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Emergence and Initial Development of *Erythroxylum pauferrense* Seedlings under Different Substrates

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HIGHLIGHTS

- The substrates provided different responses for seedling emergence.
- The use of sand and commercial substrate is not indicated for the emergence.
- Vermiculite increases the emergence rates of *E. pauferrense*.

Abstract: *Erythroxylum pauferrense* Plowman is an understory species native to Brazil, with great importance in the conservation of genetic resources and is used as a food source for animals in the regions where the species occurs. The species is currently listed in category EN (endangered). The objective of this work was to evaluate the effect of different substrates on the emergence and initial growth of *E. pauferrense*. The experiment was carried out in a protected environment, belonging to the Plant Ecology Laboratory of the Federal University of Paraíba, Areia-PB, Brazil. The substrates used were: sand; sand + vermiculite in the proportion of 1:1, 3:1 and 1:3; vegetal land; vegetal land + sand in the proportion of 1:1, 3:1 and 1:3; vegetal land + vermiculite in the proportion of 1:1, 3:1 and 1:3; vermiculite; Bioplant®; and Bioplant® + sand in a ratio of 1:1, 3:1 and 1:3. The experimental design was completely randomized, with 16 treatments (substrates) and four replications of 25 seeds. The percentage of emergence, first count, speed index, mean time, mean speed and relative emergency frequency were evaluated. The vermiculite substrate provided the best results for carrying out the emergence and vigor tests of *E. pauferrense* seedlings, being the most suitable substrate to initial growth seedlings of the species.

Keywords: forest species; Erythroxylaceae; germination; growth; vigor.

INTRODUCTION

The genus *Erythroxylum* comprises approximately 230 species, which has a wide geographic distribution [1]. In Brazil, there are about 120 species of *Erythroxylum* registered, with the highest occurrence in forest regions of the Atlantic Forest biome. It is known for having several pharmacological properties, through the presence of secondary metabolites in its composition (Barbosa et al., 2014) [2]. Among the species that make up this genus, *Erythroxylum pauferrense* Plowman is characterized as an endemic understory species that has great ecological importance for the preservation of genetic resources in environments known as 'Brejos de Altitude' [3]. It is an endemic species from the Northeast region of Brazil with distribution only in the state of Paraíba. According to the Red List of Flora of Brazil, the species is listed as "EN - in danger of extinction" [4].

Given the importance of *E. pauferrense* and the expansion of forest restoration in forest fragments degraded by antropic activity, there is an increase in the demand to produce seedlings. Give this, the propagation of forest species occurs mainly through seeds, which highlights the importance of studies on factors that affect the physiological quality of seeds, such as germination and vigor [5].

To have success in the production of seedlings, it is necessary to obtain seeds with good quality [6]. To assess the quality of seeds, the germination test is indicated, however there are still few studies to elucidate the quality of seeds of forest species [6].

Environmental stimuli cause seed germination, with a series of reactions that precede the disruption of the seed coat [7]. There are essential factors for carrying out the germination test, such as temperature and substrate [8]. Thus, the conditions imposed during the seed germination phase can be manipulated to obtain greater uniformity and germination speed, which results in more vigorous seedlings [5].

The substrate helps to supply water to the seeds, maintaining a good ratio between water availability and aeration [9]. For the choice of substrate, some parameters such as seed size, its need for light and water and the characteristics of the species must be considered during the installation, counting and evaluation of the seedlings [9,10]. In addition, some desirable aspects of the substrate are root growth, good absorption and retention of water and nutrients, adequate cation exchange capacity, and low cost [7, 11].

The variation in the responses of forest seeds to the use of different substrates indicates the need for studies to better adjust the conditions established for seedling germination and development [12]. Different substrates for *Tachigali vulgaris*, found that the best substrate was vermiculite, promoting good germination performance and the development of normal seedlings [9]. In another study, the use of sand as a substrate for carrying out germination tests in *Myracrodruon urundeuva* is recommended [5]. Thus, we formulate the hypothesis that the different substrates tested promote distinct responses in germination and initial development of *E. pauferrense*. The objective of this work was to indicate ideal substrates for germination and seed vigor tests, and to determine the most suitable substrate to produce *E. pauferrense* seedlings.

