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Adaptation Of the exudate pH Test for seeds of *Astronium urundeuva* (M.Allemão) Engl.

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A B S T R A C T

The need for rapid tests to assess the vigor of forest seeds for conservation and species propagation purposes has become increasingly significant. The objective was to adapt the exudate pH test for evaluating seeds of *Astronium urundeuva*, popularly known as “aroeira-do-sertão”. The study was conducted at the Forest Seed Analysis Laboratory of the Department of Forest Science at the Federal Rural University of Pernambuco, using five seed lots. The initial characterization of the lots included the following tests: moisture content, thousand-seed weight, first germination count, germination speed index, and germination percentage. Three seed quantities (25, 30, and 40 seeds per lot), three volumes of deionized water (50, 75, and 100 mL), and four evaluation periods (3, 6, 12, and 24 hours) were tested, totaling 36 treatments. A completely randomized design (CRD) was applied in a triple factorial scheme (number of seeds × water volume × time), with four replicates per lot. The data were subjected to analysis of variance (ANOVA), and mean comparisons were performed using Tukey’s test ($p \leq 0.05$) through the Rbio software. The results demonstrated that the exudate pH test is effective for evaluating the vigor of *A. urundeuva* seeds. The most suitable methodology involved 50 mL of distilled water, 12 hours of imbibition, and 30 seeds. These approaches are efficient and require smaller amounts of water, time, and seeds, while still providing reliable results

Keywords: *Myracrodruon urundeuva*, physiological quality, rapid tests, vigor, forest seeds

Adaptação do teste de pH do exsudato para sementes de *Astronium urundeuva* (M.Allemão) Engl.

R E S U M O

A necessidade de testes rápidos para avaliar o vigor de sementes florestais com fins de conservação e multiplicação de espécies é cada vez mais significativa. Objetivou-se adaptar o teste de pH do exsudato para a avaliação de sementes de *Astronium urundeuva*, popularmente conhecida como “aroeirado-sertão”. O estudo foi conduzido no Laboratório de Análise de Sementes Florestais do Departamento de Ciência Florestal da Universidade Federal Rural de Pernambuco, utilizando cinco lotes de sementes. A caracterização inicial dos lotes incluiu os seguintes testes: teor de água, peso de mil sementes, primeira contagem de germinação, índice de velocidade de germinação e porcentagem de germinação. Foram testadas três quantidades de sementes (25, 30 e 40 sementes por lote), três volumes de água deionizada (50, 75 e 100 mL) e quatro períodos de avaliação (3, 6, 12 e 24 horas), totalizando 36 tratamentos. Foi aplicado um delineamento inteiramente casualizado (DIC), em esquema fatorial triplo (número de sementes × volume de água × tempo), com quatro repetições por lote. Os dados foram submetidos à análise de variância (ANOVA), e as comparações de médias foram realizadas pelo teste de Tukey ($p \leq 0{,}05$) por meio do programa Rbio. Os resultados demonstraram que o teste de pH do exsudato é eficiente para avaliar o vigor de sementes de *A. urundeuva*. A metodologia mais adequada envolveu 50 mL de água destilada, 12 horas de embebição e 30 sementes. Essas abordagens são eficientes e exigem menores quantidades de água, tempo e sementes, ao mesmo tempo em que fornecem resultados confiáveis.

Palavras-chave: *Myracrodruon urundeuva*, qualidade fisiológica, testes rápidos, vigor, sementes florestais.

.Introduction

Astronium urundeuva (M. Allemão) Engl., whose basionym is *Myracrodruon urundeuva* (Mitchell and Daly, 2017; Silva-Luz et al., 2020), belongs to the family Anacardiaceae and is widely known in Brazil as “aroeira-do-sertão.” This species has a broad ecological distribution, occurring across several Brazilian biomes, including the Caatinga, Cerrado, Atlantic Forest, Pampa, and Pantanal (Pareyn et al., 2018; Lorenzi, 1992). Its wide geographic range reflects a high degree of ecological plasticity, enabling its establishment under contrasting environmental conditions, including regions characterized by seasonal drought and soil limitations.

In ecological terms, *A. urundeuva* plays an important role in forest structure and successional dynamics, particularly in dry forests and riparian environments. Recent studies have shown that the species can form monodominant stands in certain ecosystems, influencing biodiversity patterns and ecosystem functioning (Souza et al., 2024). These characteristics reinforce its importance in restoration programs, especially in degraded landscapes where native species are required to reestablish ecological processes and improve environmental resilience (Lázaro-González et al., 2023).

It is a dioecious species, with separate male and female individuals, and produces drupe-type fruits containing orthodox seeds measuring between 2 mm and 4 mm. Due to its multiple uses, *A. urundeuva* holds considerable ecological, economic, and cultural value, being widely used for timber, medicinal purposes, apiculture, and landscaping (Dantas and Kiill, 2023). Its wood is highly valued for durability, while its medicinal properties have been traditionally explored in different regions of Brazil. Such multifunctionality increases the demand for high-quality seeds, especially for commercial propagation and restoration initiatives.

