Macronutrient Omission Affects the Seedling Performance of *Eugenia dysenterica* DC., an Important Fruiting Species of the Cerrado Biome

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Abstract

Cagaita (*Eugenia dysenterica* DC.) seedling production is currently important for the restoration of degraded areas of the Cerrado biome and to supply the demand for its fruits for regional food production. The plants of Cagaita have been described in the literature as resistant to nutrient-poor soils, but the studies remain inconclusive. Therefore, we tested the hypothesis that initial *E. dysenterica* seedling development could be affected by the lack of specific nutrients. We planted seedlings of this species in complete hydroponic solution and under individual macronutrient omission of N, P, K, Ca, Mg and S. The development of macronutrient deficiency symptoms was visually monitored for 120 days after transplantation in response to the treatment solution, and growth characteristics and tissue contents of different macronutrients were determined. The omissions of Mg and P were the treatments with the strongest effects on seedling growth. The most commonly observed deficiency symptoms were chlorosis and necrosis, in addition to leaf purpling (P deprivation), impaired root hair development (Ca and S deprivation) and a reduced number of leaves (Mg and P deprivation). The macronutrients Ca and N were most accumulated in the shoots and roots of plants subjected to complete nutrient solution, showing the importance of the availability of these nutrients in the initial stages of seedling development for the production of healthy Cagaita plants, with greater viability in the field.

Keywords: nutrient deprivation, hydroponics, growth, nutrient stress

1. Introduction

Eugenia dysenterica DC., commonly known as Cagaiteira, is a fruiting species that is native to the Cerrado and has potential for cultivation in traditional farming systems (*e.g.*, Camilo et al., 2013). Interest in this species has been increasing due to its ornamental and economic value (Almeida, 1998). The latter is related to the direct use of its fruits as raw materials in the food industry (*e.g.*, Santos et al., 2012) or of its wood to supply fence posts or cork. The chemical composition and nutritional value of the fruits, due to the levels of vitamins, minerals and phenolic and antioxidant compounds, have attracted the attention of researchers in the area (Sousa et al., 2018). Recently, polyphenols extracted from plants of the Myrtaceae family and specifically *E. dysenterica* were found to be effective in attenuating dyslipidaemia and inflammation (Donado-Pestana et al., 2018a; Donado-Pestana et al., 2018b). Because this plant is virtually widespread throughout the Cerrado, particularly in the central Cerrado region, its seedlings have been used in forest restoration programmes in this biome. Some studies have also demonstrated the medicinal potential of fruits (*e.g.*, Moraes et al., 2008), latex (*e.g.*, Marinho et al., 2011), leaf extracts (*e.g.*, Silva et al., 2011) and juice essential oils of species of the *Eugenia* genus (*e.g.*, Ogunwande et al., 2005; Santos et al., 2004).

Although there is a potential and growing market for fruits of regional interest, *E. dysenterica* remains underexploited, and its fruits are used only for extractive and predatory functions (Ribeiro et al., 2008). The impact on the population structure of this species throughout the Cerrado has been confirmed by genetic studies, which have indicated a reduced and geographic distribution-dependent gene flow (Telles et al., 2001; Zucchi et al., 2005). Therefore, the large-scale production and distribution of healthy *E. dysenterica* seedlings for the establishment of commercial farming areas play an important role not only in the generation of income but also in the implementation of conservation strategies, decreasing pressure on areas of natural occurrence of this

species. Currently, however, limited knowledge on the nutrient requirements of *E. dysenterica* plants in the initial stages of their growth has hindered the production of seedlings of this species and, thus, their establishment in the field. Deficiency or excess problems are frequent due to nutrient application without scientific criteria (Resende et al., 2010).

Although Naves et al. (2002) have suggested that *E. dysenterica* plants show high adaptation to nutrient-poor soils, with high acidity, the presence of toxic aluminium and a large water deficit in some months, studies have shown that the type of soil on which the population grows may affect the tree phenotype (Telles et al., 2001) and that the occurrence of *E. dysenterica* in Cerrado areas may be strongly affected by the soil patterns (mineral occurrence) (Coelho et al., 2016). These studies indicate, therefore, that some nutrient limitations or even excesses may not be tolerated by *E. dysenterica* seedlings.

An understanding of the adequate macronutrient demand, especially during the critical seedling production phase, which is the initial development stage, is a fundamental step in this process (Munson, 2018) because it may guarantee the large-scale production of healthy seedlings with the potential to withstand adverse field conditions (Bessa et al., 2016). Nutrients affect plant growth because they work as the building blocks of organic matter, cofactors or signalling molecules (Coruzzi & Bush, 2001). Thus, macronutrient deficiency or even toxicity promote metabolic disorders. A shortage of K, for example, drastically reduces plant growth and productivity (Hafsi et al., 2014), whereas a lack of Mg affects the activity of several enzymes and the structural stabilisation of tissues (Guo et al., 2016). Conversely, a shortage of Mn directly affects the integrity of photosystem II (Schmidt et al., 2016). The metabolic problems generated by nutrient limitations, or even toxicity, manifest as symptoms, especially if the nutritional disorder is severe. These symptoms include reduced growth, leaf yellowing and/or purpling, necrosis or distorted growth of plant parts (Fernández-Escobar et al., 2016). Each mineral nutrient induces specific symptoms. Thus, visual symptoms help to identify nutritional disorders in plants. Visual diagnosis, however, may become rather complicated when more than one nutrient is deficient or when the deficiency of one nutrient is induced by the excess of another (Marschner, 2012). Therefore, a successful diagnosis also requires the analysis of plant anatomical and physiological traits and of data on growing and climatic conditions (Shear & Faust, 1980). Thus, knowledge of symptoms, as a complementary tool in the diagnosis of deficiencies or toxicity, has great practical importance for the identification and rapid resolution of nutritional problems.

