



## *In vitro* growth of the bromeliad *Alcantarea imperialis* (Carrière) Harms with different concentrations of nitrogen

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**ABSTRACT.** In various ecosystems, many plants have been reduced in number or even eliminated from natural habitats, including the endangered ornamental bromeliad *Alcantarea imperialis* (Carrière) Harms. The *in vitro* culture is a tool that has been used for conservation of endangered species and widely used for the production of ornamental plants. This study aimed at investigating the influence of different nitrogen concentrations on the growth of plantlets of the bromeliad *A. imperialis* grown *in vitro*. Seedlings of *A. imperialis* were cultured on Murashige and Skoog medium, supplemented with different concentrations of nitrogen (0.00, 3.75, 7.50, 15.00, 30.00, 60.00, 90.00, 120.00, and 175.00 mM) at 30 mmol m<sup>-2</sup> s<sup>-1</sup>, at 12 hour photoperiod and 26±2°C for six months. The results showed that plants grown with 15.00 and 60.00 mM N showed the best growth.

**Keywords:** bromeliaceae, conservation, endangered, macronutrients, nutrition, ornamental.

## Crescimento *in vitro* da bromélia *Alcantarea imperialis* (Carrière) Harms com diferentes concentrações de nitrogênio

**RESUMO.** Em vários ecossistemas, o número de plantas está se reduzindo ou até mesmo erradicadas de seus habitats naturais, e uma dessas espécies que está em perigo é a bromélia ornamental *Alcantarea imperialis* (Carrière) Harms. O cultivo *in vitro* é uma ferramenta que tem sido utilizada na conservação de espécies em extinção e tem sido amplamente utilizado para a produção de plantas ornamentais. Este trabalho teve como objetivo investigar a influência de diferentes concentrações de nitrogênio sobre o crescimento da bromélia *A. imperialis* cultivadas *in vitro*. Plântulas de *A. imperialis* foram cultivadas em meio de Murashige e Skoog, suplementado com diferentes concentrações de nitrogênio (0,00; 3,75; 7,50; 15,00; 30,00; 60,00; 90,00; 120,00 e 175,00 mM) em 30 mmol m<sup>-2</sup> s<sup>-1</sup>, em 12h fotoperíodo, a 26±2°C durante seis meses. Os resultados mostraram que as plantas crescidas com 15,00 e 60,00 mM de N proporcionam o melhor crescimento.

**Palavras-chave:** Bromeliaceae, conservação, ameaçada, macronutrientes, nutrição, ornamental.

### Introduction

The Bromeliaceae form a group of 58 genera and around 3,248 species (LUTHER, 2010). In various ecosystems, many bromeliads have been reduced in number or even eliminated due to habitat destruction as a result of anthropic activities, such as increasing deforestation and the occurrence of selective extraction (ROCHA et al., 2004). One of these species, which is threatened with extinction, is the ornamental bromeliad *Alcantarea imperialis* (Carrière) Harms, that is rupicole and endemic to “Parque Nacional da Serra dos Órgãos” in the State of Rio de Janeiro, Brazil (BERT, 2007). To reach its majestic appearance, this species needs some decades, sometimes up to 40 years to become an adult plant (CARVALHO, 1997), and the slowness

of its development follows from the small amount of nutrients available in the environment in which it lives (MAUN, 1994).

The *in vitro* culture is a tool that has been used in recent years for the conservation of endangered species (SARASAN et al., 2006), since it offers several advantages such as a large number of plants in a short period of time as opposed to the methods of natural propagation, and plants free of virus and bacteria (KAVAND et al., 2011). Moreover, it has been widely used for the production of ornamental plants (KANASHIRO et al., 2009). According to these authors, if the aim is to preserve the genetic heritage of the species, micropropagation should be started from the seed, which should be collected from specimens from different localities, and an important aspect of *in vitro* culture is the supply of mineral medium.

The additional macro- and micronutrients in the culture medium is essential for *in vitro* systems (KANASHIRO et al., 2009). A balance between nitrogen (N), phosphorus (P) and calcium (Ca) is indispensable for morphogenesis and plant growth (RAMAGE; WILLIAMS, 2002).

