Original Paper Physiological and morphological responses of seedlings of *Guazuma ulmifolia* (Malvaceae) grown under phosphorus levels

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Abstract

Phosphorus (P) is one of the nutrients most required by plants, especially in the initial phase, however, there are species whose growth effects promoted by phosphorus are still unknown. Therefore, this study aimed to test whether increasing levels of phosphorus influence the physiological and morphological behavior and the quality of seedlings of *Guazuma ulmifolia*. For that purpose, the seedlings were grown in a substrate produced only with Dystrophic RED LATOSOL (Oxisol) under a completely randomized design and subjected to five levels of phosphorus (0, 100, 200, 300, and 400 mg dm⁻³). At 120 days, the biometric and physiological evaluations were performed, and the chlorophyll indices, biomass, allometric relationships, Dickson Quality Index, and the phosphorus content were determined. The data were subjected to analysis of variance by the F-test and subsequently to regression analysis and Pearson's linear correlation. Phosphorus fertilization promoted significant gains ($p \le 0.05$) for all variables morphological e physiological, except for the specific leaf area and allometric relationships. The phosphorus content was positively correlated with all tested variables. Based on the results obtained, it is concluded that the seedlings responded positively to phosphorus fertilization. Furthermore, phosphorus fertilization promoted better physiological and morphological performance and higher seedling quality.

Key words: fertilizer, mutambo, phosphorus, specie.

Resumo

O fósforo (P) é um dos nutrientes mais exigidos pelas plantas, principalmente, na fase inicial, no entanto, existem espécies cujos efeitos de crescimento promovidos pelo fósforo ainda são desconhecidos. Portanto, este trabalho teve como objetivo testar se níveis crescentes de fósforo influenciam no comportamento fisiológico e morfológico e na qualidade das mudas de *Guazuma ulmifolia*. Para tanto, as mudas foram cultivadas em substrato produzido apenas com LATOSSOLO VERMELHO distrófico, em delineamento inteiramente casualizado e submetido a cinco níveis de fósforo (0, 100, 200, 300 e 400 mg dm⁻³). Aos 120 dias, as avaliações biométricas e fisiológicas foram realizadas, e os índices de clorofila, biomassa, relações alométricas, Índice de Qualidade de Dickson e o teor de fósforo foram determinados. Os dados foram submetidos à análise de variância pelo teste F e posteriormente à análise de regressão e correlação linear de Pearson. A fertilização com fósforo promoveu ganhos significativos ($p \le 0,05$) à todas as variáveis morfológicas e fisiológicas, exceto para a área foliar específica e relações alométricas. O conteúdo de fósforo foi positivamente correlacionado com todas as variáveis testadas. Com base nos resultados obtidos, conclui-se que as mudas responderam positivamente à fertilização com fósforo. Além disso, a fertilização com fósforo promoveu melhor desempenho fisiológico e morfológico e maior qualidade das mudas.

Palavras-chave: fertilizante, mutamba, fósforo, espécies.



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Introduction

The production of quality seedlings is related to a series of factors, among which fertilization is one of the most important since it is performed to promote plant nutrition: when appropriate and balanced, it provides higher plant growth and development (Natale et al. 2018). Among mineral fertilizations, phosphorus fertilization is extremely important as it relates to vital plant processes, such as photosynthesis and respiration. Furthermore, phosphorus (P) is a constituent of nucleic acids, phospholipids, proteins, phosphate esters, and adenosine triphosphate (Dechen & Nachtigall 2007). Therefore, P participates directly in several plant physiological processes and provides energy to trigger several cellular metabolic processes. Thus, this nutrient is essential in plant growth and development (Malhotra et al. 2018), especially in the early development stage.

P is one of the nutrients that deserve attention in seedling production, not only for the role it plays in plants but also for the low availability of this element in the soil under natural conditions (Silva et al. 2018). This occurs because the soils of tropical regions are naturally acid and highly weathered. Soils with these characteristics show high P adsorption capacity, which results in the low availability of this nutrient to plants (Guedes et al. 2016). Considering that this condition compromises the initial growth of plants, since many still die in the juvenile stage and because they do not reach the adult stage, this interferes with seed dispersal and natural production of new individuals. thus affecting the distribution of the species. It is essential to understand how P availability affects the morphological and physiological performance of seedlings of forest species, as this can help in their preservation, preventing possible future extinctions.

