SCIENTIA PLENA
www.scientiaplena.org.br
VOL. 20, NUM. 04 2024
doi: 10.14808/sci.plena.2024.040205

Production of high-quality seedlings of *Handroanthus spongiosus* (Bignoniaceae), native to a seasonally dry tropical forest

Produção de mudas de alta qualidade de *Handroanthus spongiosus* (Bignoniaceae), nativa de floresta tropical sazonalmente seca

J. J. Silva[1]; C. R. Pelacani[2]; R. A. Gomes[3]; J. C. Silva[3]; D. Signor[4]; B. F. Dantas[5*]

[1]Pesquisador de Pós-Doutorado, Embrapa Semiárido, 52302-970, Petrolina-Pernambuco, Brasil.
[2]Departamento de Ciências Biológicas, Universidade Estadual de Feira de Santana, 44036-900, Feira de Santana-Bahia, Brasil.
[3]Programa de Pós-Graduação em Recursos Genéticos Vegetais, Universidade Estadual de Feira de Santana, 44036-900, Feira de Santana-Bahia, Brasil.
[4]Laboratório de Matéria Orgânica, Embrapa Semiárido, 52302-970, Petrolina-Pernambuco, Brasil.
[5]Laboratório de Tecnologia de Sementes, Embrapa Semiárido, 52302-970, Petrolina-Pernambuco, Brasil.

**barbara.dantas@embrapa.br*
(Recebido em 02 de dezembro de 2023; aceito em 22 de abril de 2024)

Studies of the use of alternative substrates have enabled producing seedlings of various species with high quality and low cost. The objective of this work was to evaluate the best cultivation conditions for production of *Handroanthus spongiosus* seedlings. The seeds were collected from five different populations in the state of Pernambuco, Brazil, and cultivated in six substrates: soil, sand, soil + vermiculite, soil + goat manure, soil + vermiculite + goat manure, and soil + goat manure + 30% biochar. We evaluated the emergence, shoot and root length, stem base diameter, shoot and root dry mass, and Dickson quality index. The substrates with addition of organic matter favored increased shoot length, stem diameter and dry mass. The incorporation of goat manure and biochar in the substrates favored the quality of the *H. spongiosus* seedlings.
Keywords: Caatinga, sete-cascas, organic matter.

Estudos com a utilização de substratos alternativos têm possibilitado a produção de mudas de diversas espécies com alta qualidade e baixo custo. O objetivo deste trabalho foi avaliar as melhores condições de cultivo para produção de mudas de *Handroanthus spongiosus*. As sementes foram coletadas em cinco populações diferentes no estado de Pernambuco, Brasil, e cultivadas em seis substratos: solo, areia, solo + vermiculita, solo + esterco caprino, solo + vermiculita + esterco caprino e solo + esterco caprino + 30% de biocarvão. Foram avaliados a emergência, comprimento da parte aérea e da raiz, diâmetro da base do caule, massa seca da parte aérea e da raiz e índice de qualidade de Dickson. Os substratos com adição de matéria orgânica favoreceram o aumento do comprimento da parte aérea, do diâmetro do caule e da massa seca. A incorporação de esterco caprino e biocarvão nos substratos favoreceu a qualidade das mudas de *H. spongiosus*.
Palavras-chave: Caatinga, sete-cascas, matéria orgânica.

1. INTRODUCTION

The strategies for production of high-quality seedlings of native species occurring in seasonally dry tropical forests (SDTF) are still incipient. The main obstacles to this production are finding adequate substrate formulations that favor development of seedlings [1] the variability of the emergence responses in relation to the origin of the seeds [2] and the different spatial scales between and within populations [3, 4]. This variability can also serve as an important positive characteristic, because the plants produced may have better resistance to various environmental factors, a situation little explored for the species analyzed here.

The use of alternative substrates as inputs from existing processes of farms, as well as the byproducts of the timber industry, can increase the autonomy of farmers and make their activity more sustainable [5]. The inclusion of animal manure in substrates can supply the majority of nutrients necessary for good seedling development [6]. However, there is no single substrate that is ideal for all species [7, 8, 5].

The Northeast region of Brazil concentrates 94.5% of the national production of goat products [9], meaning a plentiful supply of goat manure exists for use as input to formulate substrates for production of seedlings. Another input found widely in this region and that can potentially be used to prepare or enrich substrates for seedling production is the waste from the production of wine and other grape derivatives. The Vale do São Francisco region accounts for the country's second largest wine production, with approximately 500 hectares of vineyards, producing millions of liters of wine annually (almost 15% of national output), only behind the state of Rio Grande do Sul [10].

Therefore, developing strategies are important to favor the production of seedlings of native species at low cost, accessible to nursery owners and smallholders. Among these native species is *Handroanthus spongiosus* (Rizzini) S. Grose (Bignoniaceae), endemic to the Caatinga and variously known by the common names "ipê-cascudo", "ipê-amarelo", "sete-cascas" or "pau-d'arco" [11, 12]. It occurs mainly in sandy soils and can reach a height of 8 meters [11, 12]. It is on the IUCN red list of threatened species [13] as well as the corresponding list of the Brazilian Ministry of the Environment [14]. It can be used for recomposition of native vegetation in degraded areas and for urban afforestation [11, 12, 14]. We did not find any previous studies on strategies and protocols to produce *H. spongiosus* seedlings.

Based on the endangered conservation status of the species, the strong anthropization of dry tropical forests, especially in the Caatinga biome, and the lack of information on the emergence and quality of seedlings, we formulated the following two research questions: (i) Do *H. spongiosus* seeds have different response when collected from different tree stands and germinated in substrates with different formulations? (ii) What is the response of *H. spongiosus* seedlings growth and development to different substrates?

2. Material and Methods

2.1 Description of the study area

The *H. spongiosus* seeds were collected in a SDTF area in the state of Pernambuco, Brazil, and voucher specimens of the botanical materials were deposited in the herbarium of Feira de Santana State University (HUEFS), located in the municipality of Feira de Santana, Bahia State. The collection sites were located in the villages of Pau Ferro (8°55'57.4" S, 40°43'19.8" W, 431 m) (HUEFS - 243297) and Caiçara (9° 07' 24.5" S, 40° 23' 16.1" W, 393 m) (HUEFS - 252490), the experimental farm of Embrapa Semiárid research unit (Embrapa Semiárido) (9° 8' 8.9" S, 40° 18' 33.6" W, 365 m) (HUEFS - 259090), and the district of Cristália (8°5'56.3" S, 40°19'27.1"W, 403 m) (HUEFS - 259093), all located in the municipality of Petrolina, Pernambuco. The fifth collection site was located in the village of Jutaí (8° 33' 35.7" S, 40° 12' 1.9" W, 418 m) (HUEFS - 259094) in the municipality of Lagoa Grande, Pernambuco (Figure 1).

