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In vitro propagation of *Ananas comosus* var. *ananassoides* (Baker) Coppens & F. Leal (Bromeliaceae)

Propagação *in vitro* de *Ananas comosus* var. *ananassoides* (Baker) Coppens & F. Leal (Bromeliaceae)

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Abstract

Ananas comosus var. *ananassoides* (Baker) Coppens & F. Leal is a terricole ornamental bromeliad native of the Cerrado. Since this biome is threatened of extinction, studies about the cultivation of seedlings of this species are important and the *in vitro* culture can be a useful tool for its propagation. This work aimed to multiply *A. comosus* var. *ananassoides in vitro,* by means of nodal segments, and with the culture medium containing different nitrogen (N) concentrations. In obtaining the nodal segments, the plants remained *in vitro* in the dark for three months. In the other experiment, the seedlings were cultured *in vitro* in MS medium containing different N concentrations for six months. The results showed that 92% of the plants presented stem etiolation in the dark, each plant generating three to four nodes, and these nodes gave rise to healthy plants. Moreover, *in vitro* multiplication with 15 mM and 30 mM N was found to be possible, yielding 1.5% and 3% multiplication, respectively, with each plant generating one or two buds. It is concluded that the production of plants by nodal segments is indicated for the *in vitro* multiplication of this species and it is suggested to culture it in 15 mM N.

Additional keywords: Bromeliad; *in vitro* culture; multiplication; nitrogen; stem etiolation.

Resumo

Ananas comosus var. *ananassoides* (Baker) Coppens & F. Leal é uma bromélia ornamental terrícola e nativa do cerrado. Como este bioma se encontra ameaçado de extinção, estudos sobre o cultivo de mudas desta espécie são importantes, e a cultura *in vitro* pode ser uma ferramenta útil para sua propagação. Este trabalho teve como objetivo multiplicar *in vitro A. comosus* var. *ananassoides* por meio de segmentos nodais, e com meio de cultura contendo diferentes concentrações de nitrogênio (N). Na obtenção dos segmentos nodais, as plantas permaneceram *in vitro,* no escuro, por três meses. No outro experimento, as plântulas foram cultivadas *in vitro,* em meio MS contendo diferentes concentrações de N, por seis meses. Os resultados mostraram que 92% das plantas apresentaram estiolamento caulinar no escuro, gerando de três a quatro nós cada planta, e os nós originaram plantas saudáveis. Além disso, observou-se ser possível a multiplicação *in vitro* com 15 mM e 30 mM de N, obtendo-se 1,5% e 3% de multiplicação, respectivamente, sendo que cada planta gerou de um a dois brotos. Conclui-se que a produção de plantas por segmentos nodais é indicada para a multiplicação *in vitro* desta espécie e sugere-se o seu cultivo em 15 mM de N.

Palavras-chave adicionais: Bromélia; cultivo *in vitro*; estiolamento caulinar; multiplicação; nitrogênio.

Introduction

Ananas comosus var. *ananassoides* (Baker) Coppens & F. Leal is a terricole bromeliad used in ornamentation, having its inflorescence exported to European countries (Paula & Silva, 2004; Carvalho et al., 2009; Souza et al., 2012). This species, popularly known as "Abacaxizinho do Cerrado", is native from Cerrado (Proença & Sajo, 2007). As this biome is threatened with extinction, being among the 34 global biodiversity hotspots (Myers et al., 2000; Orme et al., 2005), studies and conservation measures of its species are necessary.

One way of conserving a species can be through propagation studies and the *in vitro* cultivation is one of the methods used for multiplication of species threatened by extinction and also for the commercial production of plants, proving to be an important tool in biotechnological research (Engelmann, 1991; Fay, 1994; Sarasan et al., 2006; Vieira et al., 2009; Carvalho et al., 2012) and in physiological studies related to plant development (Suzuki et al., 2010).

