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NaCl Stress Causes Changes in Photosynthetic Pigments, Proteins, and Other Metabolic Components in the Leaves of a True Mangrove, *Bruguiera parviflora*, in Hydroponic Cultures

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We studied salt stress-induced biochemical changes in young, hydroponically grown plants of mangrove, *Bruguiera parviflora* (Rhizophoraceae). Our focus was on the effect of NaCl (applied at 100, 200, 400, or 500 mM) on leaf pigments, total soluble proteins, total free amino acids, carbohydrates, polyphenols, and proline. The total Chl content increased for 14 d after treatment with 100 mM NaCl, then gradually stabilized. At 400 mM, the total Chl content slowly decreased over the 45-d test period. However, the Chl *a*:*b* ratio remained unchanged in isolated chloroplasts and in leaf tissue. Percent changes in the carotenoids content followed the same trend as for Chl, except for a 1.5-fold decrease during the 400-mM NaCl treatment, compared with the control. The total sugar content increased by 2.5-fold by Day 45 after treatment with 400 mM NaCl, whereas the starch content measured in the same treatment decreased by 40 to 45%. Leaf protein content decreased as salinity increased, which suggests either a possible disruption in the protein synthesis mechanism or, more likely, an increase in proteolytic activity. The total amino-acid pool increased steadily, by four-fold, in the 45-d, 400-mM treatment. Both proline and polyphenols accumulated with increasing levels of salinity, which confirms the role of proline as a stress-induced protective metabolite in the adaptive process of this species. Our results showed that a true mangrove such as *B. parviflora* can easily be sustained and propagated under low-salinity conditions. At high levels of salinity (~400 mM, beyond which they could not survive), the plants became adapted to salt stress after two to three weeks. During this adaptive period, changes in pigment and protein levels also occurred. The accumulation of proline and polyphenols played a key role in the plant's stress-induced adjustment to NaCl under hydroponic culture conditions.

Keywords: *Bruguiera parviflora*, hydroponic culture, mangrove, proline accumulation, salt-stress

The rich biological diversity in the mangrove ecosystems of tropical and subtropical inter-tidal zones constitutes an economic resource for coastal people. The mangrove is an ecologically important component that protects the adjacent land from erosion by tidal waves, cyclones, and storms (Banijbatana, 1957). These trees also prevent the accumulation of terrigenous nutrients that affect nearby reefs (Dubinsky and Stambler, 1996). The survival of most of these mangroves is now threatened, largely due to human interference that has resulted in changes in tidal patterns and salinity gradients. Investigations of salinization and desalinization are likely to yield valuable information on salt adaptability by mangroves.

Bruguiera parviflora (Rhizophoraceae) is a major member in the tropical mangrove ecosystem. This family of mangrove has a viviparous mode of germination and is tolerant to changes in salinity gradients. The effects of salt on mangroves have been studied in relation to their leaf structure and their rates of transpiration, stomatal conductance, and photosynthesis (Lovelock et al., 1992; Moorthy and Kathiresan, 1999; Theuri et al., 1999; Santiago et al., 2000). Recently, Tanaka et al. (2000) reported that the Na^+/H^+ antiporter catalyzes the exchange of Na^+ for H^+ across the vacuolar membrane of cells in *Bruguiera sexangula*. This mechanism offers tolerance to the ionic stress imposed by NaCl, and is important for promoting cellular salinity adjustments. In addition, Hotta et al. (2000) have suggested that this mechanism of salt-stress adaptation in this species is linked to changes in vacuolar size.

It is usually assumed that NaCl affects photosynthetic activity. However, Ball and Farquhar (1984) found that, unlike in the case of salt-sensitive *Aegiceras corniculatum*, photosynthesis rates are barely affected by salinity in high-salt-tolerant mangroves such as *Avicennia marina*. The depressed carbon assimilation observed in the former can be attributed mainly to a reduction in stomatal opening. Furthermore, one of the biochemical

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mechanisms by which mangroves counter the high osmolarity of salt is the accumulation of compatible solutes (Takemura et al., 2000). Although pinitol and manitol are the most common compatible solutes in a number of mangrove species (Popp et al., 1985), proline (found in *Xylocarpus* sp.), methylated quaternary ammonium compounds (in *Avicennia* sp.), and carbohydrates (from *Acanthus ilicifolius*, *Heritiera littoralis*, and *Hibiscus tiliaceus*) are the dominant osmoregulating compounds. Glycinebetaine, the most common compatible solute that protects a plants photosynthetic machinery (Papageorgiou and Murata, 1995), also has been found in some mangroves such as *A. marina* (Ashihara et al., 1997).

Several mangrove species in the coastal forests have been severely affected by recent cyclones, and are being conserved via mass propagation (Das et al., 1997). In this paper, we present a study on the effects of induced NaCl stress in the hydroponic culture of *B. parviflora*. Our focus was on the pigments, proteins, free amino acids, proline, polyphenols, sugars, and starch, and our objective was to gain some insight into the changes in metabolic compositions associated with salt adaptation.