To test the hypothesis that the initial growth of *E. dysenterica* seedlings may be affected by the absence of specific macronutrients, we used the hydroponic method, which makes it possible to control the nutrients in nutrient solution, to test the effect of macronutrient omission of the growth and nutrient balance of these seedlings and to observe the visual symptoms triggered by these omission conditions.

2. Materials and Methods

2.1 Seedling Production and Growth

E. dysenterica fruits were collected from a natural population of this species located in a stretch of Cerrado *sensu stricto* at Gameleira Farm, municipality of Montes Claros, Goiás (16°06′20″ S and 51°17′11″ W at 592 m above sea level). Because the seeds of this species are typically recalcitrant (*e.g.*, Silva et al., 2017b), they were removed from the fruits by manual pulping and immediately placed in plastic trays with autoclaved sand. At 30 days after sowing, the seedlings were transferred to the hydroponic cultivation system, planted in aerated plastic pots (8-L capacity) and kept in a greenhouse.

During the first 30 days in hydroponic solution, the seedlings were grown in Hoagland and Arnon (1950) nutrient solution at ½ ionic strength of the treatment solutions. After this adaptation period, these solutions were replaced with the treatment solutions consisting of complete nutrient solution or nutrient solution with individual nutrient omission (N, P, K, Ca, Mg and S).

The solutions were prepared with deionised water, adjusting the pH to 5.5±0.5, which was corrected daily with HCl and NaOH solutions. The solutions were always replaced when they reached 30% initial electrical conductivity.

The experimental design was completely randomised, and 06 macronutrient omission treatments and a control treatment, consisting of complete nutrient solution, were tested. Each treatment was assessed in four replicates, each represented by a pot with two seedlings, totalling 56 experimental units.

2.2 Nutrient Deficiency Symptoms

The plants were observed daily to detect visual symptoms of physiological disorders. The seedlings were periodically photographed to describe the appearance of macronutrient deficiency using a qualitative method.

2.3 Vegetative Growth and Macronutrient Content

Growth was biometrically assessed at 30, 60, 90 and 120 days after transplanting (DAT) the seedlings in the hydroponic system. The study parameters were stem and root length, stem diameter and number of leaves. After the last study period, the plants were separated into root, stem and leaves and dried in a convection oven at 65 ºC to a constant weight to determine the dry matter weight.

After drying, the plants were ground in a Wiley mill with a 20-mesh sieve to determine the root and shoot content of N, P, K, Ca, Mg and S, according to the method of Malavolta (1997).

2.4 Statistical Analysis

The calculated means of quantitative parameters were subjected to analysis of variance and the Tukey test at 5% probability using the software environment R 3.4.4 (R Core Team, 2018).

3. Results

3.1 Deficiency Symptoms and Macronutrient Content

During all assessment stages, from 30 to 120 DAT, *E. dysenterica* plants grown in complete nutrient solution (with macronutrients) developed healthy leaves without symptoms of malnutrition. In these plants, the roots also developed normally, indicating effective nutrient uptake from the solution (Figure 1).
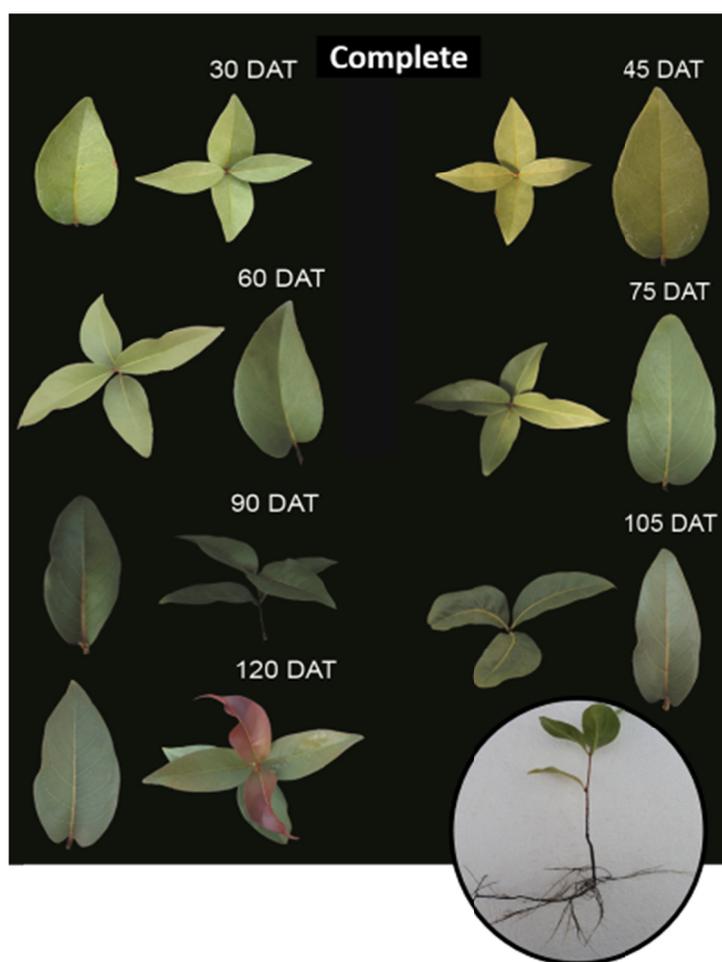

Figure 1. Visual appearance of leaves and shoots of *Eugenia dysenterica* DC. seedlings grown in a hydroponic system using complete nutrient solution (with N, P, K, Ca, Mg and S). Records were acquired at 30, 45, 60, 75, 90, 105 and 120 days after transplantation (DAT) into the nutrient solution

