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Original Article

Silviculture

Nitrogen and Potassium Fertilization in the Initial Growth of *Annona crassiflora* Mart.

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ABSTRACT

Annona crassiflora Mart. presents medicinal and food potential, and is also used in the recovery of degraded areas, however, little is known about its nutritional requirements. The aim of this work was to analyze the initial growth of *A. crassiflora* submitted to nitrogen and potassium fertilization. The experiment was conducted with a complete randomized block design with four replicates, each consisting of five nitrogen and potassium doses (0, 50, 100, 150 and 200 mg dm^{-3}). Results were submitted to regression analysis. The species showed significant response for nitrogen fertilization regarding biometric variables, biomass, DQI and nutritional contents at doses of 100 to 200 mg dm^{-3}. On the other hand, potassium only influenced DQI, nutritional content and accumulation of *A. crassiflora* seedlings.

Keywords: seedlings, nitrogen doses, potassium doses.

1. INTRODUCTION

Annona crassiflora Mart. belongs to the Annonaceae family, popularly known in Brazil as "marolo" or "Araticum" (Botrel et al., 2016). This species stands out among the native fruits of the Cerrado biome due to its socioeconomic importance (Ribeiro et al., 2016a). Moreover, it represents a promising alternative for family farming due to its extractive exploitation; a sector that is quickly expanding in the current Brazilian agricultural scenario (Valadares et al., 2015). This species also has great medicinal and food potential, as it is recommended for treating diarrhea, rheumatism and as anti-tumor. In addition, it has been used in cosmetics against scalp infections, as it contains compounds with therapeutic indications (Bailão et al., 2015).

However, with the rapid expansion of agriculture in Cerrado regions accompanied by reduction in native vegetation, many species of this domain are threatened, including *A. crassiflora*. Thus, in order to minimize environmental impacts and promote maintenance of the native vegetation, programs for the recovery of these disturbed areas are necessary (Ribeiro et al., 2016b). Accordingly, it is also necessary to understand the silvicultural behavior of this species, mainly in relation to fertilization, considering that little is known about the behavior of this species in Cerrado regions.

Soils of the Brazilian Cerrado are highly weathered with low natural fertility, high acidity, low cation exchange capacity, low organic matter and high aluminum concentration (Farias et al., 2016). All these factors influence seedling production of some species to a certain extent. Therefore, studies have used nitrogen and potassium fertilizations to increase seedling production in Brazilian Cerrado regions.

Nitrogen (N) acts in the enzymatic system of plants. It is an essential component of the ribulose 1,5-bisphosphate carboxylase/oxygenase (RuBP carboxylase - "Rubisco") enzyme, a key enzyme for carbon fixation, participating in several physiological processes directly related to cell division and stretching, resulting from the performance of the assimilatory system (Mendes et al., 2013).

Potassium (K) acts in several metabolic functions of plants. It is present in cell cytoplasm and is considered the largest osmotic cellular cationic agent. It also regulates stomata opening and closing, acts in controlling assimilation of the internal CO_2 concentration in chloroplasts, in photosynthesis, activating enzymes, and distributing/storing carbohydrates (sucrose) through the membrane and synthesis of proteins (Sousa et al., 2014).

Even with knowledge on the importance of nitrogen and potassium fertilization, information about the effect of these nutrients on the initial growth of native Cerrado species is scarce, especially for *A. crassiflora*. Thus, the aim of this study was to evaluate nitrogen and potassium fertilization in the growth and establishment of *A. crassiflora* during the nursery/seedling phase.

2. MATERIAL AND METHODS

The experiment was conducted in greenhouse belonging to the Federal Institute of Goiás - Rio Verde Campus. The species used in the experiment was *Annona crassiflora*, and seeds were collected in the municipality of Felixlândia - Minas Gerais.

