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Photosynthetic pigments of three species of bromeliads cultured *in vitro* with different concentrations of nitrogen

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Workshop Information

I Workshop of Plant Biology (I Workshop de Biologia Vegetal) was held in the Bioscience Institute – UNESP, campus of Rio Claro, Brazil, during August 20 and 21, 2012. Workshop was a scientific event organized by Post-graduate students from that Institute aiming to integrate Post-graduate and Graduate students from different areas related to Plant Biology (Anatomy, Ecology, Evolution, Morphology, Physiology, and transitional areas) from different Universities. Workshop Organization offered a large number of speaking activities, scientific discussions, and extra short-courses to improve the knowledge and formation of students in Plant Biology.

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INTRODUCTION

Ananas ananassoides belongs to Bromeliaceae. It is a terrestrial plant which develops directly on the ground or even on the litter, usually in open fields under high light, in environments of sandy soil and tropical climate (Proença and Sajo 2007). It is endemic of the "Cerrado". Another bromeliad *Alcantarea imperialis* occurs mainly in the steep rocky cliffs of the Atlantic Forest of Rio de Janeiro, in large groups, with the either rupicole or saxicolous habit (Naves, 2001). *Nidularium minutum*, is also an example of Bromeliaceae in the Atlantic Forest, but with terrestrial habit (Moreira et al. 2005).

The *in vitro* procedure has been used for the production of commercial plants and the mineral supply of culture medium is important. The culture media used for the major works are based on modified formulations of Murashige and Skoog (Kanashiro 2005). According to Raven et al. (2007), nitrogen is one of the most important nutrients for plants it is the main component of amino acids, proteins, nucleic acids, chlorophyll and coenzymes. Wherever there is a deficiency, the nitrogen is translocated from older leaves, which show chlorosis, to younger leaves, which have a slow growth.

Thus, the present work aimed to evaluate the photosynthetic pigments content of bromeliads *A. ananssoides, A. imperialis and N. minutum* grown *in vitro* with different concentrations of nitrogen.

MATERIAL AND METHODS

The work was performed at the Laboratory of the Research Center for Ornamental Plants, of Institute of Botany, of Department of the Environment of the State of Sao Paulo/Brazil. Seeds from *Alcantarea imperialis, Ananas ananassoides* and *Nidularium minutum* were surperficially disinfected with 70% alcohol for 5 minutes, then placed in a solution of sodium hypochlorite at 2%, plus two drops of Tween 20 for one hour. The process is terminated with four washes with sterile distilled water. After the disinfection, the seeds of *A. imperialis* were placed in Petri dishes with containing Murashige and Skoog (1962) medium (MS) with macronutrients reduced to 75% and *N. minutum* and *A. ananassoides* in MS with macronutrients reduced to 50% (MS/2) every medium supplemented

with 3% sucrose and 5 g L^{-1}. These dishes were maintained in culture room with 12h photoperiod, photosynthetically active radiation of 30 µmol m^{-2} s^{-1} and a temperature of 26±2 °C until attainment of seedlings.

Seedlings were transferred to 250 mL (*A. imperialis* and *N. minutum*) and 360 mL (*A. ananassoides*) flasks that contained 40 mL (*A. ananassoides*) and 30 mL (*A.imperialis* and *N. minutum*) of MS with different concentrations of N (0 mM, 7.5 mM, 15 mM, 30 mM, 60 mM-original MS, 120 mM and 175 mM). Were used five (*N. minutum* and *A. ananassoides*) or ten (*A. imperialis*) seedlings per flask. The flasks were maintained for six months under the same conditions described above for the germination process. After this time, the quantity of photosynthetic pigments in both group of plants were assigned.

The averages were calculated and submitted to ANOVA and compared by means of Tukey test at 5% probability.

RESULTS AND DISCUSSION

Only in *Alcantarea imperialis* plants grown at 175 mM concentration of N showed a high mortality rate (70%) hampering the analysis of the parameters. As for the results of photosynthetic pigments of *A. imperialis*, the chlorophyll *a* analysis showed a significant gradual increase in plants from 15 mM to 120 mM N (Figure 1a), of *N. minutum* (Figure 1b) plants grown at 60 and 120 mM N showed the highest values of chlorophyll a, but for *A. ananassoides* the highest chlorophyll a values were observed in the range 7.5 to 60 mM N (Figure 1c). At 7.5 mM N the plants of *A. imperialis* showed a decrease in the concentrations of chlorophyll a, b and carotenoids (Figure 1a). This can be explained probably by the presence of red colored leaves in 90% of the plants, and this coloration is probably due to the presence of anthocyanins, which use N in their biosynthesis (Shu and Xie 2010).

