Breaking dormancy, alternative substrates, and the production of *Samanea tubulosa* (Benth.) seedlings

Quebra de dormência, substratos alternativos e a produção de mudas de *Samanea tubulosa* (Benth.)

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We investigated different dormancy breaking methods and substrates for *Samanea tubulosa* seedlings. Four substrate formulations were tested: SCO: commercial substrate; BBA: decomposed babassu biomass + soil (1:1); BBU: buriti biomass + soil (1:1); and BCA: carnauba bagana + soil (1:1), and three dormancy breaking methods: EM: mechanical scarification; IA: immersion in water; and EQ: chemical scarification. The highest seedling emergence was in BCA substrate with chemical scarification. BCA and SCO provided the highest average seedling height, root length, root volume, root density, diameter at neck height, number of leaves, aboveground dry mass, belowground dry mass, and Dickson quality index values. Therefore, BCA can be used as a substrate for the production of *S. tubulosa* seedlings, and the immersion in water method is optimal when combined with a BBU substrate.
Keywords: Agroecology, organic waste, sustainability.

Investigamos diferentes métodos de quebra de dormência e substratos para mudas de *Samanea tubulosa*. Quatro formulações de substratos foram testadas: SCO: substrato comercial; BBA: biomassa decomposta de babaçu + solo (1:1); BBU: biomassa de buriti + solo (1:1); e BCA: bagana de carnaúba + solo (1:1), e três métodos de quebra de dormência: EM: escarificação mecânica; IA: imersão em água; e EQ: escarificação química. A maior emergência de plântulas ocorreu com um substrato de BCA com escarificação química. O substrato de BCA e SCO proporcionaram os maiores valores médios de altura, comprimento e volume radiculares, diâmetro radicular, diâmetro na altura do colo, número de folhas, massa seca da parte aérea, massa seca radicular e índice de qualidade de Dickson. Portanto, BCA pode ser usado como substrato para a produção de mudas de *S. tubulosa*, e o método de imersão em água é ideal quando combinado com um substrato de BBU.
Palavras chave: Agroecologia, resíduos orgânicos, sustentabilidade.

1. INTRODUCTION

Unsustainable agricultural practices for land use and management conducted by farmers in the humid tropics, such as along the periphery of the Amazon in the Brazilian state of Maranhão, are factors related [1] that increase soil degradation [2]. In addition, the practice of itinerant cutting and burning has accelerated deforestation in the Amazon [3, 4].

The use of native species is an important alternative in the recovery of degraded areas, as it allows the protection and enrichment of the soil, the control of erosion, less loss of water through evaporation and greater biological activity [5]. Among these species, *Samanea tubulosa* (Benth.) Barneby & JW Grimes has been used in the restoration and maintenance of ecosystems in degraded areas [6, 7], being characterized as a typical tree in the Cerrado biome, reaching up to 28 m in height and 1 m in diameter, widely used in forestry, ornamentation and as part of animal nutrition [8, 9].

According to Dutra et al. (2013) [10], mechanical scarification, chemical scarification (usually with sulfuric acid), and hot water immersion are the notable methods for breaking the dormancy of seeds of forest species. These methods aim to dissolve the waxy cuticular layer, reducing the hardness of the integument and allowing an accelerated and uniform seed germination process.

Seed dormancy is one of the main obstacles in the production of *S. tubulosa* seedlings, and results in seed loss, longer production times, and seedling unevenness [11]. Dormancy consists of delaying seed germination, which guarantees, from an ecological point of view, the distribution in time and space, however, for seedling production the attribute is not desirable, as there may be a low percentage of germination [12].

The use of commercial substrates that are based on expanded vermiculite and organic materials [13] is costly [7, 14], however, decomposed organic residues and fractions from Brazilian pre-Amazonian palms, such as babassu (*Attalea speciosa* Mart.), buriti (*Mauritia vinifera* Mart.), and carnauba (*Copernicia prunifera* Mill.), can reduce production costs without compromising seedling quality [14, 15, 16, 17, 18]. The aim of this study was to investigate three dormancy breaking methods (namely, mechanical scarification, immersion in water, and chemical scarification) and different substrates for *S. tubulosa* seedling production.

