Production of *Bertholletia excelsa* Humb. & Bonpl., (Lecythidaceae) seedlings in microenvironments under different substrates

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ABSTRACT: The lack of technological options for forest concessionaires to produce seedlings of native tree species in the Peruvian Amazon is a bottleneck for reforestation in the region. In this study, a new technology for seedlings production of low cost and accessible to producers was developed. Thus, the objective of this study was to evaluate the efficiency of using buckets as microenvironments under different substrates for the initial growth of *Bertholletia excelsa* seedlings. Forty days after germinating, the seedlings were transplanted to 115 cm³ tubes containing a standard substrate composed by sand + sawdust + charred sawdust in the proportion of 1:1:1 v/v. Afterwards, the tubes were settled in buckets (microenvironments) under a 100% shade net. The experimental design used in this study was fully randomized and the treatments were T1 = sand, T2 = sand + sawdust, T3 = decomposed Brazil nut shell and T4 = forest soil. The substrate T2 promoted the shoot length, root length, leaves number, shoot dry mass, root dry mass, total dry mass, and slenderness index in *B. excelsa* seedlings. Quality of *B. excelsa* seedlings produced in microenvironments was similar among treatments, regardless of the used substrate.

Key words: Brazil nut; forest concessionaires; Peruvian Amazon; reforestation; seedlings quality

Produção de mudas de Bertholletia excelsa Humb. & Bonpl., (Lecythidaceae) em microambientes e diferentes substratos

RESUMO: A falta de opções tecnológicas de concessionários de florestas para a produção de mudas de espécies nativas na Amazônia peruana é um gargalo para o reflorestamento na região. Neste estudo, uma nova tecnologia de produção de mudas de baixo custo e acessível a produtores foi desenvolvida. Assim, o objetivo neste trabalho foi avaliar a eficiência do uso de baldes como microambientes e substratos para o crescimento inicial de plântulas de *Bertholletia excelsa*. Aos 40 dias após a emergência, as plântulas foram repicadas para tubetes com capacidade de 115 cm³, contento um substrato padrão composto por areia + serragem + serragem carbonizada na proporção (1:1:1 v/v). Posteriormente, os tubetes foram acondicionadas em baldes (microambientes) com telado a 100% de sombra. O delineamento experimental foi inteiramente ao acaso e os tratamentos foram: T1 = areia, T2 = areia + serragem, T3 = casca de castanha decomposta e T4 = solo de floresta. O substrato T2 favoreceu o comprimento de parte aérea, raiz, número de folhas, massa seca da parte aérea, raízes e total, além do índice de esbeltez de mudas de *B. excelsa*. A qualidade das mudas de *B. excelsa* produzidas em microambientes foi semelhante entre os tratamentos, independente do substrato utilizado.

Palavras-chave: castanheira-do-brasil; concessionários florestais; Amazônia peruana; reflorestamento; qualidade de mudas

Introduction

Seedlings production is one of the most important activities in the process of setting forest stands, since the seedlings quality implies in the success of these forest stands. Projects on the recovery of degraded areas, commercial reforestation, and harvesting and conservation of native commercial species largely depend on the production of high quality seedlings (Dionisio et al., 2017; Marques et al., 2018b). In this way, it is essential the research and development of techniques to make it viable the seedlings production of native commercial species under low cost.

Bertholletia excelsa Humb. & Bonpl., (Lecythidaceae), commonly known as Brazil nut, is a tree species of great economic importance in the Amazon region (Albuquerque et al., 2015; Thomas et al., 2015; Santos et al., 2017; Nogueira et al., 2018). This species promotes employment and income for thousands of workers through the trade and local consumption of its seeds (Tonini, 2011; Scoles et al., 2016; Costa et al., 2017). *B. excelsa* seeds present high commercial and nutritional value and are internationally traded as the Brazil nut denomination (Wadt et al., 2015; Baldoni et al., 2017; Wadt et al., 2018).