One of the most important is the nutrient N, the main component of amino acids, proteins, nucleic acids, chlorophyll and coenzymes (SILVA JÚNIOR et al., 2013). According to White and Brown (2010), in their review on mineral nutrition, the N is mentioned as the primary macronutrient essential for plant development. While on deficiency, it is translocated from older leaves, which show chlorosis, to the young leaves that have a lower development (TAIZ; ZEIGER, 2010). N deficiency limits the production, but in excess, it may lead to yield losses, waste of inputs, and causes environmental pollution. Diagnostic indicators are needed to assess N status so that the optimized yields can be as efficient as possible and chlorophyll meter readings showed a good relationship with N fertilizer application (WIEDENFELD et al., 2009).

According to Caldas et al. (1998) full strength formulation of the Murashige and Skoog (1962) medium (MS) is predominantly used in plant tissue cultures. Nevertheless, for some species, a dilution of the macronutrient medium may be more suitable. Certain bromeliads, as the species *Ananas comosus*, has satisfactory growth at dilution MS/5 (12.00 mM N) (TAMAKI et al., 2007). Similarly, the species *Ananas ananassoides* and *Nidularium minutum* exhibit increased photosynthetic pigments in different concentrations of N, 7.50 to 60.00 mM N and 60.00 to 120.00 mM N, respectively (KURITA et al., 2012), ie. quite varied amounts present in the formulation of full strength MS.

This investigation aimed at evaluating the effects of different nitrogen concentrations on the *in vitro* growth of the ornamental bromeliad *A. imperialis* plantlets.

## Material and methods

### Establishing *in vitro* culture

The basal and apical appendages of the seeds were removed and the seeds were immersed in 70% ethanol for 5 minutes, followed by immersion in a 0.1% solution of the fungicide benomyl (Benlate®) for 15 min. Subsequently, the material was immersed in a 2.5% commercial solution of sodium hypochlorite (v/v) containing 2 drops of Tween 20® for 1 hour with continuous shaking. After disinfection, the seeds were germinated on Petri dishes (50 seeds per plate) containing Murashige

and Skoog (1962) medium (MS) with macronutrients reduced to 75%, supplemented with 3% sucrose, 0.1 mg L<sup>-1</sup> of thiamine-HCl, 100 mg L<sup>-1</sup> of *myo*-inositol and 5 g L<sup>-1</sup> of agar with pH adjusted to 5.8. Cultures were maintained in a growth room at 26 ± 2°C and 30 µmol m<sup>-2</sup> s<sup>-1</sup> light photons provided by cold white lamps during 12 hours of light regime for one month.

### Experimental design of *in vitro* culture at a different concentration of nitrogen

The plantlets were transferred to 250 mL vessels containing 40 mL of MS medium modified (0.00, 3.75, 7.50, 15.00, 30.00, 60.00- Control concentration, 90.00, 120.00, and 175.00 mM N) (Table 1), prior to the adjustment of pH to 5.8 and prior to autoclaving.

**Table 1.** Composition of ionic balance in modified Murashige and Skoog (1962) medium with different nitrogen concentrations (0.00, 3.75, 7.50, 15.00, 30.00, 60.00, 90.00, 120.00, and 175.00 mM N).

| Salt<br>(mM)                         | Nitrogen (mM) |      |      |       |       |       |       |        |        |
|--------------------------------------|---------------|------|------|-------|-------|-------|-------|--------|--------|
|                                      | 0.00          | 3.75 | 7.50 | 15.00 | 30.00 | 60.00 | 90.00 | 120.00 | 175.00 |
| KNO <sub>3</sub>                     | 0.00          | 3.75 | 3.75 | 3.75  | 3.75  | 3.75  | 3.75  | 3.75   | 3.75   |
| NH <sub>4</sub> NO <sub>3</sub>      | 0.00          | 0.00 | 1.85 | 5.62  | 13.12 | 28.12 | 43.12 | 58.12  | 85.62  |
| KH <sub>2</sub> PO <sub>4</sub>      | 1.25          | 1.25 | 1.25 | 1.25  | 1.25  | 1.25  | 1.25  | 1.25   | 1.25   |
| KCl                                  | 9.40          | 0.00 | 0.00 | 0.00  | 0.00  | 0.00  | 0.00  | 0.00   | 0.00   |
| K <sub>2</sub> SO <sub>4</sub>       | 9.40          | 0.00 | 0.00 | 0.00  | 0.00  | 0.00  | 0.00  | 0.00   | 0.00   |
| CaCl <sub>2</sub> ·2H <sub>2</sub> O | 3.00          | 3.00 | 3.00 | 3.00  | 3.00  | 3.00  | 3.00  | 3.00   | 3.00   |

