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Technical note

DOES STORAGE OF *Eugenia stipitata* SEEDS AFFECT THEIR GERMINATION AND EFFICACY OF THE TETRAZOLIUM VIABILITY TEST?

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ABSTRACT

Eugenia stipitata is a fruit species from the Amazon that is important in traditional medicine due to its therapeutic properties. Estimates of seed viability in this species are still scarce, and it is necessary to develop rapid tests to determine the quality of the seeds. The aim of this study was to determine the applicability of the tetrazolium test to estimate viability and germination in seeds of *E. stipitata* with and without storage. The design was completely randomised in a 2 x 2 factorial scheme with 4 replications. The results of the tetrazolium and germination tests showed that seeds of *E. stipitata* lose their viability when stored for six months in water at ambient temperature, so neither small nor large seeds stored for that time in water are recommended for use with the tetrazolium test. Small, freshly harvested, unstored seeds show a germination potential of 100%. The tetrazolium test is efficient in determining the viability of small and large seeds of *Eugenia stipitate* with no storage.
Additional keywords: Araçá-boi, medicinal plants, seed quality

RESUMEN

¿Cómo influye el almacenamiento de semillas de *Eugenia stipitata* en la prueba del tetrazolio y en la germinación?

Eugenia stipitata es una especie frutal de la Amazonía, con importancia en la medicina tradicional por sus propiedades terapéuticas. Las estimaciones de viabilidad de semillas de esta especie aún son escasas, por lo que es necesario desarrollar pruebas rápidas para conocer la calidad de las semillas. En vista de lo anterior, el objetivo fue determinar la aplicabilidad de la prueba de tetrazolio para estimar la viabilidad y germinación de semillas de *E. stipitata* con y sin almacenamiento. El diseño fue completamente al azar, en arreglo factorial 2 x 2, con 4 repeticiones. Los resultados de las pruebas de tetrazolio y germinación demuestran que las semillas de *E. stipitata* pierden su viabilidad cuando se almacenan durante seis meses en agua a temperatura ambiente, por lo que las semillas pequeñas o grandes de *E. stipitata* almacenadas durante ese tiempo no están indicadas para realizar la prueba de tetrazolio. Las semillas pequeñas recién cosechadas sin almacenamiento exhiben un potencial de germinación del 100%. La prueba de tetrazolio es eficiente para determinar la viabilidad de semillas pequeñas y grandes de *E. stipitata* sin almacenamiento.
Palabras clave adicionales: Araçá-boi, calidad de la semilla, especies medicinales, viabilidad

INTRODUCTION

Originally from the Peruvian Amazon and western region of the Brazilian Amazon, *Eugenia stipitata* (araçá-boi) is a fruit species that in Peru, Bolivia, Ecuador, Colombia and Brazil is generally cultivated on a small scale (Moura et al., 2016), and serves as an alternative income for the local population of the northern Amazon, whether by marketing the fruit or the production of seeds and/or seedlings (Silva et al., 2016).

Despite the importance of the species in the Amazon region, research on seeds of native fruit species is scarce, especially regarding storing and maintaining seed viability over a long period; quality seeds must be stored safely and correctly in order to maintain their physiological quality (Souza et al., 2019).

In practical terms, the longevity of recalcitrant seeds, such as those of *E. stipitata*, is the most important factor when storing the seeds. The causes of the loss of viability in recalcitrant seeds when stored under wet conditions (water) are unknown, however, results presented in the review

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by Gomes et al. (2006) suggest this may be a result of their germinative metabolism, since once fully hydrated, the seeds germinate, and wet storage becomes a viable option.

This is why seeds that are sensitive to drying need to be stored with a sufficient supply of water to prevent dehydration (Mata and Moreno, 2005) and maintain the mechanisms linked to root protrusion and germination (Magnitskiy and Plaza, 2007). According to Gentil and Ferreira (1999), the seeds of *E. stipitata* are sensitive to drying, and have a lethal water content of around 26 %, when drying the seeds markedly reduces germination.

