Anais da Academia Brasileira de Ciências (2019) 91(4): e20180734
(Annals of the Brazilian Academy of Sciences)
Printed version ISSN 0001-3765 / Online version ISSN 1678-2690
http://dx.doi.org/10.1590/0001-3765201920180734
www.scielo.br/aabc | www.fb.com/aabcjournal

Exudate - phenolphthalein pH test for evaluation of validity in seeds of *Libidibia ferrea*

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Manuscript received on July 23, 2018; accepted for publication on December 28, 2018

How to cite: SOUTO PC, GONÇALVES EP, VIANA JS, SILVA JCA, FERREIRA DTRG AND RALPH LN. 2019. Exudate - phenolphthalein pH test for evaluation of validity in seeds of *Libidibia ferrea*. An Acad Bras Cienc 91: e20180734. DOI 10.1590/0001-3765201920180734.

Abstract: Seed companies are looking for promising, quick and effective alternatives to determine the physiological quality of seeds. The objective of the current work was to study the efficiency of the exudate - phenolphthalein pH test to evaluate the seeds of two lots of *Libidibia ferrea* (Mart. ex Tul.) L. P. Queiroz var. *ferrea*. The statistical design for the the exudate - phenolphthalein pH test was completely randomized with four replicates of 50 seeds in a factorial design (2 x 5), two seed lots and five soaking periods (30, 60, 90, 120, and 150 minutes), respectively, using two constant temperatures (25 and 30°C). The percentage of viability and germination of the seeds did not differ in the temperatures of 25 and 30°C and in the soaking periods by the exudate - phenolphthalein pH test. Thus, it is recommended that the test be conducted for at least 30 minutes in distilled and deionized water at the constant temperature of 25 or 30°C to evaluate the vigor of the *Libidibia ferrea.*

Key words: extinction, staining, periods of imbibition, quick tests.

INTRODUCTION

Libidibia ferrea (Mart. ex Tul.) L. P. Queiroz var. *ferrea*, formerly *Caesalpinia ferrea* Mart. ex Tul., commonly known as pau-ferro, is a tree species belonging to the Fabaceae family and it is distributed in the North and Northeast of Brazil, standing out in the states of Pernambuco and Ceará. The species has many uses, and can be used in traditional medicine, landscaping, restoration of degraded areas and forage production for feeding animals during periods of drought (Lorenzi 2008, Queiroz 2009).

The use of good quality seeds is considered a determinant factor to success of the forest enterprise, being the germinative capacity the main indicative of vigorous seeds that reflects directly on the quality of the seedlings and the success of a reforestation (Pereira et al. 2015).

The studies on the physiological potential of seeds of native species are indispensable, since they provide information that serves as subsidies for the

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formation of seedlings directed to the commercial plantations, revegetation of areas of extractivism and environmental preservation (Guedes et al. 2011).

Seed technologists have been researching for several years tests that determine a true physiological seed quality. The application of vigor tests on seeds of forest species is a practice that allows to estimate and compare lots for different objectives. A simplicity allied with the good results make them use promising in several fields of research (Santos et al. 2009).

Currently vigor tests that start from the principle of cellular membranes integrity have been gaining special attention because they are fast, reliable, and are in their early stages. Therefore it helps to make decisions so that preventive and corrective measures can be taken to reduce or minimize their effects on the physiological quality of the seed (Ramos et al. 2012).

In this sense, the exudate - phenolphthalein pH test is based on membrane permeability and involves both leaching of metabolites and integument integrity. During the process of seed imbibition there is leaching of sugars, organic acids and H + ions that reduce the pH of the seed exudate leaving it more acidic (Santos et al. 2011).

For the forest seeds species the rapid tests of vigor become of fundamental importance for several advantages, they present low operational cost, homogeneity of the conditions during the test and mainly speed of the results, which makes possible a quick response on the viability of seeds, especially for those that require a long period to complete the germination process, facilitating the decision by seed and seedlings producers.

