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Adequacy of the electrical conductivity test on *Libidibia ferrea* seeds (Mart. Ex Tul.) L. P. Queiroz var. *ferrea* classified with different masses

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Abstract

This study aimed to adapt the methodology of the electrical conductivity test to detect differences in vigor between seeds of *Libidibia ferrea* of different masses. The seeds were classified according to their individual mass: small seeds ≤ 0.14 g, medium seeds 0.15 ≤ x ≤ 0.19, large seeds ≥ 0.20 g. Subsequently, seed morphology (length, width, thickness, water content, and weight of 1000 seeds), and the initial physiological potential of the seeds (germination, first count, average time, average speed, germination rate coefficient, germination and emergence rate index, emergence percentage, seedling length, and dry mass) were determined. The electrical conductivity was conducted in a 3 × 12 factorial scheme with three seed masses and 12 immersion periods (2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, and 24 h), in volumes of 50 and 75 mL of water, with 25 and 50 seeds, respectively. The physical and physiological variables of the seeds were highly correlated with the electrical conductivity test. The seed mass of *L. ferrea* has a direct relationship with its physiological potential, with the seeds of mass ≥ 0.20 g classified with high vigor; the seeds of mass 0.15 ≤ x ≤ 0.19 with intermediate vigor; and the seeds of mass ≤ 0.14 g with low vigor. The electrical conductivity test is efficient in the evaluation of the physiological potential of *L. ferrea* seeds of different masses when conducted with 25 seeds soaked in 50 mL of distilled water for 4 h.

Keywords: Jucá, Physiological potential, Seed classification, Seed size, Vigor test.
Abbreviations: WTS_weight of one thousand seeds; L_length; W_width; T_ thickness; WC_ water content; G_ germination; FGC_ first germination count; AGT_ average germination time; AGS_ average germination speed; CSG_ coefficient speed germination; GSI_ germination speed index; E_ emergence; ESI_ emergence speed index; RLFC_ root length in the first count; SLFC_ shoot length in the first count; RL_ root length; SL_ shoot length; TLS_ total length of seedling; DMR_ dry mass of the root; DMS_dry mass of the shoot; TDM_ total dry mass of seedlings; DMC_ dry mass of the cotyledons; EC_ electrical conductivity; CV_ coefficient of variation; SM_ seed masses; P_periods.

Introduction

Libidibia ferrea (Mart. Ex Tul.) L. P. Queiroz var. *ferrea,* popularly known as 'pau ferro' and/or 'jucá', is a legume belonging to the family Fabaceae-Caesalpionoideae, that is widely distributed in the northeast region of Brazil, more specifically in the Caatinga tree and shrub regions. It is a tree that is used for economic, environmental, nutritional, and landscape purposes (Lorenzi, 2002; Câmara et al., 2008).
The classification of seeds according to mass has been a useful practice. According to Oliveira et al. (2016) and Soleymani (2019), it assists in the identification of the ideal class of seed to produce seedlings of high vigor. Although there is much research related to the effect of seed mass on the physiological potential of many cultivated species, for forest seeds, this subject is unfamiliar.
To obtain more consistent information about the physiological potential of seeds, vigor tests are performed in addition to germination tests. However, there is no universally accepted vigor test to determine the physiological potential of a given species, or a genus (Alves et al., 2016). This becomes even more evident when it comes to the seeds of forest species, because the wide biomorphological variability of both fruits and seeds has hindered the establishment of techniques for seed quality and production (Martins et al., 2009).
Electrical conductivity is a vigor test routinely used in seed analysis laboratories, as it has a high correlation with the emergence of seedlings in the field (Leite et al., 2019). However, the efficacy of this test is dependent on some factors, such as temperature, soak period, water quantity,

and seed quantity and mass (Bewley et al., 2013), which reinforces the need to improve and adjust the methodology of this rapid vigor test. However, for *L. ferrea* seeds this methodology is not yet established, only the tetrazolium (Carvalho et al., 2017) and exudate pH (Souto et al., 2019) tests are standardized in the literature.

The effect of seed mass on the adjustment of vigor methodologies has been investigated by some researchers, for example, Oliveira et al. (2016), who observed by the electrical conductivity test the stratification of vigor in the seeds of *Acacia mangium* Willd., with low performance of small seeds and high vigor of large seeds. On the other hand, the electrical conductivity test was not efficient in separating seed lots of different sizes in *Piptadenia moniliformis* Benth. (Azeredo et al., 2016).

Research aimed at identifying the relationship between seed mass and physiological potential is fundamental in adjusting vigor methodology, so that the performance of seeds in the field can be estimated consistently. This helps producers, nurseries, and seed technologists to choose seed lots of superior quality. The present study aimed to determine which methodology of the electrical conductivity test was best suited to detecting differences in the vigor of *L. ferrea* seeds of different mass.

Results

Physical characterization of seeds

A statistical analysis of the separation of seed lots in different masses revealed a significant difference in the variables studied, with emphasis on the large seed lot, which resulted in the highest values for the average weight of one thousand seeds, length, width, and thickness (Table 1). The initial water content of the seeds in each studied lot (Table 2) was uniform (10.3 to 10.6%), varying on average 0.3%.

