

## Evaluation of macronutrient demand in calla lily (*Zantedeschia aethiopica*)

Daniella N.M. Carneiro<sup>1</sup>, Livia L. Coelho<sup>3</sup>, Patrícia D.O. Paiva<sup>2\*</sup>, Elka F.A. Almeida<sup>4</sup>, Leandro F. Carneiro<sup>1</sup>

<sup>1</sup>Federal University of Goiás, Jataí, GO, Brazil

<sup>2</sup>Federal University of Lavras, Department of Agriculture, University Campus. CEP: 37200-000 Lavras, MG, Brazil

<sup>3</sup>University of Copenhagen, Department of Plant and Environmental Sciences, Section for Crop Sciences. Højbakkegård Allé 13, DK-2630, Tåstrup, Denmark

<sup>4</sup>Agricultural Research Company of Minas Gerais, EPAMIG-CTSM-FERN, BR 494, Km 2, Colônia do Bengo, CTAN, São João Del Rei, MG, Brazil

\*Corresponding author: [patriciapaiva@dag.ufla.br](mailto:patriciapaiva@dag.ufla.br)

### Abstract

*Zantedeschia* species are important plants in the flower market, but there is insufficient information available on nutrient absorption and accumulation for the various developmental stages of these plants. Therefore, we aimed to evaluate macronutrient accumulation in *Zantedeschia aethiopica*. Following acclimatization, micropropagated shoots were cultivated in pots with coconut fiber as a substrate and were fertilized with a modified version of Malavolta solution, as described. For each tested plot, evaluations were performed every 30 days for 12 months. We evaluated the dry mass of each plant part (leaves, rhizomes, roots and flower stalks) and then calculated the dry mass accumulation and the nutrient contents. A randomized block study design was applied, with 4 replicates, totaling 48 plots. The aerial parts of the plants showed macronutrient content and accumulation in the sequences  $K^+ > N > Ca^{2+} > S > P > Mg$  and  $K > N > P > Ca > S > Mg$ , respectively, with the highest values being observed between 265 and 328 days after planting. The aerial parts showed greater accumulation of dry mass and macronutrients, whereas the flowers did not display significant macronutrient accumulation. The highest rates of macronutrient accumulation and growth occurred during the 210 days after transplanting, which corresponds to the pre-blooming period, indicating that fertilizers must be divided and applied at their highest levels prior to blooming.

**Keywords:** *Zantedeschia*, calla lily, nutrient accumulation, cut flowers, plant nutrition, greenhouse, potted plant.

**Abbreviations:** N-nitrogen, P-phosphorus,  $K^+$ -potassium,  $Ca^{2+}$ -calcium,  $Mg^{+2}$ -magnesium, S-sulfur, MS-dry mass, PA-aerial parts.

### Introduction

Among the cut flowers popular around the world, *Zantedeschia* species are among the plants produced at the highest levels (Landgraf and Paiva, 2009). However, growers of these flowers are strongly affected by *Pectobacterium carotovorum* infections, which are related to higher levels of water in the soil and fertilizers, particularly those that act as sources of nitrogen and phosphorus (Bloomz, 2004; Gracia-Graza et al., 2004; Fernandes et al., 2012). The application of balanced fertilization is the main way to increase the productivity and quality of floral stems of calla lilies (Almeida et al., 2012). Nutritional needs are determined by the amounts of nutrients accumulated by plants during their lifecycle, depending on their productivity (Mengel and Kirkby, 2001). Providing high levels of nitrogen to plants of the genus *Zantedeschia* may stimulate vegetative growth or blooming (Carneiro, 2012). However, *Zantedeschia* plants grown under low-nitrogen concentrations show reduced growth and flowering (Devecchi and Remotti, 2003; Fernandes et al., 2012; Almeida et al. 2015). Based on the progression of nutrient absorption, it is possible to identify those periods during which plants show the greatest nutritional requirements (Pedrosa, 2000; Malavolta, 2006). This has allowed the identification of the best times to supply

nutrients for cultivation, along with a means to improve fertilizer management, which decreases fertilizer loss and reduces the risk of toxicity while also preventing the delivery of doses below the requirements for cultivation (Mateus, 2010; Almeida et al. 2015). Addressing the lack of information about plants of the genus *Zantedeschia* and their high potential for cultivation, this work evaluated the absorption of macronutrients in *Zantedeschia aethiopica* and identified periods of increased nutrient demand.

