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Bertholletia excelsa: growth and quality of seedlings produced under different environmental conditions

Bertholletia excelsa: crescimento e qualidade de mudas produzidas sob diferentes condições ambientais

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ABSTRACT

Bertholletia excelsa is a tree species economically important in Peru, Brazil, and Bolivia. In the Peruvian Amazon, seedlings production is a bottleneck faced by concessionaires for reforestation. Thus, the objective of this work was to evaluate initial growth and quality of *B. excelsa* seedlings in two production environments. Thirty days after germination seedlings were transplanted to 115-cm³ small tubes with standard substrate and arranged in two environments under 60% shading. The experiment was fully randomized with the treatments "forest nursery" (T1) and "sub-irrigation chamber" (T2) and four repetitions of 10 plants each. ANOVA followed by the Tukey test were applied over means (p < 0.05). The production environment had effect on the development of *B. excelsa* seedlings. Higher growth rates in height, shoot dry mass, root dry mass, and total dry mass were observed in T2. *B. excelsa* seedlings produced in the sub-irrigation chamber presented higher Dickson quality index with 135 days.

Keywords: Dickson quality index, forest concessionaires, Peruvian Amazon, forest nurseries.

RESUMO

Bertholletia excelsa é uma espécie arbórea economicamente importante no Peru, Brasil e Bolívia. Na Amazônia peruana, a produção de mudas é um gargalo enfrentado por concessionários para o reflorestamento. Assim, o objetivo deste trabalho foi avaliar o crescimento inicial e a qualidade de mudas de *B. excelsa* em dois ambientes de produção. Trinta dias após a germinação as mudas foram transplantadas para tubetes de 115 cm³ com substrato padrão para em seguida serem arranjadas em dois ambientes a 60% de sombreamento. O experimento foi inteiramente casualizado com os tratamentos "viveiro florestal" (T1) e "câmara de sub-irrigação" (T2) com quatro repetições de 10 plantas cada. ANOVA seguida pelo teste de Tukey foram aplicados sobre as médias (p < 0,05). O ambiente de produção teve efeito no desenvolvimento de mudas de *B. excelsa*. Maiores taxas de crescimento em altura, massa seca da parte aérea, massa seca da raiz e massa seca total foram observadas no T2. Mudas de *B. excelsa* produzidas na câmara de sub-irrigação apresentaram maiores índices de qualidade de Dickson com 135 dias.

Palavras-chave: Índice de qualidade de Dickson, concessionários florestais, Amazônia peruana, viveiros florestais.

1 INTRODUCTION

Bertholletia excelsa Humb. Bonpl. (Lecythidaceae), commonly known as Brazil nut, is a tree species of great economic importance in the Amazon region (Santos et al., 2017; Picanço e Costa, 2019). The extractive activities related to this species in the Amazon brings employment and incomes for thousands of workers through trade and consume of its seeds (Wadt et al., 2018), which present high commercial and nutritional values. Nevertheless, *B. excelsa* has had decreases in its natural populations due to seed overharvesting and habitat fragmentation (Sujii et al., 2015). *B. excelsa* seeds present high commercial and nutritional value and are internationally traded as the Brazil nut denomination (Baldoni et al., 2017; Wadt et al., 2018).

Plantings of *B. excelsa* became an urgent need, which includes the production of high quality seedlings. Seedlings survival in field conditions is a crucial phase in planting that can be determined by the ways how seedlings were produced in forest nurseries. A given high quality seedling must have characteristics that allow maximum survival rates and high initial growth when planted in the field (Dionisio et al., 2019a; Dionisio et al., 2019b). Parameters based on morphological and physiological features are the most commonly used to calculate indexes of seedlings quality, due to the fact that they are easily recognizable by nursery workers (Auca et al., 2018).

The production of quality seedlings has been a bottleneck for planting *B. excelsa* since it depends on several factors as seed origin, shading, wetness, temperature, substrate, management, and adequate recipients to accommodate seedlings (Gutiérrez et al., 2010; Auca et al., 2018). Some of the quality indexes used to rank seedlings produced in forest nurseries are the robustness index (the relation shoot/stem collar diameter), lignification index (the relation total dry weight/total wet weight), Dickson quality index, the relation plant height/root length, and the relation dry shoot mass/dry root mass (Dickson et al., 1960; Reyes et al., 2014). These indexes indicate the possible responses of seedlings in growth and survival once they are planted in the field.

