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Physiological Potential Evaluation of *Myracrodruon urundeuva* Stored Diaspores

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Abstract

The conservation of seeds in an appropriate location combined with the utilization of rapid tests for monitoring the physiological aspects is essential for quality seedlings. Thus, the study aimed to evaluate the behavior of *Myracrodruon urundeuva* diaspore under different periods and storage environments, and verify the testing of individual exudate pH in the physiological monitoring. The experimental design was completely randomized, factorial 2 × 8 (8 periods of storage and 2 storage environments) with four replications of 25 diaspores, using the Tukey test at 5% probability for average comparisons. After analysis of variance, it was performed regression analysis for the quantitative factor and simple Pearson correlation test. The variables analyzed were moisture content, seedling emergence (E) emergence velocity index (IVE), mean emergence time (TME) and the pH of the individual exudate. The results of E, IVE, TME tests indicated that the *Myracrodruon urundeuva* diaspores could be stored for ten months in room fridge, with low vigor loss. The pH test individual exudate showed lower estimates of simple Pearson correlation with the emergence test, not monitoring the physiological potential of *Myracrodruon urundeuva* diaspores.

Keywords: storage, *ex situ* conservation, exudate pH

1. Introduction

Myracrodruon urundeuva Allemão, belonging to the family Anacardiaceae, is popularly known as aroeira, aroeira-do-sertão, aroeira-do-campo, aroeira-da-serra, aroeira-preta, urindeúva, arendiúva and urundeúva (Lorenzi, 2008). It is an arboreal species occurring in the Northeast, Central West and Southeast (Minas Gerais and São Paulo) of Brazil (Silva-Luz & Pirani, 2014). Due to the difficult extraction of the seeds, the fruit is sown, and there is no impediment to germination (Pereira, 2011).

This species has an important socioeconomic value because it presents logging and medicinal potential, as in treatments of hemorrhages, respiratory and urinary infections and disorders in the digestive system, with anti-inflammatory and cicatrizing effects (Silva-Luz & Pirani, 2014).

According to Pereira (2011) *Myracrodruon urundeuva* shows rapid growth when produced in nursery and has high resistance in the establishment stage in the field, being indicated for reforestation. For the regeneration of forests and maintenance of plant diversity, germination is considered a vital ecophysiological process and knowledge about this aspect is essential for the recovery of degraded areas to occur satisfactorily (Garcia & Diniz, 2003; Araújo, 2007). The knowledge of the physiological potential of the seeds before sowing is the means of avoiding damages due to the low germination or the uneven germination (Luiz, 1999).

The importance of storing seeds and/or diaspores as germplasm, ensures the conservation of species of great importance, since it preserves the physiological quality and maintains the genetic variability of endangered species, which serves as a subsidy for programs to reintroduce extinct populations (Degan, 2001). During storage, the seeds and/or diaspores undergo deterioration, independent of the species, whose speed is variable, since they depend on the environmental conditions and the characteristics of the seeds themselves (Vieira, 2002).

According to Marcos Filho and Kikuti (2006) the vigor assessment is necessary to estimate seed performance potential when environmental conditions deviate from the most appropriate ones. For this it is necessary the aid

of tests that aim to detect the qualitative performance of the forest seeds. To determine the viability of the seeds in a quick and practical way, the pH test of the exudate can be used. This test is based on the chemical reactions that occur in the deterioration process and may determine the reduction of seed viability (Piña-Rodrigues, 2004).

Normally, the low quality of the seeds is related to the disintegration of the membrane system, and during imbibition and consequently reorganization of the membrane system, there is release of the cytoplasmic content and exudation of organic acids, sugars, ions, among others. The release of H^+ helps to acidify the medium, and since the low pH has negative effects on the enzymatic activity, the germination of seeds ends up being impaired. Thus, the test detects pH differences in viable and non-viable seed exudates through the use of indicator solutions, such as phenolphthalein and sodium carbonate (Marcos Filho, 1987).