The soil used in the study is classified as haplortox (EMBRAPA, 2006). Samples were collected from 5 different points in the 0-20 cm depth layer, representing a composite sample for chemical and physical characterization (EMBRAPA, 2009). Physical analysis was performed for granulometric determination of fine air-dried soil according to the pipette method (EMBRAPA, 1997). The granulometric analysis indicated that the soil used consisted of 50% clay, 18% silt and 32% sand (Table 1); while the chemical analysis (Table 1) showed that the soil base saturation in natural condition was around 12%, calcium, magnesium and phosphorus were below recommended levels, and saturation was increased by bases according to treatments. After fertilization, soil presented adequate attributes for cultivation.

Two different experiments were carried out; one using nitrogen doses and another with potassium doses. The experimental design was completely randomized and composed of 5 nitrogen and potassium doses (0, 50, 100, 150, 200 mg dm^{-3}) with 4 replicates, corresponding to a total of 20 experimental plots for each nutrient.

Soil acidity was corrected with limestone and incubated for a period of 20 days seeking to achieve V = 60%, according to van Raij (1981).

The suggested basic fertilization was performed in all experimental units according to the following doses and sources: 300 mg of phosphorus/dm^3 of

Table 1. Soil chemical and texture characteristics for *A. crassiflora* seedlings before and after fertilization.

Attributes	Natural	K 0	K 50	K 100	K 150	K 200	Nitrogen
pH ($CaCl_2$)	4.30	4.80	4.80	4.80	4.70	4.80	5.30
K (cmolc dm^{-3})	0.11	0.23	0.32	0.40	0.51	0.51	0.45
Ca^{2+} (cmolc dm^{-3})	0.40	1.67	1.99	1.92	2.24	1.85	1.79
Mg^{2+} (cmolc dm^{-3})	0.10	1.47	1.70	1.51	1.97	1.44	1.30
Al^{3+} (cmolc dm^{-3})	0.30	0.04	0.07	0.08	0.05	0.03	0.02
H+Al (cmolc dm^{-3})	4.50	4.17	4.93	4.88	4.93	4.17	3.14
P (Mel) (mg dm^{-3})	6.00	15.54	15.06	17.56	13.42	15.01	11.13
SB (cmolc dm^{-3})	0.61	3.37	4.01	3.83	4.72	3.80	3.54
t (cmolc dm^{-3})	0.91	3.41	4.08	3.91	4.77	3.83	3.56
T (cmolc dm^{-3})	5.11	7.54	8.94	8.71	9.65	7.97	6.68
V(%)	11.99	44.69	44.85	43.97	48.91	47.68	52.99
Clay	50	50	50	50	50	50	50
Silt	18	18	18	18	18	18	18
Sand	32	32	32	32	32	32	32

Natural: original non-fertilized soil; K0 to K200: soil with basic fertilization, with different potassium levels (from 0 to 200 mg dm^{-3}); Nitrogen: soil fertilized by nitrogen treatments; pH: potential of hydrogen; K: potassium; Ca: calcium; Mg: magnesium; CEC: cation exchange capacity; Al: aluminum; H+Al: hydrogen plus aluminum; P: phosphorus; SB: Sum of bases; t: effective CEC; T: CEC at pH 7.0; V (%): Base Saturation.

soil (triple superphosphate and monoammonium phosphate); 40 mg of sulfur/dm^3 of soil (ammonium sulfate and potassium sulphate); 0.5 mg of boron/$dm^3$of soil (boric acid P.A.); 1.5mg of copper/$dm^3$of soil (copper sulfate P.A.); 3.0 mg of manganese/dm^3 of soil (manganese sulphate P.A.); 5.0 mg of zinc/$dm^3$of soil (zinc sulfate P.A.) and 0.1 mg of molybdenum/dm^3 of soil (ammonium molybdate P.A.); and 200 mg of nitrogen/dm^3 of soil (monoammonium phosphate and urea) and 180 mg of potassium/dm^3 of soil (potassium chloride) when necessary (Carlos et al., 2014).