MATERIAL AND METHODS

Propagules of *B. parviflora* were collected from the Bhitarkanika mangrove forest of Orissa, India (latitude 20°4′N to 20°8′N; longitude 86°45′E to 87°50′E). The seedlings were raised in a greenhouse under PAR of 12,120 to 12,360 µmole $m^{-2}s^{-1}$, and were watered with non-saline and non-brackish water. We then selected two-month-old, healthy seedlings for hydroponic culture in a Hoagland's nutrient medium (Hoagland and Arnon, 1940). These cultures were aerated continuously with an air bubbler, and were maintained in a culture room at 22 ± 2°C, 80% RH, 14-h photoperiod, and a light intensity of 300 µmole $m^{-2}s^{-1}$. Different concentrations of NaCl (100, 200, 400, or 500 mM) were added to the Hoagland solutions (pH ~5.8 to 6.0). To prevent fungal growth, the culture medium was changed every 7 d. At Days 0, 7, 14, 21, 30, and 45, we harvested the fourth pair of leaves from the tops of the shoots on the NaCl-treated and control plants to estimate their various biochemical parameters.

Extraction and Estimation of Pigment Contents

Using a mortar and pestle, we thoroughly homogenized 0.5-g samples of fresh, healthy leaves in chilled 100% N,N-dimethylformamide (DMF), holding them in the dark at 4°C. The homogenates were then centrifuged at 8,800*g* for 10 min, and the supernatants collected. We recorded the absorption spectra at 663.8 and 646.8 nm, using a Jasco V-530 UV-visible spectrophotometer to estimate the total chlorophyll content. Chl *a* and Chl *b* ratios were calculated according to the procedure of Porra et al. (1989). To estimate total carotenoid content, we homogenized 0.5-g samples of leaf tissues in chilled 80% acetone. The homogenates were centrifuged at 4°C in the dark at 8,800*g* for 10 min, and absorbances of the acetone extracts were measured at 663, 645, and 470 nm. The total carotenoid content was calculated according to the method of Arnon (1949).

Extraction and Estimation of Total Leaf Protein Content

Total leaf protein was extracted via the acetone-TCA precipitation method of Damerval et al. (1986). We used a pre-chilled mortar and pestle to homogenize 0.5-g leaf tissue samples in ice-cold 10% TCA, then incubated them overnight at 4°C. The homogenates were centrifuged at 8,800*g* for 10 min. Afterward, the pellets were washed with 100% acetone to remove the pigments. Pigment-free pellets were successively washed with 80% ethanol, 3:1 (v:v) ethanol:chloroform, 3:1 (v:v) ethanol:ether, and ether to remove the phenolic compounds. The washed pellets were suspended in a known volume of 0.1 N NaOH to estimate the amount of protein, following the method of Lowry et al. (1951). Proteins in the unknown samples were estimated at 750 nm using de-fatted bovine serum albumin (fraction V) as a standard; the results were expressed on the basis of grams per unit dry weight (g/dw).

Estimation of Total Soluble Sugars, Reducing Sugars, and Starch

To extract soluble sugars, reducing sugars, and starch, we homogenized 1 g of leaf tissue in 80% ethanol, then re-fluxed the samples for 15 min in a 60°C water bath, and centrifuged them at 4,400*g* for 10 min. The pellets were re-extracted twice with 80% ethanol, and the supernatants were pooled. Pigments were removed from the supernatant by adding 1 to 2 mL of saturated neutral lead acetate, then precipitating them out with a slight excess of Na_2HPO_4. The supernatant was filtered through Whatman No.1 filter

paper. We added 0.2 mL of 0.3N $Ba(OH)_2$ per mL of the filtrate and mixed it well. Afterward, 0.2 mL of $ZnSO_4$ was added, and the mixture was shaken thoroughly and filtered through Whatman No. 1 filter paper after 10 min. Through this process, the proteins were precipitated by $Zn(OH)_2$ to yield a protein-free sugar extract (Kumar and Sharma, 1995). The pellet that remained after the soluble sugars were extracted was solubilized in 52% perchloric acid for determination of starch content (McCready et al., 1950).

Total soluble sugars and starch were estimated according to the anthrone-sulphuric acid method of McCready et al., (1950), with some modification (Aarrouf et al., 1999). We used 0.2% anthrone in concentrated H_2SO_4 as the reagent. Spectrophotometric readings were taken at 630 nm, and a standard curve was plotted with 0 to 100 μg of pure glucose. The concentration of starch was determined by multiplying the obtained value by 0.9 for converting the glucose value to starch content (Aarrouf et al., 1999). We adapted the alkaline copper methods of Nelson (1944) and Somogyi (1945) for estimating the amount of reducing sugars, using an arsenomolybdate reagent. After absorbance was recorded at 510 nm, the reducing-sugar content was determined from a standard curve prepared against pure glucose (0 to 50 μg).

Estimation of Total Free Amino Acid Contents

We used the method of Moore and Stein (1948) to extract and analyze free amino acids. Leaves (0.5-g samples) were homogenized in 70% ethanol in a pestle and mortar. The homogenate was centrifuged at 4,400*g* for 10 min and the supernatant was taken. This extraction was repeated four or five times, and the supernatants were combined. An appropriate volume (5 to 10 mL) of this ethanolic extract was evaporated to dryness in a boiling water bath, and the residue was dissolved in 5 mL of 0.2 M citrate buffer (pH 5.0). We placed a 2-mL sample from the above in a test tube, and added 1 ml of a ninhydrin reagent (1:1 4% ninhydrin in methyl cellosolve and 0.2 M acetate buffer). The samples were boiled for 20 min and cooled. Afterward, the volume was made up to 10 mL with distilled water, and absorbance was read at 570 nm. Total free amino acids were calculated from a standard curve prepared against glycine (0 to 100 μg).

