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Growth, nutrient concentration and principal component analysis of Cagaita (*Eugenia dysenterica* DC.) seedlings grown in nutrient solution

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Abstract

Cagaita (*Eugenia dysenterica* DC.) is a native fruit tree with high economical potential from the Brazilian Cerrado. However, little is known about the essential nutritional demands of its seedlings. To determine the nutrient demands of Cagaita, a greenhouse experiment was performed, in which plants were grown under hydroponic condition to assess the growth (length and diameter of stems, number of leaves, number of nodes, volume and length of roots, area of the leaf and crown and total dry weight of the leaves, stems and roots) and nutrient concentration (N, P, K, Ca, Mg, S, B, Cu, Fe, Mn and Zn) at different time points after the plant were transferred into a nutrient solution. The seedling growth presented linear behavior until 180 days after transplantation. The total plant dry weight was 6.54 g after 180 days of transfer into the nutrient solution. The N content was positively correlated with the total dry weight and leaf area, whereas B was negatively correlated with the length of the stem and number of leaves. Macro and micronutrient concentrations presented the following order: N>Ca>K>P>Mg>S, Fe>Mn>B>Zn>Cu. A principal component analysis of the different sampling times provided important information used to define the growth variables.

Keywords: fruit tree; greenhouse; hydroponics; macronutrient; micronutrient.
Abbreviations: DAT_days after transfer, SD_stem diameter, SL_stem length, RV_root volume, RL_root length, NL_number of leaves, NN_number of nodes, LA_leaf area, CA_crown area, LDW_leaf dry weight, SDW_stem dry weight, RDW_root dry weight, TDW_total dry weight, PCA_principal component analysis

Introduction

The native fruit tree species of the Cerrado have high potential for cultivation in traditional systems. One of such a trees is the cagaita (*Eugenia dysenterica* DC.), a fruit tree belonging to the family Myrtaceae. The cagaita presents slow growth and medium heights, reaching 5 m on average at 12 years of age. It primarily grow in the central regions of Brazil and has ornamental, industrial and economic potential (Souza et al., 2008).This species is found in grouped populations, especially in Red-Yellow Latosols that have higher average contents of potassium, calcium, magnesium, manganese and clay compared with other soils, where this species does not occur (Duarte et al., 2006). Although there is a growing potential market for this fruit tree, it is not currently exploited by farmers, which is mainly because of the lack of scientific knowledge on the species because its fruits are primarily collected in an extractive and predatory manner. One of the limiting factors for the production of this species is the large-scale production of seedlings and establishment of orchards because there is a limited knowledge on the methods of fertilization, and problems have been observed in relation to nutrient deficiencies or excesses caused by fertilization applied without scientific criteria (Resende et al., 2010). The application of fertilizers in high doses to reach high crop yields is common. However, this practice results in the low use efficiency of the nutrients present in the chemical fertilizers (Zheng et al., 2014). These nutritional demands are limiting factors for the production of native plants, especially for fruit trees such as *E. dysenterica*. To establish quality seedlings in the field, the appropriate nutritional demand must be determined, especially during the plant's early development. This stage is fundamental for seedling establishment and may enable the large-scale acquisition of trees such as cagaita by farmers, which may occur through production by the farmer or by acquisition through nurseries of native fruit tree species. Many of native trees such as cagaita have potential to endure adverse field conditions and commercialization and have contributed to the recovery of degraded areas.

In general, nutrient uptake follows the pattern of growth curves (dry weight accumulation). The nutrient uptake rates can be expressed as response curves according to plant age. This can also be used to indicate the periods when nutrient uptake is higher and additional nutrients are required. Nutrient response curves are; therefore, important tools for crop management and fertilization (Vidigal et al., 2009). Plant species present specific nutritional demands, and the uptake of different nutrients varies during different vegetative stages. In the present study, we attempted to clarify the cultivation seasons of cagaita plants that have the greatest effect on growth, biomass accumulation and macro and

micronutrient uptake at the seedling stage of production. The present study aimed to characterize the growth and nutrient accumulation characteristics and analyze the principal components of cagaita seedlings grown in nutrient solution.

Results and Discussion

Evaluation of plant growth

Variation in the stem diameter (SD), stem length (SL), root volume (RV), root length (RL), number of leaves (NL), number of nodes (NN), leaf area (LA), crown area (CA) and leaf, stem, root and total dry weight (TDW) according to the sampling time (DAT) are presented in Fig 3 and Fig 4. All of the plant growth parameters increased linearly during the experimental period. The average increase per month was 0.393 mm for SD, 6.60 cm for SL, 1.425 mL for RV, 47.27 cm for RL, 4.197 units for NL, 2.103 units for NN, 62.348 mm^2 for LA and 207.39 cm^2 for CA (Fig 3). The highest values for all variables were reached at 180 DAT at 3.17 mm, 37.75 cm, 10.78 mL, 47.27 cm, 22.95 units, 11.51 units, 327.15 mm^2 and 1792.6 cm^2 for SD, SL, RV, RL, NL, NN, LA and CA, respectively (Fig 3).

