µg g^{-1} fr. wt)
0.0	10.44 a	6.14 a	6.99 a
2.5	15.97 b	9.86 b	9.21 b
3.0	16.05 b	9.84 b	10.73 b
3.5	13.01 b	8.28 b	9.98 b
4.0	9.36 a	5.28 a	9.51 b

Mean within the column followed by the same lower case letter are not significant at the 0.05 percent level of probability.

Table 3. The effect of different concentrations of NaCl on water potential and solute accumulation in salvinia.

NaCl conc. (g/l)	Molality (mol/kg)	Water potential (-MPa)	Soluble sugar (mg g^{-1} fr. wt)
0.0	1.03 a	-0.47 a	33.91 a
2.5	1.66 b	-0.76 b	59.45 b
3.0	2.06 c	-0.95 c	39.27 a
3.5	1.88 bc	-0.86 bc	38.92 a
4.0	1.87 bc	-0.86 bc	30.58 a

Mean within the column followed by the same lower case letter are not significant at the 0.05 percent level of probability.

TABLE 4. UPTAKE OF Na^+ BY SALVINIA AFTER SEVEN DAYS EXPOSURE TO DIFFERENT NACL CONCENTRATIONS

NaCl concentration (g/l)	Uptake Na^+ (µg/g)
0.0	43.38 a
2.5	119.51 b
3.0	130.32 bc
3.5	134.68 bc
4.0	171.02 c

Mean within the column followed by the same lower case letter are not significant at the 0.05 percent level of probability.

growing at 4.0 g/L in comparison to those grown at 2.5 g/L but not to the other salt treatments. The increase in Na^+ accumulation in the plant tissue had a detrimental effect on salvinia at 4.0 g/L, indicated by a significant reduction in plant growth and negative value for CO_2 assimilation. Salvinia continued to function and grow at NaCl concentrations lower than 4.0 g/l. Salvinia tolerance likely follows the general trend of plant avoidance of high NaCl concentrations, which involves compartmentalization of the salt ions in the vacuole for osmotic adjustment and detoxification of the cytoplasm (Ungar 1991, Zhu 2001).

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