In view of the previous considerations, several studies aimed to evaluate the effects of P fertilization on the production of seedlings of numerous species. The results obtained vary as the plants have distinct needs and also variable quality standards. However, in general, P fertilization has promoted positive effects on the early growth of many species (Freitas *et al.* 2017a; Andrade *et al.* 2018; Moreira *et al.* 2018; Santos *et al.* 2019). Although P fertilization benefits are widely publicized, there are still no studies of this nature for some species of food and medicinal importance, such as *Guazuma ulmifolia* Lam. (Malvaceae), commonly known in Brazil as *mutambo*.

Guazuma ulmifolia is widely distributed throughout Mexico. Central. and South America. and in the Brazilian territory, it occurs in all phytogeographic domains (Colli-Silva 2019). In medicinal terms, the bark extract is antimicrobial, antioxidant, and cardioprotective (Santos et al. 2018b). The plant also shows activity against parasites such as Leishmania brasiliensis, L. infantum, and Trypanosoma cruzi (Calixto Júnior et al. 2016). The tea or juice of the leaves of G. ulmifolia has been indicated by several doctors in Brazil and Venezuela as an alternative treatment for HIV patients (Gouveia 2018; Folha de São Paulo 2018; Singer 2018; Macedo 2019). The fruits are consumed by birds and primates (Carvalho 2007), highlighting their importance for reforestation programs (Calzavara et al. 2017), and can also be consumed by humans due to their high dietary fiber content and bioactive phenolic compounds (Pereira et al. 2020).

As seen above, the species has the potential for wide exploitation by the industrial sector, either as food or as a therapeutic plant (Pereira *et al.* 2019). However, silvicultural studies for this species are still scarce. Therefore, this study aimed to evaluate the growth and development of seedlings of *G. ulmifolia* produced with different levels of P to test the hypothesis that the seedlings of *G. ulmifolia*, when produced with P fertilization, show better physiological and morphological performance and, as a consequence, higher quality.

Material and Methods

Study area and growing conditions

The present study was conducted in a plant nursery of the Goiano Federal Institute - Campus Rio Verde, municipality of Rio Verde, Goiás, Brazil, under a completely randomized design composed of five treatments (0, 100, 200, 300, and 400 mg dm⁻³ of P) and four replications. The soil used as substrate was a dystrophic RED LATOSOL (Oxisol) (Santos *et al.* 2018a) collected at the 0.0 to 0.20 m depth layer, used for its low P content. Samples from this soil were collected and sent to the Solotech Cerrado LLC Laboratory for chemical characterization. The result of the natural conditions of the soil used in this study is shown in Table 1.

Base saturation was increased to 60% (Raij 1997) with the soil correctives Ca and Mg carbonate at a 4:1 ratio. Monoammonium phosphate (MAP) was used as a P source, and basic fertilization (mg dm⁻³) was applied ten days before establishing the

	cmol	_c dm ⁻³			mg dm ⁻³	%	g dm ⁻³			
Ca	Mg	Al	Κ	pН	Р	V	O.M	Clay	Sand	Silt
1.06	0.49	0.05	0.32	5.1	2.6	47	41.3	48	40	12

Table 1 – Chemical and physical characterization of the natural soil used as substrate for the production of seedlings of *Guazuma ulmifolia* Lam. (Malvaceae).

Ca = Calcium; Mg = Magnesium; Al = Aluminium; pH = Hydrogen potential; P = Phosphorus; K = Potassium; V = Base saturation; O.M = Organic matter. KCl 1N for Ca, Mg, and Al extraction; and the colorimetric method for organic matter. Mehlich 1 for P and K extraction.

treatments. Basic fertilization is generally applied so that the absence of other nutrients does not interfere with the treatments of the one tested, in this case, those of P. Therefore, basic fertilization (mg dm⁻³) was performed 30 days after liming by providing 180 mg dm⁻³ nitrogen (N), 150 mg dm⁻³ potassium, 40 mg dm⁻³ sulfur, 1.33 mg dm⁻³ copper, 0.81 mg dm⁻³ boron, and 4 mg dm⁻³ zinc, using as sources: urea and ammonium sulfate, potassium sulfate and potassium chloride, copper sulfate, boric acid, and zinc sulfate, respectively (Carlos *et al.* 2015). Since the MAP has both N and P in its constitution, a balancing was performed to match the amount of N in all P treatments.