Regarding symptoms of nutrient deficiency in plants subjected to macronutrient omission treatments, N deficiency symptoms were the first to appear (detected at 30 DAT). Widespread chlorosis was observed in the older leaves, progressing to initial necrosis detected at 45 DAT. At 75 DAT, chlorosis/necrosis became more

frequent in plants and also occurred in younger leaves. Necrosis was always observed first in the leaf apex (region farthest from the petiole) and then spread to the blade. The roots, especially in the last two study periods (105 and 120 DAT), showed a high incidence of root hairs and reduced pigmentation (lighter tissues tending towards opacity) (Figure 2a).
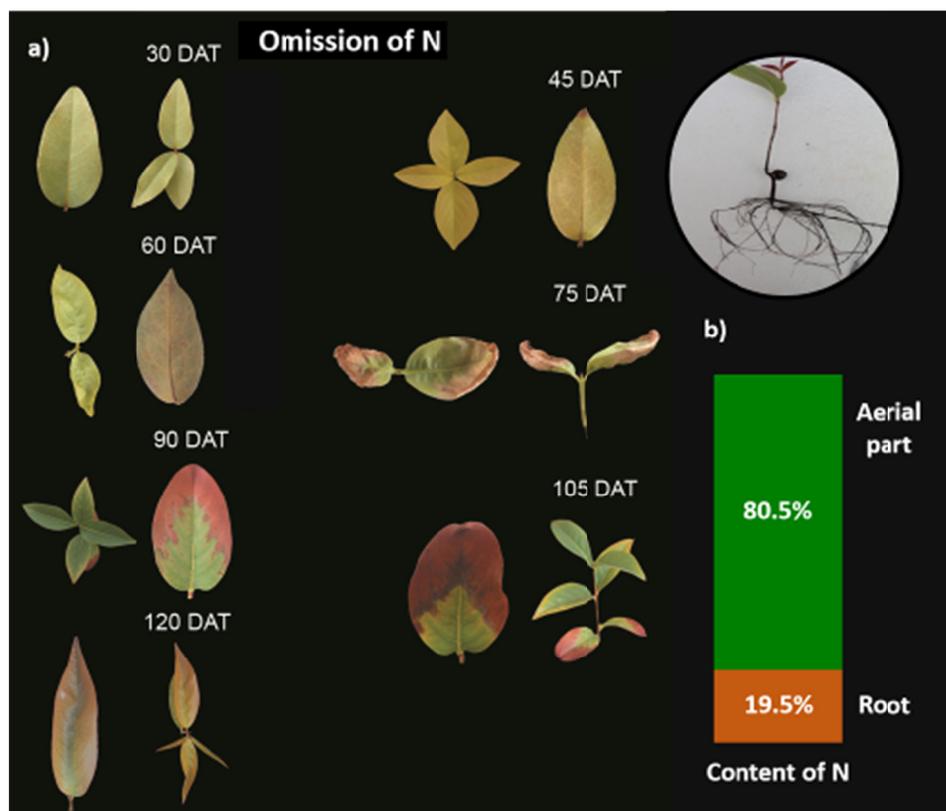

Figure 2. Visual aspect of N deficiency symptoms in leaves and shoots of *Eugenia dysenterica* DC. seedlings grown in a hydroponic system using nutrient solution without N. Records were acquired at 30, 45, 60, 75, 90, 105 and 120 days after transplantation (DAT) into the nutrient solution (a). Shoot and root N partitioning of plants subjected to N omission (b)

Plants grown under N omission primarily accumulated N in shoots (80.5%), and a small portion of this nutrient was allocated to roots (Figure 2b).

Ca deficiency in *E. dysenterica* roots was observed starting at 45 DAT with the onset of the first chlorotic spots (Figure 3a), although the leaf curling symptom was only detected in younger leaves starting at 75 DAT, followed by chlorosis and subsequent necrosis of these leaves. The roots showed the development of a long primary root, with few secondary roots. These roots were very dark, with no root hairs, especially starting at 60 DAT. Most of the Ca that accumulated in plant tissues was detected in shoots (65.3%; Figure 3b).
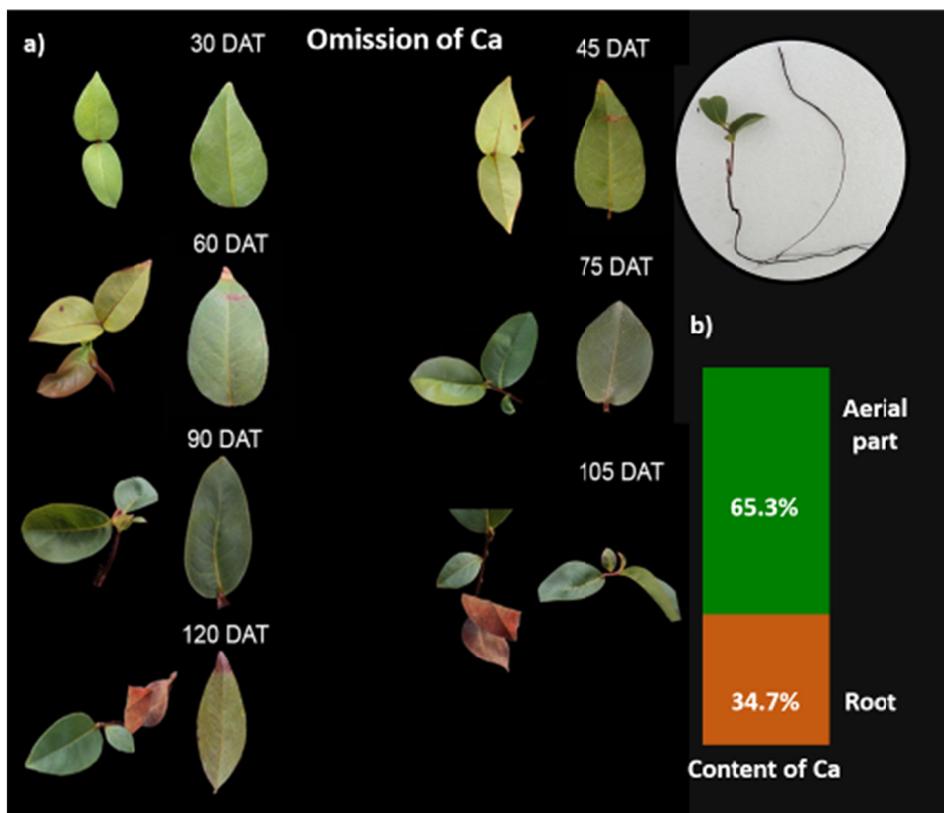
Figure 3. Visual aspect of Ca deficiency symptoms in leaves and shoots of *Eugenia dysenterica* DC. seedlings grown in a hydroponic system using nutrient solution without Ca. Records were acquired at 30, 45, 60, 75, 90, 105 and 120 days after transplantation (DAT) into nutrient solution (a). Shoot and root Ca partitioning of plants subjected to Ca omission (b)