Assessment of initial physiological potential

The seeds, independent of their mass, had similar germination (Table 2), with values of ~90%, indicating a good physiological quality in the studied seed lots. In the evaluation of the first count (FGC) and germination speed represented by average time (AGT), average speed (MGV), and germination speed coefficient (GSC) (Table 2), it was found that the seeds ≥ 0.20 g had the lowest values, which did not differ in turn from medium-mass seeds (0.15 ≤ x ≤ 0.19 g).

As for the germination speed index (GSI) and emergence speed (ESI), as well as the emergence (E%) (Table 2), it was not possible to detect significant variations in seed performance in the different mass groups, revealing them to be less sensitive in the stratification of lots as to their physiological potential.

Analyzing the data of seedlings obtained in the first germination count (Table 3), it was found that the evaluation by root length (RLFC) was efficient in the ranking of the lots, indicating superiority for the larger seeds, which in turn did not differ from the medium mass seeds.

In the second test count, performed 14 days after sowing (Table 3), it was possible to confirm again the superiority of the larger (≥ 0.20 g) and medium (0.15 ≤ x ≤ 0.19 g) seed lots, using the variables root length (RL), shoot length (SL), and total length (TLS). However, the dry mass of the roots (DMR), shoots (DMS), and cotyledons (DMC), as well as the total dry mass (TDM) of seedlings were more sensitive in predicting differences in vigor, and were able to classify the seeds of high vigor (large), intermediate vigor (medium), and low seed vigor (small seeds).

Among the variables analyzed, the length of the shoot (SLFC) performed in the first count (Table 3) was not effective in detecting different levels of physiological potential, showing little sensitivity in the evaluation of the vigor of *L. ferrea* seeds of different mass.

Test of electrical conductivity

In relation to electrical conductivity, a significant interaction was observed between the seed mass and the periods of immersion (Table 4 and Table 5). The electrical conductivity in the volume of 50 mL ranged from 8.3 to 168.8 µs cm^{-1} g^{-1}, while in the volume of 75 mL, this amplitude was smaller, ranging from 3.7 to 92.8 µs cm^{-1} $g^{-1,}$ after 2 and 24 h of immersion, respectively. The combinations of water volume (50 and 75 mL) and seed quantity (25 and 50 seeds) facilitated the stratification of three levels of vigor in *L. ferrea* seeds of different mass, with variations in the periods of immersion (Table 4).

In all the studied combinations, it was observed that the large seeds were more vigorous, followed by medium seeds with intermediate vigor. The lot represented by the small seeds presented higher values of electrical conductivity and were classified as low vigor seeds (Table 4 and Table 5).

The 50 mL of water with 25 and 50 seeds allowed us to distinguish seed lots of low (small seeds) and high (large seeds) vigor (Table 4), in the first 2 h of soaking. On the other hand, when the volume of water was increased to 75 mL, vigor stratification was only possible after 4 h of immersion, independent of the number of seeds used. In the stratification, the superiority of large and medium seeds and the low performance of small seeds were always verified (Table 5).

The combination of a period of 4 h with 50 mL (Table 4) and 6 h with 75 mL (Table 5), independent of the number of seeds used, provided greater sensitivity in detecting differences in physiological potential, making it possible to separate lots into three different levels of vigor. These results were similar to those obtained for the weight of one thousand seeds (WTS), seed length and width, root dry mass (DMR), shoot (DMS), total (TDM), and cotyledon mass (DMC), verifying the superior performance of the lot represented by the seeds of mass ≥ 0.20 g, intermediate performance in the seeds with mass 0.15 ≤ x ≤ 0.19 g, and low vigor in the seeds of mass ≤ 0.14 g (Table 1 and Table 3).

The concentration of leachate in the lot represented by seeds of superior quality (large seeds) was 27,3 µs cm^{-1} g^{-1} and 23,3 µs cm^{-1} g^{-1} for 25 and 50 seeds, respectively, after 4 h of immersion in the 50 mL volume, and 23,4 µs cm^{-1} g^{-1} (25 seeds) and 14.5 µs cm^{-1} g^{-1} (50 seeds) for 6 h in 75 mL of water, reflecting directly in the weight, length, and width of seeds, as well as in the accumulation of dry mass of the seedlings. Therefore, there is a relationship between the amount of electrolytes released by the seeds and the development of the seedlings, as was potentially observed by the accumulation of dry mass of the seedlings (Table 3).

Pearson's correlation analysis

Germination (G) was moderately and negatively correlated with electrical conductivity (25 seeds/4 h/50 mL; -0.57). However, it was possible to obtain a strong negative correlation with EC for 4 h and 6 h in volumes of 50 and 75 mL, respectively, with 25 and 50 seeds. Most of the vigor

Table 1. Weight of one thousand seeds (WTS), length (L), width (W), and thickness (T) of *Libidibia ferrea* seeds classified with different masses.