This is why obtaining information on seed tolerance to drying relative to germination power is of great importance, since the germination test is the most-used official parameter to evaluate the physiological quality of seed batches (Souza et al., 2017; Menegatti et al., 2019; Smiderle et al., 2021). The above authors, however, attributed serious limitations to the germination test. In addition to the delay when carrying out the test, it is not precise in identifying the factors that affect seed quality, and the results may be altered by the presence of biotic or abiotic factors.

Evaluating the physiological quality of seeds using rapid tests that can provide reproducible results has been a constant aim of seed technologists (França and Krzyzanowski, 2022). Among the indirect tests that are considered rapid, the tetrazolium test is important: in addition to evaluating viability and vigour, in some cases it allows the factors that influence seed quality to be identified, such as mechanical damage and the damage caused by drying, insects and deterioration (Paraíso et al., 2019).

The tetrazolium test is based on the change in color of the seed tissue in the presence of a tetrazolium salt solution, which is reduced by the dehydrogenase enzymes of living tissue, resulting in a compound known as formazan that is carmine-red in color. Dead or very deteriorated tissue remains uncolored.

The staining pattern of the tissue can be used to identify viable and non-viable seeds, and those of high and low vigour, which are considered viable (França and Krzyzanowski, 2022). Bright red or bright pink staining develops when the absorbed tetrazolium solution interferes with the reduction processes in respiration of the living cells by accepting a hydrogen ion (França and Krzyzanowski, 2019).

The tissues of a viable embryo absorb the tetrazolium solution slowly and tend to develop lighter staining than do decayed embryos, which take on a strong carmine-red color. The presence of unstained, i.e. dead, tissue is usually characterised by a white or yellowish color and flaccid texture.

Given the above, the aim of this study was to determine the applicability of the tetrazolium test in estimating viability and germination in stored and unstored seeds of *E. stipitata*.

MATERIALS AND METHODS

Experimental area. The study was conducted at the Seed Analysis Laboratory (LAS) and greenhouse of Embrapa Roraima. The species used was araçá-boi (*E. stipitata* McVaugh ssp. *sororia* McVaugh, Myrtaceae). The fruit was collected manually from trees of the Germplasm collection of the Federal University of Roraima (UFRR), Cauamé Campus, in Boa Vista, Roraima, at 2°52'14" N; 60°42'47" W (Figure 1).

Figure 1. Location and distribution of parent trees in the Araçá-boi (*Eugenia stipitata* McVaugh ssp. *sororia* McVaugh, Myrtaceae) Germplasm Collection, Boa Vista, Roraima.

The climate in Boa Vista is type Am (tropical monsoon). The average annual temperature is 25.4 °C, with an annual rainfall of 1808 mm.

Experimental design and conduct of the study. After harvesting the fruit of *E. stipitata*, post-harvest management was carried out as per Moura et al. (2016), following which the seeds were processed as recommended by Silva et al. (2016).

Seeds whose external appearance was intact were separated into two weight classes, small and large, based on individual fresh weight that was determined using a precision balance. Seeds with a weight between 0.80 and 1.20 g were classified as small, with those from 1.40 to 2.50 g being classified as large.

The water content of the seeds was determined for each weight class. Two 5-g seed samples were placed in an oven (105 ± 3 ºC) where they were kept for 24 hours. To test for a very long storing time the seeds were kept for six months in 5.0-L plastic buckets filled to the top with distilled water, with the water changed at regular seven-day intervals.

The experimental design was completely randomized in a 2 x 2 factorial scheme with four replications, each of 10 seeds. The first factor comprised the two seed classes (small and large) and the second factor consisted of the two storage periods (0 and 6 months).

The recently collected seeds, i.e. with zero storage, and those with six months storage were then subjected to 30 hours immersion in a 1% solution of 2,3,5-triphenyl-tetrazolium chloride (pH 6.5 to 7.0) in a 50-mL plastic container. The materials were then placed in a Biochemical Oxygen Demand (BOD) chamber set to 26ºC in the absence of light for both experiments.

Following immersion in the solution, the seeds were drained and washed under running water. The seeds remained submerged in water in a controlled environment at 22 ºC until it was time to evaluate the staining. A bench desk magnifier (6x) with a fluorescent lamp was used in both experiments to aid in observing the details of the seeds.