According to Araldi and Coelho (2015) it is possible to quickly separate lots of seeds of *Araucaria angustifolia* (Bertol.) Kuntze (araucaria) with embryos excised and soaked for 30 minutes in water by the exudate - phenolphthalein pH test. The test also promoted efficacy in determining the viability of soybean (*Glycine max* L.) seed lots (Barros and Marcos Filho 1990), corn (Santana et al. 1998, Cabrera and Peske 2002) and wheat (Amaral and Peske 1984).

For the forest species the methodologies are limited and scarce, due to the great morphological diversity of their seeds. In the Manual of Procedures for Analysis of Forest Seeds (Lima Junior 2011) and in the Rules for Seed Analysis (Brasil 2009) only some forest species have defined methodologies, however, no rules are cited for the analysis of *L. ferrea* seeds.

In view of this, the present work aimed to study the efficiency of the exudate – phenolphthalein pH test to evaluate the seeds of two lots of *L. ferrea*.

MATERIALS AND METHODS

LOCAL OF THE EXPERIMENT

The experiment was conducted at the Laboratory of seed analysis (LAS) of the Federal Rural University of Pernambuco / Academic Unit of Garanhuns (UFRPE / UAG), in Garanhuns-PE. Obtaining fruits - Ironwood fruits were harvested in two locations with lot 1 coming from the town of Areia-PB and 2 from Paranatama-PE, in 2015.

FRUIT PROCESSING

The processing consisted of the removal of the seeds by lateral opening of the fruits with the aid of a hammer. After the processing, manual sorting was carried out in order to obtain more uniform lots, eliminating seedlings contaminated by pathogens or with injuries caused by pest attack. Subsequently, the seeds of both batches were placed in plastic bags and kept in a cold room (10°C) until the beginning of the tests.

DETERMINATION OF THE WATER CONTENT

Was determined by the stove method at 105 ± 3°C for 24 hours, according to the recommendations of the Rules for Seed Analysis (Brasil 2009) and the results obtained were expressed as mean percentage per batch.

WEIGHT OF ONE THOUSAND SEEDS

The seeds were weighed in an analytical balance with an accuracy of 0.001g using 8 subsamples of 100 seeds for each batch. The averages obtained from the 8 replicates / lot were multiplied by 10, resulting in the values of the statistical analysis (Brasil 2009). Before the tests were installed, seeds were chemically scarified with sulfuric acid for 15 minutes (Medeiros Filho et al. 2005) and then washed in running water for 20 minutes.

GERMINATION TEST

Was carried out on the paper towel substrate of the germitest type in roll form, previously sterilized for 20 minutes in a stove at 105 ± 3°C and moistered with distilled water in the amount equivalent to 2,5 times the paper dry weight. After distribution of the seeds on the substrate, the rolls were placed in germinating chambers of the type Biochemical Demand Oxygen (B.O.D.) regulated at a constant temperature of 30°C. For each lot 200 seeds were used, consisting of four replicates of 50 seeds. The first count started on the sixth day and the final count was performed on the 14th day (Biruel et al. 2007) after sowing, using normal seedlings (Brasil 2009) as germination criteria, and the results were expressed as a percentage. Normal seedlings were counted daily to obtain the germination speed index (IVG) (Maguire 1962).

ROOT AND SHOOT LENGTH OF SEEDLINGS

At the end of the germination test, root and shoot of normal seedlings of each sub-sample were measured using a ruler graduated in centimeters (Krzyzanowski et al. 1999).

DRY MASS OF THE ROOT, AERIAL PART AND COTYLEDONS OF SEEDLINGS

In order to identify the initial performance of the normal seedlings of each lot, the dry mass of the root, aerial part and cotyledons of seedlings was determined by drying in a forced ventilation stoven regulated to 80°C for a period of 24 hours (Nakagawa 1999).

