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In vitro growth of *Nidularium minutum* and *Ananas ananassoides* with changes in the amount of culture media for flash

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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.

Scientific Committee

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INTRODUCTION

The Atlantic forest has a high rate of endemism with several endangered species (Sugiyama 2010) and among these species is the bromeliad *Nidularium minutum* Mez, endemic on the Serra de Paranapicacaba, located in São Paulo. This bromeliad appears as vulnerable on the list published in the Red Book of Endangered Plant Species (Mamede et al. 2007). *Ananas ananassoides* (Baker) L.B. Smith. belonging to Bromeliaceae, is a terrestrial plant and develops directly into 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 on the Cerrado, and is popularly known as "abacaxizinho cerrado" (Proença and Sajo 2007). Because of this biome is threatened with extinction, it has been included among the 34 global biodiversity hotspots (areas of high biodiversity, endemism and risk of extinction) (Sugiyama 2010).

Among the forms of plant preservation, *in vitro* culture of bromeliads has been considered an effective strategy to propagate genetic material from rare and endangered species, with the goal of ensuring the survival of this material in nature. But to be successful with this technique, one must take into account what or which products to be developed, chosen on the basis of demand and prices, always looking the lowest cost / benefit (Stancato 2001). Thus, an important factor in costs reduction for *in vitro* seedling production is the use of appropriate volume of culture medium per bottle that provides an adequate development of micropropagated plants.

In this context, this work aimed to determine the lowest amount of culture medium for *in vitro* growth of *N. minutum* and *A. ananassoides* that would provide a proper plant growth.

MATERIAL AND METHODS

The work was performed in the Research Center for Ornamental Plants Laboratory, Institute of Botany, Environment Department of the São Paulo State. The seeds of both species were superficially disinfected in 70% alcohol for 5 minutes and in a 2% sodium hypochlorite solution plus two drops of Tween 20® for one hour under continuous shaking. After the disinfection, 100 seeds of each species were

deposited on Petri dish containing 20 mL of Murashige and Skoog (1962) culture medium (MS-1962) at 50% of the original composition of macronutrients (MS/2) plus 3% sucrose and 5 g/L agar. The seeds were kept in culture room with a photoperiod of 12h, 30 µmol.m^{-2}.s^{-1} and a temperature of 26±2 °C for two months.

N. minutum plants were transferred to 250 mL bottles containing different amounts of MS/2, in three treatments: T1=20 ml, T2= 40 mL and T3=80 mL. In each bottle were placed five plants and four bottles were used per treatment. The plants were kept for four months in a culture room with the same conditions described above. *A. ananassoides* plants were placed in 360 mL flasks containing the same medium described above, and distributed in two treatments: M1= 30 mL and M2= 60 mL, the remainder being equal to the *N. minutum*.

The following biometric parameters were evaluated: number of leaves and roots, length of shoot and root, fresh mass and dry mass of shoot and root and photosynthetic pigments (chlorophyll a, b and carotenoids). The content of chlorophyll and carotenoids were analyzed according to methodology described by Lichtenthaler (1987).

The averages were calculated and subjected to variance analysis and compared by Tukey test at 5% probability.

RESULTS AND DISCUSSION

N. minutum plants grown in flasks containing 80 mL of culture medium exhibited morphological changes, such as no formation of rosettes, which is present in the plants from other treatments, as well as the plant in natural environment. It was unable to determine the exactly number of roots, because they were aggregated, making it difficult to separate for counting so as not to cause damage. The number of leaves for *N. minutum* showed a higher amount in T3, but for *A. ananassoides* there were no differences in the number of leaves and roots between treatments (Table 1).

Table 1. Biometric analyzes of root and shoot of *Nidularium minutum* and *Ananas ananassoides in vitro* cultured with different quantities of culture medium.

Treatment	Leaf Number	Root Number	Shoot Length (cm)	Root Length (cm)
T1 - *N. minutum*	10,1 b	----	7,20 a	7,00 a
T2 - *N. minutum*	10,8 b	----	8,29 a	6,01 a
T3 - *N. minutum*	20,9 a	----	4,15 b	2,08 b
M1 - *A. ananassoides*	8,0 a	14,0 a	9,30 a	5,20 a
M2 - *A. ananassoides*	8,0 a	13,0 a	7,80 b	4,80 a

T1 = 20 mL; T2 = 40 mL; T3 = 80 mL; M1 = 30 mL; M2 = 60 mL;
Different letters vertically within the same species indicate significant differences by the Tukey test at 5%.

In relation of root and shoot length, the treatments with the highest values were T1 and T2 in *N. minutum* (Table 1), corroborating *A. ananassoides* in study that showed a higher length of shoots in M1 , but no differences were observed in roots length (Table 1).