### Results and Discussion

#### Accumulation of dry matter

The total dry matter production increased until 284 days after transplantation, with accumulation of 102.7 g plant<sup>-1</sup> (Figure 1). In contrast, Fonseca and Segeren (2013) observed a reduction of dry matter accumulation in *Z. elliottiana* at the end of the cycle, and in *Z. rehmannii*, the accumulation was continuous, revealing different behaviors in each of these species. Regarding the dry mass distribution in different organs of the plants, the aerial part showed the greatest amount of accumulated dry mass, containing 47% of the total

**Table 1.** Macronutrient levels in the shoots of greenhouse-cultivated *Zantedeschia* plants during the growth period.

Days after replanting	N	P	K	Ca	Mg	S
	-----g.Kg <sup>-1</sup> -----					
30	35.95 a	6.10 c	44.28 a	7.20 b	3.38 a	5.25 b
60	27.08 b	5.58 c	46.00 a	6.75 b	2.90 b	5.18 b
90	25.58 b	5.65 c	45.53 a	7.80 a	3.05 b	6.08 b
120	23.03 c	5.38 c	45.95 a	6.65 b	2.98 b	6.05 b
150	19.93 c	5.90 c	28.18 c	7.43 b	3.20 a	7.43 a
180	19.63 c	5.35 c	25.35 c	7.53 b	3.23 a	6.98 a
210	21.33 c	4.68 d	31.48 c	7.10 b	3.38 a	5.83 b
240	36.13 a	5.80 c	35.48 b	8.40 a	2.68 c	7.00 a
270	29.05 b	7.78 b	36.85 b	8.93 a	2.48 c	6.90 a
300	26.48 b	8.78 a	36.50 b	8.08 a	2.48 c	7.33 a
330	25.48 b	7.65 b	36.25 b	8.48 a	2.40 c	5.95 b
360	23.98 b	7.15 b	36.28 b	8.25 a	2.63 c	7.50 a
Average	25.9	6.2	37.0	7.72	2.81	6.47
C.V. (%)	9.5	7.1	11.8	10.3	5.4	11.0

dry matter, with 38% allocated to the rhizomes and 12% to the roots. The inflorescences showed the lowest accumulation, at 3%. Casierra-Posada et al. (2012) observed that plants of *Z. aethiopica* grown in a nutrient solution mainly accumulated dry mass in the roots, where 60% of the total dry mass was found, similar to when the roots + rhizomes were considered. Additionally, 33% was found in the aerial part of the shoots (stems and leaves) and 7% in inflorescences. The production of flowers began at 210 days after transplantation and peaked at 280 days after transplantation. Concurrent with the increased production of flowers, there was a slow increase in shoot dry weight as well as reduced dry matter accumulation in the roots and rhizomes, explained by the losses due to transport to the inflorescences. After the flowering period, from 300 days onward, we observed a reduction in plant growth and gradual senescence of the leaves, beginning in mature leaves and spreading to younger leaves, followed by an increase in rhizome dry mass. Fonseca and Segeren (2013) also observed leaf senescence at the end of the vegetative growth period, associated with an increase in the growth of tubers in *Zantedeschia*.

#### Concentration of macronutrients

The concentrations of macronutrients in shoots during the trial period and after 360 days after transplantation followed the order  $K^+ > N > Ca^{+2} > S > P > Mg^{+2}$  (Table 1). Almeida et al. (2009) evaluated the effect of silicon on the development of *Zantedeschia* plants and observed slightly higher levels of N (36.32 g kg<sup>-1</sup>) and Ca<sup>+2</sup> (12.66 g kg<sup>-1</sup>) in leaves but lower levels of P (3.46 g kg<sup>-1</sup>), K<sup>+</sup> (24.75 g kg<sup>-1</sup>) and Mg<sup>+2</sup> (1.68 g kg<sup>-1</sup>). These values were lower in plants that did not receive silicon.

