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Salt tolerance in aquatic macrophytes: ionic relation and interaction

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Abstract

Effects of seawater salinity (SWS) and pure NaCl on the intracellular contents of Na^+, K^+, Mg^{2+}, Ca^{2+}, chlorophylls (Chl) and carotenoids (Car) were studied in three submerged aquatic macrophytes, *Hydrilla verticillata*, *Najas indica* and *Najas gramenia*, which differed in their tolerance to salinity. NaCl resulted in significant increase in Chl/Car ratio in the salt-sensitive *H. verticillata* and moderately salt-tolerant *N. indica*, but not in the salt-tolerant *N. gramenia*. SWS treatment did not result in any significant change in the ratio. The intracellular content of Na^+ increased significantly in all the test plants upon exposure to both NaCl and SWS. The content of K^+ decreased significantly in these plants upon salinity treatment, except in *N. gramenia*. The contents of Ca^{2+} and Mg^{2+} decreased significantly upon NaCl treatment and remained unchanged or increased upon SWS treatment. No relationship between salt tolerance and K^+/Na^+ ratio was observed. The maintenance of a minimal level of K^+ was observed to be the most probable requirement of salt tolerance in aquatic macrophytes.

Additional key words: calcium protection, carotenoids, chlorophylls, *Hydrilla verticillata*, *Najas gramenia*, *Najas indica*, oxidative stress, seawater salinity, sodium chloride.

Introduction

There is pressing need for improved salt-tolerant plants to bring the unutilized or poorly utilized saline lands into proper cultivation to take care of the need of the earth's increasing population (Holmberg and Bulow 1998). But, a clear understanding of the adaptive mechanism by which the salt-tolerant plants cope with the presence of salts in their environment is still incomplete. Salinity in nature is represented by the cations of alkali (K^+ and Na^+) and alkaline earth metals (Ca^{2+} and Mg^{2+}), while chloride forms the most abundant anion followed by SO_4^{2-} (Egan and Ungar 1998). The relative levels of these cations and anions vary greatly, but Na^+ and Cl^- remain the major contributors. Living organisms maintain a cellular inorganic ionic composition very different from that of the external environment, and the regulation of ion transport across cell membranes is an essential factor in salt tolerance in plants (*e.g.* Flowers *et al.* 1977, Greenway and Munns 1980, Leidi and Sarz 1997). In this context it has been observed that the intracellular Cl^- concentration in plants parallels the fluctuation of salinity, but that of K^+ and Na^+ varies depending upon the plant species (Kirst 1989), suggesting that tolerance to salinity probably lies in the regulation of the transport of Na^+ and K^+ (Alfocea *et al.* 1993, Blits *et al.* 1993, Glenn *et al.* 1994, Kohl 1997, Leidi and Sarz 1997, Patnaik and Debata 1997, Reddy and Iyenger 1999). Most work related to salinity tolerance has been carried out using pure NaCl as salt stress, ignoring the other ionic components of salinity. Also, little study has been carried out in macrophytes from aquatic environment.

In light of the above, the present study reports changes in the intracellular concentration of Na^+, K^+, Mg^{2+} and Ca^{2+} in three submerged aquatic macrophytes differing in salinity tolerance, *Hydrilla verticillata*, *Najas indica* and *Najas gramenia*, upon exposure to salinity. For the salinity treatment, artificial seawater (referred as seawater salinity, SWS) was also used besides pure NaCl. The toxicity of the salinity treatment was assessed by studying the changes in the contents of chlorophylls and carotenoids.

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Abbreviations: Car - carotenoids; Chl - chlorophyll; SWS - seawater salinity.

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Materials and methods

The test plants for the study were collected from a brackish water lagoon, the Lake Chilka. *N. gramenia* is a salt-tolerant species and grows luxuriantly in 20 ‰ seawater. *N. indica* is moderately tolerant to salinity. *H. verticillata* represents a freshwater species sensitive to salinity. *N. gramenia* and *N. indica* grow well also in freshwater. The plants were grown under sunlight, separately in large tanks in freshwater supplemented with nutrients.

