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Ethanol test as a determinant of physiological potential of forest species seeds: The case of *Plathymenia reticulata* Benth. (Leguminosae–Mimosoideae), a tree of interest for Atlantic Forest restoration

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ABSTRACT: The ethanol test supplies information on physiological problems related to seed deterioration. The test optimization is crucial for its application in the seed analysis routine. This study aimed to evaluate vigor test for *Plathymenia reticulata* seeds based on ethanol production in order to establish execution guidelines and verify its relationship with other seed vigor tests. Seed lots were subjected to germination and vigor tests, and seed coat evaluation. Ethanol production assessment was carried out based on two trials testing water volumes for imbibition (0.25; 0.5; 0.75; 1.00; and 1.25 mL), reading periods (2, 4, 6, 8, 24, and 48 h), and seed quantities (5, 10, and 15). There was a distinction in physiological potential between the lots, indicated by the ethanol test and other traditionally used tests. The ethanol test was efficient for vigor assessment in *Plathymenia* seeds, as were the other vigor tests, when conducted with 15 seeds imbibed in a volume of water 2.5 times their mass for 48 h. The vigor test based on ethanol production proved reliable and it is recommended for use in a system of seed production and commercialization in nurseries and forestry companies.

Index terms: ethylometer, germination, *Plathymenia reticulata,* statistical modeling.

RESUMO: O teste do etanol fornece informações sobre problemas fisiológicos relacionados à deterioração das sementes. A otimização do teste é fundamental para sua aplicação na rotina de análise de sementes. O objetivo foi avaliar o teste de vigor de sementes de *Plathymenia reticulata* com base na produção de etanol, a fim de estabelecer diretrizes de execução e relações com outros testes de vigor. Os lotes de sementes foram submetidos aos testes de germinação e vigor e avaliação do tegumento das sementes. A avaliação da produção de etanol foi realizada com base em dois ensaios testando volumes de água para embebição (0,25; 0,5; 0,75; 1,00; e 1,25 mL), períodos de leitura (2, 4, 6, 8, 24 e 48 h) e quantidades de sementes (5, 10 e 15). O teste do etanol foi eficiente para avaliação do vigor em sementes de *P. reticulata*, assim como os demais testes de vigor, quando realizados com 15 sementes embebidas em volume de água 2,5 vezes sua massa por 48 h. O teste de vigor baseado na produção de etanol mostrou-se confiável e é recomendado para uso em sistema de produção e comercialização de sementes em viveiros e empresas florestais.

Termos para indexação: etilômetro, germinação, *Plathymenia reticulata*, modelagem estatística.

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INTRODUCTION

The Atlantic Forest is an important tropical biome that is vital to Brazil from an environmental, social, and economic perspective. Recognized as one of 25 biodiversity hotspots present on the planet (Esser et al., 2019), due to the intensity of exploitation, urbanization and broadening of agricultural frontiers, a large area of its forest cover has been reduced and fragmented. Thus, currently, forest restoration efforts are considered paramount for the conservation of Atlantic Forest and alignment of Brazil with global agendas for the reduction of greenhouse gases (Londe et al., 2021).

Within the context of restoration, the propagation of native forest species plays a significant role in the efficiency of initiatives directed at reestablishing the intended ecological dynamics through restoration (Cornelius and Miccolis, 2018; Conceição et al., 2019). Most of the native trees are almost exclusively propagated through seeds; therefore, the deployment of plantations requires special attention to the genetic and physiological quality of seeds that will give rise to seedlings aimed at restoration.

The main component of a seed quality control program is the evaluation of physiological quality since, besides estimating the performance of seeds in the field, it provides information that identifies and solves problems during the production process (Ebone et al., 2019). Thus, vigor testing can supply sensitive indices for the broad characterization of physiological quality to complement information from the germination test (Virgens et al., 2019).

In seed quality control programs, quick reliable tests are considered indispensable for assessing seed quality and have become the target of attention of technologists, seed producers, and researchers who need to forecast seed performance (Kodde et al., 2012). The first in the sequence of events during seed deterioration is degradation of the mitochondrial membrane. Therefore, the ideal vigor test should be able to identify reduced seed vigor in the first stages, detecting subtle changes between lots (Barbosa et al., 2021).