Subculture to news culture media were carried out after three months. After six months, 20 plants, from each concentration, were assigned to biometric analyses (length of the longest root, numbers of leaves and length of the shoots), besides amounts of fresh and dry weight (FW and DW) of both roots and shoots, as well as the quantity of photosynthetic pigments in both group of plants. The experiment was carried out in a completely randomized design, being repeated three times to confirm the results.

### Photosynthetic pigment content

Three replicates of 0.50 g of fresh weight of leaves were harvested for determination of photosynthetic pigments. Fresh leaf tissue (0.50 g) was homogenized with 5 mL of cold pure acetone. The homogenate was filtered (Whatman paper, number 1) and its solid residue was washed up to a final volume of 25 mL. Chlorophyll concentration of the filtrate was measured spectrophotometrically and calculated according to Lichtenthaler (1987).

### Leaf mineral analysis

All dried leaves of the 100 plantlets, from each concentration, were analyzed and the leaf's macro and micronutrients were determined by using the method for mineral analysis described by Malavolta

et al. (1992). Briefly, for N determination, samples were subjected to a sulfuric digestion and analyzed by the micro-Kjeldahl method by using an N distillator. For potassium (K), calcium (Ca), magnesium (Mg), sulfur (S) and phosphorus (P), samples were subjected to nitric-perchloric digestion. After digestion, Ca and Mg were determined by atomic emission spectrometry, and K was determined by flame photometry. A digital spectrophotometer was used for the determination of S by barium turbidimetry and P by colorimetry.

### Statistical analysis

The design of all the treatments used in all the experiments was completely randomized. Data were submitted to variance analysis, with variation among means compared by using the Tukey test at  $p < 0.05$ .

### Results and discussion

Nitrogen concentrations from 0.00 mM to 175.00 mM of N were sufficient to set a range of concentrations that favored the the growth of *A. imperialis* *in vitro* for six months. Only plants grown at 175.00 mM concentration of N showed a high mortality rate (70%) and 80% of plants grown in 30.00 mM N have multiplied, impeding the analysis of the parameters, because these plants have lower growth when compared to the others that had not multiplied in this concentration. Furthermore, in natural environment, this species rarely emits shoots, as most plants in this family (BÁRBARA et al., 2009), which implies a possible influence of some abiotic stressor on plants grown at 30.00 mM N. In the analysis of shoots, differences were verified in the number of leaves of plants grown in different treatments, with each plant grown at a concentration of 0.00 mM N exhibiting around five leaves, which increased gradually with increasing nitrogen source, until each plant had around 25 leaves in 90.00 mM N (Table 2) after six months of cultivation. This result was observed by Santos et al. (2010) for other bromeliad, which observed more leaves in *Acanthostachys strobilacea* cultivated with full strength MS (60.00 mM N).