Estimation of Free Proline Content

Free proline content was estimated through the method of Bates et al. (1973). Fresh leaf samples (0.5 g) were extracted in 3% sulphosalicylic acid, and the homogenates were filtered through Whatman No. 2 filter paper. To the 2-mL filtrate, we added 2 mL of the acid ninhydrin reagent and 2 mL of glacial acetic acid. The solution was allowed to react at 100°C for 1 h, then the reaction was terminated in an ice bath. This reaction mixture was extracted with 4 mL of toluene and mixed vigorously in a vortex mixer for 15 to 20 s. The chromophore containing toluene was then aspirated from the aqueous phase and warmed to room temperature. Absorbance was measured at 520 nm, with toluene serving as the blank. The concentration of proline was calculated from a standard curve using L-proline (Sigma; St. Louis, MO, USA) of 0 to 100 μg.

Estimation of Total Polyphenol Content

Total polyphenols were analyzed according to the procedures of Chandler and Dodds (1983), as modified by Singleton and Rossi (1965). Fresh leaves (0.5 g) were homogenized in 5 mL of 80% ethanol, using a chilled pestle and mortar, with subsequent centrifugation at 8,800*g* for 20 min. The supernatant was preserved and the residue re-extracted with 2.5 mL of 80% ethanol. After being centrifuged, the supernatants were pooled and evaporated to dryness. The residue was dissolved in 5 mL of distilled water, and 3-mL aliquots were taken in test tubes. A Folin-Ciocalteau reagent (0.5 mL of 1N) was added and kept for 3 min, then 2 mL of 20% freshly made Na_2CO_3 solution was added to each tube and mixed thoroughly. The solution was boiled in a water bath for exactly 1 min, cooled, and absorbance read at 650 nm against a reagent used as a blank. From the standard curve, prepared with 10 to 100 μg of catechol, the concentrations of phenol in the test samples were calculated.

All biochemical estimations were carried out using the leaf tissue of seedlings of the same age. Each estimation included leaves from several plants occupying the same position within the plant. Standard deviations were computed from the values of two or three independent, replicated experiments.

RESULTS AND DISCUSSION

Under hydroponic-culture conditions, seedlings of *B. parviflora* tolerated applications of NaCl up to 400 mM, and were maintained for >45 d. For plants treated with 500 mM, their leaves began to fall after

just a week or so. Because those plants survived for only 14 d, our data for that treatment are limited to that time period. All the other experiments were monitored for the entire 45-d period.

Photosynthetic Pigments

The total contents of Chl and carotenoids varied significantly when different NaCl concentrations were applied (Table 1). Total Chl, on a dry-weight basis, decreased from 6.82 mg/g to 5.82 mg/g at the 400-mM level. In contrast, we found that total Chl content increased sharply (~50%) over 14 d with the 100-mM NaCl treatment, before it plateaued (Fig. 1). The untreated controls showed a profile similar to that with the 100-mM treatment; the Chl amount increased during the first 14 d and thereafter remained mostly unchanged. Compared with the 400-mM level, the Chl amount from the controls did not show any increase but, rather, decreased slightly during the entire 45-d test period. The Chl *a:b* ratio in the control plants remained at 3.0 and increased only marginally during this time. For plants treated with high concentrations of salt, the ratio was 3.2. We conclude, therefore, that high salt concentrations do not affect this ratio even though the total Chl contents increased at low levels and decreased at high concentrations.

Table 1. Effects of NaCl concentration and length of treatment period on pigment levels of *B. parviflora* (values are mean ± SE).

Duration of Treatment (day)	Concentration of NaCl (mM)	Total Chl (mg/g dw)	Carotenoids (mg/g dw)
0	0	4.56 ± 0.37	2.02 ± 0.17
7	0	4.77 ± 0.25	1.88 ± 0.19
	100	6.31 ± 0.14	2.48 ± 0.18
	200	6.22 ± 0.23	2.34 ± 0.22
	400	5.14 ± 0.34	2.12 ± 0.24
14	0	7.07 ± 0.29	2.74 ± 0.28
	100	7.58 ± 0.36	3.02 ± 0.18
	200	5.53 ± 0.22	2.15 ± 0.19
	400	4.73 ± 0.36	1.97 ± 0.23
21	0	7.19 ± 0.20	2.78 ± 0.32
	100	7.88 ± 0.40	3.10 ± 0.10
	200	5.95 ± 0.17	2.26 ± 0.12
	400	4.71 ± 0.21	2.26 ± 0.23
30	0	7.72 ± 0.38	2.73 ± 0.09
	100	8.09 ± 0.20	2.85 ± 0.18
	200	5.64 ± 0.22	2.07 ± 0.22
	400	4.67 ± 0.21	2.03 ± 0.31
45	0	7.76 ± 0.29	2.75 ± 0.38
	100	7.53 ± 0.34	2.62 ± 0.38
	200	4.95 ± 0.17	2.05 ± 0.27
	400	4.44 ± 0.27	1.71 ± 0.31

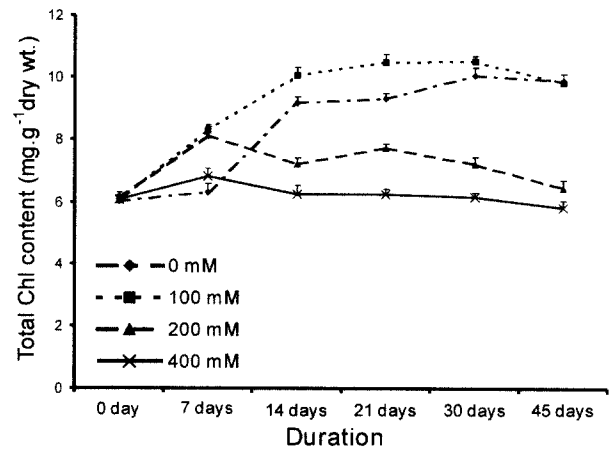

Figure 1. Effect of NaCl concentration on total chlorophyll content in leaves of *B. parviflora*. Contents were estimated as a function of the number of days of NaCl treatment. Total chlorophyll is expressed as mg/g dw.

