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Salinity Tolerance and Growth Analysis of the Cyanobacterium *Anabaena doliolum*

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Salinity is one of the most deleterious environmental factors for global agriculture. Secondary salinization from irrigation is an increasingly serious and costly problem. Approximately 30 to 50% of the worldwide irrigated land has been affected by salinity (Maas and Hoffman 1977). Cyanobacteria have been applied with success for the reclamation of saline soils. Because of the potential economic implications, much interest is currently being devoted to the mechanism of salt-adaptation/tolerance of cyanobacteria (Borowitzka 1986). Salinity inhibits general protein synthesis (Hsiao 1973), induces specific stress proteins (Hagemann et al. 1990) and increases chlorophyll (Ferreira and Shaw 1989) and protein degradation by stimulating protease activity (Davies 1982).

Very few studies have dealt with the functional approach to plant growth analysis in determining the salinity effect and whatever reports are available, concern higher plants (Cramer et al. 1990). Our earlier study (Rai 1990) showed that photosynthetic activity of the freshwater cyanobacterium *Aphanothece stagnina* was markedly affected at various salinities and that NaCl reduced chlorophyll content maximally, followed by CO_2-fixation and ribulose biphosphate carboxylase activity. Similar reduction in chlorophyll in response to NaCl has also been reported by others (Robinson et al. 1983). It has been surmised that drastic reduction in photosynthetic activity may primarily be due to degradation of chlorophyll (Rai 1990), as the light harvesting efficiency is associated with increased photosynthetic pigment contents of the cell.

In the present study, we addressed the probable question: what are the individual processes, influenced by high external salinity, that cause reduction in growth of the cyanobacterium Anabaena doliolum?

MATERIALS AND METHODS

Anabaena doliolum Bharadwaja (local isolate in axenic population), a salt-sensitive freshwater strain, was selected for experimentation. The cyanobacterium was routinely grown at 30°C in BG-11 medium without combined nitrogen source in batch culture under continuous illumination (70 $\mu Em^{-2}.s^{-1}$). Mid-logarithmic phase cultures washed with the growth medium (BG-11), were resuspended in the same nutrient solution containing various

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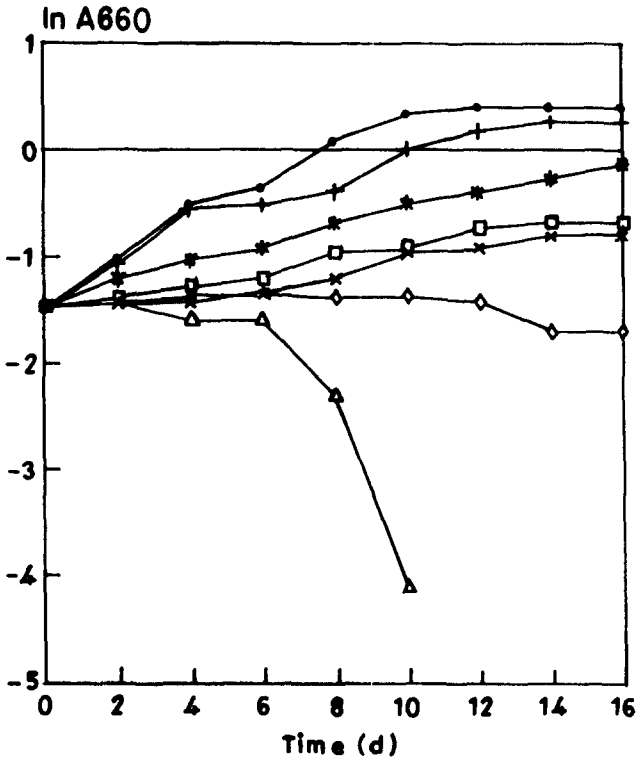

Figure 1. The effect of NaCl on the growth of *Anabaena doliolum* in relation to time (day). •, control; +, 100; *, 150; x, 200; □, 250; ◇, 300; Δ, 500mM NaCl.

level of NaCl. Growth was determined daily in terms of absorbancy of cultures at 650 nm, and also from the increments in dry weight measured after oven drying of the material at 70°C for 48 hr.

Chlorophyll was determined in methanolic extract (Mackinney 1941) and protein by the Lowry procedure (Lowry et al. 1951) using bovine serum albumin as a standard. Average filament length (based on 100 measurements) and heterocyst frequency were estimated as previously described (Rai 1976).

Each experiment was done at least thrice with triplicate samples in each. Standard deviation rarely exceeded 15% of the mean. Results presented are from individual experiments. Data were analyzed using the stepwise regression. Relative growth rate (day-1), relative protein and relative chlorophyll contents (μg.mL-1) were derived by dividing the absolute weight by the initial weight using the equation:

$$1/W \times (dW/dT)$$

where W and T represent weight (μg) and time (day), respectively.