Seed masses							
	Small		Medium		Large		CV (%)
WTS (g)	127.8	c	173.5	b	217.1	a	1.0
L (mm)	7.6	c	9.5	b	10.4	a	1.4
W (mm)	5.8	c	6.3	b	6.5	a	0.8
T (mm)	4.0	b	4.3	a	4.5	a	1.8

*Comparison of means within each line (Tukey test, $p < 0.05$).

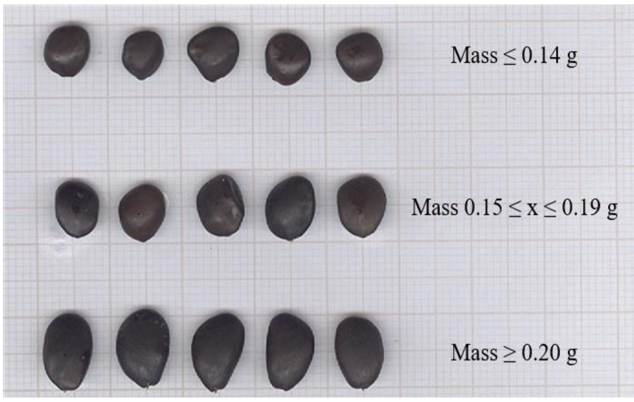

Fig 1. *Libidibia ferrea* seeds classified with different masses (Small: ≤ 0.14 g; Medium: 0.15 ≤ x ≤ 0.19 g; and Large: ≥ 0.20 g).

Table 2. Water content (WC), germination (G), first germination count (FGC), average time (AGT), average speed (AGS), speed coefficient (SGC), germination speed index (GSI), emergence (E), and emergence speed index (ESI) of *Libidibia ferrea* seedlings classified with different masses.

Seed masses							
	Small		Medium		Large		CV (%)
WC (%)	10.3		10.6		10.5		-
G (%)	89.0	a	94.0	a	95.0	a	5.5
FGC (%)	69.0	a	51.0	ab	40.0	b	19.3
AGT (days)	5.8	a	6.3	b	6.4	b	3.0
AGS (days $^{-1}$)	0.2	a	0.1	b	0.1	b	3.4
SGC (%)	17.4	a	15.8	b	15.7	b	3.2
GSI	4.1	a	3.8	a	3.8	a	8.9
E (%)	100.0	a	100.0	a	100.0	a	0.0
ESI	4.0	a	4.0	a	4.1	a	4.9

*Comparison of means within each line (Tukey test, $p < 0.05$).

Table 3. Root length (RLFC) and shoot (SLFC) in the first germination count, root length (RL), shoot (SL) and total of seedling (TLS) in the second germination count, dry mass of the root (DMR), shoot (DMS), total (TDM), and cotyledons (DMC) of *Libidibia ferrea* seedlings from seeds classified with different masses.

Seed masses							
	Small		Medium		Large		CV (%)
RLFC (cm pl^{-1})	4.00	b	4.50	ab	4.80	a	6.60
SLFC (cm pl^{-1})	3.60	a	3.30	ab	4.10	a	12.00
RL (cm pl^{-1})	6.30	b	8.20	ab	8.80	a	6.70
SL (cm pl^{-1})	8.70	b	10.00	ab	10.40	a	3.30
TLS (cm pl^{-1})	15.00	b	18.10	ab	19.20	a	5.20
DMR (g pl^{-1})	0.007	c	0.009	b	0.011	a	6.50
DMS (g pl^{-1})	0.01	c	0.02	b	0.03	a	7.50
TDM (g pl^{-1})	0.02	c	0.03	b	0.04	a	6.40
DMC (g pl^{-1})	0.01	c	0.02	b	0.03	a	5.00

*Comparison of means within each line (Tukey test, $p < 0.05$).

Table 4. Electrical conductivity (µs cm^{-1} g^{-1}) of *Libidibia ferrea* seeds classified with different masses in samples of 25 and 50 seeds, soaked in 50 mL of water in different periods of immersion.

	25 seeds						50 seeds					
Periods (P)	Seed masses (SM)											
	Small		Medium		Large		Small		Medium		Large	
50 mL of water												
2h	29	A b	16	A ab	10	A a	19	A b	11	A ab	8	A a
4h	74	B c	52	B b	27	A a	60	B c	35	B b	23	B a
6h	113	C c	80	C b	48	B a	89	C c	61	C b	40	C a
8h	131	D c	105	D b	65	BC a	113	D c	78	D b	58	D a
10h	142	DE c	116	DE b	82	CD a	125	D c	96	E b	76	E a
12h	145	DE c	124	EF b	95	DE a	140	E c	102	EF b	86	EF a
14h	145	DEF c	127	EF b	99	DE a	144	EF c	109	EFG b	95	FG a
16h	157	EFG c	128	EF b	110	EF a	145	EF c	114	FG b	100	FGH a
18h	163	FG c	131	EF b	113	EF a	149	EF c	117	GH b	106	GHI a
20h	165	G c	133	EF b	120	F a	150	EF c	128	HI b	113	HI a
22h	165	G c	135	F b	121	F a	152	EF c	129	HI b	114	I a
24h	167	G c	137	F b	121	F a	156	F c	132	I b	115	I a
F (SM)			486^{**}						633^{**}			
F (P)			319^{**}						536^{**}			
F (SM x P)			3^{**}						4^{**}			
CV (%)			7						6			

* For each combination of seed quantity and water volume, the means followed by the same lowercase letter in the row (seed masses) and uppercase in the column (periods of immersion) do not differ statistically by the Tukey test, ** and * significant at 1% and 5%, respectively, by test F.