There was a difference in tissue staining based on the categories established in the tetrazolium test (Delouche et al., 1976; Bhéring et al., 1996). Category 1 (viable): embryo with a pink coloration and tissue with a normal and firm appearance; Category 2 (unviable): seeds with the white, yellowish or cream coloration characteristic of dead tissue; Category 3 (unviable): seeds with strong carmine-red coloration, characteristic of decaying tissue (Figure 2).

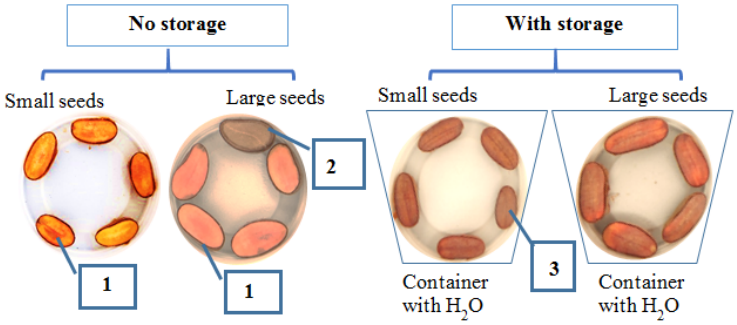

Figure 2. Staining in small and large seeds of *Eugenia stipitata* submitted to the 1 % tetrazolium tes at zero time (no storage) and at six months (with storage).

After completing the tetrazolium test, a new batch of the seeds were sown to a depth of 1.0 cm in plastic trays of 30 cm x 40 cm x 10 cm containing fine sand, and kept in a greenhouse during the experimental period at an average temperature of 25 ± 5°C and relative humidity 60-70 %. The sand substrate was irrigated twice a day by hand to maintain the moisture level.

At the end of the germination test (180 days after sowing), the percentage seed germination was determined. The criterion adopted to evaluate seed viability and germination was the emergence of at least one centimetre of the primary root, with the results expressed as a percentage. From these data, it was possible to obtain the information regarding the germination (%) and dead seeds (%)

to be compare later with the results of the tetrazolium test.

Statistical analysis. The data were subjected to analysis of variance and means separation by the Tukey's test. A multivariate analysis to study the effect of the treatments were performed as well. The R statistical software (R Core Team, 2018) was used for all analyses.

RESULTS AND DISCUSSION

The analysis of variance revealed a significant effect from the interaction between the factors: storage and no storage and the classes of *E. stipitata* seeds for each of the variables. Based on the results shown in Figure 3 for small and large stored seeds of *E. stipitata* subjected to immersion in 1 % tetrazolium solution, the seeds exhibited contrasting results for staining compared with the unstored seeds of both classes.

The percentage viability of *E. stipitata* seeds in both seed classes stored for six months showed that the tetrazolium test could not be recommended due to the divergent results obtained in the germination test of the stored seeds (Table 1).

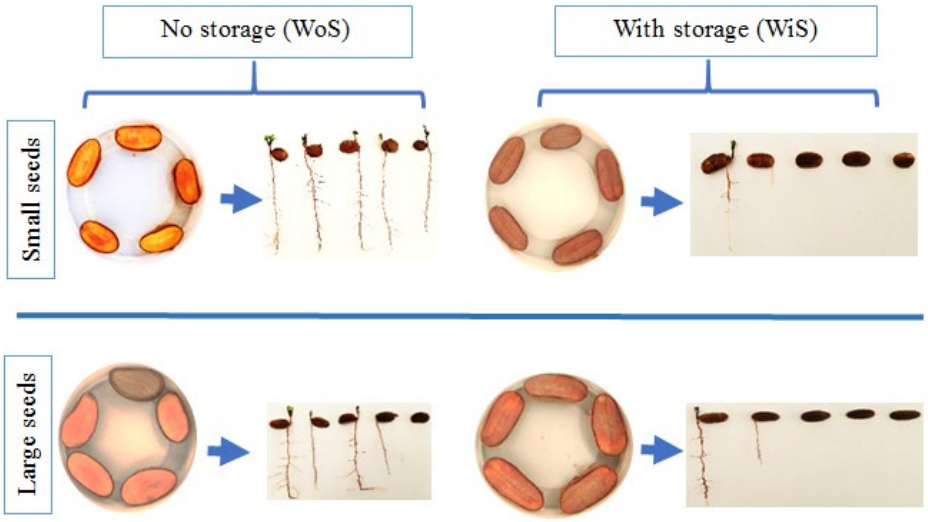

Figure 3. Staining in small and large seeds of *Eugenia stipitata* with and without storage, following immersion in 1 % tetrazolium solution, showing the morphological appearance of germination and seedlings, respectively.