To attain the minimum values predicted in quality indexes of seedlings, it is necessary to know the recipient size and volume where they are produced, the correct matter management, fertilizing, and the solar radiation control (Marana et al., 2015). In addition, an adequate environment for seedling production is essential to decrease seedling development time necessary in the forest nursery and consequently the production cost.

In this context, we hypothesized that *B. excelsa* seedlings have effects caused by the production environment conditions, so seedlings from sub-irrigation chambers would present higher quality in their morphological features. For testing this hypothesis, we defined the objective of this work: to assess the morphological quality of *B. excelsa* seedlings produced in sub-irrigation chambers in comparison to conventional forest nurseries.

2 MATERIAL AND METHODS

All experimental procedures of this study were carried out in the research campus of the Research Institute of the Peruvian Amazon (IIAP), in Puerto Maldonado district, Madre de Dios department, Peru (12º39’04” S, 69º19’17” W) under the project coordinated by the same institution entitled “Increase of the technological and genetics knowledge of Brazil nut (*Bertholletia excelsa*) aiming its domestication in Madre de Dios region, Peru”.

To evaluate initial growth, *Bertholletia excelsa* seedlings were selected 30 days after germination and transplanted to 115-cm^3 small tubes having a standard substrate composed by sand, sawdust, and charred sawdust in equal proportions (1:1:1 *v/v*). Seedlings were previously standardized by size in order to have a pair of fully open leaves and average height of 12 cm. Before seedlings transplant, part of their roots were cut (at 4 cm from the end) as well as their shoot (2 cm from the apex) to induce seedlings lignification and growth (Auca et al., 2018). After been transplanted, the small tubes were conditioned in two growing environments, both covered by a 60% shading mesh, which consisted in two treatments: forest nursery (T1) and sub-irrigation chamber (T2) (Table 1). Thirty days after the seedlings had been transplanted to the two environments, the first height measurement was done. Further assessments were done each 15 days, totaling eight evaluations (30, 45, 60, 75, 90, 105, 120, and 135 days). A data logger was used for the daily measurements of temperature and humidity. Eight measurements were taken every day in the interval of 8:00 AM to 18:00 PM in both environments (Figure 1).

Table 1. Growth of *Bertholletia excelsa* seedlings during 135 days under the effect of two treatments, number of repetitions, and number of seedlings per repetition.

Treatments	Environment	#Repetitions	#Seedlings
T1	Forest nursery	4	10
T2	Sub-irrigation chamber	4	10

Seedlings initial growth was assessed through the variables: a) shoot length (SL), b) root length (RL), c) stem collar diameter, d) root collar diameter, e) shoot dry mass (SDM), f) root dry mass (RDM), g) total dry mass (TDM), h) shoot length/root length relation (SL/RL), i) shoot dry mass/root dry mass relation (SDM/RDM), j) robustness index, l) lignification index, and m) Dickson quality index (DQI). The higher DQI, lignification, and robustness indexes the better is the seedling quality. Seedlings quality was classified in low, medium, and high, according to pre-defined intervals suggested by Reyes et al. (2014). To measure stem collar diameter, stem length, and root length, a digital caliper (precision = 0.01 mm) and a ruler graded in millimeters were used, respectively. *Bertholletia excelsa* growth in height was measured through cumulative growth and growth rate.

To measure dry mass, seedlings were split in shoots and roots through precise cut at the stem collar. Both parts were put separately in Kraft paper bags, identified, and dried in an oven at 70 °C during 72 hours up to reach a constant mass. Immediately after this, samplings were weighted in an analytical balance (precision = 0.001 g) to obtain shoot and root dry mass. SL/RL relation predicts the success of planting and there must be a balance and rate between the aerial part and the root system of the plants. It is determined by the equation 1:

$$SL/RL = \frac{\text{Shoot length (cm)}}{\text{Root length (cm)}} \qquad (1)$$

SDM/RDM relation indicates the development of plants in forest nursery. It is determined by the equation 2:

$$SDM/RDM = \frac{\text{Shoot dry mass (g)}}{\text{Root dry mass (g)}} \qquad (2)$$

The robustness index was calculated as the relation between shoot length and root collar diameter, according to the equation 3:

$$\text{Robustness index} = \frac{\text{Shoot length (cm)}}{\text{Root collar diameter (mm)}} \qquad (3)$$