A vigor test based on loss of mitochondrial membrane integrity is the ethanol test, which supplies important information on physiological problems related to deterioration, given that more deteriorated seeds produce more ethanol than less deteriorated seeds (Buckley and Buckley, 2009). This test has demonstrated as a promising method compatible with other vigor tests on cabbage (Kodde et al., 2012), malted barley (Buckley et al., 2016), corn (Onwimol et al., 2019), melon (Ornellas et al., 2020), red rice (Barbosa et al., 2021), watermelon, pepper, and radish (Kucukhuseyin et al., 2021). Optimization of ethanol analysis protocols may be an efficient alternative to determine the physiological potential of seeds. The procedures are relatively simple, quick, and cost-effective, and can be reproduced.

The use of a test with such characteristics may contribute to the advance of seed technology, which is especially interesting in the growing but small sector of seedlings and seeds of native forest species. In light of the above, the present study aimed to establish guidelines for vigor testing based on ethanol production and evaluate its efficiency. The biological model used in the present study was *Plathymenia reticulata* Benth., a native tree of two Brazilian biodiversity hotspots, namely the Atlantic Forest and the Cerrado. This species is widely employed in cabruca-cocoa agroforestry systems, in commercial planting initiatives, and mainly in forest restoration and it is considered strategic from the ecological and economic standpoint.

MATERIAL AND METHODS

Obtaining lots with different levels of vigor

Plathymenia reticulata seeds were obtained from collected fruits that were not yet ripe, in 12 matrices, marked by the Floresta Viva Institute, in the municipality of Uruçuca, Bahia, Brazil (14° 27’ 46,5” S and 39° 02’ 42,7” O). The seeds were processed by extracting them from the legume and the surrounding membrane.

To obtain lots with different levels of vigor, which were necessary to test the sensitivity of the ethanol test, the seeds were subjected to artificial aging. In this process, they were homogenized in a single lot and manually divided

into four portions. Three of the portions were subsequently placed in transparent plastic boxes on a coupled aluminum screen (11.0×11.0×3.0 cm), with 40 mL of water at the end and stored in a forced-air system oven at 42 °C for 24, 48, and 72 h, respectively. A control sample was maintained without aging. The samples were denominated lot 1, which was not aged; lot 2, aged for 24 h; lot 3, aged for 48 h; and lot 4, aged for 72 h.

Scanning electron microscope

After obtaining the lots, anatomic analysis of the seeds was carried out at the level of seed integument structure using a scanning electron microscope to verify the effect of the different aging treatments for the reduction of vigor and the formation of lots. Transverse cuts were made in the median region of the seed surrounding the area of the hilum using a steel blade. The obtained material was covered with gold using a Bal-Tec sputter coater, model SCD-050, without prior fixation and dehydration, as it presented as dry.

Thicknesses of the cuticle thickness (CT), palisade thickness (PT), and hourglass thickness (HT) were measured in the seeds of all the lots using image processing software of the scanning electron microscope (SEM - FEI Company, model Quanta 250) and the values were recorded in µm.

Subsequently, moisture content was determined, and the physiological quality was evaluated for each seed lot as per the processes described below.

Moisture content (MC): it was obtained using the oven-dry method at 105 °C for 24 h (Brasil, 2009). The results were expressed as a percentage (on wet basis) for each lot.

Germination test (GT): in conformity with the Instructions for Forest Seed Testing (Brasil, 2013), in the germination test, four sub samples of 25 seeds per lot were used. The seeds were distributed on germination paper imbibed in distilled water at a proportion of 2.5 times the weight of dry paper, and placed in a germination chamber at 25 °C, without controlling the photoperiod, which occurred according to the length of the day. Counts were carried out on the fifth and sixteenth days after sowing. The results were expressed as percentage of normal seedlings, calculating first count and final germination values.

Germination speed index (GSI): it was determined based on the daily germination count and calculated using equation 1, where Gn is the number of germinated seeds on the nth day and N is the number of days after sowing (Maguire, 1962).

Seedling emergence in the greenhouse (SE): four replications of 25 seeds for each lot were planted on polyethylene trays using MaxFertil substrate, produced with composted *Pinus* sp. shell, exfoliated vermiculite, and basis fertilizer. Daily irrigation was carried out by supplying water until reaching substrate retention capacity. Assessment was conducted on the seventh and sixteenth day after assembling the test, considering seedlings with primary leaf edges that no longer touched as emerged. The results were expressed as a percentage.