Despite its relevance, there is still a limited number of studies focused on the physiological quality and vigor assessment of *A. urundeuva* seeds. This limitation represents a critical gap, as seed quality is one of the main factors determining germination success, seedling establishment, and long-term survival in restoration projects (Vasconcelos et al., 2020; Nascimento et al., 2022). In native forest species, variability in seed vigor can significantly affect the efficiency of restoration practices, particularly under environmental stress conditions such as water deficit and high temperatures (Silva, V. A. et al., 2023).

In recent years, ecological restoration has gained prominence as a global strategy to mitigate environmental degradation, biodiversity loss, and climate change impacts. In this context, the success of revegetation programs depends not only on species selection but also on the quality of propagules used, including seeds and seedlings (Lázaro-González et al., 2023). Studies have emphasized that inadequate seed quality can compromise establishment rates, increase costs, and reduce the effectiveness of restoration efforts, especially in large-scale initiatives (Souza et al., 2024).

The evaluation of seed vigor is therefore essential for ensuring the success of restoration and reforestation programs. Reliable vigor tests allow better decision-making regarding seed lot selection, storage conditions, and sowing strategies. Moreover, they contribute to optimizing nursery practices and improving the allocation of resources in seedling production systems (Medeiros et al., 2020; Vasconcelos et al., 2020). This is particularly relevant for native species, whose seeds are often available in limited quantities and may exhibit variable physiological behavior.

Traditionally, seed viability is assessed using the germination test, conducted under controlled laboratory conditions according to standardized procedures (Brasil, 2009). Although widely used, this method has limitations, as it evaluates seed performance under optimal conditions that may not represent field environments. Consequently, germination tests may overestimate seed quality and fail to detect differences in vigor among seed lots (Gagliardi and Marcos Filho, 2011; Cruz et al., 2020).

These limitations have driven the development of alternative and complementary methods capable of providing faster and more realistic assessments of seed physiological quality. Rapid tests have become increasingly important, as they reduce the time required for analysis and allow more efficient decision-making in seed technology and restoration programs (Brandani, 2017). Additionally, many of these methods are non-destructive, preserving seed viability while providing valuable information about membrane integrity and metabolic activity (ElMasry et al., 2020; Medeiros et al., 2020).

Among the rapid tests commonly used, the electrical conductivity test stands out for its simplicity and efficiency. It is based on the quantification of electrolytes released during seed imbibition, which reflects membrane deterioration and seed vigor (Torres et al., 2015; Azeredo et al., 2016). However, recent studies have highlighted the need for alternative approaches that can complement or refine vigor assessment, particularly for native forest species with specific physiological characteristics.

In this context, the exudate pH test has emerged as a promising tool for evaluating seed vigor. This method is based on changes in the pH of the imbibition solution caused by the release of organic acids and other solutes, which are associated with membrane integrity and metabolic activity (Souto et al., 2019; Melo, 2021). Due to its rapid execution, low cost, and ease of interpretation, the exudate pH test has potential for routine application in seed analysis laboratories.

Recent advances in seed technology have also incorporated complementary techniques such as X-ray imaging and biological treatments, allowing a more comprehensive evaluation of seed quality. These approaches integrate physical, physiological, and sanitary analyses, improving the accuracy of vigor assessment and supporting better decision-making (Moura et al., 2022; Silva et al., 2025). For *A. urundeuva*, such developments highlight the importance of establishing standardized and efficient methodologies tailored to the species.

Furthermore, the growing demand for restoration of degraded ecosystems, particularly in semiarid and tropical regions, has increased the need for rapid diagnostic tools capable of supporting large-scale operations. Efficient evaluation methods are essential for ensuring the use of high-quality seeds and maximizing restoration success under challenging environmental conditions (Lázaro-González et al., 2023; Souza et al., 2024).

Given this scenario, the development and validation of rapid vigor tests for native species such as *A. urundeuva* become highly relevant. These methods can contribute to improving seed quality control, optimizing restoration strategies, and enhancing the resilience of ecosystems under recovery.

Therefore, the objective of this study was to adapt and evaluate the exudate pH test as a rapid and efficient method for assessing the physiological vigor of *Astronium urundeuva* seeds. The study aims to contribute to the standardization of methodologies for native forest species, supporting conservation, restoration, and reforestation programs.

By improving the rapid diagnosis of seed vigor, this research also contributes to current discussions on ecological restoration efficiency, resilience of native species, and the rational use of propagules in environmentally sensitive landscapes.

Materials and methods

The study was conducted at the Forest Seed Analysis Laboratory, part of the Department of Forest Science at the Federal Rural University of Pernambuco (UFRPE). Five seed lots were evaluated (Table 1 and Figure 1).

Table 1. Identification, origin, and collection date of the five evaluated seed lots. Where: Pernambuco (PE) and Paraíba (PB).