Approximately 6 cm long tips of the plants were transferred in 250 cm^3 conical flasks containing 200 cm^3 standard nutrient medium and kept in dark in a growth chamber for 9 h at 26 ± 1 °C. The tips in the flasks were then exposed to irradiance of 200 $\mu mol\ m^{-2}\ s^{-1}$ and treated with either pure NaCl or SWS in concentrations 0.5, 1.0, 1.5, 2.0 and 2.5 %. 3.05 % SWS contains 2.08 % NaCl, 0.94 % $MgCl_2 . 6\ H_2O$, 0.132 % $CaCl_2 . 2\ H_2O$, 0.06 % KCl and other salts (Parsons *et al.* 1984). In the case of ion interaction study, the standard nutrient solution was replaced by distilled water in both the control and the treated sets. After exposure for 9 or 12 h, as required, the plant tips were recovered and used for analysis.

The contents of chlorophylls and carotenoids in the plant tissue were determined following the procedure of Wellburn (1994). The exposed tips were washed in distilled water and blotted properly. Leaves (20 mg) were suspended in 5 cm^3 methanol and kept in dark in a refrigerator for 24 h. The absorbance of the extract was read at 666, 653 and 470 nm on an spectrophotometer (*DU-68*, *Beckman*, Fullerton, USA).

For estimation of tissue Na^+, K^+ , Mg^{2+} and Ca^{2+} contents the plant tips (exposed for 9 h) were washed in distilled water several times, gently blotted, wrapped in aluminium foil, and dried in oven for 24 h. The dried tips were weighed and digested separately in 5 cm^3 of 2:1 nitric acid and perchloric acid in long corning test tubes over a heater until the solution became clear. The digested solutions were allowed to cool and then made-up volume using de-ionized water (*Millipore*). The concentrations of Na^+, K^+, Mg^{2+} and Ca^{2+} in the solutions were determined on an Atomic Absorption Spectrophotometer (*AA20BQ*, *Varian*, Mulgrave, Australia).

The ion interaction study was performed only in the salt sensitive *H. verticillata*. For the purpose, the tips of the plant after incubation in dark were transferred to distilled water. They were then treated with 1.5 % NaCl, alone and in combination with $MgCl_2$, $CaCl_2$, and KCl separately in concentration equivalent to that present in seawater containing 1.5 % NaCl. The tips only in distilled water served as control.

The data presented are means of 5 or more replicates. The significance of differences was tested by Duncan's multiple range test for unequal sample size (Blis 1967).

Results

The ratio of chlorophylls to carotenoids (Chl/Car) significantly increased in all the test plants upon NaCl treatment when compared to the control value; the maximum increase was observed at 1.5 % treatment (Table 1). The increase, however, was much more pronounced in *H. verticillata*, followed by *N. indica* and *N. gramenia*. The ratio also increased upon SWS treatment in *H. verticillata* and *N. indica*, but only insignificantly. In *N. gramenia*, in contrast, Chl/Car ratio decreased upon SWS treatment, but the decrease was significant only at 2 % treatment.

The Chl/Car ratio also increased in *H. verticillata* upon NaCl treatment in distilled water (Table 2). The Chl/Car ratio in the NaCl treated set supplemented with Ca^{2+} did not differ significantly from that in control. The effect of NaCl was also ameliorated by the presence of Mg^{2+}, but to a lesser extent.

Table 1. Effect of 12 h NaCl and SWS treatments on Chl/Car ratio in the test plants. Mean of at least 5 determination ± standard deviation. The mean values of control and treatment sets of a species with at least one common letter are not significantly different from each other ($P \leq 0.05$), as determined by Duncan's multiple range test.