Electrical conductivity (EC): four replications of 25 seeds per lot were weighed on scales accurate to 0.001 g, and imbibed in 100 mL disposable cups containing 75 mL of deionized water at 25 °C for 24 h. After the imbibing period and uniformization of the electrolytes leached into the solution, electrical conductivity of the solution was determined through reading on a conductivity meter and the results were expressed in $\mu S.cm^{-1}.g^{-1}$ (Baalbaki et al., 2009).

Evaluation of ethanol production (EE): two trials were carried out with four replications per lot. The seeds were placed in 30 mL glass vials, and the quantity of water established as being sufficient for metabolism activation was added to each trial. The vials were closed with a rubber lid and metal seals using a manual flip off crimper and maintained at 40 °C for different periods according to the trial (Buckley and Huang, 2011).

In trial I, 10 seeds were used with five volumes of water for imbibition (0.25; 0.5; 0.75; 1.0; and 1.25 mL) and six reading periods (2, 4, 6, 8, 24, and 48 h). In trial II, three quantities of seeds (5, 10, and 15) were used with a volume of water for imbibition proportional to 2.5 times the weight of the seeds, for the same six reading periods.

The ethanol concentration in the vial was measured in the pre-established periods with a DrägerAlcotest® 6810 modified ethylometer, with a coupled metallic needle, which is operated through a suction mechanism of one gas aliquot from the interior of the vial, expressed in $\mu g.L^{-1}$.

Statistical analysis

Statistical modeling was carried out to identify the ethanol prediction as a result of the tested factors (seed quantity, volume of water for imbibition, and reading periods), adjusted with gamma distribution and identity function. Two models were formulated and differentiated in the fraction of the explicative variables component; one model with factor interaction (model 1) and the other without interaction (model 2). The fit of the models was verified using the Akaike information criterion (AIC) (Akaike, 1974).

To evaluate the seed lots, grouping analysis was conducted using the Euclidian measure of dissimilarity and Ward's method of hierarchical clustering. The group number was defined using the *Pseudo t2* index. A heat map was associated with the cluster analysis, in which purple, green, and white, respectively, indicate values above, below, and similar to the mean and color intensity matches the magnitude of comparison. Subsequently, the percentage contribution of each variable was estimated to form groups using the method of Singh (1981). All statistical analyses were performed on R (R Core Team, 2019).

RESULTS AND DISCUSSION

The artificial aging imposed on the lots resulted in anatomical alteration of the seeds (Figure 1). There was reduction of a cuticle or similar structure is located on the out surface of the seed coat from lots 3 and 4 (Table 1), which were subjected to aging for 48 and 72 h, respectively. The seeds moisture content (Table 1) increased proportionally to the amount of time they remained in humid conditions, resulting in an increase of 15.3 percentage points between the unaged lot (seed lot – 1) and the seeds subjected to 72 h of aging (seed lot – 4).

Cluster analysis with heat map (Figure 2) resulted in the formation of two groups, namely group 1 composed of seed lot – 1 and seed lot – 2 and group 2 composed of seed lot – 4 and seed lot – 3. The contrast pattern between the groups on the heat map is visually clear between the seed lots for most variables, with different color intensities within each group. Moreover, the lots are associated with different variables according to their standard of physiological potential and a general reduction in vigor was observed in the direction of the lots with longer aging time. Group 1 presented better results for electrical conductivity (EC), moisture content (MC), germination speed index (GSI), first germination count (FGC), germination percentage (GP), seedling emergence (SE), cuticle thickness (CT), and palisade thickness (PT) in comparison to group 2. The electrical conductivity (EC) tests detected a progressive increase in electrolyte leaching according to the period in which the seeds were maintained under the stressful conditions inherent to accelerated aging. In relation to the set of variables, two groups were also formed, with group I made up of electrical conductivity (EC), moisture content (MC), and hourglass thickness (HT) and group II consisting of the remaining variables (Figure 2).

Analysis of the Singh contribution revealed that the variables of first germination count (FGC) (40.35%), electrical conductivity (EC) (35.29%), moisture content (MC) (10.01 %), and seedling emergence (SE) (9.48%) presented greater lot discrimination power when compared to germination speed index (GSI) (2.47%), germination percentage (GP) (1.18%), cuticle thickness (CT) (0.85%), palisade thickness (PT) (0.35%) and hourglass thickness (HT) (0.02%) (Figure 3).

