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ORIGINAL ARTICLE

Germination of *Bixa orellana* L. under nursery conditions

Germinação de Bixa orellana L. em condições de viveiro

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ABSTRACT: The species *Bixa orellana* L. is native to the Amazon and is in high demand due to the pharmacological properties of its fruits. However, research on germination and seedling production is incipient. The objective of this study was to evaluate the germination percentage with and without mechanical scarification of *Bixa orellana* L. seeds associated with commercial substrate or sand. The experiment was carried out in a completely randomized design, in a 2x2 factorial arrangement, with four treatments and four replications each. The germination rate and number of normal seedlings per treatment were determined. In addition to these parameters, the germination speed index, root and shoot length, and fresh and dry biomass of the seedlings were evaluated. The germination rate and normal and abnormal seedlings were expressed in percentage. In relation to the other parameters, the data were submitted to analysis of variance and the means were compared by the Scott-Knott test ($p<0.01$), with application of the Pearson correlation matrix ($p<0.01$) to the parameters. The germination rate showed higher values in sand (83%) and in commercial substrate (82%), and higher germination speed index when the seeds were scarified. The germination percentage was higher when sand was used and when the seeds were scarified. Thus, under nursery conditions, scarification was favorable to seed germination in both substrates.

RESUMO: *A espécie Bixa orellana L. é nativa da Amazônia e tem grande apreciação no mercado devido às propriedades farmacológicas dos seus frutos. Porém, as pesquisas em relação a germinação e produção de mudas são incipientes. Objetivou-se avaliar a porcentagem de germinação com e sem escarificação mecânica de sementes de Bixa orellana L. associadas a substrato comercial ou areia. O ensaio foi conduzido em delineamento experimental inteiramente casualizado, em arranjo bifatorial 2x2, com quatro tratamentos e quatro repetições cada. Foram determinados a taxa de germinação e número de plântulas normais por tratamento. Além desses, foram avaliados o índice de velocidade de germinação, comprimento de raiz e de parte aérea, biomassa fresca e biomassa seca das mudas. A taxa de germinação e de plântulas normais e anormais foram expressos em porcentagem. Em relação aos demais parâmetros, os dados foram submetidos à análise de variância e as médias foram comparadas pelo teste de Scott-Knott (p<0,01), sendo aplicada a matriz de correlação de Pearson (p<0,01) aos parâmetros avaliados. A taxa de germinação apresentou valores superiores em areia (83%) e em substrato comercial (82%) e índice de velocidade de germinação superior quando as sementes foram escarificadas. Verificou-se que a porcentagem de germinação foi maior quando utilizada a areia e quando as sementes foram escarificadas. Dessa forma, em condições de viveiro a escarificação foi favorável à germinação das sementes em ambos os substratos.*

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1 Introduction

According to APG IV (APG, 2016), *Bixa orellana* L., also known as urucum or colorau in Brazil (and achiote in some other countries), belongs to the family Bixaceae, order Malvales, clade Malvids. The species can be considered a tree or shrub, and is native to Tropical America (Vilar *et al.*, 2014).

The uses of the seeds are diverse, including the production dyes, resulting in high economic and industrial value, especially in the cosmetics industry (Fontana *et al.*, 2016). They can also be employed in the production of foods, giving better appearance and nutritional quality to products, besides having several medicinal properties (Vieira, 2018). Thus, the species is a potential source of income for smallholders. Finally, because of its fast growth and rusticity, if is also used for the recovery of degraded areas (Ferronato *et al.*, 2015).

Seeds are the main source of plantlet production, because of their easy availability, low propagation cost and possibility of storage for long periods before sowing. Lima (2014) pointed out that the seeds of *Bixa orellana* are considered orthodox because they have tolerance to desiccation.

The good health of the seeds used is essential to assure the morphological and physiological quality of the seedlings. Besides biotic variables, some abiotic variables can influence the final quality of the seedlings, such as light, temperature and humidity, among others (Oliveira *et al.*, 2016; Silva *et al.*, 2017). According to Gomes & Paiva (2011), some species, even under favorable conditions of temperature, light, oxygen and water, have difficulty germinating, so they are classified as dormant.

