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Research article

Salinity induced physiological and biochemical changes in the freshly separated cyanobionts of *Azolla microphylla* and *Azolla caroliniana*

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ABSTRACT

Freshly separated cyanobionts of *Azolla microphylla* and *Azolla caroliniana* plants exposed to salinity showed decline in the cellular constituents such as chlorophyll (23.1 and 38.9%) and protein (12.9 and 19.3%). However, an increase in the carotenoid and sugar content was observed. Exposure to salinity stress reduced the heterocyst frequency (35.4 and 57.2%) and nitrogenase activity (37.7 and 46.3%) of the cyanobionts. Increase in the activity of antioxidant enzymes such as super oxide dismutase (50.6 and 11.5%), ascorbate peroxidase (63.7 and 57.9%), catalase (94.2 and 22.5%) as well as non-enzymatic antioxidant proline (18.8 and 13.3%) was also observed in response to salinity. The cyanobionts exhibited significant increase in the intracellular Na^+ level and reduced intracellular K^+/Na^+ and Ca^{2+}/Na^+ ratio in response to salinity. The results demonstrate the adverse impact of salinity on the freshly separated cyanobionts as similar to free living cyanobacteria. These results may be helpful in the critical evaluation of salinity tolerance mechanism of the cyanobiont and its interaction with the host.

1. Introduction

The free floating aquatic fern *Azolla* has the ability to fix atmospheric nitrogen due to the presence of symbiotic association with the nitrogen fixing cyanobacterium *Anabaena azollae* (Peters, 1978). Because of the high rates of nitrogen fixing potential the system is considered to be economically important (Stewart et al., 1987). The *Azolla-Anabaena* system is unique and has great agronomic significance due to high productivity, nitrogen fixation and photosynthesis. Singh (1989) highlighted the importance of *Azolla* as biofertilizer in rice cultivation. Besides the traditional use as biofertilizer the plant has several other uses such as green manure, fish and animal feed, water purifier and hydrogen gas producer (Wagner, 1997).

Increasing soil salinity is however, serious impediment in the popularization of this system as biofertilizer. There are several reports on the impact of salinity on *Azolla* and free living cyanobacteria (Rai and Rai, 1999; Masood et al., 2006; Rai et al., 2014). However, there have been no attempts to understand the effect of salinity on the physiological and biochemical response of the cyanobionts. On the other hand, the salinity tolerance mechanism in free living cyanobacteria has been worked out in considerable detail (Moisander et al., 2002; Chris et al., 2006; Srivastava et al., 2005, 2008). Pabby et al. (2003) isolated the cyanobionts from different strains of *Azolla* and characterized them physiologically. Understanding the physiological response of the cyanobiont to salinity is therefore important to unravel the salinity tolerance mechanism operating in this system. This will further pave way for the intervention of advanced molecular biological tools to work out the mechanisms operating under salinity stress conditions in the cyanobionts. Therefore in the present study the physiological and biochemical response of freshly separated cyanobionts from two different species of *Azolla* viz. *Azolla microphylla* and *Azolla caroliniana* exposed to salinity was studied.

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2. Results

2.1. Cellular constituents and nitrogenase activity of freshly separated cyanobionts

The freshly separated cyanobionts from *A. microphylla* and *A. caroliniana* showed contrasting pattern with respect to the cellular constituents (Fig. 1a&b). Cyanobionts of *A. caroliniana* showed much drastic reduction in the content of chlorophyll ($P > 0.05$) as compared to *A. microphylla*. Compared with the control, the observed decrease in the chlorophyll content was 23.1 and 38.9% in the cyanobionts of *A. microphylla* and *A. caroliniana*, respectively. On the other hand cellular constituents such as carotenoid increased in response to salinity exposure and the increase was less pronounced in case of the cyanobionts isolated from *A. caroliniana*. Increase in the carotenoid content was 67.4% in the cyanobionts of *A. microphylla* as compared to 29% increase in the cyanobionts of *A. caroliniana*. Similarly the cyanobionts of *A. microphylla* showed significant increase in the sugar content by 2.5 fold. However, increase in the sugar content in the cyanobionts of *A. caroliniana* was non-significant. Fig. 1c shows the heterocyst frequency of the cyanobionts in response to salinity stress imposed on *Azolla* plants. Freshly separated cyanobionts of *A. microphylla* and *A. caroliniana* showed decrease in the heterocyst frequency by 35.4 and 57.2%, respectively as compared to their control ($P > 0.05$). The nitrogenase activity was maximally observed in the cyanobionts of *A. microphylla* and *A. caroliniana* not exposed to salinity (Fig 1c). However, the nitrogenase activity reduced by 37.7 and 46.3%, respectively in the cyanobionts ($P > 0.05$).

2.2. Proline accumulation and antioxidant enzyme activity of freshly separated cyanobionts

Fig. 2a shows the changes in the proline concentration of freshly separated cyanobionts. Increase in the concentration of proline was non-significant in case of both the category of cyanobionts. In case of the cyanobionts of *A. microphylla* the increase was however more pronounced. Fig. 2b shows the SOD, APX and CAT activity of freshly separated cyanobionts. Significant increase in the level of antioxidant enzyme such as SOD, APX and CAT was observed in cyanobionts of *A. microphylla* exposed to salt ($P > 0.05$). In contrast, the level of increase in the enzyme activity was non-significant in the cyanobionts of *A. caroliniana*.