2. MATERIAL AND METHODS

Location and climate

The experiment was conducted from April to June 2018 at the Center for Agricultural and Environmental Sciences (CCAA) of the Federal University of Maranhão (UFMA), Chapadinha, Maranhão, Brazil (3°44′12.62″S, 43°19′03.51″E, and 105 m above sea level). The region's climate is classified as humid tropical, with an average annual rainfall of 1613.2 mm and an average annual temperature of 26.9 °C [19].

Experimental design, treatments, and characterization procedures

The study was conducted with a completely randomized design in a 4×3 factorial arrangement (four substrates and three dormancy breaking methods) with five replicates containing five plants each, totaling 25 plants per treatment and 300 plants in total, corresponding to 60 experimental units. Four substrate formulations were tested: SCO: commercial substrate; BBA: decomposed babassu biomass + soil (1:1); BBU: buriti biomass + soil (1:1); and BCA: carnauba bagana + soil (1:1), and three dormancy breaking methods: EM: mechanical scarification; IA: immersion in water; and EQ: chemical scarification.

SCO was composed of pine bark and vermiculite (Plantmax HT®) and was used as a control, and soil used in the formulation of BBA, BBU, and BCA was collected from the 0-20 cm soil layer in the UFMA/CCAA experimental area. The soil was classified as a dystrophic Yellow Latosol [20], contained 560, 190, and 250 g kg^{-1} of sand, silt, and clay, respectively, had an "average" textural classification, and a degree of flocculation of 77 g 100 g^{-1}.

The soil and substrate samples were sent to the Soil Science Laboratory of the Federal University of Ceará, Fortaleza, to measure global density, particle density, and total porosity (Table 1) according to the procedures described by Schmitz et al. (2002) [21], as well as pH, organic matter content, nitrogen, phosphorus, potassium, calcium, magnesium, and sulfur (Table 2) by the methodology described by MAPA (2007) [22].

Table 1. Physical characterization of the substrates.

Substrates	Density (g cm^{-3})		Porosity (%)
	Global	Particle	
SCO	0.56	0.85	34.43
BBA	1.08	2.41	55.12
BBU	1.21	2.27	63.41
BCA	0.89	2.20	60.02
Solo	1.44	2.67	45.99

SCO: commercial substrate; BBA: babassu biomass + soil (1:1); BBU: buriti biomass + soil (1:1); and BCA: carnauba bagana + soil (1:1).

Table 2. Chemical characterization of the substrates.

Substrates	pH	M.O.	N	P	K	Ca	Mg	S
		g kg^{-1}	g kg^{-1}	mg kg^{-1}	————————cmol$_c$ kg^{-1}————————			
SCO	4.9	--	3.08	55.3	2.89	22.50	3.40	29.40
BBA	5.0	1.58	1.74	13.0	2.09	3.8	2.25	9.20
BBU	6.0	1.52	7.53	19.5	1.10	5.8	2.30	10.00
BCA	5.0	84.52	6.13	17.5	1.00	5.2	1.35	7.90
Solo	5.06	15.41	0.63	3.01	0.07	0.88	0.30	1.50

SCO: commercial substrate; BBA: babassu biomass + soil (1:1); BBU: buriti biomass + soil (1:1); and BCA: carnauba bagana + soil (1:1).

The *S. tubulosa* seeds were purchased from local stores. EM was performed using No. 180 sandpaper until the shiny surface of the seed coat was removed, IA was conducted in distilled water at room temperature for 24 h, and EQ was performed using concentrated sulfuric acid (H_2SO_4) for 5 min, followed by washing for 5 min in running water.

Experimental procedure

Seeds were distributed in polyethylene bags (12×20 cm) with two seeds per bag, in which they were buried at a depth of 1 cm for a period of 60 days. The bags were placed on a wooden bench in a greenhouse under controlled conditions. Daily watering was carried out to ensure adequate moisture within the substrate.