#### Accumulation of macronutrients

##### Nitrogen

Nitrogen was the nutrient showing the second highest content, with a maximum value of 1.86 g plant<sup>-1</sup> (Figure 2). The accumulation of N in the plants increased during the first 283 days and then decreased until the 360th day. Of the total N content in plants, 58% was found in the shoots, 12% in the rhizomes, 9.6% in the roots and 20% in the inflorescences. However, the periods of greatest demand for the shoots and inflorescences were similar, between 231 and 283 days. In the rhizomes and roots, the period between 250 and 360 days showed the highest demand for nitrogen.

##### Phosphorus

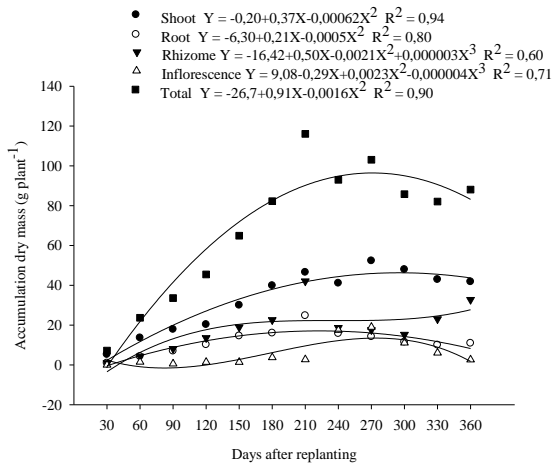
The phosphorus requirements of the plants were constant until 210 days after transplantation, and the greatest accumulation of this nutrient was observed at 328 days (Figure 3), at 0.63 g plant<sup>-1</sup>. The aerial parts accounted for 53% of total P; the rhizomes, 26.6%; the roots, 12.6%; and the inflorescences, 5%. Scagel and Schreiner (2006) found that the supply of P is related to increases in dry matter in the tubers of *Zantedeschia* cultivars 'Pot of Gold' and 'Majestic Red'. In the shoots, no increase was observed until 240 days, and the greatest demand was observed between 240 and 270 days. In contrast, the accumulation in the rhizomes continued until the 360th day, totaling 0.20 g plant<sup>-1</sup>.

##### Potassium

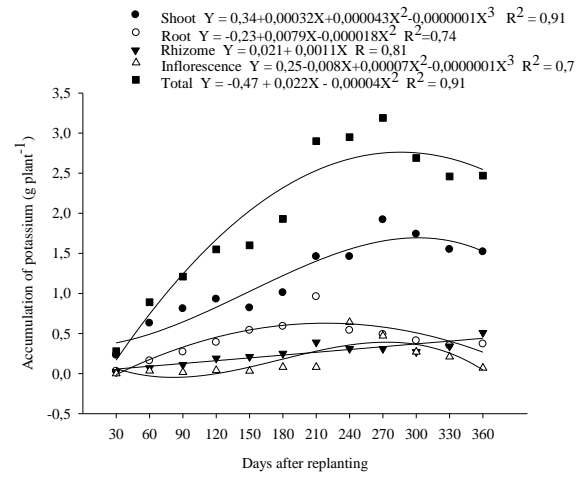
Potassium (Figure 4) was the most abundant nutrient accumulated in *Zantedeschia aethiopica* plants, presenting a peak of 2.55 g plant<sup>-1</sup> on the 275th day. Regarding the total potassium accumulation, 60% was found in the shoots, 9.7% in the rhizomes, 15% in the roots and 14.7% in the inflorescences. The higher requirement for potassium in crops that store organic compounds in underground reserve organs, such as *Zantedeschia* rhizomes, is explained by the important role of this nutrient in the transport of assimilates from the leaves to the reserve organs (Meurer, 2006). The greatest demand for potassium in the plants occurred between 180 and 210 days, when an accumulation of 0.97 g was observed. The period of highest demand varied among shoots (180-270 days), roots (300-360 days), rhizomes (90-180 days) and inflorescences (210-260 days). Comparing the high-demand periods for N, P and K<sup>+</sup>, we observed that, although the accumulation of nitrogen in the plants indicated a higher demand for this nutrient from day 231 to day 283, demand for potassium was higher from day 283 to day 275, and the increase in phosphorus accumulation occurred only later, on day 328.