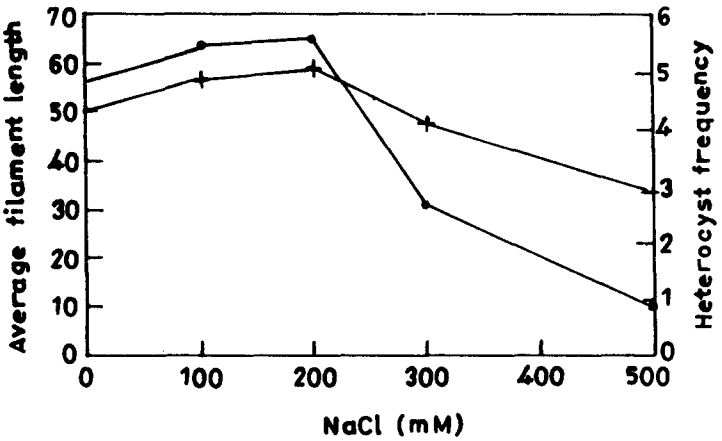

Figure 2. The effect of NaCl on average filament length (number of cells per filament, •) and heterocyst frequency (%, +) of *Anabaena doliolum*

RESULTS AND DISCUSSION

Growth was inhibited by all the salinity treatments (Fig. 1). Even on day 2 of incubation, probably when cell reserves exhausted, the growth rate of salt-stressed cells was substantially lower than those of the control. However, after about 8 days of incubation with 150 mM NaCl, the cells appeared to grow faster than the control. This lag period might be due to the adaptation of the organism to the salt. The cells survived in 300 mM NaCl but appeared to decay at 500 mM NaCl. Division time 2.77 day under control remained unaltered when the cells were grown with 100 mM NaCl but increased to 4.62, 5.77 and 9.9 days at 150, 200, 250 mM NaCl level, respectively.

In the filamentous cyanobacteria like *Anabaena,* the average filament length and heterocyst frequency in a clonal population appears to be constant when grown in particular media and alteration in them can be caused by changing the composition of the nutrient solution. With increase in NaCl concentration (beyond 200 mM), filaments were shorter and had less heterocysts (Fig.2). N_2-fixation is the only system providing nitrogen to the cells under the present set of condition. The reduction in heterocyst number is indirect evidence for reduced N_2-fixation. The N-starvation in the cells would result in reduction in protein synthesis (Ownby et al. 1979) and ultimately a decrease in growth of the cyanobacterial population.

Relative growth rate declined in all the NaCl concentrations below that of control in the begining (Fig. 3). By day 4, the relative growth of control and 100 mM NaCl-treated cells dropped, whereas it remained almost steady up to day 10 to 12 in cells treated with 150 to 250 mM NaCl, indicating for delayed growth. The relative growth of cells incubated with 300 and 500 mM NaCl declined below that of O, by day 14 and 4, respectively, which probably showed the degeneration of the cells.

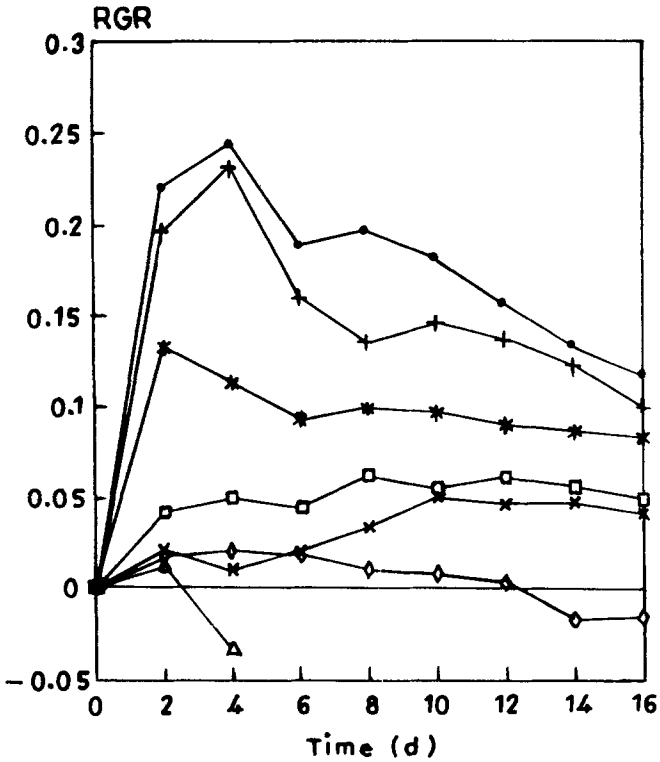

Figure 3. The effect of NaCl on the relative growth rate (day $^{-1}$) of *Anabaena doliolum* over time. Symbols are the same as for Figure 1.