Lignification index relates the plant's total dry mass with the total wet mass, giving the lignification percentage. It is determined by the equation 4:

$$\text{Lignification index} = \left(\frac{\text{Total dry mass (g)}}{\text{Total wet mass (g)}}\right) \text{x } 100 \qquad (4)$$

To determine DQI, seedlings were separated from shoots and roots and not considered as part of the total dry mass (Siqueira et al., 2018). The index is given by the equation 5:

$$\text{DQI} = \frac{\text{Total dry mass (g)}}{\frac{\text{Shoot length (cm)}}{\text{Stem collar diameter (mm)}} + \frac{\text{Shoot dry mass (g)}}{\text{Root dry mass (g)}}} \qquad (5)$$

To verify the three assumptions for applying an analysis of variance (ANOVA), the data were firstly checked for: a) normality with the Shapiro-Wilk test ($p > 0.05$), b) homoscedasticity through the Bartlett test ($p > 0.05$), and the independence among experimental units. Once attending to these assumptions, the data were submitted to ANOVA, which was run with the software R, version 3.5.2. When detected significant differences, means were compared through the Student t-test ($p < 0.05$). ANOVA and regression analysis were used for the variables cumulative growth and growth rate, where, in each treatment, the equations were adjusted to the data obtained in relation to the time after transplanting. The model for each variable was selected once considered the significance of the parameter coefficients and the highest determination coefficient (R^2). The Student t-test was used to test the regression coefficients (p <0.05).

3 RESULTS AND DISCUSSION

Every day temperature monitoring can be observed in Figure 1. Treatment effects over cumulative growth and growth rate were detected after adjusting the linear and quadratic models, respectively (Figures 2A and 2B). Forty-five days after transplanted, *B. excelsa* seedlings produced in sub-irrigation chamber (T2) presented higher growth rate in height (p = 0.001). After 135 days *B. excelsa* seedlings presented average height of 27.1 ± 6.4 and 18.0 ± 3.7 in sub-irrigation chamber and in forest nursery, respectively.

Figure 1. Mean values of temperature (A) and relative humidity (B) in the tested environments, forest nursery (T1) and sub-irrigation chamber (T2) for growing seedlings of *Bertholletia excelsa* from the age of 30 to 135 days.

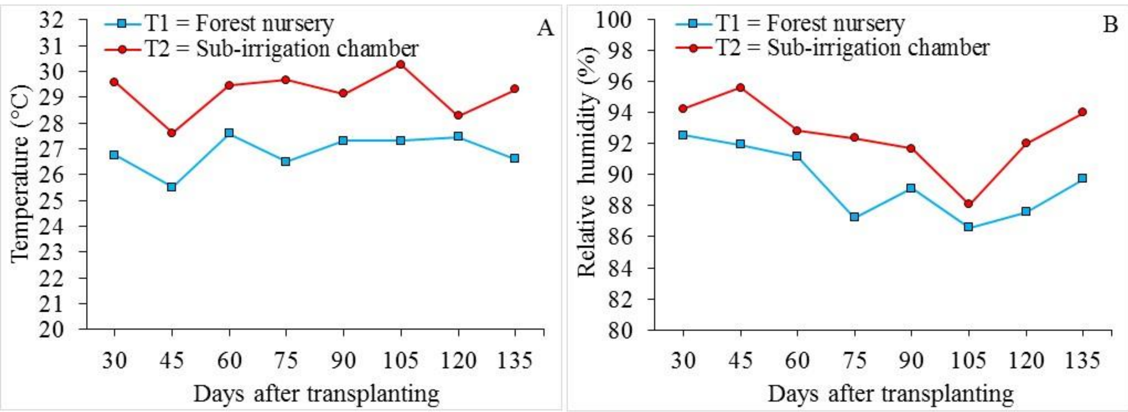
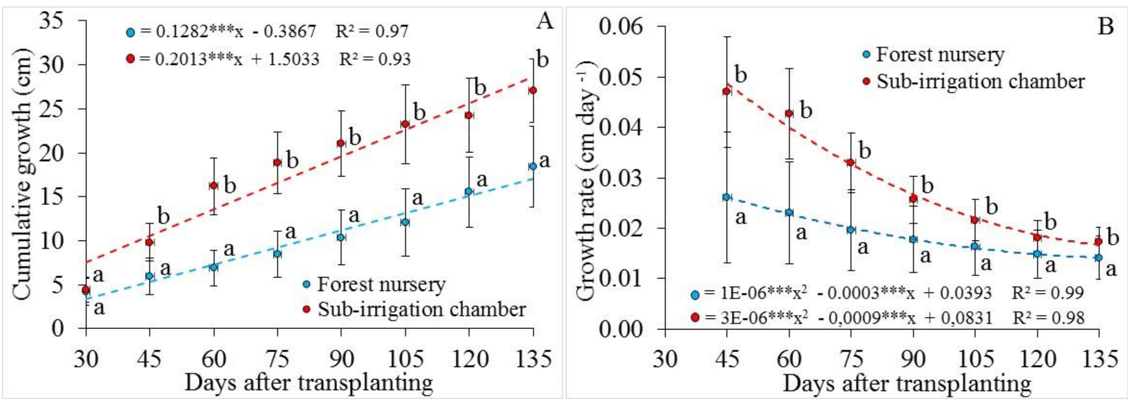