The *in vitro* vegetative propagation, also called micropropagation, has been an instrument used in

several studies on the cultivation of bromeliads (Souza et al., 2009; Carvalho et al., 2012; Dias et al., 2013; Andrade & Tamaki, 2016), whether these are of commercial interest, endemic, rare and/or threatened by extinction (Mercier & Kerbauy, 1995; 1996; Arrabal et al., 2002; Rodrigues et al., 2004; Rech-Filho et al., 2005; Kurita, 2011; Kurita et al., 2014; Andrade & Tamaki, 2016).

Among the micropropagation methods, it can be mentioned the multiplication by stem etiolation (Kiss et al., 1995; Tamaki et al, 2007; Santos, 2009; Dias et al., 2011) and the use of different concentrations of nitrogen (N) (Araujo et al., 2009; Kurita, 2011; Dias et al., 2013; Kurita & Tamaki, 2014; Andrade & Tamaki, 2016), which could be applied to the bromeliad *A. comosus* var. *ananassoides*, since there is little information available about the production of seedlings of this species and the best conditions for culturing them.

Tamaki et al. (2007) reported that the use of plant growth regulators in the production of pineapple seedlings, through nodal segments from plants cultured *in vitro*, subject to etiolation conditions such as lack of light, is unnecessary, as observed by Barboza & Caldas (2001), Moreira et al. (2003) and Santos et al. (2010), suggesting that it is possible to use this method with other species.

The choice of the most suitable culture medium is fundamental for establishing the cultivation, since mineral nutrition implies the growth and development of plants (Bunn et al., 2011). Among the formulations of the culture medium described in the literature, the most used for *in vitro* culture is the one developed by Murashige & Skoog (1962) (MS) (Werner et al., 2010). Some studies have reported that modifications in the MS medium, such as changes in N concentration, may favor the multiplication of some plants, as in *Alcantarea imperialis* (Carrière) Harms (Kurita & Tamaki, 2014).

Maldaner et al. (2006), working with *Pfaffia glomerata* (Spreng.) Pedersen *in vitro*, observed that the reduction of the N concentration to 50% of the MS medium, associated with an increase in the sucrose dose to 45 g L^{-1}, favored an increase in height, in the number of nodal segments and buds, as well as in the biomass production of *P. glomerata*. Thus, the present work aimed to evaluate the multiplication and the *in vitro* development of the bromeliad *A. comosus* var. *ananassoides* with the use of different concentrations of nitrogen and by stem etiolation.

Material and methods

Plant material

The work was carried out in the Laboratory of the Ornamental Plants Research Center, of the Institute of Botany (IBt) belonging to the Environment Secretariat of the State of São Paulo. In the experiments, seeds of *Ananas comosus* var. *ananassoides* (Baker) Coppens & F. Leal collected in the Biological Reserve of Mogi-Guaçu, belonging to the IBt, were used. The fruits were manually opened and their seeds were packed in brown paper bags and stored under refrigeration conditions at 10 °C.

For the *in vitro* germination, about 650 seeds were subjected to surface disinfestation in a 250 mL flask containing 70% alcohol for 5 minutes, being, after rinsing, placed with 2% sodium hypochlorite solution, plus two drops of Tween 20, for one hour, under stirring. Subsequently, in a sterile air-flow chamber, the hypochlorite solution was withdrawn and the process was terminated with four consecutive washes of the seeds with sterile distilled water. After disinfestation, the seeds were deposited in Petri dishes containing only agar and 3% sucrose. The pH of the medium was adjusted to 5.8 before the addition of 5 g L^{-1} agar. Medium sterilization was performed at 120 °C for 15 minutes. The seeds were deposited in 9-cm-diameter Petri dishes containing 20 mL of culture medium each, with 25 seeds per dish. These were kept in culture room with photoperiod of 12 hours, photosynthetically active radiation (PAR) of 30 μmol m^{-2} s^{-1} and temperature of 26±2 °C until the seedlings were obtained, which took 30 days.