Lots	City	State	Date colleted	Latitude	Longitude
1	Sertânia	PE	15/10/2020	-8.066448471	-37.2824614
2	Petrolina	PE	26/08/2021	-9.202241811	-40.45448629
3	Petrolina	PE	13/09/2022	-9.116970237	-40.67197536
4	Cachoeira dos Índios	PB	25/10/2022	-6.983060072	-38.68289508
5	Cajazeiras	PB	06/10/2022	-7.01831283	-38.5806159

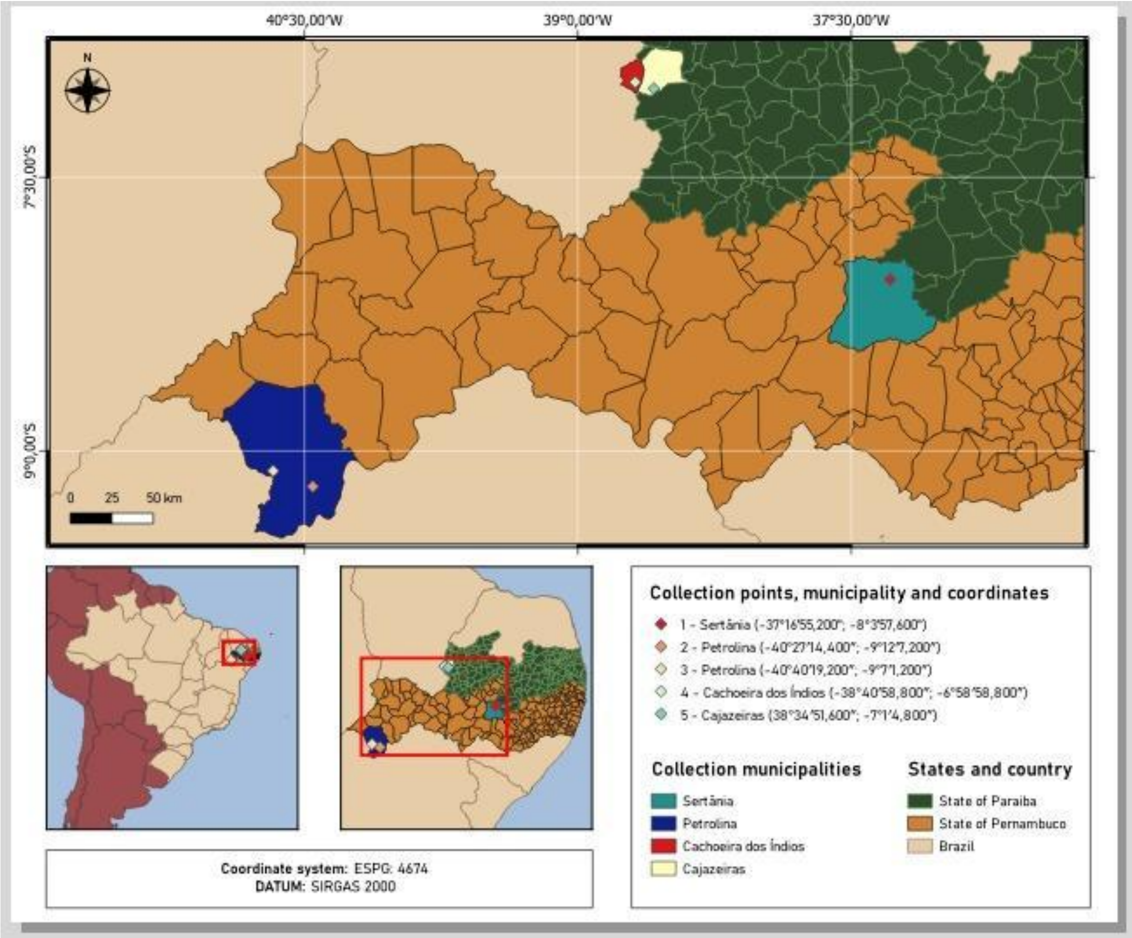

Figure 1. Identification and collection origin of the five *Astronium urundeuva* (M. Allemão) Engl. seed lots used in pH test. Locations: Pernambuco (PE) and Paraíba (PB).

The seeds were donated by the Seed Network of the São Francisco Integration Project (PISF) from the Ecological and Environmental Monitoring Center of the Federal University of the São Francisco Valley (NEMA/UNIVASF). The seeds underwent processing, including the removal of seeds that were visibly empty, immature, damaged by insects or injuries, and other impurities.

For the evaluation of the thousand seed weight, 800 seeds were divided into eight repetitions of 100 seeds each. The material was weighed on an analytical balance with a precision of 0.0001 g (Shimadzu ATX224) to determine the wet mass. The calculation of the thousand seed weight followed the formula described in the Rules for Seed Analysis (RAS) (Brasil, 2009).

The seed moisture content was determined according to the methodology of the Rules for Seed Analysis (RAS) (Brasil, 2009), which involves the initial weighing of the wet material followed by its dehydration in an oven at 105 ± 3 °C for 24 hours, using aluminum capsules. After drying, the moisture content was calculated by the difference between the mass of the wet and dry material.

The germination test was preceded by a seed cleaning process to prevent contamination. The seeds were immersed in a 2% sodium hypochlorite solution for 3 minutes. Subsequently, they were distributed in four repetitions of 25 seeds per lot. The material was placed in Gerbox containers, containing two sheets of Germitest paper that had been previously autoclaved and moistened with distilled water in a proportion of 2.5 times the weight of the dry paper (Brasil 2013).