The dry weights at 180 DAT were 2.9466 g, 1.3022 g, 2.82 g and 6.5484 g, and the unit increment of masses of dry matter were 0.0208 g, 0.0106 g, 0.0142 g and 0.045 g (Fig 4) and dry weight accumulation per month were 0.624 g, 0.318 g, 0.426 g and 1.371 g for the leaf, stem, root and total plant dry matter, respectively. Leaves accounted for most of the dry weight of *E. dysenterica* plants during the initial stage of seedling growth, which may have been related to their role as a sink for nutrients taken up by the plant from the nutrient solution. The source:sink ratio of different crops is an important tool for explaining the potential production of plants (Mendes et al., 2007). Rosane et al. (2013) also observed a higher dry weight percentage for leaves than for stems or roots in star fruit trees. The linear behavior observed for the quantified growth variables may be explained by the early developmental stages, which has been previously observed (Costa et al., 2012; Rosane et al., 2013). In addition, linear growth may have resulted from the availability of nutrients in the nutrient solution during growth because the nutrient solution is a homogeneous medium, in which nutrients are available as ions in adequate amounts (Puga et al., 2010).

Macronutrient concentrations in plant tissues

The maximum uptake of each nutrient was dependent on the time of seedling transplantation in to the nutrient solution. Nitrogen (N) had the highest concentration at the whole plant level and reached 7.82 g kg^{-1}on average at 180 DAT. This increased uptake may have been caused by the importance of N for plants. N is a component of aminoacids and protein and is present in important N compounds, such as chlorophyll and nucleic acids (Malavolta, 2006). The leaf N concentration had an average value of 2.97 g kg^{-1} and was not influenced by the sampling time, whereas at the stem and root the highest N concentrations were 1.62 at 180 DAT, and 3.24 g kg^{-1} at 164 DAT, respectively. A higher N concentration (12.47 g kg^{-1}) at 180 DAT was also observed in the shoots of pequi plants (*Caryocar brasiliense* Camb.) (Carlos et al., 2014). The N demand of the banana cultivar prata-anã (Musa spp. cv. AAB) is constant during most of its growth cycle, especially at the vegetative stage (Silva et al., 2013). N is the most important nutrient for drupaceous species; however, in excess, N may delay fruit tree maturation and decrease qualitative parameters (Rombolà et al., 2012). N fertilization has been observed to promote the vegetative growth of *Prunus armeniaca* L., although excess amounts of N did not increase fruit size and production (Milosevic et al., 2013).

The highest phosphorus (P) concentration occurred in the leaves (0.63 g kg^{-1}) at 145 DAT, stems (0.47 g kg^{-1}) at 180 DAT, and roots (0.57 g kg^{-1}) at 159 DAT (Fig 5). The higher accumulation of P in leaves was likely because of the high P mobility within the plant (Dominghetti et al., 2014), with P transported from older to younger tissues during P deficiency. A higher P concentration in leaves (2.63 g kg^{-1}) was also found in 10-week-old *Pilea sinofasciata* plants grown in non-mineral soils (Zheng et al., 2014).

The highest potassium (K) concentration was observed in the roots at 116 DAT (1.41 g kg^{-1}). The highest K concentration occurred in the leaves (1.32 g kg^{-1}) at 180 DAT and stems (1.04 g kg^{-1}) at 117 DAT (Fig 5). Higher K concentration in roots has been reported in species with underground reserve organs (Cecílio Filho and Peixoto, 2013). K is the second most important nutrient for a number of plant species. Its concentration in crops varies with the location, year and species as well as according to the uptake of other nutrients. However, differences in K uptake are also related to the length and density of root hairs (Christian et al., 2014), which may explain the higher K concentration in the roots of *E. dysenterica* observed in the present study.

Leaf calcium (Ca) concentrations were not influenced by the sampling time and presented an average value of 1.71 g kg^{-1}. The stem (1.04 g kg^{-1}) and root (0.52 g kg^{-1}) Ca concentrations were highest at 66 DAT and 180 DAT, respectively (Fig 5). Ca presented the second-highest concentration in seedlings of *E. dysenterica*, with an average of 3.53 g kg^{-1} for the entire plant (leaf, stem and root). In fruit trees, Ca directly affects fruit quality, softening, stability and shelf life, and low contents result in increased respiration and consequently affect fruit shelf life (Malavolta, 2006; Aular and Natale, 2013). Ca is also considered one of the most important nutrients for plants because it is associated with plant defense, and it may influence the growth, anatomy, morphology and chemical composition of crop species (Nagadze et al., 2014).

Magnesium (Mg) concentrations in the leaves and stem were not influenced by the sampling time and presented average values of 0.44 and 0.36 g kg^{-1}, respectively. The root Mg concentration was highest at 178 DAT (0.29 g kg^{-1}) (Fig 5). Nascimento et al. (2011) observed leaf Mg concentrations of 2.70 g kg^{-1} and 2.77 g kg^{-1} in seedlings of the Woodard and Bluebelle cultivars of *Vaccinium myrtillus* L. grown in semi-hydroponics, respectively.