To overcome dormancy, several techniques can be used, depending on the type of dormancy of each species, either endogenous or exogenous (Fowler & Bianchetti, 2000). Baskin & Baskin (2008) classified embryonic dormancy as endogenous, while dormancy imposed by seed coats (exogenous) is classified as physical (tegumentary), chemical or mechanical. All the treatments to break dormancy are called pre-germinative, and aim to increase the germination rate and standardization of seedlings (Brasil, 2009).

Such treatments can contribute to seedling quality indicators, such as good initial growth and subsequently high survival of seedlings in the field. Thus, these indicators are associated with physiological and morphological parameters, such as survival and germination rate (Gomes & Paiva, 2011; Fronza & Hamann, 2015).

The substrate used directly influences the quality of the seedlings, because a good substrate provides physical and chemical properties that favor development in the initial phase (Fronza & Hamann, 2015). Several substrates have been used, such as charcoal, paper, cloth and sand, along with commercial substrates (Ferraz *et al.*, 2020). To be classified as ideal, whatever its composition, the substrate must have basic aeration and drainage conditions for germination to occur.

In this sense, scarification can have a greater positive influence on germination than substrate choice, since the seed is not influenced by its chemical quality. Therefore, the objective of this work was to evaluate the germination of *Bixa orellana* seeds with different methods of breaking dormancy and different substrates under nursery conditions.

2 Materials and Methods

The study was carried out at the Federal University of Rondônia, in the city of Rolim de Moura, located in the southwestern Amazon (latitude 11° 43' 18.59" S, longitude 61° 46' 23.39" W, and altitude at sea level of 261 m.). The climate in the region is classified as Monsoon, with average annual temperatures ranging between 24 ºC and 26 ºC (Alvares *et al.*, 2013; Mascarenhas *et al.*, 2020).

The seeds of *Bixa orellana* were collected from plants located on a private rural property in the municipality of Rolim de Moura, in January 2017. They were submitted to treatment to break dormancy using mechanical scarification with medium grain sandpaper. The seeds were placed between sheets of sandpaper and rubbed with gentle pressure. Subsequently, they were submitted to asepsis in a beaker containing 200 mL of sodium hypochlorite solution in water (2.5% NaClO) and 3 drops of neutral liquid detergent, stirred for 3 minutes, after which the seeds were triple washed with autoclaved water (Almeida, 2015). The process was conducted under nursery conditions in March 2017. The nurseries were built in wooden frames of 5 m², covered with polyethylene mesh with 50% permeability to sunlight.

The seeds were placed in drained black polyethylene trays with dimensions of 0.52 m × 0.35 m × 0.10 m (length × width × height), containing commercial substrate or coarse sand, arranging the seeds about 2 cm apart to ensure homogeneous distribution. During the experiment, the substrates were moistened by hand daily until the end of germination.

The experimental design used was entirely randomized in a 2 x 2 factorial arrangement, with the first factor consisting of two treatments to overcome dormancy (with or without mechanical scarification with medium grain sandpaper), and the second factor consisting of different substrates (coarse sand submitted to solarization for seven days (Ghini *et al.*, 2003) or commercial substrate based on pine bark).

In each treatment four repetitions were established, so it was necessary to separate eight 50-seed subsamples from two 400-seed lots.

The parameters evaluated were germination rate (GR), germination speed index (GSI), classification seedlings as normal and abnormal, seedling length and fresh and dry biomass. The germination rate (GR) was measured according to Rules for Seed Analysis - RSA (Brasil, 2009). The seeds were considered germinated when emission of the radicle was observed. The germination speed index (GSI) was measured according to Maguire (1962), as the ratio between the sum of seeds that germinated on a day and the respective number of days it took to germinate, considering emission of the radicle.

For the classification of normal and abnormal, the seedlings that presented a defined root system and shoot, intact or with small defects, were considered normal, and those that did not present a root system or shoot were considered abnormal (damaged, defective or deteriorated) (Brasil, 2009).

To evaluate the fresh biomass, a semi-analytical balance (0.01g) was used. For dry biomass, the root system and shoot were placed in kraft paper bags and placed in an oven at 50°C ± 1 °C until weight stabilization (about 72 hours). Subsequently, a 0.0001 g precision balance was used to weigh them.

The germination rate and normal and abnormal seedlings were expressed in percentage. For the other parameters, the data were submitted to tests for normality of residuals and homogeneity of variances. After confirming the homoscedasticity and normality of the data, they were submitted to analysis of variance ($p<0.01$). When there were differences among the variation sources, the means were compared using the Scott-Knott test ($p<0.01$). In addition, Pearson's correlation matrix was applied ($p<0.01$) to the evaluated parameters. The statistical program used in all cases was Assistat 7.7.