Seed collection and dormancy breaking

The seeds of *G. ulmifolia* were collected from ten parent plants located in the orchard of the University Federal of Lavras, municipality of Lavras, state of *Minas Gerais*, between August and September. Seed dormancy was overcome with hot water at 70 °C for approximately 30 minutes or until the water temperature was reduced to 50 °C (Nunes *et al.* 2006). Sowing was performed in non-perforated 4-liter pots 45 days after acidity correction. Irrigation was performed daily, maintaining the substrate at 60% of field capacity by weighing the pots with the plants and directly irrigating the soil.

Biometric assessments

The biometric evaluations were performed 120 days after sowing by measuring the plant height (H) with a millimeter rule, between the stem and stem apex, while the base diameter (D) was obtained with a digital caliper. The number of fully expanded leaves (NF) was also counted.

Gas exchange assessments

At 115 days after sowing, the gas exchange analyses were performed using a portable

infrared gas analyzer (Model Li-6800xt, Li-Cor, Nebraska, USA), with constant irradiance with photosynthetically active radiation (PAR) of 1.500 µmol photons m⁻² s⁻¹, atmospheric concentration of CO₂ (Ca) de ~400 µmol mol⁻¹, temperature of ~25 °C and humidity of ~50%, between 8:00 a.m. and 11 a.m., the gas exchange analyses were determined on a leaf of the second pair from the apex of the plant, by evaluating the variables: photosynthetic rate [A, µmol (CO₂) m⁻² s⁻¹)], transpiration rate [E, mmol (H₂O) m⁻² s⁻¹], stomatal conductance [gs, mol (H₂O) m⁻² s⁻¹]. The electron transport rate (ETR, µmol m⁻² s⁻¹) was established through the fluorescence of the chlorophyll *a* from seedling leaves.

Chlorophyll Index

At 115 days after sowing, the photosynthetic pigments were analyzed by measuring the Falker® Chlorophyll Index (Chl a and Chl b) on the same leaf in which the gas exchange analyses were performed. A ClorofiLOG chlorophyll meter was used for this analysis (model CFL1030, Falker Automação Agrícola, Porto Alegre, BRA). The total chlorophyll index was obtained through the sum of chlorophyll a and b.

Biomass and allometric ratios

At 120 days after sowing, the seedlings were removed from the pots, cleaned, separated into leaves, stems, and roots, and dried in a forced-air oven at 65 °C until constant weight. Finally, the material was weighed in a digital balance, thus obtaining the biomass variables: leaves dry mass (LDM), stem dry mass (SDM), and root dry mass (RDM). The sum of these three variables resulted in the total dry mass (TDM), after which the allometric relationships were also calculated: foliar mass ratio (LDM/TDM, g g⁻¹), stem mass ratio (RDM/TDM, g g⁻¹).

Dickson Quality Index

The leaf area (LA) was calculated with photographic records made with Smartphone (12 megapixels) of the leaves from each experimental unit, obtained when the experiment was dismantled, using the software Image J \otimes (HIN, Bethesda, Maryland, USA). With the LA and LDM data, it was possible to calculate the specific leaf area (SLA) through the formula: LA/LDM. Based on the growth and biomass data, it was also possible to calculate the Dickson Quality Index (DQI) (Dickson *et al.* 1960), following the formula below:

$$DQI = TDM / (H/D) + (APDM/RDM)$$

TDM = total dry mass; H = height; D = diameter; APDM = aerial part dry mass; RDM = root dry mass.

P content

The shoot P content of the seedlings (leaves and stems) was determined by colorimetry following the methodology by Santos *et al.* (2009), and the reading was performed by spectrophotometry.

Statistical analysis

The data obtained were tested regarding the assumptions of normality and homogeneity, and posteriorly subjected to analysis of variance (ANOVA) and when the results were significant (F test, p < 0.05) it was evaluated linear and quadratic regression analysis to verify the adjustment of the data to the models. The choice of the model occurred according to the significance of each equation and determination coefficient (R²). Such statistical analyzes were performed using software SISVAR (Ferreira 2014). Pearson's linear correlation coefficient was calculated by involving the variables with each other, utilizing software BioEstat (Ayres *et al.* 2007).

