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CONTROLLED RELEASE FERTILIZER AND CONTAINER VOLUME IMPROVE THE SEEDLING MORPHOPHYSIOLOGY OF *Lonchocarpus muehlbergianus* HASSL.

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Resumo

Fertilizante de liberação controlada e volume de recipiente melhoram a morfofisiologia de mudas de Lonchocarpus muehlbergianus Hassl. O objetivo do presente estudo foi avaliar o efeito de diferentes volumes de recipientes e das concentrações de fertilizante de liberação controlada (FLC) no crescimento de mudas de *Lonchocarpus muehlbergianus* produzidas em viveiro. O delineamento experimental utilizado foi blocos ao acaso com parcela subdividida, em esquema fatorial (2x5), sendo dois volumes de recipientes (100 e 175 cm³), e cinco concentrações de FLC (0, 3, 6, 9 e 12 g L^{-1}), em seis repetições. Os dados foram submetidos à análise de variância (ANOVA) e, quando significativo, foram comparados pelo teste de Tukey (p<0,05) ou regressão polinomial. Aos 180 dias após a emergência das plântulas, foram avaliados os seguintes atributos: altura (H), diâmetro do coleto (DC), relação H/DC, massa seca da parte aérea (MSPA), massa seca radicular (MSRA), índice de qualidade de Dickson (IQD), teor relativo de clorofila (ICF), teor de clorofila *a*, *b* e carotenoides, e fluorescência da clorofila *a*. A espécie é responsiva a fertilização, bem como ao volume do recipiente, obtendo resultados superiores quando produzidas em 175 cm³ e a adição de 9,6 a 12 g L^{-1} de FLC, pois proporcionam de maneira geral, as maiores médias para as variáveis morfofisiológicas analisadas, possibilitando mudas com qualidade comercial e mais rápida expedição do viveiro.
Palavras-chave: Fertilização, qualidade de mudas, tubetes, viveiro florestal.

Abstract

The aim of this study was to evaluate the effect of different container volumes and controlled-release fertilizer (CRF) concentrations on the growth of *Lonchocarpus muehlbergianus* seedlings produced in a nursery. The used experimental design was of randomized blocks with split plots, in a factorial scheme (2x5), with two container volumes (100 and 175 cm³) and five CRF concentrations (0, 3, 6, 9 and 12 g L^{-1}), with six replicates. The data were subjected to analysis of variance (ANOVA) and, when significant, were compared by Tukey's test (p<0.05) or polynomial regression. At 180 days after the seedling emergence, the following attributes were evaluated: height (H), stem diameter (DC), H/SC ratio, shoot dry mass (MSPA), root dry mass (MSRA), Dickson's quality index (DQI), relative chlorophyll content (ICF), chlorophyll *a* and *b* and carotenoid content and chlorophyll *a* fluorescence. The species is responsive to fertilization as well as to the container volume, obtaining superior results when produced in 175 cm³ and the addition of 9.6 to 12 g L^{-1} of CCRF, as they generally provide the highest averages for the morphophysiological variables analyzed, enabling seedlings with commercial quality and faster shipment from the nursery.
Keywords: Fertilization, seediling quality, tubes, forest nursery.

INTRODUCTION

The seedling quality has been the focus of studies for the area restoration and the forest enrichment, considering that native species present different patterns of growth and development of the root system (GRIEBELER *et al*., 2021). The species *Lonchocarpus muehlbergianus* Hassl., belongs to the Fabaceae family, popularly known as *farinha-seca*, *rabo-de-bugio*, *embira-de-sapo* and *timbó*. It occurs naturally in some Brazilian states, such as Minas Gerais and Mato Grosso do Sul, extending to Rio Grande do Sul, mainly in the Semideciduous Broadleaf Forest of the Paraná river basin (LORENZI, 2000).

Due to its biological nitrogen fixation characteristics, the species *L. muehlbergianus* is considered an alternative for use in plantations aimed at restoring forests, especially in riparian areas of the Atlantic Forest (MOREIRA *et al*., 2014). It has high potential for economic exploitation, since its wood, classified as moderately heavy (density 0.72 g cm^{-3}), can be used for tool handles, crates, firewood, among others, and is also used in landscaping in general, once it is ornamental, due to its flowering (LORENZI, 2000).

According to Abreu *et al.* (2015), there is a large increase in the demand for projects to recover degraded areas and for ecological restoration, requiring an increase in the production of seedlings of native forest species, of different species, that are adapted to the conditions of the planting sites, with quality and attractive prices.

