

SCIENTIFIC ARTICLE

Gerbera leaf nutritional status as a cut flower cultivated with different doses of nitrogen and calcium

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Abstract

Vegetative tissue analyses might help to diagnose nutritional status of a crop. Foliage diagnosis is an effective method to set up balanced fertilization procedures. The current work aims to evaluate nutrient contents of two gerbera leaf sampling periods as a cut flower cultivated in pots with different doses of nitrogen (N) and calcium (Ca) under greenhouse conditions. The experiment was carried out in a protected environment, shelter type, in a container containing substrate in a 3x3+1 factorial scheme. Three doses of N (0.07, 0.15 or 0.2 g L⁻¹) or three doses of Ca (0.02, 0.03 or 0.04 g L⁻¹) were applied every 15 days. Control plants did receive neither N nor Ca. The substrate was analyzed before and after cultivation, and at the end of production years, as well as completely expanded leaves were collected for nutrient analyses. There was not significant interaction between N and Ca doses. Regarding the sampling period or doses of N and Ca, the determined macronutrient concentrations were in the following order of N>K>Ca>P>S>Mg. The micronutrient concentrations were in the following order of Fe>Mn>B>Zn>Cu. Except for K, all the other nutrient concentrations were in the recommended range for gerberas production.

Keywords: cut flower, *Gerbera hybrida* H., nutrient contents, substrate.

Resumo

Estado nutricional foliar da gérbera de corte cultivada com diferentes doses de nitrogênio e cálcio

A análise química do tecido vegetal serve como forma de diagnosticar o estado nutricional da cultura. Neste sentido, o diagnóstico foliar torna-se uma ferramenta para se estabelecer programas racionais de adubação. O objetivo do trabalho foi avaliar o teor foliar de nutrientes, ao término do primeiro e segundo ano de produção, de gérbera de corte cultivada com diferentes doses de nitrogênio (N) e cálcio (Ca). O experimento foi conduzido em ambiente protegido, tipo abrigo, em recipiente contendo substrato em esquema fatorial 3x3+1, sendo três doses de N (0,07; 0,15 e 0,2 g L⁻¹) e de três doses de Ca (0,02; 0,03 e 0,04 g L⁻¹), aplicadas a cada 15 dias, além do controle, sem adubação com N e Ca. O substrato foi analisado antes e após o cultivo, e ao término dos anos de produção, realizou-se a coleta de folhas completamente expandidas para análise nutricional. Não houve efeito significativo da interação entre as doses de N e Ca. O teor foliar de potássio (K) encontrou-se abaixo do sugerido na literatura. Independente do período de coleta das folhas e das diferentes doses de N e de Ca, a concentração dos macronutrientes seguiu a ordem decrescente: N>K>Ca>P>S>Mg, e para os micronutrientes: Fe>Mn>B>Zn>Cu. Os teores foliares dos macronutrientes, com exceção do K, e os teores foliares dos micronutrientes, ao término do primeiro e segundo ano de produção, estão de acordo com o recomendado na literatura para gérbera de corte.

Palavras-chave: flor de corte, *Gerbera hybrida* H., substrato, teor de nutrientes.

Introduction

Gerbera (*Gerbera hybrida* H.) is a commercial flower that is cultivated as a cut flower or in pots, and it is highly enjoyable in the flowers market and ornamental plants in Brazil, offering great diversity of colors, good durability, and resistance to transportation (Santos et al., 2017).

According to Malavolta (2006), plant nutrition and fertilization are vital components to promote good quality resulting in adequate returns to economically important crops. Thus, nitrogen is one of the key nutrients required by plants, since it is involved in essential biochemical functions, such as chlorophyll synthesis, amino acid constituents, amides, proteins, nucleic acids, and nucleotides. On the

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other hand, calcium has a central role in cell division and nitrogen absorption at vegetative growth points, as well as being a crucial constituent of the cell wall (Taiz and Zeiger, 2013).

