

BRIEF COMMUNICATION

Effect of salinity on chlorophyll and proline contents in three aquatic macrophytes

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The role of proline in imparting tolerance to salinity was investigated in *Hydrilla verticillata*, *Najas indica* and *Najas gramenia*. The plants were exposed to different concentrations of NaCl and artificial sea water (SWS) separately. The chlorophyll (Chl) *a/b* ratio decreased significantly in all the three plant species in both NaCl and SWS treatments, comparatively more in former than the latter. NaCl resulted in drastic decrease in this ratio in salt sensitive *H. verticillata* and *N. indica*, but in somewhat lesser decrease in salt resistant *N. gramenia*. Proline content increased at both NaCl and SWS treatments, especially at the latter. However, in *H. verticillata* proline content at 1.5 and 2.5 % NaCl decreased. It was concluded that proline cannot be used as a biochemical marker of salt tolerance in aquatic plants, however, the decrease in Chl *a/b* ratio in response to NaCl may be used as an index of salt sensitivity in this ecological group of plants.

Additional key words: *Hydrilla verticillata*, *Najas indica*, *Najas gramenia*, salt tolerance, sea water, sodium chloride.

The concentration of proline increases rapidly in the tissues of many plants as a response to drought and salinity (Hanson and Hitz 1982, Voetberg 1991, Thomas *et al.* 1992, Colmer *et al.* 1996, Lin and Kao 1996, Martinez *et al.* 1996, Jain *et al.* 1996) suggesting that proline plays a pivotal role in imparting plants tolerance to

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Abbreviations: Chl - chlorophyll; PAR - photosynthetically active radiation; SWS - artificial sea water.

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stresses that lower the water potential of ambient environment. Nevertheless, the levels of proline in plants have been observed to be enhanced even by heavy metals and UV light (Alia and Saradhi 1993, Saradhi *et al.* 1995) that do not lead to osmotic stress or create low water potential. Thus the induction of proline accumulation in plants under drought or salinity stress is although well known, but not a well understood phenomenon, and its role in providing plants tolerance to salinity continues to be controversial. Besides, the relationship between salinity tolerance and proline accumulation has so far been studied mostly in terrestrial plants, and often pure NaCl has been used, which is unlikely in natural environment. Therefore, in the present work involvement of proline in salinity tolerance was studied in three aquatic macrophytes *Hydrilla verticillata*, *Najas indica* and *Najas gramenia* differing in resistance to salinity. For the salinity treatment, besides pure NaCl, artificial sea water has been used representing the natural salinity condition. The relative degree of tolerance of the macrophytes to salinity has been assessed by studying the changes in Chl *a/b* ratio.

The plants were collected from their natural environment and grown separately in large tanks in fresh-water under sun light. For the purpose of the experiment, approximately 7 cm long tips of the plants were collected. Six such tips of each plant were transferred into 250 cm³ conical flasks containing 200 cm³ of standard nutrient solution. The flasks were incubated in dark for 12 h at 26 ± 1 °C. The tips were then exposed to light (irradiance of 200 $\mu\text{mol(PAR)} \text{ m}^{-2} \text{ s}^{-1}$ and treated immediately either with pure NaCl solution or artificial sea water. The temperature was maintained at 27 ± 0.5 °C. After 12 h of exposure, the contents of Chl and proline were measured. Chl *a* and Chl *b* contents were measured following the procedure of Wellburn (1994): 20 mg of the leaf tissue was suspended in 5 cm³ of methanol and kept in dark in refrigerator for 24 h; the absorbance of the extract was read at wavelengths 653 and 666 nm with a spectrophotometer (DU-68, Beckman, USA). The content of proline was determined according to Bates *et al.* (1973): 500 mg of the plant tips were homogenized in 3 % aqueous sulfosalicylic acid and the homogenate was centrifuged at 10 000 g. The reaction mixture consisting of 2 cm³ supernatant, 2 cm³ acid ninhydrin (1.2 g ninhydrin in 30 cm³ glacial acetic acid and 20 cm³ 6 M orthophosphoric acid) and 2 cm³ of glacial acetic acid was boiled at 100 °C for 1 h. After termination of the reaction in ice bath the reaction mixture was extracted with 4 cm³ toluene and the absorbance was read at 520 nm. The data presented are means \pm standard deviation (sd) of at least 5 determinations. The significance of differences were tested by Student's *t*-test ($P < 0.05$) or Duncan's multiple range test for unequal sample size (Bliss 1967).

