http://journal.unoeste.br/index.php/ca/index
DOI: 10.5747/ca.2022.v18.n1.a480
ISSN on-line 1809-8215

Colloquium Agrariae

Submetido: 29/10/2021 Revisado: 16/12/2021 Aceito: 17/03/2022

Seed germination of *Astronium urundeuva* (M. Allemão) Engl. and *Anadenanthera colubrina* (Vell.) Brenan in different substrates

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Abstract
The present study evaluated the effect of different substrates on the germination and initial development of *Astronium urundeuva* and *Anadenanthera colubrina*. The substrates were: washed sand, vegetable soil, and vermiculite for *A. urundeuva* experiment; washed sand and vermiculite for *A. colubrina* experiment. The following variables were analyzed: percentage of emergence, emergence speed index, average emergence time, average emergence speed, synchronization index, first emergency count, and the relative frequency of emergence. For initial development, shoot and root length, number of leaves, and shoot, root and total dry mass were evaluated. The highest percentages of emergence for *A. urundeuva* (52%) and for *A. colubrina* (50.5%) were observed in vermiculite substrate. The use of vermiculite as substrate favored germination and initial development of *A. urundeuva* and *A. colubrina*.
Keywords: Aroeira-do-sertão; angico; Caatinga; substrate; seed technology.

Germinação de sementes de *Astronium urundeuva* (M. Allemão) Engl. e *Anadenanthera colubrina* (Vell.) Brenan em diferentes substratos

Resumo
O presente estudo avaliou o efeito de diferentes substratos na germinação e no desenvolvimento inicial de *Astronium urundeuva* e *Anadenanthera colubrina*. Foram testados os substratos areia lavada, terra vegetal e vermiculita na germinação e desenvolvimento inicial de *A. urundeuva* e areia lavada e vermiculita na germinação e desenvolvimento inicial de *A. colubrina*. As seguintes variáveis foram analisadas: porcentagem de emergência, índice de velocidade de emergência, tempo médio de emergência, velocidade média de emergência, índice de sincronização, primeira contagem de emergência e a frequência relativa de emergência. Para o desenvolvimento inicial foram avaliados o comprimento da parte aérea e da raiz, o número de folhas e as massas secas da parte aérea, raízes e total. As maiores porcentagens de emergência para *A. urundeuva* (52%) e para *A. colubrina* (50,5%) foram observadas em substrato vermiculita. A utilização de vermiculita como substrato favoreceu a germinação e o desenvolvimento inicial de *A. urundeuva* e *A. colubrina*.
Palavras-chave: Aroeira-do-sertão; angico; Caatinga; substrato; tecnologia de sementes.

Introduction

The Caatinga is an exclusively Brazilian biome, which occupies approximately 11% of the national area, occurring from Maranhão to the north of Minas Gerais state. There is a predominance of xerophyte and deciduous species, with the presence of trees, shrubs, and herbaceous vegetation, besides cactus, orchids, and bromeliads (MAIA, 2012). One of the most remarkable characteristic of Caatinga is the high plant endemism, which is seriously threatened by anthropic activities (RIBEIRO *et al.*, 2015). The illegal deforestation to produce stakes, posts, forage, firewood, charcoal and to convert natural areas into agricultural land and pasture put several species on risk of extinction (BISPO *et al.*, 2017; FAGGIN *et al.*, 2017). In this context, up to 50% of the Caatinga are already changed and the losses of vegetal cover compromise the biodiversity of this biome (SAMPAIO, 2010; DRUMOND *et al.*, 2016; SCHULZ *et al.*, 2017).