##### Calcium

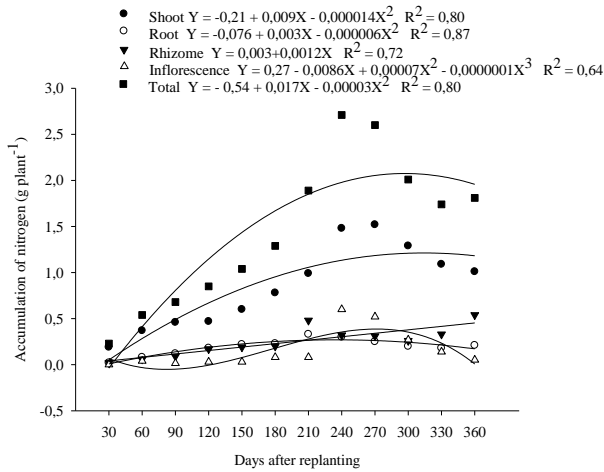
Calcium is the nutrient required by *Zantedeschia* plants at the third highest level, reaching a maximum level of 0.60 g plant<sup>-1</sup> on day 321st. The calcium requirement in the shoots corresponded to 61% of the total accumulated in the entire plant (Figure 5); in the rhizomes, roots and inflorescences, the percentages were 18.6, 13.0 and 6.7%, respectively. Accumulation in the rhizomes continued until day 360, and there were no changes in calcium demand during different



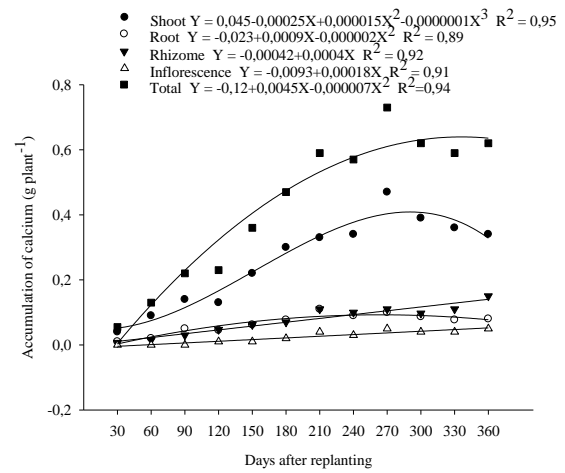
**Fig 1.** Dry matter accumulation in *Zantedeschia* plants.



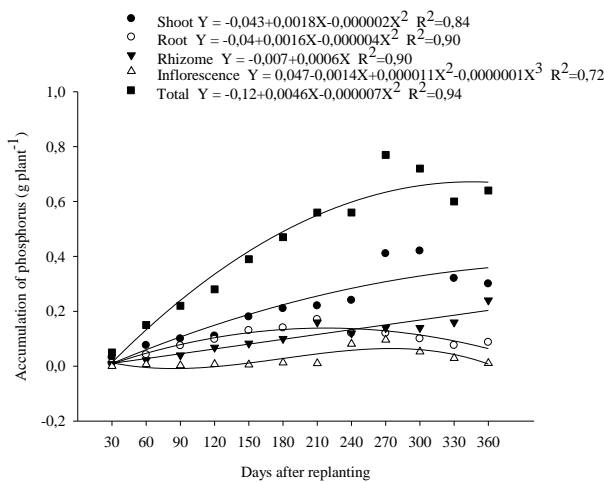
**Fig 4.** Potassium accumulation in *Zantedeschia* plants.