3 Results and Discussion

Germination started in all treatments on the 9th day after sowing. When comparing the treatments T1 (without scarification on sand) with 30% germination rate and T3 (with scarification on sand) with 83%, an increase of 53 percentage points in germination rate was observed. On the other hand, T2 (without scarification on commercial substrate), with 43%, compared to T4 (with scarification on commercial substrate), with 82%, there was an increase of 39 percentage points.

Table 1. Germination rate (%) and Germination Speed Index (GSI) of *Bixa orellana* seeds for treatments without and with scarification on different substrates under nursery conditions. Rolim de Moura, RO.

Tabela 1. Taxa de germinação (%) e Índice de Velocidade de Germinação (GSI) de sementes de *Bixa orellana* para os tratamentos sem e com escarificação em diferentes substratos em condições de viveiro. Rolim de Moura, RO.

Treatments	Germination (%)	GSI
T1	30.0	5.0
T2	43.0	7.0
T3	83.0	15.0
T4	82.0	13.0

T1: without scarification in sand; T2: without scarification in commercial substrate; T3: with scarification in sand; T4: with scarification in commercial substrate.

T1: sem escarificação em areia; T2: sem escarificação em substrato comercial; T3: com escarificação em areia; T4: com escarificação em substrato comercial.

Thus, the type of substrate had little influence on germination (commercial substrate increased germination by 6%), while scarification benefited germination (47% increase). In this sense, Mafra (2021) found that scarification had similar behavior when evaluating the seeds of *Abrus precatorius* L. germinated in sand. He observed germination rates on the order of 85% when the seeds were mechanically scarified, thus obtaining the highest germination rate.

These higher values may be related to the fact that scarification changes the tegument, facilitating contact of the embryo with water and oxygen, thus promoting seed germination (Saldanha *et al.*, 2017).

Regarding the germination speed index of *B. orellana* seeds, the values were higher when submitted to seed scarification, both in sand and commercial substrate (Table 1).

On day 10 after sowing, all treatments achieved maximum germination, with treatments T3 and T4 (with scarification), tending to have highest values, with germination of 63 seeds each (Figure 1).

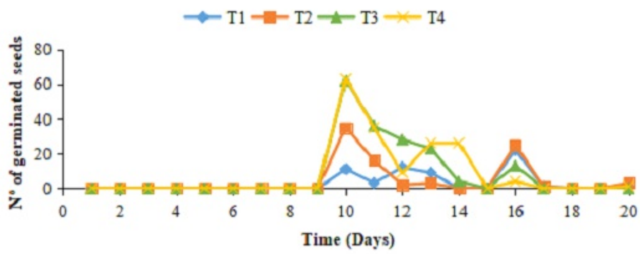

Figure 1. Number of germinated seeds of *Bixa orellana* as a function of time under nursery conditions. T1: not scarified in sand, T2: not scarified in commercial substrate, T3: scarified in sand, T4: scarified in commercial substrate. Rolim de Moura, RO.

Figura 1. Número de sementes germinadas de *Bixa orellana* em função do tempo submetida às condições de viveiro. T1: não escarificado em areia, T2: não escarificado em substrato comercial, T3: escarificado em areia, T4: escarificado em substrato. Rolim de Moura, RO.

Seeds need water and oxygen for their germination and scarification facilitates the contact of the seeds with these. Pinheiro (2021) reported that when choosing a substrate, one should take into account the water retention capacity, porosity and composition. However, Abreu *et al.* (2005) observed that the substrate for germination does not exert much influence, because the seeds use their reserves to germinate and nourish the seedlings in their initial growth, in contrast to our results.

In all treatments of the seeds that germinated, the seedlings were 100% normal, and there was an interaction between scarification and substrates for the parameters root and shoot length (Table 2).

Similar to our results, Silva *et al.* (2015) found there was no difference in root length of *Euterpe oleracea* Mart. between scarified and non-scarified seeds. Also in this sense, Rocha *et al.* (2017) corroborated that scarification methods are efficient for breaking dormancy, because by breaking the tegument barrier, the first phase of germination begins (water soaking).

Table 2. Root and shoot length of *Bixa orellana* seedlings with and without scarification and on different substrates under nursery conditions. Rolim de Moura, RO.