Nutrient analysis of plant tissues aids to diagnosis the nutritional status of a crop. Leaf tissue analyses reflect the effects of soil-plant-climate interactions and managements that might be a strategic tool to set up rational fertilization programs for adequate nutrient supply based on the quantitative variation of elements in plant tissues (Damasceno et al., 2011). However, in Brazil, there is slight information on nitrogen (N) and calcium (Ca) fertilization for gerberas as cut flower grown in containers with organic substrate. Thus, the choice to study these nutrients is due to their functions for plants growth (Milani et al., 2019).

Therefore, the objective of the present work is to evaluate the leaf nutrient contents at the end of the first and second production years of cut gerberas cultivated in containers with different doses of nitrogen and calcium.

Material and Methods

The experiment was conducted during 28 months in a protected environment, covered with transparent low density polyethylene film. Every day at 30-minute intervals the room temperature and relative humidity were recorded on a thermo-higrograph (Klimalogg Pro®).

Average room temperature and relative humidity varied according to the season. In the summer months the average temperature was 26.1 °C and relative humidity was 75.1%. They were 20.1 °C and 80.2%, respectively in the autumn months and they were 15.4 °C and 81.8%, respectively during the winter months. Throughout the spring period the average temperature was 21.2°C and the relative humidity was 76.2%. Along the summer period when the light intensity was beyond 60 thousand Lux (Mercurio, 2002), an aluminized thermo-reflective net with 50% shading is used.

Sixteen-week-old gerbera seedlings of the cultivar Dino with an average of seven full developed leaves were purchased from a specialized plantlet grower. The floral stalk and capitulum and the ligulas of the capitulum, referred to as petals, was considered a complete inflorescence. The plantlets were transplanted into plastic containers (one plantlet/pot) of 2.8 L volume with 17.6 mm top diameter, 14 mm base diameter and a height of 15 cm. The pots were filled up to 2.5 L volume with a *Pinus* spp. bark-based commercial substrate.

The experiment was conducted in a completely randomized block design in a factorial 3x3+1, with four replicates and eight plants as experimental unit. The first factor was three doses of N (0.07, 0.15 or 0.2 g L⁻¹) and second factor three doses of Ca (0.02, 0.03 or 0.04 g L⁻¹) and a control treatment (without any addition) had been combined. A total of 320 plants were disposed in a density of 11 plants m⁻². The doses of N and Ca were based in Hulshof (2008).

Each pot was irrigated by means of a drip irrigation system at a flow rate of 0.46 L h⁻¹. During the autumn and

winter seasons, the plants were irrigated three times a week for about three minutes in only one cycle a day. In the spring season the plants were irrigated every day and again for one single cycle of three minutes. In the summer season the plants were also irrigated every day, but then divided in three times a day in a total of seven minutes of water delivery. The irrigation water presented an average pH of 6.53 and 116.20 µS cm⁻¹ conductivity.

After 27 days of transplanting (DOT), fertilization of the pots started and ended after 833 DOT. The fertilizers were all diluted in water and 50 mL were manually applied to every pot at 15 days intervals. The doses of N and Ca were applied in the form ammonium nitrate (30% total N: 15% NO₃⁻ and 15% NH₄⁺) and calcium nitrate (15.5% NO₃⁻ plus 19% Ca). At the end of the experiment the total amounts of N for each pot were 3.94, 8.58 and 11.83 g L⁻¹. The total amounts of Ca applied to every experimental unit were 0.93, 186 and 2.55 g L⁻¹. All the experimental units received a complementary fertilization of potassium (K), phosphorus (P) and magnesium (Mg) during the growth period aggregate of 0.08, 0.32 and 0.02 g L⁻¹, respectively.