The treatments were conducted in B.O.D. germination chambers, with the temperature set at 30 °C and a photoperiod of 8 hours daily (Brasil 2013). Seeds that produced normal seedlings, showing all essential structures, were considered germinated. The results were expressed as a percentage (%).

The first count test was performed concurrently with the germination test, counting the number of normal seedlings formed up to the fifth day after sowing, according to the protocol by Bhering et al. (2006). The germination speed index (GSI) was calculated based on the daily number of seeds germinated until stabilization, using the formula proposed by Maguire (1962).

For the adjustment of the exudate pH test, different seed quantities (25, 30, and 40 seeds from each lot), volumes (50, 75, and 100 mL) of distilled water, and imbibition periods (3, 6, 12, and 24 hours) were evaluated. All treatments were conducted in a completely randomized design (CRD) with a total of 4 repetitions, each consisting of 25 seeds. Before the tests, the seeds were disinfected in 2% sodium hypochlorite for 3 minutes, according to Brazil (2013). Subsequently, the seeds were placed in beakers containing the respective volumes of distilled water according to the treatments.

The trays containing the samples were then wrapped in aluminum foil and placed in a B.O.D. chamber, following the previously mentioned periods, under a constant temperature of 25 °C and a daily photoperiod of 12 hours (Avelino et al., 2018). Subsequently, the material was measured using a bench pH meter. The data were then subjected to analysis of variance (ANOVA). Afterward, mean comparison was performed using Tukey's test ($p \leq 0.05$) with the Rbio program (Bhering et al., 2017).**Results**

In the initial characterization of the lots, the data revealed (Table 2) that there was no statistical difference (5% significance) in the percentage of germinated seeds, germination speed index, root length, and dry mass of the seedlings among the evaluated lots. In the first count test, which measures the speed of translocation of assimilates from the seeds to the development of the normal seedling, it was observed that there was no similarity between lots 1 and 2 compared to lot 5.

However, in the final germination count, it was observed that there was a speed increase, with germination rates exceeding 70% for all lots. In terms of shoot length, only lots 1 and 5 were not statistically similar. There was a statistical difference in total length between lots 3 and 5.

Table 2. Water content (WC), 1000-seed weight (SW), germination (G), first count (FC), germination speed index (GSI), seedling length (SL), root length (RL), total length (TL), and dry matter (DM) of seedlings from five lots of *Astronium urundeuva* seeds.

Lots	**WC (%)**	**SW (g)**	**G (%)**	**FC (%)**	**GSI**	**SL (cm)**	**RL (cm)**	**TL (cm)**	**DM (g/p)**
1	13 a	10.33 c	87 a	2 b	7.1 a	2 b	0.5 a	2.5 b	0.9 a
2	9 a	9.61 d	71 a	3 b	7.1 a	2.4 ab	0.4 a	2.8 ab	0.9 a
3	10 a	11.83 a	78 a	3.3 ab	9.10 a	2.4 ab	0.4 a	2.8 b	0.9 a
4	11 a	10.73 b	72 a	5 ab	9.90 a	2.6 ab	0.5 a	3.1 ab	0.9 a
5	9 a	8.84 e	79 a	11 a	14.30 a	3 a	0.6 a	3.5 a	0.9 a
C.V. (%)	**21.85**	**-**	**19.45**	**92.54**	**41.5**	**12.2**	**19.8**	**11.47**	**2.56**

Averages followed by the same letters do not differ according to Tukey's test at 5% probability.

In relation to the adjustment of the exudate pH test, the results indicated that the most promising treatments, using a volume of 50 mL, were those with 30 seeds soaked for 12 hours or with 40 seeds soaked for 6 hours (Figure 2). These treatments showed performance similar to the germination test, standing out as ideal conditions for the procedure.