Figure 2. Cumulative growth in height (A) and growth rate in height (B) of *Bertholletia excelsa* seedlings in forest nursery (T1) and sub-irrigation chamber (T2) as a function of time after transplanting. *** Significant at 0.1% probability by the F-test.

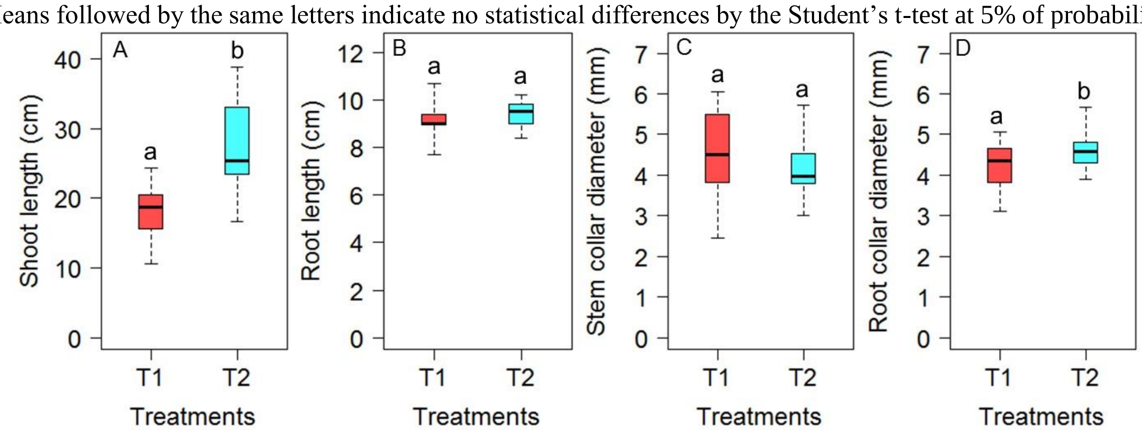


Growth rate in height had a linear increase in relation to time in both production environments, forest nursery (T1) and sub-irrigation chamber (T2) (Figure 2). However, 45 days after transplanted, seedlings in T2 had higher increment in height than those produced in T1 (Figure 3). Such increase can be related to the higher temperatures observed in T2 (Figure 1). Five months after transplanted, seedlings produced in T2 attained the proper height to be planted in the field (27.1 cm), while the T1's seedlings did not reach this size during the same period. According to Müller (1981), *B. excelsa* seedlings are ready to be planted in the field at the height of 25 cm and 16 leaves. These features are commonly observed between 4 and 8 months after seedlings transplanting. Considering the values recommended by Müller (1981) for the variable height, only *B. excelsa* seedlings produced in T2 would have high quality (Table 2).

Precocity and uniformity are important characteristics in seedling production, since the more time seedlings remain in the forest nursery the higher is the cost production. Financial costs have been a variable seriously considered in tree seedlings production over the last years. The need to reduce costs has driven managers to use more technology for improving ways of nursery production (Ribeiro et al., 2018).

Bertholletia excelsa seedlings produced in T2 presented larger shoot length (27.1 ± 6.4) and root collar diameter (4.7 ± 0.1) than seedlings produced in the forest nursery (T1) (p = 0.001) (Figures 3A and 3D).