MATERIAL AND METHODS

The experiment was carried out in a greenhouse belonging to the Plant Ecology Laboratory of the Department of Phytotechnics and Environmental Sciences, Center for Agricultural Sciences, Federal University of Paraíba, municipality of Areia, Paraíba, Brazil (6°57'57.16"S, 35°42'54.13"O). The region has a tropical climate (hot and humid), classified as As [13]. During the survey, the air temperature and the relative humidity were 27.2 °C and 55.1%, respectively. The measurements were performed with the aid of a portable digital thermo-hygrometer (Minipa, model MT-241A).

E. pauferrense seeds were collected from 20 adult matrices in the Mata do Pau-Ferro State Park, municipality of Areia, Paraíba, Brazil. For processing, the pulp was manually removed from the fruits and the seeds were kept in the shade for a period of 24 hours, for natural drying. For disinfestation, the seeds were subjected to a 2% sodium hypochlorite solution for five minutes.

Sowing took place in plastic trays perforated at the bottom, with dimensions of 8 x 48 x 30 cm in depth, length, and width, respectively. Seeding was carried out on the following substrates (treatments): sand (T_1); sand + vermiculite in the proportion of 1:1 (T_2), 3:1 (T_3) and 1:3 (T_4); vegetal land (T_5); vegetal land + sand in the proportion of 1:1 (T_6), 3:1 (T_7) and 1:3 (T_8); vegetal land + vermiculite in the proportion of 1:1 (T_9), 3:1 (T_{10}) and 1:3 (T_{11}); vermiculite (T_{12}); Bioplant® (T_{13}); and Bioplant® + sand in a ratio of 1:1 (T_{14}), 3:1 (T_{15}) and 1:3 (T_{16}). The substrates were irrigated daily, keeping up to 60% of their retention capacity [9].

To determine the emergence, the number of seedlings emerged daily from the 12th to the 23rd day after sowing was observed, considering the emergence of cotyledons as an evaluation criterion, with the results expressed in percentage. The first emergence count (FEC) corresponded to the percentage of normal seedlings emerged [9], with values recorded on the 12th day after sowing. The emergence speed index (ESI) was determined by the daily count of normal seedlings emerged during the 12-day period (from the 12th to the 23rd day after sowing) [14]. The mean emergency time (MT), the mean emergency speed and the relative emergency frequency were determined from the daily count of the number of seedlings emerged [15].

The length and dry mass of the seedlings were measured at the end of emergence (23 days after sowing), and the cotyledons were removed, and the shoot and primary root of normal seedlings were measured with a graduated ruler in centimeters, with results expressed in cm $seedlings^{-1}$. Then, the seedlings were dried in an oven with forced air circulation at 65 °C until they reached constant weight and the dry mass of the aerial part and root were determined with the aid of an analytical balance (0.0001). The results were expressed in g mol^{-1}.

The experimental design was completely randomized, with 16 treatments (substrates) and four replications of 25 seeds. Data were subjected to analysis of variance (F test) and means were grouped by Scott-Knott test at 5% probability. Statistical analyzes were performed using the software R® *v.4.0.0* [16].

RESULTS

According to the results presented in Table 1, there were significant differences for percentage of seedling emergence, count of first emergence and emergence speed index between the combinations of substrates used.

Treatments T_9 (vegetal land + 1:1 vermiculite) and T_{12} (vermiculite) provided the highest percentage of emergence (Table 1). The lowest emergence percentages in treatments T_1 (sand), T_2, T_3 (sand + vermiculite 1:1 and 3:1, respectively), T_{10} (vegetal land + vermiculite 3: 1) and T_{13} (Bioplant®) (Table 1).

Regarding the first emergency count (FEC), the highest values were recorded in treatments T_{12} (vermiculite) and T_4 (sand + vermiculite 1:3), with 47% and 38%, respectively, while the lowest percentage of emergence in the first counts were observed in treatments T_1 (sand); T_2 and T_3 (sand + vermiculite, 1:1 and 3:1); T_5 (vegetal land); T_6 and T_7 (vegetal land + sand, 1:1 and 3:1); T_{10} (vegetal land + vermiculite 3:1); T_{13} (Bioplant®); T_{14}, T_{15} and T_{16} (Bioplant® + sand, 1:1, 3:1 and 1:3) (Table 1).