The whole-plant sulfur (S) concentration (leaf, stem and root) was 0.7146 g kg^{-1} at 180 DAT. The roots had the highest S concentrations at 0.35 g kg^{-1} at 158 DAT. Leaf and stem S concentrations were highest at 137 DAT (0.13 g kg^{-1}) and 180 DAT (0.22 g kg^{-1}), respectively. The effect of S in plants has received little attention because its limitations are less frequent in agricultural soils; however, S deficiencies have been previously reported (Divito and Sadras, 2014).
Macronutrients concentrations in *E. dysenterica* seedlings occurred in the following order at 180 DAT: N>Ca>K>P>Mg>S. The macronutrient concentration in *Averrhoa carambola* L. root stocks occurred in the following order: N>K>Ca>Mg>S>P (Rosane et al., 2013). Also the macronutrient concentration in *Annona muricata* L. seedlings occurred in the following order: K>N>Ca>Mg>P (São José et al., 2014).

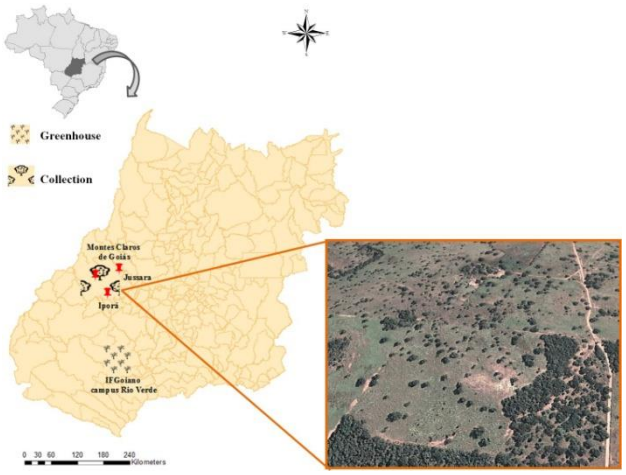

Fig 1. Map showing the location of the cagaita fruit (*Eugenia dysenterica* DC.) collection area and experimental site. The detailed section shows an aerial view of the vegetation of the collection site (Cerrado strict sensu vegetation).

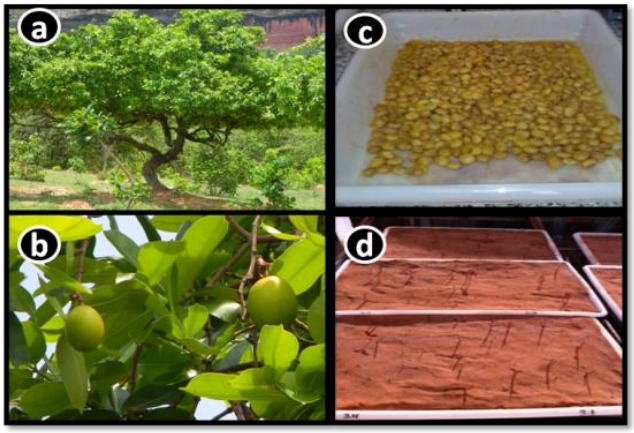

Fig 2. Tree (a), fruit (b), depulped seeds (c) and cagaita seedling (*Eugenia dysenterica* DC.) emergence in trays containing sand as substrate (d).

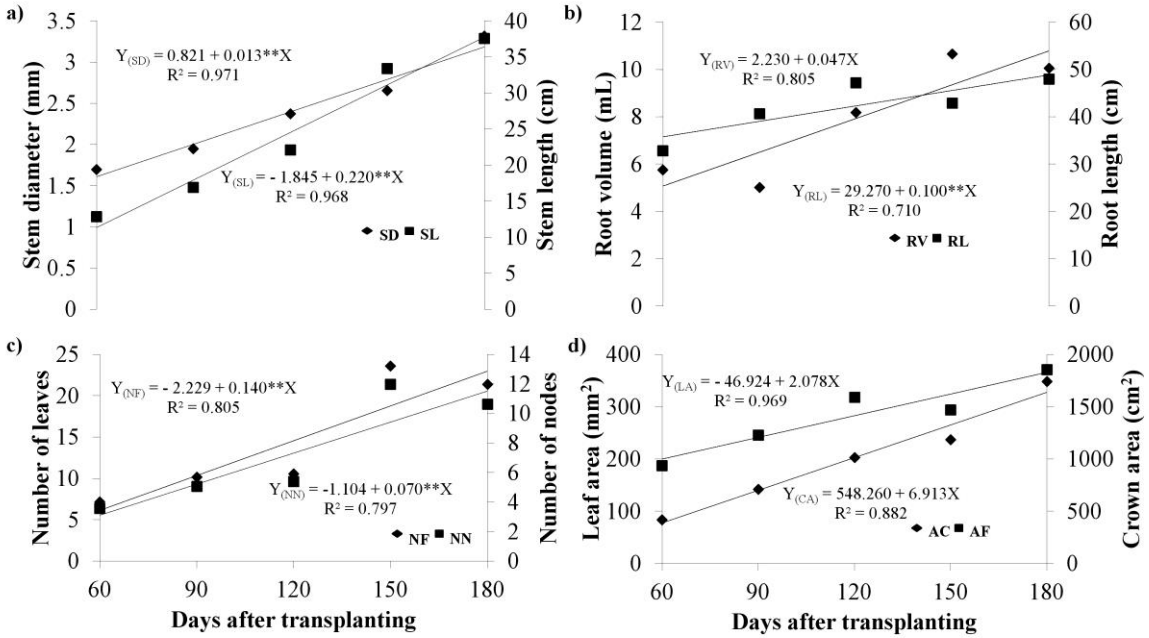

Fig 3. Growth parameters of cagaita (*Eugenia dysenterica* DC.) seedlings at different days after transplanting: a) stem diameter (SD) and stem length (SL), b) root volume (RV) and root length (RL), c) number of leaves (NL) and number of nodes (NN) and d) leaf area (LA) and crown area (CA) ** Significant according to the F test ($p<0.01$).