The knowledge of basic technological methods to be used to estimate the viability of seeds of forest species helps seed technologists and seedlings producers to better understand the processes involved in seed germination. In view of the above, the relevance of information on the seeds of forest species in relation to the physiological aspects. In this sense, the present study had as objectives to evaluate the behavior of diaspores of *Myracrodruon urundeuva* under different periods and storage environments.

2. Material and Methods

The research was conducted during the period from June 2012 to October 2013, in Serra Talhada, Pernambuco. The municipality is located in the semi-arid region of the microregion of the Pajeú Valley, located between the geographic coordinates, 07°59′S and 38°50′W and average altitude of 431 m, with climate of type Bwh according to the classification of Koppen, called semiarid, hot and dry, with summer rains (Melo, 2008).

The diaspores of Myracrodruon urundeuva (Lot 570) were purchased from the Petrolina-PE Degraded Areas Recovery Center (CRAD) in November 2011. From the collection period to the experiment setup, the diaspores were kept in plastic bags at a temperature of 7 °C.

After the acquisition of the diaspores, they went through the process of beneficiation and manual selection. Subsequently they were packed in previously identified Kraft paper packages, containing samples necessary to carry out the tests during the experiment. It was stored in natural and refrigerator conditions, whose temperature and relative humidity were monitored with INCOTERM® gital thermohygrometer.

The experiment was evaluated bimonthly for 14 months (0, 2, 4, 6, 8, 10, 12 and 14 months), being analyzed the variables: water content, seedling emergence (E), emergency speed index IVE), mean time of emergency (SMT), and the rapid exudate pH test.

2.1 Water Content

The water content was determined by the greenhouse method at 105±3 °C for 24 hours (Brasil, 2009), using two samples with 25 diaspores, placed in metal containers with a lid (3.5 cm in diameter). The results were expressed as percentage in the wet basis.

2.2 Seedling Emergency Test (E)

The emergency test was installed in a nursery (50% light interception), with four replicates of 25 diaspores sown in trays of expanded polystyrene with 200 cells (16 cm^3) containing commercial coconut fiber substrate, according to recommendations of Pacheco (2006). Daily irrigations were performed to maintain substrate moisture (60%). The counts made daily from the day of sowing (fith day) until the 25th day, had as emergency criteria the appearance of the first pair of true leaves, being the results expressed in percentage (Brasil, 2009).

2.3 Emergency Speed Index (IVE)

The emergency speed index was performed simultaneously to the emergency test. The seedlings were evaluated daily, at the same time, from the day the first seedling emerged, continuing until the 25th day. For the calculation of IVE, we used the Equation 1 proposed by Maguire (1962):

$$IVE = \frac{E_1 + E_2 + \cdots + E_n}{N_1 + N_2 + \cdots + N_n} \quad (1)$$

Where, IVE = Emergency speed index; E = number of seedlings emerged in each repetition daily; N = number of days elapsed after sowing.

2.4 Average Time of Emergency (TME)

The estimate of the average time of emergency was obtained by daily counting the emerged seedlings by the 25th day after sowing, as proposed by Labouriau (1983), the Equation 2:

$$t = \frac{\sum n_i.t_i}{\sum n_i} \quad (2)$$

Where, t = time interval; n_i = number of seedlings emerged on day i (i = 1, 2, ... 25); t_i = days after the onset of the emergency.

2.5 PH Test of the Individual Exudate

In the execution of the pH test of the individual exudate two indicator solutions were used: (1) phenolphthalein solution, composed of 1 g of phenolphthalein dissolved in 100 ml of absolute alcohol, plus the addition of 100 ml of distilled and boiled water; (2) solution of sodium carbonate, composed of 8.5 g L^{-1} of the solute dissolved in distilled water and boiled. The concentrations of the indicator solutions were based on the analyzes performed by Matos (2009).