The reproductive period was considered as starting after 91 DOT when 80% of the plants displayed a perceptible floral button of ± 1 cm. In meanwhile, macro and micronutrients supply were recalculated, and the plants started to receive them. The total amounts of K, P and Mg for each experimental unit were 1.63, 8.77 and 0.61 g L⁻¹, respectively. Additionally, 5.1 g L⁻¹ Rexolin® (water soluble micronutrients) were applied to the experimental units. Rexolin® contains 11.6% K₂O, 1.28% S, 0.86% Mg, 2.1% B, 0.36% Cu, 2.66% Fe, 2.48% Mn, 0.036% Mo and 3.38% Zn. The source for K was K chloride (KCl containing 60% K₂O) for P the source was monopotassium phosphate (MKP) containing 52% P₂O₅ and 34% K₂O and for magnesium the source was magnesium sulphate containing 9% Mg.

The substrate was analyzed before and after 28 months of gerberas cultivation period. Samples of every treatment and block were analyzed at the Substrate Laboratory of the Faculty of Agronomy (UFRGS). Conductivity (mS cm⁻¹) and pH in water were determined via dilution method 1:5 (Brasil, 2007). Physical analyses such as dry density (kg m⁻³), total porosity (%), aeration space (%), available water (%) and remaining water (%) were determined according to De Boodt and Verdonck (1972).

At the end of the first and second year of trial (384 and 849 DOT), fifteen completely expanded leaves were sampled (adapted from the Comissão de Química e Fertilidade do Solo – RS/SC, 2016) and taken to the laboratory to determine macro and micronutrients contents according to the methodology of Tedesco et al. (1995).

Data for total nutrient contents were analyzed as trifactorial because sampling of leaves was considered a factor of variation besides the doses of N and Ca. When the double interactions (periods versus doses of N or periods versus doses of Ca) were not significant only the main effects were evaluated. The data were submitted to analysis of variance at $p < 0.05$ and regression analysis for N and Ca doses. The statistical program SISVAR (Ferreira,

2021). was used for the statistical analyses and linear Pearson correlation was determined for nutrient contents of gerbera leaves at 5% significance using the software Action (Estatcamp, 2021).

Results and Discussion

There was not significant effect on the interaction (doses of N versus doses of Ca) for the chemical and physical variables of the substrates. Therefore, only the main effects were determined. Concerning the chemical characteristics

of the substrate, the conductivity was influenced by the tested doses of N and Ca fertilization (Figure 1A and B). The tested concentrations of N and Ca also affected the pH values (Figure 1C), indicating that the gerbera cultivation parameters changed compared to the beginning of cultivation since the pH value was 6.37 and the salinity was 0.28 mS cm⁻¹. The conductivity of the substrate had a similar increasing trend for N and Ca indicating that their highest doses (0.2 g L⁻¹ N and 0.04 g L⁻¹ Ca, at 15 days intervals) presented the highest values of conductivity: 0.81 and 0.78 mS cm⁻¹, respectively.