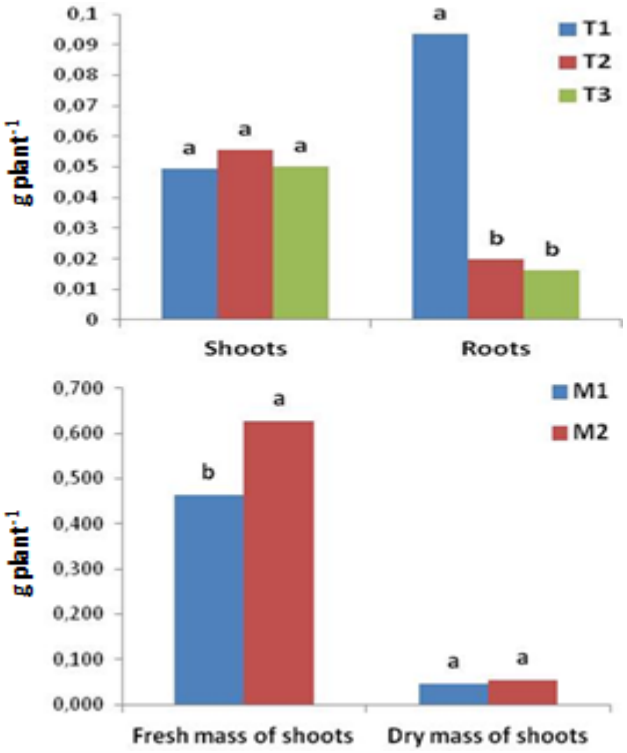

Figure 2. Shoots and roots dry mass of *Nidularium minutum* Mez. (a) and *Ananas ananassoides* (Baker) L. B. Smith. (b) *in vitro* cultured with different quantities of culture medium (T1=20 ml; T2=40 mL; T3=80 mL, M1= 30 mL and M2=60 mL). Different letters indicate significant differences by Tukey test at 5% within the same parameter.

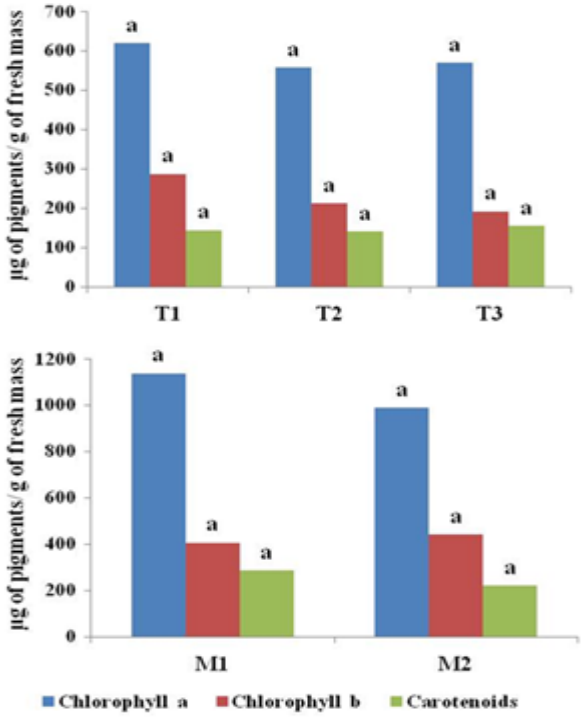

Figure 3. Quantity of photosynthetic pigments (chlorophyll a, b, carotenoids) in *Nidularium minutum* Mez (a) and *Ananas ananassoides* (Baker) L. B. Smith. (b) *in vitro* cultured with different quantities of culture medium (T1=20 ml; T2=40 mL; T3=80 mL, M1= 30 mL and M2=60 mL). Different letters indicate significant differences by Tukey test at 5% for the same pigment.

It was observed that *N. minutum* plants grown in T1 showed the highest amount of roots dry mass than the other two treatments (Figure 2a). For *A. ananassoides*, the shoot fresh mass showed a higher value in plants grown in M2, suggesting a higher accumulation of water in this treatment (Figure 2b).

Analyses of photosynthetic pigments showed no differences between treatments (Figure 3a and Figure 3b), suggesting that the plants are in proper nutritional status.

CONCLUSIONS

These results suggest that is possible the growth with 20-40 mL of culture medium per bottle for *N. minutum* and with 30-60 mL for *A. ananassoides.* This difference in the amount of culture medium from these bromeliads is due to the fact that *A. ananassoides* grow faster than *N. minutum*, so larger flasks were used and consequently more culture medium, but the amounts of culture medium indicated in this work are equivalents among species. With this reduction is possible to keep an *in vitro* collection for a longest time, reducing spending on the culture maintenance.

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Review process and quality of English writing is responsibility of Workshop Scientific Committee.