The diaspores of *Myracrodruon urundeuva* were placed to be soaked in individualized disposable cups (50 mL), containing 10 mL of boiled distilled water, at 25 °C for the soaking times of 30; 60 and 90 minutes, with four replicates of 25 diaspores, the time being 30 minutes based on the study by Santana (1998). After these periods a drop of each indicator solution was added to the set, homogenizing with the aid of disposable plastic rods. The reading was carried out immediately upon the contact of the indicator solutions with the soaking solution.

The interpretation of the results was based on the staining of the imbibition solutions. The imbibition solutions that remained in pink purple indicated *viable seeds* (basic medium), while those that remained light pink and colorless indicated *non-viable seeds* (acid medium), the results being expressed as a percentage (Santana, 1998).

2.6 Statistical Analysis

The experimental design was completely randomized in an 8 × 2 factorial scheme, consisting of the combination of eight storage periods (0, 2, 4, 6, 8, 10, 12 and 14 months) and two storage environments (natural and refrigerated), for the variables PE, IVE and TME.

For the pH of the individual exudate, the factorial scheme was 8 × 3 × 2, ie eight storage periods, three soak times (30, 60 and 90 minutes) and two environments. Evaluations were performed using four replicates of 25 diaspores. The data of water content, temperature and humidity of the storage environments were not analyzed statistically.

Data were submitted to analysis of variance, and the means were compared by the Tukey test at 5% probability. Subsequently, the regression analysis was performed for the quantitative factor (storage periods) and Pearson's simple correlation test. Statistical analyzes were performed using the SISVAR 5.3 computer program (Ferreira, 2010).

3. Results and Discussion

The water content of the diaspores decreased in all periods and environments (Table 1), which may be related to the type of packaging in which the diaspores were stored, that is, paper bags. The permeable package allowed the variation of the water content of the diaspores according to the relative humidity of the air, because they were hygroscopic with oscillations in water contents. According to Medeiros and Eira (2006) orthodox seeds can be dehydrated to very low values of water, ranging from 5 to 7%, without loss of viability. Therefore, the diaspores of *Myracrodruon urundeuva* were kept at levels acceptable for storage for 14 months in the two environments studied (Table 1).

Probably, this decrease in the water content of the stored diaspores was influenced by the atmospheric conditions of the storage environments. During storage, the diaspores of *Myracrodruon urundeuva* were exposed to an average air temperature and humidity of 27.28 °C and 38.38% in natural environment and 7.28 °C and 47.28% in refrigerator environment, as the conditions of low humidity are characteristics of this region, possibly storage in more humid places could lead to the degradation of the seeds. The reduction in the water content of the seeds causes the decrease of the metabolic activity, which prolongs the viability of the seeds (Fowler, 2000). This decrease in the respiratory process and enzymatic metabolism leads to less consumption of the seed reserves before they are put to germinate, and these reserves will be translocated to the initial seedling.

Table 1. Water content (%), temperature (°C) and relative humidity (%) of *Myracrodruon urundeuva* diaspores exposed to different periods and storage environments

Storage Periods (Months)	Water content (%)		Temperature (°C)		Relative humidity from the air (%)	
			Storage Environments			
	Natural	Refrigerator	Natural	Refrigerator	Natural	Refrigerator
0	8.0	8.0	25.7	8.2	40.6	45.8
2	7.5	7.8	27.5	7.0	37.3	47.6
4	7.3	7.5	29.5	6.7	35.4	48.5
6	7.2	6.9	28.2	7.6	36.7	48.2
8	6.0	6.3	26.5	8.0	37.7	45.9
10	5.7	6.1	27.2	5.8	43.6	49.9
12	5.7	5.6	25.7	6.9	39.0	46.2
14	5.1	5.3	27.0	8.1	37.2	46.3

There was interaction between the environments and storage periods ($P < 0.05$) for seedling emergence variables (E), emergency velocity index (IRS) and mean time of emergence (EMS), with the means adjusted to the equation of first degree in natural environment and second degree equation in refrigerator environment for E and of third degree for IVE and TME (Table 2 and Figure 1).