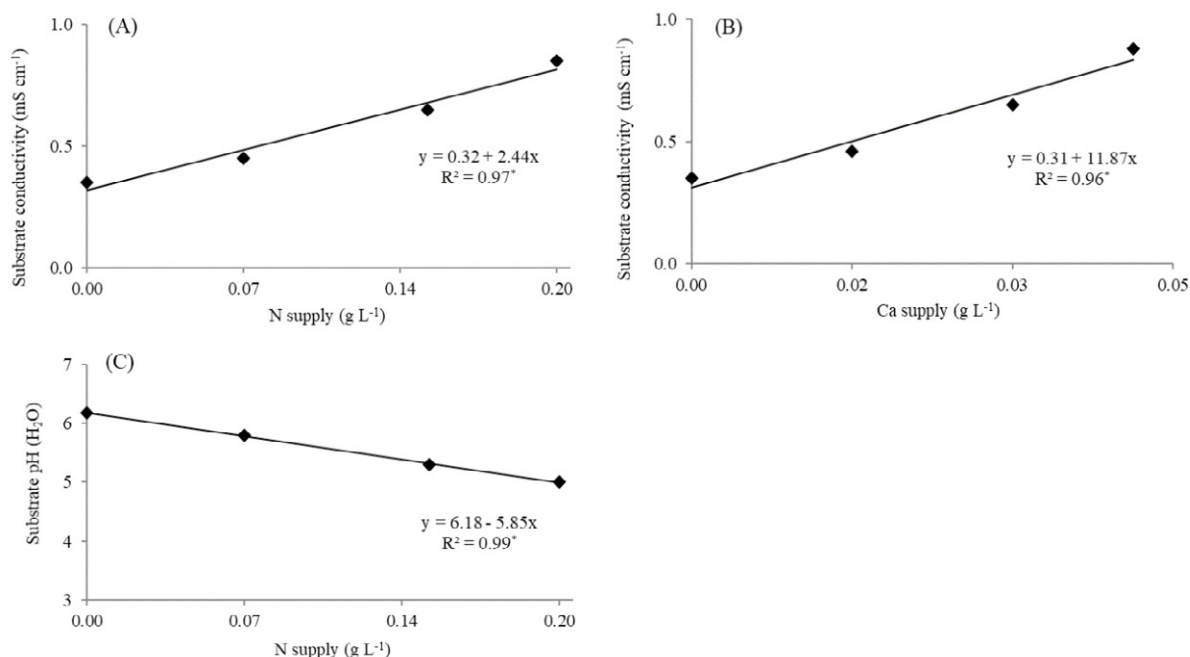


Figure 1. Substrate chemical characteristics after 28 months of gerbera cultivation. Substrate conductivity (A) and substrate pH (B) in the function of N doses supply. Substrate conductivity (C) in the function of Ca doses supply. The N and Ca were supplied at 15 days intervals.

*Significant at 5% probability

The obtained results are in accordance with those of Mercurio (2002) who recommends that substrate for gerbera as cut flower should have a conductivity below 0.9 mS cm⁻¹ in 1:5 diluted solutions. The pH value of the substrate realized opposite conductivity trend for N doses (Figure 1C). A linear negative adjustment was noticed with the highest doses of N resulted in a lower pH value (4.98). This is probably might attributed to using ammonium nitrate reduced the pH value of the substrate. Also, ammonia nitrogen after biological oxidation by the nitrification process results in soil acidification. Calcium doses did not present a significant polynomial adjustment. According to Mercurio (2002), the ideal pH for cut gerbera cultivation is situated

between 5.5 and 6.0. Ludwig et al. (2020) concluded that substrates with extreme pH values, below 5.0 or over 7.0 are not adequate for gerbera plants. Inadequate pH could influence the availability of nutrients since at pH values below 5.0 deficiency symptoms of N, K, Ca Mg and B might come into sight. On the contrary, at pH values over 6.5 problems with the availability of P, Fe, Mn, Zn and Cu might be expected (Kämpf, 2005).

With regards to physical properties of the substrates, not significant polynomial adjustment for N and Ca doses could be calculated. However, there were variations on the average values determined before and after the end of cultivation period of the gerberas, which is a predictable result when using organic substrates (Table 1).

Table 1. Physical characteristics of the substrate at the beginning of the experiment and after 28 months of gerbera cultivation for cut flower cv. *Dino*.

Physical characteristics of the substrate	At experimental set up	After 28 months of cultivation
DD (kg m ⁻³)	342.76	337.96
TP (%)	77.46	86.23
AS (%)	27.22	37.22
AW (%)	14.60	10.50
RW (%)	35.65	38

DD = dry density; TP = total porosity; AS = aeration space; AW = available water; RW = remaining water.

Dry density (DD) of the substrate at the experimental set up had an average value of 342.76 kg m⁻³ and it reduced to 337.96 kg m⁻³ after 28 months experiment duration. These values of DD are in accordance with Kämpf (2005) who recommended that DD values varying from 250 to 400 kg m⁻³ per pot.

The total porosity (TP) was increased almost by 10% after gerbera cultivation. The TP value of 86.23% is very close to that value suggested by De Boodt and Verdonck (1972) as ideal for a substrate (85% of its volume as pores).