One of the main forms of *B. excelsa* propagation is by sexual reproduction, but its seeds present integumentary dormancy. Germination is slow, irregular, and often in low percentages, even under favorable conditions. Because of this, the tree species dormancy makes seedlings production difficult, mainly due to the irregular and long period of germination (Coelho et al., 2010). Germination represents an important phase of the tree species life cycle, which is influenced by factors such as temperature, substrate, wetness, and light availability (Cosmo et al., 2017), as well as dormancy.

In Madre de Dios, Southeastern Peru, native forest concessionaires develop activities of timber harvesting and, by law requirements, are required to remediate forests losses through plating of native tree species. Such concessionaires face hurdles both to acquire and to produce tree seedlings. They also face the obstacle to overcome difficult access to the concession areas and their long distances to the districts where tree seedlings could be acquired. Alternatively, concessionaires aim to produce their own seedlings in the concession areas, but they do not count on adequate infrastructure and technical assistance for that.

In this sense, the Research Institute of the Peruvian Amazon (IIAP), in collaboration with concessionaires, traditional communities, and riverside residents, seeks to research and develop alternative technologies to produce high quality tree species seedlings, under low cost, and according to the reality of the communities from the region. In this context, *B. excelsa* is one of the main tree species used by these communities both for reforestation and in agroforest systems.

The objective of this study was to evaluate the efficiency of using buckets as microenvironments under different substrates in the initial growth of *B. excelsa* seedlings.

Material and Methods

Study area and seedlings production

This study was carried out in the nursery of tree seedlings from the Research Institute of the Peruvian Amazon (IIAP), in Madre de Dios district, Peru (2°49'11" N, 60/40'24" W).

Seeds were extracted from fruits originated from different *Bertholletia excelsa* trees immediately after the fruit dispersal in two study areas belonging to IIAP. Seed extraction from the fruits (a hard-shelled fruit containing several tegument seeds) was done manually using a machete. Tegument extraction was done carefully with a lathe to avoid seed damage. After removing their tegument, seeds were treated with the fungicide Vitavax®-300, composed by carboxin 200 g kg^{-1} and captan 200 g kg^{-1}, before being sowed in seedbeds with washed sand as substrate.

Forty days after germination, the seedlings were transplanted to 115 cm³ tubes all containing the same standard substrate composed of sand, sawdust, and charred sawdust in the proportion of 1:1:1 v/v. The standard substrate is the one normally used to produce *B. excelsa* seedlings. Later, the tubes were settled in buckets (microenvironments) under a 100% shade net (Figure 1) in order to test microenvironments created by different substrates and their effect on seedlings development. Each bucket received an amount of substrate in order to accommodate the tubes carefully (Figure 1). The bucket substrate varied according to four different treatments to maintain *B. excelsa* seedlings (Table 1).

After preparing substrates and filling buckets (microenvironment), they received the tubes with seedlings, previously standardized by size in order to have the two cotyledons open and an average height of 5.0 cm. Shoots and roots were cut to induce seedling lignification and growth (Figure 1).

Table 1. Different substrates tested to produce *Bertholletia excelsa* seedlings in microenvironments and their proportions.

Treatments	Substrates	Proportion
T1	Sand	7 l/bucket
T2	Sand + sawdust	7 l/bucket (1:1 v/v)
T3	Decomposed Brazil nut shell	7 l/bucket
T4	Forest soil	7 l/bucket

Figure 1. Process of *Bertholletia excelsa* seedlings production in microenvironments. A) Seedling ready for transplanting; B) transplanted seedling; C) shoot and root system cut; D) seedlings ready to be transplanted in tubes.

Data collection and data analysis

To evaluate the initial growth of *B. excelsa* seedlings in microenvironments under different substrates, the following

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variables were tested: shoot length (SL), root length (RL), stem collar diameter (SCD), leaves number (LN), Dickson quality index (DQI), slenderness index (SI), lignification index (LI), shoot dry mass (SDM), root dry mass (RDM), and total dry mass (TDM). Ratios were also used for analyses such as the shoot length/root length ratio (SL/RL) and the shoot dry mass/root dry mass ratio (SDM/RDM).