The emergence of seedlings (E) comparing natural storage and refrigerator conditions presented a significant difference in the 8th; 10 and 12 months of storage (Table 2). In the storage periods, the diaspores conditioned in natural environment had a reduction of the emergence of the seedlings from the 6th month of storage, indicating reduction of vigor. While the diaspores kept in refrigerators, they maintained the emergency potential for 12 months, which reached a maximum emergency value of 75% in the 10th month of storage (Table 2). The behavior for this variable was represented by quadratic equation for the refrigerator environment and linear for the natural environment (Figure 1A). Possibly these alternations in the results of E may be related to the water content of each storage period, and to certain characteristics of the species itself, such as the oil content present in the seed. According to Guedes (2012) a moderate increase in temperature due to the respiratory process, may destabilize the lipids and increase the rate of deterioration, reducing vigor.

Table 2. Emergency-(E%); emergency speed index-IVE, mean emergency time-TME of *Myracrodruon urundeuva* diaspores exposed to different periods and environments (natural-N and fridge-G) of storage

Storage Periods (Months)	E (%)		IVE		TME (days)	
	N	G	N	G	N	G
0	46 aA	46 aB	1.37 aA	1.37 aB	9 aC	9 aC
2	47 aA	46 aB	0.91 aAB	1.23 aBC	15 aAB	12 bAB
4	48 aA	47 aB	0.99 aAB	1.11 aBC	15 aAB	13 bA
6	40 aA	51 aAB	0.85 aAB	1.21 aBC	13 aB	14 aA
8	33 bA	64 aAB	1.29 aAB	1.74 aB	8 bC	13 aA
10	30 bA	75 aA	0.69 bAB	3.24 aA	13 aB	6 bD
12	28 bA	49 aAB	0.65 bAB	1.82 aB	13 aB	10 bBC
14	26 aA	14 aC	0.42 aB	0.41 aC	16 aA	10 bBC
CV (%)	---------- 27.97 ----------		---------- 32.30 ----------		---------- 10.49 ----------	

Note. * Means followed by the same letters, uppercase in the column and lowercase in the lines and, do not differ among themselves by the Tukey test at 5% probability.

Regarding the rate of emergence (IVE), it was observed that the diaspores conserved in the refrigerator for the period of 10 and 12 months had an average higher than the diaspores conserved in the natural environment, differing statistically from the other periods of storage (Table 2). The behavior for this variable was represented by cubic equation for both storage environments (Figure 1B). In the wild, IVE did not differ statistically from the control (0 months) between periods in which the diaspores were stored (Figure 1B). When comparing the environments, IVE differed in the 10th and 12th month of storage.

For the TME, the diaspores presented in a refrigerator environment showed a six-day interval between the emergence of the last seedlings and the emergence of the first seedling, in the 10th month of storage, differing statistically from the other periods. Regarding the natural environment, the 8th month of storage was the one that presented the shortest emergency time interval of about eight days, similar to non-stored diaspores (Table 2). According to Marcos Filho (2005) the reduction of the growth rate of the seedlings, increase in the permeability of the cytoplasmic membrane, reduction of the activity of some enzymes, greater susceptibility to stress, changes in respiration, alteration in dietary reserves, alteration in color, alteration in the synthesis rate of the organic compounds are some events that can occur with the increase of the period of storage.

In general, refrigerator storage for the *Myracrodruon urundeuva* diaspora maintained viability for 10 months. Souza (2007) reported that the conditions most favorable to the germination of this species occurred when the diaspores were stored in a cold chamber, conserving the germinability and vigor of the seeds for 180 days. According to the results observed by Guedes (2012), storage and refrigeration were important to minimize seedling emergence in this species. The authors observed that in the refrigerator environment there was greater storage of the diaspores when packed in paper packaging for 240 days of storage.