The decrease in Chl content at high NaCl concentrations (200 and 400 mM) might be due to the disruption of some chloroplasts or to changes in the lipid protein ratio of pigment-protein complexes, as well as because of increased chlorophyllase activity (Iyengar and Reddy, 1996). Likewise, high salinity decreases Chl content along with soluble and hydrolysable sugars, and cytosolic proteins in *Vicia faba* (Gadallah, 1999). Because the Chl *a:b* ratio was unaffected by NaCl treatment in *B. parviflora*, it appears that the light harvesting complex (LHC) of the thylakoid membranes was not much altered by this stress. Nevertheless, treatment with NaCl seemed to evoke some structural alteration of the leaf. Future anatomical studies under these conditions would provide information on the nature of those changes.

We observed a similar trend in carotenoid contents (Table 1). At high salt concentrations (200 and 400 mM), carotenoid levels did not increase, but a small rise was seen with 100 mM NaCl (Fig. 2). Therefore, we assume that high salt concentrations do not enhance either carotenoid or Chl contents, even though the low concentration can enhance the pigment content of the leaves. In rice, the potential efficiency of PSII was almost completely unaffected by salinity, but the conversion of photochemical energy or the actual quantum yield declined with higher salt concentrations (DionisioSese and Tobita, 2000). Although salinity stress causes substantial reductions in the rates of car-

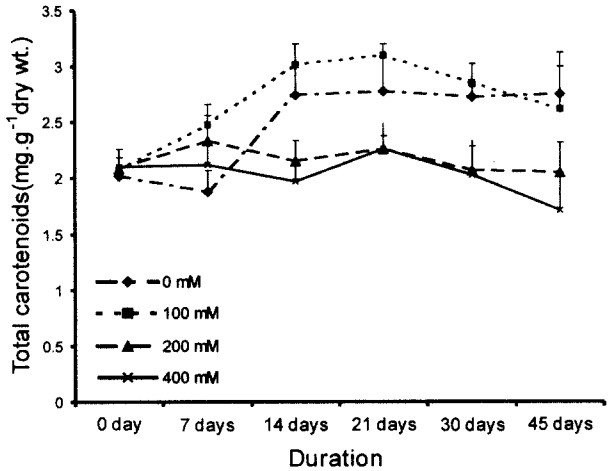

Figure 2. Changes in carotenoid levels due to treatment with different concentrations of NaCl in *B. parviflora*.

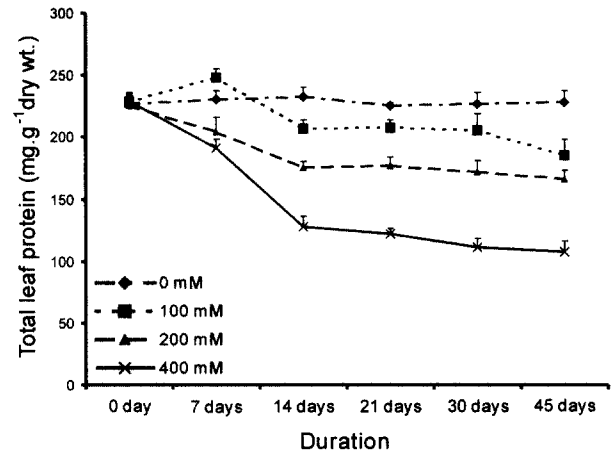

Figure 3. Changes in total soluble leaf protein content due to NaCl treatment in *B. parviflora*. Contents were estimated as function of the number of days of NaCl treatment.

bon assimilation and stomatal conductance in rice (DionisioSese and Tobita, 2000), this has not been found for all mangrove species (Ball and Farquhar, 1984).

Total Leaf Protein, Free Amino Acids, and Proline

Protein content was reduced in treated plants, but hardly at all in the controls (Fig. 1). The maximum decrease, recorded for the 400-mM treatment, was 1.5-fold lower than that measured in the controls (from 226.47 mg/g dw to 107.74 mg/g dw). A salt-induced decline in soluble protein content continued for the first 14 d of treatment, and then the level remained stable (Fig. 3). Total protein contents gradually decreased with increasing concentrations of NaCl. This decrease may have resulted from an adverse effect of NaCl on protein synthesis or proteolysis.

Several proteases are known to be induced by salt stress (Hassanein, 1999). Although protein contents can decline at higher salt concentrations, Sugihara et al. (2000) found that the level of a 33-kDa manganese-stabilizing protein actually increased in *Bruguiera gymnorrhiza*. Our profile analyses of cytosolic as well as plasticidic proteins during NaCl treatment in *B. parviflora* might, therefore, provide valuable information on the nature of the adjustment during salt stress in this species. Treatment with NaCl imposes both osmotic and ionic effects. The former prevents water movement in the cytosol, which leads to a rapid increase in intercellular salt concentration. With the latter, an influx of Na^+ ions through the potassium/Na^+ channels increases the salt concentration in the cytosol, thereby effecting an irreversible ionic effect that can inactivate PSI and PSII activities (see Allakhverdiev et al., 2000). NaCl stress appears to be both osmotic and ionic in *B. parviflora*.