Table 5. Electrical conductivity (µs cm^{-1} g^{-1}) of *Libidibia ferrea* seeds classified with different masses in samples of 25 and 50 seeds, soaked in 75 mL of water in different periods of immersion.

	25 seeds						50 seeds					
Periods (P)	Seed masses (SM)											
	Small		Medium		Large		Small		Medium		Large	
75 mL of water												
2h	12	A a	6	A a	5	A a	9	A a	5	A a	4	A a
4h	35	B b	21	AB a	15	AB a	27	B b	17	AB a	10	AB a
6h	54	C c	35	BC b	23	BC a	38	B c	25	B b	15	ABC a
8h	66	CD c	45	CD b	34	C a	54	C c	37	C b	18	BC a
10h	77	DE c	54	DE b	38	CD a	64	CD c	48	CD b	25	CD a
12h	84	EF c	65	EF b	52	DE a	70	DE c	57	DE b	37	DE a
14h	87	EF c	74	FG b	59	EF a	80	EF c	63	EF b	42	EF a
16h	90	EF b	75	FG a	65	EF a	83	FG c	68	EFG b	49	FG a
18h	91	EF c	80	FG b	67	F a	88	FG c	73	FG b	58	GH a
20h	91	EF b	80	G a	71	F a	91	FG c	75	G b	61	GH a
22h	92	EF b	81	G a	71	F a	92	FG c	76	G b	63	H a
24h	93	F b	84	G b	72	F a	92	G c	77	G b	63	H a
F (SM)			181^{**}						387^{**}			
F (P)			183^{**}						293^{**}			
F (SM x P)			2^{*}						4^{**}			
CV (%)			11						10			

* For each combination of seed quantity and water volume, the means followed by the same lowercase letter in the row (seed masses) and uppercase in the column (periods of immersion) do not differ statistically by the Tukey test, ** and * significant at 1% and 5%, respectively, by test F.

indicators (WTS, L, W, T, RLFC, RL, SL, TLS, DMR, DMS, DMC, and TDM) indicated that the higher the seed mass, and the growth and accumulation of dry mass of seedlings, the lower the EC value. On the other hand, the emergence, GSI, and ESI did not correlate significantly with the electrical conductivity test (Table 6).

Discussion

The results proved that there is a direct relationship between the weight of one thousand seeds, the length and width of the seeds, and seed mass (Table 1). The water content of the seeds (Table 2) was within the maximum limit (2%) required for the performance of tests that evaluate the physiological potential of the seeds, indicating safe and reliable results (Marcos-Filho, 2015).

According to Zareian et al. (2013), when assessing the germination of seeds according to the mass, the results are often not significant because the amount of reserve present in the seeds may be sufficient to initiate the germination process. Thus, depending on the germination criterion used,

Table 6. Pearson correlation coefficients between variables on physical and physiological characteristics of *Libidibia ferrea* classified with seeds of different masses.

	EC 25S/4H/50mL	EC 50S/4H/50mL	EC 25S/6H/75mL	EC 50S/6H/75mL
WTS	-0.94**	-0.97**	-0.89**	-0.90**
L	-0.89**	-0.96**	-0.88**	-0.89**
W	-0.87**	-0.94**	-0.90**	-0.80**
T	-0.84**	-0.90**	-0.76**	-0.86**
G	-0.57*	-0.36^{ns}	-0.39^{ns}	-0.31^{ns}
GSI	0.24^{ns}	0.50^{ns}	0.32^{ns}	0.49^{ns}
E	-0.38^{ns}	-0.35^{ns}	-0.50^{ns}	-0.10^{ns}
ESI	-0.31^{ns}	-0.36^{ns}	-0.53^{ns}	-0.27^{ns}
RLFC	-0.71**	-0.74**	-0.69*	-0.82**
SLFC	-0.49^{ns}	-0.28^{ns}	-0.27^{ns}	-0.45^{ns}
RL	-0.81**	-0.86**	-0.79**	-0.71**
SL	-0.84**	-0.91**	-0.82**	-0.72**
TLS	0.83**	-0.89**	-0.81**	-0.72**
DMR	-0.89**	-0.93**	-0.85**	-0.78**
DMS	-0.90**	-0.93**	-0.90**	0.81**
DMC	-0.94**	-0.93**	-0.85**	-0.89**
TDM	-0.90**	-0.94**	-0.90**	-0.81**
EC:25S/4H/50mL		0.87**	0.84**	0.80**
EC:50S/4H/50Ml			0.93**	0.88**
EC:25S/6H/75Ml				0.76**
EC:50S/6H/75Ml				