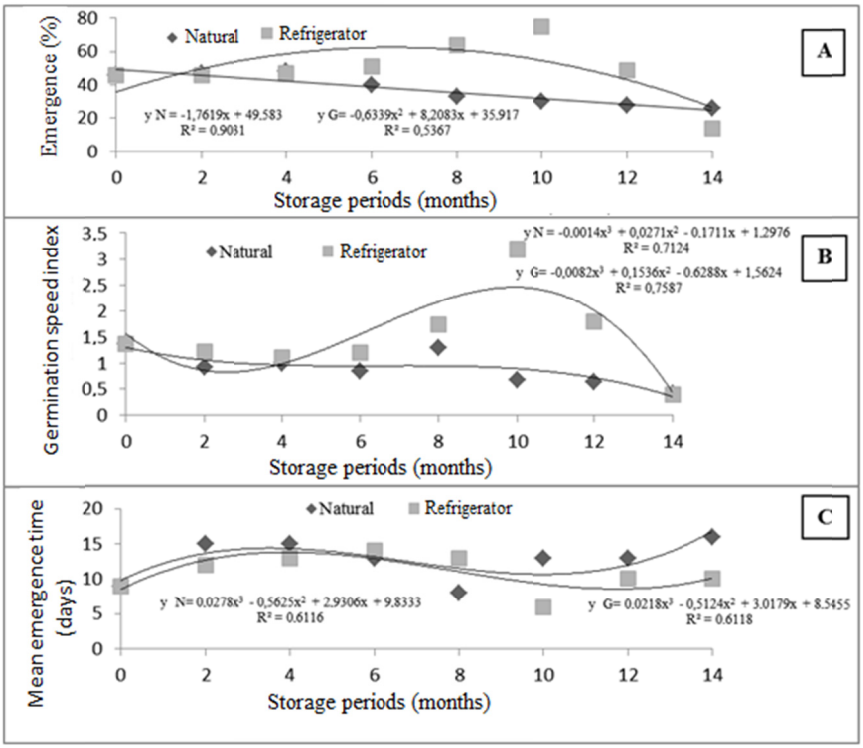

Figure 1. Emergence (A), germination speed index (B), mean emergence time (C) of seedlings from *Myracrodruon urundeuva* diaspores submitted to different periods and environments storage (values differ by the F test)

In the results of the pH test of the exudate by the individual method with diaspores of *Myracrodruon urundeuva*, there was an interaction effect between storage period, imbibition time and environment (Table 3). Regardless of the environment, the pH test results of the exudate remained unchanged when the diaspores were conserved for 2; 4 and 8 months of storage, in all the soaking periods studied. However, there was a significant difference between the storage periods and the periods of imbibition previously mentioned when the conservation environments of the *Myracrodruon urundeuva* diaspores were compared. The diaspores stored for 2 and 10 months and immersed in water for 30 minutes, stored for 6 months and immersed for 60 minutes and stored for 4 to 12 months and immersed for 90 minutes, presented lower averages of viable diaspores in the natural environment, when compared to those stored in a refrigerator environment (Table 3).

For the environment under natural conditions, pH testing of the individual exudate indicated that the 30 minute imbibition time maintained viability for a longer time, with up to 6 months of storage. During 60 and 90 minutes

of imbibition the diaspores showed viability for 4 and 2 months, respectively. These results obtained by the pH test overestimated the percentages of diaspores with physiological percentage of originating normal seedlings in relation to those obtained by the emergency test.

Decreases in viability at different imbibition times were also verified in a refrigerator environment. The diaspores stored for 2 and 6 months with subsequent imbibition for 30 minutes indicated a viability potential of 99 and 85% of diaspores, respectively, but this was not verified by the emergency test which presented for these storage periods 47 and 51% of emergency (Table 2), being those of low and medium physiological potential. At 60 and 90 minutes of imbibition the diaspores were separated at different levels of viability, with the ones with the best physiological potential being the ones that were stored for 2 months (Table 3), differently from the one observed by the emergency test, which evidenced the 10th month of storage of higher physiological potential (Figure 1A).