For evaluation of ethanol production (Trial I), the imbibition volume and reading period were significant factors in the proposed models (with and without interaction). However, the lower AIC value of model 2 (without interaction between the factors) qualifies it as more adequate for explaining the behavior of the ethanol response variable (Table 2).

When comparing significance among the reading periods, only the 48-h period was significant (Table 3), whereas all the levels among the volume factor were significant except 0.25 mL. Thus, the factors that were not statistically significant to the model were removed from the analysis and only the factors and their respective levels relevant (significant) for elucidation of the selected model were considered.

Plathymenia seeds produce ethanol in an anaerobic environment, whereby emission increased with the imbibition volume and the reading period, and was null for the 8 h period, regardless of the vigor level of the lot and imbibition volume, as well as for lot 1 at a volume below 1.0 mL.

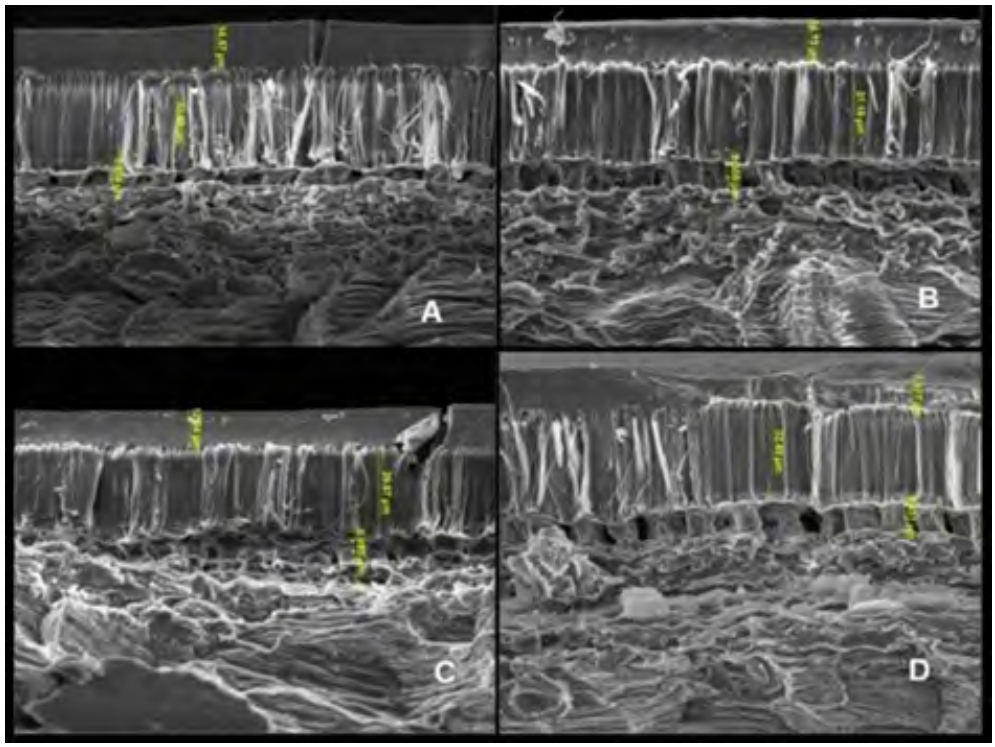

Figure 1. Scanning electron microscopy the integument of four *Plathymenia* seed lots. A: lot 1, B: lot 2, C: lot 3, and D: lot 4. Notes in yellow indicate the measurements of the layers of the cuticle (external), palisade cells (intermediate), and hourglass cells (internal).

Table 1. Anatomical characterization (thicknesses of the cuticle thickness (CT), palisade thickness (PT), and hourglass thickness (HT)) and moisture content (MC) for the four *Plathymenia* seed lots.

Lots	CT	PT	HT	MC
	---------- µm ----------			%
1	15.12	33.51	6.75	21.2
2	16.55	32.43	7.26	26.9
3	13.81	30.50	6.88	32.9
4	11.92	32.58	7.26	36.5
CV (%)	13.7	3.9	3.7	-

The ethanol quantity produced by the seeds depends on the vigor level. In lot 4, production increased sharply (795.0 $\mu g.L^{-1}$) when compared to lots 1, 2, and 3, which, in the same period (48 h) and at the same volume (1.25 mL), presented emissions of 17.0 $\mu g.L^{-1}$, 95.0 $\mu g.L^{-1}$, and 130.0 $\mu g.L^{-1}$, respectively. The same pattern occurred for the other volumes, although lots 1 and 4 showed a greater contrast and lots 2 and 3 were the most similar to each other (Figure 4).