Due to the morphophysiological dormancy, seeds were arranged in sowing equipment using sand over a layer of gravel substrate. A layer of carbonized rice husk was added on the top of seeds for period of approximate eight months. Soon after seeds were removed from the sowing equipment, gibberellic acid (GA_3) at 500 ppm was applied for a period of 24 hours at room temperature for complete imbibition. Subsequently, seeds were rinsed in hypochlorite and placed to germinate in vermiculite, according to the modified methodology of Silva et al. (2007) and Braga et al. (2014).

After radicle protrusion, pre-germinated seeds were transplanted by sowing a single seed in each 3.5-liter pot containing soil and the established treatments. Irrigation was maintained at 60% of field capacity according to the International Association of Engineering Geology (IAEG, 1979).

During the nursery phase, corresponding to the period between January and August, the morphological parameters evaluated were: plant height between neck and stem apex measured with the help of a millimeter ruler, with results expressed in cm; and stem diameter using a digital caliper, with results expressed in mm.

Seedlings were removed from pots at the end of experiments and separated into stems, leaves and roots. Subsequently, they were dried in a forced air circulation oven at 65ºC until reaching constant weight. After drying, the material was weighed in an analytical digital scale to obtain the biomass. Then, the dry mass of leaves (DML), stem (DMSt), roots (DMR), shoots (DMSh) and total dry mass (TDM) were determined, and results were expressed in g $plant^{-1}$. With these parameters, the following seedling quality indexes were calculated: height-to-diameter ratio (H/D), root-to-shoot ratio (R/S) and Dickson Quality Index (DQI), according to Dickson et al. (1960).

For quantification of contents and nutritional accumulation of shoot and root systems after being dried, the plant material was milled in a Willye type mill and 0.1 g of N and 0.5 g of K were weighed for extraction of each macronutrient. N extraction was carried out by wet digestion using nitrogen distiller and determined by titration, while for K extraction, the material was calcined in a muffle furnace and readings were performed by flame emission photometer (EMBRAPA, 2009).

Results were submitted to analysis of variance at 0.05% probability level and regression analysis was performed when effect was found using the SISVAR 5.3 software (Ferreira, 2011).

3. RESULTS

Nitrogen fertilization had significant influence on all variables analyzed, except for height-to-diameter ratio (H/D) and root-to-shoot ratio (R/S).

Results for diameter, height and number of leaves are presented in Figure 1, with the first two adjustments using quadratic equations and the last using linear equation. The highest estimated measurements for height and diameter were 7.97 cm and 9.07 mm obtained for doses of N 94.5 and 89 mg dm^{-3}, respectively. Maximum leaf yield was 5.23 leaves for the N dose of 200 mg dm^{-3}. Considering that nitrogen is one of the nutrients most required by plants in higher amounts, being directly related to growth, plants well-nourished in N have great capacity to assimilate CO_2 and to synthesize carbohydrates during photosynthesis.

Dry mass data presented quadratic adjustments, in which doses that presented the highest biomass yields were the following: 89.5 mg of N per dm^{-3} of soil for DMSh; 87.85 mg of N per dm^{-3} of soil for DML; 91.66 mg of N per dm^{-3} of soil for DMSt; 94.5 mg of N per dm^{-3} of soil for DMR; and 92 mg of N per dm^{-3} of soil for TDM (Figure 2).