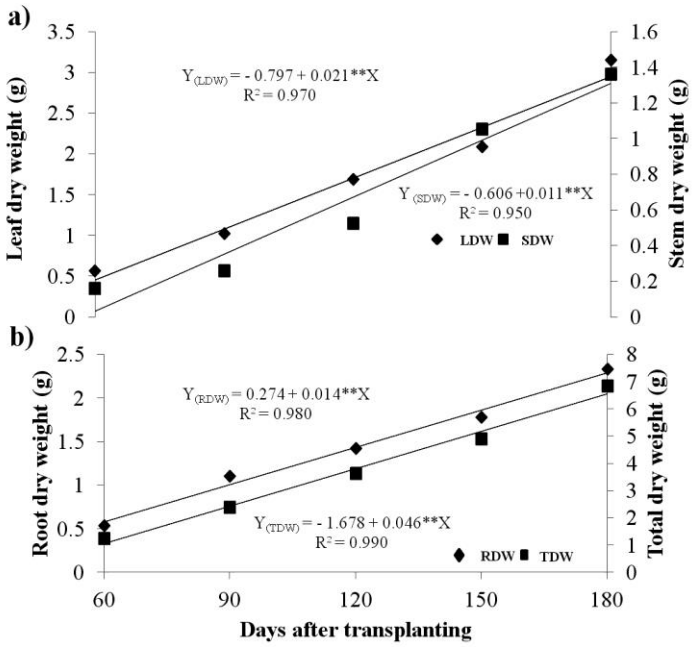

Fig 4. Leaf dry weight (LDW), stem dry weight (SDW), root dry weight (RDW) and total dry weight (TDW), of cagaita (*Eugenia dysenterica* DC.) seedlings at different days after transplanting. ** Significant according to the F test ($p<0.01$).

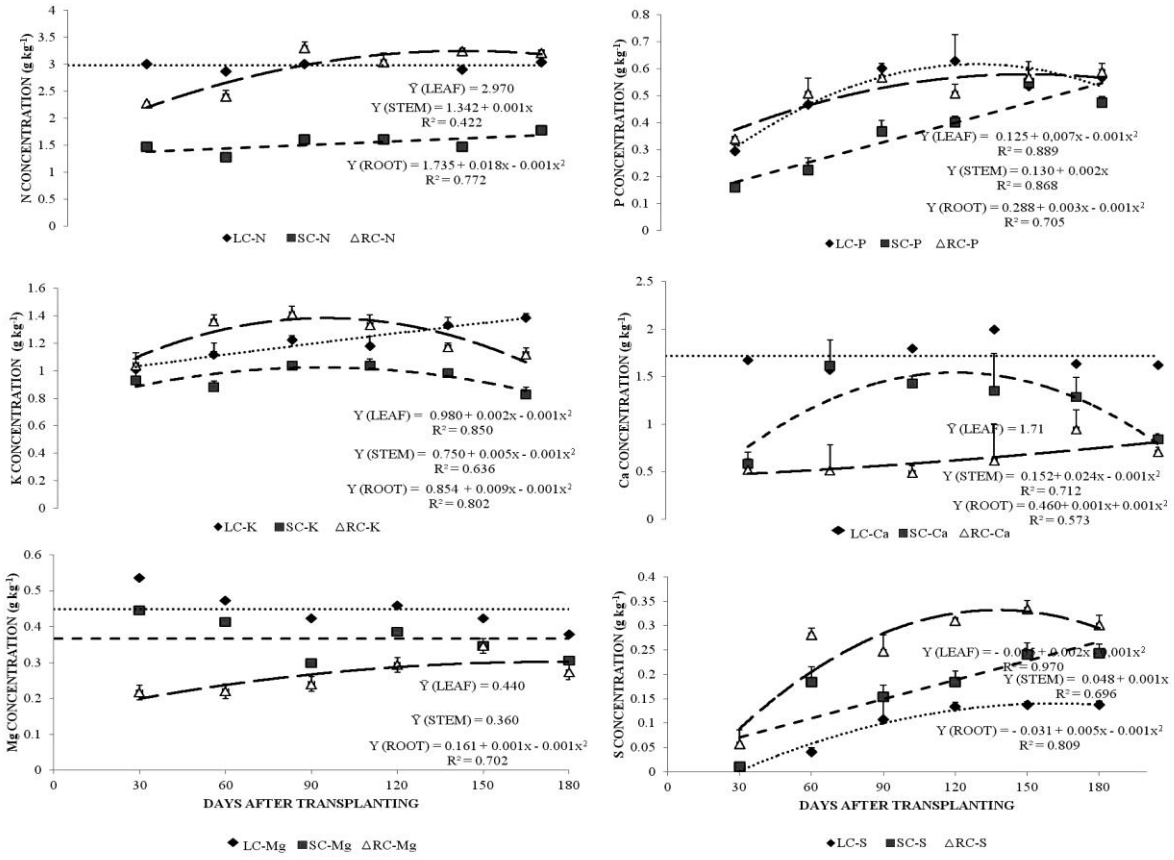

Fig 5. Macronutrient (N, P, Ca, K, Mg and S) concentrations in the leaves, stems and roots of cagaita (*Eugenia dysenterica* DC) seedlings at different days after transplanting.