**Table 2.** Numbers of leaves (NL), shoot length (SL) (cm) and amounts of shoots fresh weight (SFW) (g) and dry weight (SDW) (g) of plants *Alcantarea imperialis* (Carrière) Harms after six months of cultivation in different concentrations of nitrogen.

|         | Treatments (mM N) |         |         |         |         |         |         |        |
|---------|-------------------|---------|---------|---------|---------|---------|---------|--------|
|         | 0.00              | 3.75    | 7.50    | 15.00   | 30.00   | 60.00   | 90.00   | 120.00 |
| NL      | 5d                | 10c     | 12c     | 13b     | 13b     | 14b     | 25a     | 15b    |
| LS (cm) | 2.67f             | 6.70c   | 8.05a   | 6.95bc  | 7.74ab  | 8.59a   | 4.35c   | 5.71d  |
| SFW (g) | 0.059c            | 0.167b  | 0.440ab | 0.453ab | 0.370ab | 0.713a  | 0.315ab | 0.254b |
| SDW (g) | 0.007c            | 0.016bc | 0.100a  | 0.067a  | 0.028b  | 0.050ab | 0.031b  | 0.024b |

Letters compare the values in the row and different letters indicate significant differences by Tukey test at 5%.

With respect to shoot length, smaller plants were obtained at a concentration of 0.00 mM N ( $2.67 \pm 0.29$  cm); plants reached a height of 8.59 cm at 60.00 mM N, and then showed a reduction in concentrations of 90.00 and 120.00 mM N ( $4.35 \pm 0.58$  cm and  $5.71 \pm 0.90$  cm, respectively) (Table 2). Santos et al. (2010) worked with acclimation of the bromeliad *A. strobilacea* (epiphyte / saxicolous) at different dilutions of macronutrients of the MS medium (60.00, 30.00, 12.00, 6.00, 1.00, and 0.60 mM N) and observed a longer shoot length after eight months with cultivation using complete MS (60.00 mM N). This result is similar to that obtained for *A. imperialis*, which, although having rupicole habit, had greater shoot growth at 60.00 mM N.

As for the weight, FW of shoot was significantly higher in plants grown at 7.50 mM N up to 90.00 mM N, with the highest value found at 60.00 mM N ( $0.713 \pm 0.352$  g), compared to other concentrations (Table 2). The DW of shoot was higher in the 7.50 mM N treatment ( $0.100 \pm 0.060$ g), 15.00 mM N ( $0.067 \pm 0.020$  g), and 60.00 mM N ( $0.05 \pm 0.01$  g). Plants grown under shortage of N showed no mortality, but had the lowest average of fresh and dry weight ( $0.059 \pm 0.007$  and  $0.0050 \pm 0.0007$  g, respectively). Kanashiro et al. (2007) examined the growth of the bromeliad *A. blanchetiana* cultured *in vitro* for 120 days with different concentrations of nitrogen ( $7.50 \leq N \leq 120.00$  mM) and detected a decrease in fresh and dry weight of shoots with increasing nitrogen concentration, even with 120.00 mM N, result that was opposite to that observed in the present study. However, Dijk and Eck (1995) studied the *in vitro* growth of three species of orchids of the same genus and habit, for five months, and noticed different behaviors with the increase of N supply in the medium (0.00 - 12.00 mM N). The biomass production decreased in *Dactylorhiza incarnata*, increased in *D. praetermissa* and remained stable in *D. majalis*. Thus, plants of different species, but within the same genus and habit, may have different responses in relation to the increase in nitrogen fertilization.

Regarding the root, at 0.00, 90.00 and 120.00 mM N, root length values were lower ( $1.05 \pm 0.70$ ,  $0.80 \pm 0.35$  and  $1.13 \pm 0.71$  cm, respectively) than at 7.50 mM N ( $5.08 \pm 0.87$  cm) (Table 3), and at 3.75 mM N, the plants had thinner roots (data not shown).

**Table 3.** Length of roots (LR) (cm) contents of roots fresh weight (RFW) (g) and dry weight (RDW) (g) and ratio of RFW/RDW of plants *Alcantarea imperialis* (Carrière) Harms after six months of cultivation in different concentrations of nitrogen.

|                  | Treatments (mM N) |         |         |         |         |        |        |         |
|------------------|-------------------|---------|---------|---------|---------|--------|--------|---------|
|                  | 0.00              | 3.75    | 7.50    | 15.00   | 30.00   | 60.00  | 90.00  | 120.00  |
| LR (cm)          | 1.05c             | 2.96b   | 5.08a   | 2.24b   | 2.05b   | 2.95b  | 0.80c  | 1.13c   |
| RFW (g)          | 0.020ef           | 0.054cd | 0.090ab | 0.048de | 0.084bc | 0.108a | 0.006f | 0.022ef |
| RDW (g)          | 0.002d            | 0.006cd | 0.013a  | 0.007cd | 0.010bc | 0.014a | 0.001d | 0.004d  |
| Ratio of RFW/RDW | 9.414a            | 8.551ab | 7.640b  | 5.887c  | 7.993ab | 7.600b | 6.281c | 6.030c  |

Letters compare the values in the row and different letters indicate significant differences by Tukey test at 5%.