Results

The supply of P promoted a significant increase ($p \le 0.05$) in the shoot P content of the seedlings of *G. ulmifolia*. The 400 mg dm⁻³ level promoted the highest value among the tested levels, reaching 14 g of P kg of plant tissue (Fig. 1).

P fertilization promoted a significant effect on the chlorophyll indices of the seedlings of *G. ulmifolia*. The data obtained fit the quadratic



Figure 1 – Phosphorus content of seedlings of *Guazuma ulmifolia* Lam. (Malvaceae) as a function of phosphorus levels at 120 days after sowing.

model, and the 400 mg dm⁻³ P level promoted the highest values for these variables, reaching the indices of 31.5 for chlorophyll a, 10 for chlorophyll b, 41.5 for total chlorophylls (Fig. 2a-c). On the other hand, for chl a/chl b ratio, there was a decrease in the function of increasing doses of P, with a dose of 200 mg dm⁻³ being the one that promoted the highest chl a/chl b ratio; 3.72 (Fig. 2d).

The P levels significantly influenced the physiological variables. The data obtained fit the quadratic model, although, the highest level tested (400 mg dm⁻³) was also the one that promoted the highest values for these variables, reaching 17 and 93.5 μ mol m⁻² s⁻¹ for *A* and ETR, respectively (Fig. 3a-d), and 0.41 mol m⁻² s⁻¹ for gs and 0.01 mmol m⁻² s⁻¹ for E (Fig. 3b-c).

The biometric variables also showed significant gains with the phosphorus levels. The highest P level tested, 400 mg dm⁻³, promoted the highest values, and the plants showed, on average, 49 cm height, 9 mm diameter, 22 leaves, and 800 cm² of leaf area (Fig. 4a-d). However, for specific leaf area, P levels do not promote the difference.

The biomass variables were significantly affected by the P levels. For all biomass variables obtained, the highest values were found at the highest P level tested, 400 mg dm⁻³. The seedlings showed, on average, 10.5 g of LDM, 4.6 g of SDM, 35 g of RDM, and 50 g of TDM (Fig. 5a-d). However, for the allometric relationships, the treatments promoted no differences.

P fertilization promoted a significant effect on the DQI of the seedlings of *G. ulmifolia*. The equation that best fit was the linear model, and the highest P level tested, 400 mg dm⁻³, promoted the highest index, 9 (Fig. 6).



Figure 2 – a-d. Falker® chlorophyll indices of seedlings of *Guazuma ulmifolia* Lam. (Malvaceae) as a function of phosphorus levels at 115 days after sowing – a.chlorophyll a; b.chlorophyll b; c.total of chlorophylls; d. ratio between chlorophyll a and b.



Figure 3 – a-d. Physiological variables of seedlings of *Guazuma ulmifolia* Lam. (Malvaceae) as a function of phosphorus levels at 115 days after sowing – a. photosynthetic rate; b. stomatal conductance; c. transpiration rate; d. electron transport rate.



Figure 4 – a-d. Biometric variables of seedlings of *Guazuma ulmifolia* Lam. (Malvaceae) as a function of phosphorus levels at 120 days after sowing – a. height; b. diameter; c. number of leaves; d. leaf area.



Figure 5 – a-d. Biomass variables of seedlings of *Guazuma ulmifolia* Lam. (Malvaceae) as a function of phosphorus levels at 120 days after sowing – a. leaf dry mass; b. stem dry mass; c. root dry mass; d. total dry mass.



Figure 6 – Dickson Quality Index of seedlings of *Guazuma ulmifolia* Lam. (Malvaceae) as a function of phosphorus levels at 120 days after sowing.

All significant correlations found were positive. And, the P level was strongly correlated with all studied variables. The photosynthetic rate correlated with the shoot P level of the seedlings as well as with the variables of growth, biomass accumulation, chlorophylls, and DQI. The DQI also showed to be highly correlated with the biometric variables and biomass, while what the ETR correlated with the chlorophylls *a* and *b* and with all physiological variables (Tab. 2).