Chl *a/b* ratio decreased significantly in all the three tested species in response to salinity treatment (Fig. 1). The pattern of decrease in the Chl *a/b* ratio was more or less similar in *H. verticillata* and *N. indica*; the ratio decreased to a minimum at 1.5 % NaCl and increased in response to higher concentrations. In more tolerant *N. gramenia* the lowest Chl *a/b* was observed at 2 % NaCl treatment and the decrease observed (87.5 % of the control) was much less when compared to that obtained at 1.5 % treatment in *H. verticillata* (65.4 %) and *N. indica* (71.9 %). Thus the less

decrease in the Chl *a/b* ratio in response to NaCl was found in the species resistant to salinity and collected from more saline water than the other two species.

Although SWS treatment resulted in significant decrease in Chl *a/b* ratio, the effect in general was much less than that produced by pure NaCl; in none of the SWS treatments the decrease in Chl *a/b* ratio was less than to 90 % of the control value (Fig. 1). The effects of NaCl and SWS differed significantly in *H. verticillata* and *N. indica*. In *N. gramenia* the effect of the two treatments did not differ significantly at higher concentrations, probably due to higher NaCl tolerance in this plant species. The toxic effect of NaCl might be ameliorated by the other ions present in sea water. In fact, Ca^{2+} has been reported to do so (Colmer *et al.* 1996), although no direct report is available for protection against chlorophyll degradation.

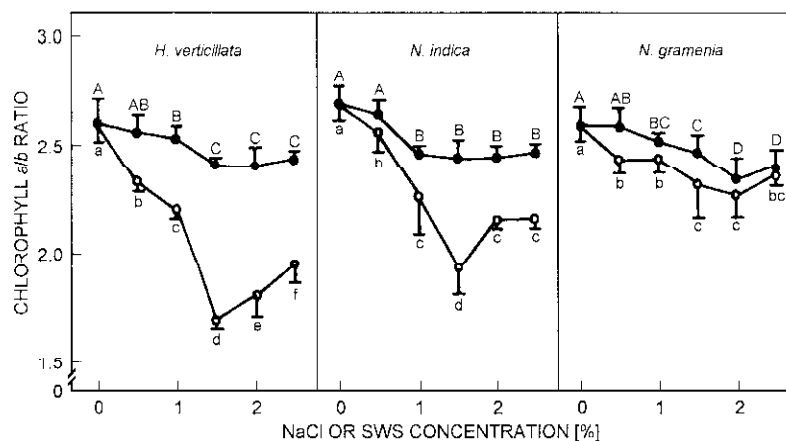


Fig. 1. Changes in Chl *a/b* ratio in *Hydrilla verticillata*, *Najas indica* and *N. gramenia* after treatment with different concentrations of NaCl (open circles) and SWS (closed circles) for 12 h. Means \pm sd; $n = 5$ or more. Two means marked by the same letter (A, B, C, D for SWS and a, b, c, d, e, f for NaCl) are not significantly different at $P < 0.05$ as determined by Duncan's multiple range test.

The decrease in Chl *a/b* ratio is considered to be a symptom indicating stress conditions, particularly oxidative stress (*e.g.* Shaw 1995). The decrease in Chl *a/b* ratio is because of unequal destruction of chlorophylls, comparatively more of Chl *a* than of Chl *b*, as a result of their reaction with singlet oxygen produced when the acceptor side of PS 2 is inhibited (Barber 1994). NaCl has been reported to influence several physiological parameters in plants including the activity of Rubisco (Bongi and Loreto 1989, Kalaji and Pietkiewicz 1993, Solomon *et al.* 1994), and thus might lead to acceptor side mediated photodamage of chlorophylls in the present study.

All the three tested plant species showed enhancement in the content of proline in their tissues under salinity treatment (Fig. 2). Nevertheless, they differed greatly from each other in background tissue levels as well as in relative increase in the osmoticum in their tissues in response to the treatments. In *H. verticillata* the content of proline increased at 0.5 % NaCl, but decreased at 2.5 % NaCl. In *N. indica* and

N. gramenia content of proline increased with increasing NaCl concentration, but proline increase observed at 0.5 % NaCl was insignificant. At 1.5 % NaCl, *N. gramenia* showed greater accumulation of proline when compared to *N. indica*.