Among the species in Caatinga, "aroeira-do-sertão" (*Astronium urundeuva* (M. Allemão) Engl., basinomy *Myracrodruon urundeuva* M. Allemão (MITCHELL; DALLY, 2017; SILVA-LUZ *et al.*, 2020)) belonging to Anacardiaceae family and "angico" (*Anadenanthera colubrina* (Vell.) Brenan) belonging to Fabaceae family have a great ecological and economical importance. All of them are tree species that stand out for their high wood quality, medicinal and pharmacological importance, and ornamental and reforestation uses (ARAÚJO *et al.*, 2015; LORENZI, 2008; MAIA, 2012; GALVÃO *et al.*, 2018). Seeds are the main means of propagation of forest species such as *A. urundeuva* and *A. colubrina*, being widely used in planting and for the production of seedlings in nurseries. Thus, studies of various environmental factors, such as water, temperature, oxygen, and substrate, which interfere in the germination process, are fundamental for seed technology (CARVALHO; NAKAGAWA, 2012; BASKIN; BASKIN, 2014; PIÑA-RODRIGUES *et al.*, 2015).

Mastery of propagation techniques is a key factor in the production of seedlings of forest species, especially species from the Caatinga, as studies about germination are still scarce (RODRIGUES *et al.*, 2007). Choosing the right substrate is essential for seed germination, directly influencing the seedling production process in nurseries (SCALON *et al.*, 2012; OLIVEIRA *et al.*, 2012). The type of substrate enables different conditions of aeration, humidity, support, retention of water, and pathogen infestation, favouring or not the process of germination (BASKIN; BASKIN, 2014; PIÑA-RODRIGUES *et al.*, 2015). This will ensure a better quality of seedlings of native forest species, being a fundamental process for the conservation of threatened species and the recovery of degraded areas (RODRIGUES *et al.*, 2007; SCALON *et al.*, 2012; OLIVEIRA *et al.*, 2012).

In this context, the recognition of factors that influences germination, important step of plant development, allows the increase of percentage, speed, and uniformity of germination, in order to produce vigorous seedlings with reduced time and budget (PACHECO *et al.*, 2006; OLIVEIRA *et al.*, 2014). Taking into account the importance of the studied species and the need of production of seedlings, our aim was to characterize the germination of Caatinga species, evaluating the influence of different substrates on germination and initial development of *A. urundeuva* and *A. colubrina* seedlings.

Material and Methods

Plant Material

Seeds of *A. urundeuva* and *A. colubrine* were collected from trees of a Caatinga fragment located in Guanambi, BA, Brazil (14° 12' 26'' S; 42° 46' 55'' W). The collection of seeds was performed manually, between the months of october and november 2018. The seeds were processed manually, taken to the laboratory, and stored for three months in transparent plastic bags at room temperature (25 ± 2 °C) until the beginning of the experiment. The experiment was performed in a greenhouse of Federal University of Sergipe located in São Cristóvão, SE, Brazil (10°55'34" S; 37°06'08" W).

Experiment I: Seedling emergence and initial development of *A. urundeuva* in different substrates

Seeds of *A. urundeuva* were sown in styrofoam trays containing different substrates: washed sand (T1), humus substrate (T2), and vermiculite (T3). Throughout the 30-day experiment, the trays were kept well-watered and the number of germinated seeds was evaluated daily, adopting as a germination criterion the emergence of cotyledons and the emergence of hypocotyl.

The analyzed variables were seedling emergence (%E) corresponding to the total percentage of seeds germinated until the 30th day after sowing, considering normal seedlings, according to Brasil (2009), emergence speed index:

$$ESI = \frac{G_1 + G_2 + \cdots + G_n}{N_1 + N_2 + \cdots + N_n}$$

where G_1, G_2, G_3, ..., G_n correspond to the number of seeds germinated in the first, second, third until the last count and N_1, N_2, N_3, ..., N_n correspond to the number of days from the sowing to last count (MAGUIRE, 1962); average emergence time (LABOURIAU; VALADARES, 1976); average speed emergence; and the emergency synchronization (LABOURIAU; PACHECO, 1978) were determined using GerminaQuant software version 1.0 (MARQUES *et al.*, 2015). It was also determined from the first emergence count - corresponding to the percentage accumulation of normal seedlings to the 6th day after onset of the test. In addition, graphic analysis of the relative frequency of emergence was performed $[F_r = n_i / \Sigma n_i]$.