For the second trial (Trial II), the tested factors (number of seeds and reading period) were significant in the proposed models. The interaction between them was significant (model 1). The lowest AIC value was also obtained in model 1 (Table 4), with an agreement between the Deviance criterion and the Akaike information criterion, that is, that function with less deviation also had the lowest AIC value, which facilitated selection of the most parsimonious model for evaluating the physiological quality of *Plathymenia* seeds.

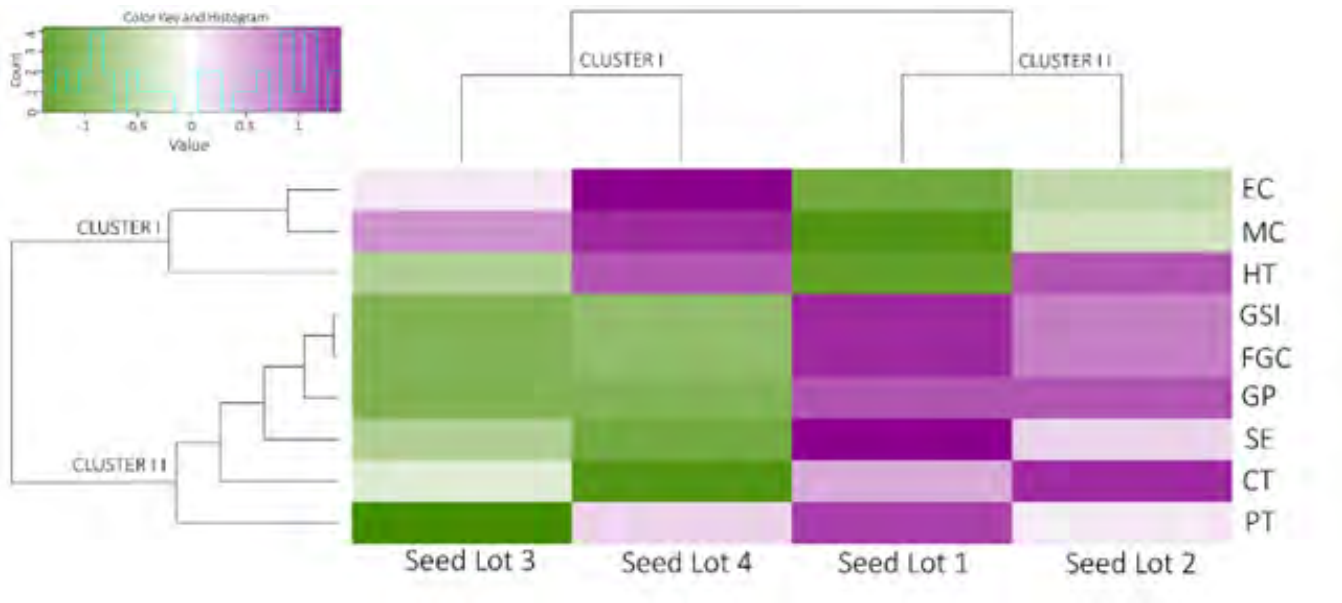

Figure 2. Heatmap grouping with physiological characterization for four batches of *P. reticulata* seeds according to Electrical conductivity (EC); Moisture content (MC); Hourglass thickness (HT); Germination speed index (GSI); First germination count (FGC); Germination percentage (GP); Seedling emergence (SE); Cuticle thickness (CT); Palisade thickness (PT).