Multiplication by stem etiolation

In the experiment of multiplication by stem etiolation, 100 seedlings were used, which were transferred to 350 mL flasks (10 seedlings per flask), containing 30 mL of MS medium with 50% of the macronutrient concentrations of the original formulation (MS/2), supplemented with 30 g L^{-1} of sucrose and 5 g L^{-1} of agar. They remained for two months under the same light conditions in culture room for germination. The generated plants had their leaves cut, leaving about 1 cm of leaves from the stem base, and then were transferred to 360 mL flasks containing 30 mL of MS/2 medium (five plants per flask). The flasks remained in the dark for three months. After this period, the plants were analyzed about the number of specimens etiolated and the number of nodes per plant. The generated nodes were transferred to 360 mL flasks containing 30 mL of MS/2 medium (three nodes per flask) and kept in culture room with 12 h photoperiod, PAR of 30 μmol m^{-2} s^{-1} and temperature of 26 ± 2 ºC, aiming to follow the emergence of new plants for a period of three months. After this period, the generated plants were quantified.

Multiplication with different concentrations of nitrogen (N)

In the experiment of multiplication with different nitrogen (N) concentrations, the seedlings obtained were transferred to 350 mL flasks, containing 30 mL of MS medium modified with different N compositions (0 mM, 7.5 mM, 15 mM, 30 mM, original MS - 60 mM, 120 mM and 175 mM) (Table 1), supplemented with 30 g L^{-1} sucrose and 5 g L^{-1} agar. Each treatment consisted of 15 flasks and five seedlings were deposited in each flask, which were kept under aseptic conditions in

culture room with 12 h photoperiod, PAR of 30 µmol m^{-2} s^{-1} and mean temperature of 26±2 °C for six months. Three months after the transfer initiation, they were placed in new culture medium (subcultured) with the same concentrations, to guarantee the same nutritional content due to the consumption of nutrients of the culture medium by the plants. After six months, the following parameters were analyzed: number of multiplied plants; number of buds per plant; number of leaves and roots; length of shoots and roots; fresh and dry mass of shoots and roots; and quantifications of photosynthetic pigments.

Table 1 - Salts concentrations in the treatments with 0, 7.5, 15, 30, 60, 120 and 175 mM of nitrogen (N), changed from original Murashige & Skoog medium (1962) (MS).

Salts ($g L^{-1}$)	Treatments (mM of N)						
	0	7.5	15	30	60*	120	175
KNO_3	-	0.38	0.38	0.38	0.38	0.38	0.38
NH_4NO_3	-	0.15	0.45	1.05	2.25	4.65	6.85
$CaCl_2$ $2H_2O$	0.44	0.44	0.44	0.44	0.44	0.44	0.44
$MgSO_4$ $7H_2O$	0.37	0.37	0.37	0.37	0.37	0.37	0.37
KH_2PO_4	0.17	0.17	0.17	0.17	0.17	0.17	0.17
KCl	0.70	-	-	-	-	-	-
K_2SO_4	0.82	-	-	-	-	-	-

* Corresponds to the concentration present in the original MS medium.

Photosynthetic pigments content

The photosynthetic pigments extraction process was based on Lichtenthaler (1987), where cold acetone and triplicate plant material previously stored at -20 ºC were used. 0.5 g of fresh leaf mass were ground in 3 mL of cold acetone. The sample was filtered through funnels previously lined with filter paper moistened with cold acetone. After the entire liquid was filtered, the filter paper was washed with acetone until the deposited material lost its green color, being collected in 25 mL volumetric flasks. Thereafter, pure acetone was added until completion of 25 mL. The samples were stored on ice in test tubes and capped with aluminum foil and transparent PVC film until reading. The test tubes were removed from ice and subjected to spectrophotometry. The absorbance (*A*) of chlorophyll *a* was read at 661.6 nm, chlorophyll *b* at 644.8 nm and carotenoids at 470 nm. The concentrations of chlorophyll *a* (*Ca*), chlorophyll *b* (*Cb*) and carotenoids (*C*) were obtained by equations: $Ca = 11.24\ A_{661.6} - 2.04\ A_{644.8}$; $Cb = 20.13\ A_{644.8} - 4.19\ A_{661.6}$; $C = (1000\ A_{470} - 1.90\ Ca - 63.14\ Cb)\ 214^{-1}$. The chlorophyll and carotenoid contents of the tissues were expressed in µg of the pigment per gram of fresh leaf mass (µg g^{-1}).