A reduction of available water (AW) was observed after the end of the gerbera's cultivation period. The AW values ranged between 25% and 35% are considered ideal for gerbera cultivation (De Boodt and Verdonck, 1972). The aeration space (AS) realized an increase of 10% by the end of cultivation period. The average value of AS was 37.22% which is higher than those values (20% to 30%) suggested by De Boodt and Verdonck (1972). They recommend that the remaining water of the substrate should be between 20% and 30% and the substrate of the present experiment had an average value of 35.65% at the experimental set up and 38% after the end of the cultivation period.

The variations of substrate physical characteristics after finishing the experiment might be due to the long

cultivation period resulting in a rearrangement of particles either because of higher accommodation of the substrate or because of its decomposition. The main effects of that reorganization are increases on macropore volume, boosting aeration space, and ultra-micro-pores, enhancing the remaining water so both characteristics raises total porosity (Schafer et al., 2008).

As for the gerbera leaf contents of macro and micronutrients, the triple interaction (sampling period x nitrogen doses x calcium doses) as well as the double interaction (doses of nitrogen x doses of calcium) were not significant. When double interactions (sampling period x nitrogen doses and sampling period x calcium doses) were not significant only the main effect of the fertilizer doses were examined.

Regarding foliar content of N, a significant effect of the interaction of sampling periods and N doses was observed. Analyzing the N doses on every period, illustrated that it was possible to determine in two periods a significant second order adjustment of the observed values. After 489 DOT, the maximum N contents of 26.1 g kg⁻¹ were recorded when 0.14 g N L⁻¹ substrate was applied every 15 days. After 849 DOT, the maximum content (34.34 g kg⁻¹) was noticed when 0.13 g L⁻¹ of N was supplied to the gerberas every 15 days (Figure 2A).