A review on nitrogen and its effect on roots developed by Garnett et al. (2009) demonstrated that the root growth at high concentrations of N is reduced, which was also noticed in this work. Probably, this reduced growth in the treatments of 90.00 and 120.00 mM N was due to a certain toxicity to the plant when subjected to these concentrations (TAIZ; ZEIGER, 2010), once it is a rupicole species adapted to low availability of nutrients. Under low N availability, root growth is greater, as shown by Bonifas and Lindquist (2009) studying the effect of N supply on the morphology of corn and velvetleaf roots, as N supply decreases. Velvetleaf may change its root morphology more than corn by investing a greater fraction of total biomass to production of thin roots. Thin roots are important because they are the main pathways for nutrient absorption and because they get in contact with a larger volume of soil per unit of root volume. Plants with longer roots and vigorous lateral roots will have a larger areas of contact with the growth medium and, therefore, a high potential to uptake nutrients (ZHANG et al., 2010).

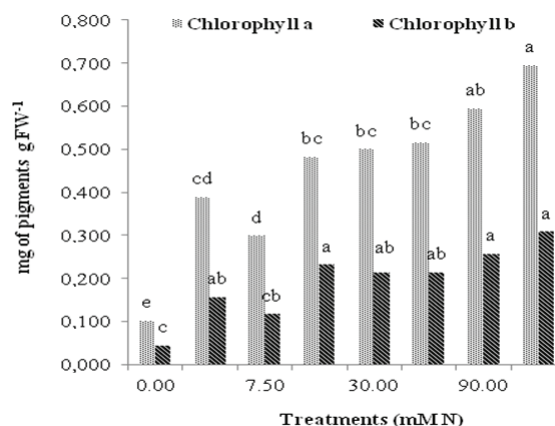
The greater root growth at low N concentrations was also explained by Tamaki and Mercier (2007) when they studied the signaling between root and shoot in the bromeliad *A. comosus*, and observed the accumulation of auxin IAA (approximately 2.5-fold) in roots cultivated without N, which suggests that the hormone might have moved down from the leaves to the roots, communicating N-shortage. Marschner (2012) and Davies (2010) showed that auxin is one of the hormones responsible for cell elongation, and that the roots are more sensitive to it, thus possibly inducing the elongation of roots at low concentrations of N. Consequently, under nitrogen limitation, the IAA might affect root growth and development, which probably should have occurred with *A. imperialis* at low concentrations of N in this work.

Considering the results of fresh and dry weight of root, values were higher in 60.00 mM N ( $0.108 \pm 0.028$  g FW and  $0.014 \pm 0.004$  g DW) followed by 7.50 mM N ( $0.090 \pm 0.003$  g FW and  $0.013 \pm 0.002$

g DW) (Table 3). By observing the fresh: dry weight ratio, higher values were observed in plants grown at 0.00 and 3.75 mM N, due to the lower amount of dry weight, indicating that elongated roots have larger amount of water, confirming that the root length was greater at low concentrations of N.

As for the results of photosynthetic pigments, the chlorophyll analysis showed a significant gradual increase in plants from 7.50 mM to 120.00 mM N, reaching at this latter concentration  $0,690 \pm 0,060$  mg g FW<sup>-1</sup> chlorophyll *a* and  $0,310 \pm 0,040$  mg g FW<sup>-1</sup> chlorophyll *b*. The plants grown at 0.00 mM N concentration had the lowest amount of pigments,  $0,100 \pm 0,002$  mg g FW<sup>-1</sup> chlorophyll *a* and  $0,040 \pm 0,008$  mg g FW<sup>-1</sup> chlorophyll *b* (Figure 1).