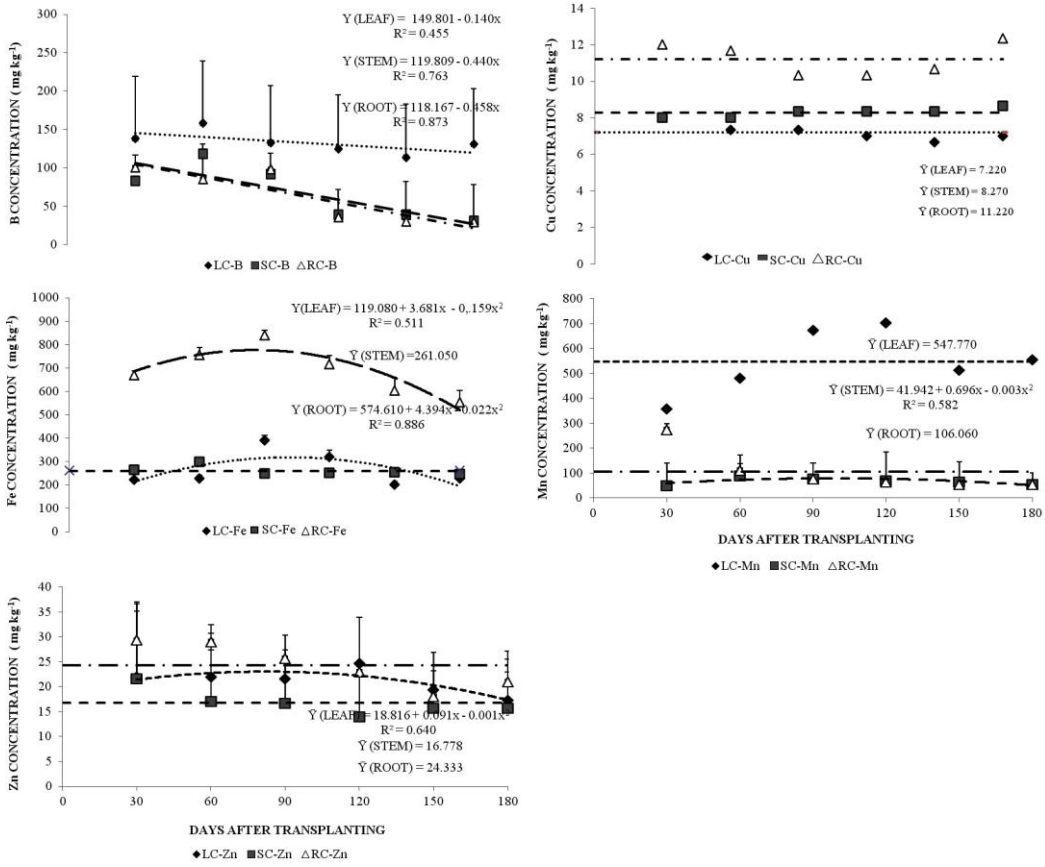

Fig 6. Micronutrient (B, Cu, Fe, Mn and Zn) concentrations in theleaves, stems and roots of cagaita (*Eugenia dysenterica* DC) seedlings at different days after transplanting.