Discussion

The results found in the present study show that the increasing phosphorus levels promoted better physiological and morphological performance and higher-quality seedlings of G. ulmifolia. The specie showed to be highly responsive to the application of P to the soil since a significant influence was verified for all studied variables, except for the specific leaf area and allometric relationships. Among the P levels tested, the 400 mg dm⁻³ level promoted the highest values for all variables. This high P requirement in the early stage is associated with the pioneer character of the specie (Herrera-Peraza et al. 2016), and for showing a rapid growth (Silva et al. 2016). As a function of the improvement in soil fertility, there is greater investment in seedling growth as the seedlings require greater amounts of nutrients to meet the nutritional demand, allowing an increase in the biomass production potential of species with a high early growth (Silva et al. 1997).

The greater P uptake by the seedlings allowed them to increase the Falker® indices of chlorophylls a and b, the number of leaves, and the leaf area. The higher indices of leaf photosynthetic pigments (chlorophylls) can be attributed to the increased P concentration in the tissues, which, in theory, accelerated the energetic metabolism and cell division (Marschner 2012), also stimulating the increase in the leaf area. Chlorophylls are extremely important as they are responsible for light capture during photosynthesis, resulting in the excitement of the electrons used to boost the reduction of nicotinamide adenine dinucleotide phosphate (NADP) and chemical energy in the form of adenosine triphosphate (ATP) (Croft et al. 2017). The number of leaves and the leaf area are related to the ability to intercept solar radiation and boost CO₂ assimilation with allowing to accumulate dry matter (Crous et al. 2015; Huang et al. 2016). Berghetti et al. (2019) and Silva et al. (2020) found similar results to the present study, also verifying higher chlorophyll indices and increased leaf area in seedlings of Cordia trichotoma and Eucalyptus urophylla x Eucalyptus grandis, respectively, with the increase in the P levels.

With higher chlorophyll indices, number of leaves, leaf area, and shoot P concentration, the seedlings of G. ulmifolia were able to increase the efficiency photosynthetic, showing higher stomatal conductance, transpiration, electron transport rate, and higher photosynthetic rates. Berghetti et al. (2019, 2021) also verified that the high concentrations of chlorophylls a and b and the increase in the leaf area as a function of the high P availability reflected higher photosynthetic rates. This increase in the photosynthetic process of the seedlings is also essential as plants with an adequate leaf P supply show an increase in CO₂ assimilation, carboxylation efficiency, and in the photochemical process, influencing the gain of biomass by plants (Warren 2011). In this case, the seedlings of G. ulmifolia could specifically invest in height and diameter. Considering that P deficiency is one of the greatest limitations in the development of forest species (Zhu et al. 2018) since this nutrient plays a central role in plant growth, several studies have demonstrated that tropical tree species respond positively to the increase in the P levels, which is largely due to the low P levels in tropical soils (Fernandes et al. 2013).

The seedlings of *G. ulmifolia* also accumulated biomass as a function of the phosphorus levels. The results obtained in this study show that the seedlings showed greater vigor at higher P levels, indicating that the specie requires high P levels during its early growth, such as occurs with *Senna macranthera* (DC. *ex* Collad.) H.S. Irwin & Barneby (Cruz *et al.* 2011), *Cassia grandis* L. f. (Andrade *et al.* 2018), and *Dalbergia nigra* (Vell.)

Table 2 – Pearson's linear correlation coefficient (r) between the variables of seedlings of *Guazuma ulmifolia* Lam. (Malvaceae) subjected to phosphorus levels. H = height (cm); D = diameter (mm); NF = number of leaves; LA = leaf area (cm²); LDM = leaf dry mass (g); SDM = stem dry mass (g); RDM = root dry mass (g); DQI = Dickson Quality Index; Chl *a* = Chlorophyll *a*; Chl *b* = Chlorophyll *b*; *A* = photosynthetic rate (µmol (CO₂) m⁻² s⁻¹); gs = stomatal conductance (mol (H₂O) m⁻² s⁻¹); E = transpiration rate (mmol (H₂O) m⁻² s⁻¹); ETR = electron transport rate (µmol m⁻² s⁻¹); P = shoot phosphorus content (g Kg⁻¹).