Tabela 2. Comprimento de raiz e da parte aérea das mudas de *Bixa orellana* com e sem escarificação e em diferentes substratos submetidas a condições de viveiro. Rolim de Moura, RO.

Treatment	Root Length (cm)		Shoot Length (cm)	
	Sand	Commercial Substrate	Sand	Commercial Substrate
Not scarified	6,61 aB	10,23 aA	5,22 aB	9,38 bA
Scarified	5,85 bA	6,24 bA	5,76 aB	11,24 aA

Different letters, lower case in the column and upper case in the row, different from each other, by the Scott-Knott test at 5% probability of error.

Letras diferentes, minúsculas na coluna e maiúsculas na linha diferente entre si, pelo teste de Scott-Knott a 5% de probabilidade de erro.

The values of shoot length presented a different trend than the root length, that is, the highest values were verified when scarification was performed and commercial substrate was used. However, further regarding these conditions, the difference in root length and shoot length was 8.32% for non-scarified seeds, while for scarified seeds this difference was 80.00%. This indicates that, even though the length of the shoot was smaller when the seeds were not scarified, the seedlings presented a better balance between the shoot and the root system. Thus, when the seedlings are taken to the field, the occurrence of toppling is reduced when the root system is proportional to the shoot. In addition, if there is greater surface contact, there will be greater transpiration of the plant (Taiz & Zeiger, 2013).

In addition, Nogueira *et al.* (2012), when analyzing the seeds of *Mimosa caesalpiniifolia* Benth, which have tegumentary dormancy like *B. orellan*a, obtained greater length of the shoot when they used scarification and a substrate based on pine bark, similar behavior to the present study.

The growth of the root and shoot systems in commercial substrate was superior compared to sand. This result can be explained by the aeration, porosity and nutrients provided by this substrate. Ferreira *et al.* (2009), using different substrates, observed similar root length and shoot length when using commercial substrates based on pine bark. Additionally, Silva *et al.* (2017) stated that suitable substrates promote better growth and development of the root system because they contain physicochemical characteristics that allow a balance of aeration, water retention, nutrient uptake, and oxygen for seedlings. However, it is important to emphasize that the physical properties have the greatest influence at the beginning of seedling growth, when there is still a nutritional supply by the cotyledons, while in the post-emergence period, it is necessary for the substrate to be in balance with respect to physical and chemical characteristics. Commercial substrates have both attributes and are considered satisfactory for this purpose (Lameira *et al.*, 2021).

In relation to the fresh mass of the root system of *B. orellana* seedlings there was a significant interaction between scarified and non-scarified seeds, in the different substrates. However, the values of shoot fresh mass were not influenced by the treatments (Table 3).

Table 3. Fresh biomass of the root system and shoot of *Bixa orellana* seedlings with and without scarification and on different substrates under nursery conditions. Rolim de Moura, RO.

Tabela 3. Biomassa fresca do sistema radicular e da parte aérea das mudas de *Bixa orellana* com e sem escarificação e em diferentes substratos submetidas a condições de viveiro. Rolim de Moura, RO.

Treatment	Root Fresh Biomass (g)		Shoot Fresh Biomass (g)	
	Sand	Commercial Substrate	Sand	Commercial Substrate
Not scarified	0.0460 aB*	0.1410 aA	0.0770 ns**	0.3222 ns
Scarified	0.0382 aA	0.0416 bA	0.0489 ns	0.2489 ns

*Different letters, lowercase in the column and uppercase in the row, differ from each other by the Scott-Knott test at 5% probability. **non-significant by the Scott-Knott test at 5% probability.

*Letras diferentes, minúsculas na coluna e maiúsculas na linha diferente entre si, pelo teste de Scott-Knott a 5% de probabilidade de erro. **não significativa pelo teste de Scott-Knott a 5% de probabilidade de erro.

We suggest that these results were influenced by the properties of the commercial substrate, because this substrate has higher water retention than sand, providing greater availability for the plants for a longer time. This constitutes, a priori, an indispensable factor for the production of seedlings, given that water constitutes more than 80% of plant tissues, and the root system is the protagonist in the absorption and distribution of water to other organs of the plant (Taiz & Zeiger, 2013).

Regarding root dry mass and shoot dry mass, a joint effect of scarification and no scarification was observed on these parameters (Table 4).