Os valores do eixo vertical na seriam com vírgula, já foi corrigido



**Figure 1.** Photosynthetic pigments (chlorophyll *a* and *b*) in *Alcantarea imperialis* (Carrière) Harms after six months of cultivation in different concentrations of nitrogen. Different letters compare the amount of the same type of pigment between treatments and significant difference according to Tukey's test at 5% probability.

With respect to chlorophyll, four nitrogen atoms are necessary for its composition, suggesting that this molecule could demonstrate the nitrogen status of plants (SALEEM et al., 2010, TAIZ; ZEIGER 2010). At 7.50 mM N, plants of *A. imperialis* showed a decrease in the concentrations of chlorophyll *a* and *b* (Figure 1). This is probably explained by the presence of red colored leaves in 90% of the plants, which is probably due to the presence of anthocyanins, which use N in their biosynthesis, because these pigments derive from phenylalanine (SHI; XIE, 2010). These authors studied *Arabidopsis thaliana* grown in medium with different concentrations of N and observed that, as the concentration of total N was reduced (9.40 mM nitrate), the formation of anthocyanins increased, as

observed in this study with *A. imperialis*. At concentrations of 15.00 to 60.00 mM N, plants showed similar chlorophyll contents, implying that such a concentration range was sufficient for the growth of this species (Figure 1). This results corroborate Tamaki et al. (2007) who cultivated plants of *A. comosus* from stunted stem nodes segments in different dilutions of the MS medium (60.00 mM N - MS, 30.00 mM N - MS/2, 12.00 mM N - MS/5, 6.00 mM N - MS/10, 1.00 mM N - MS/60 and 0.60 mM N - MS/100) for 90 days. The authors observed that in the treatments MS, MS/2, and MS/5, there were no significant differences in chlorophyll content among treatments, showing that the amount of nitrogen in MS/5 was sufficient for normal development of the plant, since 12.00 mM N (MS/5) was similar to 15.00 mM N used in this work. These authors mentioned that the concentrations of chlorophyll can be used as an estimate of total nitrogen content and hence the plant nutritional status, a fact corroborated by Saleem et al. (2010) and Agüera et al. (2010). Thus, the high chlorophyll content in 120.00 mM N may be due to the highest amount of nitrogen in this concentration. Saleem et al. (2010) showed that leaf chlorophyll contents have increased linearly with increment of N levels up to 150 kg ha<sup>-1</sup>, both in greenhouse and field trials while leaf area indices continued to increase up to the highest application rate (200 kg N ha<sup>-1</sup>). Therefore, the increase in the concentration of N supplied to the plant can increase its photosynthetic capacity (HIKOSAKA; OSONE, 2009).

At high concentrations of N, as 175.00 mM N, most plants of *A. imperialis* have not survived, which suggest a toxicity to the plant when subjected to this concentration. Guo et al. (2010) observed in plants of *Abies fabri*, an endemic conifer dominant in mountain regions of China, that excessive nitrogen supply had negative effects on plant growth. According to Taiz and Zeiger (2010), when minerals are excessive in the soil, it becomes saline, and plant growth, in general, is reduced. In salt stress, water is osmotically retained in the saline solution. So, if the concentration of salt in the soil is higher, the water becomes less available, causing the same effect as the water deficit stress. Among such effects is the reduction in growth and in photosynthesis due to stomatal closure (TAIZ; ZEIGER, 2010). According to Benzing (2000), many native bromeliads are adapted to environments with limited nutrients, making it possible to assume that the same occurred

with the rupicole *A. imperialis* because the results suggested that, at concentrations from 15.00 to 60.00 mM N, the *in vitro* growth was satisfactory.

Plants maintained at concentrations of 90.00 and 120.00 mM N had a high accumulation of photosynthetic pigments (Figure 1) and an increased number of leaves. According to Prado (2008), the chlorophyll content in leaves increases with linear increase of N. Kanashiro et al. (2007) show that higher concentrations of N probably extended the lifespan of older leaves, unlike those grown in reduced concentrations, which withdrew nitrogen from older leaves, causing senescence, and transporting nitrogen to the new leaves.