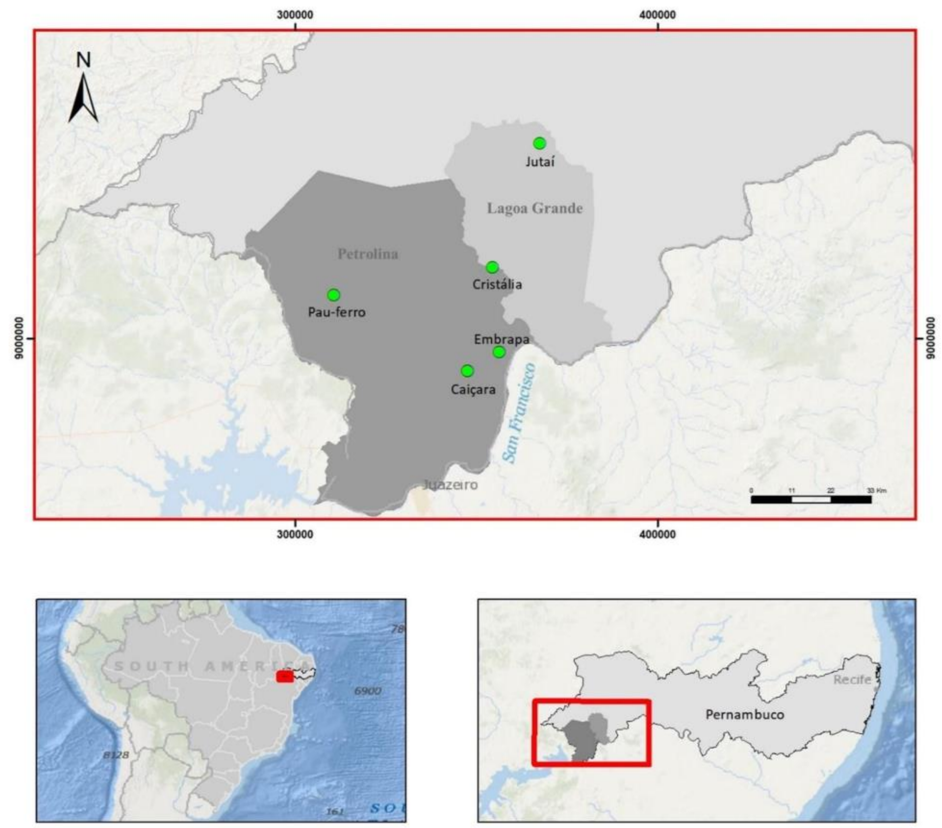

Figure 1. Location of the study area. Sites of collection of seeds of Handroanthus spongiosus (Rizzini) S. Grose (Bignoniaceae) in the state of Pernambuco, Brazil.

The predominant vegetation in this region is anthropized Caatinga remnant, with the presence of semideciduous xerophytic plants. The region has low rainfall, with yearly average of 435 mm, along with high rates of potential evapotranspiration (1520.9 mm), average annual temperature of 26 °C and relative humidity of 60% [15]. The climate is classified as BSh [16], with the presence of a strong rainy season in the summer (November to April) and a dry season from May to September. The soils of the Caatinga are characterized as shallow, stony and poor in organic matter on their surface [17].

2.2 Collection of seeds and water content measurement

At each collection site we selected eight parent trees for collection of the fruits with seeds, with the aid of a trimmer and tarp. The selection of the parent trees was based on size, vigor and health, with a minimum distance between the trees of 20 m. The seeds were obtained from ripe fruits (brown color), since the process of seed dispersion starts at the moment of physiological maturity. After opening the fruits, the seeds were gathered to form a single lot representing each site. Around 600 seeds were collected from each tree, totaling 4800 seeds per site. Then the water content was determined by oven drying the seeds at 105 ± 3 °C for 24 h using two subsamples of 50 seeds for each sampling site [18]. Quiescent seeds were stored in a cold chamber at 10 ± 3 °C and relative humidity of 60 ± 4% until conduction of the experiment [19].

2.3 Germination and vigor seeds of seeds

The seed germination percentage was evaluated with subsamples of four repetitions of 50 seeds collected from each sampling site. The seeds were distributed between three sheets of Germitest paper, moistened with distilled water in a proportion of 2.5 times the weight of the dry paper, placed individually in polyethylene bags and incubated in a BOD chamber (biochemical oxygen demand) at constant temperature of 25 ± 1 °C and 12 h photoperiod for 14 days [18]. Seeds with radicle length greater than or equal to 2.5 mm were considered germinated for calculation of the germination percentage (%G).

The seeds vigor (SV) was determined by electrical conductivity test with four repetitions of 50 seeds. The seeds were placed in beakers with 75 mL of distilled water and then incubated at a constant temperature of 30 ± 1 °C for 24 h [20]. After this interval, the electrical conductivity was read (expressed in $\mu S.cm^{-1}.g^{-1}$) in a solution containing the seeds with a Digimed model DM-31 benchtop conductivity meter for aqueous solutions, with a sensor (electrode) [21].

2.4 Production of seedlings

The experiment was structured in a 6 x 5 factorial scheme (6 cultivation conditions x 5 collection sites) in a completely randomized design with four repetitions of 25 seeds per treatment, under a sunscreen (50% light interception) in the nursery sector of the Embrapa Semiárido research unit (09° 04’ 16.4” S, 40° 19’, 5.37” W, 379 m).

Five seeds were sown in each polyethylene bag (15 x 20 cm) at a depth of 1.0 cm, filled with six substrate types (v/v): soil (So); sand (SD); soil + vermiculite (1:1, SoV); soil + goat manure (1:1, SoM); soil + vermiculite + goat manure (1:1:1, SoVM); and soil + goat manure + 30% biochar (1:1:1, SoMB). The substrates in all the bags were irrigated daily at intervals of 1 hour for 1 minute with an automatic spraying mechanism.

The soil used was classified as Ultisol (Argissolo Amarelo, according to Brazilian Soil System Classification), collected in the upper layer (0-20 cm) in the experimental field of Embrapa Semiárido. Before incorporation into the substrates, the soil and sand were sieved to remove impurities (twigs and leaves). We also used in the substrate formulation containing coarse “E” expanded vermiculite, biochar and goat manure.

Biochar was produced from grape vine production residues. Biochar production was carried out in a metal barrel. The barrel containing the raw material was closed and placed inside a brickwork oven, surrounded by dry mango trees branches that were burned for around 30 minutes, while the material inside the barrel was pyrolyzed at reduced atmospheric oxygen concentration. The barrel lid had four holes with 5 mm diameter, in order to allow the escape of gases produced during pyrolysis. In order to be used as substrate in the experiments, the biochar was ground and sieved through a 0.2 mm mesh.

The goat manure used was obtained from the semiarid Embrapa experimental farm, left for a week under irrigation to remove excess ammonia.

The substrates and/or seedlings were irrigated daily, during the 180 days of trail, at intervals of 1 hour for 1 minute with an automatic spraying mechanism.