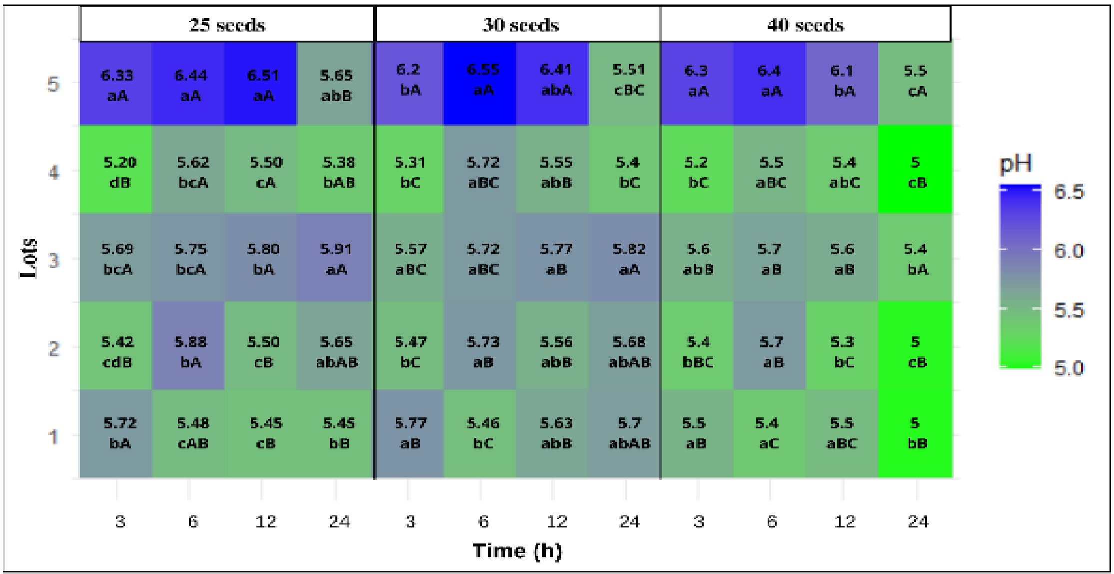

Figure 2. Heatmap representing the exudate pH of 5 seed lots of *Astronium urundeuva* (M. Allemão) Engl. after pre-conditioning in 50 mL volumes of distilled water with groups of 25, 30, and 40 seeds during the periods of 3, 6, 12, and 24 hours. Means followed by the same uppercase letters in the row (comparison between soaking times) and lowercase letters in the column (comparison between lots in the column) do not differ significantly from each other, according to Tukey's test at 5% probability.
Source**:** author, 2024.

In the 75 mL + 25 seeds treatments, the ANOVA results for the groups were not significant at 5% probability between soaking times and seed lots. Therefore, comparisons were made separately. As shown in Table 3, there was no statistical difference according to the soaking time, nor between the seed lots.

Table 3. Analysis of the pH of the exudate from 5 batches of *Astronium urundeuva* (M. Allemão) Engl. seeds after preconditioning in 75 mL of distilled water with groups of 25 seeds for periods of 3, 6, 12 and 24 hours.

Treatments			
75 mL + 25 seeds			
Time (h)	**pH**	**Lots**	**pH**
3	6.5 a	1	5.8 a
6	5.8 a	2	6 a
12	8.5 a	3	5.9 a
24	5.8 a	4	5.6 a
		5	9.8 a

Averages followed by the same letters do not differ according to Tukey's test at 5% probability.

However, the treatments involving soaking times of 12 hours, for both the 30- and 40-seed groups, showed results similar to those observed in the germination test (Figure 3).

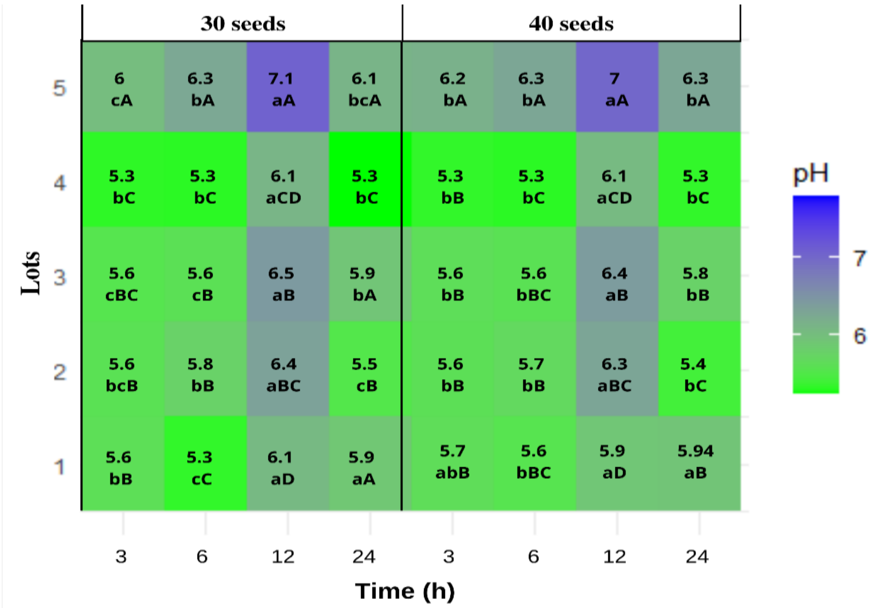

Figure 3. Heatmap representing the exudate pH of 5 lots of *Astronium urundeuva* (M. Allemão) Engl. seeds after pre-conditioning in 75 mL volumes of distilled water with groups of 30 and 40 seeds during 3, 6, 12, and 24 hours. Means followed by the same uppercase letters in the row (comparison between imbibition times) and lowercase letters in the column (comparison between lots in the column) do not differ from each other by Tukey's test at 5% probability.

Source: author, 2024.

1

For the treatments with a 100 mL volume, all seed groups with a 24-hour imbibition period showed results closer to the germination test. However, this volume showed a significant statistical differentiation for lot 5 compared to the other lots in all the applied treatments (Figure 4).