In the process of producing seedlings in a nursery, choosing the right container is of great importance once its size directly influences quality and final cost. Larger volume tubes reduce the time required to produce seedlings in the nursery and produce better quality seedlings (WALTER *et al*., 2022), depending on the species' growth rate.

Fertilization is a common practice in nurseries and can be carried out in several ways, including base fertilization, i.e., mixed with the substrate (NAVROSKI *et al*., 2018). Controlled-release fertilizers (CRFs) provide higher quality seedlings produced in the nursery, compared to conventional fertilizers (ZAVASCHI *et al*., 2014). This practice is necessary, as the substrate does not always have the capacity to provide all the nutrients for plant growth and development, requiring supplementation with mineral fertilizer.

The gradual release of nutrients occurs due to the presence of a polymer that coats them, allowing the controlled release of ions, reducing leaching and reducing the effects of salinity. In this way, nutrients are gradually made available to plants, depending on irrigation and temperature (NAVROSKI *et al*., 2018). It is essential that the fertilizer provides nutrients uniformly, so that the plants grow properly and the fertilizer remains in the substrate for a certain period of time.

The application of CRF in the plant fertilization can help improve the efficiency of the nutrient use and reduce the nutrient losses, helping minimize the environmental pollution (LAWRENCIA *et al*., 2021). In studies carried out by Cabreira *et al*. (2021), with *Inga laurina*, and Filho *et al.* (2018) with *Enterolobium contortisiliquum*, it was observed superiority in the growth characteristics of the seedlings produced with controlled-release fertilizer.

Thus, the present study aimed to evaluate the effect of different container volumes and concentrations of controlled-release fertilizer on the growth of *Lonchocarpus muehlbergianus* seedlings produced in nursery.

MATERIAL AND METHODS

The experiment was conducted at the Forest Research Center, part of the Department of Agricultural Research and Diagnosis (DDPA/SEAPDR) (South latitude 29° 39' 37.28" and West longitude 53° 54' 28.84"), in Santa Maria/RS state. The fruits were collected from five selected matrices in the same municipality, and the seeds were subsequently processed and stored in a cold chamber with controlled temperature and humidity (6 to 9°C and RH of 35 to 60%) until the experiment was carried out.

The seeds were sown manually in december in polypropylene tubes with circular cross-section and conical shape, with volumetric capacity of 100 and 175 cm^3, with two seeds placed in each 100 cm^3 tube and three seeds in the 175 cm^3 tubes. After 30 days of sowing, thinning was performed, leaving only one seedling per tube. During the experiment, irrigation was performed with an application of 6 ± 1 mm day^{-1}. The tubes were placed in trays and filled with commercial substrate Carolina Soil® and compacted manually. Subsequently, the trays were taken to the greenhouse where they remained for 180 days after the emergence of the seedlings. The commercial substrate Carolina Soil® has an electrical conductivity of 0.7 mS cm^{-1} and is composed of Sphagnum peat, expanded vermiculite, carbonized rice husk, added nitrogen, phosphorus and potassium (NPK), dolomitic limestone and agricultural gypsum, according to the manufacturer. The CRF used was Osmocote® Plus, in the formulation 15-09-12 (NPK) in different concentrations (0, 3, 6, 9 and 12 g L^{-1}). According to the manufacturer, nutrients are released within eight months at an average temperature of 21°C.

The evaluations of the morphological and physiological characteristics of the seedlings were carried out 180 days after seedling emergence, measuring the height of the aerial part (H) with the aid of a graduated ruler (cm), the diameter of the stem (DC) with a digital caliper (mm), the H/DC ratio, the dry mass of the aerial part (MSPA), the dry mass of the root (MSRA), the Dickson quality index (DQI), the relative chlorophyll content (RCC), chlorophyll a, b and carotenoids and chlorophyll a fluorescence.

To determine the dry mass, the samples of the aerial part and root were separated with pruning shears, and the roots were washed in running water with the aid of a sieve, and then placed in paper bags for drying in an oven at 60°C with forced air circulation until reaching a constant mass. After that, the material was weighed on an electronic scale with a precision of 0.001 g. The Dickson quality index (Dickson *et al*., 1960) was obtained using Equation 1:

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$$\mathrm{DQI} = \frac{\mathrm{MST}}{[(\mathrm{H/DC})+(\mathrm{MSPA/MSRA})]} \qquad \text{(Eq. 1)}$$

Where: DQI = Dickson quality index; MST: total dry mass (g); H: Height of the aerial part (cm); DC: Diameter of the stem (mm); MSPA: Dry mass of the aerial part (g); MSRA: Dry mass of the root (g).