To measure dry mass, seedlings were split in shoots and roots by a cut at the stem collar diameter. Both parts were put separately in Kraft paper bags, identified and dried in an oven at 65 °C for 48 hours up to obtain a constant mass. After this period, samplings were weighted in an analytical balance with precision of 0.001 g to have shoot and root dry mass as well as their ratios measured. Stem collar diameter was measured using a digital caliper with precision of 0.01 mm, and shoot and root lengths were measured with a ruler graded in millimeters.

To determine DQI, seeds were separated from shoots and roots and not considered as part of the total dry mass. The DQI was calculated according to equation 1:

$$DQI = \frac{TDM}{\dfrac{SL}{SCD}} + \frac{SDM}{RDM} \quad (1)$$

where: TDM = total dry mass; SL = shoot length; SCD: stem collar diameter; SDM = shoot dry mass; RDM = root dry mass.

The experimental design used in this study was fully randomized with four treatments (substrates) and four replicates of 12 seedlings each. The experimental data were initially tested for residual normality through the Shapiro-Wilk test ($p > 0.05$) and for homogeneity through the Bartlett test ($p > 0.05$). Besides these tests, independence between experimental units was checked to fulfill requirements to perform an analysis of variance (ANOVA) (Banzatto & Kronka, 2006).

ANOVA was performed with the collected data using the software R, version 3.3.3. Once detected significant differences, the post-hoc Tukey test ($p < 0.05$) was applied to compare means.

Results and Discussion

There was a significant statistical difference (ANOVA) in at least one pair of means in all studied variables, except in stem collar diameter (SCD), Dickson quality index (DQI), and in shoot dry mass/root dry mass ratio (SDM/RDM, Table 2).

Bertholletia excelsa seedlings attained the highest shoot lengths (SL) in T2 (sand + sawdust) (40.34 ± 3.42 cm, $F_{3;12} = 5.145$, $p = 0.016$), differing from T3 (decomposed Brazil nut shell) and T4 (forest soil). T2 had also a positive effect on root length (RL) (19.51 ± 4.83 cm, $F_{3;12} = 6.275$, $p = 0.008$), leaves number (LN) (7.59 ± 0.74 cm, $F_{3;12} = 4.826$, $p = 0.0199$), shoot dry mass (SDM) (1.99 ± 0.08 cm, $F_{3;12} = 6.302$, $p = 0.008$), and root dry mass (RDM) (0.34 ± 0.06 cm, $F_{3;12} = 11.1$, $p = 0.001$) (Figures 3B, 3D, 3E, and 3F, respectively). SCD did not present significant differences in the substrates ($F_{3;12} = 1.762$, $p = 0.208$) (Figure 2).
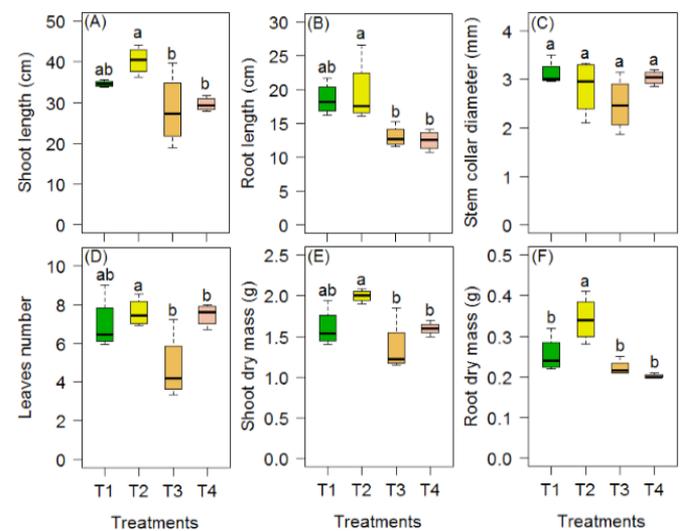

Figure 2. Mean values for shoot length, root length, stem collar diameter, leaves number, shoot dry mass, and root dry mass of *Bertholletia excelsa* seedlings produced in microenvironments under four different substrates. Thick horizontal lines show the mean, while boxes represent interquartile intervals, and the dotted lines the extreme values. Letters indicate significant differences ($p < 0.05$) in ANOVA with the Tukey post-hoc test among treatments.