THE EXUDATE - PHENOLPHTHALEIN pH TEST

Was conducted in two experiments, one of them being carried out at a constant temperature of 25°C and the other at 30°C. For each experiments, four replicates of 25 seeds and five soaking periods (30, 60, 90, 120 and 150 minutes) were used. In the preparation of the indicator solutions, 1 g of phenolphthalein was dissolved in 100 mL of absolute ethyl alcohol, and after this process 100 mL of distilled and boiled water were added. The sodium carbonate solution was made by mixing 0.8 g of sodium carbonate in 1000 mL of distilled water and boiled. The seeds were distributed individually in disposable cups (50 mL) and immersed in 8 mL of distilled water, after which the respective temperatures and immersion periods were submitted. After the soaking periods, a drop of the phenolphthalein and sodium carbonate solutions were added into the beakers containing each individualized seed, then those beakers were shaken slightly to homogenize the two solutions according to the methodology described by Cabrera and Peske (2002) been reading was carried out immediately. The evaluations were made based on staining of the soaking solution, being considered viable seeds, capable of forming normal seedlings, those in which the soaking solution was light pink and dark pink, while the colorless solution would indicate non-viable seeds (dead), the results being expressed as percentage of viable seeds. For the purpose of comparison with previous test, a germination test was executed with the same seeds from the exudate - phenolphthalein pH test.

STATISTICAL ANALYSIS

The statistical design used for the results of water content, weight of one thousand seeds, germination test, first germination test, germination speed index, seedling root and shoot length and dry mass of cotyledons, as well as root and aerial part of seedlings, was completely randomized, consisting of two treatments (two lots) with four replications. The results were submitted to analysis of variance by the F test and the means compared by the Tukey test at the 5% probability level. The experimental design of the exudates phenolphthalein pH test was completely randomized in a factorial scheme (2 x 5), being two seed lots and five soaking periods, respectively. The results were submitted to analysis of variance by the F test and the means were compared by the Tukey test at the 5% probability level and the polynomial regression analysis, by testing the linear and quadratic models, considering in order to explain the higher significant coefficient of determination (R^2). The exudate - phenolphthalein pH test values were correlated with the initial batch characterization tests by Pearson's simple correlation analysis with the application of the t test at 1 and 5% probability.

RESULTS AND DISCUSSION

The water content of the *L. ferrea* seeds differed between the lots (Table I), in which the seeds of lot 1 obtained the highest water content, around 9.68%, while the seeds from lot 2 found with 8.60% of water content. However, the variation of the water content between the lots was 1.08% which is considered acceptable for the tests, with less than 2% permissible, ensuring the reliability of the results (Marcos Filho 1999).

According to the data in Table I, statistical differences were observed among the lots for the first germination count, showing that lot 2 showed 26% germinated seeds in the first count, and could be characterized as the one with the highest physiological potential, whereas lot 1 obtained only 7% of normal seedlings on the sixth day after sowing.

In high-vigor seeds, there is a higher velocity in the metabolic activities, promoting faster and uniform emission of the primary root in the germination process, high growth rates and seedling production with a larger initial size (Schuch et al. 1999).

The seed lots did not differentiate between germination percentage and germination speed index (GSI) (Table I). However, this does not mean that in adverse field conditions the two lots acquire similar behavior to those observed in the germination test, since it is carried out under controlled conditions of temperature, substrate and humidity, which contribute to maximum expression of vigor and germination (Brasil 2009). Regarding IVG, the results can be explained by the fact that the seeds have very close germination percentage values, which tend to reduce the sensitivity of the vigor tests evaluated in conjunction with the germination test.

The highest values of weight of one thousand seeds were verified in the seeds of lot 2 (144.54g), while the seeds of lot 1 obtained an average weight of 127.00g (Table I). Therefore, it is believed that the fast germination of the *L. ferrea* seeds coming from lot 2 is directly related to its weight and comes from the hypothesis that larger seeds are formed by the greater deposition of storage substances during the maturation phase, which results in well-formed embryos and a higher level of hormones, and are therefore potentially more vigorous (Carvalho and Nakagawa 2012).