To determine the relative chlorophyll content (ICF), two plants from each treatment replicate were selected, with measurements taken on the last expanded leaf from the apical meristem using a Clorofilog chlorophyll meter (Falker®).

To determine the chlorophyll content with dimethyl sulfoxide (DMSO) (WELLBURN, 1994), six seedlings from each treatment were selected and a leaf disc (8.5 mm in diameter) was removed from the first expanded leaf and incubated in DMSO saturated with $CaCO_3$ for 48 hours in tubes kept in the dark at room temperature of 25ºC. After the incubation period, the absorbance of the samples was determined in a spectrophotometer (Model Cirrus 80) at 665, 649 and 480 nm using a quartz cuvette with a 10 mm optical path. The wavelengths and equations for calculating the concentrations of chlorophyll *a*, *b* and carotenoids were based on the methodology described by Wellburn (1994).

To evaluate the fluorescence of chlorophyll *a*, six plants from each treatment were selected, with average height and stem diameter characteristics. Measurements were taken on the last expanded leaf of each plant; for this purpose, the leaves were adapted to the dark for 30 minutes before reading. The analyses were performed with the Junior PAM fluorometer (Walz®). The following characteristics were evaluated: minimum fluorescence (F_0), maximum fluorescence (F_m) and variable fluorescence (F_v), aiming to estimate the maximum quantum yield of photosystem II (F_v/F_m) (MISHRA, 2018).

The experimental design used was a randomized block design (DBA) with a split plot, in a factorial scheme (2x5), with two container volumes (100 and 175 cm³), combined with five concentrations of CRF (0, 3, 6, 9 and 12 g L^{-1}). Six replicates were used per treatment, with an experimental unit consisting of 10 plants, one per tube.

The data were subjected to the Bartlett and Kolmogorov-Smirnov tests to verify the homogeneity of variances and the normality of errors, respectively, using the SAS software, version 9.0 (SAS INSTITUTE, 2003). Subsequently, they were subjected to analysis of variance (ANOVA) and, when significant, compared by the Tukey test ($p<0.05$) or polynomial regression. The maximum technical efficiency dose (MTE) was determined for the quadratic equations with a significant effect. In the analyses, the statistical software Sisvar 5.6 was used (FERREIRA, 2019).

RESULTS

There was a significant interaction ($p<0.05$) between volumes and concentrations of CRF in the substrate, for the variables height of the aerial part (H) and diameter of the stem (DC) (Figure 1A and 1B) of *Lonchocarpus muehlbergianus* seedlings. The quadratic behavior and the determination of the maximum technical efficiency (MET) indicate that the combination between 100 cm³ of substrate resulted in the highest H occurred when 10.9 g L^{-1} (H = 16.65 cm) was used, increasing to 12.1 g L^{-1} (H = 19.16 cm) for the 175 cm³ tube. While for the DC the MTE for FLC was estimated at 10.8 g L^{-1} (4.65 mm) for the 100 cm³ tube and 12.1 g L^{-1} (5.72 mm) for the 175 cm³ tube.

For the H/DC ratio (Figure 1C and 1D), there was a significant influence for the isolated factors (container volumes and CRF concentrations). The H/DC ratio showed an average of 3.4 for the production of *L. muehlbergianus* seedlings in tubes filled with 100 cm³ of substrate, compared to seedlings grown in tubes with 175 cm³ of substrate (3.2).

The dry mass of the aerial part (MSPA) and dry mass of the root (MSRA) showed significant responses to the container volumes and to CRF concentrations, with interaction between them (Figures 1E and 1F). The estimated MTE for the variable MSPA was 16.4 g L^{-1} for the 100 cm³ tube (1.730 g) and 20.7 g L^{-1} (2.910 g) for the 175 cm³ tube. For MSRA it was 11.2 g L^{-1} (1.289 g) for the 100 cm³ tube and 12.6 g L^{-1} (1.863 g) of CRF for the 175 cm³ tube.

The Dickson Quality Index responded in a positive quadratic manner to the container volume and CRF concentrations (Figure 1G). The estimated MTE for the CRF was 11.7 g L^{-1} (CRF = 0.6) for the 100 cm³ tube and 13.5 g L^{-1} (CRF = 0.9) of CRF for the 175 cm³ tube.

As for the relative chlorophyll contents (ICF), there was a significant influence ($p<0.05$) only of the CRF concentrations (Figure 2A), with quadratic behavior in response to the CRF concentrations. The estimated MTE for this variable was 11.5 g L^{-1} (ICF = 36.82) of CRF in the substrate.