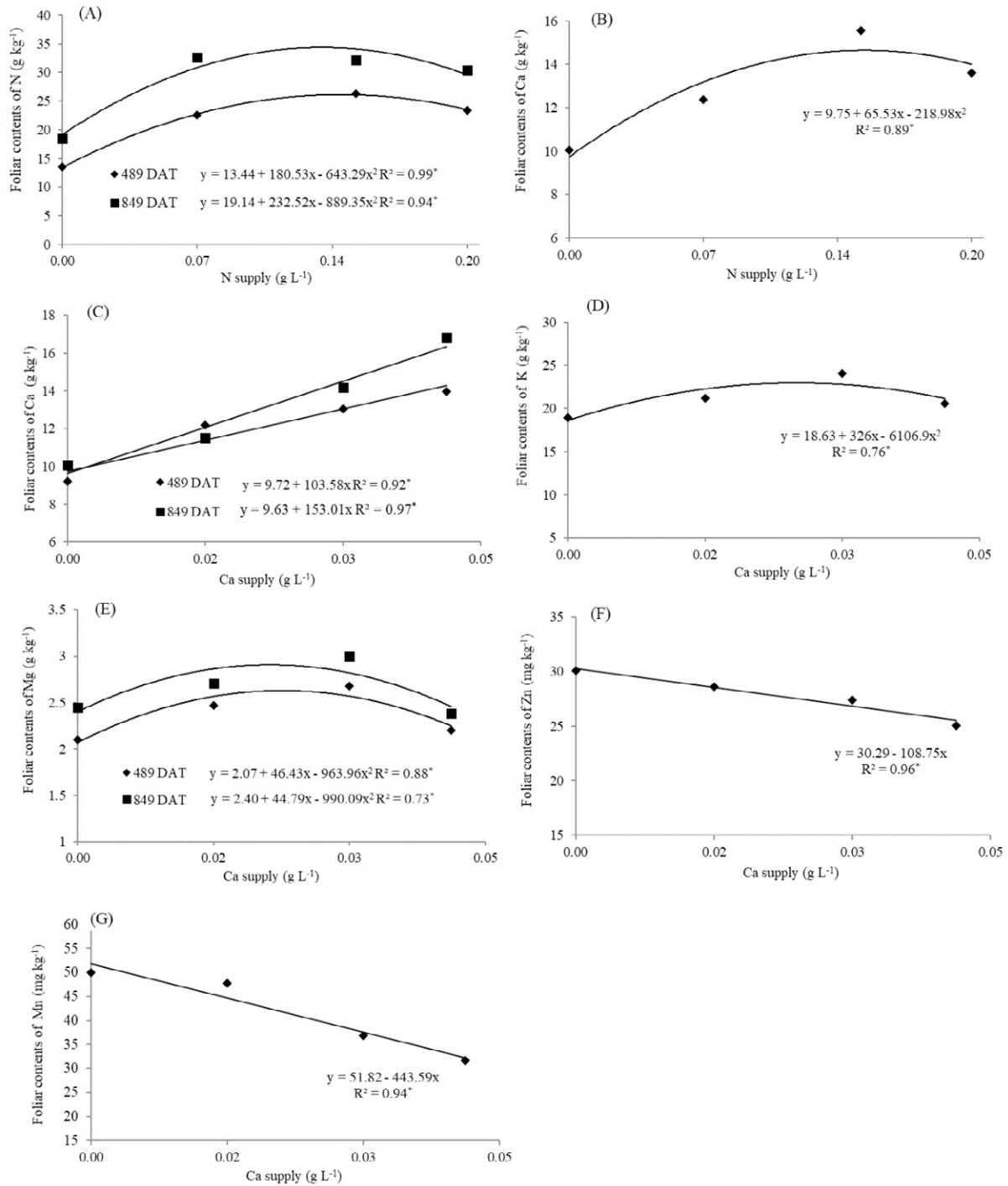


Figure 2. Gerberas foliar content of N (A) and Ca (B) in the function of N doses supply and contents of Ca (C), K (D), Mg (E), Zn (F), Mn (G) in the function of Ca doses supply. The N and Ca were supplied at 15 days intervals. *Significant at 5% probability.

These results are in accordance to those of Hulshof (2008) who indicated that N concentration ranged between 22 and 36 g kg⁻¹. These values are slightly higher than those suggested by Mercurio (2002) who mentioned value of 27 to 31 g kg⁻¹. In the control plants the average N contents was 13.44 g kg⁻¹ after 489 DOT and 19.14 g kg⁻¹ after 849 DOT (Figure 2A). These values indicated that the substrate was the source for N nutrient with low C/N ratio. In these plants, since the foliar N content was below the recommended for gerberas, symptoms of N deficiency could be visualized (smaller and yellowish leaves). There was not significant interaction between periods of retrieval of samples and doses of Ca for leaf contents of nitrogen. The analyses of Ca doses indicated that there was not significant polynomial adjustment. The average value of N was equal to 25.12 g kg⁻¹, which is in accordance to the recommendations for gerberas production. Malavolta (2006) stated that added Ca has no effect on the foliar contents of N.

The foliar content of Ca showed not significant interaction between periods of sample retrieval and doses of N. The regression analysis for N doses resulted in a significant second order adjustment (Figure 2B). With a dose of 0.15 g L⁻¹ of N at every 15 days the highest Ca contents was equal to 14.65 g kg⁻¹. The synergistic effect might be attributed to the elevation of the cationic exchange capacity of the roots promoted by N, allowing the plants to absorb more Ca (Malavolta, 2006).

On the other hand, the foliar content of Ca was influenced significantly by the interaction of the periods of sample retrieval and the Ca doses (Figure 2C). After 489 and 849 DOT, the leaves Ca contents was 13.86 g kg⁻¹ and 15.75 g kg⁻¹, respectively, at 0.04 g L⁻¹ of Ca applied every 15 days. These values are considered adequate for gerberas growth (Hulshof, 2008) and slightly below the lowest limit of 16.6 g kg⁻¹ of Ca which was indicated by Mercurio (2002).

In the control plants an average concentration of 9.68 g kg⁻¹ of Ca was found in the leaves, indicating that the substrate was the source of Ca for gerbera plants. In the control plants symptoms of Ca deficiency as described by Hulshof (2008) were observed (wrinkled and curled new leaves and afterwards chlorotic and with necrosis at the edges and apex).