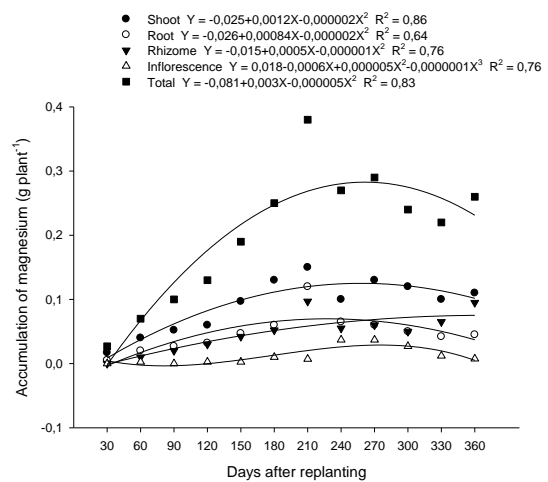
**Fig 2.** Nitrogen accumulation in *Zantedeschia* plants.



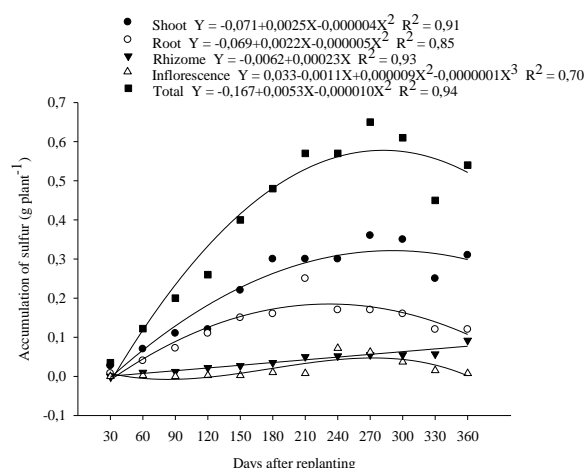
**Fig 5.** Calcium accumulation in *Zantedeschia* plants.



**Fig 3.** Phosphorus accumulation in *Zantedeschia* plants.



**Fig 6.** Magnesium accumulation in *Zantedeschia* plants.



**Fig 7.** Sulfur accumulation in *Zantedeschia* plants.



**Fig 8.** Side view of a *Zantedeschia* plant after 210 days of growth.

periods, which suggests that calcium was not redistributed from the leaves to the rhizomes or from the rhizomes to meet the needs of the growing shoots, leaves and flowers.

### Magnesium

Magnesium was the nutrient required in the lowest amounts, showing a maximum accumulation of  $0.37 \text{ g plant}^{-1}$  on day 300 (Figure 6). The aerial parts of the plants did not require any magnesium supply until day 120, with the greatest demand occurring between days 120 and 210, and an increment of  $1.17 \text{ mg plant}^{-1}$  per day. The inflorescences showed higher accumulation rates between days 210 and 240, at  $1.0 \text{ mg plant}^{-1}$  per day. The root demand peaked between days 90 and 210, and the demand of the rhizomes remained constant throughout the testing period. Because this nutrient is required by many enzymes to transfer phosphates and is a component of important molecules, more  $\text{Mg}^{+2}$  is required by the aerial parts of the plants than by the other plant organs (Taiz and Zeiger, 2004).

### Sulfur

Sulfur showed the highest total accumulation on 265th day, at  $0.55 \text{ g plant}^{-1}$  (Figure 7). The highest accumulation rate was observed from day 120 to day 210, at  $0.31 \text{ g plant}^{-1}$  (56% of the maximum accumulated in the entire plant). During this

period of greatest accumulation, the shoots accumulated 55%, the roots 26%, the rhizomes 8% and the inflorescences 10% of the total S accumulated by the plant. In *Zantedeschia* plants, N and S are the nutrients required at the highest levels, and deficiencies in these nutrients show symptoms at early stages (Almeida et al., 2012). All nutrients showed increased accumulation in the rhizomes on day 360, which was accompanied by rhizome growth. This increase can most likely be attributed to the redistribution of these macronutrients from the aerial parts, considering that leaf senescence was also observed in this period. At the end of the life cycle of calla lilies (*Zantedeschia* sp.), the plants undergo full senescence of their leaves and experience dormancy (Carneiro et al., 2011; Clark et al., 1991). However, in *Z. aethiopica*, only partial senescence of the leaves was observed without dormancy, apart from the reduction of plant growth and flower production and increased rhizome growth.