		0.0 %	0.5 %	1.0 %	1.5 %	2.0 %	2.5 %
H. verticillata	NaCl	5.23 ± 0.23^{c}	5.15 ± 0.68^{c}	6.40 ± 1.47^{c}	11.03 ± 1.64^{a}	9.18 ± 2.06^{b}	8.35 ± 1.86^{b}
	SWS		5.25 ± 0.43^{c}	5.33 ± 0.31^{c}	5.48 ± 0.32^{c}	5.82 ± 0.76^{c}	6.16 ± 0.61^{c}
N. indica	NaCl	5.12 ± 0.32^{d}	5.10 ± 0.34^{d}	7.32 ± 1.57^{b}	10.07 ± 1.04^{a}	6.17 ± 0.42^{c}	6.06 ± 0.50^{c}
	SWS		5.24 ± 0.62^{d}	5.33 ± 0.40^{d}	5.22 ± 0.62^{d}	5.22 ± 0.58^{d}	5.47 ± 0.52^{d}
N. gramenia	NaCl	4.94 ± 0.33^{bcd}	4.95 ± 0.23^{bcd}	5.06 ± 0.37^{abc}	5.32 ± 0.35^{a}	5.14 ± 0.36^{ab}	5.26 ± 0.41^{a}
	SWS		4.85 ± 0.32^{cde}	4.73 ± 0.31^{de}	4.69 ± 0.28^{de}	4.62 ± 0.23^{e}	4.77 ± 0.16^{cde}

Table 2. Influence of Ca^{2+}, Mg^{2+} and K^+ (in distilled water) on the NaCl induced changes in Chl/Car ratio in *H. verticillata*. Means of at least 6 determination ± SD. The mean values with at least one common letter are not significantly different from each other ($P \leq 0.05$).

	Control	1.5 % NaCl	NaCl + $CaCl_2$	NaCl + $MgCl_2$	NaCl + KCl
Chl/Car ratio	5.08 ± 0.41^c	16.55 ± 2.84^a	5.70 ± 3.03^{bc}	7.94 ± 1.49^b	14.88 ± 0.72^a