Figure 3. Mean values of shoot length (A), root length (B), stem collar diameter (C), and root collar diameter (D) of *Bertholletia excelsa* seedlings produced in forest nursery (T1) and sub-irrigation chamber (T2). The thicker horizontal line, boxes, and dashed lines represent mean, interquartile interval, and extreme values, respectively. Means followed by the same letters indicate no statistical differences by the Student's t-test at 5% of probability.



One of the main issues in tree plantings for forest restoration is the seedlings quality. Seedlings production usually has infrastructure hurdles observed in isolated regions, where

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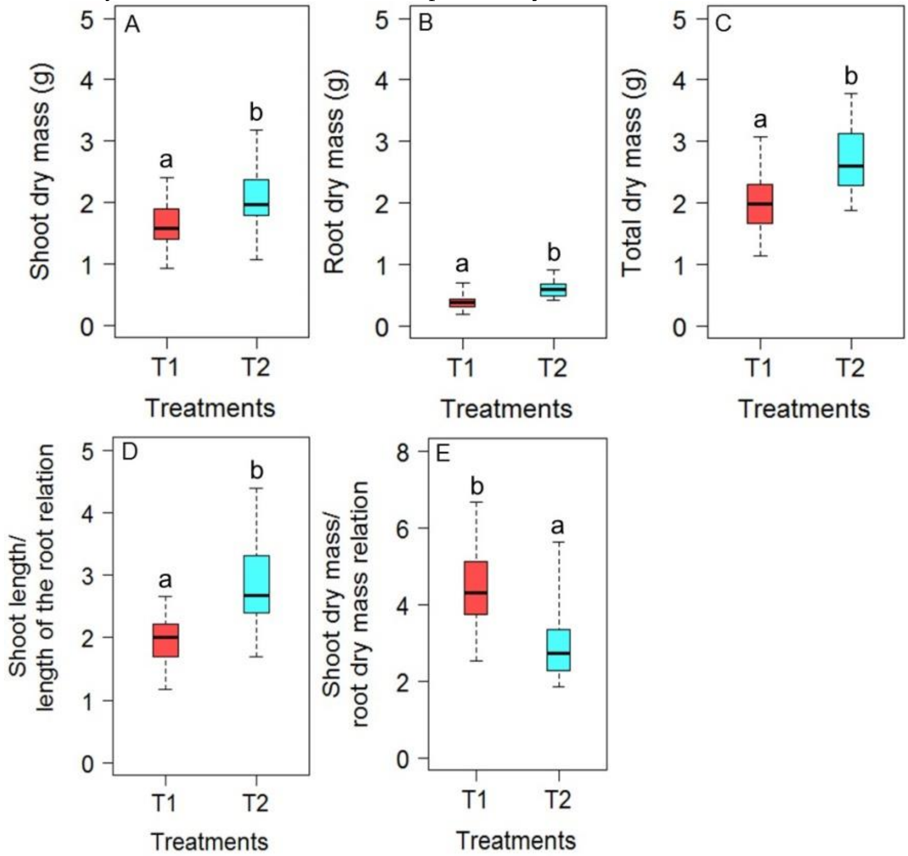
access to technology and agricultural inputs by concessionaires is more difficult and expensive. Forest concessionaires that usually have poor infrastructure in remote areas, far from large production centers, demand for low-cost alternatives to produce high quality seedlings. The environmental factors and silvicultural techniques employed are crucial to reduce cost and time to establish a tree planting (Lima Filho et al., 2019).

Root length (p = 0.22) and stem collar diameter (p = 0.229) did not present significant differences between the two production environments. T1 presented higher shoot dry mass (2.2 ± 0.5), root dry mass (0.74 ± 0.1), total dry mass (2.9 ± 0.5) while T2 had the higher relation SDM/RDM (4.4 ± 1.1, p = 0.001, Figure 4).

Figure 4. Mean values of shoot dry mass (A), root dry mass (B), total dry mass (C), relation of shoot length/root length (D), and relation shoot dry mass/root dry mass (5) of *Bertholletia excelsa* seedlings produced in forest nursery (T1) and sub-irrigation chamber (T2). The thicker horizontal line, boxes, and dashed lines represent mean, interquartile interval, and extreme values, respectively. Means followed by the same letters indicate no statistical differences by the Student's t-test at 5% of probability.