### Accumulation (total)

The accumulation of nutrients in the plants on day 360 showed the sequence  $\text{K}^+ > \text{N} > \text{P} > \text{Ca}^{+2} > \text{S} > \text{Mg}^{+2}$ , corresponding to average contents of 2.47, 1.81, 0.64, 0.62, 0.54 and  $0.26 \text{ g plant}^{-1}$ , respectively (Table 1). This sequence was similar to those reported by Fonseca and Segeren (2013) and Gómez et al. (2010) for cultivated *Zantedeschia elliottiana*. Generally, dividing the maximum contents of macronutrients accumulated in the whole plant by the total volume of the vessel ( $16 \text{ dm}^3$ ), under ideal conditions (assuming there is no loss of nutrients), the applied macronutrient fertilizer should be sufficient to ensure average concentrations of 130 mg N, 40 mg P, 160 mg  $\text{K}^+$ ,  $37.7 \text{ Ca}^{+2}$ , 23 mg  $\text{Mg}^{+2}$ , and 34 mg S per  $\text{dm}^3$  substrate. These results indicate that the macronutrient fertilization performed in the present study was sufficient to ensure the growth and development of the plants, as no symptoms of deficiency and/or toxicity were observed, except for the accumulation of  $\text{K}^+$ , the levels of which were lower than the applied levels. Accumulation curves were established for each macronutrient, divided based on plant age and plant structure. Thus, considering the absorption of nutrients during the cycle for the determined dry mass production, it was possible to establish an adequate plan for fertilization that avoids shortages or excesses in supply, which can affect growth and productivity. The results of this study provide guidance regarding the supply of nutrients in adequate amounts, ensuring satisfactory initial development of seedlings and nutritional balance. Figure 8 shows a clear side view of a healthy and mature plant.

### Materials and Methods

#### Plant material and experimental conditions

Plants were obtained from *in vitro* propagation (Ribeiro, 2014), and acclimatized in plastic trays containing Plantmax® under intermittent mist irrigation for 60 days (Fernandes et al., 2012). Then, the plants were transferred to plastic pots ( $16 \text{ dm}^3$ ) containing a coconut fiber substrate (Golden Mix® Granulated PM-11 - Amafibra®) until they reached 50 cm in height, when they were transferred to experimental plots. Because the calla lily grows better in partial shade (Almeida and Paiva, 2012), the plants were grown in a greenhouse built with 80% shade screen, for reducing the temperature inside the greenhouse. During the experimental period, the average day/night temperature was  $26/18^\circ\text{C}$ , the relative humidity was 85%, and the photoperiod was 12 h. Humidity was monitored daily, and manual irrigation was performed. The

quantity of water supplied varied with plant growth and environmental conditions throughout the experiment. The applied fertilizer was modified from the formulation of Malavolta (2006), and it contained the following (in mg dm<sup>-3</sup>): N-500; P-200; K-150; Ca-200; Mg-50; S-50; B-0.3; Cu-1.3; Fe-5.0; Mn-1.25; Mo-0.1 and Zn-1.9. The macronutrient fertilization was split into 12 equal doses provided every 30 days and was applied in nutrient solutions. Micronutrients were applied in a single dose during the first fertilization treatment, immediately after seedling transplantation.

### Treatments

The treatments consisted of 12 months of plant collection, with evaluations conducted every 30 days. Inflorescences were evaluated daily, and harvesting was performed according to commercial standards. The experiment was performed in a randomized block design with four replications, totaling 48 plots. In the first month, because the plants were very small, 8 plants per plot were collected to provide sufficient material (dry mass) for chemical analysis. At the other assessments, 3 plants were collected.