At the end of the experimental period, 20 seedlings of each treatment were randomly chosen, and it was evaluated number of leaves, height of the seedling (cm), and length of main root (cm) with the aid of a millimeter ruler. The seedlings were placed in paper bags and dried in an oven with air circulation forced at 70 °C, until reaching constant weight. After that, dry mass of aerial part (g), roots (g), and total (g) were determined, using a scale with a precision of 0.001 g. The experiment had a completely randomized design, with three treatments (different substrates) and 10 replicates. Each replicate was composed by 25 seeds, totalling 250 seeds per treatment.

Experiment II: Seedling emergence and initial development of *A. colubrina* in different substrates

Seeds of *A. colubrina* were sown in styrofoam trays containing different substrates: washed sand (T1) and vermiculite (T2). Throughout the 15-day experiment, the trays were kept well-watered and the seedling emergence was counted daily, considering the emergence of cotyledons.

The analyzed variables were seedling emergence (%E) corresponding to the total percentage of seeds germinated until the 15th day after sowing, considering normal seedlings, according to Brasil (2009); emergence speed index (MAGUIRE, 1962); average emergence time (LABOURIAU; VALADARES, 1976); average speed emergence; and the emergency synchronization (LABOURIAU; PACHECO, 1978), determined using GerminaQuant software version 1.0 (MARQUES *et al.*, 2015). It was also determined to the first emergence count - corresponding to the accumulated percentage of normal seedlings until the 5th day after onset of the test. In addition, graphic analysis of the relative frequency of germination was performed $[F_r = n_i / \Sigma n_i]$.

At the end of the experimental period, 20 seedlings of each treatment were randomly chosen, and it was evaluated number of leaves, height of the seedling (cm), and length of main root (cm) with the aid of a millimeter ruler. The seedlings were placed in paper bags and dried in an oven with air circulation forced at 70 °C, until reaching constant weight. After that, dry mass of the aerial part (g), roots (g), and total (g) were determined, using a scale with a precision of 0.001 g. The experiment had a completely randomized design, with two treatments (different substrates) and eight replicates. Each replicate was composed by 25 seeds, totalling 200 seeds per treatment.

Statistical analyses

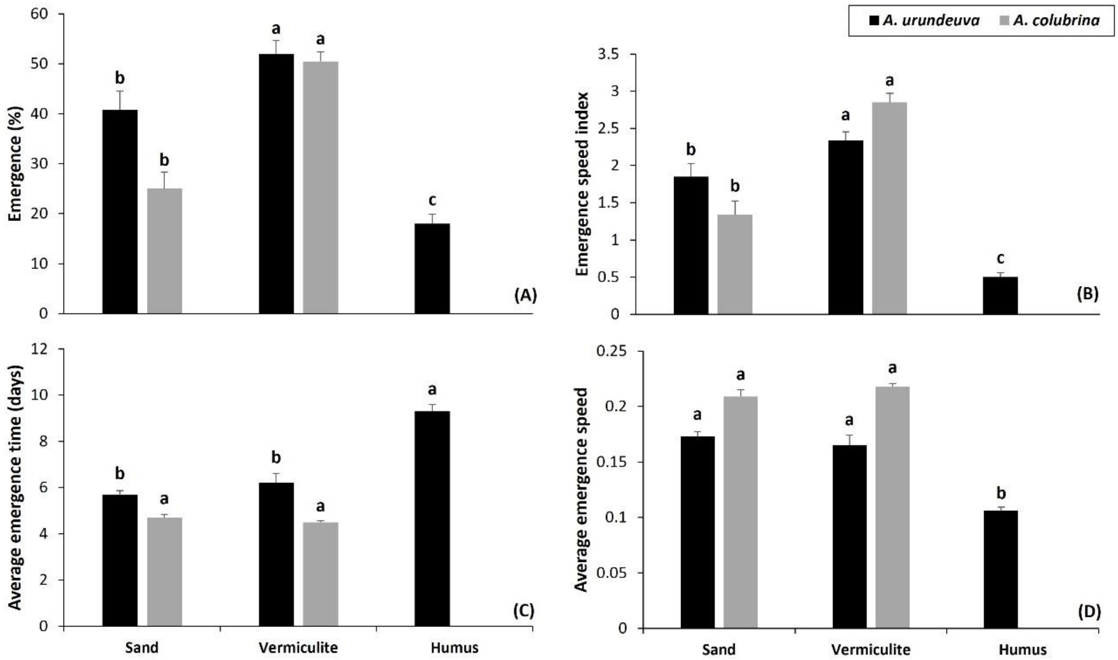
Data were tested for normality by Shapiro-Wilk test in Past software. As long the data presented a normal distribution, they were subjected to an one-way ANOVA and the means were compared by Tukey's test ($p<0.05$), with the exception of *A. colubrine* in which the comparison was performed with teste T (LSD) ($p<0.05$). These analyses were run using SISVAR software (FERREIRA, 2005).