The count of emerged seeds was performed until 30 days after sowing (DAS), considering the seeds that contained the cotyledons fully open above the substrate. Subsequently, thinning was done, leaving only one plant per bag. The emergency percentage (%E) was calculated based on these data. At 60 DAS were measured: seedling height (SH, measured from the neck to the insertion of the last leaf), root length (RL), diameter at neck height (DNH), number of leaves (NL), root density (RD), aboveground dry mass (ADM), belowground dry mass (BDM), and total dry mass (TDM).

For the height and length measurements, rulers graduated in mm were used, for the diameter, a digital caliper was used, and to determine ADM and BDM, fresh material was placed in a forced-air-circulation oven for 72 h and then weighed on a precision scale. The Dickson quality index (DQI) was determined according to Equation 1 [23].

$$DQI = TDM/(HP/DSD+SDM/DMRS) \qquad (1)$$

Where: TDM= total dry mass (g); HP= plant height (cm); DSD= stem diameter (cm); SDM= shoot dry matter (g): DMRS= dry mass of the root system (g).

Statistical analysis

The data were subjected to an analysis of variance (Equation 2) followed by a Tukey test. Associations among the variables were assessed using Pearson's linear correlation coefficient (r) (Equation 3) and classified according to Gavioli et al. (2019) [24], where $0 < r \leq 0.2$: no correlation; $0.2 < r \leq 0.4$: weak correlation; $0.4 < r \leq 0.6$: moderate correlation; $0.6 < r \leq 0.8$: strong correlation; and $0.8 < r \leq 1$: very strong correlation. $P < 0.05$ was set as statistical significance. Analyses were performed using Infostat 2018d statistical software [25], and figures were plotted using SigmaPlot 14.0.

$$Ye_{ij} = \mu + M_i + S_j + M_i \times S_j + Erro_{ij} \tag{2}$$

Where Ye_{ij} is the response variable, "μ" is the overall average, M_i is the i-th method of breaking dormancy, S_j is the i-th substrate, $M_i \times S_j$ is the interaction between the M_i-th dormancy breaking method and the S_j-th substrate, and $Erro_{ij}$ is the random error.

$$r = \frac{1}{n-1} \sum_{i=1}^{n} \left(\frac{x_i - \bar{x}}{S_x} \right) \cdot \left(\frac{y_i - \bar{y}}{S_y} \right) \tag{3}$$

Where x and y are the sample means of x and y, respectively, and Sx and Sy are the sample standard deviations of x and y, respectively.

3. RESULTS

There was a significant interaction effect between substrate type and dormancy breaking method on seedling emergence (Table 3). Using SCO, EM resulted in the highest seedling emergence (70.07%). BCA and BBA provided the highest seedling emergence using EQ, with respective values of 78.08 and 56.05%. Using BBU, the best method for breaking dormancy was IA, with 50.05% seedling emergence.

Table 3. Emergence of Samanea tubulosa *seedlings using different substrates and dormancy breaking methods.*

Variable	Substrate	Method			CV (%)	P value		
		EM	IA	EQ		Substrate	Method	S x M
EP (%)	SCO	70.07Aa	55.08Ba	40.01Cc	10.51	<0.0001	<0.0001	<0.0001
	BBA	24.06Bb	20.01Bb	56.05Ab				
	BBU	30.01Bb	50.05Aa	30.05Bd				
	BCA	32.05Bb	20.04Cb	78.08Aa				

Averages followed by uppercase letters in the row and lowercase letters in the column did not significantly differ according to a Tukey test at 5% significance. CV, coefficient of variation; S × M, substrate × dormancy breaking method; EM, mechanical scarification; IA, immersion in water; EQ, chemical scarification; SCO, commercial substrate; BBA, babassu biomass; BBU, buriti biomass; BCA, carnauba bagana; EP, seedling emergence.

There was no significant interaction effect between dormancy breaking method and substrate (P > 0.05) on SH, RL, root volume (RV), or RD, suggesting that these factors acted independently (Table 4).

Table 4. Effect of substrate (S), dormancy breaking method (M), and substrate × dormancy breaking method (S × M) on seedling height, root length, root volume, and root density.