However, seeds from lot 2 provided a higher growth rate of seedlings (10.92 cm) than lot 1 (9.77 cm) (Table I), and it could be characterized, according to this parameter, as a larger batch force. Since seedlings, or parts of them, of higher average lengths are the most vigorous (Nakagawa 1999) because of the greater ability to transform the reserve supply of the storage tissues and the greater incorporation of these through the embryonic axis (Dan et al. 1987).

TABLE I
Water content, weight of one thousand, first germination count, germination, germination speed index, root length (RL), shoot length (SL), root dry mass (RDM), shoot dry mass (SDM) and cotyledons dry mass (CDM) of *Libidibia ferrea* seeds.

	LOT		
	1	2	CV (%)
Water content (%)	9.68a	8.60b	3.43
Weight of one thousand seeds (g)	127.00b	144.54a	4.31
First germination count (%)	7.00b	26.00a	25.75
Germination (%)	94.00a	95.00a	3.21
Germination speed index	6.030a	6.360a	4.10
Root length (cm)	7.11a	8.34a	9.41
Shoot length (cm)	9.77b	10.92a	1.83
Root dry mass (g)	0.0100a	0.0100a	0.00
Shoot dry mass (g)	0.0200a	0.0200a	0.00
Cotyledons dry mass (g)	0.0100a	0.0100a	39.28

* Means followed by the same lowercase letter in the line do not differ statistically from each other by the Tukey test at 5% probability.

The data obtained by the exudate - phenolphthalein pH test (percentage of viability and germination) did not fit any of the usual regression models (linear and quadratic).

The percentage of viability and germination of the two seed lots of *L. ferrea* did not differentiate as a function of the soaking periods at 25°C (Figure 1), and there was not possibility of stratifying the lots as to the level of vigor. Corroborating with most of the results obtained from the initial characterization evaluated by the germination test such as germination rate, germination percentage, seedling root length, root dry matter, aerial part and seedling cotyledons (Table I).

In the present study the significance in the vigor only confirms the hypothesis that the lots do not actually have them. The viability of the seeds remained high and did not differ even in the longer periods of imbibition, proving the high physiological potential of both seed lots.

It is assumed that the evaluated seeds had similarity to the physiological potential and that in this way the exudate - phenolphthalein pH test could not differentiate the lots. These results show the importance of analyzing seeds of narrow and broad physiological potential, in order to detect the efficiency, sensitivity and reliability of the tests used.

When the exudate - phenolphthalein pH was tested at 30°C (Figure 2), it was observed a similar behavior regarding on the viability and germination of the seeds at 25°C (Figure 1), noting that the seeds of both lots actually had similar physiological potentials.

The fact that there were no differences between seed lots does not imply that the methodology used is ineffective, since the initial event of the deterioration process refers to the destabilization of the cell membrane system and that the tests that evaluate this condition, such as the exudate - phenolphthalein pH test, may be considered more sensitive to differences in vigor between seed lots (Santos et al. 2011).

The vigor tests should provide fast, accurate, low-cost results and losses, thus, the pH exudate - phenolphthalein pH test in *L. ferrea* seeds soaked for at least 30 minutes in distilled and deionized water at the constant temperatures of 25 or 30°C provided an estimate of viability, being of fundamental importance when it is aimed to commercializate of the seeds.