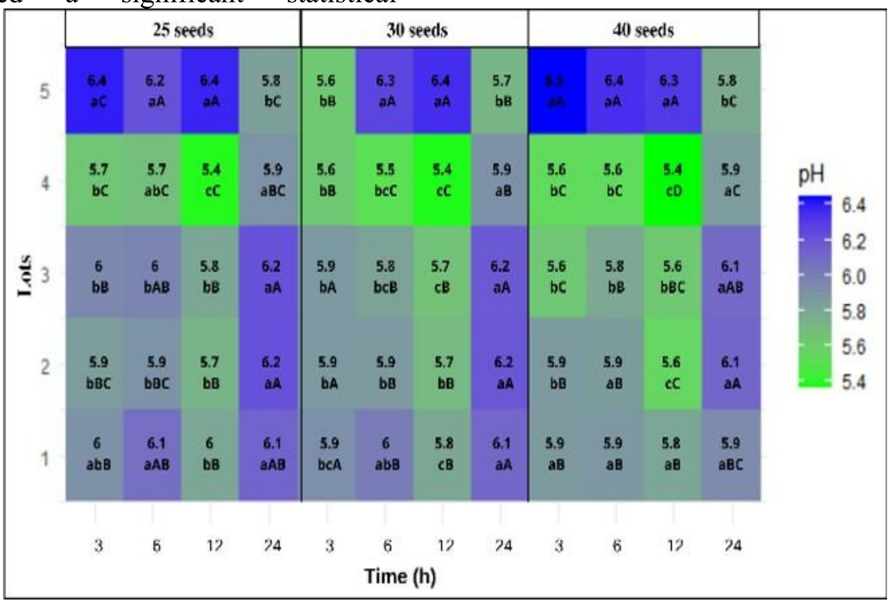

Figure 4. Heatmap representing the exudate pH of 5 seed lots of *Astronium urundeuva* (M. Allemão) Engl. after pre-conditioning in 100 mL volumes of distilled water with groups of 25, 30, and 40 seeds during the periods of 3, 6, 12, and 24 hours. Means followed by the same uppercase letters in the row (comparison between imbibition times) and lowercase letters in the column (comparison between lots in the column) do not differ from each other, according to Tukey's test at 5% probability.

Source: author, 2024.

Discussion

The analysis of thousand-seed weight revealed a statistically significant difference among the evaluated seed lots, with lot 3 presenting the highest value and lot 5 the lowest. This variation may reflect genetic and environmental influences during seed development, including nutrient availability, water conditions, and maternal plant vigor, which directly affect seed filling and reserve accumulation (Nascimento et al., 2022; Marcos Filho, 2015).

In native forest species, such variability is particularly relevant, as seed collection often occurs under heterogeneous environmental conditions, resulting in differences in physiological quality among lots (Carvalho and Nakagawa, 2012; Ferraz and Calvi, 2011).

This heterogeneity represents an important limitation for seed standardization, especially in restoration programs that depend on seeds collected from different regions and environmental conditions. From a practical perspective, differences in thousand-seed weight are not merely descriptive attributes of the lots, but indicators of possible variation in reserve availability and initial seedling performance.

Heavier seeds tend to have a greater amount of stored compounds, which may favor the early stages of germination and seedling establishment, particularly under stressful conditions. However, this relationship is not necessarily direct or universal, since seed performance also depends on membrane integrity, metabolic activation, and tolerance to environmental constraints.

Thus, the significant difference detected among lots reinforces the need to interpret seed quality as a multidimensional attribute, rather than as a function of a single biometric parameter. Despite these differences, no significant variation was observed in germination percentage, which remained above 70% for all lots, indicating high viability.

However, differences observed in the first germination count and shoot length highlight a well documented limitation of germination tests: their reduced sensitivity in detecting differences in seed vigor (Silva, V. A. et al., 2023; Marcos Filho, 2015). Seeds may present similar final germination percentages while differing significantly in vigor, particularly in terms of germination speed and seedling uniformity, which are critical under field conditions.

The use of vigor tests as complementary tools to germination has been widely recommended, particularly for detecting differences among seed lots with similar viability (Krzyzanowski et al., 2020; Vieira et al., 1994; Hampton and TeKrony, 1995). This distinction between viability and vigor is especially important for native forest species destined for restoration programs.

Under field conditions, successful establishment rarely depends only on the capacity to germinate, but also on the speed and uniformity with which seedlings emerge and occupy the site. In operational settings, lots with similar final germination may behave very differently after sowing, which can compromise stand formation, increase the need for re-sowing, and reduce the efficiency of restoration investments.

Therefore, the lack of significant differences in final germination should not be interpreted as evidence of equivalent physiological performance among all lots. The absence of significant differences in germination speed index, root length, and seedling dry mass suggests a certain degree of physiological similarity among the evaluated lots.

However, it is important to consider that these parameters may not be sufficiently sensitive to detect early stages of deterioration, particularly those associated with membrane disorganization. Membrane integrity is a key factor in seed vigor and is one of the first systems affected during deterioration processes (Delouche and Baskin, 1973; Bewley et al., 2013).

Therefore, relying exclusively on these parameters may lead to an overestimation of seed quality, especially under controlled laboratory conditions. Another relevant point is that controlled laboratory tests tend to reduce the expression of environmental stressors that would normally intensify the performance differences among seed lots.

Under ideal moisture, temperature, and substrate conditions, seeds with moderate physiological impairment may still complete germination successfully. In contrast, under nursery or field conditions, even subtle differences in membrane organization, reserve mobilization, and metabolic efficiency may become more evident.