Small stored seeds of *E. stipitata* immersed in the tetrazolium solution showed 100 % carmine-red staining of the reserve tissue (Figure 3), presenting an advanced stage of deterioration, with insufficient staining to differentiate and determine seed viability or the efficiency of the tetrazolium test itself (Table 1).

In contrast, Figure 3 shows large seeds after storage presenting carmine-red staining with small spots of pink to light-red color throughout the tissue. At the same time, the mean value for percentage germination in both seed classes was 40 % (Table 1); these results suggest inadequate tissue differentiation, with the seeds unreliably categorized as viable, unviable, deteriorated and/or dead (Figure 2).

Studies in the literature indicate an initial water content of 50 to 60 % in seeds of *Eugenia stipitata* (Calvi et al., 2016; Anjos and Ferraz, 1999; Gentil and Ferreira, 1999). The initial water content in small and large seeds of *Eugenia stipitata* was 49.02 and 51.8 %, respectively; after six months of storage the respective values were 50.1 and 51.1 %. So, no important changes in water content occurred. These results are within the range reported by the above authors, showing that the initial water content did not affect germination percentage in the treatments of the present study.

Table 1. Mean values for germination (G, %), dead seeds (D, %) and efficiency of the tetrazolium test (EF, %) in two classes of *Eugenia stipitata* seeds (small and large) both with storage (WS) and without storage (NS), immersed in 1 % tetrazolium solution

	G (%)		D (%)		EF (%)	
Classes	NS	WS	NS	WS	NS	WS
Small	100 aA	40 aB	0 bB	60 aA	100 aA	0 aB
Large	80 bA	40 aB	20 aB	60 aA	100 aA	0 aB
CV (%)	3.94		10.94		10.20	

For any class and seed size, summation of G plus D equals 100 %. Mean values followed by the same lowercase letter in a column and uppercase letter on a row do not differ by Tukey's test ($P \leq 0,05$).

Small, unstored seeds of *E. stipitata* (Figure 3) showed 100 % of the tissue with a uniform pink or red staining, typical of viable tissue. In addition, the results of the germination test were proportional to the mean values obtained for the efficiency of the tetrazolium test (Table 1).

Sáenz et al. (2021) also found a greater number of stained, unstored seeds of *Himatanthus sucuuba*, indicating 100% viability and germination.

On the other hand, 80 % of the large, unstored seeds of *E. stipitata* (Figure 3) showed the uniform pink or red staining typical of healthy tissue, while only 20 % showed the white or yellowish staining of dead tissue.

The parameters established for the tetrazolium test proved to be efficient in evaluating the viability of unstored seeds of *E. stipitata*, since the above characteristics show that both the tetrazolium and germination tests agree as to the results for seed viability, i.e. depending on the damage caused to the embryo, either a normal or abnormal seedling may develop: a characteristic previously reported in the germination test of one batch of seeds (Ministério da Agricultura, Pecuária e Abastecimento, 2009). The tetrazolium test should therefore be used to determine seed viability when necessary, since the results are obtained in less time compared to the germination test, which, for the species of the present study, requires 180 days after sowing.

Consequently, multivariate analysis to study the effect of the treatments on the joint behaviour of the variables under evaluation resulted in a first principal component (PC 1) that explains 98.8% of the total variance of the data, and a second principal component (PC 2), which explains 1.2%, giving a total of 100% for the first two principal components (Figure 4).

The percentage of accumulated variance is an empirical criterion recommended when choosing the number of principal components to represent the variance of the data; components that can explain at least 70% of the total variance should be used (Silva et al., 2022), as shown in Figure 4.

The first quadrant (Figure 4) includes the viability and efficiency variables from the tetrazolium test, which is considered highly efficient and responsive (ER), showing values above the mean for the two cartesian axes. In the fourth quadrant, germination was classified as non-efficient and responsive (NER), i.e. with values below the mean of the abscissa axis and above that of the ordinate axis, showing high efficiency for germination and low efficiency for usage.