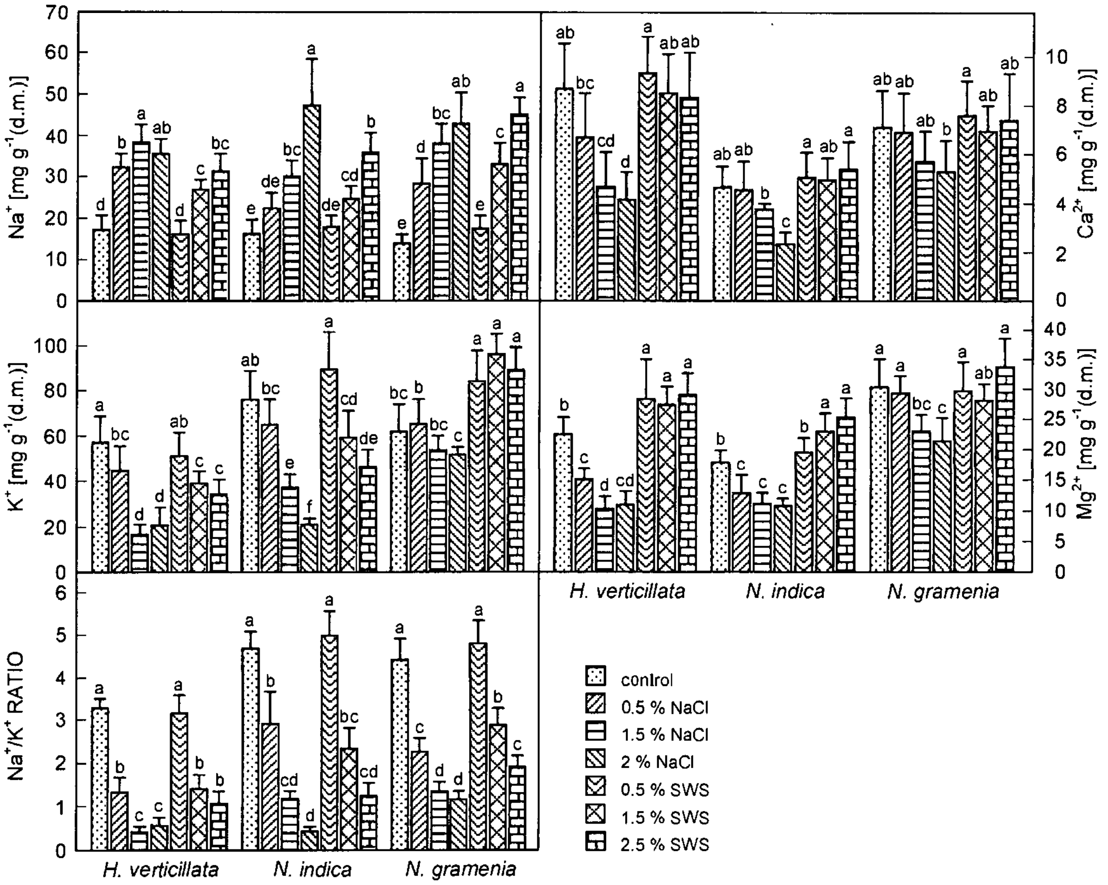

Fig. 1. Changes in the intracellular levels of Na^+, K^+, $Ca^{2+}$and Mg^{2+}, and K^+/Na^+ ratio in the test plants after 9 h of exposure to different concentrations of NaCl and SWS. The data are means of 5 determination; the vertical bars represent standard deviations. The mean values of control and treatment sets of a species marked with at least one common letter are not significantly different at $P \leq 0.05$.

The intracellular content of Na^+ in response to both NaCl and SWS treatments increased significantly in all the test plants when compared to control (Fig. 1). The increase was in a concentration dependent manner, except in *H. verticillata* in response to NaCl in which the accumulation at 2 % NaCl treatment was less than that at 1.5 % NaCl treatment. The Na^+ accumulation in *H. verticillata* was higher in response to NaCl than in response to SWS containing the same amount of NaCl. In *N. gramenia*, and also in *N. indica*, on the other hand the accumulation was similar in both the treatments.

In contrast to Na^+ the K^+ content decreased significantly in a concentration dependent manner in *H. verticillata* and *N. indica* in response to both NaCl and SWS treatments (Fig. 1). The decrease was, however, more in response to NaCl than in response to SWS in both the plants. *N. gramenia* on the other hand exhibited significant increase in the content of K^+ upon SWS treatment; the increase was more or less same in all the treatment concentrations. The content of K^+ although decreased in response to NaCl, the decrease was only insignificant.

The intracellular content of Ca^{2+} decreased significantly in *N. indica*, and especially in *H. verticillata* in response to NaCl treatment (Fig. 1), but remained unchanged upon SWS treatment. In *N. gramenia* the content of Ca^{2+} remained unchanged. When compared to Ca^{2+}, the intracellular level of Mg^{2+} was more susceptible to NaCl treatment; the content of Mg^{2+} decreased significantly even at 0.5 % treatment *in H. verticillata* and *N. indica* (Fig. 1). The content of Mg^{2+} also decreased significantly in *N. gramenia* at 1.5 and 2 % NaCl. In SWS treatment, the intracellular content of Mg^{2+} increased in *H. verticillata* and *N. indica*, unlike the content of Ca^{2+}. The increase was higher in *H. verticillata* than *in N. indica.* In *N. gramenia*, however, the Mg^{2+} content remained statistically unchanged, like that of Ca^{2+}.

The contents of all the cations were different in the three test plants, but they did not show any relationship with their salt tolerance. The ratio of K^+ to Na^+ (Fig. 1) also did not reveal any such relationship. The ratio decreased significantly in all the test species in response to both NaCl and SWS treatments. The decrease was more pronounced *in H. verticillata* and *N. indica* than in *N. gramenia*, particularly in NaCl treatment.