Plant survival in the absence of N (0.00 mM N) can occur probably because of the reserves in the seeds (FERREIRA; BORGHETTI, 2004). In this work it is suggested that a part of these reserves was used for germination, as well as during the initial growth of the plantlets. The other part was used to ensure the survival of the plants grown at 0.00 mM N, indicating that this species could grow in an environment poor in N. In this sense, plants grown at 0.00 mM N showed some characteristics distinct from plants growing in media containing the nutrient, such as reduced size, fewer leaves and chlorosis.

The plants were shown to be resistant to changes in the availability of nitrogen, even in the absence or excess of such nutrient for a six-month *in vitro* cultivation.

As for macronutrient analysis, it could be seen that, by increasing N, there was an endogenous increase of this element, with the highest amount observed at 15.00 mM and 60.00 mM N ( $966.72 \pm 60.75 \mu\text{g N per g FW}^{-1}$  and  $1054.23 \pm 61.79 \mu\text{g N per g FW}^{-1}$ , respectively) (Table 4).

**Table 4.** Concentration of nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg) and sulphur (S) ( $\mu\text{g nutrient per g FW}^{-1}$ ) in *Alcantarea imperialis* (Carrière) Harms after six months of cultivation in different concentrations of nitrogen.

| Treatments<br>(mM N) | Macronutrients ( $\mu\text{g of nutrient per g FW}^{-1}$ ) |         |          |         |         |         |
|----------------------|--|---------|----------|---------|---------|---------|
|                      | (N)  | (P)     | (K)      | (Ca)    | (Mg)    | (S)     |
| 7.50                 | 795.71b  | 379.80a | 3902.27a | 335.86a | 101.01a | 225.25a |
| 15.00                | 966.72a  | 225.98b | 2376.75b | 197.04b | 62.14b  | 176.76b |
| 30.00                | 821.62b  | 108.29c | 1254.32c | 95.05c  | 29.73c  | 82.79c  |
| 60.00                | 1054.23a   | 92.25c  | 1061.01c | 81.81c  | 24.15c  | 65.45d  |

Letters compare the values in the row and different letters indicate significant differences by Tukey test at 5%.

Analysis of foliar nutrients suggested an interference of N with the amount of some nutrients such as P, K, Ca, Mg and S, because their endogenous concentrations increased as N was reduced (Table 4), and since the amount of

these nutrients supplied in the culture medium was the same as in all treatments (Table 1).

Regarding the endogenous concentrations of P, K, Ca and Mg, higher values were registered in plants grown at 7.50 mM N, and lower endogenous values were observed in plants grown at 30.0 mM and 60.0 mM N (Table 4). Aranda-Peres et al. (2009) examined the importance of Ca for three species of bromeliads (*Vriesea friburguensis*, *Vriesea hieroglyphica* and *Vriesea unilateralis*) cultivated *in vitro*. They observed that the increase of Ca in the culture medium led to an increased concentration of N. However, this was not observed in *A. imperialis*. This fact might be related to the rupicole habit of the species studied, because it grows in a limited nutrient environment (BENZING, 2000).

As for S, plants grown at 7.50 mM N had the highest endogenous concentration of S ( $225.25 \pm 5.826.44 \mu\text{g S per g FW}^{-1}$ ), and those grown at 60.00 mM N showed the lowest one ( $65.45 \pm 0.94 \mu\text{g S per g FW}^{-1}$ ) (Table 4).

## Conclusion

This study suggests that the main effects of different concentrations of N, 15.00 and 60.00 mM, in modified MS medium, promoted an increase in the *in vitro* growth of *A. imperialis*, and also demonstrated that N may influence endogenous levels of P, K, Ca, Mg and S. This work suggested the importance of making amendments in the mineral composition of the culture medium in order to improve growth during the process of micropropagation of the endangered ornamental bromeliad *A. imperialis*.

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