Statistical Analysis

The experimental design was completely randomized and each experimental unit consisted of five flasks containing five plants and three replicates for each treatment. For the statistical analyses, the means were calculated and submitted to variance analysis, being compared by Tukey test at 5% probability.

Results and discussion

Multiplication by stem etiolation

Of the 100 plants used in the stem etiolation experiment, 92% showed stem elongation in the absence of light, generating three to four nodes per plant in three months at *in vitro* culture (Table 2).

Of the 92 etiolated plants, 376 nodal segments were obtained, of which 24.2% (91 nodes) originated only a single plant, 31.7% (119 nodes) gave rise of two to six plants per nodal segment and 44.1% (166 nodes) did not develop (Table 3).

Of the nodal segments that provided more than one plant, it was obtained a total of 405 plants developed in three months of *in vitro* culture, in culture room, with the absence of plant growth regulators. Similarly, Santos et al. (2010), in a work on the micropropagation of the bromeliad *Acanthostachys strobilacea* (Schultz F.) Klotzsch cultured in MS with 1/5 of the concentration of the macronutrients, verified that the middle and basal segments give rise to more than one plant per segment, without the use of plant growth regulators, as in the present work.

Tabela 2 - Distribution in nodal segments numbers per etiolated plant of *Ananas comosus* var. *ananassoides* (Baker) Coppens & F.Leal, after three months kept in the absence of light.

Nodal segments numbers	00	01	02	03	04	05	06	07	10	11	Total of plants
Number of plants that developed nodal segments	08	01	04	33	27	19	03	02	01	02	100

Table 3 - Nodal segments number of *Ananas comosus* var. *ananassoides* (Baker) & Coppens F. Leal which developed one plant, more than one plant per segment, and not developed, with the respective percentages, after six months of *in vitro* culture.

	Developed a plant	Developed more than one plant (2 – 6)	Have not developed a plant	Total of segments obtained
Nodal segments numbers	91	119	166	376
Relative frequency (%)	24.7	31.7	44.1	100

Kiss et al. (1995) propagated nodal segments of *Ananas comosus* (L.) Merril, cultivar Smooth Cayenne, *in vitro* with two types of cytokinins (benzyladenine-BA and kinetin-K) with different concentrations (0≤BA≤40 µm and 0≤K≤50 µm) in N6 culture medium (Chu, 1978), verifying that it is possible to use this method for the micropropagation of this species. However, Tamaki et al. (2007) reported that the use of plant growth regulators in the production of seedlings of this same species by stem etiolation is unnecessary, as observed in the present study for *A. comosus* var. *ananassoides*.

In many studies, the use of plant growth regulators is associated with the induction of somaclonal variations in plant tissues (Pierik, 1987; Zaffari et al., 2002; Joyce et al., 2003; Zhao et al., 2005). These changes are not relevant when it is sought to obtain plants with selected phenotypes (Tamaki et al., 2007) or when the aim is the conservation of a species.

In relation to the total yield of plants obtained from the stem etiolation method used in the present study, around four new plants were obtained from a matrix plant, in six months of *in vitro* culture in MS/2 medium. Santos et al. (2010) obtained superior results in their work with *A. strobilacea*, about 80 plants from a matrix plant, in a year of *in vitro* and *ex vitro* culture. With these results, there is a need for further studies to improve the technique with *A. comosus* var. *ananassoides*, aiming to increase the amount of individuals generated by the matrix plant, without the use of growth regulators.

Thus, the possible production of seedlings verified in *A. comosus* var. *ananassoides* may serve for future physiological research, in addition to contributing to the propagation and conservation of this species.