Results and Discussion

Experiments I and II: Vermiculite improves the emergence and initial development of *A. urundeuva* and *A. colubrina*

The use of vermiculite promoted the emergence, showing higher results compared to washed sand and humus substrate (*A. urundeuva*) and washed sand (*A. colubrina*) (Figure 1 A and B). Thus, for *A. urundeuva* and *A. colubrina* seeds sown in vermiculite substrate, it was observed values of 52% and 50.5% for emergence and 2.34 and 2.85 for emergence speed index, respectively (Figure 1 A and B). For average emergence time and average speed emergence of *A. urundeuva*, vermiculite and washed sand significantly differed from humus substrate, which was not observed for *A. colubrina* (Figure 1 C and D). Higher emergence percentage values and emergence speed index may be related to a better uniformity of moisture distribution in the vermiculite substrate, as this factor is essential during the germination process (GUEDES *et al.,* 2011).

Figure 1. Seedling emergence (A), emergence speed index- ESI (B), average emergence time (C), and average speed emergence (D) of *A. urundeuva* (black columns) and *A. colubrina* (gray columns) seeds germinated on different substrates.



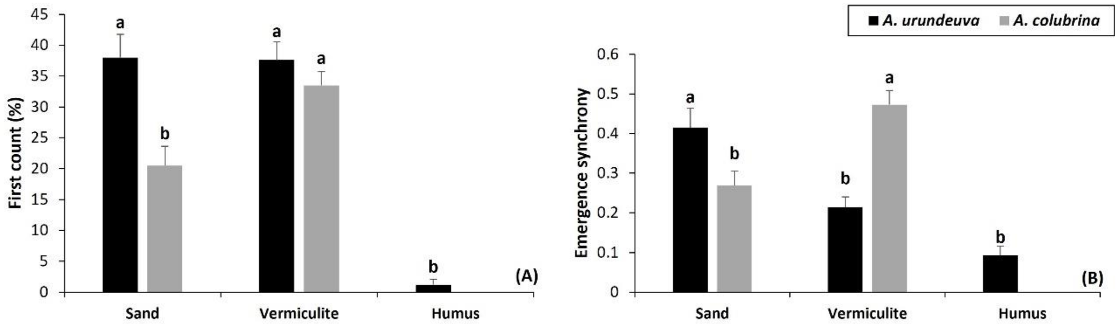
Different letters among the black bars indicate a significant difference (p<0.05), based on Tukey test. Different letters among the gray bars indicate a significant difference (p<0.05), based on T (LSD). Columns indicate the mean and bars indicate the standard error (n=10 for *A. urundeuva* and n=8 for *A. colubrina*).

The use of vermiculite has enhanced germinative responses of tree species, as observed for *Parapiptadenia* rigida and *Anadenanthera peregrina*, by Mondo *et al.* (2008) and Miranda *et al.* (2012), respectively. In fact, the positive of vermiculite on characteristics related to the emergence of *A. urundeuva* and *A. colubrina* is in agreement with the results previously obtained by Bandeira *et al.* (2017), Pacheco *et al.* (2006), Oliveira *et al.* (2012), and Dorneles *et al.* (2013). Studies with other species of the Caatinga have demonstrated the efficiency of vermiculite in seed emergence, as for example, the work by Guedes *et al.* (2010) with *Amburana cearensis* and the work of Martins *et al.* (2012) with *Tabebuia chrysotricha*.