Leaf symptoms of K deficiency were observed starting at 45 DAT. The seedlings developed necrotic spots throughout the blade, although the leaves developed yellowing symptoms only at 90 DAT, starting from the margin and progressing towards the midrib (Figure 4a). At 105 DAT, necrotic points became spots, and the roots were long, highly pigmented and had root hairs. Regarding K partitioning, 70% of this element was found in shoots (Figure 4b).
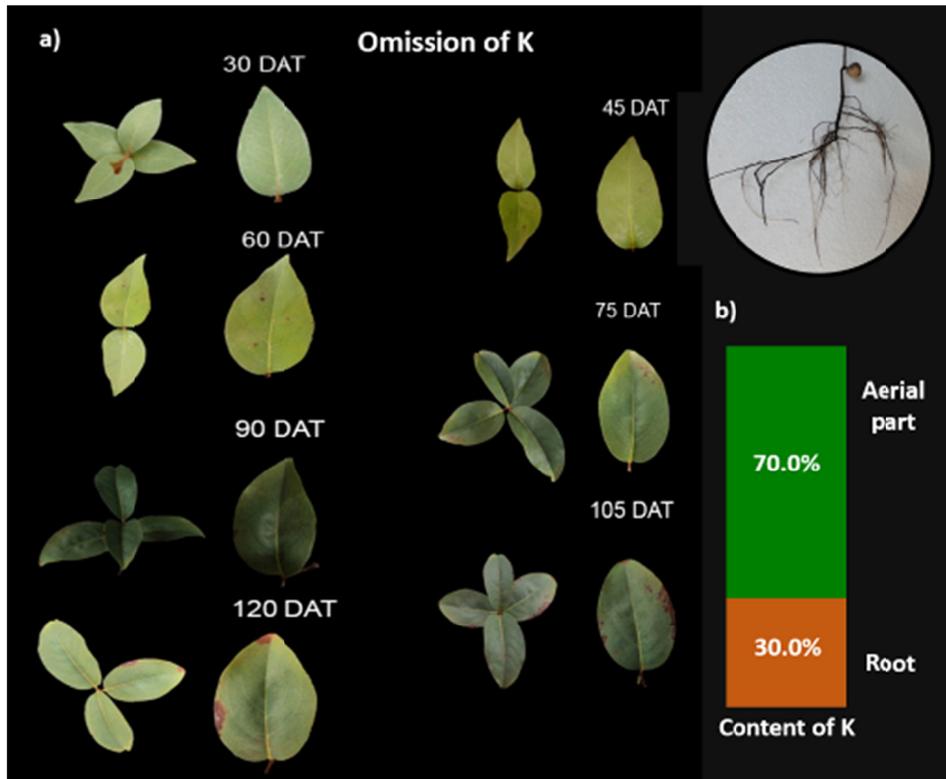
Figure 4. Visual aspect of K deficiency symptoms in leaves and shoots of *Eugenia dysenterica* DC. seedlings grown in a hydroponic system using nutrient solution without K. Records were acquired at 30, 45, 60, 75, 90, 105 and 120 days after transplantation (DAT) into nutrient solution (a). Shoot and root K portioning of plants subjected to K omission (b)