Regarding the emergence speed index (EST), results were obtained similar to FEC, in which seeds submitted to treatment T_{12} (vermiculite) presented the highest values, while the lowest values were obtained in treatments T_1 (sand); T_2 and T_3 (sand + vermiculite, 1:1 and 3:1); T_5 (vegetal land); T_6 and T_7 (vegetal land + sand, 1:1 and 3:1); T_{10} (vegetal land + vermiculite 3:1); T_{13} (Bioplant®); T_{14}, T_{15} and T_{16} (Bioplant® + sand, 1:1, 3:1 and 1:3) (Table 1).

Table 1. Emergence, first count (FEC) and emergence speed index (ESI) in *Erythroxylum pauferrense* seedlings under different substrates.

Treatmens	Emergency (%)	FEC (%)	ESI
T_1	23 d	5 c	0.976 d
T_2	24 d	7 c	1.229 d
T_3	20 d	4 c	0.832 d
T_4	65 b	38 a	5.781 b
T_5	45 c	2 c	1.497 d
T_6	43 c	2 c	1.444 d
T_7	49 c	6 c	1.974 d
T_8	70 b	17 b	3.514 c
T_9	80 a	14 b	3.736 c
T_{10}	30 d	2 c	0.992 d
T_{11}	68 b	18 b	4.267 c
T_{12}	86 a	47 a	7.912 a
T_{13}	20 d	4 c	1.508 d
T_{14}	67 b	8 c	2.556 d
T_{15}	39 c	4 c	1.396 d
T_{16}	65 b	5 c	2.520 d
CV (%)	4.69	6.55	4.97

In Table 2, it is observed that the seedlings obtained in treatments T_7, T_8 (vegetal land + sand, 3:1 and 1:3) and T_{12} (vermiculite) had greater root length. The greatest length of the aerial part of the seedlings was registered in treatments T_2 and T_4 (sand + vermiculite, 1:1 and 1:3); T_6, T_7 and T_8 (vegetal land + sand, 1:1, 3:1 and 1:3); T_9, T_{10} and T_{11} (vegetal land + vermiculite, 1:1, 3:1 and 1:3); T_{12} (vermiculite); T_{14}, T_{15} and T_{16} (Bioplant® + sand, 1:1, 3:1 and 1:3). On the other hand, the shortest aerial part length was obtained in the seedlings of the other treatments tested (Table 2).

The dry mass of the roots of the seedlings presented similar results to the length of the roots, in which the highest values were verified in the treatments T_7 (vegetal land + sand 3:1) and T_{12} (vermiculite), with 0.0030g and 0.0031g, respectively (Table 2). Regarding the dry mass of the aerial part of the plants, it was observed that the treatments T_2 (sand + vermiculite 1:1) and T_{12} (vermiculite) provided the highest dry matter contents, with 0.0098 g and 0.0109 g, respectively (Table 2).

Table 2. Length and dry mass of root and shoot of *Erythroxylum pauferrense* seedlings under different substrates.

Treatments	-------Length (cm)-------		-------Dry mass (g)-------	
	Root	Shoot	Root	Shoot
T_1	0.9050 c	1.9025 b	0.0011 c	0.0064 c
T_2	1.0975 c	4.6125 a	0.0009 c	0.0098 a
T_3	0.4500 c	1.1000 b	0.0015 c	0.0050 c
T_4	1.5875 b	4.2725 a	0.0012 c	0.0081 b
T_5	0.8924 c	1.8025 b	0.0014 c	0.0061 c
T_6	1.3600 c	3.1925 a	0.0013 c	0.0066 c
T_7	2.9575 a	4.1800 a	0.0030 a	0.0068 c
T_8	2.7775 a	4.5850 a	0.0021 b	0.0084 b
T_9	1.7200 b	4.3975 a	0.0011 c	0.0077 b
T_{10}	0.8650 c	2.7925 a	0.0009 c	0.0058 c
T_{11}	1.6550 b	4.4175 a	0.0011 c	0.0085 b
T_{12}	2.6275 a	5.0550 a	0.0031 a	0.0109 a
T_{13}	0.7125 c	1.9125 b	0.0021 b	0.0040 c
T_{14}	2.0125 b	4.2500 a	0.0012 c	0.0070 c
T_{15}	1.1075 c	3.9700 a	0.0009 c	0.0061 c
T_{16}	1.7525 b	3.7200 a	0.0011 c	0.0060 c
CV (%)	4.53	4.15	9.22	6.84