The chlorophyll content with DMSO (Figures 2B, 2C and 2D) varied between the treatments, and for chlorophyll *a*, the model that best fitted was the linear one, with an increase in the means with the addition of

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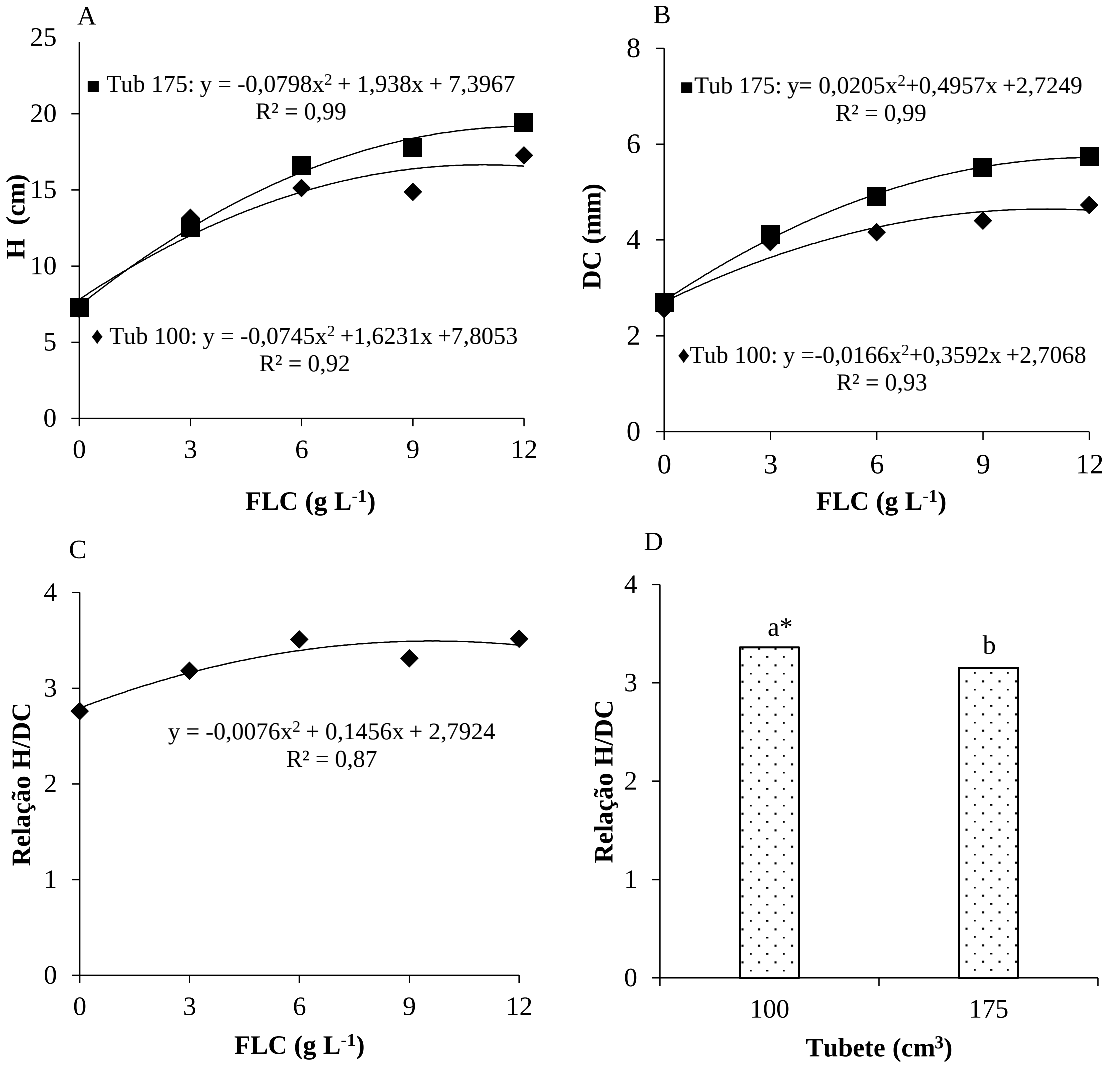
different concentrations of CRF in the substrate. The other pigments (chlorophyll *b* and carotenoids) demonstrated quadratic behavior in relation to the CRF concentrations, and high means were observed for the plants produced in tubes with a volume of 100 cm^3 for the three contents of photosynthetic pigments.