Symptoms of P deficiency also became visible starting at 75 DAT when the older leaves developed purpling. This purpling was followed by leaf necrosis (Figure 5a). These plants also showed stunted stem growth with a reduced number of leaves and leaf nodes. Most tissue P was detected in roots (81.4%), and only a small part was allocated to shoots (18.6%; Figure 5b).
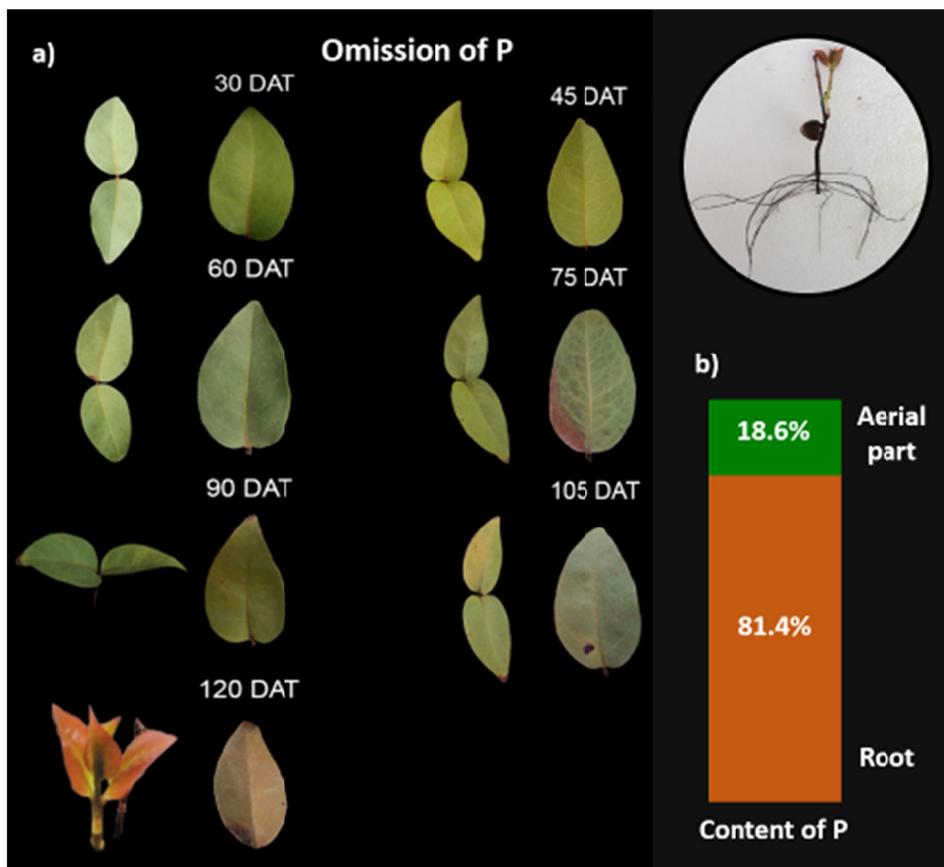
Figure 5. Visual aspect of P deficiency symptoms in leaves, shoots and roots of *Eugenia dysenterica* DC. seedlings grown in a hydroponic system using nutrient solution without P. Records were acquired at 30, 45, 60, 75, 90, 105 and 120 days after transplantation (DAT) into nutrient solution (a). Shoot and root P partitioning of plants subjected to P omission (b)

Mg deficiency symptoms were detected at 75 DAT when the leaves showed widespread yellowing. Necrotic points were also detected throughout the blade. The leaves showed a pale appearance starting at 75 DAT and a marked decrease in plant growth with a reduced number of leaves and leaf nodes. The roots showed little branching with reduced secondary root development (Figure 6a). Most tissue Mg was found in the shoots of these plants (66.7%; Figure 6b).
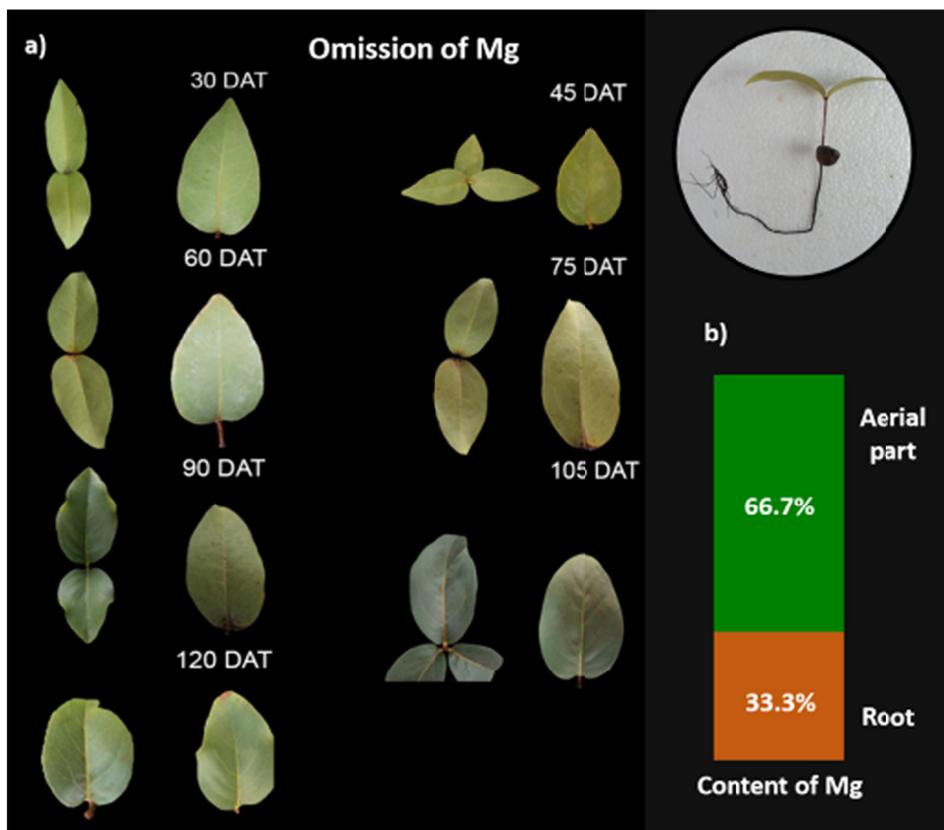
Figure 6. Visual aspect of Mg deficiency symptoms in leaves and shoots of *Eugenia dysenterica* DC. seedlings grown in a hydroponic system using nutrient solution without Mg. Records were acquired at 30, 45, 60, 75, 90, 105 and 120 days after transplantation (DAT) into nutrient solution (a). Shoot and root Mg partitioning of plants subjected to Mg omission (b)