### Traits measured

Every 30 days, the leaves, rhizomes and roots were separated and washed in distilled water. The same procedure was performed for inflorescences. The material was then packed in paper bags and dried in a forced-air oven at 70°C until it reached a constant weight. The dry mass of the shoots, rhizomes, roots and stalks was measured, as was the total dry mass, which was obtained from the sum of the dry mass of each plant part. The inflorescences were evaluated to determine dry mass according to the harvest season, using the data accumulated for each plant during the experimental period. Following the dry mass determination, the various parts (leaves, rhizomes, roots and stalks) were ground in a Willey-type mill and stored in plastic bags for subsequent chemical analyses. After nitro-perchloric digestion of the samples (Zaroski and Burau, 1977), the nutrient concentrations were determined as follows: Ca and Mg via atomic absorption spectrophotometry, K via flame photometry (Malavolta, 2006), P via colorimetry (Braga and Defelipo, 1974), and S via turbidimetry (Blanchar et al., 1965). Total N was determined using the semi-micro Kjeldahl method following sulfuric acid digestion (Bremner and Mulvaney, 1982). To determine the accumulation of macronutrients in the plants, the content of each plant was compared to the corresponding dry mass.

### Statistical analysis

The obtained data were subjected to analysis of variance. Significant F-test results ( $P \leq 0.05$ ) were subjected to polynomial regression analysis, and the mean levels of nutrients in shoots were compared using the Scott-Knott test at the 5% level using System for Analysis of Variance for Balanced Data (Sisvar) software (Ferreira, 2011).

### Conclusions

The content of macronutrients in the aerial parts of the plants followed the sequence  $K > N > Ca > S > P > Mg$ . The accumulation of nutrients followed the sequence  $K > N > P > Ca > S > Mg$ , with the highest values being observed between days 265 and 328. The aerial parts showed higher dry matter and macronutrient accumulation than the roots and rhizomes. Inflorescences do

not represent an important structure for macronutrient extraction. The highest rates of increase in the accumulation of macronutrients and growth occurred before 210 days after transplantation (Figure 8), corresponding to the pre-blooming period, indicating that fertilization must be supplied in larger doses during the phases prior to floral induction.