Variable	CV (%)	P value		
		Substrate	Method	S x M
Seedling height	9.79	<0.0001	0.0027	0.1097
Root length	14.24	<0.0001	0.1686	0.1659
Root volume	30.05	<0.0001	0.2655	0.1330
Root density	27.44	<0.0001	0.6210	0.7223

Substrates based on BCA and SCO had the highest average SH, with values of 15.30 and 14.44 cm, respectively, which did not significantly differ (P > 0.05) (Figure 1A). Seedling RLs in BCA and BBU were similar to those in the control (SCO) (Figure 1B), and SCO (2.63 mL) and BCA (2.11 mL) had the highest average RVs, which did not significantly differ (Figure 1C). BCA (0.17 g mL^{-1}), SCO (0.15 g mL^{-1}), and BBU (0.15 g mL^{-1}) gave the best results for RD (Figure 1D) and did not significantly differ. The BBA-based substrate gave the lowest average SH, RL, RV, and RD values.
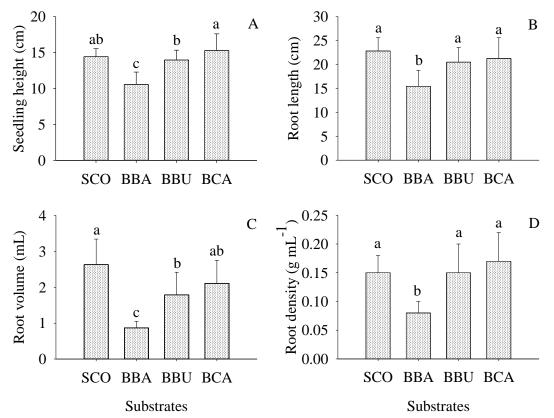

Figure 1. Seedling height (A), root length (B), root volume (C), and root density (D) of Samanea tubulosa *seedlings in different substrates. Averages followed by equal letters did not significantly differ according to a Tukey test at 5% significance. Bars indicate standard deviation.*

There was a significant interaction effect between substrate type and dormancy breaking method on DNH, NL, ADM, BDM, and DQI (Table 5). SCO and BCA provided the highest average DNH, regardless of the dormancy breaking method employed. There was no difference between methods using the BBA-based substrate, which had the lowest averages. The BBU substrate gave higher DNH using the EQ method than EM (Table 5).

The use of SCO, BCA, and BBU resulted in the highest NL, with no differences between the dormancy breaking methods. Using BBA, EM provided a higher NL than AI (Table 5). There were no significant differences in ADM among the methods, regardless of the type of substrate used, except for BCA, which provided higher ADM using the AI method than EQ. BCA had the highest values, regardless of the dormancy breaking method employed.

The highest BDM values were found using SCO and BCA, which were not significantly different. Using BCA, the best methods were EM and IA, while using BBU, the best dormancy breaking method was EQ. SCO and BCA gave the highest DQI values and did not significantly differ, regardless of the method employed (Table 5). BBA and BBU had the lowest values, and only by using the EQ method was the BBU substrate as successful as SCO and BCA.

We found significant, positive correlations (P < 0.05 and P < 0.01) among all of the variables under study (Table 6), in contrast to Gavioli et al. (2019) [24], who only reported weak correlations among them.

SH was very strongly correlated with ADM and TDM and strongly correlated with RL, RV, BDM, DQI, and RD. NL was only weakly correlated with the other variables (Table 6).

Table 5. Diameter at neck height, number of leaves, aboveground dry mass, belowground dry mass, and Dickson quality index values of seedlings using different substrates and dormancy breaking methods.