According to the data found in Figure 1, it can be observed that the T_1 treatment (sand) presented a relative emergency frequency above 40% on the seventh day after sowing, showing uniformity in seedling emergence. For treatment T_2 (sand + vermiculite, 1:1) the relative frequency was greater than 30% on the sixth day (Figure 1). On the other hand, for treatment T_{12} (vermiculite) the relative frequency of emergence was greater than 20% on the fifth day after sowing, decreasing later. However, this treatment provided longer mean emergence time and mean emergence speed, with 4.082 and 0.208, respectively (Figure 1).

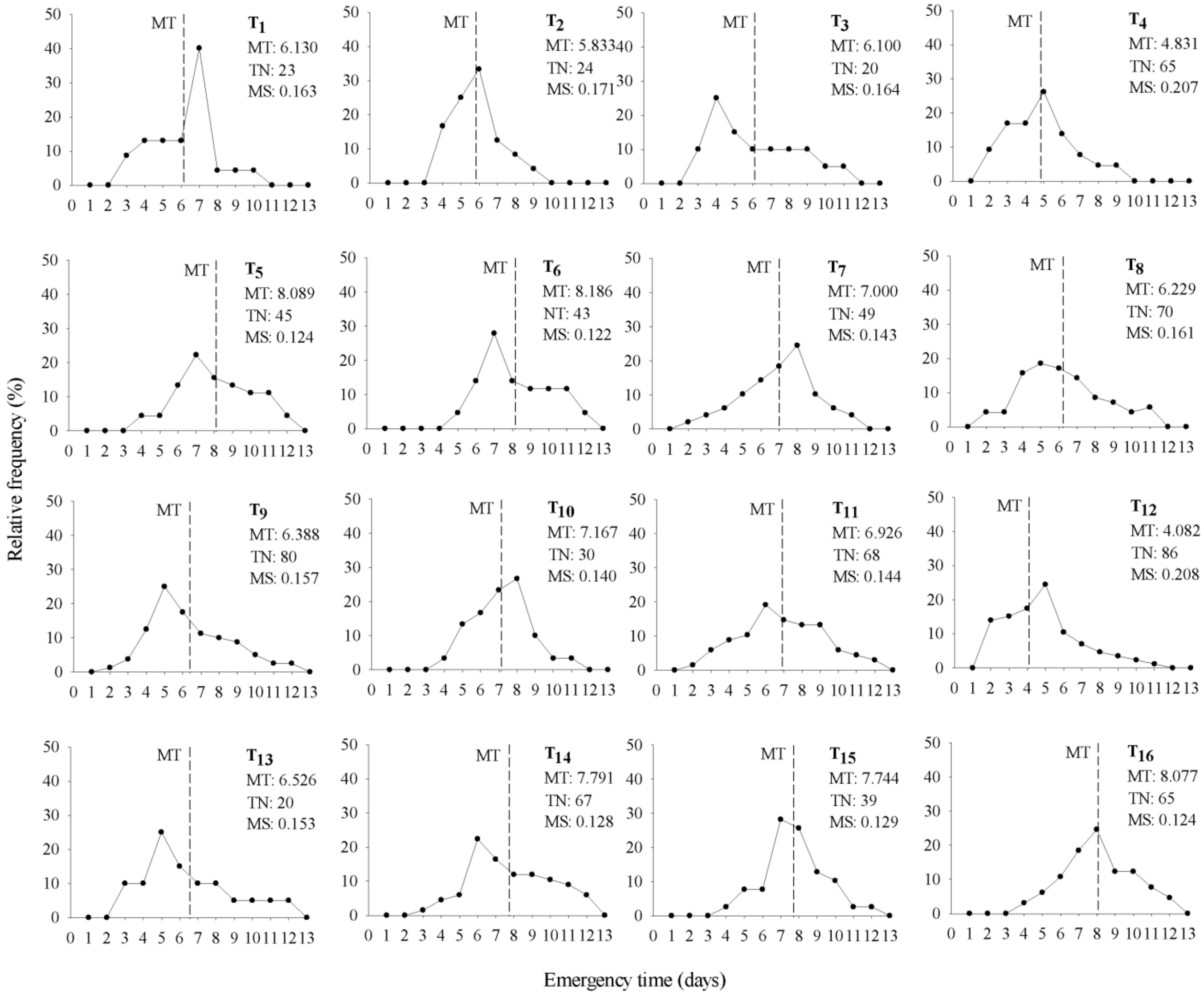

Figure 1. Distribution of the relative frequency of emergence of Erythroxylum pauferrense seedlings under different substrates. MT: mean emergency time; TN: total number of emerged seeds; MS: mean emergency speed.

DISCUSSION

The results obtained for T_9 and T_{12} indicate that these substrates provide good moisture conditions for the emergence of seedlings, since, to activate the emergence metabolism, it is necessary that the seed water content increase, therefore, the water absorption it is directly related to the emergence of the radicle [17].

Substrates that have sand in their composition reduced the percentage of emergence, which may have occurred due to the easy drainage of water in these substrates, causing the substrate surface to dry out and, therefore, be harmful to seed germination. Distinct results were found in their study with *Peltophorum dubium*, showing that substrates with larger particles have a lower degree of compaction and, therefore, favor the emergence of seedlings [18]. However, the results found with seeds of *Vochysia tucanorum* and *Archontophoenix cunninghamiana* agree with the present study, indicating the efficiency of vermiculite as a substrate for the emergence of seeds of forest species [19,20].

T_{12} (vermiculite) provided the expression of the maximum physiological potential of the seeds. This substrate has physicochemical characteristics that favor the emergence and development of seedlings, such as aeration, water retention and seed distribution. The efficiency of this substrate in the germination uniformity was also observed in other studies with seeds of *Hibiscus sabdariffa* [21] and *Amburana cearensis* [22]. However, the treatments with the lowest PCE values may have been characterized by low substrate moisture, making seed swelling difficult [17]. These results indicate that the most suitable substrate is the one that promotes a faster and more uniform emergence [23].