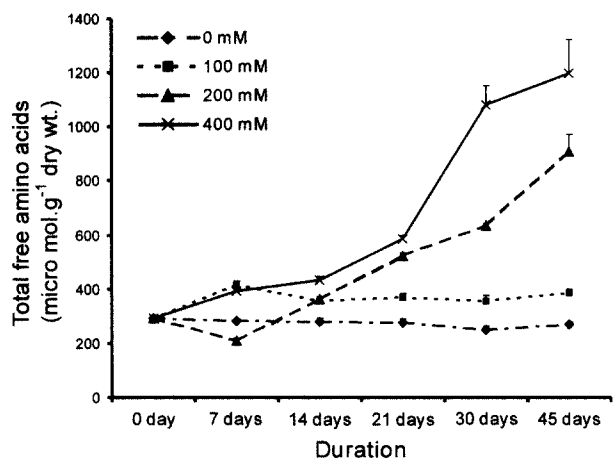

Figure 4. Effect of NaCl concentration on the total free amino acid level in leaves, estimated as a function of the number of days of NaCl treatment.

The pool of free amino acids did not change very much in the control samples over the test period. At the 100-mM treatment level, total amino acids increased only slightly, whereas their increase was steady at 200 and 400 mM. After 45 d, the content measured at 400 mM was four-fold greater than for the control (Fig. 4). Such an accumulation of free amino acids was also reported by Gilbert et al. (1998), who showed that leaves of *Coleus blumei* accumulate

a great amount of free amino acids, but have a reduction in protein content during an extended period of high-saline exposure. Thus, the increase in the total amino-acid pool and the decrease in protein content reflect the mode of adjustment to salinity stress by *B. parviflora*. Although this species shows tolerance to salt, other mangrove species, e.g., *A. corniculatum* are less adaptable to high levels (i.e., 500 mM) of NaCl in hydroponic culture (data not shown).

At increasing exogenous salt concentrations, Na^+, Cl^-, sugar, amino acids, and quaternary ammonium compounds accumulate in the leaves and roots of hydroponic *Zea mays* (AbElBaki et al., 2000). Proline contents in the leaves of many plant species also increase in response to several stresses, including salt (Aziz et al., 1999; Lee and Liu, 1999; Hernandez et al., 2000). Because this accumulation seems to protect the thylakoids from photoinhibitory damage (Alia et al., 1997), we monitored proline levels in leaves of *B. parviflora*. At 400 mM NaCl, the proline content strongly increased for up to 14 d, then remained steady at that high level (Fig. 5). For our controls, however, the level was unchanged during the entire test period. At 100 and 200 mM, proline increased gradually, but the levels were less than that measured in response to 400 mM.

The accumulation of proline as either a compatible osmolyte in NaCl stress or for an ion ratio imbalance has been reported in *Ulva faciata* (Liu et al., 2000). Accumulations also occur when the activity of proline dehydrogenase, a catabolic enzyme, is inhibited (Alia and Saradhi 1993; Lee and Liu, 1999). Proline, a major solute, can also accumulate because of salt stress in the leaves, stems, and roots of sub-antarctic *Pringlea antiscorbutica*. There, levels have been measured that are up to two to three times higher than the initial level in the cytoplasm compared with the vacuole (Aubert et al., 1999). The extent of proline accumulation induced by salt stress varies among species. In tissue-culture experiments with excised tomato shoots, treatment with NaCl did not cause a significant increase in proline content (Aziz et al., 1999). However, in our *B. parviflora* study, proline synthesis in response to salt stress increased to a maximum level by 14 d. In other words, under hydroponic culture, the plants required 14 d to adjust to the change in the external NaCl content; after which, the cellular content level remained steady. Thus, it appears that proline accumulation is a major salt-stress adaptive mechanism in *B. parviflora*.

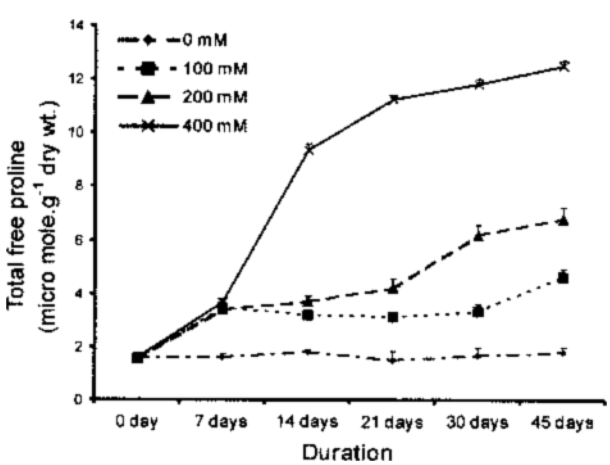

Figure 5. Accumulation of proline in leaves of *B. parviflora* due to treatment with different concentrations of NaCl. Proline contents were estimated as a function of the number of days of NaCl treatment.