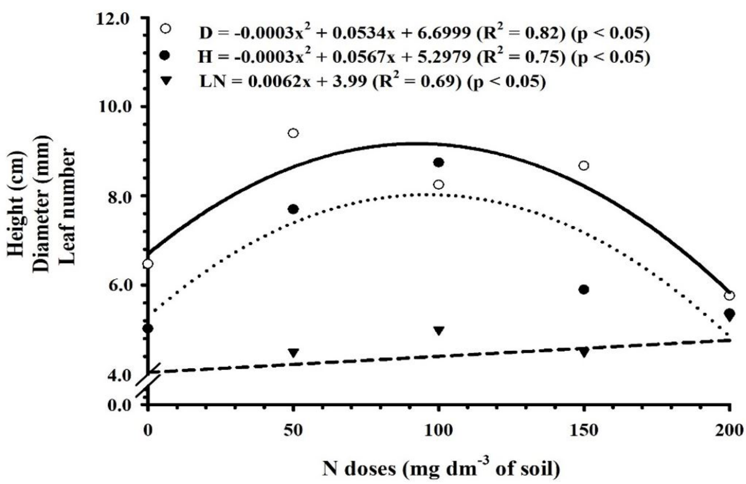

Figure 1. Height, diameter and number of *A. crassiflora* leaves in response to nitrogen fertilization.

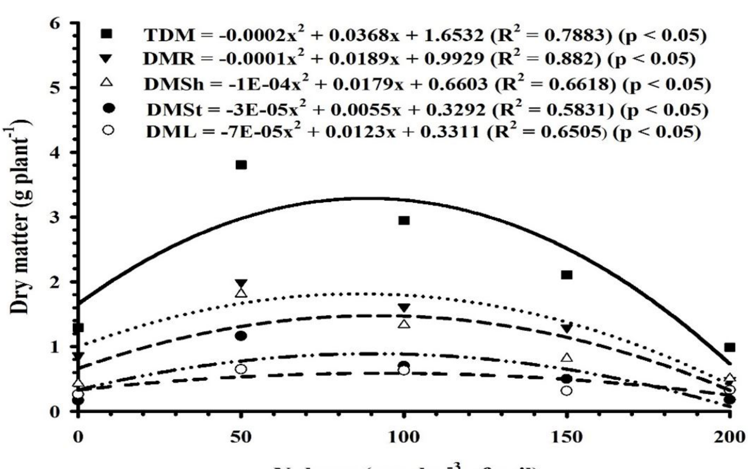

Figure 2. Shoot, leaf, stem, root dry mass and total dry mass of *A. crassiflora* plants submitted to different nitrogen fertilization doses.

Among the morphological attributes that determine the quality of seedlings, only the Dickson Quality Index (DQI) was significant, reaching maximum value of 2.05 at N dose of 95 mg per dm^3 of soil, adjusted for the quadratic regression model according to Figure 3.

Significant effect for the N content in stem was observed with maximum estimated concentration of 32.29 g kg^{-1} obtained at dose of 200 mg dm^{-3} with linear adjustment, and 33.58 g kg^{-1} in roots obtained at dose of 144.25 mg dm^{-3} with quadratic adjustment. No significant differences were found for the N content in leaves (Figure 4A).

In relation to nutrient accumulation (Figure 4B), significant effect was only observed for leaves and roots, in which leaves presented maximum accumulation of 18.14 mg $plant^{-1}$ for N dose of 87.76 mg dm^{-3}, and maximum accumulation for roots was 60.11 mg $plant^{-1}$ at dose of 97.65 mg dm^{-3}. No significant difference was observed for stem.

For potassium fertilization, no statistical difference was found for the evaluated characteristics, except for DQI, in which dose of 150 mg dm^{-3} was higher; however, there was no regression adjustment for this variable (Table 2). The behavior of this species suggests that lower K mobilization occurred during the initial growth stages for the plant's biochemical reactions, thus negatively influencing the growth and quality of seedlings, and with only significant difference ($p<0.05$) for accumulation and allocation of the nutritional content.