Table 2. Analysis of variance (ANOVA) for the variables shoot length (SL), root length (RL), stem collar diameter (SCD), leaves number (LN), Dickson quality index (DQI), slenderness index (SI), lignification index (LI), shoot dry mass (SDM), root dry mass (RDM), total dry mass (TDM), shoot length/root length ratio (SL/RL), and shoot dry mass/root dry mass ratio (SDM/RDM) in microenvironments under four different substrates for germination and initial growth of *Bertholletia excelsa* seedlings.

SV	DF	Mean Quadrats					
		SL	RL	SCD	LN	DQI	SI
Treat.	3	119.75*	52.6**	0.3137ns	7.016*	0.00042ns	15.758**
Error	12	23.28	8.38	0.178	1.454	0.0001702	1.493
CV (%)		14.52	18.18	14.70	18.03	12.54	10.48
		LI	SDM	RDM	TDM	SL/RL	SDM/RDM
Treat.	3	0.00227*	0.27709*	0.0153***	0.4054**	0.181ns	3.463**
Error	12	0.000525	0.04397	0.001377	0.0548	0.1227	0.435
CV (%)		5.73	12.78	14.52	12.34	16.42	10.06

nsNon-significant; *significant ($p < 0.05$); ** significant ($p < 0.01$); *** significant ($p < 0.001$); Treat. = treatment; SV = source of variation; DF = degree of freedom; CV (%) = coefficient of variation.

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Growth for the variables SL, RL, LN, SDM, and RDM in substrates with sand indicates the advantage of using such component in substrates to produce higher quality seedlings. The presence of sand + sawdust probably increased features such as aeration, substrate structure, and water retention, which permitted better seedlings development.

Adequate substrates must promote the root system development and must have physical-chemical characteristics to permit aeration, water and nutrients retention, and oxygen for seedlings (Dutra et al., 2014; Santos et al., 2014; Silva et al., 2017; Siqueira et al., 2018). Besides these features, adequate substrates must also be free of pathogens to ensure seeds germination. Another important factor observed in the experiment was the substrate humidity. Along the whole evaluation, time seedlings were irrigated only when the experiment was established. Further irrigations were not needed because the microenvironment created inside the bucket promoted an evapotranspiration decrease that, consequently, resulted in an increase of water retention.

In relation to TDM of *B. excelsa* seedlings, T2 presented a significantly higher mean (2.34 ± 0.13 cm, $F_{3; 12} = 7.403$, $p = 0.005$) than T3 and T4, but no differences were observed when compared to T1 (Figure 3A).

It is important to stress that, although T2 (sand + sawdust) promoted good results in seedling strength, based on the performance of *B. excelsa* seedlings (shoot length and main root), T2 did not present significant differences in DQI ($F_{3; 12} = 2.488$, $p = 0.11$) in relation to the other treatments (T1 - sand, T3 - decomposed Brazil nut shell, and T4 - forest soil) (Figure

3B). The higher is DQI the better is seedling, since the index is a quality indicator (Siqueira et al., 2018).

This result can come from three factors: a) evaluation time might not be enough to detect significant difference in growth among treatments; b) seedlings could still be fed by seed reserves (endosperm), and; c) the combination of the two first factors.

Sand has been reported as an excellent substrate to maintain viability of stored seeds of *B. excelsa* (Silva et al., 2009) and germination of many tree species (Dionisio et al., 2017; Marques et al., 2018a).

Sand presents great drainage, it is useful as physical conditioner in substrate mixtures, low costly, and easy to be acquired when compared to other sources for substrates (Brito et al., 2009). Another advantage of using sand as substrate is its possible reuse, since sand can be disinfected in the autoclave. However, sand alone as a substrate cannot favor seedling development (Sousa et al., 2014). This reinforces the possibility that, in this study, seedlings maintained their metabolism using their own reserves (endosperm).