For the first emergency count, it was observed that the vermiculite substrate was superior to the humus substrate for *A. urundeuva* and the sand for *A. colubrina* (Figure 2 A). According to Carvalho and Nakagawa (2012), the first count evaluation can be used as a vigor test, since the germination speed is reduced over time, as the seeds deteriorate. Thus, the highest results in the first count evaluation obtained in this experiment for vermiculite may indicate that this substrate favored seed germination.

For the emergency synchronization index, the sand substrate was superior to vermiculite and humus for *A. urundeuva*, whereas for *A. colubrina* the vermiculite substrate showed greater synchronization when compared to sand (Figure 2 B). This can also be observed in the relative emergency frequency, where germination of *A. urundeuva* and *A. colubrina* occurred in a shorter period of time in sand and vermiculite substrates, respectively (Figure 3 A, B, and C).

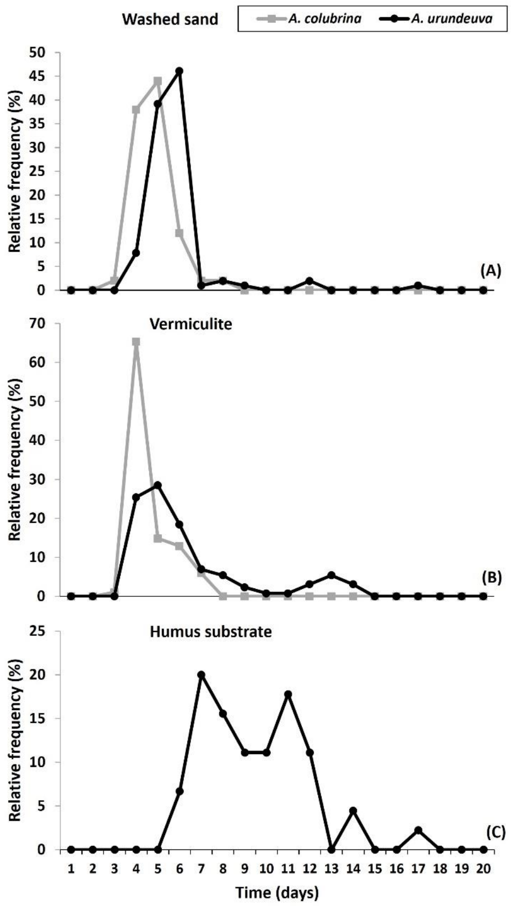
Figure 2. First count (A) and germination synchronization of A. urundeuva (black columns) and A. colubrina (gray columns) seeds germinated on different substrates.



Different letters among the black bars indicate a significant difference (p<0.05), based on Tukey test. Different letters among the gray bars indicate a significant difference (p<0.05), based on T (LSD). Columns indicate the mean and bars indicate the standard error (n=10 for *A. urundeuva* and n=8 for *A. colubrina*).

The strategy of increasing synchronization and seeking a unimodal distribution of relative emergency frequency is directly related to the successful production of forest species seedlings in nurseries (ROSSATTO; KOLB, 2010). However, in a natural environment, less synchronization and a multimodal relative frequency distribution can be an evolutionary strategy to increase the chances of at least some seeds germinating in favorable conditions for seedling development (FERREIRA; BORGHETTI, 2004).

Figure 3. Relative emergence frequency (%) of *A. urundeuva* and *A. colubrina* seeds germinated on different substrates.



Similar responses were found for the initial development of *A. urundeuva* and *A. colubrina*. Seedlings of *A. urundeuva* grown in vermiculite showed higher results of height (2.38 cm), length of main root (4.08 cm), number of leaves (2.85), and shoot fresh mass (0.043 g) in comparison to washed sand and humus substrate (Table 1). The other characteristics did not show statistical differences.

Table 1. Height (cm), length of main root (cm), number of leaves, root dry mass (g), shoot dry mass (g) and total dry mass of *A. urundeuva* seedlings after 30 days growing in different substrates.