Multiplication with different concentrations of nitrogen

In the experiment of multiplication with different concentrations of N, all plants survived at the concentration changes of 0 mM, 7.5 mM, 15 mM, 30 mM, 60 mM, 120 mM and 175 mM N in the nutrient media for six months, but the multiplication was only observed in two concentrations, with the appearance of a bud in a plant at the concentration of 15 mM N and two buds in two plants at the concentration of 30 mM N; in the other concentrations, there were no buds. However, Maldaner et al. (2006), studying the *in vitro* growth of *Pfaffia glomerata* (Spreng.) Pedersen at different N concentrations, reported that the reduction of N to 50% of that MS medium standard, associated with an increase in the sucrose dose to 45 g L^{-1}, favored the emergence of buds, as well as the production of biomass. The same was observed by Kurita & Tamaki (2014), working with the bromeliad *Alcantarea imperialis* (Carrière) Harms with different N concentrations (0 ≤ N ≥ 175 mM), where 80% of the plants grown at the concentration of 30 mM N generated from two to five buds per plant. Nonetheless, the change in the N concentration in the MS culture medium, alone, was not sufficient to generate buds in plants of *Ananas comosus* var. *ananassoides*.

Regarding the shoot growth data of the plants grown at different concentrations of N for six months, in general, the lowest avareges were observed only in the treatment with 0 mM N (Table 4). There was a difference in the amount of leaves between the treatments used, about eight leaves at the 0 mM N concentration, nine leaves at the 15 mM N concentration and approximately 10 leaves at the concentrations of 7.5 mM, 30 mM, 60 MM, 120 mM and 175 mM N (Table 4). This variation in the number of leaves was also observed by Kanashiro (2005), in his work with the bromeliad *Aechmea blanchetiana* (Baker) L.B. Smith., when analyzing its *in vitro* growth at different concentrations of nitrogen (7.5≤N≥120 mM). The same author also verified that the increase of the nitrogen concentration induced the increase of the amount of leaves, as it seems to have occurred with *A. comosus* var. *ananassoides* in the present study.

Regarding shoot length (Table 4), the smaller leaves were obtained in the plants at the concentrations of 0 mM (5.40±0.70 cm) and 175 mM N (6.67±1.01 cm); the other treatments presented plants with an average of 7.81 cm shoot length. Grossi (2000), when culturing *in vitro* the bromeliad *Aechmea nudicaulis* (L.) Griseb. at the concentrations of 1.78, 7.5 and 30 mM N of the MS, found that this species presented a higher increase in shoot length at the concentration of 7.5 mM N after 5 months of cultivation. Notwithstanding, Russowski & Nicoloso (2003) analyzed the effect of different concentrations of nitrogen (0 ≤ N ≤ 150%) of the MS medium on nodal segments of *Pfaffia glomerata* (Spreng.) Pedersen (Brazilian Ginseng) and observed that after 15 days, the shoot height was higher in 100% N (60 mM N), with a decrease in length at higher concentrations. In *A. comosus* var. *ananassoides*, there also seems to be a tendency to reduce leaf length at concentrations above 60 mM N (Table 4).

Table 4 - Average values of fresh and dry weight of the shoot, length of the largest leaf and leaves number in *Ananas comosus* var. *ananassoides* (Baker) F. Leal & Coppens plant, after six months of *in vitro* culture in the nitrogen (N) concentrations of 7.5 mM, 15 mM, 30 mM, 60 mM, 120 mM and 175 mM.

Treatments (mM of N)	*Fresh mass (g plant $^{-1}$)	*Dry mass (g plant^{-1})	*Length of the largest leaf (cm)	*Number of leaves
0	0.2903 b	0.0344 b	5.40 c	08.4 c
7.5	0.6137 a	0.0590 a	7.56 ab	10.6 ab
15	0.7005 a	0.0707 a	8.30 a	09.7 b
30	0.5770 a	0.0605 a	8.33 a	10.4 ab
60	0.6533 a	0.0681 a	8.20 a	09.9 ab
120	0.6065 a	0.0629 a	7.74 ab	09.9 ab
175	0.5414 a	0.0483 a	6.67 b	10.9 a

* Means followed by the same letter in the column do not differ by Tukey test at 5% probability.