Table 3. Viability (%) of *Myracrodruon urundeuva* diaspores exposed to different periods and environments (natural and fridge) of storage, obtained by pH test of individual, in three periods of exudate soaking (30; 60 and 90 minutes)

Periods of storage (Months)	Natural environment		
	Soaking periods (Minutes)		
	30	60	90
0	73 abcBα	84 aABα	91 aAα
2	86 aAβ	88 aAα	86 abAα
4	81 abAα	80 aAα	70 bAβ
6	76 abcAα	20 dBβ	21 dBβ
8	62 bcAα	53 bAα	49 cAβ
10	29 dBβ	40 bcABα	46 cAβ
12	29 dBα	23 cdBα	71 bAβ
14	59 cAα	54 bAα	14 dBα
Periods of storage (Months)	Refrigerator Environment		
	Soaking periods (Minutes)		
	30	60	90
0	73 bcBα	84 aABα	91 aAα
2	99 aAα	99 aAα	97 aAα
4	70 bcdAα	81 abAα	84 abAα
6	85 abAα	50 cBα	40 dBα
8	60 cdeAα	62 bcAα	66 bcAα
10	51 deBα	48 cBα	70 bcAα
12	12 fBβ	25 dBα	53 cdAβ
14	44e Aβ	14 dBβ	9 eBα
CV (%)	---------- 14.95 ----------		

Note. * Means followed by the same letters, lowercase in the columns, upper case in the lines, Greek letter between the environments do not differ by Tukey test at 5% probability.

Based on the results obtained by Pearson's simple correlation test (Table 4), it was verified that the pH test of the individual exudate obtained a positive correlation coefficient with the seedling emergence test, highlighting the correlation of 30 and 60 (r = 0.50, er = 0.45, respectively) and 90 minutes of soaking in the refrigerator environment (r = 0.38). However, these coefficients were not of great significance, because they presented a low magnitude and only correlations that present values higher than 0.7 according to Martins and Domingues (2011) are acceptable for association between the variables.

These results are not in agreement with those observed by Carvalho (2002), since these researchers considered the use of the exudate pH test to estimate the viability of *Citromelo swingle* (citromelo) seeds, since it classified and differentiated the lots in 30 and 60 minutes, similar to the seedling emergence and germination tests.

Possibly, the pH test of the individual exudate was not efficient in properly detecting the viability of the diaspores due to oscillations in the water content of the seeds, since the water content decreased during storage (Table 1). According to Amaral and Peske (1984), the quality of the water used and the water content of the seed at the time of the test can affect the pH test results of the exudate, compromising its accuracy and overestimating the results, which was also verified in this study. These findings reinforce Gomes (2013) assertions that the pH test of the individual exudate did not present a linear correlation tendency with the seed germination of Terminalia argentea (captain of the weeds), due to the use of dormant seeds and seeds with absence of embryonic tissues that made it impossible to estimate the viability.

Table 4. Simple Pearson correlation coefficients (r) estimated between tests of emergency (%) and pH tests of individual exudate (%) after 30; 60 and 90 minutes soaking, of Myracroduon urundeuva diaspores subjected to different environments (natural and fridge) storage

Soaking periods (Minutes)	Individual pH	
	Natural	Refrigerator
30	0.50^{*}	0.06^{ns}
60	0.45^{**}	0.25^{ns}
90	0.22^{ns}	0.38^{*}

Note. ** r significant at 1% probability; * r significant at 5% probability; ^{ns}r not significant by the t test.

4. Conclusions

Among the storage conditions studied, the refrigerator environment was the most suitable for the conservation of the diaspores of *Myracroduon urundeuva*, for a period of ten months of storage. The pH test of the individual exudate did not monitor the physiological potential of the diaspores studied during storage.

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