A deficiency of S was detected in the initial period of exposure to omission (30 DAT), although necrosis was only observed starting at 105 DAT. The deficiency in this nutrient caused leaf fall and compromised the formation of secondary roots and root hairs (Figure 7a). Regarding the tissue partitioning of accumulated S, the highest percentage (71.4%) was detected in the roots of plants subjected to the S omission treatment (Figure 7b).
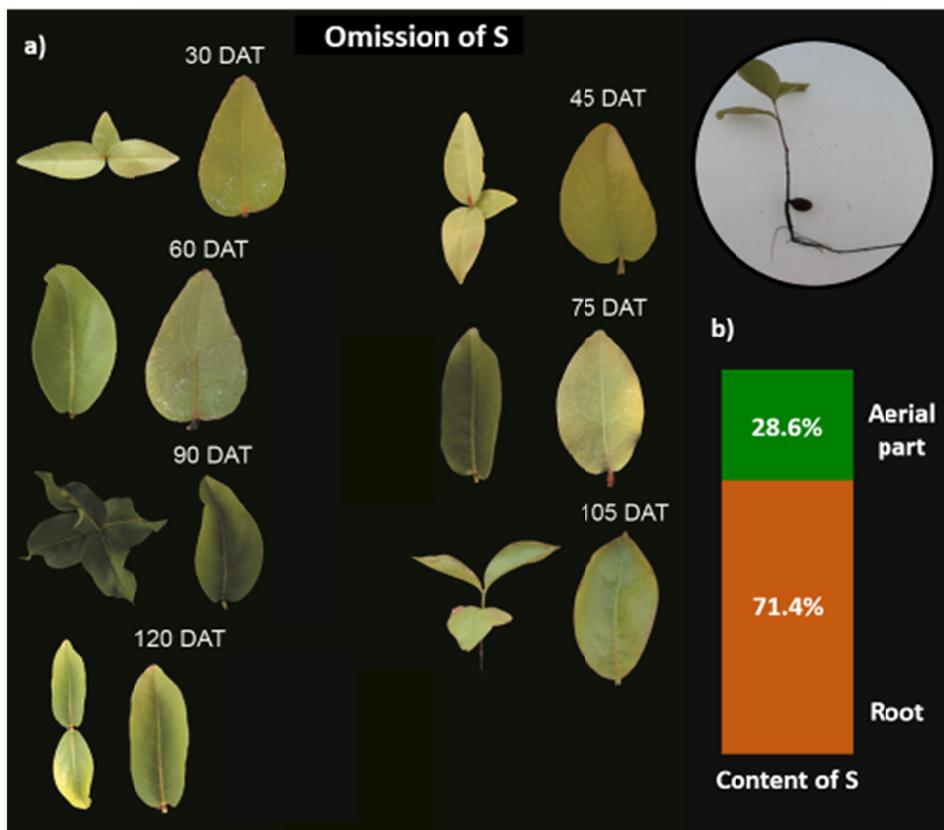
Figure 7. Visual aspect of S deficiency symptoms in leaves and shoots of *Eugenia dysenterica* DC. seedlings grown in a hydroponic system using nutrient solution without S. Records were acquired at 30, 45, 60, 75, 90, 105 and 120 days after transplantation (DAT) into nutrient solution (a). Shoot and root S partitioning of plants subjected to S omission (b)

The deficiency symptoms most commonly observed in *E. dysenterica* seedlings were chlorosis and necrosis (Figure 8). Each symptom was observed in plants subjected to the absence out of 06 nutrients tested. Only plants grown in the absence of P developed purple leaves, whereas the absence of P and Mg visibly affected the number of leaves. The development of root hairs was affected by the lack of Ca and S.
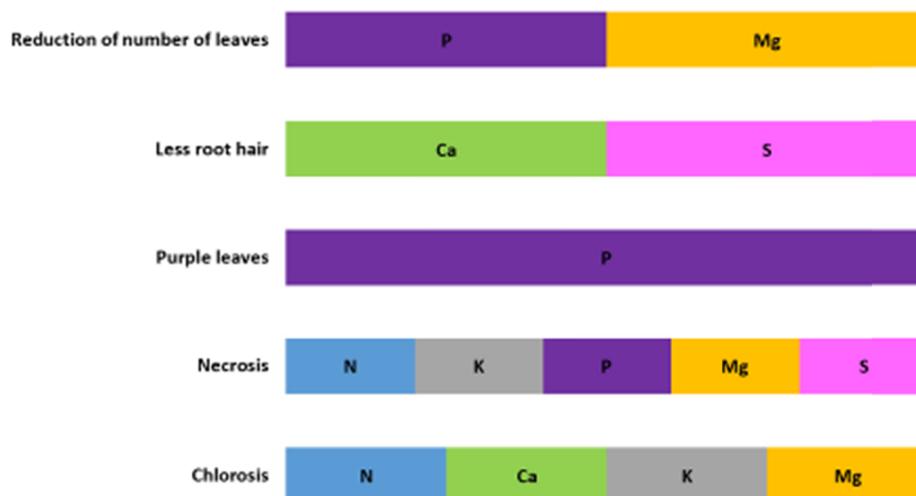

Figure 8. Main visual symptoms of nutritional deficiency observed in *Eugenia dysenterica* DC. seedlings grown in a hydroponic system using nutrient solution without N, P, K, Ca, Mg and S. The tables outline the nutrient omission treatment in which the aforementioned symptoms were assessed during seedling development