In relation to the results of fresh mass of shoots, there were no significant differences in the plants grown with 7.5 mM N up to 175 mM N, and the highest average was observed at 15 mM N (0.70±0.11 g) (Table 4). The dry mass of shoots was higher in the treatments from 15 mM N to 175 mM N, and at 15 and 60 mM N, the plants obtained the highest averages (0.07±0.01 g and 0.068±0.017 g, respectively) (Table 4). The plants cultured in the absence of N did not present mortality, but obtained the lowest averages of fresh mass and dry mass (0.29±0.07 g and 0.031± ±0.006 g, respectively). When studying the growth of the bromeliad *A. blanchetiana*, cultured *in vitro* for 120 days at different concentrations of nitrogen (7.5≤N≥120 mM), Kanashiro et al. (2007) observed that the increase of nitrogen concentration reduced the fresh and dry mass of shoots, a result opposite to that observed in *A. comosus* var. *ananassoides*. Dijik & Eck (1995) studied the *in vitro* growth of three species of orchids (of the same genus and habit) for five months and observed different behaviors among the specimens when they increased the concentration of nitrogen in the nutrient medium (from 0 to 12 mM N), with increased biomass production in *Dactylorhiza praetermissa* (Druce) Soó, while *D. majalis* (Rchb. F.) P.F. Hunt & Summerh remained stable and *D. incarnata* (L.) Soó showed biomass reduction. Thus, it can be concluded that different species, although belonging to the same genus and habit, may present different responses in relation to the increase in nitrogen fertilization.

As for the results of photosynthetic pigments, there was no significant difference between the 7.5 mM, 15 mM, 30 mM and 60 mM N treatments, however, there was a decline in the treatments with 0 mM, 120 mM and 175 mM N (Table 5). Tamaki et al. (2007) cultured *A. comosus* plants, from nodal segments of the etiolated stem axis in the absence of light, at different macronutrient dilutions of the MS medium (60 mM N – MS; 30 mM N - MS 2^{-1}; 12 mM N - MS 5^{-1}; 6 mM N - MS 10^{-1}; 1 mM N - MS 60^{-1}; and 0.6 mM N - MS 100^{-1}) for 90 days, and observed that there were no significant differences between treatments: MS, MS 2^{-1} and MS 5^{-1}, showing that the amount of N in MS 5^{-1} was sufficient for the normal development of the plant, a result similar to that observed in the bromeliad under study, *A. comosus* var. *ananassoides*.

Table 5 - Average values of photosynthetic pigments (chlorophylls a, b and carotenoids) in *Ananas comosus* var. *ananasssoides* (Baker) F. Leal & Coppens leaves, after six months of *in vitro* culture in the nitrogen (N) concentrations of 7.5 mM, 15 mM, 30 mM, 60 mM, 120 mM and 175 mM.

Treatments (mM of N)	*Chlorophyll a (μg g^{-1} fresh mass)	*Chlorophyll b (μg g^{-1} dry mass)	*Carotenoids (μg g^{-1} fresh mass)
0	366.4 d	158.7 c	99.0 b
7.5	894.2 ab	376.1 a	216.8 a
15	911.2 ab	386.9 a	209.5 a
30	858.3 ab	372.0 a	201.6 a
60	1002.9 a	446.7 a	230.8 a
120	802.9 b	354.3 ab	194.6 a
175	583.0 c	265.4 b	142.9 b

* Means followed by the same letter in the column do not differ by Tukey test at 5% probability.

Regarding the variable root length, the lowest results were obtained at the concentrations of 120 mM N (2.75±0.59 cm) and 175 mM N (1.82 ± 0.70 cm) and the highest result was found at the concentration of 7.5 mM N (8.94 ± 2.13 cm); the remaining treatments presented plants with a mean of 4.32 cm root length (Table 6). Garnetti et al. (2009), in their review work about N and its effects on roots, reported a reduction in root growth at high concentrations of N, as observed in this work. Possibly, such reduction of growth in the treatments of 120 mM and 175 mM N occurs by a certain degree of toxicity when subjected to these high concentrations. According to Marschners and Marschners (2012), minerals excess in the soil makes it saline, causing little availability of water, inducing the plant to saline and water stress, and with that, there is growth reduction.