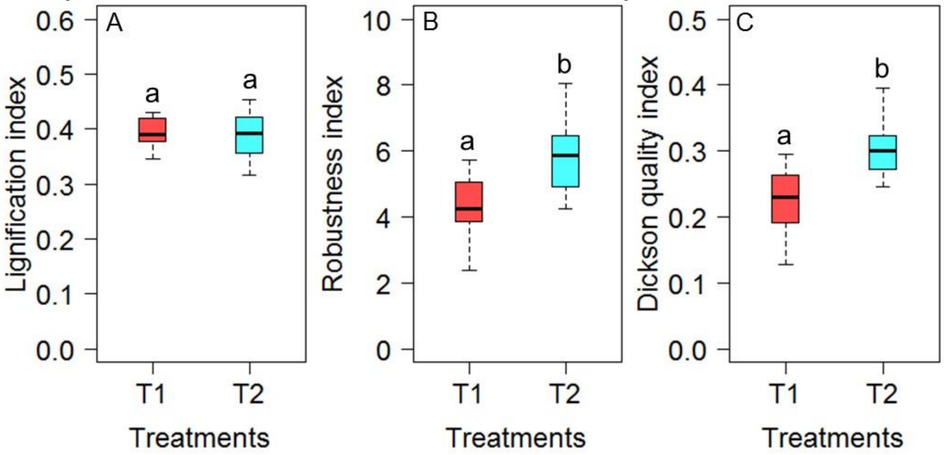


The relation SDM/RDM indicates the biomass distribution between shoot and root system (Siqueira et al., 2018). Thus, no significant differences in the relation SDM/RDM indicate that seedlings have similar distribution patterns of dry matter among organs (Marana et al., 2015). The balanced distribution between shoot dry mass and root dry mass makes possible an adequate development of the plant, decreasing risks of its felling in the field. Such values showed a better relation SDM/RDM of seedlings produced in T2. Since *B. excelsa* seedlings were produced in the same kind of recipient and substrate, it is possible to affirm that the microclimate provided by T2 had positive effects on the higher and more rapid seedlings development. The substrate temperature in the recipients is a variable dependent on time and space, with important role in physical processes and in the energy exchanges with the atmosphere, that interferes in shoot and root growth and in water and nutrients absorption (Cavalcanti et al., 2019).

Records on the substrate temperature in recipients for seedlings production are not part of the workers' daily routine in a forest nursery. Lack of data on temperature is due to the fact that this variable is not considered as a limiting factor for tree seedlings production. Nonetheless, substrate temperature is one of the properties that directly influences many processes involving plant growth (Cavalcanti et al., 2019). Besides this, substrate temperature ultimately determines evaporation and aeration, as well as the speed of chemical reactions involved on that (Nascimento et al., 2016). The substrate temperature changes in response to meteorological elements, where the difference between the air and substrate temperature triggers processes of energy transference such as conduction, convection, and radiation (Carneiro et al., 2013).

Seedlings produced in T2 obtained more shoot, root, and total biomass accumulation and less relation shoot dry mass per root dry mas (SDM/RDM) when compared to seedlings produced in T1 (Figure 4). No statistical differences were found between treatments in the lignification index ($p = 0.0802$). Treatment T2 presented the higher values for robustness index (4.4 ± 0.8, $F_{1;\,38} = 22.36$) and DQI (0.3 ± 0.04, $F_{1;\,38} = 35.88$, $p = 0.001$, Figure 5). T2, however, presented higher average DQI values than T1 ($p = 0.001$) (Figure 5, Table 2).

Figure 5. Mean values of lignification index (A), robustness index (B), and the Dickson quality index (C) of *Bertholletia excelsa* seedlings produced in forest nursery (T1) and sub-irrigation chamber (T2). The thicker horizontal line, boxes, and dashed lines represent mean, interquartile interval, and extreme values, respectively. Means followed by the same letters indicate no statistical differences by the Student's t-test at 5% of probability.



No statistical differences were observed in the lignification index, which is related to the lignification of shoot and root tissues of woody species seedlings under stress. Physical stimuli applied as stem flexion make plants to trigger morphometric responses normally associated to reduction in height and increase in stem collar diameter and dry mass of the root tissues (Volkweis et al., 2014; Dranski et al., 2015). The response to the mechanical stimulus is called thigmomorphogenesis (Jaffe, 1973), which strengthens seedlings during their rustification phase. Tissues lignification has been related to the seedlings performance and survival in the field (Dranski et al., 2015).