Variable	Substrate	Method			CV (%)	P value
		EM	IA	EQ		S x M
DNH (mm)	SCO	3.46Aa	3.28Aa	3.49Aa	13.69	0.0306
	BBA	1.93Ab	1.85Ab	2.34Ab		
	BBU	1.99Bb	2.37ABb	2.85Aab		
	BCA	3.17Aa	3.22Aa	2.92Aab		
NL	SCO	14.30Aa	13.70Aa	12.40Aa	16.27	0.0048
	BBA	10.73Aa	6.00Bb	6.66ABb		
	BBU	13.90Aa	16.92Aa	15.20Aa		
	BCA	12.80Aa	14.31Aa	12.33Aa		
ADM (g)	SCO	0.77Ab	0.82Ab	0.90Aa	23.00	0.0003
	BBA	0.21Ac	0.16Ac	0.19Ab		
	BBU	0.54Abc	0.64Ab	0.89Aa		
	BCA	1.17ABa	1.43Aa	0.93Ba		
BDM (g)	SCO	0.34Aa	0.37Aab	0.46Aa	30.07	0.0001
	BBA	0.06Ab	0.06Ac	0.07Ab		
	BBU	0.21Bab	0.20Bbc	0.40Aa		
	BCA	0.38ABa	0.49Aa	0.28Ba		
DQI	SCO	0.17Aa	0.18Aa	0.20Aa	29.75	0.0010
	BBA	0.03Ab	0.03Ab	0.03Ab		
	BBU	0.08Bb	0.09ABb	0.17Aa		
	BCA	0.21Aa	0.21Aa	0.14Aa		

Averages followed by uppercase letters in the row and lowercase letters in the column did not significantly differ according to a Tukey test at 5% significance. CV, coefficient of variation; S × M, substrate × dormancy breaking method; EM, mechanical scarification; IA, immersion in water; EQ, chemical scarification; SCO, commercial substrate; BBA, babassu biomass; BBU, buriti biomass; BCA, carnauba bagana; DNH, diameter at neck height; NL, number of leaves; ADM, aboveground dry mass; BDM, belowground dry mass; DQI, Dickson quality index.

Table 6. Pearson correlation matrix of growth and quality variables in Samanea tubulosa *seedlings in different substrates.*

	SH	RL	DNH	NL	RV	ADM	BDM	TDM	DQI
RL	0.63**								
DNH	0.56**	0.47**						Very strong	
NL	0.38**	0.33**	0.29*					Strong	
RV	0.69**	0.66**	0.65**	0.33**				Moderate	
ADM	0.81**	0.59**	0.63**	0.33**	0.77**			Weak	
BDM	0.77**	0.65**	0.65**	0.33**	0.89**	0.91**		No correlation	
TDM	0.81**	0.62**	0.65**	0.35**	0.82**	0.99**	0.96**		
DQI	0.72**	0.62**	0.76**	0.30*	0.87**	0.93**	0.97**	0.96**	
RD	0.67**	0.50**	0.37**	0.37**	0.48**	0.80**	0.78**	0.81**	0.73**

* and **: significant at 5% and 1%, respectively. SH, seedling height; RL, root length; DNH, diameter at neck height; NL, number of leaves; RV, root volume; ADM, aboveground dry mass; BDM, belowground dry mass; TDM, total dry mass; DQI, Dickson quality index; RD, root density.

4. DISCUSSION

Choosing what type of substrate to use is associated with the inherent physical and chemical characteristics of each substrate, such as porosity and density that influence water absorption and retention, as well as the availability of nutrients necessary for good seedling development Dorneller et al. (2014) [26]. In the present study, the type of substrate used for the production of *S. tubulosa* seedlings influenced the efficiency of the method for breaking seed dormancy.

EM is a simple, low-cost technique that cracks seeds in order to reduce the impermeability of the seed coat. However, the technique can damage the embryo, and affect germination and seedling emergence [27, 28]. In the present study, this method was particularly efficient in SCO by exposing the embryo and facilitating the imbibition process. Although this substrate had the lowest porosity (Table 1), suggesting lower infiltration, the amount of water was sufficient to achieve 70% seedling emergence.

Sulfuric acid accelerates the imbibition process by breaking the seed coat, which results in greater seedling emergence [29]. Using EQ, seedling emergence in BCA and BBA may have been related to their physical characteristics, because their total porosity was 14.03 and 9.13% higher, respectively, than soil with no organic residue (Table 1). In addition, according to Pagliarini et al. (2012) [30], the increase in porosity is associated with an increase in aeration and water infiltration, providing adequate moisture for the emergence of seedlings.

Oliveira et al. (2012) [29] investigated pre-germinative treatments for *S. tubulosa* seeds using washed sand (high porosity) as a substrate, and found that the highest percentage (>70%) of seedling emergence occurred with the use of concentrated sulfuric acid for 5 and 15 min, which is similar to the results of the present study.