The higher emergence speed obtained may have occurred due to the better uniformity of substrate moisture in carrying out the tests [5]. In addition to this factor, the low density of vermiculite can favor ESI and FEC, as substrates with high density limit seedling germination and development [7]. Distinct results were found, showing that the best ESI for *Peltophorum dubium* was obtained with washed sand [18].

The highest values of root and shoot length obtained reflect the vigor of the seeds in the best substrates, moreover, seedlings that group characteristics that involve greater root growth and greater shoot growth have better emergence, in addition to reaching a more effective adaptive capacity to field conditions [11].

The substrates that provided the highest root and shoot dry mass content of the seedlings were considered sufficiently porous, with efficiency for the occurrence of gas exchange, so that it did not limit root respiration either [24]. In addition to the greater capacity for aeration and water retention, a more adequate degradation of seed reserves may have favored seedling growth, being totally dependent on the chemical composition of the seeds [25].

The higher relative frequency of seedling emergence favored by substrates T_1, T_2 and T_{12} is a desirable characteristic, as in addition to promoting uniformity of emergence, it causes the formation of a good stand [24]. The results obtained in this study are different from those found in *Copaifera langsdorffi* seeds, which confirmed that germination and emergence were favored with the commercial substrate Bioplant® [30].

CONCLUSION

Pure commercial substrates sand and Bioplant®, as well as the sand + vermiculite mixture in the proportions 1:1 and 3:1, and vegetal land + vermiculite in the proportion 3:1 are not recommended for carrying out emergency tests with *E. pauferrense* seeds under the tested conditions;

The vermiculite substrate is the most efficient in promoting the emergence of *E. pauferrense* seeds under the conditions tested, being the most suitable to initial growth seedlings.

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Conflicts of Interest: The authors declare no conflict of interest.

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