Sugars and Starches

As with other cellular constituents, starch and sugar levels are affected by stress (Prado et al., 2000). In our controls, both starch and sugar dw contents increased only slightly in 45 d. During this same period, treatment with 400 mM NaCl caused a 2.5-fold increase in the total sugar content. Starch content at Day 45, in contrast, had decreased by 40 to 45% with 400 mM NaCl. At 100 mM, the total sugar content increased to a small extent and the starch level decreased, but with 200 mM of NaCl, changes in sugar and starch levels were fairly similar to that recorded at 400 mM (Table 2). This suggests that low concentrations of NaCl induce changes in sugar/starch conversion.

We also estimated the extent of changes in the ratio of reducing to non-reducing sugars in both the con-

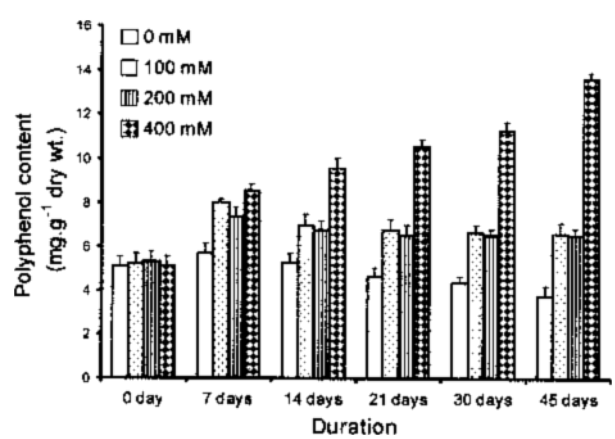

Figure 6. Histograms showing changes in total polyphenol content due to NaCl treatment in *B. parviflora*.

Table 2. Effects of NaCl concentration and length of treatment period on sugar and starch contents in leaves of *B. parviflora* (values are mean ± SE).

Duration of treatment (day)	Concentration of NaCl (mM)	Total sugar (mg/g dw)	Starch (mg/g dw)
0	0	69.39 ± 5.00	756.89 ± 3.57
7	0	70.82 ± 3.79	792.86 ± 4.33
	100	104.48 ± 3.51	755.70 ± 1.76
	200	125.60 ± 2.19	737.82 ± 4.31
	400	145.80 ± 4.60	636.32 ± 8.22
14	0	75.32 ± 4.17	808.85 ± 1.47
	100	87.43 ± 5.24	781.88 ± 4.51
	200	111.35 ± 6.07	712.34 ± 4.19
	400	137.34 ± 6.62	533.30 ± 2.44
21	0	75.17 ± 3.78	763.28 ± 2.86
	100	87.05 ± 5.08	744.89 ± 2.79
	200	108.94 ± 3.93	641.36 ± 6.02
	400	109.05 ± 5.00	495.48 ± 4.61
30	0	77.41 ± 3.78	797.23 ± 3.19
	100	105.79 ± 5.26	687.56 ± 7.26
	200	112.13 ± 5.23	514.62 ± 4.52
	400	178.62 ± 4.30	445.60 ± 4.69
45	0	71.48 ± 3.22	774.96 ± 3.39
	100	122.19 ± 5.86	532.78 ± 4.77
	200	189.59 ± 4.82	485.14 ± 5.55
	400	223.14 ± 5.20	437.77 ± 5.72

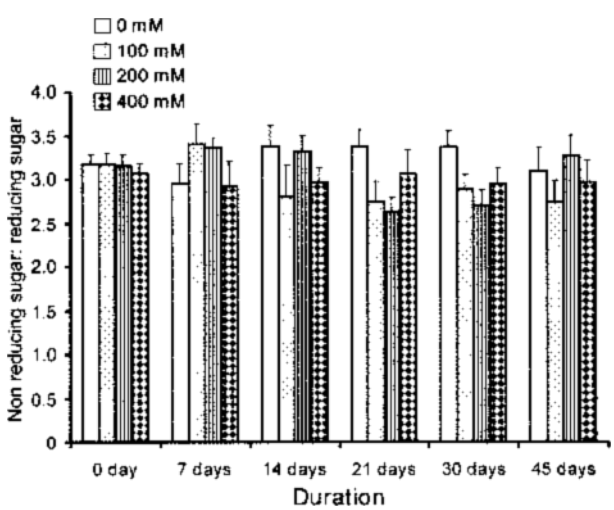

Figure 7. Histograms showing changes in the ratios of non-reducing/reducing sugar after NaCl treatment in *B. parviflora*.

trols and the 400 mM-treated plants (Fig. 7). Total water-soluble carbohydrates, and glucose, fructose, sucrose, and fructan contents increase in salt-tolerant genotypes of *Triticum aestivum* (Kerepesi and Galiba, 2000). In contrast, the contents of glucose and fructose are reduced in the cotyledons and embryonic axes of *Chenopodium quinoa* in the presence of NaCl (Prado et al., 2000). Sucrose phosphate synthase plays a vital role in the synthesis of reducing and non-reducing sugars in NaCl-tolerant rice genotypes such as CSR-1 and CSR-3 (Dubey and Singh, 1999). Although this ratio did not seem to change much in response to salt treatment in *B. parviflora,* the levels of sugar alcohols should be monitored in the future to ascertain their conversion towards salt protection in this species.