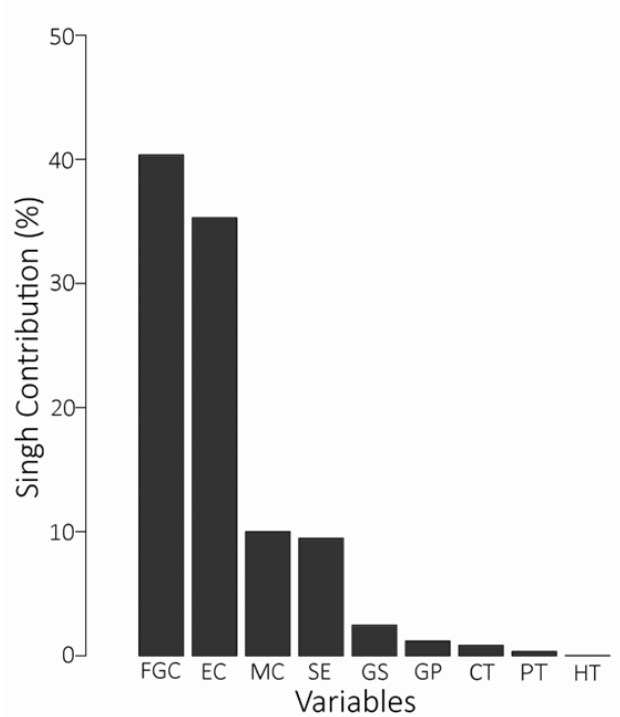

Figure 3. Relative contribution (Singh, 1981) of variables to divergence between seed lots. First germination count (FGC); Electrical conductivity (EC); Moisture content (MC); Seedling emergence (SE); Germination speed index (GSI); Germination percentage (GP); Cuticle thickness (CT); Palisade thickness (PT); Hourglass thickness (HT).

Table 2. Deviation analysis (ANODEV) for ethanol emission of *Plathymenia* seeds with two factors, modeled with interaction (model 1) and without interaction (model 2).

FV	DF	Difference DF	Model 1		Model 2	
			Deviance	F	Deviance	F
Null	0	28	39.36		39.36	
Volume	4	24	4.99	5.52*	4.99	3.37*
Period	1	23	24.47	108.36*	24.47	66.15*
Interaction	4	19	3.94	4.36*	-	
		AIC	350.50		347.70	

DF: Degree of freedom; F: Statistic of the Snedecor test; *p < 0.05.

Table 3. Estimates of coefficients, standard error of estimate and t-test value of all factors for Model 2 (no interaction).

Coefficient	Estimate	error	Valor-t
Intercepto	58.40	24.80	2.35*
Vol (0.5mL)	-1.26	32.28	0.03
Vol (0.75mL)	-38.62	25.74	-1.50
Vol (1.0mL)	-25.97	27.25	-0.95
Vol (1.25mL)	22.04	37.18	0.59
Per (48 hours)	348.57	62.90	5.54***

*p < 0.05; ***p < 0.001.

There was significance for both levels for the number of seeds and for the period of 48 h. The interactions between the period of 48 h and 10 and 15 seeds were also significant (Table 5).

The increase in the number of seeds in the samples and the reduction in the level of vigor of the lots resulted in greater production of ethanol by the seeds.

The greatest variation in emission and, therefore, sensitivity in the distinction of the lots occurred with the use of 15 seeds in the period of 48 h. Under these conditions, minimum emission was 55.0 $\mu g.L^{-1}$ (lot 1) and maximum emission was 1865.0 $\mu g.L^{-1}$ (lot 4).

The present study provides some important guidelines for the analysis of forest seed quality by defining a protocol for a vigor test with innovative and efficient technology, conditioned to an arboreal biological model. Based on the characterization carried out on the seed lots, the present findings are considered reliable since the simultaneous analysis of the variables (multivariate) demonstrates accurate separation in relation to the physiological quality of the lots used to test the experimental factors.

Anatomical evaluation indicated thickness reduction of the most external layer of the seed coat, responsible for protection and controlling of imbibition. This relationship has previously been observed in soybean seeds (Silva et al., 2008), Fabaceae such as *Plathymenia,* in which aging also caused anatomical alterations in the seed layers, followed by a reduction in germination and vigor.

During aging, *Plathymenia* seed moisture content also showed distinct patterns between the lots. However, the high-water absorption by the seeds during the process was insufficient to alter germination potential. Germination presented a low percentage contribution to the discrimination of the lots, which indicates that this variable is not sensitive enough to distinguish physiological potential. The germination test result alone does not always present values that are consistent with the true potential of the quality of a determined lot, as said test is carried out under