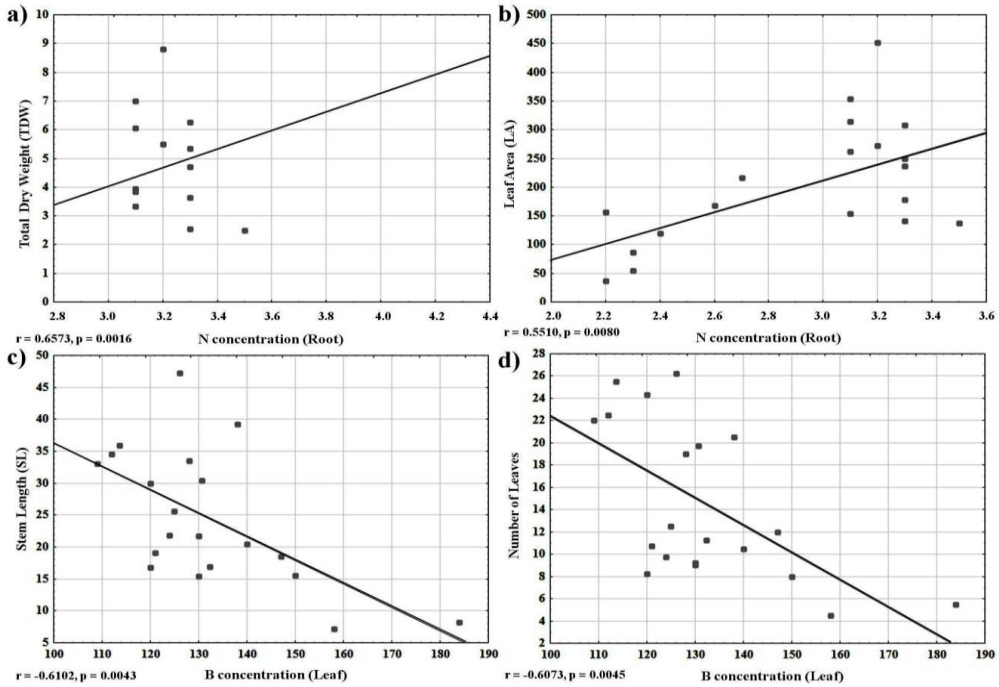

Fig 7. Correlations between the a) total dry weight and root N concentration; b) leaf area and root N concentration; c) stem length and leaf boron concentration; and d) number of leaves and leaf boron concentration.

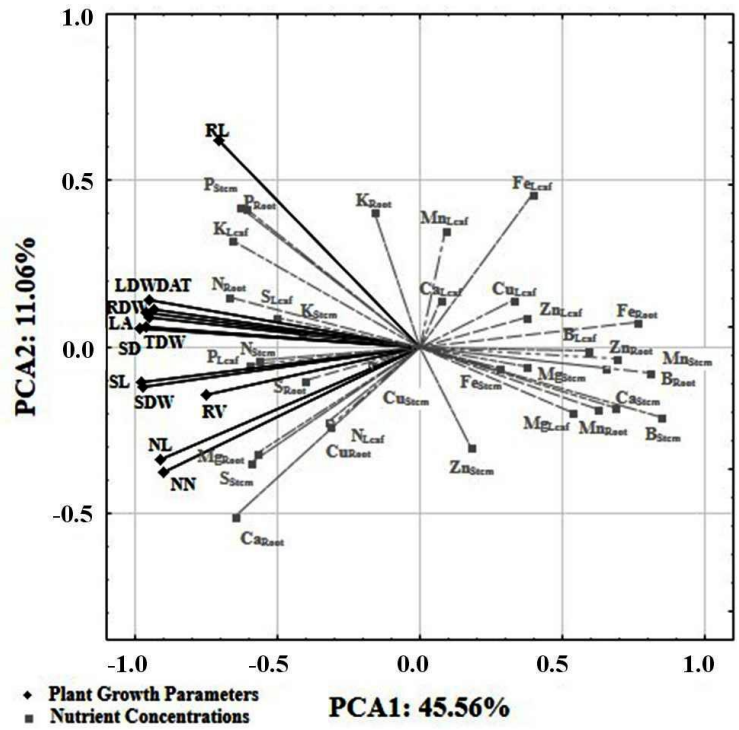

Fig 8. Biplot showing the first two variables of the principal component analysis (PCA1 and PCA2). The black lines are growth parameters evaluated and lines in gray, the concentrations of nutrients found in the tissues of plants of *Eugenia dysenterica* DC. evaluated. The size of the line is the weight parameter to define the principal component.

Micronutrient concentrations in plant tissues

The highest boron (B) concentration (124.52 mg kg^{-1}) was observed in leaves at 180 DAT. For the same sampling time, the B concentrations of the stems and roots were 40.60 and 35.67 mg kg^{-1}, respectively (Fig 6). This higher B accumulation in the leaves was likely because this nutrient is immobile in the plant and does not move from leaves or other organs to meet growth needs (Malavolta, 2006). However, B fertilization was observed to increase the number of new leaves in *Prunus persica* (L.) Batsch. seedlings, and mobility of B within the plant after leaf application has been observed (Souza et al., 2012). Sá et al. (2014) observed that B soil application increased its concentration in the fruits and leaves of *Malus domestica* more efficiently compared with leaf application. Copper (Cu) concentrations for all plant parts were not affected by the sampling time, with average values of 7.22 mg kg^{-1} in leaves, 8.27 mg kg^{-1} in stems and 11.22 mg kg^{-1} in roots (Fig 6). However, a tendency towards higher Cu concentration was observed in roots. Plants most likely take up Cu as Cu^{+} or Cu^{+2}, which have low mobility until tissue senescence, which results in delayed translocation into the shoot and consequently Cu accumulation in the root (Lehmann and Rillig, 2015).

Iron (Fe) levels in the stem were not influenced by the sampling time and had an average value of 261.05 mg kg^{-1}. Fe concentrations were higher in the roots, with the highest value (648 mg kg^{-1}) at 180 DAT. The highest leaf Fe concentration (332.23 mg kg^{-1}) was observed at 115 DAT (Fig 6). Among the micronutrients, Fe presented the highest average concentration (1175.82 mg kg^{-1}) until 180 DAT (leaf, stem and roots). Certain species of fruit trees, e.g., *Citrus aurantium* L. and *Citrus limonia* Osb, present high tolerance to the absence of Fe, although their hybrids may present sensitivity to Fe availability (Gama et al., 2015). Fe uptake by the roots is directly related to the uptake of other nutrients, such as Cu and Zn (Pestana et al., 2013; Gama et al., 2015).