** and *: significant at 1% and 5% probability at t-test respectively; ^{ns}: not significant at 5% probability by t-test.
WTS: weight of a one thousand seeds; L: length; W: width; T: thickness; G: germination; GSI: germination speed index; E: emergence; ESI: emergency speed index; RLFC: length of seedling root at first count; SRFC: length of shoot of seedlings at first count; RL: root length; SL: shoot length; TLS: total length of seedlings; DMR: dry mass of root; DMS: dry mass of shoot; DMC: dry mass of cotyledons; TDM: total dry mass of seedlings; EC: 25S/4H/50mL: electrical conductivity with 25 seeds soaked for 4 hours in 50 mL of water; EC: 50S/4H/50mL: electrical conductivity with 50 seeds soaked for 4 hours in 50 mL of water; EC: 25S/6H/75mL: electrical conductivity with 25 seeds soaked for 6 hours in 75 mL of water; EC: 50S/6H/75mL: electrical conductivity with 50 seeds soaked for 6 hours in 75 mL of water.

these differences may not be detected, as was verified in the different seed classes in the present study (Table 2).
The higher germination speed in smaller seeds when compared to larger seeds may be related to the thickness of the seed coating. This is a characteristic that can be inversely proportional to the water absorption speed; that is, the larger the seed area, the longer the time taken to obtain the minimum water content to initiate the germinative metabolic process (Carvalho and Nakagawa, 2012; Mendonça et al., 2016).
Thus, it could be said that the smaller seeds of *L. ferrea* (Table 2), because they have a larger contact surface per unit of mass, hydrated their tissues more quickly, accelerating the germination process, and consequently, required less water to induce root protrusion when compared to larger seeds. The higher germination speed in smaller seeds when compared to larger seeds was also observed by Dera et al. (2019) in *Prosopis africana* Taub.
The speed of germination (IVG) and emergence (IVE), as well as the emergence percentage, were not efficient in estimating the vigor of *L. ferrea* seeds of different masses (Table 2). Biruel et al. (2010), working with different sizes and formats of *L. ferrea* seeds, verified contradictory results, because, according to the authors, the lowest germination speed was obtained for smaller seeds. Thus, the differences observed between the studies with this species may be related to the criteria of seed classification.
In the present study, for example, the individual seed mass was chosen, while Biruel et al. (2010) used the diameter to obtain different classes. The divergence of results in tests that evaluated the physiological potential of seeds as a function of seed mass was verified in other native species, such as in *Sideroxylon obtusifolium* seeds (Roem. & Schult.) Penn. (Silva, 2015) and *Anadenanthera colubrina* (Vell.) (Bispo et al., 2017).
The length of seedlings was efficient in the stratification of the *L. ferrea* seeds. However, the dry mass of the seedlings was more sensitive in detecting the vigor levels, indicating superior performance of the seeds ≥ 0.20 g, followed by the medium and small seeds (Table 3). According to Amaro et al. (2015) and Oliveira et al. (2015), larger seeds are more vigorous, because they have more reserve material and are more skilled in the transformation of tissues, leading to seedlings with higher growth rates and accumulation of dry mass. Therefore, these results confirm the effectiveness of seedling length and dry mass testing in classifying the vigor of seed lots of *L. ferrea* of different mass.
The greater permeability of the membrane system of smaller seeds, such as the ≤ 0.14 g seeds in the present study (Table 4 and Table 5), according to Moncaleano-Escandon et al. (2013) and Marcos-Filho (2015) may be associated with the low physiological potential of these seeds. Because they are more deteriorated, they have a membrane system with reduced selective activity, increasing their permeability during the water absorption phase of the germination process, resulting in the leaching of larger amounts of electrolytes to the external medium.
According to Menezes et al. (2007), at the beginning of seed conditioning to perform the test, the electrolyte release for the soaking solution is intense in vigorous seeds or not, making it difficult to categorize the seed lots, especially those with a narrow difference in physiological potential.

However, over the period of immersion, the tendency is that the seeds of high vigor reorganize the cell membranes more quickly, resulting in the stabilization of exudates. Thus, from that moment on, the separation of seeds is possible with respect to their physiological potential. This is evidenced by the more consistent results after 4 and 6 h of immersion in volumes of 50 and 75 mL, respectively, in the present study (Table 4 and Table 5).

The strong, positive correlation between electrical conductivity and morphology, as well as the tests of length and dry mass of seedlings (Table 6) reinforce the hypothesis of Oliveira et al. (2015) that high EC values are associated with less integrity and organization of cell membranes, which classifies the seeds as less vigorous and, therefore, with a lower capacity to produce high performance seedlings due to higher degree of deterioration, as observed in seeds ≤ 0.14 g in the present study.

The absence of significant correlation between emergence, germination, and most vigor tests (Table 6) was also reported by Gonzales et al. (2011) in seeds of *Corymbia citriodora* (Hook.) K.D. Hill & L.A.S. Johnson, and Araújo et al. (2017b) in *Leucaenaleu cocephala* (Lam.) from Wit. seeds.