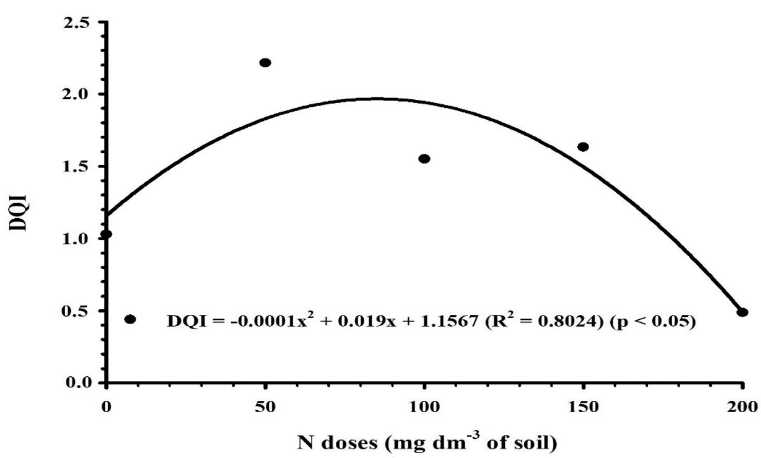

Figure 3. Dickson quality index (DQI) of *A. crassiflora* seedlings under different nitrogen fertilization doses.

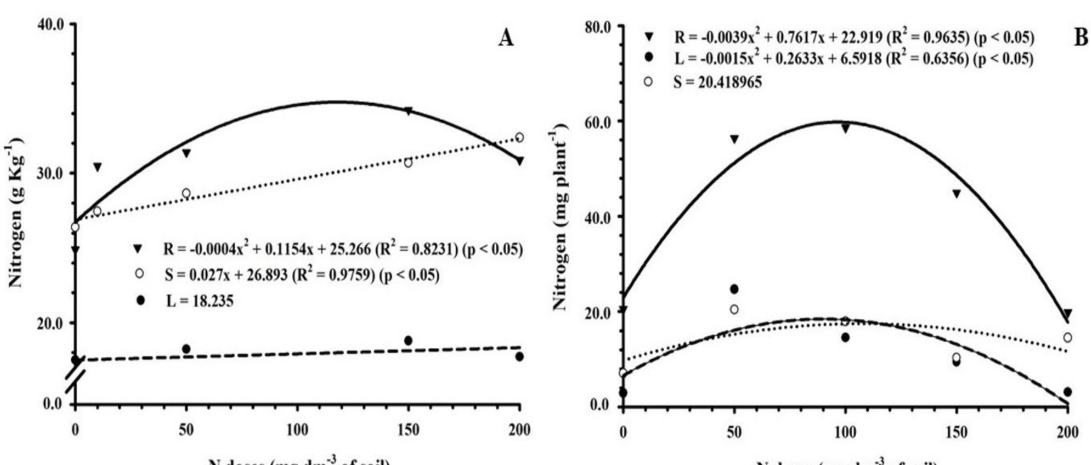

Figure 4. Nitrogen content (A) and accumulation (B) in leaves, stem and roots of *A. crassiflora* seedlings in relation to N fertilization doses.

Table 2. Mean values of biometric variables in relation to potassium doses (K). D-diameter, H-height, NL-number leaves, DML-dry mass of leaves, DMSt- stem, DMR-roots, DMSh-shoots, TDM-total dry mass, H/D-height-to-diameter ratio, R/S-root-to-shoot ratio and DQI-Dickson Quality Index.

K doses mg dm^{-3}	D mm	H cm	NL	DML g	DMSt g	DMR g	DMSh g	TDM g	R/S	H/D	DQI
0	9.4 a	12.9 a	7.5 a	1.5 a	0.9 a	3.6 a	2.4 a	6.0 a	1.3 a	0.7 a	2.8 b
50	9.2 a	7.9 a	5.8 a	2.1 a	0.8 a	1.6 a	2.9 a	4.5 a	1.2 a	1.1 a	1.4 b
100	7.5 a	7.2 a	4.5 a	1.0 a	0.8 a	3.0 a	1.8 a	4.8 a	1.5 a	0.9 a	2.1 b
150	17.8 a	14.5 a	7.8 a	3.5 a	2.5 a	8.9 a	6.0 a	14.9 a	1.4 a	1.2 a	6.9 a
200	10.8 a	10.2 a	6.3 a	0.9 a	1.2 a	2.8 a	2.1 a	4.9 a	1.3 a	1.0 a	2.3 b

Means followed by the same letter did not differ from each other (p < 0,05).