In relation to chlorophyll b, *A. imperialis* and *N. minutum* plants grown between 30 and 120 mM N showed the highest values (Figure 1a and 1b), but *A. ananassoides* showed highest values from 7.5 to 120 mM N (Figure 1c). With respect to carotenoids, *A. imperialis* had higher concentration at 120 mM N (Figure 1a) and *N. minutum* at 60 to 120 mM N (Figure 1b), for *A. ananassoides*, the results were similar to chlorophyll b. The plants grown in 0 mM of N had the lowest amounts of pigments in all species (Figure 1a to 1c). With respect to chlorophyll, four nitrogen atoms are necessary for its formation, suggesting that this molecule could demonstrate the nitrogen status of plants (Saleem et al. 2010, Taiz and Zeiger 2010).

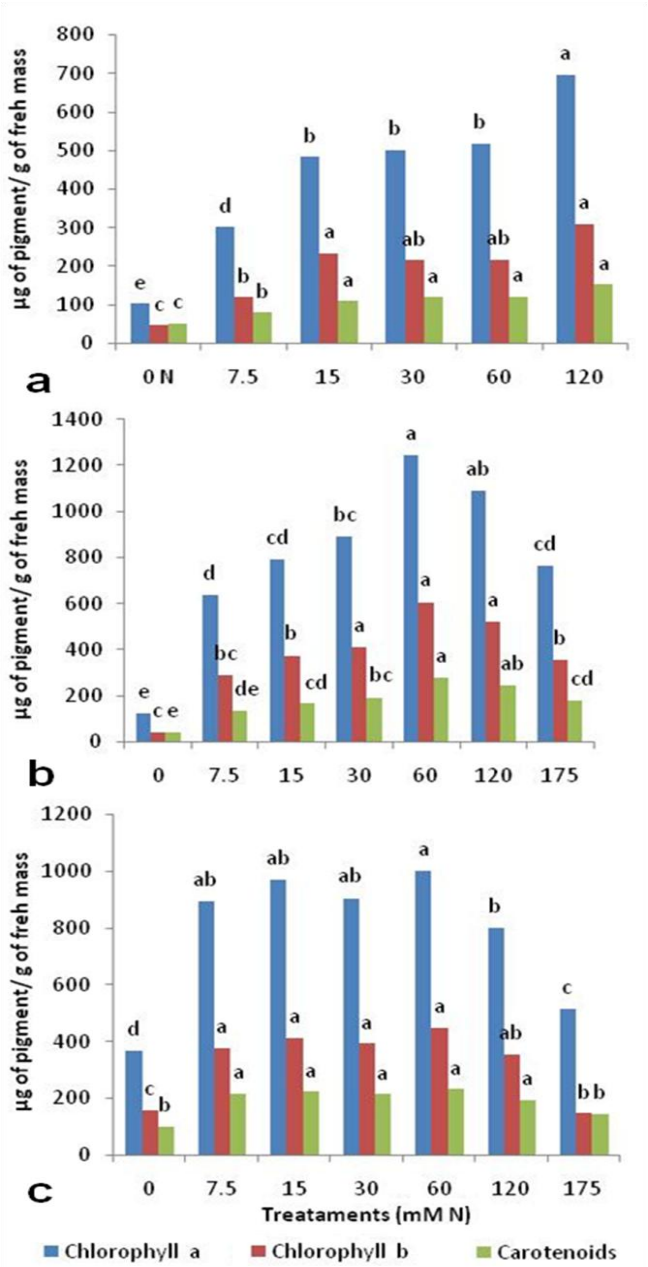

Figure 1. Quantity of photosynthetic pigments in plants of *Alcantarea imperialis* (a), *Nidularium minutum* (b) and *Ananas ananassoides* (c) after six months of cultivation in different concentrations of nitrogen. Different letters compare the quantities of the same type of pigment between the treatments and indicate that the values are significantly different according to Tukey test at 5% probability.

Possibly, the reduced growth or death in the treatment of 175 mM N, relates to a certain degree of toxicity when is subjected to this concentration. According to Taiz and Zeiger (2010), the excess of minerals in the soil becomes saline, resulting in low availability of water, inducing the plant to salt stress and water, and thus a reduction in its growth.

CONCLUSIONS

It follows that, in the case of *A. ananassoides* grown *in vitro* for six months, the concentration 7.5 to 60 mM N proved satisfactory for photosynthetic pigments

content, but *A. imperialis* is higher with 120 mM N and *N. minutum* with 60 to 120 mM N. It is suggested that *A. ananassoides,* a species from "Cerrado" and with terrestrial habit, is best adapted to variations in the environment, showing a greater range of growth compared to the others species studied.

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