It can be affirmed, therefore, that the electrical conductivity test can be applied in the evaluation of the physiological potential of *L. ferrea* seeds of different mass, due to its high correlation with most traditional vigor tests. Thus, it can be included in seed quality control programs, estimating, even in early stages, the degree of deterioration and guaranteeing the success of plants in the field.

Seed mass, sample size, water volume, and seed soaking periods are factors that considerably affect electrical conductivity and therefore need to be adjusted to standardize this methodology in different species, to obtain consistent results. Thus, the use of 25 and 50 seeds soaked in 50 mL of distilled water for at least 4 h or in a volume of 75 mL for 6 h, are suitable for carrying out electrical conductivity tests in *L. ferrea* seeds of different mass. Considering the low availability of seeds of this species and the speed at which the results are obtained (which is one of the main characteristics of the vigor tests), it is recommended that this test be conducted using a combination of 25 seeds in 50 mL for 4 h.

Materials and methods

Plant materials and experimental design

The experiment was conducted in the Plant Propagation Laboratory belonging to the Federal University of Alagoas. The mature fruit of *L. ferrea* were harvested from trees of forest fragments located in the rural zone of the municipality of Paranatama in the state of Pernambuco, Brazil (8°52' 59" S; 36°34'36'' W) in December 2017. After harvest, the fruit was transferred to the laboratory for the manual extraction of the seeds, which was performed by the lateral opening of the pods with a hammer. The seeds that were visibly healthy and free from mechanical injuries were selected for conducting the experiments.

A completely randomized experimental design was used, with four replicates.

Classification of the seeds

The seeds were visually separated into three classes (Figure 1). Later, 500 seeds of each predefined class were weighed individually with an analytical balance (precise to 0.0001g) to obtain the intervals of the mass classes, according to the methods of Alves et al. (2005), and Pagliarini et al. (2014). The seeds were classified into small (≤ 0.14 g), medium (0.15 ≤ x ≤ 0.19 g), and large (≥ 0.20 g).

Physical characterization of seeds

Weight of one thousand seeds (WTS) was carried out with eight sub-samples of 100 seeds of each seed mass (Brasil, 2009). Seed biometrics (length, width, and thickness) were performed on 200 seeds of each seed mass, with the aid of a digital caliper with an accuracy of 0.01 mm. The results were expressed in millimeters for each seed mass. Water content (WC) of seeds was determined by the oven method at 105 ± 3 °C, with four replicates of 10 seeds. The results were expressed as percentage (wet base) for each seed mass (Brasil, 2009).

To overcome the tegumentary dormancy, seeds of each class of the species under study were soaked in concentrated sulfuric acid (96% H_2SO_4) for 15 min, followed by washing in running water for 20 min for the complete removal of the acid (Araújo et al., 2017a).

Assessment of initial physiological potential

Germination test (G) - Four replications of 25 seeds were used. Seeds were placed to germinate on paper towels, previously sterilized in an autoclave and moistened with distilled water in the amount equivalent to 2.5 times the weight of dry paper. Seeds were packed in plastic bags and kept in germination chambers at 30 °C (Brasil, 2009, 2013). The evaluations were carried out according to the criteria established by Biruel et al. (2007), in which the first count was carried out on the sixth day, and the final germination count was on the 14th day after sowing, with results expressed as the average percentage of normal seedlings for each seed mass.

At the end of the germination test, the average time (AGT) (Labouriau, 1983), average speed (AGS) (Labouriau, 1970), speed coefficient (SGC) (Nichols and Heydecher, 1968), and germination speed index (GSI) (Maguire, 1962) were calculated.

Seedling length - On the first (sixth day) and last count (14 days) of the germination test, the measurements of the root and shoot of the normal seedlings of each subsample were made with the aid of a centimeter ruler. The results were expressed in centimeters per seedling for each mass of seeds (Krzyzanowski et al., 1999).

Dry mass of seedlings - The roots, shoots, and cotyledons of the normal seedlings of each replicate obtained from the final germination test count (14 days) were packed separately in 'Kraft' paper bags and placed in a forced ventilation oven for 24 hours, at 80 ºC. The results were expressed in grams/primary root, hypocotyl, cotyledon, and seedling for each seed mass (Nakagawa, 1999).

Emergence test (E) - Eight replicates of 25 seeds of each seed mass were sown 1.5 cm deep in polyethylene plastic bags containing a mixture of soil and dried bovine manure in a ratio of 2:1 (soil:manure), and kept in a greenhouse. We computed the number of seedlings that emerged until stabilization. The emergence of the hypocotyl on the substrate was the emergence criterion. The results were expressed as the percentage of seedlings that emerged for each mass of seeds.

Emergency Speed Index (ESI) - Daily counts were made of the number of seedlings that emerged until emergence stabilized, and ESI was calculated according to the formula proposed by Maguire (1962).

Electrical conductivity (EC) - Four subsamples of 25 and 50 seeds of each seed mass were weighed in an analytical balance (0.0001g accuracy), using volumes of 50 and 75 mL of distilled water and different periods of immersion (2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, and 24 h). The seeds immersed in water were kept in germination chambers at a temperature of 30 °C. The results are expressed in mS cm^{-1} g^{-1} for each seed mass (Vieira and Krzyzanowski, 1999).