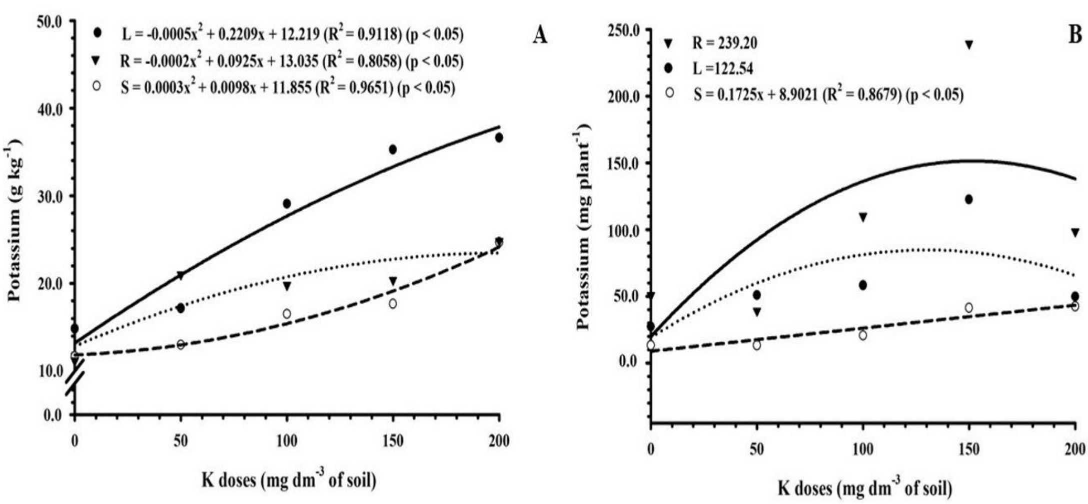

Figure 5. Potassium content (A) and accumulation (B) in leaves, stem and roots of *A. crassiflora* seedlings in relation to K fertilization doses.

In general, a trend towards higher growth rates at K dose of 150 mg dm^{-3} was observed, and the dose presented statistically higher result for DQI; a strong indication that this would be the recommended dose for the species under study.

The K content in the tissues of *A. crassiflora* plants (Figure 5A) showed quadratic behavior. The highest K content was found at dose of 200mg dm^{-3}.

Nutrient accumulations presented no significant difference. The results obtained by calculating the potassium accumulation in plants indicated the highest values at P doses of 137, 200 and 158 mg per dm^{-3} of soil for leaves, stem and roots, respectively (Figure 5B).

4. DISCUSSION

Nitrogen fertilization had significant effect on the growth and quality of *A. crassiflora* seedlings. The results obtained in this study are similar to those found by Carnevali et al. (2016) in *Stryphnodendron polyphyllum* seedlings and by other authors in pitaia (*Hylocereus undatus*) (Almeida et al., 2014), *canafistula* (*Peltophorum dubium*) (Souza et al., 2013), *gonçalo-alves* (*Astronium fraxinifolium*) (Feitosa et al., 2011), *açaí* (*Euterpe oleracea*) (Oliveira et al., 2011) and *angico-vermelho* (*Anadenanthera macrocarpa*) (Gonçalves et al., 2012). Plants submitted to nitrogen doses generally present higher photosynthetic activity during the process of CO_2 assimilation in the catalytic sites of the RuBP carboxylase enzyme in detriment of absorption, translocation and use of assimilates from senescent leaves to storage organs or growing tissues (Lötter et al., 2014).