2.5 Physical and chemical properties of the substrates

The physical and chemical characteristics of all substrates including the biochar were determined by the method described by Embrapa (2009) [22]. All the analyses were carried out in the Laboratory for Analysis of Soil, Water and Plants of Embrapa Semiárido in Petrolina, Pernambuco (PE). The total porosity and density of the soil were determined, respectively, by the stress table (stress of 6 kPa) and volumetric ring methods, and the granulometry was measured by the sieve-pipette and textural triangle method [22]. The chemical analyses consisted of determining the electrical conductivity (EC) in saturation paste; pH in water at soil-water ratio of 1:2.5; contents of Al, Ca and Mg (exchangeable, extracted with KCl 1 mol $.L^{-1}$, analyzed by titrimetry); P, K and Na (Mehlich 1 extraction); Cu, Fe, Mn and Zn (Mehlich 1 extraction and reading by atomic-absorption spectrophotometry); sum of bases (SB); and cation exchange capacity (CEC) (calcium acetate extraction), in all cases according to Embrapa (2009) [22].

The lowest EC values of the solution (water + substrates), pH, CEC and base saturation (V%) were obtained in the substrates composed of SD, So and SoV (Table 1). The EC and pH results allowed inferring the concentration of soluble salts and input of available nutrients in the substrates [23]. According to Minami and Salvador (2010) [24], EC values between 0.15 and 0.49 mS.cm^{-1} are considered low, while those greater than 3.4 mS.cm^{-1} are considered very high. Therefore, among the substrates tested in our experiments, those with the presence of organic matter (goat manure) were the richest in nutrients. However, pH values below 5.8 can increase the availability of iron and manganese and reduce the availability of nitrogen, potassium, calcium and magnesium [25], while values greater than 6.5 can cause unavailability of phosphorus, iron, zinc and copper [26]. Only the soil and SoV substrates had pH values below 6.5.

Table 1. Physical-chemical properties of the substrates produced and chemical properties of the biochar produced based on grape juice processing residues.

Parameters	SD	So	SoM	SoV	SoVM	SoMB	Biochar
EC (mS.cm^{-1})	0.21	5.63	12.45	4.55	6.87	8.58	0.82
pH	6.90	6.40	7.60	6.40	7.60	7.50	5.40
P (mg.dm^{-3})	1.43	4.88	55.30	6.61	221.80	167.86	730.23
K^+ (mg.dm^{-3})	0.04	1.40	1.10	2.00	2.10	1.80	0.95
Na^{2+} (mg.dm^{-3})	0.11	0.46	0.56	0.48	0.62	0.47	0.02
Ca^{2+} (mg.dm^{-3})	0.60	2.50	4.90	2.40	5.00	6.50	3.00
Mg^{2+} (mg.dm^{-3})	0.25	1.30	2.60	1.30	2.80	3.60	1.70
H + Al (cmol$_c$dm^{-3})	0.20	1.20	0.00	1.00	0.00	0.00	13.00
SB (cmol$_c$dm^{-3})	1.00	5.70	9.20	6.20	10.50	12.40	5.70
CEC (cmol$_c$dm^{-3})	1.20	6.90	9.20	7.10	10.50	12.40	18.60
V (%)	80.60	82.50	100.00	86.60	100.00	100.00	30.40
Cu (mg.dm^{-3})	0.59	0.76	0.99	0.80	1.05	0.78	ND
Fe (mg.dm^{-3})	90.23	14.04	23.88	34.69	68.64	54.60	ND
Mn (mg.dm^{-3})	10.64	27.36	41.60	54.75	60.10	33.54	ND
Zn (mg.dm^{-3})	1.22	1.26	4.86	7.24	7.40	1.36	ND
Soil density (mg.dm^{-3})	1.69	1.41	1.24	1.38	1.21	1.23	ND
Particle density (mg.dm^{-3})	2.68	2.47	2.53	2.53	2.38	2.35	ND
Total porosity (%)	37.03	44.57	49.73	45.72	49.24	47.53	ND
Total sand (g.kg^{-1})	971.00	627.00	615.00	571.00	522.00	608.00	ND
Silt (g.kg^{-1})	28.00	302.00	348.00	357.00	426.00	325.00	ND
Clay (g.kg^{-1})	0.88	71.18	35.88	72.58	52.18	66.98	ND
Ash (%)	ND	ND	ND	ND	ND	ND	8.37
C (%)	ND	ND	ND	ND	ND	ND	55.65
N (%)	ND	ND	ND	ND	ND	ND	1.80
H (%)	ND	ND	ND	ND	ND	ND	5.76

SD, sand; So, soil; SoM, soil + cured goat manure; SoV, soil + vermiculite; SoVM, soil + vermiculite + goat manure; SoMB, soil + goat manure + 30% biochar; EC, electrical conductivity; pH, potential of hydrogen determined in water; P, phosphorus content; Mg^{2+}, magnesium content; Ca^{2+}, calcium content; Na, sodium content; K^+, exchangeable potassium; H + Al, potential acidity; SB, sum of bases (Ca^{+2} + Mg^{+2} + Na^{+1} + K^{+1}); CEC, cation exchange capacity (H + Al + SB); V%, saturation of bases (SB/CEC)*100; Cu, copper; Fe, iron; Mg, magnesium; Zn, zinc; ND, not determined.

2.6 Emergence and growth of seedlings

The final emergence percentage (%E) was calculated 14 days after sowing (DAS). The morphological characteristics were evaluated at 180 DAS involving measurement of shoot length (SL, cm) and root length (RL, cm) with a ruler (cm) and stem base diameter (SBD, mm) with a digital pachymeter (0.01 mm). Then the seedlings were washed in tap water to remove substrate clumps and cut at the base of the stem and separated into organs (roots and shoots) to obtain the dry mass. The shoots and roots were placed in labeled brown paper bags and arranged in a forced-air oven at temperature of 65 °C for 72 h. The shoot dry mass (SDM) and root dry mass (RDM) were determined by weighing with an analytic balance (precision of 0.0001 g) and the results were expressed in grams [27]. The total dry mass (TDM) was obtained by the sum of SDM and RDM.

Based on these morphological characteristics, we evaluated the quality of the seedlings according to the collection site and substrate types by calculating the Dickson quality index (DQI) [28], according to Equation: DQI = (TDM (g))/((SL (cm))/(SBD (mm))+(SDM (g))/(RL (g))).

2.7 Statistical analyses

Initially we evaluated the normal distribution of residuals and homogeneity of variances by the Shapiro-Wilk test [29] and Levene test [30], respectively, at probability of 0.05. Then we analyzed the data by applying generalized linear models (GLM) [31]. After the GLM analysis, we investigated significant differences within the principal effects (substrates and collection sites) by pairwise comparison of the means using the post-hoc Tukey test ($P < 0.05$). The means were adjusted by the method of Šidák (1967) [32].

To evaluate the relationship of the different cultivation conditions, seed collection sites and morphological characteristics of the seedlings, we applied multiple factor analysis (MFA) utilizing the "FactoMineR" package [33] of the R software [34]. The MFA is used to analyze related multivariate characteristics and thus extract key information from the original dataset. The choice of the principal components was based on the quantity of variance explained, with a threshold of at least 70% of the total variance of the original dataset [35]. All the analyses were performed with the R software [34].

