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Nitrate reductase activity in bromeliad *Alcantarea imperialis* (Carrière) Harms cultivated *in vitro* at different concentrations of nitrate

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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

Alcantarea imperialis (Carrière) Harms, popularly known as imperial bromeliad (Versieux and Wanderley 2007), is an endemic species of the Serra dos Orgãos in the State of Rio de Janeiro. Numerous specimens of bromeliad ornamental species are illegally collected from the forest, as in *A. imperialis*, since it is a species that is in the list of endangered flora in the category "endangered" (Biodiversitas 2007).

An important aspect of in vitro culture is the supply of mineral medium, since the mineral nutrition is essential to plant growth and development (Bunn et al. 2011). ON, according to Marschner (1995), is the main component of aminoacids, proteins, nucleic acids, chlorophylls and coenzymes. While on disability, N is translocated from older leaves, presenting chlorotic in young leaves it is less developed. The two main sources of nitrogen are found in soil NO_3^- and NH_4^+ (Jackson and Volk 1995). NO_3^- absorbed by the roots is the only active process, and then reduced in the leaves and the roots by NH_4^+ nitrate reductase (NR), which is assimilated into aminoacids and proteins (Kerbauy 2008).

The objective of this study was to analyze the diurnal and nocturnal NR *A. imperialis in vitro* culture, with different concentrations of NO_3^-.

MATERIAL AND METHODS

The work was performed at the Laboratory of the Research Center for Ornamental Plants, Institute of Botany, Department of the Environment of the State of Sao Paulo. Plantlets were deposited in *in vitro* treatments with Murashige and Skoog (1962), with different concentrations of nitrate (NO_3^-) at concentrations of 5, 15, 30 and 60 mM. N Samples were collected every four hours 24 hours after the transfer, which began at 10am, in a photoperiod of 12 hours (beginning at 5 am and ending at 17h).

The activity of nitrate reductase (NR) *in vivo* was determined according to the method described by Jaworski (1971). The sheets collected were chopped in portions into 0.25 to 0.5 g, which were deposited in test tubes in which 6 ml of incubation solution were added. This consisted of 0.1 M phosphate buffer containing 1% propanol, 25 mM KNO_3 and pH 6.5. The plant tissue was kept submerged in the incubation solution

and the tubes were subjected to vacuum so as to facilitate infiltration of the buffer solution in the tissue. Afterwards, 1 ml of each tube was used and incubated at 30 °C in the dark for 90 minutes. After the incubation period, the amount of nitrite ions released into the medium infiltration was determined in aliquots of 1 ml added to 0.3 ml of 1% sulfanilamide in 3 M HCl, and 0.3 ml of N-naphthyl-ethylene 0,2-diamino%.

The determination of nitrite produced was 30 minutes (reaction time) after the addition of these reagents at the rates. After this period, the samples were analyzed by spectrophotometer at 540 nm. The mean values were subjected to analysis of variance (ANOVA) and compared by means of Tukey test at 5% probability.

RESULTS AND DISCUSSION

NR activities measured in plants grown in different *Alcantarea imperialis* NO_3^- are shown in Figure 1, where it is noticed that, in the first four hours, the plants grown in concentrations of 15 and 30 mM had the highest activity when compared to the other two concentrations. At the 8th hour, we observed a decrease in NR activity in plants grown at 15, 30 and 60 mM NO_3^-, but the concentration of 5 mM NO_3^-. There meant an increase in activity of the studied enzyme.

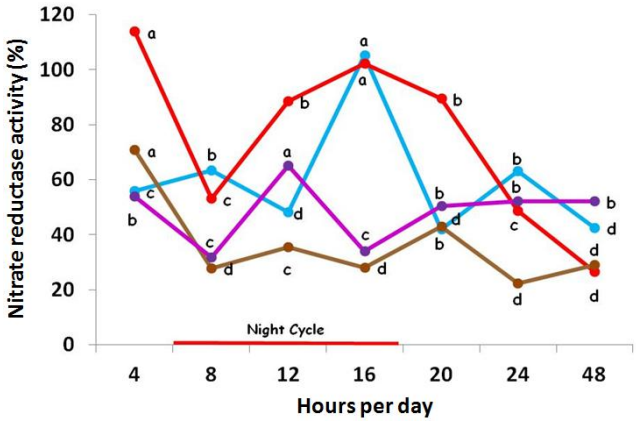

Figure 1. *In vivo* activity of nitrate reductase during the day and night cycle (24 hours), after 2 days of the beginning of the collection, in the leaves of *Alcantarea imperialis* (Carrière) Harms in different concentrations of NO_3^- (5mM-blue, 15mM-red, 30mM-brown and 60mM- purple). The red bar indicates the dark period of the collections. The enzyme activity was expressed as nmol $NO_2^-.h^{-1}.g^{-1}$ fresh mass. Different lowercase letters indicate statistical differences at 5% probability by means of Tukey test, within the same treatment.

However, when checking the results after 12 hours after being collected, plants grown in 5 mM continued to have reduced enzyme activity; a result contrary to the other treatments. Within 16 hours, the plants of the concentrations of 5 and 15 mM NO_3^- showed an increase in NR activity, decreasing thereafter. Regarding treatment with 30 mM NO_3^-, plants practically had the lowest values of NR activity over the analyzed period. The plants grown in 60 mM NO_3^- presented, in general, less NR activities than in plants grown at concentrations of 5 and 15 mM NO_3^-.

According Zonia et al. (1995), nitrate availability can positively influence the activity of NR, for the NO_3^- is the substrate of the studied enzyme which was not observed in this study because the higher activities of NR were found by using 15 mM NO_3^-.

CONCLUSIONS

It can be inferred that that the highest activity of NR in leaves of *A. imperialis* was observed in plants grown in 15 mM NO_3^-. They presented higher activities in the 4th and 12th hours."

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