Statistical analysis

The morphological data and initial physiological potential of the seeds were submitted to analysis of variance, with the averages compared by the Tukey test at a 5% level of significance. Electrical conductivity data were analyzed in a 3 × 12 factorial scheme (seed masses and periods of immersion), separately for each volume (50 and 75 mL) and seed quantity (25 and 50 seeds). The means were submitted to analysis of variance by the "F" test and compared by the Tukey test at 5 % probability. The statistical analysis was performed using the statistical program SISVAR version 5.6 (Ferreira, 2011).

Pearson's simple correlation analysis (r) was performed between the physical characterization of the seeds, the traditional vigor tests, and the electrical conductivity, grouping the three seed masses (small, medium, and large), with the significance of the "r" values determined by *t* ($p < 0.05$), using the statistical software Sigmaplot version 11.0.

Conclusion

The physical characteristics represented by the weight of one thousand seeds (WTS), length (L), and width (W) of the seeds, as well as the tests of dry mass of the root (DMR), shoot (DMS), total seedling (TDM), and cotyledon (DMC) are useful in estimating the physiological potential of *L. ferrea* seeds of different mass. Seed mass interferes with the electrical conductivity test in *L. ferrea*, but the methodology is optimized using 25 seeds soaked in 50 mL of distilled water for 4 h.

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References

Alves EU, Bruno RLL, Oliveira AP, Alves AU, Alves AU, Paula RC (2005) Influência do tamanho e da procedência de sementes de *Mimosa caesalpiniifolia* Benth. sobre a germinação e vigor. Rev Árvore. 29 (6): 877-885.

Alves CZ, Rodrigues LA, Rego CHQ, Silva JB (2016) pH of exudate test in the physiological quality of crambe seeds. Cienc Rural. 46 (6): 1014-1018.

Amaro HTR, David AMSS, Assis MO, Rodrigues BRA, Cangussú V, Oliveira MB (2015) Testes de vigor para avaliação da qualidade fisiológica de sementes de feijoeiro. Rev Ciênc Agrár. 38 (3): 383-389.

Araújo AV, Silva MAD, Ferraz APF (2017a) Superação de dormência de sementes de *Libidibia ferrea* (Mart. ex Tul.) LP Queiroz var. *ferrea.* Magistra. 29 (3/4): 298-304.

Araújo FS, Félix FC, Ferrari CS, Bruno RLA, Pacheco MV (2017b) Adequação do teste de envelhecimento acelerado para avaliação do vigor de sementes de leucena. Rev Bras Ciênc Agrár. 12 (1): 92–97.

Azeredo GA, Paula RCD, Valeri SV (2016) Electrical conductivity in *Piptadenia moniliformis* Benth. seed lots classified by size and color. Rev Árvore. 40 (5): 855-866.

Bewley JD, Bradford KJ, Hilhorst HWM, Nonogaki H (2013) Seeds: physiology of development, germination and dormancy. New York: Springer, 3: 392 p.

Biruel RP, Aguiar IB, Paula RC (2007) Germinação de sementes de pau-ferro submetidas a diferentes condições de armazenamento, escarificação química, temperatura e luz. Rev Bras Sementes. 29 (3): 134-141.

Biruel RP, Paula RCD, Aguiar IBD (2010) Germinação de sementes de *Caesalpinia leiostachya (*Benth.) Ducke (pau-ferro) classificadas pelo tamanho e pela forma. Rev Árvore. 34 (2): 197-204.

Bispo JS, Costa DCC, Gomes SEV, Oliveira GM, Matias JR, Ribeiro RC, Dantas BF (2017) Size and vigor of *Anadenanthera colubrina (*Vell.) Brenan seeds harvested in Caatinga areas. J Seed Sci. 39 (4): 363-373.

Brasil (2009) Ministério da Agricultura, Pecuária e Abastecimento. Secretaria de Defesa Agropecuária. Brasília: MAPA/ACS, 2: 395 p.

Brasil (2013) Ministério da Agricultura, Pecuária e Abastecimento. Instruções para Análise de Sementes de Espécies Florestais, de 17 de janeiro de 2013. Brasília: MAPA, 1: 98 p.

Câmara FAA, Torres SB, Guimarães IP, Oliveira MKT, Oliveira FDA (2008) Biometria de frutos e sementes e superação de dormência de jucá (*Caesalpinia ferrea* Mart. ex. Tul. (Legumisosae – Caesalpinoideae). Rev Caatinga. 21 (4): 172-1778.

Carvalho NM, Nakagawa J, (2012) Sementes: ciência, tecnologia e produção. Jaboticabal: FUNEP, 5: 590 p.

Carvalho SMC, Torres SB, Benedito CP, Nogueira NW, Souza AAT, Souza Neta MLD (2017) Viability of *Libidibia ferrea* (Mart. ex Tul.) LP Queiroz var. *ferrea*) seeds by tetrazolium test. J Seed Sci. 39 (1): 7-12.