Relative growth rate may be accounted as a function of net synthetic activity (protein synthesis and photosynthetic capacity of the cells). Relative protein content (Fig. 4) declined in the NaCl treated cells and coincided well with relative growth rate. There was a much larger decrease in relative protein content of the 200, 300 and 500 mM NaCl treated cells, whereas it was marginal with 100 mM NaCl treated cells. Even at high salt level, the protein content of the cells did not decrease over the level in the inoculum. It indicated for no degradation of the existing protein, albiet some protein was synthesized. Apte and Bhawat (1989) observed diminished methionine incorporation by *Anabaena* strains during salt stress, whereas protein synthesis was almost blocked in *Synechocystis* PCC 6803 (Hagemann et al. 1990).

Relative chlorophyll content also declined significantly in relation to NaCl concentration (Fig. 5). Cyanobacterial strains show diminished photosynthesis at extended salt concentration (Blumwald and Tel-Or 1984). Since, chlorophyll decrease is a general salt-induced response in cyanobacteria, Hagemann et al. (1989) concluded that this led to lower photosynthetic activity and might be reason for the reduced growth rate at elevated salt levels. Relative chlorophyll content in cells incubated with 100 and 200 mM NaCl increased over the inoculum but incubation with 300 and 500 mM NaCl showed a degrading trend

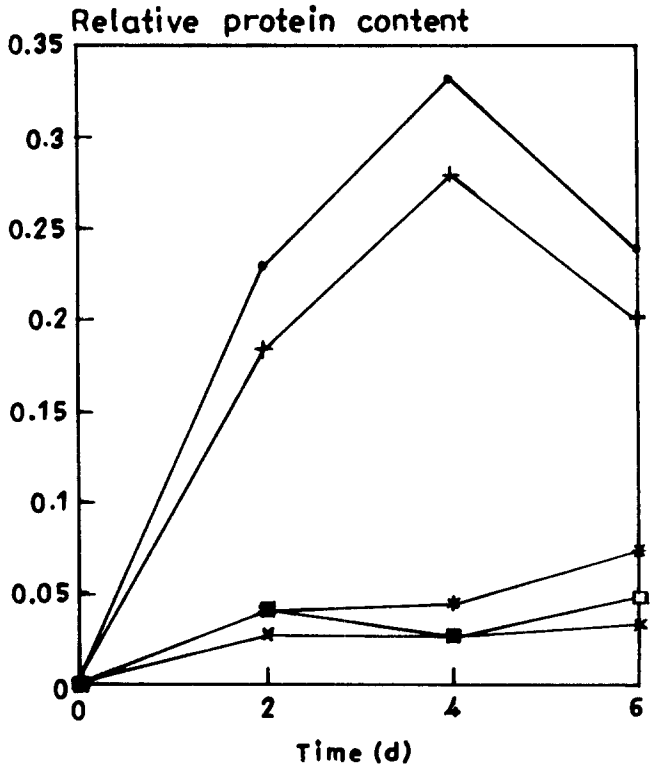

Figure 4. The effect of NaCl on the relative protein content (μg mL -1) of *Anabaena doliolum* over time (day). •, control; +, 100; *, 200; □, 300; x, 500mM NaCl.

after 4 day, not evident for protein content. A rapid degradation of proteins and chlorophylls depending on the concentration of salt has been reported by Ferreira and Shaw (1989) and Kang and Titus (1989). However, the quantitative requirement for *de novo* synthesis of proteolytic enzymes is rather small (Kang and Titus 1989). Rawson et al. (1988) and Myers et al. (1990) have concluded it unlikely that the primary inhibitory effects of salinity on growth are attributable to effects on photosynthetic processes.

There was negligible change in the protein chlorophyll ratio in all salinity treatments up to 2 day, but the ratio was sensitive to salinity influences beyond that (Fig. 6). During day 4 of salinity treatment the values for the ratio of control and 100 mM NaCl treatment remained almost the same, whereas the ratio was lower in 200, 300 and 500 mM Nacl treatments. The ratio value at higher concentrations on day 6 suddenly increased indicating the decay of chlorophyll.

The degree of correlation was assessed by linear regression. Relative growth rate (r^2=0.697), relative protein content (r^2=0.686) and protein chlorophyll ratio (r^2=0.629) was highly correlated with salt concentration, but not the relative chlorophyll content (r^2=0.548). This indicated that relative growth rate