Polyphenols

The levels of polyphenols increased significantly over time for NaCl-treated plants, e.g., from 5.14 to 13.63 mg/gdw at 400 mM. Polyphenol content increased approximately two-fold, compared with the control, by Day 21 (Fig. 6). This increase however, was not as great with the 100- and 200-mM treatments. Several plant species have shown higher levels of polyphenol in different tissue types under increasing salinity stress (Kennedy and Fillippis, 1999; Agastian et al., 2000). This stressed environment might force the cell to produce these secondary products as an adaptive mechanism against stress-induced oxidative kinase. Muthukumarasamy et al. (2000) recently reported that an increase in polyphenol in plant tissues can ameliorate the ionic stress evoked by NaCl. However, we noted that the enhanced level of polyphenol in *B. parviflora* was linked with reducing the oxidative stress-induced damage to cells, and that this enhancement was an adaptive mechanism not yet fully elucidated.

In summary, we have shown that the mangrove *B. parviflora* can be easily propagated under limited or low-salinity conditions. At high salt levels, i.e., ~400 mM, plants become adapted to salt stress after two to three weeks of exposure. During this adaptive period, changes occur in the levels of photosynthetic pigments and proteins. In addition, the large accumulation of proline and polyphenols plays a role in NaCl stress-induced adjustments during hydroponic culture. These changes in metabolite contents are apparent even at low levels of salt stress.

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LITERATURE CITED

Aarrouf J, Darbelley N, Demandre C, Razafindramboa N, Perbal G (1999) Effect of horizontal clinorotation on the root system and on lipid breakdown in rapeseed (*Brassica napus*) seedlings. Plant Cell Physiol 40: 396-405

AbElBaki GK, Siefritz F, Man HM, Welner H, Kaldenhoff R, Kaiser WM (2000) Nitrate reductase in *Zea mays* L. under salinity. Plant Cell Environ 23: 515-521

Agastian P, Kingsley SJ, Vivekanandan M (2000) Effect of salinity on photosynthesis and biochemical characteristics in mulberry genotypes. Photosynthetica 38: 287-290

Alia, Paradhasaradhi P, Mohanty P (1997) Involvement of proline in protecting thylakoids against free radical induced photodamage. J Photochem Photobiol 38: 253-257

Alia, Saradhi PP (1993) Suppression of mitochondrial electron transport activity is the prime cause behind stress induced proline accumulation. Biochem Biophys Res Commun 403: 54-58

Allakhverdiev SI, Sakamoto A, Nishiyama Y, Inaba M, Murata N (2000) Ionic and osmotic effects of NaCl-induced inactivation of photosystems I and II in *Synechococcus sp.* Plant Physiol 123: 1047-1056

Arnon DI (1949) Copper enzymes in isolated chloroplasts, polyphenol oxidase in *Beta vulgaris*. Plant Physiol 24: 1-15

Ashihara H, Adachi K, Otawa M, Yasumoto E, Fukushima Y, Kato M, Sano H, Sasamoto H, Baba S (1997) Compatible solutes and inorganic ions in the mangrove plant *Avicennia marina* and their effects on the activities of enzymes. Z Naturforsh 52c: 433-440

Aubert S, Hennion F, Bouchereau A, Gout E, Blingy R, Dome AJ (1999) Subcellular compartmentation of proline in the leaves of the sub-antarctic Kerguelen cabbage *Pringlea antiscorbutica* R-Br. *In vivo* C-13 NMR study. Plant Cell Environ 22: 255-259

Aziz A, Martin TJ, Larther F (1999) Salt stress-induced proline accumulation and changes in tyramine and polyamine levels are linked to ionic adjustment in tomato leaf discs. Plant Sci 145: 83-91

Ball MC, Farquhar GD (1984) Photosynthetic and stomatal responses of two mangrove species, *Avicennia marina* and *Aegiceras corniculatum*, to long term salinity and humidity conditions. Plant Physiol 74: 1-6

Banijbatana D (1957) Mangrove forest in Thailand, *In* Proceedings of the 9th Pacific Science Congress, Bangkok, pp 22-34

Bates LS, Waldren RP, Teare ID (1973) Rapid determination of free proline for water-stress studies. Plant Soil 39: 205-207

Chandler SF, Dodds JH (1983) The effect of phosphate, nitrogen and sucrose in the production of phenolics and solasidine in callus cultures of *Solanum laciniatum*. Plant Cell Rep 2: 105-108

Damerval C, Vienne P, Zivy M, Thiellement H (1986) Technical improvement in two-dimensional electrophoresis increase the level of genetic variation detected in wheat seedling proteins. Electrophoresis 7: 52-54

Das P, Basak UC, Das AB (1997) Restoration of mangrove vegetation in mahanadi delta of Orissa, India. Mangroves and Saltmarshes 1: 155-161

DionisioSese ML, Tobita S (2000) Effects of salinity on sodium content and photosynthetic responses of rice seedlings differing in salt tolerance. J Plant Physiol 157: 54-58

Dubey RS, Singh AK (1999) Salinity induces accumulation of soluble sugars and alters the activity of sugar metabolizing enzymes in rice plants. Biol Plant 42: 233-239

Dubinsky Z, Stambler N (1996) Eutrophication, marine pollution and coral reef. Glob Change Biol 2: 511-526

Gadallah MAA (1999) Effects of proline and glycinebetaine on *Vicia faba* response to salt stress. Biol Plant 42: 249-257

Gilbert GA, Gadush MV, Wilson C, Madore MA (1998) Amino acid accumulation in sink and source tissues of *Coleus blumei* Benth. during salinity stress. J Exp Bot 49: 107-114