### References

- Almeida EFA, Paiva PDO (2012) Copo-de-leite. In: Paiva PDO, Almeida EFA (eds) Produção de flores de corte. Editora UFLA, Lavras.
- Almeida EFA, Paiva PDO, Carvalho JG, Frazão JEM, Oliveira NP (2015) Descriptive analyses of deficiency symptoms in calla lily plants. *J Plant Nut* 38(5):260-271.
- Almeida EFA, Paiva PDO, Carvalho JG, Oliveira NP, Fonseca J (2009) Efeito do silício no desenvolvimento e na nutrição mineral de copo-de-leite. *Rev Bras Horticult Orn*. 15(2):103-113.
- Almeida EFA, Paiva PDO, Morais JEF, Santos FHSS, Rezende FA, Mara CL (2012) Diferentes doses de NPK e esterco no crescimento e produção de inflorescências em plantas de copo-de-leite (*Zantedeschia aethiopica*). *Rev Bras Hort Orn*. 18(2):129-134.
- Bloomz (2004) Guidelines for pot growers. Technical bulletin series C001/00.2004. Zantedeschia (Calla Lily) production. Callafornia Callas, Nueva Zelanda.
- Braga JM, Defelipo BV (1974) Determinação espectrofotométrica de fósforo em extratos de solos e plantas. *Rev Ceres*. 21(113):73-85.
- Blanchar RW, Rehm G, Caldwell AC (1965) Sulfur in plant material digestion with nitric and perchloric acids. *Soil Sci Soc Am Pro*. 29(1):71-72.
- Bremner JM, Mulvaney CS (1982) Nitrogen total In: Page AL (ed) Methods of soil analysis, 2nd edn. Soil Sci Soc Am Pro., Madison.
- Carneiro DNM, Santos Filho AB, Carneiro LF, Paiva PDO (2012) Callas. In: Paiva PDO, Almeida EFA(eds) Produção de flores de corte. Editora UFLA, Lavras.
- Carneiro DNM, Almeida EFA, Paiva PDO; Frazão JEM; Santos FHS; Carneiro LF (2011) Development and dry mass accumulation in calla lily at the initial cultivation stage. *Ciê Agrotec*. 35(6):1085-1092.
- Casierra-Posada F, Nieto PJ, Ulrichs C (2012) Crecimiento, producción y calidad de flores en calas (*Zantedeschia aethiopica* (L.) K. Spreng) expuestas a diferente calidad de luz. *Rev U.D.C.A Act & Divulg Cien*. 15(1):97-105.
- Clark CJ, Boldingh HL (1991) Biomass and mineral nutrient partitioning in relation to seasonal growth of *Zantedeschia*. *Sci Hort*. 47:125-135.
- Devecchi M, Remotti D (2003) Influence of fertilization on vegetative growth and flowering of the calla (*Zantedeschia aethiopica* Spreng.). *Acta Hort*. 614:541-545.
- Meurer EJ Potássio(2006). In: Fernandes MS (ed) Nutrição mineral de plantas. Editora UFV, Viçosa.
- Fernandes KD, Paiva PDO, Carvalho JG, Rezende AC, Figueiredo MA (2012). Multiple nitrogen and phosphorus deficiency in *Zantedeschia*. *Cien Agrotec*. 36 (6):631-638.
- Ferreira DF (2011) Sisvar: a computer statistical analysis system. *Cien Agrotec*. 35(6):1039-1042.
- Fonseca AS, Segeren MI (2013). Nutrient uptake in two species of calla lily (*Zantedeschia* sp.) under fertigation. *Acta Hort*. 1000:377-384.
- Gómez S, Correa CRB, Flores JCM (2010) Absorption of nutrients in *Zantedeschia elliottiana* Crystal Blush and its relationship with biomass production in the Colombian coffee-growing conditions. *Acta Agronóm*. 59(4):462-472.

- Gracia-Garza JA, Blom TJ, Brown W, Roberts DP, Schneider K, Freisen M, Gombert D (2004) Increased incidence of erwinia soft-rot on Calla Lilies in the presence of phosphorous. *Eur J Plant Pathol.* 110(3):293-298.
- Landgraf PRC, Paiva PDO (2009) Produção de flores cortadas no estado de Minas Gerais. *Ciêñ Agrotec.* 33(1):120-126.
- Malavolta E (2006) Manual de nutrição mineral de plantas. Agronômica Ceres, São Paulo.
- Mateus CMDA, Pivetta KFL, Villas Boas RL, Coan RM (2010) Análise de crescimento do amarelinho cultivado a pleno sol. *Rev Ceres.* 57(4):469-475.
- Mengel K, Kirkby E (2001) Principles of plant nutrition. 5ed. Dordrecht/Boston/London:Kluwer Academic Publishers, 849p.
- Pedrosa MW (2000) Concentração e acúmulo de nutrientes em plantas de *Gypsophila paniculata* L. cultivadas em solução nutritiva. *Rev Bras Hort Orn.* 6(1):19-30.
- Ribeiro MNO, Pasqual M, Silva AB and Rodrigues VA (2014) Propagação in vitro de copo-de-leite: sulfato de adenina e 6-benzilaminopurina. *Rev Bras Hort Orn.* 20(1):21-26.
- Scagel CF, Schreiner RP (2006) Phosphorus supply alters tuber composition, flower production, and mycorrhizal responsiveness of container grown hybrid *Zantedeschia*. *Plant and Soil.* 283:323-337.
- Taiz L, Zeiger E (2004) Mineral nutrition. In: Taiz L, Zeiger E (eds) Fisiologia vegetal, 3rd edn. Artmed, Porto Alegre.
- Zaroski RJ, Bureau RG (1977) A rapid murex-perchloric acid digestion method for multi-element tissue analysis. *Com. Soil Sci Pl Anal.* 8(5):425-436.