This helps explain why vigor tests are so important in seed technology: they capture dimensions of physiological quality that are not always fully expressed in conventional germination assays. In this context, the exudate pH test demonstrated the ability to detect differences among seed lots under specific experimental conditions, supporting its use as a vigor assessment tool. The observed influence of seed quantity and soaking duration indicates that the test is sensitive to experimental configuration, which is consistent with findings reported for other biochemical tests based on solute leakage (Dalanhol et al., 2014; Torres et al., 2015).

The inverse relationship between seed number and soaking time suggests that the concentration of released solutes plays a key role in determining pH variation, highlighting the importance of standardizing test conditions. This result has important methodological implications. A vigor test is only useful in routine practice when it combines physiological meaning with operational reproducibility. In the case of the exudate pH test, the sensitivity to seed number, water volume, and imbibition period indicates that minor procedural deviations may change the outcome substantially.

On the one hand, this sensitivity demonstrates that the method is responsive to physiological differences among lots; on the other hand, it shows that the test cannot be applied loosely without precise calibration. For this reason, standardization is not a secondary aspect of the method, but a central requirement for its scientific and practical validity. The combinations of 30 seeds for 12 hours and 40 seeds for 6 hours, using 50 mL of distilled water, produced results comparable to the germination test, indicating their suitability for vigor evaluation.

However, it is important to emphasize that this equivalence does not necessarily imply identical sensitivity. While germination tests assess the final expression of seed viability, the exudate pH test reflects early physiological changes associated with membrane integrity.

Therefore, these methods should be interpreted as complementary rather than interchangeable, as previously discussed for other vigor tests (Matthews and Powell, 2006; Vieira and Marcos Filho, 2020). Among the proposed conditions, the treatment with 30 seeds for 12 hours appears particularly promising because it combines discrimination capacity with greater operational simplicity.

In practical terms, a protocol that requires fewer seeds and an intermediate soaking period may be easier to incorporate into laboratory routines, especially when seed availability is limited or when many lots must be analyzed simultaneously. This is highly relevant for native forest species, whose seed supply is often irregular and seasonal. In such cases, reducing sample size without compromising diagnostic quality is a substantial advantage.

The 12-hour soaking period proved to be a stable condition across different treatments, suggesting its potential as a standard for the species. Similar results have been reported for other forest species, in which intermediate imbibition periods provided better discrimination among seed lots (Dalanhol et al., 2014).

However, it is important to note that the optimal soaking time may vary depending on seed size, permeability, and physiological status, which limits the direct extrapolation of these results to other species. This apparent stability at 12 hours may reflect a balance between two opposing effects. Shorter periods may be insufficient for generating detectable pH differences among lots, whereas very long periods may amplify leakage to the point of increasing noise and reducing consistency.

Intermediate imbibition periods may therefore offer a better compromise between sensitivity and analytical stability. Even so, this interpretation should be treated with caution, since the physiological behavior of seeds during imbibition is strongly species-specific and can also vary according to storage history, maturation stage, and seed coat properties. Treatments involving larger water volumes (100 mL) and longer soaking periods (24 hours) resulted in greater differentiation among seed lots, particularly highlighting the lower performance of lot 5. This behavior can be explained by increased solute leakage during prolonged imbibition, which amplifies the effects of membrane deterioration in low-vigor seeds (Ferreira et al., 2020; Bewley et al., 2013).

Although this condition may increase test sensitivity, it also introduces greater variability, which can compromise the repeatability of the method under routine laboratory conditions. Therefore, there is a trade-off between sensitivity and stability that must be considered when defining standard protocols. This trade-off is one of the most relevant outcomes of the present study. In seed testing, the most sensitive condition is not always the most appropriate one for standardization. Excessively sensitive conditions may accentuate differences among lots, but they may also make the test more unstable, more dependent on small procedural variations, and therefore less suitable for routine decision-making.

For seed analysis laboratories, a robust method is often preferable to a highly reactive one if the former generates more repeatable and interpretable results. In this sense, the proposed protocol appears to favor analytical consistency without losing practical discrimination capacity. The relationship between membrane integrity and solute leakage is well established in seed physiology. Seeds with lower physiological quality tend to release higher amounts of electrolytes and organic compounds, forming the basis of tests such as electrical conductivity and related approaches (Matthews and Powell, 2006; Torres et al., 2015; Azeredo et al., 2016).

In this study, the exudate pH test showed behavior consistent with these principles, reinforcing its physiological basis and supporting its applicability as a vigor test. Similar responses have been reported for native forest species such as *Libidibia ferrea* and *Peltogyne confertiflora* (Souto et al., 2019; Ferreira et al., 2020), suggesting that this approach may have broader applicability among native forest species. In broader terms, these results contribute to the ongoing effort to adapt classical seed physiology tools to native forest species, for which standardized methodologies are still less available than for agricultural crops. This is an important scientific contribution because native species frequently present greater variability in seed size, dormancy behavior, maturation stage, and post-harvest handling.