Table 4. Dry biomass of the root system and shoot of *Bixa orellana* seedlings with and without scarification and on different substrates under nursery conditions. Rolim de Moura, RO.

Tabela 4. Biomassa seca do sistema radicular e da parte aérea das mudas de *Bixa orellana* com e sem escarificação e em diferentes substratos submetidas a condições de viveiro. Rolim de Moura, RO.

Treatment	Root Dry Biomass (g)		Shoot Dry Biomass (g)	
	Sand	Commercial Substrate	Sand	Commercial Substrate
Not scarified	0.0107 aB	0.0186 aA	0.0224 aB	0.0651 aA
Scarified	0.0094 aA	0.0113 bA	0.0199 aB	0.0460 bA

Different letters, lowercase in the column and uppercase in the row, differ from each other by the Scott-Knott test at 5% probability.

Letras diferentes, minúsculas na coluna e maiúsculas na linha diferente entre si, pelo teste de Scott-Knott a 5% de probabilidade de erro.

There was no difference in relation to the accumulation of dry root biomass when the seeds were scarified in sand, presenting a difference of 12%, when compared to scarification. The commercial substrate, on the other hand, showed higher values in the contribution to biomass when there was no scarification. This increase in root dry biomass was 73% in comparison with the sand treatment. In turn, the dry biomass of the shoot showed higher values in the commercial substrate, and when the seeds were not scarified there was a 41% increase in biomass.

Campos *et al.* (2015), when germinating seeds of *Rollinia mucosa* Jacq. in the state of Goiás, verified the unfeasibility of mechanical scarification, both for the accumulation of dry biomass of the root and shoot, since when there was no germination pretreatment, the values were higher.

The values of root and shoot dry mass were correlated to fresh mass (Table 5). This fact can be explained by the turgor pressure in plants, caused by water, which is responsible for the expansion of plant cells, leaving them stable and rigid. Thus, water influences the biomass increment (Taiz & Zeiger, 2013). However, the opposite behavior was verified in the correlation matrix for the seeds that were scarified (Table 6).

Table 5. Pearson correlation matrix using the parameters root length (RL), shoot length (SL), root fresh mass (RFM), shoot fresh mass (SFM), root dry mass (RDM) and shoot dry (SDM) of seeds without scarification in different substrates under nursery conditions. Rolim de Moura, RO.

Tabela 5. Matriz de correlação de Pearson utilizando os parâmetros comprimento de raiz (RL), comprimento da parte aérea (CPA), massa fresca da raiz (MFR), massa fresca da parte aérea (MFPA), massa seca da raiz (MSR) e massa seca da parte aérea (MSPA), das sementes sem escarificação em diferentes substratos em condições de viveiro. Rolim de Moura, RO.

Substrates	Attributes	SL (cm)	RFM (g)	SFM (g)	RDM (g)	SDM (g)
Sand	RL	0.0901^{ns}	0.4161^{ns}	0.0320^{ns}	0.1002^{ns}	-0.0375^{ns}
	(cm)	(*0.80*)	(*0.23*)	(*0.93*)	(*0.78*)	(*0.91*)
	SL		-0.3585^{ns}	0.1433^{ns}	0.3222^{ns}	0.7761**
	(cm)		(*0.30*)	(*0.69*)	(*0.36*)	(<0.01)
	RFM			0.0788^{ns}	-0.6745*	-0.2523^{ns}
	(g)			(*0.82*)	(<0.05)	(*0.48*)
	SFM				-0.3670^{ns}	-0.1566^{ns}
	(g)				(*0.29*)	(*0.66*)
	RDM					0.3075^{ns}
	(g)					(*0.38*)
Commercial Substrate	RL	0.3975^{ns}	0.6857*	0.6908*	0.6811*	0.6673*
	(cm)	(*0.25*)	(<0.05)	(<0.05)	(<0.05)	(<0.05)
	SL		0.3845^{ns}	0.6597*	0.4729^{ns}	0.6961*
	(cm)		(*0.27*)	(<0.05)	(*0.16*)	(<0.05)
	RFM			0.7397**	0.9353**	0.6141*
	(g)			(<0.01)	(<0.01)	(<0.05)
	SFM				0.6448*	0.9565**
	(g)				(<0.05)	(<0.01)
	RDM					0.5614^{ns}
	(g)					(*0.09*)

ns: not significant; * and **: Pearson correlation coefficient significant at the 1 and 5% level; numbers in italics below each correlation value represent its probability.

ns: não significativo; * e**:coeficiente de correlação de Pearson significativo ao nível de 1 e 5%; números em itálico, abaixo do valor de cada correlação, representam sua probabilidade.