The exudate - phenolphthalein pH test was efficient at 25°C for 30 minutes in the evaluation

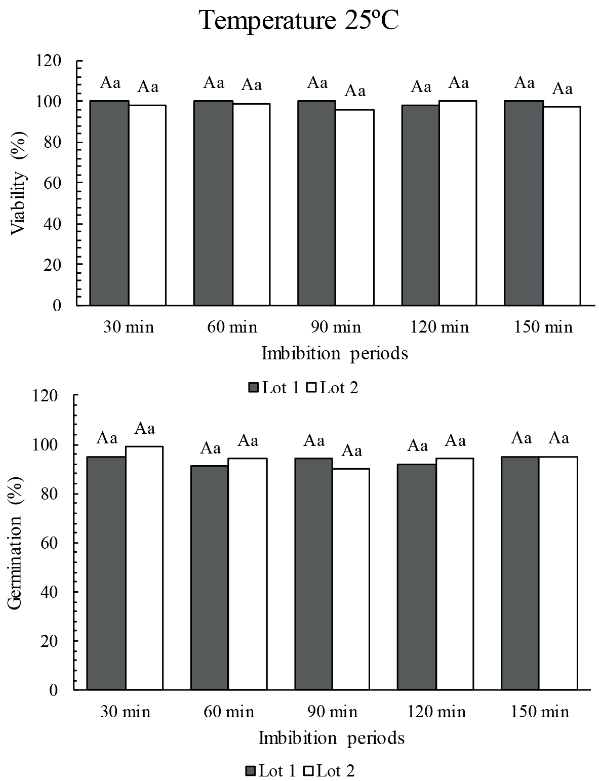

Figure 1 - Percentage of viability and germination by pH test of the individual exudate of two seed lots of *Libidibia ferrea* at 25ºC as a function of imbibition periods. *Means followed by the same lowercase letter in the line do not differ statistically from each other by the Tukey test at 5% probability.

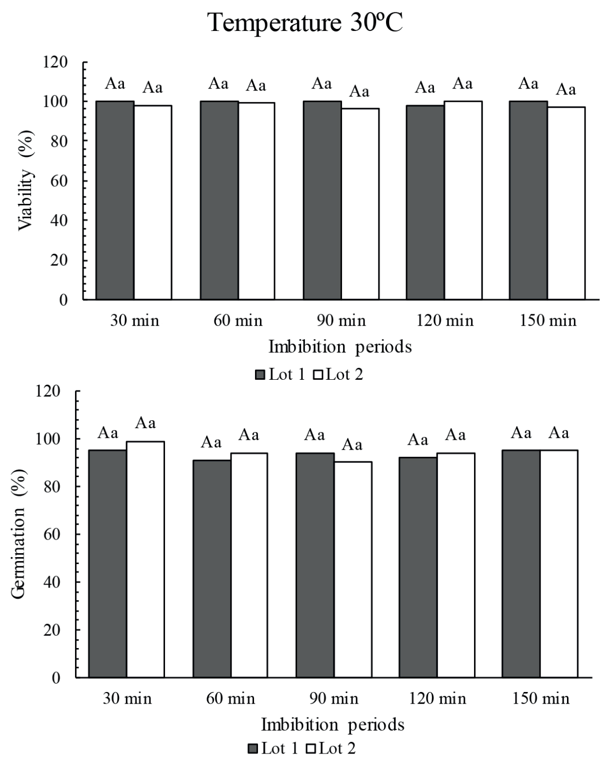

Figure 2 - Percentage of viability and germination by pH test of the exudate pH of two seed lots of *Libidibia ferrea* at 30ºC as a function of imbibition periods. *Means followed by the same lowercase letter in the line do not differ statistically from each other by the Tukey test at 5% probability.

of the physiological quality of soybean seeds (*G. max* L.) (Peske and Amaral 1986, Barros and Marcos Filho 1990). As well as, for the crambe (*Crambe abyssinica* Hochst) seeds, cultivar 'FMS Brilhante', in which the test was promising in the distinction of the plots at vigor levels at both the constant temperature of 25°C for 30 minutes and at 30°C for 45 minutes (Alves et al. 2016).

The results confirm the importance of evaluating the physiological quality of the seeds using several vigor tests in order to choose the test of greater safety and reliability. According to Santana et al. (1998), although it is an easy and fast test, the evaluation based on coloration may induce a subjective connotation to the test, when related to other factors such as seed moisture, temperature and period of imbibition, reduces its effectiveness and leads to erroneous interpretations of the results.