For foliar content of P, not significant interactions between sample retrieval and N doses as well as the Ca doses were observed. Regression analyses for N and Ca doses showed not significant polynomial adjustment. The average foliar content of P was 2.98 g kg⁻¹. This value is considered adequate according to Mercurio (2002) and Hulshof (2008). They suggested values in the range of 1.9 up to 3.5 g kg⁻¹ and 2 up to 4 g kg⁻¹ of P, respectively.

Regarding foliar content of K, also not significant interaction between sample retrieval and doses of N and Ca were observed. In the analysis of N doses, not significant polynomial adjustment could be calculated. The average value for K was 23.19 g kg⁻¹. In the analysis for Ca doses, a second order adjustment was determined (Figure 2 D). A maximum content of 22.9 g kg⁻¹ of K was obtained with a dose of 0.03 g L⁻¹ of Ca substrate. At low concentrations of

Ca²⁺ a synergistic effect of K⁺ uptake takes place. Otherwise, at high Ca²⁺ concentrations a competitive inhibitory effect on K⁺ is observed (Malavolta, 2006). Therefore, the K concentrations reduced via a competitive inhibitory effect of Ca²⁺ ions as with increasing Ca concentrations a higher content of Ca in the leaves was determined while K contents reduced after a dose of 0.03 g L⁻¹ of Ca substrate.

Mercurio (2002) suggested that the appropriate K contents in gerberas were in the range of 31 to 36 g kg⁻¹. On the other hand, Hulshof (2008) proposed K contents between 32 up to 52 g kg⁻¹. The K concentrations determined in the present experiment remained below the recommended values. However, no deficiency symptoms were observed. Taiz and Zeiger (2013) describe K deficiency as marginal chlorosis in fully developed leaves that later could evolve to necrosis on leaf apex, leaf margins and between leaf ribs.

The foliar content of Mg showed not significant interaction of leaf sampling periods and doses of N. The average value for Mg in the leaves was 2.69 g kg⁻¹. Regression analyses showed not significant polynomial adjustment for Mg content. As for the Ca doses, a significant interaction between leaf sampling periods was observed. A second order adjustment for Ca doses on every sampling period was calculated for Mg content (Figure 2E). The maximum foliar content of Mg every 15 days interval at 0.02 g L⁻¹ of Ca substrate were 2.61 and 2.9 g kg⁻¹ after 484 and 849 DOT, respectively. Even in plants in which a foliar content of Mg is reduced, the element is considered in the adequate range for gerberas: 2.0-4.0 g kg⁻¹ (Hulshof, 2008).

The foliar content of S in the gerbera leaves showed not significant interaction of periods of leaf sampling and doses of either N or Ca. The regression analysis for N and Ca showed not significant polynomial adjustment. The average S content in the gerberas was 2.83 g kg⁻¹ and it is in the range (1.5-3.0 g kg⁻¹) that recommended by Hulshof (2008).

Significant correlations between foliar contents of some macronutrients were determined after 489 days or 849 days of cultivation. These actions indicated that one nutrient was positively affected the concentration of another nutrient in the leaves of gerbera plants. After 489 DOT, the correlations were: P and K (r = 0.56), P and S (r = 0.50) and Mg and S (r = 0.51). After 849 DOT, the correlations were: P and K (r = 0.75), P and Mg (r = 0.52), P and S (r = 0.91), K and S (r = 0.80) and Mg and S (r = 0.53).

Concerning sampling retrieval and of the tested doses of N and Ca, the macronutrients concentrations in the gerbera leave plants could be arranged in the descending order of N>K>Ca>P>S>Mg. This trend was differed than that observed by Damasceno et al. (2011) who stated the following order for macronutrients of K>N>Ca>Mg>P>S. These discrepancies are expected because nutrient content might vary depending on the cultivation conditions such as soil or substrate type, temperature, humidity and even the cultivar used (Hulshof, 2008). In addition, plants of different species or genotypes might impart different absorption rates because of anatomical dissimilarities.