The highest means for the chlorophyll a content were observed with the addition of 9 g L^{-1} of FLC (13.25 μg cm^{-2}) for the 100 cm³ tube and 12 g L^{-1} (12.45 μg cm^{-2}) for the 175 cm³ tube. The maximum MTE estimated for chlorophyll b was 11.5 g L^{-1} (10.66 μg cm^{-2}) in leaves of plants grown in 100 cm³ tubes and 12 g L^{-1} (5.29 μg cm^{-2}) for the 175 cm³ tube. For carotenoids, the estimated MTE was 12.4 g L^{-1} (8.71 μg cm^{-2}) in the 100 cm³ tube and 16.4 g L^{-1} (7.56 μg cm^{-2}) for the 175 cm³ tube.



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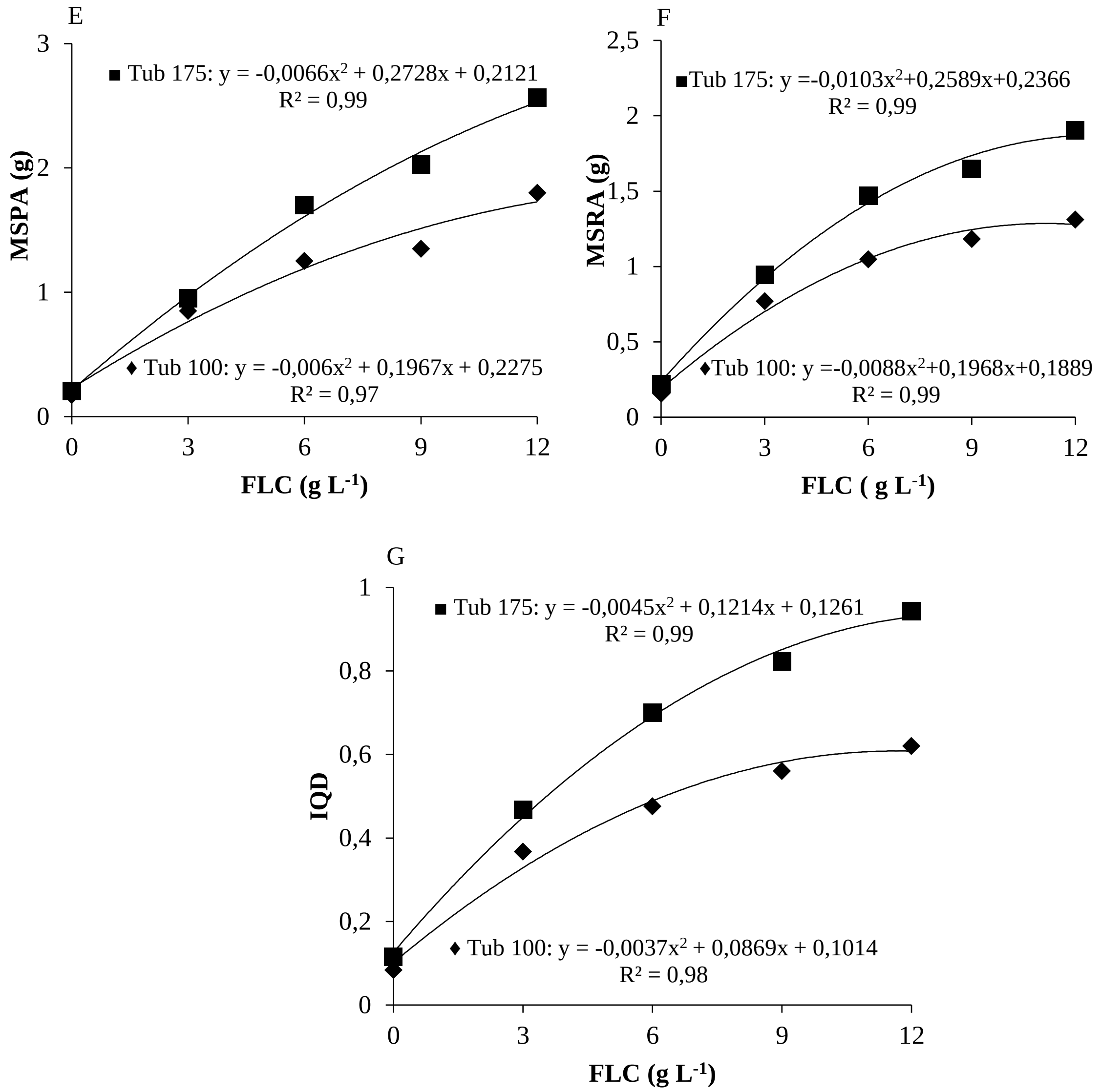

Figure 1. A) Height of the aerial part (H), (B) collar diameter (CD), (C, D) H/DC ratio, (E) dry mass of the aerial part (MSPA), (F) dry mass of the root (MRSA and (G) Dickson quality index (DQI) of Lonchocarpus *muehlbergianus* seedlings produced in different containers, as a function of the concentrations of controlled release fertilizer (CRF), at 180 days after emergence.
*Means followed by the same letter do not differ according to Tukey's test at a 5% probability of error.