Discussion

The response of Chl/Car ratio to NaCl treatments in the three plant species (Table 1) was related with their salt-tolerance; the lower was the increase in the ratio, the greater was the plant tolerance to salinity. On this basis, *N. gramenia* was the most salt-tolerant and *H. verticillata* the least salt-tolerant. The data quite agree with the salinity condition from where the three test species were collected. Thus, the parameter can be used to assess the tolerance of aquatic plants to salinity. However, only pure NaCl can be used, as SWS did not result in any significant increase in the ratio even in the salt sensitive *H. verticillata.*

General protective effect of Ca^{2+} against NaCl toxicity in terrestrial plants is known (Hyder and Greenway 1965). This was observed in our study in terms of prevention of increase in Chl/Car ratio (Table 2). Recently, Ca^{2+} has been reported to protect terrestrial plants against NaCl toxicity by improving the intracellular content of Ca^{2+} and maintaining Na^+/K^+ selectivity (Colmer *et al.* 1996, Davenport *et al.* 1997). Reports on the influence of Ca^{2+} on Na^+ toxicity in aquatic plant are scant; recently Rout and Shaw (1998) reported Ca^{2+} to enhance the accumulation of proline in aquatic macrophytes. Our results also suggest a protective effect of Mg^{2+} against Na^+ toxicity, although it may be limited only to prevention of oxidative stress (Table 2).

It is generally considered that salt resistance in glycophytes is due to limited Na^+ uptake and maintenance of high intracellular K^+ to Na^+ ratio through high K^+ selectivity (Alfocea *et al.* 1993, Greenway and Munns 1980), and that in halophytes due to accumulation of Na^+ without the requirement of maintaining high K^+ selectivity (Alfocea *et al.* 1993, Glenn *et al.* 1994, Leidi and Sarz 1997). This generalized view is, however, not reflected in the present observations on aquatic angiosperm; the intracellular content of K^+ remained unaffected in the salt-tolerant *N. gramenia* (Fig 1) in the presence of NaCl despite significant accumulation Na^+ (Fig. 1), and the content of K^+ increased significantly upon treatment with SWS, containing NaCl. This suggests that the maintenance of a minimal intracellular level of K^+ upon challenge with NaCl is probably a characteristic feature in salt-tolerant aquatic angiosperms, unlike that reported for terrestrial plants. Similar maintenance of K^+ selectivity has also been reported in the cell suspension cultures of the halophyte *Kosteletzkya virginica* (Blits *et al.* 1993).

Observation of a greater K^+/Na^+ selectivity in a few salt-tolerant plants than in their salt-sensitive counterparts, and consequently manifestation of a higher K^+/Na^+ ratio in them, has lead to suggestion of these being the features of salt adaptation (Fortmeir and Schubert 1995, Gorham *et al.* 1985, Storey and Wyn Jones 1979). The same has also been observed in salt-tolerant callus and cell lines (Binzel *et al.* 1987, Blits *et al.* 1993). Present study, however, does not suggest any relationship between salt tolerance and K^+/Na^+ ratio. Non-existence of any such relationship has also been reported in marine algae (Kirst 1989) and some terrestrial plants (Glenn *et al.* 1994, Leidi and Sarz 1997, Patnaik and Debata 1997).

The results on the changes in the contents of Ca^{2+} and Mg^{2+} upon NaCl treatment in the plants under study are in agreement with one reported for tomato cultivars, and favour the view that a lower decrease in Ca^{2+} and Mg^{2+} content under salt stress implies a higher salt-tolerance (Alfoceae *et al.* 1993). However, there are also reports of Ca^{2+} and Mg^{2+} remaining unaltered (Kohl 1997) or getting increased (Egan and Ungar 1998) in halophytes upon exposure to NaCl. Moreover, simply the ability to maintain the content of these ions may not be enough to make a plant tolerant to saline environment; *H. verticillata* and *N. indica* besides being sensitive to NaCl are also sensitive to SWS (Rout and Shaw 1998) although upon exposure to the latter the levels of Ca^{2+} and Mg^{2+} are maintained to that of the control values. Thus, the decrease in the intracellular contents of Ca^{2+} and Mg^{2+} in response to NaCl might be simply due to insufficient availability of these ions for transportation into the cells in the presence of excess NaCl.

Thus, so far the data suggest, salt tolerance in aquatic plants is most possibly due to their ability to maintain the intracellular levels of K^+ above a minimal level; the K^+/Na^+ ratio is unimportant. The osmotic adjustment is probably determined by Na^+, which enters into the cells in a concentration dependent manner. Together with Na^+, proline also might be playing some role in the osmotic adjustment (Rout and Shaw 1998).

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