3. RESULTS AND DISCUSSION

3.1 Germination (G%) and vigor (SV) of seeds

The upper and lower water content (WC) values were observed for the seeds gathered at Cristália (6.90%) and Embrapa (5.20%), respectively (Table 2). There was a statistically significant difference ($P < 0.05$) between the collection sites for germination percentage (%G) and electrical conductivity test for seed vigor (SV). The highest values of %G were found for the seeds collected at Cristália (91.5 ± 2.22) and the lowest value in Caiçara (75.0 ± 3.70). The SV is related to the integrity of the cell membranes and is measured to determine the physiological quality of seeds, where low values denote low quantities of leachates (ions, sugars, organic acids, amino acids) in the absorbed water [36]. The seeds collected at Cristália, Embrapa and Pau Ferro had the best physiological quality.

Table 2. Water content (WC), germination percentage (%G) and electrical conductivity of seeds leachate - seed vigor (SV) of Handroanthus spongiosus (Rizzini) S. Grose (Bignoniaceae), collected in november 2019 at Pau Ferro, Caiçara, Embrapa, Cristália and Jutaí, Pernambuco, Brazil.

Sites	WC (%)	%G	SV ($\mu S.cm^{-1}.g^{-1}$)
Pau Ferro	5.27	84.0 ±3.16 b	129 ± 16.93 b
Caiçara	5.26	75.0 ± 3.70 c	272 ± 22.23 a
Embrapa	5.20	84.5 ± 4.35 b	126 ± 5.62 b
Cristália	6.90	91.5 ± 2.22 a	114 ± 3.78 b
Jutaí	5.24	82.0 ± 4.24 b	229 ± 21.63 a
SW (*P*)		0.979 (0.862)	0.866 (0.014)
L (*P*)		0.475 (0.754)	1.914 (0.160)
CV%		8.68	18.49

Means followed by different lowercase letters in the columns differ statistically by the Tukey test at probability of 0.05. WC%, water content; SV, seed vigor - electrical conductivity of leachate; SW, Shapiro-Wilk test; L, Levene test; CV%, coefficient of variation; P-values between parentheses. Mean (± standard error).

3.2 Physical and chemical properties of the substrates

The low values of sum of bases (SB) in the substrates without addition of organic matter indicated that the cation exchange sites were filled, mainly by hydrogen cations (H^{+1}) and aluminum cations (Al^{+3}), which could have caused nutritional deficiency [37].

The addition of organic matter increased the quantity of magnesium (Mg^{2+}), an element in the composition of chlorophyll molecules, active in photosynthesis, respiration and synthesis of carbohydrates [38]. There also was an increase in the availability of phosphorus (P), contributing to cell growth, energy transfer in root cells, photosynthesis and cell respiration [39]. Finally, there were increases in the levels of calcium (Ca^{2+}), a nutrient essential for the development of the roots, maintenance of cell integrity and membrane permeability [23], as well as potassium (K^{+}), sodium (Na^{+}), manganese (Mn) and zinc (Zn).

The addition of the goat manure also improved the physical attributes of the substrate, by increasing the total porosity and reducing its density. The density values reflect the compaction of the substrate; indeed, it is the most direct measure of compaction [40]. According to Andrade et al. (2018) [41], compacted substrates hinder the uptake of water and nutrients by the roots and cause deficient oxygenation.

3.3 Emergence and growth of the seedlings

The morphological variables that presented normal distribution of the residuals by the Shapiro-Wilk test were emergence percentage (%E) ($P < 0.346$) and stem diameter (SBD) ($P < 0.077$), while regarding homogeneity of variances, only DQI presented homogeneous variances ($P < 0.802$) (Table 3). The GLM analysis indicated significant interaction ($P < 0.01$) of the factors evaluated with all the morphological characteristics.

Table 3. Analysis of deviance (ANODEV) of emergence percentage (%E), shoot length (SL, cm), root length (RL, cm), shoot dry mass (SDM, g), root dry mass (RDM, g), stem base diameter (SBD, mm) and Dickson quality index (DQI) of seedlings of Handroanthus spongiosus (Rizzini) S. Grose (Bignoniaceae), from seeds collected in november 2019 at Pau Ferro, Caiçara, Embrapa, Cristália and Jutaí, Pernambuco, Brazil.

Source of variation	Analysis of Deviance (ANODEV)							
	Df	%E	SL	RL	SDM	RDM	SBD	DQI
Location (L)	4	56.37**	3.187**	3.214**	17.232**	38.016**	0.687**	4.2142**
Substrate (S)	5	366.42**	11.283**	7.039**	49.107**	60.417**	2.735**	6.1032**
L*S	20	77.22*	5.082**	7.032**	40.749**	70.254*	2.057**	8.6011**
Shapiro-Wilk		0.346	< 0.001	< 0.001	< 0.001	< 0.001	0.077	< 0.001
Levene		< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.802
CV%		21.70	19.27	22.54	17.23	22.35	27.04	16.12

Df, degrees of freedom; %E, emergence percentage; RL, root length; SL, shoot length; RDM, root dry mass; SDM, shoot dry mass; SBD, stem base diameter; CV%, coefficient of variation; DQI, Dickson quality index. Significant differences were determined as $P < 0.05$ * and $P < 0.01$ ** by the F-test.

The sand substrate was the cultivation condition that most favored %E of the radicle, with values between 46% (Jutaí) and 70% (Pau Ferro) (Table 4). This higher %E in sand can be related to the lower resistance to emergence and initial development of the seedlings' organs and the better supply of water and oxygen necessary for emergence [42]. In this phase, the nutrient reserves of the seeds contribute sufficiently to the emergence, without the need for external sources of nutrients [43, 44]. The need for nutritional support from the substrate occurs with the expansion of the cotyledon, formation of the first true leaf and roots [45]. Sand alone does not provide this support, so it tends to produce seedlings with low biomass increment [46] and this was observed in our results.

Table 4. Average morphological responses observed for emergence percentage (%E), shoot length (SL, cm), root length (RL, cm) and stem base diameter (SBD, mm) in seedlings of Handroanthus spongiosus (Rizzini) S. Grose (Bignoniaceae) from seeds collected in november 2019 at Pau Ferro, Caiçara, Embrapa, Cristália and Jutaí, Pernambuco, Brazil.