	Н	D	NF	LA	LDM	SDM	RDM	DQI	Chl a	Chl b	A	gs	Е	ETR	Р
Н		0.99*	0.94*	0.96*	0.95*	$0.85^{\rm ns}$	0.87 ^{ns}	0.85*	0.82 ^{ns}	0.87 ^{ns}	0.88*	0.90*	0.81*	0.83 ^{ns}	0.87*
D			0.93*	0.95*	0.93*	0.84^{ns}	0.84 ^{ns}	0.82*	0.86 ^{ns}	0.90*	0.82*	0.92*	0.87*	$0.87^{ m ns}$	0.91*
NF				0.99*	0.99*	0.97*	0.97*	0.96*	0.76 ^{ns}	0.85 ^{ns}	0.84*	0.90*	0.86*	0.81^{ns}	0.78*
LA					0.99*	0.95*	0.95*	0.94*	0.80 ^{ns}	0.88*	0.88*	0.93*	0.71*	0.85 ^{ns}	0.82*
LDM						0.96*	0.97*	0.97*	0.72 ^{ns}	0.81 ^{ns}	0.89*	0.87*	0.61 ^{ns}	0.77^{ns}	0.75*
SDM							0.99*	0.99*	0.64 ^{ns}	0.76 ^{ns}	0.84*	0.82 ^{ns}	0.51 ^{ns}	0.73 ^{ns}	0.86*
RDM								0.99*	0.61 ^{ns}	0.73 ^{ns}	0.80*	0.80 ^{ns}	0.63 ^{ns}	0.69 ^{ns}	0.84*
DQI									0.58 ^{ns}	0.71 ^{ns}	0.87*	0.78 ^{ns}	0.98*	0.66 ^{ns}	0.81*
Chl a										0.98*	0.99*	0.95*	0.95*	0.99*	0.99*
Chl b											0.98*	0.98*	0.99*	0.99*	0.97*
A												0.94*	0.91*	0.99*	0.97*
gs													0.90*	0.97*	0.94*
Е														0.97*	0.95*
ETR															0.97*

Where: * = significant at 5% probability; ns = not significant.

Fr. All. *ex* Benth (Gonçalves *et al.* 2014; Carlos *et al.* 2018). Biomass is one of the best features to determine seedling quality as it correlates with plant vigor (Cruz *et al.* 2011), and, even if destructive, it reflects the investment made by the plant in biomass (Fernandes *et al.* 2019) and is also highly associated with the ability to survive in the field. Furthermore, the dry matter data (biomass) should be taken into account as they help reduce the erroneous classification of seedling quality, in the case of seedlings that are taller due to etiolation (Freitas *et al.* 2017a).

Consequently, the phosphorus levels influenced the Dickson Quality Index the seedlings of *G. ulmifolia*. This index determines seedling quality based on several morphological features, minimizing the possible errors that may occur by using only one or two features (Vieira *et al.* 2019). Therefore, with the results obtained, it is safe to affirm that the P levels increased the quality of the seedlings of *G. ulmifolia*. However, this was already foreseen as it also occurred for the variables needed to calculate this index, which agrees with the verified for other variables. This reinforces the high need for P that the seedlings require in the early stage in order to reach maximum growth, development, and vigor, with these being the most desirable features when the subject is seedling production.

Regarding the correlations found between the studied variables, values above 0.75 indicate that one variable allows inferring about the others (Freitas *et al.* 2017b); therefore, it is valid to say that the studied variables are strongly and positively interlinked as all significant correlations were positive. In physiological terms, the photosynthetic capacity is usually positively correlated with the P concentration, both in the soil and in the leaves (Bloomfield *et al.* 2014; Bahar *et al.* 2017), as leaf P is essential for the adjustment of net photosynthesis through the regulation of the main carbon metabolism intermediates (ATP, NADPH, and sugar phosphates, including ribulose 1-5bisphosphate) (Bahar *et al.* 2017). In terms of growth, the correlations were high because the P levels lead to growth increments and a marked development in pioneer species, in addition to improving the physiological capacity of the seedlings, which reflects in their morphological behavior (Resende *et al.* 1999).

Based on the results obtained, it is concluded that the seedlings of *G. ulmifolia* responded positively to P fertilization. The P level of 400 mg dm⁻³ promoted seedlings with better physiological and morphological performance and higher quality.

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