3.2 Vegetative Growth

Throughout the production cycle of *E. dysenterica* seedlings, Mg and P omission treatments had the strongest effects on stem development, showing the lowest mean stem lengths at 120 DAT (4.63 and 4.47 cm, respectively). Conversely, the mean stem length observed in response to treatment with complete nutrient solution was 6.86 cm (Figure 9a). Unexpectedly, N omission had no apparent effect on stem growth, and the mean stem length in this treatment (7.16 cm) was even higher than that observed in the treatment with complete nutrient solution, albeit with a large variation in stem length values, ranging from 3.8 cm to 11 cm.
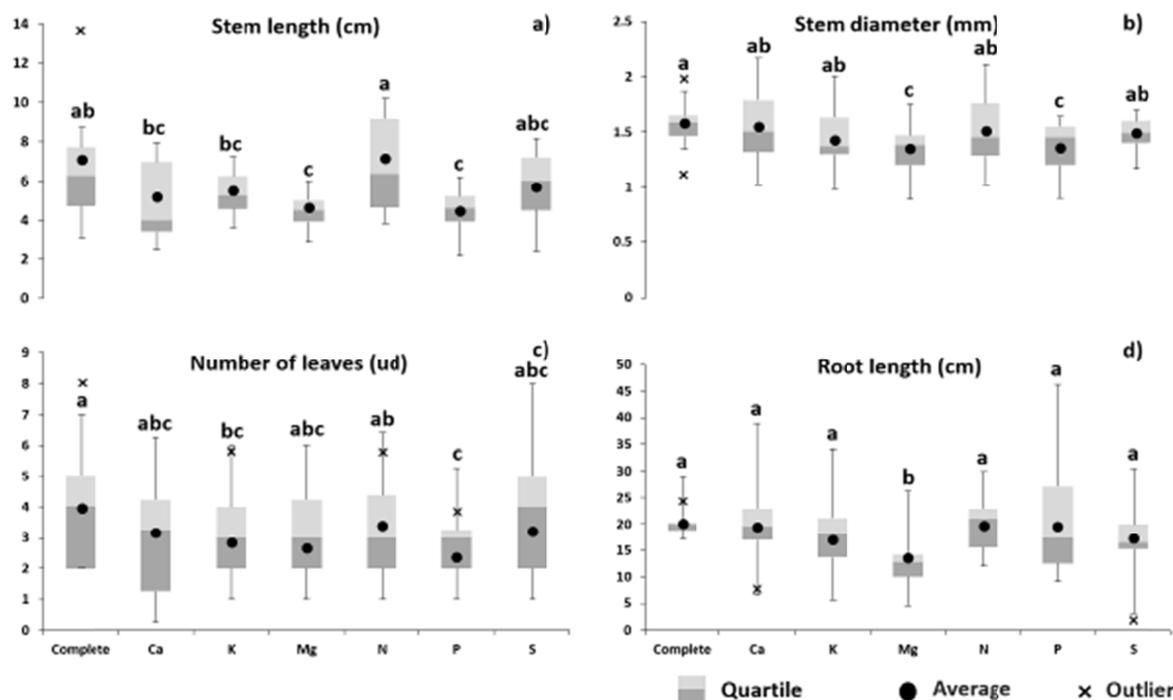

Figure 9. Effects of macronutrient omission on stem length (cm), stem diameter (mm), number of leaves and root length (cm) of *Eugenia dysenterica* DC. seedlings grown in a hydroponic system with complete nutrient solution or without Ca, K, Mg, N, P or S. Data collected at 120 days after transplantation (DAT). *Letters above black spots compare omission treatments, according to the Tukey test (5% probability)

The highest mean stem diameter was observed in the treatment with complete nutrient solution (1.58 mm mean stem diameter). Therefore, the omission of macronutrients affected the development of this structure. Similar to stem length, the lack of Mg and P ions limited stem development, and stems with a mean diameter of 1.35 and 1.36 mm, respectively, were observed in the absence of these ions (Figure 9b). The P omission treatment also affected leaf development. At 120 DAT, plants subjected to this treatment showed a mean of 2.4 leaf units, whereas plants grown in complete nutrient solution showed a mean of 3.5 leaves in this period (Figure 9c). Following the same effect pattern, Mg omission inhibited root growth. At 120 DAT, the mean root length was 13.5 cm in plants subjected to this treatment, and the mean root length was 20.12 cm in plants grown in complete nutrient solution (Figure 9d).

The values of nutrients that had accumulated in the shoots of plants grown in complete nutrient solution were arranged in the following decreasing order: Ca > N > K > S > Mg > P, and the contents of this macronutrient accumulated in the roots of plants grown in complete nutrient solution were arranged in the following decreasing order: N > Ca > K > P > Mg > S.

4. Discussion

This study reports, for the first time, that several visual characteristics may be used to detect macronutrient deficiencies in *E. dysenterica* seedlings. Purple leaves, for example, may indicate low P availability. Flavonoid accumulation in leaf tissues, especially red/purple anthocyanins, is used as an indicator of changes in plant metabolism due to nutrient deficiency (Stewart et al., 2001). Studies have shown that P deficiency may stimulate

secondary metabolism pathways related to the synthesis of bioactive compounds, such as essential oils and flavonoids (*e.g.*, Jia et al., 2015; Valentinuzzi et al., 2015). Our data indicate that *E. dysenterica* plants also react to P omission by accumulating anthocyanins in their leaves. According to Pourcel et al. (2013), anthocyanins are key regulators of stress responses and plant development.