	Substrates					
	So	SD	SoV	SoM	SoVM	SoMB
Local	Emergence (%)					
Jutaí	7 bD	46 bA	7 aD	18 bC	12 cC	32 aB
Cristália	19 aC	56 bA	7 aC	21 bB	24 bB	33 aB
Caiçara	4 cC	49 bA	8 aC	19 bB	21 bB	14 bC
Embrapa	34 aB	66 aA	4 aD	38 aB	50 aB	20 bC
Pau Ferro	24 aC	70 aA	11 aC	26 aC	34 bB	15 bC
	Shoot Length (cm)					
Jutaí	7.12 aC	5.24 aD	5.87 aD	14.90 aA	8.81 aC	11.77 aB
Cristália	4.86 aC	4.48 aC	5.94 aC	15.88 aA	6.40 aC	11.94 aB
Caiçara	4.43 aC	5.01 aB	5.63 aB	4.42 cC	6.30 aB	8.05 aA
Embrapa	6.97 aC	4.50 aD	5.78 aD	12.67 bB	8.76 aC	13.32 aA
Pau Ferro	5.30 aC	4.10 aC	6.77 aB	7.26 cB	8.90 aB	11.56 aA
	Stem base Diameter (mm)					
Jutaí	1.71 bC	1.84 aB	1.86 aB	2.59 aA	2.39 aB	2.13 bB
Cristália	1.74 bC	1.57 aC	2.07 aB	2.91 aA	2.45 aA	2.69 bA
Caiçara	1.36 bC	1.57 aC	1.78 bB	2.65 aB	2.46 aA	1.91 cB
Embrapa	2.29 aA	1.60 aB	1.72 bB	2.24 bA	2.10 aA	2.18 bA
Pau Ferro	2.43 aB	1.85 aB	2.11 aB	1.92 bB	2.53 aB	3.63 aA
	Root Length (cm)					
Jutaí	19.48 bC	23.43 aB	28.95 bA	22.44 aB	19.02 aC	14.12 cD
Cristália	28.33 aB	26.14 aB	36.97 aA	22.66 aB	13.29 bC	17.61 bC
Caiçara	15.22 cB	28.88 aA	17.38 cB	7.67 cC	9.50 cC	9.65 cC
Embrapa	26.00 aA	23.03 aB	29.73 bA	17.70 bB	15.75 aC	19.57 bB
Pau Ferro	22.77 bA	22.65 aA	23.97 bA	12.04 bB	18.31 aA	22.58 aA

SD, sand; So, soil; SoM, soil + goat manure; SoV, soil + vermiculite; SoVM, soil + vermiculite + goat manure; SoMB, soil + goat manure + 30% biochar. Means followed by different lowercase letters in the columns and different uppercase letters in the rows do not differ statistically by the Tukey test at probability of 0.05.

During the experiment (december/2020-may/2021), we observed the death of the seedlings cultivated in sand and soil alone. This may have occurred due to the low relative humidity (67%), low levels of rainfall (244 mm) and high temperatures (27 °C) at the experiment site, which caused rapid drying and an increase in the temperature of the substrates after irrigation. Moreover, the soil substrate suffered from compaction in the initial days of the experiment, hampering the emergence and growth of the seedlings.

The addition of goat manure and biochar to the substrates favors the growth of the shoot (SL) and stem diameter (SBD) of the seedlings. The highest SL values were observed in the seedlings from seeds collected at Cristália (15.88 cm) and Jutaí (14.90 cm) cultivated in SoM (Table 4; Figure 2). On the other hand, the largest SBD values were found in seedlings from seeds gathered at Pau Ferro (3.63 mm) and Cristália (2.91 mm), cultivated in SoMB and SoM, respectively. The root lengths (RL) were higher on seedlings grown in the soil, sand and SoV substrates, with the standouts being the seedlings grown in SoV from seeds collected at Cristália (36.97 cm), Embrapa (29.73 cm) and Jutaí (28.95 cm). However, it is noticed that the volume of roots is smaller in these substrates in relation to substrates with the addition of manure and biochar.

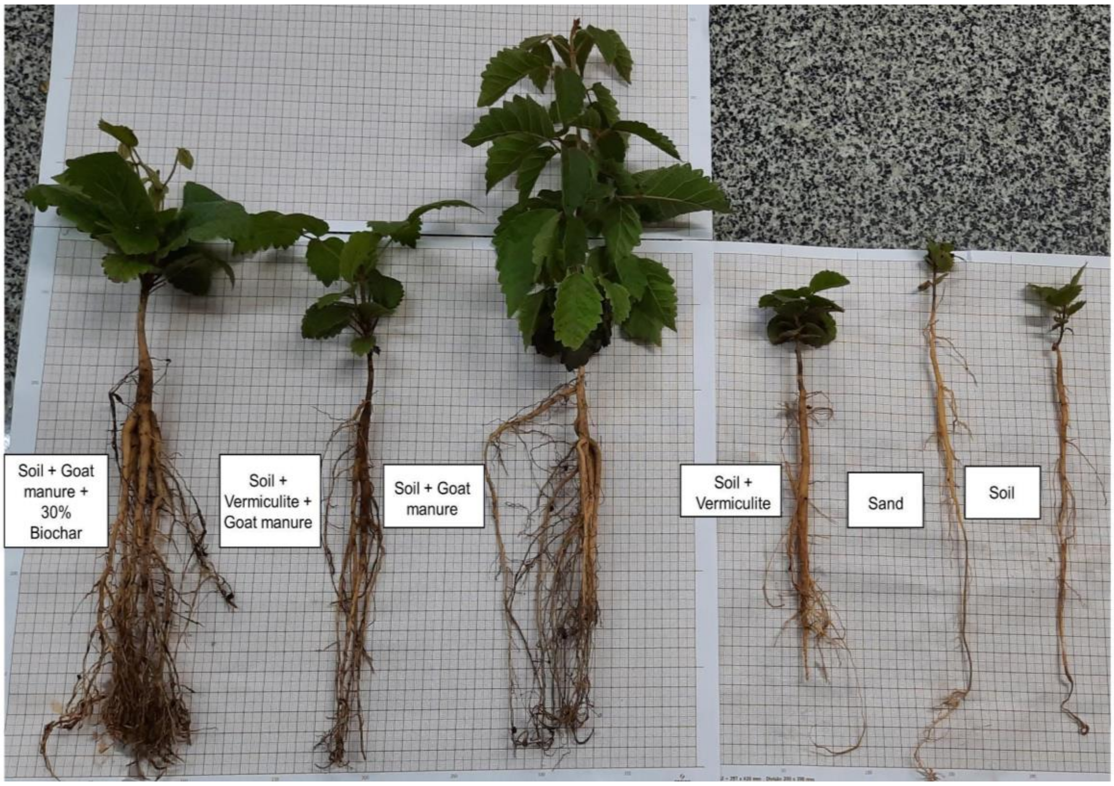

Figure 2. Seedlings of Handroanthus spongiosus (Rizzini) S. Grose (Bignoniaceae) formed from seeds collected in november 2019 at Pau Ferro and 180 days after sowing (DAS) in different cultivation conditions.

The dry mass production increased with addition of manure (Table 5), associated with the accumulation of nutrients extracted from these substrates during the development of the plants, since manure is a good source of macro and micronutrients [23].

Definition of the time for seedlings to remain in nurseries generally depends only on shoot length and stem diameter, without paying attention to the size of the roots [47]. However, it is intuitive that well-developed roots favor the formation of healthy seedlings, by fortifying the entire structure, increasing the chances for growth and development in the field, especially in arid and semiarid regions where erratic rainfall is among the most restrictive factors for establishment [48].

The Dickson quality index (DQI) is considered a good indicator of seedling quality because it reflects the balance in distribution of dry biomass and growth between the shoots and roots, enabling the choice of seedlings better able to adapt to adverse field conditions [47]. Higher values of this index are associated with greater chance of survival and establishment of seedlings in the field [49, 50].

The greatest Dickson quality indices were obtained for the seedlings originating from seeds collected at Pau Ferro and cultivated in SoMB (0.996) and those from seeds gathered at Embrapa and cultivated in SoVM (0.991).