The manganese (Mn) concentration at the whole plant level (leaf, stem and roots) was 658.18 mg kg^{-1} at 180 DAT, and an influence of sampling time was not observed, with the overall average at 547.77 mg kg^{-1}. There was a tendency for higher Mn concentration in leaves. The highest Mn concentration in the stem was observed at 111 DAT (111.83 mg kg^{-1}) and for roots the average concentration was 106.06 mg kg^{-1} (Fig 6). In *A. carambola*, the highest Mn concentration was observed in roots following 120 days of cultivation (211 mg kg^{-1}), and the lowest was observed in the stems (53 mg kg^{-1}) and leaves (175 mg kg^{-1}) (Hernandes et al., 2011).

The highest zinc (Zn) concentration was observed in roots and stems, having average of 24.33 mg kg^{-1} and 16.78 mg kg^{-1}, respectively. The highest Zn concentrations in the leaves (23.3 mg kg^{-1}) were observed at 98 DAT (Fig 6). A high concentration of Zn in roots may affect the growth of certain plants. Marichali et al. (2014) observed a suppression of root elongation because of excess Zn in *Coriandrum sativum* L., which may be associated with a significant loss of cell viability at the root tip. In cagaita fruits, micronutrient concentration presented the following order of demand at 180 DAT: Fe>Mn>B>Zn>Cu. Micronutrients in fruits of *Olea europaea*. L. cv Picholine have been reported to accumulate in the following order: Fe>B>Zn>Mn (Tekaya et al., 2014).

Nitrogen and boron correlation with growth variables

All of the quantified variables were correlated. The growth parameters presenting the highest correlation coefficients were also correlated, e.g., LA × leaf dry weight (r =0.99), SL × NL (r = 0.93), NN × NL (r = 0.97). Nutrient concentrations were correlated with the growth parameters; however, lower correlation coefficients were obtained (Fig 7). The TDW and

LA were positively correlated with root N concentration (Fig 7); thus, as the root N concentration increased, the TDW and LA also increased. N concentration and leaf expansion are directly related to vegetative growth (Ata-Ul-Karim et al., 2014). Thus, LA has been used as a new indicator of N in crops (Zhao et al., 2014). Studies of *Ricinus communis* L. showed that increasing N application rates resulted in increased LA and dry weight (Lima et al., 2014). Other correlations were negative, such as those for SL and NL with leaf B concentration (Fig 7); thus, SL and NL decreased with increasing B concentrations. A negative correlation between leaf B concentration and growth/productivity may have occurred because of the difficulty in removing B from the leaf cuticle or pectic layer of the cell wall, which does not perform metabolic functions, leading to an over estimation of leaf B concentrations (Boaretto et al., 1997). In addition, no evidence was observed to support a detrimental role of this nutrient on plant development through the interruption of metabolic processes or changes to the stability of the cell wall (Reid, 2010). The B may have a high affinity for several main metabolites, such as ribose, and likely forms strong complexes with these metabolites; thus inactivating them. This reaction suggests that excess B may interfere with transcription/translation and consequently cell division and expansion in certain plants (Nozawa et al., 2006). B is associated with IAA oxidase activity and could form complexes with their inhibitors, leaving free this enzyme to oxidize the IAA, regulating their endogenous levels (Jarvis et al., 1983). Therefore, it is suggested that B content can influence the concentrations of auxin in plants and consequently their growth.

Principal component analysis

A principal component analysis (PCA) was performed to clarify the relationship between the different evaluated variables, and the results are presented in Fig 8. The PCA1 and PCA2 explained 45.56% and 11.06% of the total variance, respectively. In the plot, the weights of the different variables are presented with arrows and are shown to vary with distance from the origin. The different growth parameters and sampling times, referred to as DAT, were observed to have the same approximate weight in the definition of the scores, except for RV, which had a lower weight. All of the nutrient concentrations presented lower weights than the growth variables; thus, they contributed less to explain the data variance. The biplot enabled the evaluation of the correlation level among the quantified variables, with lines presenting the same direction being more closely correlated. All of the growth parameters were disposed in the opposite direction to a significant proportion of the nutrient concentrations; thus, growth parameters were better correlated with each other than with nutrient concentrations. Variables such as DAT, LA, RDW (root dry weight), TDW and SD were similar and were highly correlated. However, N, P, K and S concentrations were highly correlated with the growth. Root Ca concentrations were also positively correlated with the quantified growth parameters. Cambrollé et al. (2015) observed that the Ca concentration in plant tissues was positively correlated with the reproductive capacity and seeds per fruit in *Glaucium flavum* Crantz. The concentrations of certain micronutrients, such as Zn, B, Cu, Fe and Mn, were negatively correlated with the growth parameters, suggesting that these nutrients may have been supplied in amounts exceeding the beneficial threshold for the growth of *E. dysenterica*. Nutrient excess may directly affect plant growth and physiology (Malavolta, 2006). High quantities of metals such as Zn and Cu may induce oxidative stress, resulting in changes in the capacity of certain antioxidant enzymes, such as catalase, peroxidase, superoxide dismutase, as well as glutathione-ascorbate cycle enzymes (Remans et al., 2012). Woody plants such as *Populus cathayana* Bartr present symptoms of micronutrient toxicity (e.g., Mn) from doses as low as 5 mM (Lei et al., 2007). However, *Vitis vinifera* Linn. can tolerate Mn stress between 15-30 mM without presenting pronounced decreases in LA and NL (Yao et al., 2012), indicating that this species is tolerant to high levels of Mn. However, specific studies are needed to establish the toxic nutrient levels for *E. dysenterica*. We suggest further plant growth studies for this species to evaluate nutrient concentrations in other developmental stages, because the sampling time (DAT; 30, 60, 90, 120, 150 and 180 days) as well as the principal components (PCA1 and PCA2) significantly explained most of the analyzed variables. Therefore, the sampling time and principal components were effective in explaining the growth parameters and many of the nutrient concentrations.