As a result, methods that are well consolidated for crop seeds often need careful adjustment before they can be transferred to forest species. The present study advances this process by showing that the exudate pH test can be physiologically meaningful and operationally feasible for *A. urundeuva*. Rapid vigor tests have gained increasing importance due to their efficiency and ability to support decision-making in seed technology and restoration programs (Powell, 1986; Marcos Filho, 2015). From an applied perspective, the results obtained in this study indicate that the exudate pH test can contribute to improving seed quality assessment by providing faster responses compared to traditional methods.

However, it is important to avoid overgeneralization. Although high seed vigor is associated with improved establishment potential, restoration success depends on multiple interacting factors, including soil conditions, climate variability, species interactions, and management practices (Palma and Laurance, 2015; Pedrini et al., 2020; Lázaro-González et al., 2023). From the perspective of the Sustainable Development Goals (SDGs), this applied relevance is particularly associated with SDG 15 (Life on Land), because improving the physiological assessment of native seeds can support the production of higherquality propagules for restoration and conservation actions. There is also an indirect connection with SDG 13 (Climate Action), since the success of ecological restoration contributes to increasing ecosystem resilience and to the recovery of degraded landscapes that play an important role in climate regulation.

In addition, the use of faster and less resourceintensive seed testing protocols is aligned, in a more indirect way, with SDG 12 (Responsible Consumption and Production), as it favors more efficient use of water, time, and biological material in laboratory routines. These connections should be interpreted carefully: the present study does not measure restoration outcomes directly, but it does provide a technical contribution that may strengthen the seed-quality component of broader environmental strategies.

The relationship between seed vigor and seedling establishment is widely recognized, but it is not always linear or predictable. While highvigor seeds generally produce more robust seedlings, field performance may be influenced by environmental variability and biotic interactions. In this sense, the absence of field validation represents an important limitation of the present study.

Future research should investigate the correlation between exudate pH results and seedling emergence under field conditions, as well as long-term establishment and survival. Another limitation is related to the number of seed lots evaluated.

Although five lots were used, they may not represent the full range of variability of the species, particularly considering its wide geographic distribution. Additionally, factors such as seed storage conditions, age, and genetic origin were not explicitly controlled, which may influence the observed responses. These aspects should be addressed in future studies to strengthen the robustness and generalizability of the method.

It is also worth noting that the proposed discussion of ODS/SDGs and restoration relevance should not obscure the methodological nature of the present contribution.

The central advance of this study is not the demonstration of restoration success itself, but the refinement of a rapid vigor test for a native forest species of ecological and practical interest. This distinction is important because it preserves the analytical rigor of the study while still recognizing its potential contribution to wider environmental agendas.

Furthermore, the sensitivity of the exudate pH test to experimental conditions highlights the need for careful standardization. Small variations in seed number, water volume, or soaking time may significantly affect the results, which may limit its reproducibility across laboratories.

Therefore, the establishment of standardized protocols is essential before recommending large-scale application. Future work could also explore integrated validation strategies, combining the exudate pH test with electrical conductivity, image-based analysis, emergence tests, and storage studies. Such an approach would help clarify whether the method is best used as a screening tool, a confirmatory assay, or part of a broader decision framework for seed lot classification.

In the context of native forest species, where biological material is often limited and operational decisions must be made efficiently, the development of complementary diagnostic systems may be more valuable than reliance on any single isolated test.

Overall, the results demonstrate that the exudate pH test is a promising tool for evaluating the physiological quality of *Astronium urundeuva* seeds. Its main advantages include rapid execution, low cost, and reduced use of biological material. However, its application should be considered complementary to other vigor tests, and further validation under field conditions is necessary to confirm its practical relevance in ecological restoration programs.

Conclusion

The exudate pH test proved to be an effective, rapid, and practical method for evaluating the physiological quality of *Astronium urundeuva* seeds. The technique was sensitive in distinguishing differences among seed lots, allowing faster vigor assessment compared to traditional methods, which are often more time-consuming and less responsive to subtle variations in physiological quality.

The most suitable condition for standardizing the test was 50 mL of distilled water, 12 hours of soaking, and 30 seeds per repetition, ensuring consistent and reliable results while reducing the use of time, water, and biological material. This optimization represents an important advantage for routine laboratory analyses, particularly when working with native species whose seeds are often available in limited quantities.

The methodology demonstrated strong practical applicability for seed analysis laboratories and ecological restoration programs involving native forest species. In scenarios where rapid decision-making is required, such as large-scale restoration initiatives, the use of efficient and low-cost methods can significantly improve operational performance and increase the likelihood of successful seedling establishment.

In addition, the exudate pH test can be used as a complementary tool to germination and other vigor tests, contributing to a more comprehensive and reliable assessment of seed quality. Its integration into seed quality evaluation protocols may enhance the selection of high-vigor seed lots, optimizing the use of propagules in restoration and conservation strategies.

Future studies should further investigate the performance of this method under different storage conditions, seed origins, and environmental contexts. Additionally, research focusing on the relationship between exudate pH results and field emergence, seedling establishment, and long-term plant performance would be valuable to validate its applicability under real restoration conditions. Expanding the use of this methodology to other native forest species may also contribute to the development of standardized and efficient protocols for seed vigor evaluation in diverse ecological contexts.

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Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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