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Plant physiology in response to salinity stress in Azolla pinnata

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Abstract

Azolla pinnata plants were exposed to salinity treatment (0-40 mM). Salinity treatment reduced growth and altered the chl a/b ratio in all the treatments. Significant reduction in the total chlorophyll content was also noticed. The results suggest NaCl induced depression in plant growth of A. pinnata to be a function of decreased chlorophyll content which in turn could reduce the photosynthesis related physiological variables.

Key words: *Azolla pinnata*, salinity, chlorophyll, growth

Introduction

Azolla, an aquatic fern has a global distribution and occurs in fresh water habitats of tropical, sub-tropical and warm temperate regions. It is also used as a biofertilizer in rice paddy fields due its ability to fix atmospheric nitrogen. The dinitrogen fixing ability of the association is due to the cyanobiont *Anabaena-azollae* that inhabits the dorsal leaf lobe cavity of the host *Azolla* (1). However, increasing soil salinity has become a serious threat to agriculture worldwide. Approximately 100 million hectares of the worldwide land has been adversely affected by salinity (2). Salt exposure has an adverse effect on *Azolla* plants because the host plants supply photosynthates and ATP for N_2 fixation by the cyanobiont (3). Understanding the physiological bases of salinity tolerance and adaptation could help us to a great extent in selecting plants tolerant to salinity stress. Therefore, in the present communication we describe the findings on growth behavior and chlorophyll content of *A. pinnta* pants exposed to various levels of salinity.

Materials and methods

Azolla pinnata plants were collected locally from paddy fields. Plants were washed and cleaned of contaminating organisms. The plants were surface sterilized with a solution of mercuric chloride (0.1 % for 30 s) and were dipped immediately into a large volume of sterile distilled water. Plants were then transferred into dishes containing combined-N free 2/5 strength sterile Hoagland's nutrient solution with added micronutrients (4) and 0.04 mM ferrous ion as Fe-EDTA, pH 5.6. The cultures were grown at 26^0C, under a 16:8 (light: dark) photoperiod with light from a combination of incandescent and cool white light fluorescent lamps at a photon fluence rate of 95 μmol $m^{-2}s^{-1}$. Cultures were routinely transferred into fresh medium twice a week to maintain plants in a sterile state. Log phase cultures were used

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for experiments. Growth was estimated as biomass yield of the plants (in terms of dry weight) and was measured after drying the samples at 90 ^{0}C to constant weight. Chlorophyll was extracted using 80% acetone and the amount of chlorophyll a and b was determined using specific absorption coefficients of Lichtenthaler (5). The experiments have been conducted in triplicate with triplicate samples.

Results and discussion

Figure 1 shows the effect of different concentrations of NaCl on the growth of *A. pinnata*. All the NaCl concentrations reduced the growth of the plants as evident from the decreased dry weight. *A. pinnata* plants grew well up to 30 mM NaCl, but growth was almost completely inhibited at 40 mM NaCl. The dry weight of salt stressed *A. pinnata* plants was reduced by 26.7, 40 and 60 % at 10, 20 and 30 mM NaCl respectively on the fourth day after incubation. Although, the plants contributed to the dry weight the morphological appearance was pale and yellow at higher concentrations of NaCl. Some plants failed to grow but decayed during growth. Our results are comparable to the observations made by Rajarathinam and Padhya (6) and Rai and Rai (7) who found that NaCl is toxic to the growth of *A. pinnta* plants. Reduction in growth may be due to osmotic injury or specific ion toxicity due to the entry of salt (8, 9). Excess salt decreases leaf water potential leading to reduced water and nutrient uptake by the plants (10).