The water content obtained a negative correlation coefficient (r = -0.63) ($p < 0.05$) and the seedling length test positive (r = 0.67) ($p < 0.05$) with the percentage of germination by the exudate - phenolphthalein pH test at 25°C (Table II). When correlating, those variables evidenced to be affected by a extrinsic factor in common, certainly the quality of the batches analyzed. However, it was not possible to verify significant correlations between the other initial physiological quality tests with exudate - phenolphthalein pH test.

The negative correlation between the water content of the seeds and the percentage of germination by the exudate - phenolphthalein pH test shows that one variable depends on the other and

TABLE II

Pearson's simple correlation coefficients (r) estimated between water content (WC), weight of one thousand seeds (WTS), first germination count (FGC), germination speed index (GSI), germination (G), root length (RL), shoot length (SL), root dry mass (RDM), shoot dry mass (SDM), cotyledons dry matter (CDM) of seedlings and percentage of viability (Viab) and germination (GpH) of the exsudate pH test on *Libidibia ferrea* seeds at 25ºC.

	Viab (%)	GpH (%)	WTS (g)	WC (%)	FGC (%)	GSI	G (%)	RL (cm)	SL (cm)	RDM (g)	SDM (g)	CDM (g)
Viab	-	-0.16^{ns}	-0.07^{ns}	0.54^{ns}	-0.57^{ns}	-0.36^{ns}	-0.16^{ns}	-0.61^{ns}	-0.57^{ns}	0.00^{ns}	0.00^{ns}	0.45^{ns}
GpH		-	-0.17^{ns}	-0.64^{*}	0.40^{ns}	0.16^{ns}	0.13^{ns}	0.48^{ns}	0.67^{*}	0.00^{ns}	0.00^{ns}	-0.23^{ns}
WTS			-	0.57^{ns}	-0.54^{ns}	-0.02^{ns}	0.24^{ns}	-0.56^{ns}	-0.57^{ns}	0.00^{ns}	0.00^{ns}	-0.06^{ns}
WC				-	-0.85^{**}	-0.43^{ns}	-0.22^{ns}	-0.53^{ns}	-0.90^{**}	0.00^{ns}	0.00^{ns}	0.31^{ns}
FGC					-	0.49^{ns}	0.18^{ns}	0.65^{*}	0.92^{**}	0.00^{ns}	0.00^{ns}	-0.13^{ns}
GSI						-	0.87^{**}	0.54^{ns}	0.60^{ns}	0.00^{ns}	0.00^{ns}	-0.71^{*}
G							-	0.33^{ns}	0.36^{ns}	0.00^{ns}	0.00^{ns}	-0.83^{**}
RL								-	0.79^{**}	0.00^{ns}	0.00^{ns}	-0.52^{ns}
SL									-	0.00^{ns}	0.00^{ns}	-0.38^{ns}
RDM										-	0.00^{**}	0.00^{ns}
SDM											-	0.00^{ns}
CDM												-

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

that the same are inversely proportional. However, the significance of the correlation between these variables was expected to be positive since the higher the moisture content, within certain limits, the higher the germination percentage of the seeds.

The positive correlation between seedling length and germination percentage of seeds submitted to the exudate - phenolphthalein pH test is consistent and indicates the proportional increase of both variables. Thus, the greater number of normal seedlings, obviously from more vigorous seeds, will reflect the emergence of higher growth rate seedlings, and this is due to the greater translocation of the stored reserves to the growth of the embryonic axis (Dan et al. 1987).

Although these correlations were significant, however, they showed little practical importance due to their low magnitude, since according to Martins and Domingues (2011) only values higher than 0.7 are acceptable for association between the variables.

Pearson's simple correlation coefficients were not significant for most of the characteristics analyzed in the physiological quality standards and the exudate - phenolphthalein pH test at 30°C (Table III), except for the germination speed index (GSI) and the percentage of viable seeds, in which the correlation showed to be significantly negative ($r = -0.63$) ($p < 0.05$), being a correlation of low practical importance.