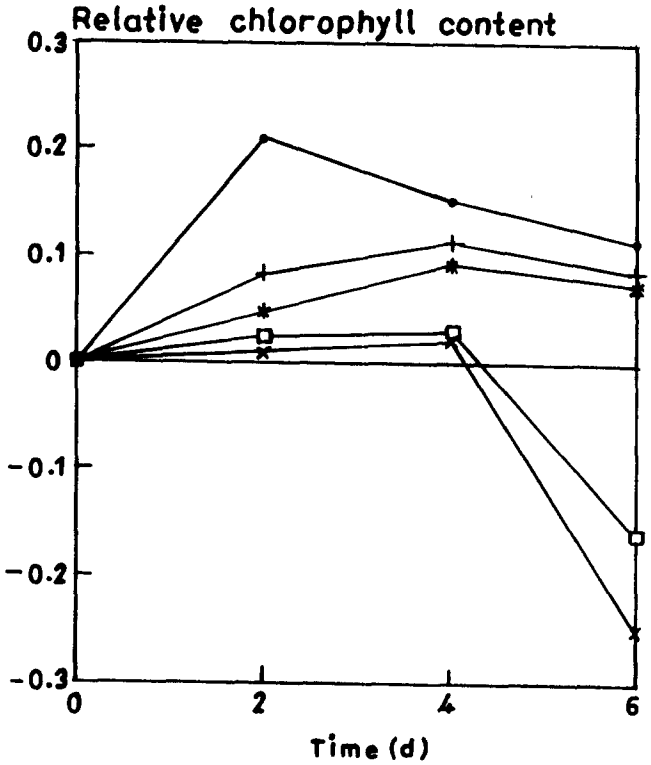

Figure 5. The effect of NaCl on the relative chlorophyll content (μg mL $^{-1}$) of *Anabaena doliolum* over time (day). Symbols are the same as for Figure 4.

and relative protein content were almost similarly affected by the concentration of NaCl.

We also found that relative protein content of salt stressed cells was highly correlated with relative growth rate (r^2=0.955) but not with relative chlorophyll content. It may be concluded that salinity affects the growth of *A. doliolum* primarily by a reduction in protein synthesis rather than a decline in chlorophyll content. We have reported that chlorophyll content was significantly affected and that it played a determinental role in photosynthetic activity of the salt-stressed *A. stagnina* cells (Rai 1990). This data, however, would suggest that protein content is a more important factor than photosynthesis in determining relative growth rates of salt-stressed *A.doliolum* cells. Cramer et al. (1990) suggested net assimilation rate as the most sensitive process to salinity in determining the growth rate. Salinity increases respiration in plants (Bloom and Epstein 1984). Thus, not only a decreased photosynthesis, that salinity affects the growth of A. doliolum primarily by a reduction in protein synthesis but also an increase in maintenance respiration can effectively lower the assimilation rate.

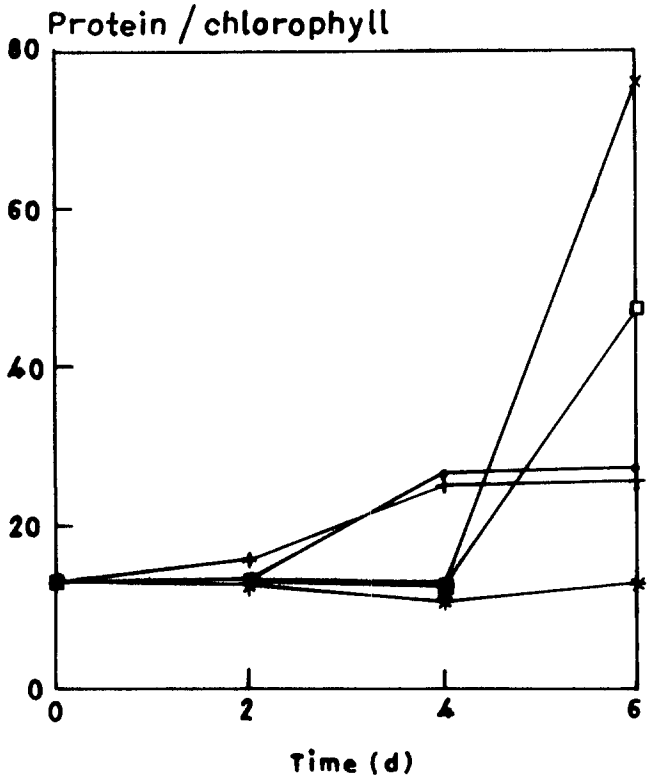

Figure 6. The effect of NaCl on the protein chlorophyll ratio of *Anabaena doliolum* over time (day). Symbols are the same as for Figure 4.

To date there has been no definite study that has clearly uncoupled growth from protein synthesis and photosynthesis. Although the primary limiting process for salt toxicity remains elusive, the protein turnover during salt-stress could be important in the toxicity of plants to saline condition. Hurkman et al. (1989) have noted a change in the levels of translatable mRNA with salt-treatment indicating altered gene regulation by salt-stress. It remains an open question whether growth is inhibited by reduction in protein synthesis or vice-versa. However, Hagemann et al. (1990) suggested the limited growth rate as one reason for the reduced demand of protein synthesis.

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