2.3. Intracellular ion content of freshly separated cyanobionts

Intracellular Na^+, K^+ and Ca^{2+} content of freshly separated *Anabaena azollae* from their respective host plants exposed to salinity was studied. Our results showed that salinity (90 mM) increased the intracellular Na^+ content of the cyanobionts of both *A. caroliniana* and *A. microphylla* in a differential manner (143 and 106 times of control). Highest increase in the concentration of the intracellular Na^+ content was observed in the cyanobionts isolated from *A. caroliniana*. Salinity also resulted in reduced intracellular K^+ content of the cyanobionts of *A. caroliniana* (Fig 3). Salinity treatment led to increased intracellular Ca^{2+} content in the cyanobionts of *A. microphylla* as compared to the cyanobionts of *A. caroliniana*. Increase in the intracellular Ca^{2+} content was more than 2 fold in cyanobionts of *A. microphylla* and *A. caroliniana*. The ratio of K^+/Na^+ as well as Ca^{2+}/Na^+ was also found to reduce considerably in the cyanobionts of *A. caroliniana* as compared to the cyanobionts of *A. microphylla* (Fig. 4).

Intracellular Na^+ accumulation has been reported to induce ion toxicity and physiological disturbances in the system. Therefore, a correlation analysis was performed with respect to Na^+ accumulation and physiological parameters in the cyanobionts (Table 1 A&B & Supplementary file 1). Decrease in the chlorophyll content is negatively correlated with the intracellular Na^+ content of the cyanobionts of *A. microphylla* ($r = -0.899$) and significantly in *A. caroliniana* ($r = -0.965$). Similarly the protein content of *A.*

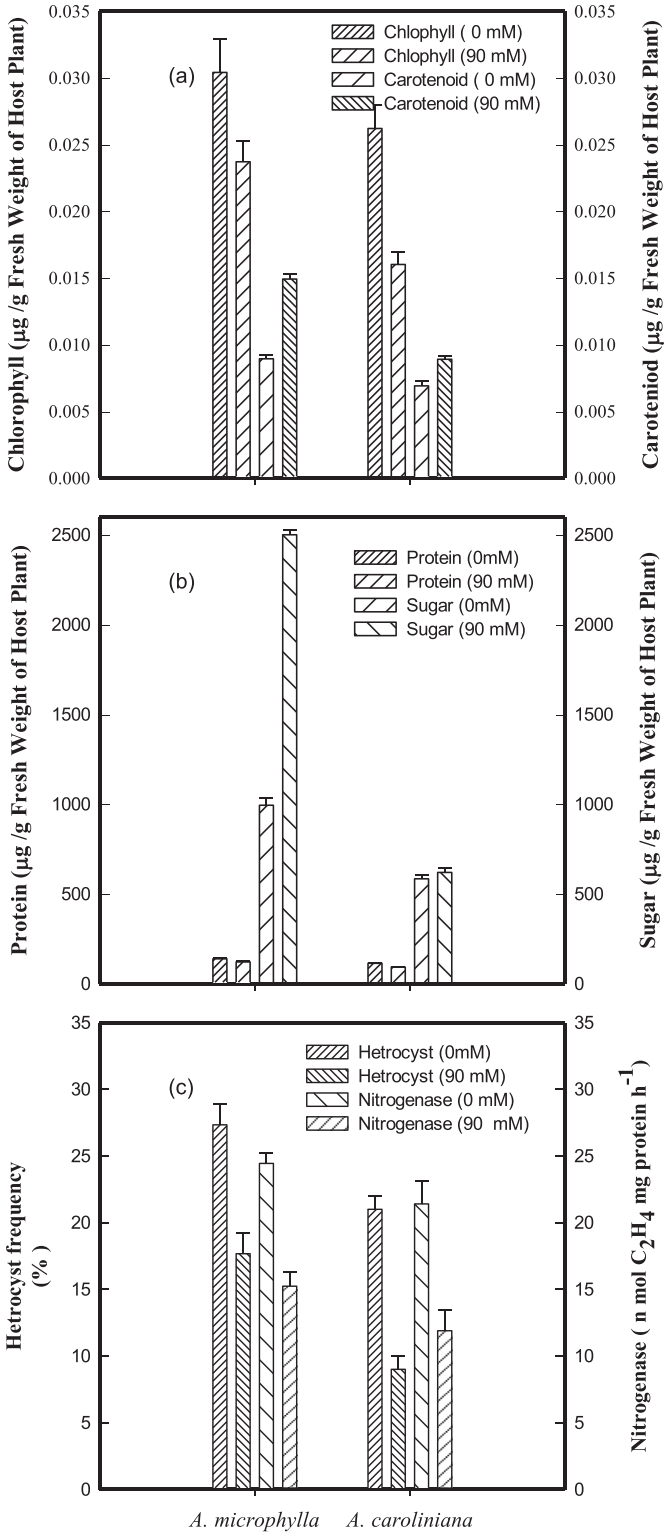

Fig. 1. (a & b) Cellular constituents, (c) heterocyst frequency and nitrogenase activity of the freshly separated cyanobionts from *A. microphylla* and *A. caroliniana* exposed to NaCl.