Figura 1. (A) Altura da parte aérea (H), (B) diâmetro do coleto (DC), (C, D) relação H/DC, (E) massa seca da parte aérea (MSPA), (F) massa seca radicular (MSRA e (G) índice de qualidade de Dickson (IQD) de mudas de *Lonchocarpus muehlbergianus* produzidas em diferentes recipientes, em função das concentrações de fertilizante de liberação controlada (FLC), aos 180 dias após a emergência.
*Médias seguidas pela mesma letra não diferem entre si pelo teste de Tukey a 5 % de probabilidade de erro.

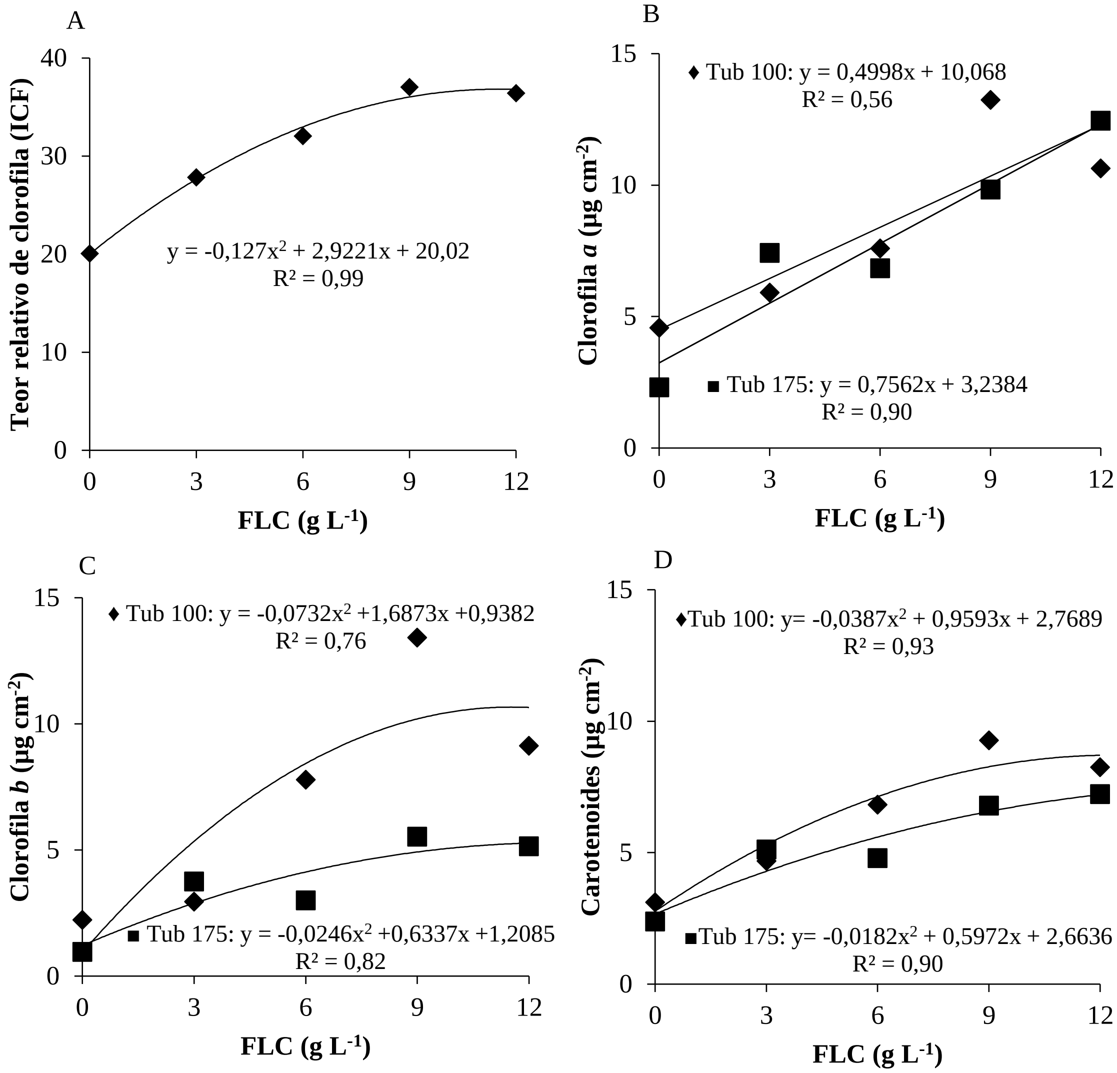

Figure 2. (A) Relative chlorophyll content (RCC), (B) chlorophyll *a*, (C) chlorophyll *b* and (D) carotenoids of *Lonchocarpus muehlbergianus* seedlings grown in different containers, as a function of controlled release fertilizer (CRF) concentrations, at 180 days after the emergence.

Figura 2. (A) Teor relativo de clorofila (ICF), (B) clorofila *a*, (C) clorofila *b* e (D) carotenoides de mudas de *Lonchocarpus muehlbergianus* produzidas em diferentes recipientes, em função das concentrações de fertilizante de liberação controlada (FLC), aos 180 dias após a emergência.

The chlorophyll fluorescence analysis showed that there was no significant influence of the container volume and CRF concentration factors for the variables F_0, F_m, and F_v, nor there was interaction between them. However, the averages of the F_v/F_m ratio (photochemical efficiency) showed a statistical difference in response to the container volumes (Table 1).

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Table 1. Maximum photochemical efficiency (F_v/F_m) of *Lonchocarpus muehlbergianus* seedlings grown in different containers, as a function of the controlled release fertilizer (CRF) concentrations, at 180 days after emergence.

Tabela 1. Eficiência fotoquímica máxima (F_v/F_m) de mudas de *Lonchocarpus muehlbergianus* produzidas em diferentes recipientes, em função das concentrações de fertilizante de liberação controlada (FLC), aos 180 dias após a emergência.

Tube (cm³)	**FLC (g L^{-1})**				
	0	**3**	**6**	**9**	**12**
100	0,52 A*b**	0,56 Aab	0,54 Aab	0,71 Aa	0,52 Bb
175	0,55 Aa	0,62 Aa	0,62 Aa	0,60 Aa	0,66 Aa

* Averages in rows followed by the same uppercase (row) and lowercase (column) letter do not differ from each other by the Tukey test at 5% probability of error.

DISCUSSION