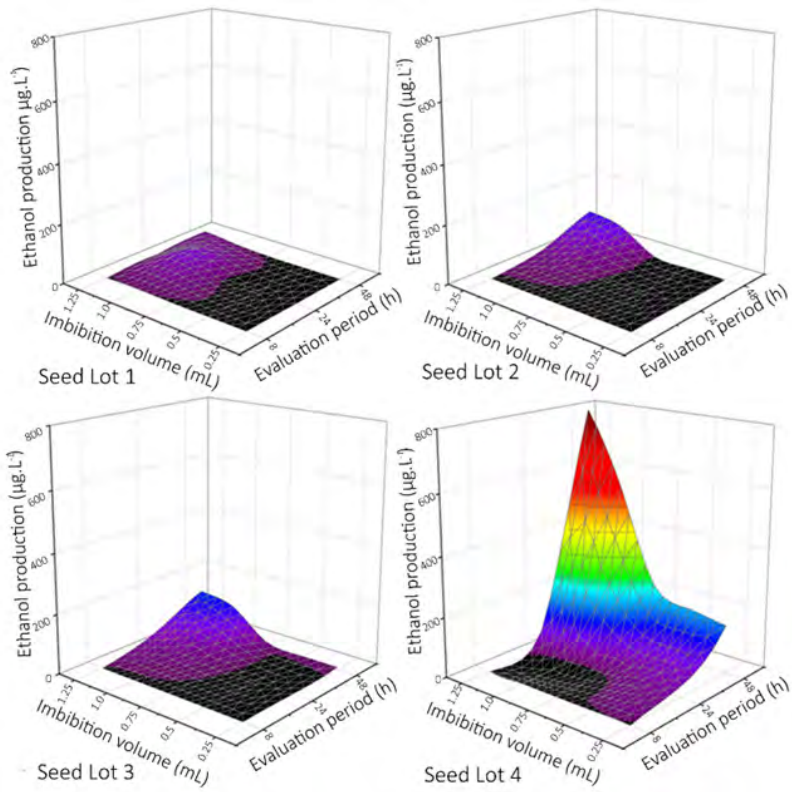

Figure 4. Ethanol production (µg.L^{-1}) as a function of four *Plathymenia* seed lots, imbibition volumes, and reading periods.

Table 4. Deviation analysis (ANODEV) for ethanol production of *Plathymenia* seeds with two factors, modeled with interaction (Model 1) and without interaction (Model 2).

FV	DF	Difference DF	Model 1		Model 2	
			Deviance	F	Deviance	F
Null	0	23	56.21		56.209	
Seeds	2	21	41.92	138.78***	41.92	38.75***
Period	1	20	4.27	28.26***	4.27	10.02*
Interaction	2	18	6.84	22.64***		
		AIC	276.7		297.6	

DF: Degree of freedom; F: Statistic of the Snedecor test; *p < 0.05 ***p < 0.001.

optimum conditions, making it impossible to detect small variations in performance potential (Hampton and Tekrony, 1995), which can lead to overestimation of lot quality. Therefore, this information should be complemented with vigor test results.

The high percentage contribution of first count and electrical conductivity to discriminating the lots demonstrates the potential of these variables as important tools for the separation of lots with high physiological potential. The electrical conductivity test is a biochemical test based on the principle that a process of deterioration leads to leaching of the cellular constituents due to the loss of integrity of the cellular membrane systems and reduced membrane repair speed (Ebone et al., 2019; Ma et al., 2020). When evaluating electrical conductivity, we observed that the quantity of leachates increased progressively in the direction of the more degraded lots. Inversely proportional to the damage

Table 5. Estimates of coefficients, standard error of estimate and t-test value of all factors for Model 1 (with interaction).

Coefficient	Estimate	Error	Value t
Intercept	22.5	4.37	5.14***
Seeds (10)	20.0	9.34	2.14*
Seeds (15)	375.0	77.36	4.84***
Period (48 hours)	32.5	11.54	2.81*
Seeds (10): Per (48 hours)	157.5	47.35	3.32**
Seeds (15): Per (48 hours)	1435.0	370.70	3.87**

*p < 0.05; **p < 0.01; ***p < 0.001.

to cellular membranes (Barbosa et al., 2021). The electrical conductivity increase indicates that there was a gradual reduction in seed vigor, this being observed at the level of membrane systems. Seeds with low vigor and, therefore, high electrical conductivity (Ma et al., 2020), perform poorly in the resumption of metabolic activities towards germination. This is due to the low number of mitochondria during metabolism reactivation and the consequent low rate of respiration and energy production (Borella et al., 2013), leading to the results found for first count and seedling emergence, which also exhibit relevant lot discrimination power.

After ascertaining a reduction in vigor of the formed lots, the demands of a vigor test can be met by comparing lots with differences in performance potential. This can be done by measuring endogenous ethanol in partially imbibed seeds as it is a volatile organic compound produced as part of a fermentation process (Kucukhuseyin et al., 2021). The vigor test based on the production of ethanol proved sensitive to the detection of deterioration at different levels, which suggests the capacity to interfere in the physiological state of the lots and assertively discriminate them, even in lots that had their physiological quality overestimated in the germination test.