Table 6 - Average values of fresh and dry weigth of the root and length in the largest root in *Ananas comosus* var. *ananassoides* (Baker) F. Leal & Coppens plant, after six months of *in vitro* culture in the nitrogen (N) concentrations of 0 mM, 7.5 mM, 15 mM, 30 mM, 60 mM, 120 mM and 175 mM.

Treatments (mM of N)	*Fresh mass (g plant^{-1})	*Dry mass (g plant^{-1})	*Length of the largest root (cm)
0	0.0190 c	0.0078 b	4.80 b
7.5	0.0417 ab	0.0119 a	8.94 a
15	0.0291 b	0.0066 b	4.30 b
30	0.0317 b	0.0057 b	4.00 b
60	0.0502 a	0.0080 b	4.20 b
120	0.0293 b	0.0047 bc	2.75 c
175	0.0149 c	0.0022 c	1.82 c

* Means followed by the same letter in the column do not differ by Tukey test at 5% probability.

The root growth was shown to be higher when there was low N availability, as seen by Kurita & Tamaki (2014) when studying the *in vitro* culture of the bromeliad *Alcantarea imperialis* at different concentrations of N. In their study, it was verified that this species of bromeliad presented greater root growth in the treatment with 7.5 mM N (5.80 ± 0.87 cm), a result similar to that of *A. comosus* var. *ananassoides*.

Working with the bromeliad *A. comosus* cultured *in vitro* in the absence of N, Tamaki & Mercier (2007) studied the signaling between shoots and roots, observing an accumulation of indolylacetic acid in the roots, indicating that this hormone may be signaling the lack of N from the leaves to the roots. According to Davies (2010), one of the hormones responsible for cell elongation is the auxin, the roots being more sensitive to this hormone, inducing root elongation at low concentrations of N, that is, N deficiency leads the auxin to provide the signal that induces root growth, which may have occurred with *A. comosus* var. *ananassoides* at low concentrations of N.

As for the results of fresh and dry mass of roots, plants grown at the concentrations of 7.5 mM and 60 mM N had the highest mean values (0.04±0.01 g) fresh mass; 0.011±0.004 g dry mass and 0.05±0.01 g fresh mass; 0.008±0.0014 g dry mass, respectively) when compared to the other concentrations, and the lowest biomass was presented in plants grown at 175 mM N (0.015 ± 0.002 g fresh mass and 0.0022 ± 0.0009 g dry mass (Table 6). Russowski and Nicoloso (2003), working with *in vitro* culture of nodal segments of *P. glomerata* at different concentrations of nitrogen (0≤N≤150%) in the MS medium (100% with 60 mM N), observed that the concentration of 80% showed plants with higher dry mass of shoots and roots, occurring a decrease as a function of the increase of N. Possibly, the smaller amount of nitrogen in the treatment of 7.5 mM N favored the increase of the dry mass in the present work, since, according to Van Der Werf (1996), the biomass allocation to the roots and shoots is deeply influenced by the availability of N, so the reduction in N supply initially induces root growth and reduces shoot growth.

According to Benzing (2000), many native bromeliads are adapted to nutrient-deficient environments, what may be assumed to be the case of *A. comosus* var. *ananassoides*, which is a species native from Cerrado, a biome that has a soil poor in nutrients, as the results indicate that at the concentrations of 15 to 60 mM N, the *in vitro* growth was satisfactory. Therefore, the use of 15 mM N for the cultivation of this species could reduce the use of N in the culture medium and consequently the costs for its production.

Thus, it is concluded that it is possible to multiply *A. comosus* var. *ananassoides* by *in vitro* culture in MS medium modified with 15 mM and 30 mM nitrogen, but mainly by stem etiolation in MS/2 medium. This latter method is suggested for the *in vitro* multiplication of this bromeliad, in addition to its *in vitro* culture at the 15mM N concentration of the MS medium formulation.

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