Concerning micronutrients, for none-analyzed elements double interactions (sampling period versus

doses of N or Ca) were significant. Likewise, for none of the micronutrients a polynomial adjustment was observed either for N or Ca doses. The average Cu content was 9.34 mg kg⁻¹. These values in accordance with those suggested by Mercurio (2002) and Hulshof (2008) as adequate value which ranged from 8 to 12 mg kg⁻¹ and from 5 to 12 mg kg⁻¹ of Cu, respectively.

The average value of Fe in gerbera leaves in the present experiment was 308.41 mg kg⁻¹. There is no consensus about the ideal Fe concentration in gerberas. Mercurio (2002) suggested that a concentration of 450 to 500 mg kg⁻¹ of Fe while Hulshof (2008) found a range of 90 to 140 mg kg⁻¹ of Fe content in gerberas leaves.

For foliar Zn leaf content of Zn, the regression analysis showed not significant adjustment in response to doses of N. The average content of Zn in leaves was 26.73 mg kg⁻¹. In respect to Ca doses, a significant linear adjustment was viable (Figure 2F). The Zn contents of the gerbera leaves diminished as the Ca doses increases, which means that the Zn content was high in the control plants (30.29 mg kg⁻¹). Beyond that, a negative correlation was recorded ($r = - 0.69$) among leaf contents of Ca and Zn. According to Malavolta (2006) there is competitive inhibition of Ca²⁺ ions over Zn²⁺ ions. Furthermore, there is no agreement on the recommendations for ideal concentration of Zn in leaves. Mercurio (2002) suggested values of 6 to 8 mg kg⁻¹ of Zn. On the other hand, Hulshof (2008) found a range of 25 to 80 mg kg⁻¹ of Zn which is compatible with present experiment values.

Regression analyses of Mn contents showed not significant adjustment for N doses. The average value of Mn in gerbera leaves was 44.8 mg kg⁻¹. This concentration is equivalent to that recommended by Mercurio (2002) and Hulshof (2008) who indicated that Mn ideal values of 40 to 50 mg kg⁻¹ and 30 to 100 mg kg⁻¹, respectively. Ca doses presented a linear negative adjustment (Figure 2G). Increases Ca doses resulted in reduced Mn contents in the leaves. Malavolta (2006) stated that Mn²⁺ has similar chemical properties of the Ca²⁺ ion so Ca ions leads to lower absorption of Mn, justifying the negative correlation ($r = - 0.80$) of Ca and Mn contents.

There was also a significant correlation between the contents of Zn and Mn at the retrieval of leaf tissues after 489 ($r = 0.94$) and after 849 ($r = 0.88$) days after transplanting. These correlations pointed out the positive relation of these micronutrients in the gerbera leaves.

For foliar content of B, no adjustment resulted statistically significant was found for neither N nor Ca supply to the plants. The average value for B contents was 40.7 mg kg⁻¹, which is similar to that obtained by Hulshof (2008) since it ranged from 20 to 50 mg kg⁻¹.

Referring to the different doses of N and Ca, the concentrations of micronutrients in the leaves of the gerbera plants followed the descending order of Fe>Mn>B>Zn>Cu. This sequence is similar to the conclusion of Guerrero et al. (2016).

Conclusions

Regardless of the leaf sampling period and the different doses of N and Ca applied every 15 days to gerbera cultivated in containers as cut flower, the leaf contents in descending order at the end of the first and second year of production were: N>K>Ca>P>S>Mg, e: Fe>Mn>B>Zn>Cu.

Author Contribution

MM: responsible for the implementation, conduct, evaluation of the experiment and writing of the scientific article. **EMP**: responsible for the implementation, conduct, evaluation of the experiment and writing of the scientific article. **WH**: responsible for the implementation, conduct, evaluation of the experiment and writing of the scientific article. **GS**: advisor, participating in the conception and planning of the experiment and in the correction of the scientific article. **RJB**: advisor, participating in the conception and planning of the experiment and in the correction of the scientific article.

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