Dera BA, Agera SIN, Ezugwu EU (2019) Effect of seed size and acid scarification on germination and early growth of *Prosopis africana.* J Global Biosci. 8 (1): 5774-5788.

Ferreira DF (2011) Sisvar: a computer statistical analysis system. Ciênc Agrotec. 35 (6): 1039-1042.

Gonzales JLS, Valeri SVE, Paula RC (2011) Qualidade fisiológica de sementes de diferentes árvores matrizes de *Corymbia citriodora (*Hook.) K.D. Hill & L.A.S. Johnson. Sci For. 39 (90): 171-181.

Krzyzanowski FC, Vieira RD, França Neto JB (1999) Vigor de sementes: conceitos e testes. Londrina: ABRATES, 1: 218 p.

Labouriau LG (1970) On the physiology of seed germination in *Vicia graminea* Sm. – 1. An Acad Bras Cienc. 42 (2): 235-262.

Labouriau LGA (1983) A germinação das sementes. Washington: OEA, 1: 174 p.

Leite MDS, Leite TDS, Torres SB, Leal CCP, Freitas RMOD (2019) Classification of West Indian gherkin seeds vigor by respiratory activity. Rev Ciênc Agron. 50 (2): 307-311

Lorenzi H (2002) Árvores brasileiras: manual de identificação e cultivo de plantas arbóreas do Brasil. Nova Odessa: Instituto Plantarum, 4: 368 p.

Maguire JD (1962) Speed of germination aid selection and evaluation for seedling emergence and vigour. Crop Sci. 2 (2): 176-177.

Marcos-Filho J (2015) Fisiologia de sementes de plantas cultivadas. Londrina: ABRATES, 2: 660 p.

Martins L, Lago AA, Sales WRM (2009) Conservação de sementes de ipê-amarelo [*Tabebuia chrysotricha* (Mart. Ex DC.) Standl.] em função fazer teor de Água das Sementes e da temperatura do armazenamento. Rev Bras Sementes. 31 (2): 86-95.

Mendonça AVR, Freitas TAS, Souza SS, Fonseca MDS, Souza JS (2016) Morfologia de frutos e sementes e germinação de *Poincianella pyramidalis (*Tul.) L.P. Queiroz. Ci Fl. 26 (2): 375-387.

Moncaleano-Escandon J, Silva BCF, Silva SRS, Granja JAA, Alves MCJL, Pompelli MF (2013) Germination responses of *Jatropha curcas* L. seeds to storage and aging. Ind Crops Prod. 44: 684-690.

Menezes NL, Garcia DC, Bahry CA, Mattioni NM (2007) Teste de condutividade elétrica em aveia preta. Rev Bras Sementes. 29 (2): 138-142.

Nakagawa J (1999) Testes de vigor baseados no desempenho das plântulas. In: Krzyzanowski FC, Vieira RD, França – Neto JB (eds) Vigor de sementes: conceitos e testes. Londrina: Abrates, 1: 1-21.

Nichols MA, Heydecker W (1968) Two approaches to the study of germination date. ISTA. 33: 531-40.

Oliveira LM, Cavalheiro VBD, Moraes DM, Tilmann MAA, Schuch LOB (2015) Medição do CO_2 como método alternativo para a diferenciação do vigor de lotes de sementes de melancia. Cienc Rural. 45 (4): 606-611.

Oliveira DL, Smiderle OJ, Paulino PPS, Souza AG (2016) Water absorption and method improvement concerning electrical conductivity testing *Acacia mangium* (Fabaceae) seeds. Rev Biol Trop. 64 (4): 1-5.

Pagliarini MK, Nasser MD, Nasser FACM, Cavichioli JC, Castilho RMM (2014) Influência do tamanho de sementes e substratos na germinação e biometria de plântulas de jatobá. Tecnol Ciênc Agropec. 8 (5): 33-38.

Silva KB (2015) Qualidade fisiológica de sementes de *Sideroxylon obtusifolium* (Roem. & Schult.) Penn. classificadas pelo tamanho. R Bras Bioci. 13 (1): 1-4.

Soleymani A (2019) Safflower (*Carthamus tinctorius* L.) seed vigor tests for the prediction of field emergence. Ind Crops Prod. 131: 378-386.

Souto PC, Gonçalves EP, Viana JS, Silva JCA, Ferreira DTRG, Ralph LN (2019) Exudate - phenolphthalein pH test for evaluation of validity in seeds of *Libidibia ferrea*. An Acad Bras Ciênc. 91 (4): e20180734.

Vieira RD, Krzyzanowski FC (1999) Teste de condutividade elétrica. In: Krzyzanowski FC, Vieira RD, França Neto JB (Eds) Vigor de sementes: conceitos e testes, Londrina: ABRATES, 1: 1- 26.

Zareian A, Hamidi A, Sadeghi H, Jazaeri MR (2013) Effect of seed size on some germination characteristics, seedling emergence percentage and yield of three wheat (*Triticum aestivum* L.) cultivars in laboratory and field. Middle East J Sci Res. 13 (8): 1126-1131.