The first principal component is therefore highly correlated, and is better represented by the viability (V) and efficiency variables of the tetrazolium test, whereas component 2 showed a negative correlation with efficiency in the tetrazolium test and with dead seeds. Principal component 1 was positively correlated with all the variables under study, except dead seeds (Table 1 and Figure 4).

The present results underline the importance of a knowledge of the different classes of seeds shown by the tetrazolium test, both with and without storage, as well as of investigating the most efficient combinations when using the tetrazolium test for seeds of *E. stipitata*. In addition, the data allow the subsequent behaviour of this combination to be inferred for germination, even when still in the greenhouse phase.

This type of study may provide information for selecting the appropriate combinations in classes of unstored seeds, with a view to optimising the use of the tetrazolium test, and reducing the time spent in the nursery, as well as ensuring the return of the capital invested by the fruit farmer.

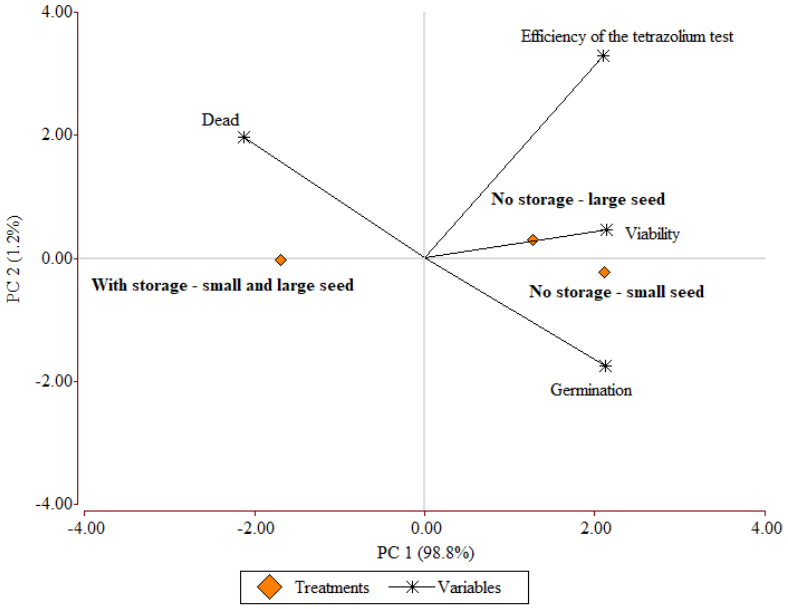

Figure 4. Principal component analysis of the variables as a function of small and large seeds of *Eugenia stipitata* with and without storage in 1 % tetrazolium solution

CONCLUSION

The tetrazolium test and the germination test show that seeds of *Eugenia stipitata* lose their viability when stored for six months in water at ambient temperature.

Neither small nor large seeds of *E. stipitata* stored for six months in water at ambient temperature are recommended for use with the tetrazolium test.

Small, unstored seeds show a germination potential of 100%.

The tetrazolium test is efficient in determining the viability of small and large seeds of *E. stipitata* with no storage.

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LITERATURE CITED

1. Anjos, A.M.G., and I.D.K. Ferraz. 1999. Morfologia, germinação e teor de água das sementes de araçá-boi (*Eugenia stipitata* ssp. sororia). Acta Amazônica 29(1): 337-348.
2. Bhéring, M.C., R.F. Silva, E.M. Alvarenga, D.N.F.S. Dias, and M.F. Pena. 1996. Avaliação da viabilidade e do vigor de sementes de feijão de vagem (*Phaseolus vulgaris* L.) pelo teste de tetrazólio. Boletim Técnico, UFV.Viçosa. 27 p.
3. Calvi, G.P., F.F. Aud, I.D.K. Ferraz, H.W. Pritchard, and I. Kranner. 2016. Analyses of several seed viability markers in individual recalcitrant seeds of *Eugenia stipitata* McVaugh with totipotent germination. Plant Biology 19(1): 6-13.
4. Delouche, J.C., T.W. Still, M. Raspet, and M. Lienhard. 1976. O teste de tetrazólio para viabilidade da semente. Brasília: Agiplan, 103p.
5. França-Neto, J.B. and F.C. Krzyzanowski. 2019. Tetrazolium: an important test for

physiological seed quality evaluation. Journal of Seed Science 41(2): 359-366.