SWS treatment induced the accumulation of proline even more than pure NaCl. In *H. verticillata* treated with SWS the content of proline increased also at 1.5 and 2.5 % SWS, unlike that observed in response to NaCl. The increase in proline accumulation under SWS treatment over that under NaCl treatment was insignificant in *N. gramenia* at 0.5 and 2.5 % concentrations (Fig. 2).

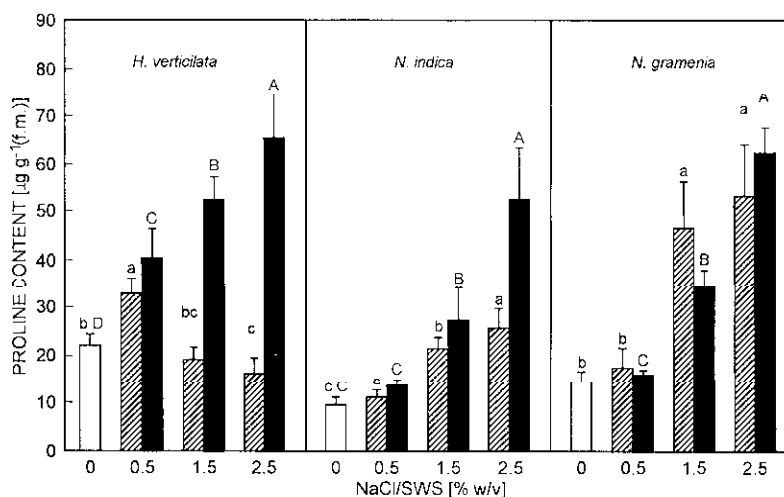


Fig. 2. Effect of 12-h NaCl and SWS treatments on the proline content of *H. verticillata*, *N. indica* and *N. gramenia*. Means of at least 5 determinations; the vertical bars represents sd. The two means of either NaCl or SWS treatment marked by the same letter (A, B, C, D for SWS and a, b, c, d, e, f for NaCl) are not significantly different at $P < 0.05$ as determined by Duncan's multiple range test.

Proline has been reported to ameliorate the deleterious effect of wide range of environmental factors like heat, frost, salt and chemicals, and has been attributed to a variety of functions such as an osmoticum, a protective agents for cytoplasmic enzymes, a reservoir of nitrogen and carbon for post-stress growth, a stabiliser of membranes and the machinery for protein synthesis, and a scavenger of free radicals (Paleg *et al.* 1984, Fukutaku and Yamada 1984, Kardpal and Rao 1985, Saradhi *et al.* 1995, Jackson and Seppelt 1995, Lin and Kao 1996, Martinez *et al.* 1996). Nevertheless, a clear-cut role of this compound in salinity tolerance, and also other stress tolerance, is still lacking and most of the suggestions regarding its involvement is based on its enhanced accumulation in response to the stress (Dix and Pearce 1981, Jain *et al.* 1987, Thomas *et al.* 1992, Jackson and Seppelt 1995, Lin and Kao 1996, Martinez *et al.* 1996). Although highly significant concentration-dependent accumulation of proline was observed in *N. gramenia* in response to NaCl, and in all of them in response to SWS (Fig. 2), the present study, however, hardly advocate for any role of the compound in imparting tolerance to salinity in the test plants. This is because *H. verticillata* despite having the highest background levels of proline in its tissue (Fig. 2), is the most sensitive species to NaCl treatment. Secondly, Chl *a/b*

ratio decreases significantly in *N. indica* at 1.5 % NaCl (Fig. 1) in spite of significant increase in proline accumulation (Fig. 2). Also, there are reports that accumulation of proline in response to salinity is not mandatory at least in aquatic plants; for example Jacob *et al.* (1991) and Jackson and Seppelt (1995) did not find any accumulation of proline in *Prasiola crispera* cultured in artificial sea water or after different exposures to salt spray. Thus, the enhancement in proline accumulation may be simply a stress effect, rather than a cause of stress tolerance as has been suggested, *e.g.*, by Hanson *et al.* (1979) and Moftah and Michel (1987).

In conclusion, the present study revealed that the toxicity of NaCl in aquatic plants can be assayed by determining Chl *a/b* ratio, and the decrease of the ratio may be used as an index of NaCl sensitivity. The study also revealed that the aquatic plants cannot be evaluated for their tolerance to salinity using proline as a biochemical marker, unlike that suggested for terrestrial plants (Martinez *et al.* 1996).

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