Therefore, *B. orellana* seedlings without scarification in commercial substrate showed superiority of root fresh biomass. In the same treatment, the shoot fresh biomass expressed greater increment and the fresh biomass of the root system and shoot were directly proportional to the results of the respective lengths (Table 5 and 6).

In summary, in the test performed under nursery conditions, germination was not significantly influenced by the substrates. However, scarification provided a germination rate and GSI 3 and 4 times higher than treatments T2 and T1, respectively.

Table 6. Pearson correlation matrix using the parameters root length (RL), shoot length (SL), root fresh mass (RFM), shoot fresh mass (SFM), root dry mass (RDM) and shoot dry mass (SDM) of scarified seeds in different substrates under nursery conditions. Rolim de Moura, RO.

Tabela 6. Matriz de correlação de Pearson utilizando os parâmetros comprimento de raiz (CR), comprimento da parte aérea (CPA), massa fresca da raiz (MFR), massa fresca da parte aérea (MFPA), massa seca da raiz (MSR) e massa seca da parte aérea (MSPA), das sementes escarificadas em diferentes substratos em condições de viveiro. Rolim de Moura, RO.

Substrates	Attributes	SL (cm)	RFM (g)	SFM (g)	RDM (g)	SDM (g)
Sand	RL	0.4062^{ns}	0.3457^{ns}	-0.0735^{ns}	0.0044^{ns}	0.0865^{ns}
	(cm)	(*0.24*)	(*0.32*)	(*0.84*)	(*0.99*)	(*0.81*)
	SL		0.4275^{ns}	0.1390^{ns}	-0.1396^{ns}	0.3713^{ns}
	(cm)		(*0.21*)	(*0.70*)	(*0.70*)	(*0.29*)
	RFM			0.0986^{ns}	-0.2633^{ns}	0.1464^{ns}
	(g)			(*0.78*)	(*0.46*)	(*0.68*)
	SFM				-0.1912^{ns}	0.1407^{ns}
	(g)				(*0.59*)	(*0.69*)
	RDM					0.5665^{ns}
	(g)					(*0.08*)
Commercial Substrate	RL	0.8840**	0.6388*	-0.4937^{ns}	0.0132^{ns}	0.6450*
	(cm)	(<0.01)	(<0.05)	(*0.14*)	(*0.97*)	(<0.05)
	SL		0.5518^{ns}	-0.4524^{ns}	-0.2829^{ns}	0.8052**
	(cm)		(*0.09*)	(*0.18*)	(*0.42*)	(<0.01)
	RFM			-0.1540^{ns}	-0.1959^{ns}	0.2171^{ns}
	(g)			(*0.67*)	(*0.58*)	(*0.54*)
	SFM				-0.0088^{ns}	-0.2436^{ns}
	(g)				(*0.98*)	(*0.49*)
	RDM					-0.0185^{ns}
	(g)					(*0.95*)

ns: not significant; * and **: Pearson correlation coefficient significant at 1% and 5% level; numbers in italics below each correlation value represent the probability.

ns: não significativo; * e**: coeficiente de correlação de Pearson significativo ao nível de 1 e 5%; números em itálico, abaixo do valor de cada correlação, representam sua probabilidade.

However, for the growth of the seedlings, the substrates made a difference. Thus, root and shoot length, root and shoot fresh mass, and root and shoot dry mass were superior in the plants grown on commercial substrate. As for the scarification of the seeds, it proved to be efficient only for the shoot length.

Based on these findings, scarification can be performed with medium texture sandpaper, because besides giving good results, it is easy and inexpensive. Regarding germination, preference should be given to seedbeds containing solarized sand with 50% shade during the dry season. The sand used as a substrate is easy to acquire and inexpensive, and also facilitates solarization.

It is also noteworthy that the seeds that were not scarified and grown in commercial substrate had greater root length. Sand did not influence root biomass production, while in the commercial substrate the absence of scarification was beneficial.

4 Conclusion

For the germination of *Bixa orellana*, sand can be used as a substrate. For the growth of seedlings, the commercial substrate was best.

For nursery seedling production, scarification is recommended to increase the germination rate.

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