Table 5. Average morphological responses observed for shoot dry mass (SDM, g), root dry mass (RDM, g) and Dickson quality index (DQI) of seedlings of Handroanthus spongiosus (Rizzini) S. Grose (Bignoniaceae) from seeds collected in november 2019 at Pau Ferro, Caiçara, Embrapa, Cristália and Jutaí, Pernambuco, Brazil.

	Substrates					
	So	SD	SoV	SoM	SoMV	SoMB
Location	Shoot Dry Mass (g)					
Jutaí	0.158 bC	0.388 aC	0.103 cC	1.415 bB	1.227 bB	2.943 aA
Cristália	0.299 bC	0.428 aC	0.463 bC	1.979 bA	1.125 bB	2.104 bA
Caiçara	0.944 aA	0.353 aB	0.186 cC	0.234 cB	0.452 cB	0.356 cB
Embrapa	0.604 aB	0.320 aC	0.642 aB	2.725 aA	2.741 aA	2.066 bA
Pau Ferro	0.170 bC	0.257 aC	0.384 bC	2.325 aB	3.220 aA	2.397 aB
	Root Dry Mass (g)					
Jutaí	0.295 cC	1.399 aB	0.460 cC	1.436 cB	1.851 cB	3.326 aA
Cristália	0.616 bC	0.984 bC	2.319 aB	3.279 aA	2.318 bB	2.893 aB
Caiçara	1.292 aA	0.794 bB	0.940 bA	0.242 dC	0.212 dC	0.295 bC
Embrapa	1.702 aC	0.875 bD	0.952 bD	2.849 aB	3.317 aA	3.629 aA
Pau Ferro	0.427 bD	0.634 cC	0.693 cC	2.130 bB	4.291 aA	2.942 aB
	Dickson Quality Index					
Jutaí	0.205 bC	0.525 aB	0.168 cD	0.372 bC	0.810 aA	0.948 aA
Cristália	0.278 bC	0.388 bB	0.915 aA	0.905 aA	0.983 aA	0.984 aA
Caiçara	0.625 aA	0.323 bB	0.335 bB	0.172 cC	0.140 bC	0.105 cC
Embrapa	0.675 aB	0.350 bC	0.425 bC	0.770 aB	0.991 aA	0.885 aA
Pau Ferro	0.222 bC	0.360 bB	0.290 bC	0.458 bB	0.988 aA	0.996 aA

SD, sand; So, soil; SoM, soil + goat manure; SoV, soil + vermiculite; SoVM, soil + vermiculite + goat manure; SoMB, soil + goat manure + 30% biochar. Means followed by different lowercase letters in the columns differ statistically and means followed by different uppercase letters in the rows do not differ statistically by the Tukey test at probability of 0.05.

The relationship of the seed collection sites, cultivation conditions and morphological characteristics of the seeds could be observed from the results of the multiple factor analysis (MFA) (Figure 3). Principal component 1 (PC1) explained 56.43% of the variability of the data while principal component 2 (PC2) explained 17.24%, for a total of 73.67%. Figure 3a shows that the characteristics which most contributed to the construction of PC1 were SL, SBD, SDM, RDM and DQI, in contrast to PC2, which received the greatest contributions from RL and %E. The seed collection sites Pau Ferro, Embrapa and Cristália together with the cultivation conditions SoM, SoVM and SoMB were positively associated with PC1. This suggests a similar performance of these three substrates, as can be seen in Figure 3b. On the other hand, the sites Jutaí and Caiçara along with the cultivation conditions So, SD and SoV were positively associated with PC2 (Figure 3b).

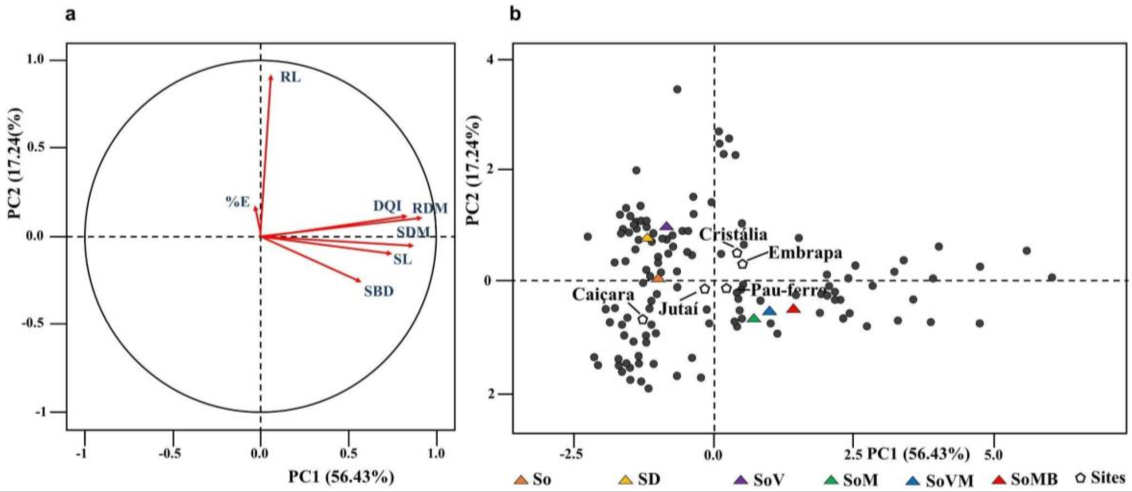

Figure 3. Multiple factor analysis related to %E and morphological characteristics of seedlings of Handroanthus spongiosus (Rizzini) S. Grose (Bignoniaceae) cultivated in different substrates from seeds collected in november 2019 at Pau Ferro, Caiçara, Embrapa, Cristália and Jutaí, Pernambuco, Brazil. Circle of correlation between the morphological responses (a); relation between seed collection locations and substrates (b). SD, sand; So, soil; SoM, soil + goat manure; SoV, soil + vermiculite; SoVM, soil + vermiculite + goat manure; SoMB, soil + goat manure + 30% biochar. The pentagons correspond to the seed collection locations; the triangles correspond to the cultivation conditions. %E, emergence percentage; SL, shoot length; RL, shoot length; SBD, stem base diameter; SDM, shoot dry mass; RDM, root dry mass; DQI, Dickson quality index.

These results corroborate those found in the univariate analysis, in which the substrates with addition of organic matter generally had the best physical-chemical attributes, promoting better morphological characteristics of the seedlings. The use of this type of analysis complements univariate analysis, enabling an overview of the interplay of the cultivation conditions, seed collection sites and morphological characteristics of the seedlings.

Therefore, the employment of agricultural wastes, such as goat manure and biochar, can favor farmers with small and medium-sized operations because these residues are easily available at zero or low cost.

The seedlings from seeds gathered at Caiçara presented different results from the others since the best yields were obtained with use of soil and sand alone. These results confirmed our hypothesis that the collection site and type of substrate used would influence the quality of the *H. spongiosus* seeds.

The addition of goat manure to the soil provided better results in the development and formation of *H. spongiosus* seedlings. Biochar has an important effect of increasing water retention in the soil or in substrates and this can be important in regions with a semiarid climate characterized by high temperatures and low rainfall concentrated in some months of the year.