It is worth mentioning that the results of the exudate - phenolphthalein pH test and the others evaluated obtained significant results but controversial correlations, or even those that were not correlated, can be justified because the tests used evaluate different aspects of physiological quality characteristics of the seeds, and there may be different responses depending on the method used. Since the preliminary tests are based on physiological characteristics of the seeds, while the phenolphthalein test is based on biochemical factors (Aosa 2009).

According to Punia et al. (2006) the exudate - phenolphthalein pH test correlated significantly with the emergence percentage and germination of *Brassica juncea* (L.) Coss. when they were

TABLE III

Pearson's simple correlation coefficients (r) estimated between water content (WC), weight of one thousand seeds (WTS), first germination count (FGC), germination speed index (GSI), germination (G), root length (RL), shoot length (SL), root dry mass (RDM), shoot dry mass (SDM), cotyledons dry mass (CDM) of seedlings and the percentage of viability (Viab) and germination (GpH) of pH test of the exudate in *Libidibia ferrea* seeds at 30ºC.

	Viab (%)	GpH (%)	WTS (g)	WC (%)	FGC (%)	GSI	G (%)	RL (cm)	SL (cm)	RDM (g)	SDM (g)	CDM (g)
Viab	-	0.16^{ns}	0.11^{ns}	-0.13^{ns}	0.11^{ns}	-0.63*	-0.59^{ns}	0.11^{ns}	0.02^{ns}	0.00^{ns}	0.00^{ns}	0.21^{ns}
GpH		-	-0.31^{ns}	0.03^{ns}	0.28^{ns}	0.15^{ns}	0.26^{ns}	0.36^{ns}	0.20^{ns}	0.00^{ns}	0.00^{ns}	-0.15^{ns}
WTS			-	0.57^{ns}	-0.54^{ns}	-0.02^{ns}	0.24^{ns}	-0.56^{ns}	-0.57^{ns}	0.00^{ns}	0.00^{ns}	-0.06^{ns}
WC				-	-0.85**	-0.43^{ns}	-0.22^{ns}	-0.53^{ns}	-0.90**	0.00^{ns}	0.00^{ns}	0.31^{ns}
FGC					-	0.49^{ns}	0.18^{ns}	0.65*	0.92**	0.00^{ns}	0.00^{ns}	-0.13^{ns}
GSI						-	0.87**	0.54^{ns}	0.60^{ns}	0.00^{ns}	0.00^{ns}	-0.71*
G							-	0.33^{ns}	0.36^{ns}	0.00^{ns}	0.00^{ns}	-0.83**
RL								-	0.79**	0.00^{ns}	0.00^{ns}	-0.52^{ns}
SL									-	0.00^{ns}	0.00^{ns}	-0.38^{ns}
RDM										-	0.00**	0.00^{ns}
SDM											-	0.00^{ns}
CDM												-

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

submitted to a soaking period of 30 minutes at 25°C. In the same way that exudate - phenolphthalein pH test showed a significant correlation with the emergence of crambe seedlings, 'FMS Brilhante', in the combinations of 30 minutes at 25°C and 45 minutes at 30°C and with germination in all soaking periods tested (15, 30 and 45 minutes) at 30°C (Alves et al. 2016).

Thus it is evident that the development of a suitable methodology to test the exudate - phenolphthalein pH test in seeds should be carried out with a wide scientific basis by the researchers, in order to adjust it to the intrinsic conditions of the species.

Most of the results from the initial physiological quality of the lots did not show significant correlations or strong dependence (Tables II and III) with the vigor tests carried out in conjunction with the standard germination test (Table I).

However, there were negative correlations of strong magnitude between seed water content and the first count (r = -0.85), as well with the seedling length (r = -0.90), and also between the seed water content and the seed percentage (r = -0.83) at 1% significance, there was also a negative correlation between germination speed index and dry mass accumulation of seedling cotyledons (r = -0.71) (p <0.05).