Furthermore, the emission of ethanol was consistent with the classes of vigor previously established in the accelerated aging test and with the results of electrical conductivity, germination speed index, and seedling emergence. As such, emission increases in the direction of more degraded lots, converging with the decline in vigor detected by said tests. In fact, a relationship between ethanol production and seed deterioration has also been reported for melon (Ornellas et al., 2020), and caupi bean (Cavalcante et al., 2019), demonstrating that ethanol is a deterioration biomarker (Colville et al., 2012). Its production results from mitochondrial cell loss (Buckley and Huang, 2011), here resulting from damage accumulated during aging and the consequent production of metabolic energy through alcoholic fermentation by converting pyruvate into ethanol (Benamar et al., 2003; Ornellas et al., 2020).

To establish the efficiency of a test, it is also important to define the conditions for said test. Thus, for the correct execution of the ethanol test and, therefore, its reliability, assessment time, and number or mass of seeds should ensure ethanol production in sufficient quantity to enable detection using the device and supply information on the state of deterioration of the lots. For cabbage seeds, a few hours of evaluation were sufficient to differentiate lots as per vigor (Kodde et al., 2012), which did not occur with *Plathymenia*, for which null or low ethanol production was observed in the short periods, even in more degraded lots.

Plathymenia is a species that presents orthodox seeds (Carvalho, 2009) with integumentary dormancy (Borges et al., 2019), plausibly suggesting that they possess protective structures that act as barriers to the entrance of water, which would make absorption in short periods difficult, resulting in low ethanol emission in such conditions. It should be emphasized that each species has unique behavior, thus indicating the need for research approaches of this magnitude for other species, especially native forest species, with the aim of formulating protocols and detecting possible patterns among this group of seeds.

When setting the number of seeds for testing, there was differentiated behavior in ethanol production in favor of a higher quantity of seeds. Considering the interactions, only 10 and 15 seeds with a period of 48 h demonstrated as significant, although it is worth mentioning that when evaluated in isolation, 15 seeds showed greater significance than the interactions. The use of 15 seeds in the 48 h-period also involved maximization of the differences between lots when compared to the use of 10 seeds and other reading periods. This greater capacity to distinguish lots establishes this condition as ideal for testing, given that it is desirable to increase ethanol emission by low vigor seeds compared to high vigor seeds in order to enhance test reliability (Buckley and Huang, 2011).

Many forest species exhibit intrinsic characteristics that demand more time and specific conditions for testing to verify the physiological quality of seed lots. Others, given the wide diversity of species and their under-representation, are not contemplated by the normative instructions guiding their analysis (Brasil, 2009; Brasil, 2013), and, as such, little or nothing is known about how germination and vigor tests should be conducted. In this case, the ethanol test may be an efficient alternative to traditional testing, resulting in time optimization to obtain results and make decisions.

For *Plathymenia,* the final count in the germination test should occur on the sixteenth day (Brasil, 2013). With the ethanol test, results enabled the distinction between lots with differences in physiological potential in a trial of 48 h, these being especially compatible with electrical conductivity, germination speed index, and seedling emergence in a greenhouse. The saving of physical space that the ethanol test provides when compared, for example, to the germination test, should also be considered. This aspect, combined with the optimization of time to obtain results, could reduce costs in the production chain of the seeds and seedlings sector, which reinforces the use of ethanol production as a vigor test.

Although it is not the focus of the present study, we also believe that the generalized linear models provide more alternatives for the distribution of variables in seed studies (Santana et al., 2018), such as that carried out here. This technique has been used in seed technology research with the aim of clarifying the relationships between the different variables evaluating physiological potential. Moreover, it has been successfully used for soybean seeds (Barbosa et al., 2013), forage grasses of the *Brachiaria* genus (Silva et al., 2017; Silva et al., 2019), and caupi beans (Mendonça et al., 2018).

CONCLUSIONS

The ethanol test is efficient for the evaluation of vigor, as it enables a sensitive lot classification compatible with other tests. For *Plathymenia*, it was recommended that the test be conducted with 15 seeds imbibed in a quantity of water 2.5 times the mass of the seeds for 48 h. The ethanol test is an excellent choice for the distinction of seed lots and provides speed and reliability to seed quality control programs aimed at forest restoration.

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