Treatments	Height	Length of main root	Number of leaves	Dry root mass	Shoot dry mass	Total dry mass
Washed sand	1.94±0.4b	2.71±1.0b	2.25±0.4b	0.04±0.002a	0.011±0.01a	0.053±0.01a
Humus substrate	1.76±0.4b	1.64±1.1c	2.60±0.5ab	0.02±0.003a	0.007±0.005a	0.03±0.007a
Vermiculite	2.38±0.3a	4.08±0.9a	2.85±0.4a	0.04±0.005a	0.011±0.008a	0.053±0.01a
CV (%)	20.3	36.5	18.6	39.8	22.0	22.2

* Different letters in the same column indicate a significant difference ($p<0.05$), based on Tukey test. Data are expressed as mean ± standard deviation (n=20).

For *A. colubrina*, seedlings grown in vermiculite showed higher values of number of leaves (1.8) and shoot dry mass (0,070g) in comparison to the washed sand (Table 2). The other characteristics did not show statistical differences.

Table 2. Height (cm), length of main root (cm), number of leaves, root dry mass (g), shoot dry mass (g) and total dry mass of *A. colubrina* seedlings after 30 days growing in different substrates.

Treatments	Height	Length of main root	Number of leaves	Dry root mass	Shoot dry mass	Total dry mass
Washed sand	2.7±0.8a	6.7±1.4a	1.5±0.5b	0.029±0.01a	0.055±0.02b	0.085±0.04a
Vermiculite	3.0±0.4a	6.4±0.9a	1.8±0.3a	0.034±0.01a	0.070±0.07a	0.105±0.03a
CV (%)	24.4	18.6	26.1	53.3	38.6	41.0

* Different letters in the same column indicate a significant difference ($p<0.05$), based on T test (LSD). Data are expressed as mean ± standard deviation (n=20).

The high-water retention capacity and adequate aeration conditions of vermiculite, combined with its physical properties, such as thickness and texture, may have contributed to the increase in the aerial part, number of leaves and root system of *A. urundeuva* and a greater number of leaves and shoot dry mass in *A. colubrina* compared to other substrate (AZERÊDO *et al.,* 2011; ARAÚJO *et al.,* 2016). Also, studies with initial development of *A. urundeuva* showed that seedlings grown in vermiculite had higher height of hypocotyl (PACHECO *et al.*, 2006).

Some studies have pointed out that the use of washed sand increases the responses of tree species during their initial development (SOUZA *et al.*, 2007; LIMA *et al.*, 2011; GUEDES *et al.*, 2011). Thus, washed sand has been widely used for the emergence of forest species (LOPES; PEREIRA, 2005; GUEDES *et al.*, 2011; LIMA *et al.*, 2011); however, the low and uneven content of water hold by this substrate leads to the need of constantly rewatering (FERRAZ; CALVI, 2010). Humus substrate has a higher content of organic material, which helps holding more water, but, this characteristic facilitates the proliferation of microorganisms and reduces the aeration, which negatively affects the emergence (RODRIGUES *et al.*, 2007; FAGUNDES *et al.* 2011).

In this context, vermiculite has been an alternative to the initial cultivation of forest species. This substrate has a great capacity of aeration and water-holding, fundamental characteristics for the seed emergence, avoiding daily rewatering (LIMA *et al.*, 2011; OLIVEIRA *et al.*, 2012; MIRANDA *et al.*, 2012). Besides that, vermiculite is an easy to handle and inert substrate, which hinders the proliferation of pathogens (PACHECO *et al.*, 2006; UGART *et al.*, 2008; MARTINS *et al.*, 2011).

Conclusion

The use of vermiculite favored the emergence and initial development of *A. urundeuva* and *A. colubrina*, in comparison to the other treatments. Future studies including image analysis through computer programs of their seeds and seedlings, as well as evaluating the initial growth for periods longer than 30 days on different substrates are essential to improve the methods of large-scale seedling production of these species.

Acknowledgements

The authors would like to thank to the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for the financial support through the scholarships. We also would like to thank the technician João dos Santos for his contributions during the experimental period.

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