In the present study, the addition of CRF to the substrate stimulated the growth of dry flour seedlings in relation to the control treatment (Figure 1). Growth is an integrative characteristic that responds to environmental factors and is crucial for plant fitness. One of the main environmental factors that influences plant growth is the supply of nutrients (OGDEN *et al*., 2018). According to the same authors, nutrients are essential for the plant growth and development, and their depletion directly affects their performance.

The quadratic trend of the variables height and stem diameter, with interaction between the factors (containers x CRF concentrations) (Figure 1) were similar to the results found by Stüpp *et al*. (2015). According to the same authors, there was quadratic behavior in the growth in height and diameter of the stem of *Mimosa scabrella* Benth (bracatinga) seedlings, also from the Fabaceae family, with the estimated MTE being 6.25 g L^{-1} of FLC (H = 23.22 cm) for the height variable and 6.80 g L^{-1} (6.54 mm) for the stem diameter, presenting a notably lower MTE when compared to *L. muehlbergianus*. In *Cabralea canjerana*, the stem diameter and root dry mass expressed the best performance of the seedlings in the largest volume tube (280 cm³) (GASPARIN *et al*., 2014), which was confirmed by the data from this study.

The results found for *L. muehlbergianus* of the H/DC ratio increased quadratically with the addition of CRF in the substrate, with the MET estimated at 9.6 g L^{-1} corresponding to values of 3.4 (100 cm³ tube) and 3.2 (175 cm³ tube), that is, these results corroborate what is indicated in the literature. Seedlings of forest species qualified for planting must present an H/DC ratio of less than 10, and may present greater survival after planting in the field (BIRCHLER *et al*., 1998). However, it is worth mentioning that each species presents its own morphophysiological characteristics, which is why the H/DC ratio cannot be considered standard for all species.

According to Garcia and Souza (2015), seedlings must have a minimum diameter in relation to height, and the lower this ratio, the better the quality of the seedlings. However, it is worth noting that it is possible to obtain higher values in the H/DC ratio without losing plant quality.

The averages of MSPA, MSRA, DQI, chlorophyll b and carotenoids showed the same trend as the variables H and DC, corroborating that the container and CRF concentrations promoted adequate growth of *L. muehlbergianus* seedlings.