These results show that as sand (which does not presents good chemical characteristics for seedling development) as the other substrates do not alter growth of *B. excelsa* seedlings. T2 promoted higher slenderness index (SI) that differed significantly (14.46 ± 1.96 cm, $F_{3; 12} = 10.55$, $p = 0.001$) from the other treatments (Figure 3C), while T3 presented higher mean in the lignification index (LI) in relation to T1, T2, and T4 (Figure 3D). The treatment T3 (decomposed Brazil nut shell) presented a lignification index significantly higher than the other treatments (0.43 ± 0.04 cm, $F_{3; 12} = 4.33$, $p = 0.0275$). This index is related to the lignification of the shoot and root tissues of woody species under stress situations.

Physical stimuli applied as stem flexion makes seedlings to trigger morphometric responses normally associated to height reduction, increase in the stem collar diameter, and increase in the dry mass of the root tissue (Volkweis et al., 2014; Dranski et al., 2015). Such response to physical stimuli is defined as thigmomorphogenesis (Jaffe, 1973), which is observed in phases where seedlings are getting more resistant to mechanical injuries. Therefore, it is possible to imply that there is a relation between seedlings tissues lignification and their growth and survival performance in the field (Dranski et al., 2015).

The ratio SDM/RDM indicates the biomass distribution between shoot and root system (Siqueira et al., 2018). SDM/RDM was significantly higher in T4 (forest soil) (7.92 ± 0.44 cm, $F_{3; 12} = 7.969$, $p = 0.00345$), which shows that seedlings presented different patterns of dry matter distribution between the two organs (Marana et al., 2015).

Figure 3. Mean values of total dry mass, Dickson quality index, slenderness index, lignification index, shoot length/root length ratio, and shoot dry mass/root dry mass ratio of *Bertholletia excelsa* seedlings produced in microenvironments under four different substrates. Thick horizontal lines show the mean, while boxes represent interquartile intervals, and the dotted lines the extreme values. Letters indicate significant differences (p < 0.05) in ANOVA with the Tukey post-hoc test among treatments.

Recommendations

Once used the standard substrate (sand, sawdust, and charred sawdust) in the 115 cm³ tubes, any of the four substrate can be successfully used in the bucket. The results showed good seedling development and quality indexes in all treatments. This means that forest concessionaires in the
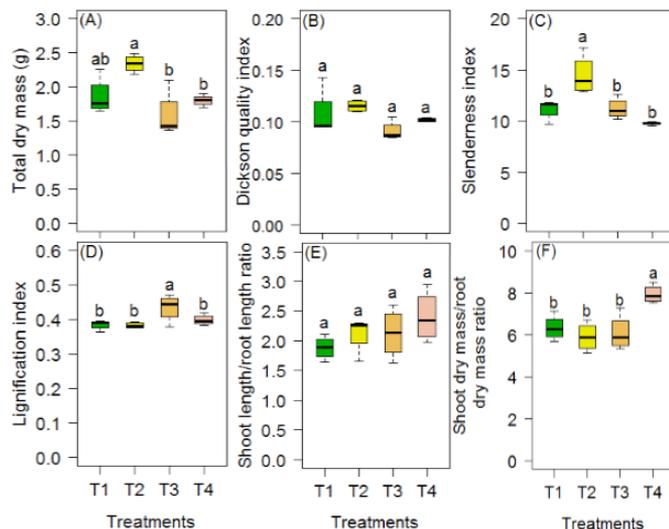
Peruvian Amazon can choose any of these four substrates tested in the present study, according to the most abundant raw material available in their properties. Thus, it is recommend the use of this technology to produce *B. excelsa* seedlings as well as other tree species seedlings when the adequate nursery infrastructure is not available. The use of microenvironments becomes important to provide seedlings protection against rodents and ants attacks and to prevent water loss through evapotranspiration that can ensure substrate wetness and consequently less irrigation. Under these working conditions, the usage of bucket becomes a viable choice, their lower necessary of substrate permits faster and cheaper seedlings transport and makes easier to handle seedlings for plantings.

Conclusion

Seedlings of *Bertholletia excelsa* produced in microenvironments under four different substrates presented good development performance, even though presenting statistical differences in some variables.

The seedlings quality was high in all treatments as it could be found in all quality indexes tested. Therefore, any of the four substrates can be used for *B. excelsa* seedlings production in microenvironments using buckets, since the standard substrate inside tubes are maintained.

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