Thus, studies involving growth analyses constitute an important indicator of the quality of *A. crassiflora* seedlings (Duarte et al., 2015), allowing describing in part the physiological and morphological behavior of the plant during a certain period of time, which would not be possible with just a simple record of the nutritional content yield (Pedó et al., 2014).

Both potassium and nitrogen showed high redistribution in plant tissues; however, when there is lack of N and K, plants stimulate the activation of innumerable morphophysiological responses in order not to be impaired, reducing the photosynthetic rate, leaf osmotic potential and shoot growth (Magadlela et al., 2014). On the other hand, it increases the root system efficiency in order to obtain access to greater volume of soil (Rajan & Veilumuthu Anandhan, 2016), which represents an important strategy for physiological, morphological and biochemical adaptation to ensure growth, cellular turgor maintenance, and absorption of sufficient water and nutrients to meet the plant needs. On the other hand, when the nitrogen and potassium content is excessive in the root system, they can cause toxicity, directly affecting growth (Saiz-Fernández et al., 2015; Deng et al., 2015). Thus, high doses can also be harmful.

Nutrient concentration in senescent leaves is entirely correlated with the presence of green leaves, and the increase in nutrient reabsorption efficiency considerably reduces N and K concentrations in green leaves. This flexibility indicates a strategy in the acquisition of biomass to reallocate photoassimilates, coordinating a series of processes related to the formation of several organs; however, adjustment in the efficiency of the internal use of nutrients is optional (Zhang et al., 2015).

A discrete response of seedlings in relation to potassium fertilization of morphological variables was also observed in vines (*Platymenia foliolosa*), where results similar to *A. crassiflora* were only found for the quality of seedlings and shoot height/diameter ratio (Duarte et al., 2015). In *Dalbergia nigra* seedlings, mathematical models appropriately adjusted to increasing K doses grown in Red-yellow Alic Latosol (LVAa), Mesotrophic Red-yellow Argisol (PVAm) and Dystrophic Red-Yellow Latosol soils (LVAd) were also not found (Gonçalves et al., 2014). This tendency also resembles the results observed by Gonçalves et al. (2010) in *Mimosa caesalpiniaefolia* seedlings using Dystrophic Red Latosol (LVd) and Red-Yellow Latosol (LVAs) soils as substrate, in which absence of effect of potassium fertilization in relation to the several evaluated growth characteristics was observed.

Compared with the obtained results, mahogany (*Swietenia macrophylla*), similar to the other species mentioned above, did not respond to K doses or even to nitrogen fertilization (Tucci et al., 2011). Although no effects were observed on seedlings, potassium supply is still important. The lack of response to this nutrient indicates that low amounts of K present in the soil are possibly sufficient to meet the plant needs. This explains the low nutritional requirement of *A. crassiflora* seedlings in terms of K (Gonçalves et al., 2014). Souza et al. (2010) and Cruz et al. (2010, 2011) found significant effect of K application on *fedegoso* seedlings (*Senna macranthera*), indicating that some forest species present greater nutritional K requirement when compared to the species under study.

The present study provides evidence of the positive effects promoted by nitrogen and potassium fertilization on *A. crassiflora* seedlings. In general, nitrogen affected the growth and quality of seedlings by altering several parameters, including N and K allocation and accumulation, which promoted an increase in height, diameter, shoot and root dry mass, which in turn promoted better Dickson quality index (DQI). Based on these variables, it was possible to evidence that the best nitrogen fertilization doses are 100 to 200 mg dm^{-3} while in the nursery stage. Although potassium fertilization showed discrete behavior for most characteristics evaluated under the conditions of this study, DQI, nutrient content and accumulation in *A. crassiflora* seedlings showed significant response.

5. CONCLUSIONS

Nitrogen fertilization positively influences the growth and quality of *Annona crassiflora* Mart. Seedlings at doses between 100 and 200mg dm^{-3}under the conditions of this study.

The best quality of *A. crassiflora* seedlings regarding potassium fertilization was found for the dose of 150 mg dm^{-3}.

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