The larger volume of substrate in the container (175 cm³) provided high averages for the MSPA and MSRA variables (Figures 1E and 1F), a fact that may be related to the volume of the containers, since larger ones provide better development of the root system as well as greater availability of nutrients, which are essential for plant growth, and consequently, allow greater growth of the aerial part and root.

According to Araujo *et al*. (2018), the results obtained from MSPA are commonly higher than from MSRA, which can directly interfere with the quality of the seedlings. For *L. muehlbergianus*, it was possible to observe this condition for the two dry mass variables analyzed, highlighting the importance of evaluating these characteristics.

The DQI of seedlings grown in 100 and 175 cm³ tubes was 0.6 and 0.9, respectively, showing that the larger volume of substrate allowed for higher averages of this variable. It is worth noting that the index has been considered a relevant indicator in the analysis of the quality of forest seedlings once it integrates relevant morphological variables and the balance in the distribution of dry mass, indicating that the higher the DQI, the better the quality of the seedling (GOMES *et al*., 2019).

The DQI has been considered a good indicator of seedling quality once it considers the robustness and stability of the plant biomass distribution in its calculation, and the higher the value, the better the seedling quality (VIEIRA *et al*., 2019). The increase in the DQI may be related to a lower H/DC ratio and higher MSRA (ARAUJO *et al*., 2018). For *L. muehlbergianus*, it was possible to observe this condition, since in comparison with the two container volumes tested (100 and 175 cm³), higher DQI averages were obtained for tubes with a volume of 175 cm³, while for the same, there were lower averages of the H/DC ratio and high values for MSRA in relation to the use of 100 cm³ tubes. In this study, it was found that *L. muehlbergianus* seedlings (Figure 1G) produced in treatments with high concentrations (9 to 12 g L^{-1}) of CRF in the substrate presented higher quality.

The ICF averages increased with the addition of up to 11.5 g L^{-1} of FLC in the substrate, with no difference between the two containers, with the averages being 30.36 for the 100 cm³ tube and 31.03 for the 175 cm³ tube. It is important to evaluate this variable because it also allows identifying if there is a deficiency of nutrients that are essential to the plant, such as nitrogen, allowing for simple and quick nutritional corrections. Chlorophyll content and nitrogen (N) concentration are related, however, the increase in chlorophyll with the increase in N levels in the soil occurs until the moment when nitrogen stops accumulating in the form of ammonium and nitrate, and is therefore not assimilated by plants (KURTZ *et al*., 2022).

Plants grown in smaller volume tubes (100 cm³) presented higher averages for photosynthetic pigments (chlorophyll *a*, *b* and carotenoids), a fact that may be associated with the greater biomass and reduced chlorophyll concentration, as the plant has a larger area for capturing light for photosynthesis, while plants with lower biomass concentrate these pigments. In addition, the reduction in the chlorophyll production can free up nitrogen resources for other uses by the plant, increasing the nitrogen allocation without compromising the carbon assimilation (CHO *et al*., 2024). Thus, the species may be plastic to different cultivation situations. Thus, despite growth limitations, *L. muehlbergianus* physiologically maintains its photosynthetic capacity. This justifies the fact that the plant did not reduce its quantum yield, based on the results of chlorophyll *a* fluorescence, in which the maximum photochemical efficiency (F_v/F_m), with the cultivation of seedlings in 175 cm³ tubes (0.66; 12 g L^{-1}) provided higher averages compared to the 100 cm³ tube (0.52; 12 g L^{-1}).

In addition, the significant difference ($p<0.05$) in F_v/F_m between the containers demonstrates that the use of a container with a smaller volume of substrate caused a stress condition. The F_v/F_m ratio has been widely used to measure stress conditions, and the decline in the F_v/F_m ratio is evidence that the photosynthetic performance of the plant is being compromised (KONO *et al*., 2021). The F_v/F_m ratio has been the most used parameter to characterize conditions that affect the photochemical efficiency of plants under stress conditions (KONO *et al*., 2021; MISHRA, 2018), the F_v/F_0 ratio has also been used as a good indicator to characterize the maximum quantum yield of photosystem II, while reduced values of the F_v/F_m ratio may indicate foliar photoinhibition (MISHRA, 2018).

CONCLUSION

- *Lonchocarpus muehlbergianus* seedlings present satisfactory morphophysiological attributes when produced in tubes with a substrate volume of 175 cm³ and fertilization between 9.6 and 12 g L^{-1} of CRF, as they generally provide the highest averages for the morphophysiological variables analyzed, enabling seedlings with commercial quality and faster shipment from the nursery.

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