Hassanein AM (1999) Alterations in protein and esterase patterns of peanut in response to salinity stress. Biol Plant 42: 241-248

Hernandez S, Deleu C, Larher F (2000) Proline accumulation by leaf tissues of tomato plants in response to salinity. Comptes Rendus de L Academie Des Sciences Serie III, Sciences de La Vie, Life Sci 323: 551-557

Hoagland DR, Arnon DI (1940) Crop production in artificial solutions and in soil with special reference to factors influencing yields and absorption of inorganic nutrients. Soil Sci 50: 463

Hotta M, Nemoto S, Mimura T (2000) Re-evaluation of role of vacuole during salt adaptation in higher plant cells. Plant Cell Physiol 41: 79

Iyengar ERR, Reddy MP (1996) Photosynthesis in high salt-tolerant plants, *In* M Pesserkali, ed, Hand book of Photosynthesis, Marshal Dekker, Baton Rouge, LA, USA, pp 56-65

Kennedy BF, De Fillippis LF (1999) Physiological and oxidative response to NaCl of the salt tolerant *Grevillea ilicifolia* and the salt sensitive *Grevillea arenaria*. J Plant Physiol 155: 746-754

Kerepesi I, Galiba G (2000) Osmotic and salt stress-induced alteration in soluble carbohydrate content in wheat seedlings. Crop Sci 40: 482-487

Kumar N, Sharma PN (1995) Effect of phosphorous deficiency stress on photosynthesis in mulberry *Morus alba* L. Ind J Exp Biol 33: 616-619

Lee TM, Liu CH (1999) Correlation of decreases in calcium contents with proline accumulation in the marine green macroalga *Ulva fasciata* exposed to elevated NaCl contents in seawater. J Exp Bot 50: 1855-1862

Liu CH, Shih MC, Lee TM (2000) Free proline levels in

Ulva (Chlorophyta) in response to hypersalinity: Elevated NaCl in seawater versus concentrated seawater. J Phycol 36: 118-119

Lovelock C, Clough BF, Woodrow E (1992) Distribution and accumulation of ultraviolet-radiation-absorbing compounds in leaves of tropical mangroves. Planta 188: 143-154

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the Folin Phenol reagent. J Biol Chem 193: 265-275

McCready RM, Guggolz J, Silviera V, Owens HS (1950) Determination of starch and amylose in vegetables. Anal Chem 22: 1156-1158

Moore S, Stein WH (1948) Photometric ninhydrin method for use in chromatography of amino acids. J Biol Chem 176: 367-388

Moorthy P, Kathiresan K (1999) Photosynthetic efficiency in Rhizophoracean mangroves with reference to compartmentalization of photosynthetic pigments. Revista Biol Trop 47: 21-25

Muthukumarasamy M, Gupta SD, Pannerselvam R (2000) Enhancement of peroxidase, polyphenol oxidase and superoxide dismutage activities by triadimefon in NaCl stressed *Raphanus sativus* L. Biol Plant 43: 317-320

Nelson N (1944) A photometric adaptation of the Somogyi method for the determination of glucose. J Biol Chem 153: 375-380

Papageorgiou GC, Murata N (1995) The unusually strong stabilizing effect of glycinebetaine on the structure and function of oxygen evolving photosystem II complex. Photosyn Res 44: 243-252

Popp M, Larther F, Weigel P (1985) Osmotic adaptation in Australian mangroves. Vegetatio 61: 247-254

Porra RJ, Thompson, WA, Kriendemann PE (1989) Determination of accurate extinction coefficients and simultaneous equations for assaying chlorophyll a and b extracted with four different solvents: Verification of the concentration of chlorophyll standards by atomic absorption spectroscopy. Biochem Biophys Acta 975: 384-394

Prado FE, Boero C, Gallardo M, Gonzalez JA (2000) Effect of NaCl on germination, growth and soluble sugar content in *Chenopodium quinoa* Willd. Seeds Bot Bull Acad Sinica 41: 27-34

Santiago LS, Lau TS, Melcher PJ, Steele OC, Goldstein G (2000) Morphological and physiological responses of Hawaiian *Hibiscus tiliaceus* population to light and salinity. Intl J Plant Sci 161: 99-106

Singleton VL, Rossi JA (1965) Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid. Am J Enal Vitric 163: 144-158

Somogyi M (1945) A new reagent for determination of sugars. J Biol Chem 160: 61-68

Sugihara K, Hanagata, N, Dubinsky Z, Baba S, Karube J (2000) Molecular characterization of cDNA encoding oxygen evolving enhancer protein 1 increased by salt treatment in the mangrove *Bruguiera gymnorrhiza*. Plant Cell Physiol 41: 1279-1285

Takemura T, Hanagata N, Sugihara K, Baba S, Karube I, Dubinsky Z (2000) Physiological and biochemical responses to salt stress in the mangrove, *Bruguiera gymnorrhiza*. Aquat Bot 68: 15-28

Tanaka Y, Fukuda A, Nakamura A, Yamada A, Saito T (2000) Molecular cloning and characterization of mangrove Na^+/H^+ antiporter cDNA. Plant Cell Physiol 41: 27

Theuri MM, Kinyamario, JI, van Speybroeck D (1999) Photosynthesis and related physiological process in two mangrove species, *Rhizophora mucronata* and *Ceriops tagal*, at Gazi Bay, Kenya. Afr J Ecol 37: 180-193