6. França-Neto, J.B., and F.C. Krzyzanowski. 2022. Use of the tetrazolium test for estimating the physiological quality of seeds. Seed Science and Technology 50(1): 31-44.
7. Gentil, D.F.O., and S.A.N. Ferreira. 1999. Viabilidade e superação da dormência em sementes de araçá-boi *(Eugenia stipitata ssp. sororia)*. Acta Amazônica 29(3): 21-31.
8. Gomes, P., I. Marquesand, and F. Martins. 2006. Germination of *Geonoma brevispatha* in laboratory and its relation to the palm spatial distribution in a swamp forest Aquatic Botany 85(4): 16-2.
9. Mata, D.I., and P. Moreno-Casasola. 2005. Effect of in situ storage, light, and moisture on the germination of two wetland tropical trees. Aquatic Botany 83(1): 206-218.
10. Magnitskiy, S.V., and G.A. Plaza. 2007. Fisiología de semillas recalcitrantes de árboles tropicales. Agronomía Colombiana 25(1): 96-103.
11. Menegatti, R.D., A.G. Souza, and V.J. Bianchi. 2019. Estimating genetic divergence between peach rootstock cultivars using multivariate techniques based on characteristics associated with seeds. Genetics and Molecular Research 1(3): 01-10.
12. Ministério da Agricultura, Pecuária e Abastecimento. 2009. Regras para análise de sementes. Brasília. 399 p. https://www.gov.br/agricultura/pt-br/assuntos/insumos-agropecuarios/arquivos-publicacoes-insumos/2946_regras_analise__sementes.pdf
13. Moura, M.L.S., E.A. Chagas, O.J. Smiderle, R. Vilaça, P.C. Chagas, E.A. Moura, and E.E. Farias. 2016. Biometric characterization, water absorption curve and vigor on araçá-boi seeds. International Journal of Plant Biology 7(2): 6265.
14. Paraíso, H.A., D.S. Brandão Junio, R.I.S. Avelar, C.A. Costa, L.S.P. Gomes, and W.M. Nascimento. 2019. Adjustments in the tetrazolium test methodology for assessing the physiological quality of chickpea seeds. Journal of Seed Science 41(1): 007-012.
15. R Core Team. 2018. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/
16. Sáenz Ramírez, L.H., A.M.S. Mendes, A.M. Imakawa, P.T.B. Sampaio, and M.G.G. Melo. 2021. Viability of stored seeds of *Himatanthus sucuuba* Wood by tetrazolium test. Ciência Florestal 31(1): 333-349.
17. Silva, M.L., E.A. Chagas, R. Vilaça, O.J. Smiderle, E.A. Moura, and Chagas, P.C. 2016. Osmopriming duration in Araçá-boi seeds germination. Revista Agro@mbiente On-line 10(1):17 - 21.
18. Silva, J.N., E.A. Alves, M.L.S. Medeiros, G.V.G. Pádua, M.J. Silva, M.H.B.S. Rodrigues et al. 2022. Caracterização morfológica de frutos e sementes em uma população natural de *Hymenaea martiana* Hayne. Scientia Forestali, 50(4): e3929.
19. Smiderle, O.J., and A.G. Souza. 2021. Do scarification and seed soaking periods promote maximum vigor in seedlings of *Hymenaea courbaril*? Journal of Seed Science 43(1): e202143030.
20. Souza, O.M., O.J. Smiderle, A.G. Souza, E.A. Chagas, P.C. Chagas, C.G. Bacelar Lima, and B.S. Morais. 2017. Influência do tamanho da semente na germinação e vigor de plântulas de populações de camu-camu. Scientia Agropecuaria 8(2): 119-125.
21. Souza, A.G., O.J. Smiderle, and C.A. Pedrozo. 2019. Long-time storage *Pochota fendleri* seeds with different packaging. Advances in Horticultural Science 33(3): 327-332.