Materials and Methods

Obtaining plant material and cultivation in nutrient solution

The experiment was conducted in a greenhouse at the Laboratory of Plant Tissue Culture (Laboratório de Cultura de Tecidos Vegetais) of the Federal Institute of Goiás (Instituto Federal Goiano), Rio Verde Campus (17° 48' 15.9'' S, 50° 54' 19.5" W) between February and August 2012. The Cagaita fruits were collected from the Gameleira Farm, which is located at the municipality of Montes Claros, Goiás, Brazil (16° 06'20'' S, 51° 17' 11" W, 592 m altitude) (Fig 1).

Fruits were collected from five to eight matrix plants located in close proximity in the permanent preservation area of the farm. For pulping and seed collection, the fruits were macerated manually on sieves and washed under running water for seed separation. Damaged seeds were discarded, and intact seeds were surface sterilized with 5% commercial sodium hypochlorite for 3 minutes, washed in distilled water and placed in trays covered with towel paper at ambient temperatures for the removal of excess water. Following pulping, the seed tegument was removed to speed up and standardize germination and seedling formation. Sowing was performed on plastic trays (50×35×8 cm) containing washed sand as substrate (Fig 2). Thirty days following sowing, when the seedlings presented three to four fully expanded leaves, the plants were transferred to eight liter pots containing half-strength Hoagland and Arnon (1950) nutrient solution for 45 days.

Plants were grown under an average irradiance of 230 µmol m^2 s^{-1}. The nutrient solution was maintained under constant aeration by compressed air, and the pH was adjusted daily to 5.5 ± 0.5 by the addition of HCl or NaOH as needed. The nutrient solution was changed when electrical conductivity presented a 30% decrease from its initial value.
A randomized block experimental design was used, with six treatments and four replicates per treatment. Each treatment had a different sampling time occurring in 30-day intervals beginning with seedling transference into the nutrient solution (e.g., 30, 60, 90, 120, 150 and 180 days after transplanting). Each experimental plot consisted of one eight liter pot containing nutrient solution and two plants.

Measurement of traits

At 60, 90, 120, 150 and 180 days after transfer (DAT) into the nutrient solution, the following plant growth parameters were determined: length and diameter of the stem, number of leaves, number of nodes, volume of the roots, length of the largest root and area of the leaf and crown. Leaf area was measured by analyzing leaf images using *Image J* open-access software (http://rsbweb.nih.gov/ij/download.html) (Ferreira and Rasband, 2010). The plants were harvested and separated into leaf, stem and root samples, which were dried in a forced-air circulation oven at 65°C until a constant weight was reached, and the dry weights were then measured. Following drying, the samples harvested at the different evaluated sampling times (30, 60, 90, 120, 150 and 180 days after transplanting) and were ground using a Wiley-type Mill equipped with a 20 mesh sieve Then the nutrient concentrations were determined (N, P, K, Ca, Mg, S, Fe, Cu, Zn, B and Mn) according to Malavolta et al. (1997).

Statistical analyzes

The obtained data were subjected to variance and regression analyses using Sisvar 5.3 software (Ferreira, 2010). Regression models were selected based on the highest coefficients of determination and significance of the regression coefficients according to the t-test at $p \leq 0.05$. Because of the large number of parameters quantified in the experiment and high level of correlation among them, a principal component analysis was performed to decrease the number of variables (Wold et al., 1987; Jeong et al., 2015). Different variables were also evaluated depending on the sampling time (DAT) and principal components. These analyses were performed using the R statistics package (R Core Team, 2013). The normality of the residuals was confirmed using the Shapiro-Wilk test.

Conclusion

The growth of *E. dysenterica* seedlings varied linearly until 180 DAT. The TDW of the seedlings was 6.54 g at the end of the experiment. The N was positively correlated with TDW and LA, whereas B was negatively correlated with SL and NL. The macronutrient and micronutrient concentrations were observed in the following order: N>Ca>K>P>Mg>S and Fe>Mn>B>Zn>Cu, respectively. Two principal components (PCA1 and PCA2) explained 56.65 % of all variation. The principal component analysis of the sampling times (DAT) was important and could define most of the analyzed parameters, especially the growth parameters.

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