The presence of chlorosis or necrosis, which generally manifested as leaf spots or points, was the most common nutrient deficiency symptom in *E. dysenterica* seedlings. Chlorosis may be explained by two causal factors. First, under deficiency, nutrients that are already allocated to leaves are transported to other plant parts, and these nutrients are described as phloem-mobile. In such cases, chlorosis usually starts in younger leaves (Uchida, 2000). A second reason for chlorosis may be the low nutrient concentrations, which are unable to meet the leaf nutrient demands for growth because the quantity already allocated to leaves is insufficient. In such cases, chlorosis is particularly detected in younger leaves, which are deficient in phloem-immobile or poorly mobile nutrients. The development of necrosis may have merely represented a worsening of the chlorosis symptom, as occurs under N deficiency, although necrosis may be caused by the accumulation of basic amino acids (ornithine, citrulline and arginine) in other cases. These amino acids are decarboxylated, thereby increasing the concentration of putrescine (Ruiz & Moyano, 2017; Epstein & Bloom, 2006), a nitrogenous compound that is toxic to plants and responsible, for example, for the necrotic spots that appear on K-deficient leaves (*e.g.*, Costa et al., 2017; Silva et al., 2017a).

Although studies suggest that *E. dysenterica* plants are highly adapted to soils with medium or low (Naves et al., 2002) fertility, our results confirmed the hypothesis that seedling growth of this species may be affected by the unavailability of specific nutrients. This effect is unsurprising because the lack of nutrients, either macronutrients, significantly alters nutrient uptake and transport in plant cells, primarily affecting proteins, which play a key role in deficiency-compensation responses (Nouri et al., 2016). However, our results showed that growth characteristics in *E. dysenterica* plants are primarily affected by limitations in the macronutrients Mg and P. After K, Mg is the second most abundant cation in plant cells. Many physiological processes, such as enzymatic activities and ribosomal subunit aggregation, depend on Mg (Gerendás & Führs, 2013). In plants, Mg is the central atom of chlorophyll molecules. More than 300 enzymes are Mg-dependent, and changes in the concentration of Mg significantly affect the membrane potential. Kobayashi and Tanoi (2015) reported that starch accumulation and the development of chlorosis associated with reactive oxygen species generation occur in young and mature leaves under Mg deficiency. These problems may further affect photosynthesis and lead to a decrease in biomass. Sugar often accumulates in leaves under Mg deficiency because this nutrient is involved in carbohydrate transport from source to sink organs (Farhat et al., 2016). In turn, Peng et al. (2015) demonstrated that the levels of the photosynthesis-related enzymes ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO), RuBisCO activase and ferredoxin-NADP(+) reductase significantly decline under Mg deficiency.

E. dysenterica seedling growth was affected by the lack of P, most likely because, under this condition, plants experience drastic cellular changes in metabolism, physiology, hormonal balance and gene expression (Ha & Tran, 2014) because this is an essential nutrient involved in several fundamental biochemical processes, such as lipid metabolism and nucleic acid and cell membrane biosynthesis.

N, P, K, Ca, Mg, S, B, Cu, Zn and Mo differentially accumulated in *E. dysenterica* plant shoots, most likely due to competition or inhibition effects between nutrients because the presence of one element may affect plant uptake of another due to competition for the same absorption site (Epstein & Bloom, 2006). Several shoot and root nutrient concentrations were higher in plants subjected to omission treatments than in plants grown in complete nutrient solutions, indicating an actual metabolic adjustment of *E. dysenterica* plants to adapt to and survive the stress caused by nutrient deficiency. According to Kulcheski et al. (2015), plants develop physiological and even molecular adaptive responses to cope with the lack of a nutrient, increasing the uptake of a specific ion in the absence of another. Knowledge concerning these adaptation mechanisms may lead to the development of important tools for plant improvement. Recent studies have shown, for example, the involvement of noncoding microRNAs in stress responses, including nutritional stress (Kulcheski et al., 2015; Jeong & Green et al., 2013).

Plants that were grown in complete nutrient solution accumulated higher concentrations of Ca and N in shoots and roots. These nutrients are highly demanded by perennial and deciduous tree species, such as *E. dysenterica*. Aerts and Chapin (2000) showed that deciduous perennial species store more N in leaves than perennial evergreen, herbaceous or grass species, whereas Martre et al. (2003) modelled N accumulation and protein composition in wheat plants and generated key indicators showing that plant N accumulation is primarily regulated by the source. Our data corroborate these findings because plants grown in complete nutrient solution had higher values of the variables mean number of leaves and root length. Because leaf carbon accumulation is

linearly related to leaf N content (Evans, 1989), plants in the initial growth stage, such as those evaluated in this study, must demand high N amounts to regulate carbon metabolism and amino acid and protein synthesis (Kulcheski et al., 2015). In a previous study, Bessa et al. (2016) found, at 180 DAT, that *E. dysenterica* seedlings demanded a high N and Ca supply. *Anacardium othonianum* Rizz., another fruit tree species native to the Cerrado, also showed an increase in N and Ca accumulation relative to other nutrients at 180 DAT (Bessa et al., 2013). In this study, Ca was essential for seedling growth. According to Tang et al. (2017), this element usually accounts for 0.1 to 5% of plant shoot dry matter. Although this element is abundant in nature, its deficiency occurs in rapidly growing tissues because it is an essential component of cell walls and of other cellular structures (Yamamoto et al., 2011).

5. Conclusion

Limitations of the macronutrients Mg and P are weakly tolerated by *E. dysenterica* seedlings. Therefore, farmers growing seedlings of this fruit tree should supply these nutrients. Chlorosis and necrosis symptoms are commonly observed in plants of this species under macronutrient deficiency and therefore can be used by seedling growers as signs of inaccessibility to nutrients. Plants grown in complete nutrient solution accumulated the highest shoot and root contents of the macronutrients Ca and N, thus demonstrating the importance of the availability of these nutrients in the initial stages of seedling development in this species for the production of healthy plants, representing a strategy to maintain higher numbers of this fruit tree in the Cerrado biome.

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