The results corroborated with the values observed for the seeds of lot 2 that were low water content (Table I) and that even though they were able to promote a higher percentage of normal seedlings at the first germination count. However, a positive correlation was expected between seedling length and seed moisture content, since the same seeds mentioned above also gave rise to higher growth rate seedlings, such as the germination speed index and Percentage of germination with the deposition of dry mass content of the cotyledons.

The first germination test had a positive correlation of moderate and strong magnitude (Tables II and III) with root length (r = 0.65) (p <0.05) and seedling aerial part (r = 0.92) (p <0.01), respectively. Proving similarity with the vigor tests carried out in conjunction with the germination test (Table I), in which the seeds of lot 2 germinated faster in the first count, being more vigorous, and consequently able to originate higher seedlings.

In this way, the faster germination and establishment of seedlings favor the development of vegetative structures, since according to Vieira et al. (1994) the seeds that have high vigor mobilize rapidly their storage energy, providing greater initial growth with vigorous development of the root system and aerial part of the seedlings.

The germination speed index from the initial quality of the lots correlated positively with the percentage of germination (r = 0.87) (p <0.01), as well as the length of the root with shoot seedling (r = 0.79) (p <0.01). Although there is a significant correlation between the dry mass of the root system and the aerial part of the seedlings (r = 0.00) at 1% probability (Tables II and III), this coefficient of very low magnitude is negligible, and has no practical importance and also low dependence between the variables, which evidences the low accuracy of the results (Martins and Domingues 2011).

According to Abbade and Takaki (2014), the germination speed index of the seeds of *Tabebuia roseoalba* (Ridl.) correlates positively with the percentage of germinated seeds, assigning coefficient of strong magnitude (r = 0.94) at 5% reliability. The same authors also found a significant correlation between root length and shoot size (r = 0.94*), as observed for the *L. ferrea* seeds of the present study.

The results showing the trend of variation comparable to each other, serve to provide information to make decisions regarding the selection of lots. In addition, they should be analyzed and interpreted in conjunction with those obtained from the various vigor tests.

According to Marcos Filho et al. (1984), the exclusive use of correlation analysis in the evaluation of the comparative efficiency of the vigor tests can trigger the obtaining of inconsistent and misleading information. It may lead to incorrect or incomplete interpretations, since the data can correlate positively or negatively only because they have similar trends of variation. In this way, the classification capacity of the lots, based on tests of comparison of means, has been better adjusted in studies on the efficiency of the tests of vigor, being in agreement with the results obtained in the present work.

CONCLUSIONS

The first count and seedling length tests of seedlings are efficient to evaluate the physiological potential of *Libidibia ferrea* seeds.

The exudate - phenolphthalein pH test by the individual method can be conducted for at least 30 minutes in distilled and deionized water at the constant temperature of 25 or 30°C to determine the vigor of the ironwood seeds.

ACKNOWLEDGMENTS

To the support of CAPES - Coordenação de Aperfeiçoamento de Pessoal de Nível Superior, CNPq - Conselho Nacional de Desenvolvimento Científico e Tecnológico and FACEPE - Fundação de Amparo a Ciência e Tecnologia do Estado de Pernambuco which is acknowledged with immense gratitude.

AUTHOR CONTRIBUTIONS

Priscila Cordeiro Souto performed the experiments, analyzed the data, prepared figures and tables and wrote the paper. Edilma Pereira Gonçalves and Jeandson Silva Viana conceive and designed the experiments, contributed reagents/materials/analysis tools, analyzed the data, prepared figures and tables, wrote the paper and reviewed drafts of the paper. Júlio César de Almeida Silva and Lidiana Nayara Ralph performed all experimental procedures. Débora Teresa da Rocha Gomes Ferreira - conducted the statistic, data analysis and reviewed drafts of the paper. All authors critically revised the manuscript and approved the final version.

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