IE for 24 h was most effective in BBU, which had >50% seedling emergence probably because of its highly porous nature (63.41%). This provided greater infiltration, optimized seed soaking, and weakened the integument. In this respect, BBU stands out from the other substrates studied, because in addition to being found in abundance in the Amazon and pre-Amazon regions, AI can be used to break dormancy in seeds with hard integuments. This would bring great economic benefits to the seedling producer because the raw material is widely available and cheap, in contrast to the sulfuric acid used in EQ, which has restricted use and is expensive, in addition to harming the environment. The IA method increases the permeability of the seed tissue owing to wear or softening, thus facilitating the diffusion of water into the embryo [31].

Although BCA only had low levels of nitrogen in its composition (Table 2), it contained high levels of organic matter that may have provided a controlled release of nitrogen during seedling development and affected their size (Figure 1A). According to Guerrini and Trigueiro (2004) [32], organic matter is a fundamental component in the substrates. It is capable of increasing the water

retention capacity, improving the physical characteristics of the substrate, and providing nutrients for the seedlings, in addition to being closely related to organic carbon.

BBA gave the lowest average RL (Figure 1B) probably because of its low levels of phosphorus (Table 2), which is closely linked to root growth. The highest averages for RV and RD (Figures 1C and 1D) were attributed to a large root mass, given the very strong and strong significant correlations, respectively, between these variables (Table 6). The high phosphorous levels in BCA, SCO, and BBU probably increased RD.

SCO and BCA gave the best DNH results, probably because of its strong correlation with RV (Table 6), suggesting that increased root growth results in greater stem thickening.

NL is an important parameter that is used in choosing the best seedlings in terms of photosynthetic capacity [33]. The results obtained using SCO, BCA, and BBU can be attributed to the levels of nutrients present in each substrate, particularly nitrogen and potassium that are needed in cell division and directly affect seedling growth and development, which are reflected in the NL.

The NL and ADM results were related to the levels of nitrogen and phosphorus present in the BCA substrate (Table 2). According to Faria et al. (2016) [34], these are the most important nutrients during the initial phase of seedling growth, because they play important roles in enzymatic processes, cell maintenance and turgidity, energy storage, and cell growth and elongation. Organic matter is an important component of BCA because it improves the physical, chemical, and biological properties of the substrate.

The high BDM values of plants grown in SCO and BCA can be attributed to their phosphorus concentrations, given their effects on root development, as well as their physical characteristics such as density and porosity, because high porosity and low density provide greater water retention and favor root development [35].

SCO and BCA had macronutrient levels that were adequate for plant development, as reflected in their high DQI values (Table 5). DQI is a measure of seedling quality, and seedlings with a high DQI value have high phytomass, vigor, and, consequently, greater production capacity [36].

SH was very strongly correlated with ADM and TDM (Table 5), indicating that seedling size is positively related to ADM and TDM values. This may have been due to greater light and carbon dioxide capture, resulting in higher photosynthetic activity.

The very strong correlations found between DQI and ADM, BDM, and TDM were probably caused by the dependence of this index on TDM and the ADM/BDM ratio used in calculating its value. Therefore, *S. tubulosa* seedlings with high TDM values and good ADM/BDM ratios had high DQI values.

NL was weakly correlated with all of the growth variables (SH, RL, DNH, RV, ADM, BDM, and TDM) and seedling quality (DQI), indicating that NL alone cannot explain the responses of the other variables studied [33].

5. CONCLUSIONS

Carnauba bagana may be used as an alternative substrate to the commercial substrate for the production of *S. tubulosa* (Benth.) seedlings; when combined with the chemical scarification method using sulfuric acid, it accelerated the emergence of seedlings. When buriti biomass was used as the substrate, the method of immersion in water for 24 h was satisfactory for overcoming the tegumentary dormancy of *S. tubulosa* seeds. The use of babassu biomass as the substrate was not suitable for the production of *S. tubulosa* seedlings.

6. ACKNOWLEDGMENTS

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