In environments where there is no formation of organic matter on the surface that favors the emergence and recruitment of seedlings, as is the case of the Caatinga, an alternative would be the production of seedlings in a nursery using goat manure in the preparation of the substrate.

Direct sowing in a substrate with only sand allows rapid emergence, however it produces smaller seedlings with a low increase in biomass. Substrates with goat manure, despite not favoring a quick emergence, produce larger seedlings with a greater increase in biomass and with better distribution between shoots and roots.

We did not evaluate the performance of the seedlings after transplantation in the field, only at the nursery area, so more studies are necessary focused on the survival and growth of *H. spongiosus* in field conditions.

4. CONCLUSION

The results presented here can help support the production of seedlings for restoration of degraded seasonal dry tropical forests, and can be used as a basis for production of seedlings of other species. The addition of goat manure and biochar in the formulation of the substrates can improve the development of *H. spongiosus* seedlings and increase the economic benefits in nursery operations, because these inputs are locally abundant. The determination of the best places to collect seeds will facilitate decisions on the implementation of *H. spongiosus* nurseries for production of high-quality seedling.

5. ACKNOWLEDGMENTS

This study was financed in part by and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brazil (CAPES), FACEPE: PROCESSO Nº BFP-0072-5.02/23 and Embrapa Semiárido.

6. REFERENCES BIBLIOGRPHIC

1. Shalizi MN, Goldfarb B, Burney OT, Shear TH. Effects of five growing media and two fertilizer levels on polybag—Raised Camden whitegum (*Eucalyptus benthamii* Maiden & Cambage) seedling morphology and drought hardiness. Forests. 2019;10(7):543. doi: 10.3390/f10070543
2. Khurana EKTA, Singh JS. Ecology of seed and seedling growth for conservation and restoration of tropical dry forest: a review. Environl Conserv. 2001;28(1):39-52. doi: 10.1017/S0376892901000042
3. Mitchell J, Johnston IG, Bassel GW. Variability in seeds: biological, ecological, and agricultural implications. J exp bot. 2017;68(4):809-17. doi: 10.1093/jxb/erw397
4. Vargas-Figueroa JA, Torres-González AM. Germination and seed conservation of a pioneer species, *Tecoma stans* (Bignoniaceae), from tropical dry forest of Colombia. Rev Biol Trop. 2018;66(2):918-36. doi: 10.15517/rbt.v66i2.33423
5. Madrid-Aispuro RE, Prieto-Ruíz JÁ, Aldrete A, Hernández-Díaz JC, Wehenkel C, Chávez-Simental JA, et al. Alternative substrates and fertilization doses in the production of *Pinus cembroides* Zucc. in nursery. Forests. 2020;11(1):71. doi: 10.3390/f11010071
6. Vukobratović M, Lončarić Z, Vukobratović Ž, Mužić M. Use of composted manure as substrate for lettuce and cucumber seedlings. Waste Biomass Valori. 2018;9(1):25-31. doi: 10.1007/s12649-016-9755-2
7. Ribeiro HM, Romero AM, Pereira H, Borges P, Cabral F, Vasconcelos E. Evaluation of a compost obtained from forestry wastes and solid phase of pig slurry as a substrate for seedlings production. Bioresour technol. 2007;98(17):3294-7. doi: 10.1016/j.biortech.2006.07.002
8. Cáceres R, Marfà O. Diagnosis of the fertility of compost-based growing media: Method comparison and monitoring in pot plant cultivation. Sci Hortic. 2013;164:213-20. doi: 10.1016/j.scienta.2013.09.032
9. Viana MP, Silva JD, Lima AMC, Alves FSF, Pinheiro RR, Costa DF, et al. Risk factors associated with *Leptospira* sp. seroprevalence in goats in Northeast of Brazil. Prev Vet Med. 2022;208:105751. doi: 10.1016/j.prevetmed.2022.105751
10. Pereira GE, Biasoto ACT. Vinhos tropicais brasileiros em busca de certificação. Cadernos do Semiárido: Riquezas e Oportunidades. 2015;1:14-5.
11. Gentry AH. Bignoniaceae: Part II (tribe Tecomeae). Flora Neotrop. 1992;25:1-370.
12. Lohmann LG, Fonseca LHM, Kaehler M, Farias-Singer R, Firetti F, Silva-Castro MM. Bignoniaceae in Flora e Funga do Brasil [Internet]. Rio de Janeiro: Reflora Jardim Botânico do Rio de Janeiro; 2020 [cited 2022 Feb 03]. Available in: http://reflora.jbrj.gov.br/reflora/floradobrasil/FB112305
13. Sistema de Informação Sobre a Biodiversidade Brasileira (SiBBr). Ficha de espécies [Internet]; [cited 2021 feb. 17]. Available in: https://ferramentas.sibbr.gov.br/ficha/bin/view/especie/*handroanthus_spongiosus*
14. Brasil. Ministério do Meio Ambiente, Gabinete do Ministro. Portaria n° 561, de 15 de dezembro de 2021. Institui a lista de espécies nativas ameaçadas de extinção, como incentivo ao uso em métodos de recomposição de vegetação nativa em áreas degradadas ou alteradas. Brasília (DF): Diário Oficial da União; 2021. Available from: https://www.in.gov.br/en/web/dou/-/portaria-mma-n-561-de-15-de-dezembro-de-2021-367747322