The Dickson quality index (DQI), as well as the lignification and robustness index, is considered as seedling quality indicator. In DQI, the seedling vitality and balance of the biomass distribution are taken into account (Lima Filho et al., 2019). Many authors consider DQI as the main indicator of seedlings' quality standard. This is because the fact that in the DQI's formula is taken into account robustness and the balance of seedlings' biomass distribution, that combines variables of growth and biometric relations (Dickson, 1960; Binotto et al., 2010; Siqueira et al., 2018). Binotto et al. (2010) observed that the dry mass variables followed by the stem collar diameter are the most strongly correlated variables to the DQI index.

The seedling stage is probably the most vulnerable in a plant's life-cycle. In natural conditions, the establishment of a given seedling and the following reproductive success can be interrupted due to multiple motivations: premature emergence, germination in non-appropriate depths and emergence in environments under strong competitive pressure for survival and reproduction. Hence, seedlings must have effective mechanisms to detect changes in the environmental conditions in order to continue its development process (Batlla & Benech-Arnold, 2014). According to the qualitity intervals suggested by Reyes et al. (2014), *B. excelsa* seedlings presented high quality for the variables shoot length, stem collar diameter, and robustness index in both production environments. DQI was classified as medium for both treatments.

Table 2. Mean values of the morphological and qualitative variables of *Bertholletia excelsa* seedlings produced under two treatments, forest nursery (T1) and sub-irrigation chamber (T2).

Variable	Mean		Quality and interval*		
	T1	T2	High	Medium	Low
Shoot length (cm)	18 ± 3.7	27.1 ± 6.4	15-25	10-14.9	< 10.0
Stem collar diameter (mm)	4.5 ± 1.2	4.2 ± 0.7	≥ 4.0	2.5-3.9	< 2.5
Relations of shoot length/root length	2.0 ± 0.4	2.9 ± 0.7	≤ 2.0	2.1-2.5	> 2.5
Relation shoot dry mass/root dry mass	4.4 ± 1.1	3.0 ± 1.1	1.5-2.0	2.1-2.5	> 2.5
Lignification index	38.4 ± 4.4	41.1 ± 5.0	Nd.	Nd.	Nd.
Robustness index	4.4 ± 0.8	5.8 ± 1.1	≤ 6.0	6.1-8.0	> 8.0
Dickson quality index	0.2 ± 0.04	0.3 ± 0.04	≥ 0.5	0.49-0.20	< 0.20

*For conifer species (Reyes et al., 2014)
Nd.= not determined

B. excelsa seedlings had positive quality under the production environment provided by the sub-irrigation chamber. Therefore, we recommend the application of this technology in situations without availability of adequate infrastructure for tree seedlings production. The use of sub-irrigation chamber for *B. excelsa* seedlings production is also essential to avoid rodents and ants attacks as well as to impede water losses due to evapotranspiration, maintaining the substrate wetness and reducing the irrigation frequency. It is fundamental the use of little tubes as recipients under these working conditions, because the substrate volume transported will be substantially smaller.

Based on the last measure values, seedlings produced in both environments are still growing rapidly in height (Figure 1). One of the main issues found in forest nurseries of native species is the fact that seedlings cannot be planted before the best time for that. A consequence of this longer time inside the forest nursery can be the reduction in the root phisiological activity, root deformations, and necrosis due to lack of space and nutrients in small recipients (Lamhamedi et al., 1998; Lopes et al., 2014). Therefore, with the data of the last measure in height and root collar diameter in addition with the values of dry mass, relation SDM/RDM, and DQI (Table 2), it is possible to say that seedlings produced in the sub-irrigation chamber presented better performance than seedlings produced in the forest nursery. This technology can also be recommended for foresters aiming the production of *B. excelsa* seedlings with higher quality in shorter time.

4 CONCLUSIONS

Bertholletia excelsa seedlings produced in the sub-irrigation chamber presented higher Dickson quality index than seedlings produced in the forest nursery at 135 days.

The sub-irrigation chamber showed to be the best environment production of B. excelsa seedlings for forest concessionaires in the Peruvian Amazon. In this sense, sub-irrigation chambers can also be used to produce *B. excelsa* seedlings in other countries such as Bolivia and Brazil.

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