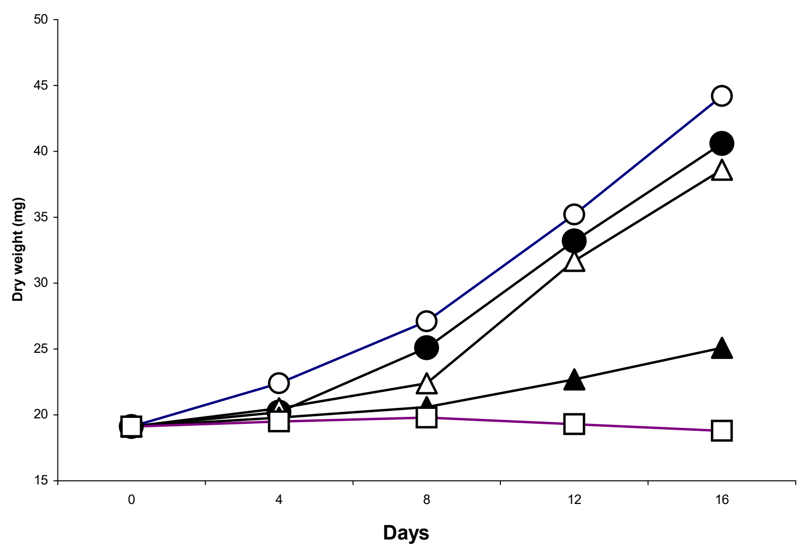

Figure 1. Effect of different concentrations of NaCl on dry weight accumulation in *Azolla pinnata*

The chlorophyll content of *A. pinnata* plants exposed to NaCl followed declining trend. Immediately after salt exposure (8 h) the chlorophyll content of plants reduced by 13.8, 25.9, 51.5 and 68.2 % at 10, 20, 30 and 40 mM NaCl (Table 1). Chl a/b ratio was significantly lower at higher concentrations of NaCl especially 30 and 40 mM. However, chl a/b ratio was slightly better at lower concentrations of NaCl such as 10 and 20 mM. The chl a/b ratio was maximum in control (1.72). However, it reduced to 1.44 and 1.31 at 10 and 20 mM NaCl. At higher concentrations the chl a/b ratio reduced further and the values recorded were 0.985 and 0.792, respectively. The reduction in chlorophyll content as well as altered chl a/b ratio at

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higher concentrations might be due to salt accumulation by plants. It could also be due to reduced chl a content. It appears that presence of NaCl in the growth medium adversely affected chlorophyll content and alteration in chl a/b ratio in *A. pinnata* plants. This could lead to reduced photosynthetic efficiency of *A. pinnata* plants exposed to salinity. Reduction in chlorophyll content in *Azolla* plants due to salinity treatment has been reported earlier by Rai and Rai (11). They correlated higher photosynthetic rates in *Azolla* plants to adaptation to salt. Decreased chlorophyll content in cotton plants exposed to salinity is correlated with decreased photosynthetic performance. Decreased chlorophyll content is correlated with higher reduction of photosynthesis in cotton (12). We also do not rule out the possibility of NaCl interacting synergistically with other physiological and biochemical components there by leading to impairment in plant growth and other physiological parameters.

Table. 1. The chlorophyll content (mg.g FW^{-}1) and its ratio in *Azolla pinnata* plants exposed to salinity stress. Values are mean of three replicates ± SD

NaCl (mM)	Chl a	Chl b	Total chlorophyll	Chl a/b
0	0.36± 0.006	0.209± 0.007	0.569± 0.008	1.72±0.02
10	0.29± 0.004	0.201±0.001	0.491±0.006	1.44±0.09
20	0.24± 0.002	0.182±0.003	0.422±0.004	1.31±0.05
30	0.14± 0.004	0.142±0,002	0.282±0,006	0.985±0.04
40	0.08± 0,002	0.101±0.002	0.181±0.002	0.792±0.02

Conclusions

Azolla pinnata plants exposed to salinity showed reduction in biomass, chlorophyll content and altered chl a/b ratio which could lead to reduced photosynthetic efficiency and impaired plant growth. Elucidation of physiological response to salinity is important in this organism because the problem of salinity is increasing and potential use of *Azolla* as biofertilizer in saline environment needs to be investigated in detail.

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