15. Leal IR, Silva JMC, Tabarelli M, Lacher TE. Changing the course of biodiversity conservation in the Caatinga of northeastern Brazil. Conserv Biol. 2005;19(3):701-6. doi: 10.1111/j.1523-1739.2005.00703.x
16. Alvares CA, Stape JL, Sentelhas PC, Gonçalves JDM, Sparovek G. Köppen's climate classification map for Brazil. Meteorol Z. 2013;22(6):711-28.
17. Queiroz LP, Cardoso D, Fernandes MF, Moro M. Diversity and evolution of flowering plants of the Caatinga domain. In: Silva JC, Leal I, Tabarelli M, editors. Caatinga: The largest Tropical Dry Forest Region in South America. Springer; 2017. p. 23-63. doi: 10.1007/978-3-319-68339-3_2
18. Brasil. Ministério da Agricultura, Pecuária e Abastecimento. Instruções para análise de sementes de espécies florestais. Brasília (DF): MAPA; 2013. Available from: https://www.gov.br/agricultura/pt-br/assuntos/insumos-agropecuarios/insumos-agricolas/sementes-e-mudas/publicacoes-sementes-e-mudas/instrucoes-para-analise-de-sementes-de-especies-florestais/view
19. Silva JJ, Alencar SS, Gomes RA, Matias JR, Pelacani CR, Dantas BF. Conservation and physiological quality of *Handroanthus spongiosus* (Rizzini) S. Grose (Bignoniaceae) seeds. J Seed Sci. 2022;44:e202244007. doi: 10.1590/2317-1545v44257812
20. Ferreira LBDS, Fernandes NA, Aquino LCD, Silva ARD, Nascimento WM, Leão-Araújo ÉF. Temperature and seed moisture content affect electrical conductivity test in pea seeds. J Seed Sci. 2017;39:410-6. doi: 10.1590/2317-1545v39n4181021
21. Vieira RD, Krzyzanowski FC. Testes de condutividade elétrica. In: Krzyzanowski FC, Vieira RD, França-Neto JB. Vigor de sementes: Conceitos e testes. Londrina (PR): ABRATES; 1999.
22. Embrapa. Manual de análises químicas de solos, plantas e fertilizantes da embrapa. 2ed rev ampl. Distrito Federal: Embrapa Informação Tecnológica; 2009.
23. Antunes LF, Vaz AFS, Martelleto LAP, Leal MAA, Alves RS, Ferreira TS, et al. Sustainable organic substrate production using millicompost in combination with different plant residues for the cultivation of *Passiflora edulis* seedlings. Environ Technol Inno. 2022;28:102612. doi: 10.1016/j.eti.2022.102612
24. Minami K, Salvador ED. Substrato para plantas. Piracicaba (SP): Degaspari; 2010.
25. Stöcker CM, Monteiro AB, Silva DR, Kunde RJ, Morselli TBGA. Substratos alternativos para a produção de mudas de alface (*Lactuca sativa* L.) em sistema orgânico. Rev Jornada Pós-Graduação e Pesquisa-Congrega Urcamp. 2016;1113-22.
26. Noulas C, Tziouvalekas M, Karyotis T. Zinc in soils, water and food crops. J Trace Elem Medd Biol. 2018;49:252-60. doi: 10.1016/j.jtemb.2018.02.009
27. Nakagawa J. Testes de vigor baseados em desempenho de plântulas. In: Krzyzanowski FC, Vieira RD, França-Neto JB, Marcos-Filho J. Vigor de sementes: Conceitos e testes. 2rd ed. Londrina (PR): ABRATES; 2020.
28. Dickson A, Leaf AL, Hosner JF. Quality appraisal of white spruce and white pine seedling stock in nurseries. For Chron. 1960;36(1):10-3. doi: 10.5558/tfc36010-1
29. Shapiro SS, Wilk MB. An Analysis of variance test for normality. Biometrika. 1965;52(3):591-11.
30. Levene H. Robust tests for equality of variances. In: Olkin I, editor. Contributions to probability and statistics: Essays in honor of harold hoteling. Redwood (CA): Stanford University Press; 1960.
31. Carvalho FJ, Santana DGD, Araújo LBD. Why analyze germination experiments using Generalized Linear Models? J Seed Sci. 2018;40:281-7. doi: 10.1590/2317-1545v40n3185259
32. Šidàk Z. Rectangular confidence region for the means of multivariate normal distributions. J Am Stat Assoc. 1967;62(318):626-33. doi: 10.1080/01621459.1967.10482935
33. Lê S, Josse J, Husson F. FactoMineR: an R package for multivariate analysis. J Stat Softw. 2008;25:1-18. doi: 10.18637/jss.v025.i01
34. R Core Team. 2020. The R Project for statistical computing. Available in: https://www.R-project.org/.
35. Rencher AC, Christensen WF. Methods of multivariate analysis. 3rd ed. Nova Jersey (US): John Wiley & Sons; 2012.
36. Lima JJP, Freitas MND, Guimarães RM, Vieira AR, Ávila MAB. Accelerated aging and electrical conductivity tests in crambe seeds. Ciênc Agrotec. 2015;39:7-14. doi: 10.1590/S1413-70542015000100001
37. Almeida ROPO, Sánchez LE. Indicadores da qualidade do substrato para monitoramento de áreas de mineração revegetadas. Floresta Ambiente. 2015;22:153-63. doi: 10.1590/2179-8087.072714
38. Peng YY, Liao LL, Liu S, Nie MM, Li J, Zhang LD, et al. Magnesium deficiency triggers SGR–mediated chlorophyll degradation for magnesium remobilization. Plant physiol. 2019;181(1):262-75. doi: 10.1104/pp.19.00610
39. Berti CLF, Kamada T, Silva MP, Menezes JFS, Oliveira ACS. Crescimento de mudas de baru em substrato enriquecido com nitrogênio, fósforo e potássio. Rev Cult Agron. 2017;26(2):191-202. doi: 10.32929/2446-8355.2017v26n2p191-202

40. Gao Y. Soil-water retention behavior of compacted soil with different densities over a wide suction range and its prediction. Comput Geotech. 2017;91:17-26. doi: 10.1016/j.compgeo.2017.06.016
41. Andrade GF, Paniz FP, Martins Junior AC, Rocha BA, Lobato AKS, Rodrigues JL, et al. Agricultural use of Samarco's spilled mud assessed by rice cultivation: A promising residue use?. Chemosphere. 2018;193:892-902. doi: 10.1016/j.chemosphere.2017.11.099
42. Pedrosa TO, Xavier EG, Souza RF, Silva AG, Gomes ES. Emergência e desenvolvimento inicial de plântulas de *Anacardium humile* em função de diferentes substratos. Ipê Agron J. 2020;4(2):1-7. doi: 10.37951/2595-6906.2020v4i2.6376
43. Mayer AM, Poljakoff-Mayber A. The germination of seeds. New York (US): Pergamon Press; 1963.
44. Bewley JD, Black M. Seeds: Physiology of development and germination. 2rd ed. New York (US): Plenum Press; 1994.
45. Corte VB, Borges EEL, Pontes CA, Leite ITA, Ventrella MC, Mathias AA. Mobilization of the reserves during germination of seeds and growth of seedlings of *Caesalpinia peltophoroides* Benth (Leguminosae-Caesalpinoideae). Rev Árvore. 2006;30:941-9. doi: 10.1590/S0100-67622006000600009
46. Barros DI, Nunes HV, Quirino MS, Silva HD, Oliveira LB, Santos LF, et al. Assessment of different substrates used for seedling production in passion fruit (*Passiflora edulis* var. flavicarpa). J Exp Agric Int. 2022;44:12-5. doi: 10.9734/jeai/2022/v44i230793
47. Güney D, Bayraktar A, Fahrettin ATAR, Turna İ. Effects of root undercutting, fertilization and thinning on seedling growth and quality of oriental beech (*Fagus orientalis* Lipsky) seedlings. Artvin Çoruh Üniversitesi Orman Fakültesi Dergisi. 2020;21(2):214-22.
48. Schenk HJ, Jackson RB. Rooting depths, lateral root spreads and below-ground/above-ground allometries of plants in water-limited ecosystems. J Ecol. 2002;90:480-94.
49. Santos CC, Jorge HPG, Dias LGF, Vieira MDC. Shading levels and substrates affect morphophysiological responses and quality of *Anadenanthera peregrina* (L.) speg seedlings. Floresta Ambiente. 2020;27(2):e20190119. doi: 10.1590/2179-8087.011919
50. Zuffo AM, Steiner F, Busch A, Aguilera JG, Alvarez RDCF. Limestone and topdress nitrogen application improves the quality of *Peltophorum dubium* (Sprengel) Taubert (Fabaceae-Caesalpinioideae) seedlings. Ciência Florestal. 2021;31(3):1306-22. doi: 10.5902/1980509837974