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Adaptation of tetrazolium test methodology to estimate the viability of *Eugenia stipitata* McVaugh ssp. *sororia* McVaugh seeds[1]

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ABSTRACT – (Adaptation of tetrazolium test methodology to estimate the viability of *Eugenia stipitata* McVaugh ssp. *sororia* McVaugh seeds). The tetrazolium test is a fast and effective method to determine seed viability. In this context, the objective was to adapt the methodology of the tetrazolium test, in relation to preconditioning, to determine the viability of araçá-boi (*Eugenia stipitata* McVaugh ssp. *sororia* McVaugh) under different times of immersion in solution and seed size. The seeds of *E. stipitata* ssp. *sororia* need to go through preconditioning, fractionation being an indispensable technique to be performed before immersion of the seed in the tetrazolium solution, as it allows the contact of the salt with the tissue to be analyzed, which, when feasible, staining occurs (formation of the substance triphenyl-formazan). The immersion time of 26 hours in the tetrazolium solution (1% at 26 °C) is indicated for determining the viability of *E. stipitata* ssp. *sororia* seeds, regardless of the seed mass classes used.
Keywords: araçá-boi, germination, native fruit tree, seed analysis

RESUMO – (Adaptação da metodologia do teste de tetrazólio para estimar a viabilidade de sementes de *Eugenia stipitata* McVaugh ssp. *sororia* McVaugh). O teste de tetrazólio é um método rápido e eficaz para obter a viabilidade de sementes. Neste contexto, objetivou-se adequar a metodologia do teste de tetrazólio, em relação ao pré-condicionamento, para determinar a viabilidade de sementes de araçá-boi (*Eugenia stipitata* McVaugh ssp. *sororia* McVaugh) sob diferente tempo de imersão em solução e tamanho de semente. As sementes de *E. stipitata* ssp. *sororia* necessitam-se passar pelo pré-condicionamento, sendo o fracionamento uma técnica indispensável de ser realizada antes da imersão da semente na solução de tetrazólio, pois permite o contato do sal com o tecido a ser analisado, que quando viável ocorre a coloração (formação da substância trifenil-formazan). O tempo de imersão de 26 horas na solução de tetrazólio (1% a 26 °C) é indicado na determinação da viabilidade de sementes de *Eugenia stipitata* ssp. *sororia.* independentemente da classe de semente utilizada.
Palavras-chave: análise de sementes, araçá-boi, fruteira nativa, germinação

Introduction

Araçá-boi (*Eugenia stipitata* McVaugh ssp. *sororia* McVaugh, Myrtaceae) is one of the native fruit trees of the Amazon and has economic potential for farmers in northern Brazil, because the processed fruit can be used to prepare juices, yogurts, ice cream, liqueurs and wines and the pulp can be used to improve the nutritional quality of other foods with phenolic compounds and antioxidant activity, which contribute to human health (Neri-Numa *et al.* 2013, Baldini *et al.* 2017, Sousa *et al.* 2018, Araújo *et al.* 2021).

In addition, seeds of this species are important materials for study, hence being a source of information for seminiferous propagation (Anjos & Ferraz 1999, Mendes & Mendonça 2020), as it is the main form of propagation of the species.

Seeds of this genus are recalcitrant, which makes storage more difficult (Gentil & Ferreira 1999). In addition, they

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present physical and physiological numbness, due to the impermeability of the integument to water and gases or the presence of inhibitors, such as abscisic acid and phenolic compounds (Pinedo *et al. 1*981, Sert *et al. 2*009) requiring up to 180 days to complete the traditional (Brasil 2013).

To reduce this time, it is necessary to adopt dormancy overcoming techniques, such as partial removal of the seed coat and osmopriming in KNO_3 solution, to obtain germination with 48 to 70 days after sowing, respectively (Mendes & Mendonça 2012, Silva *et al.* 2016). However, Paiva *et al.* (2017) reported that the results no longer coincide with the actual condition of the seed lot of the species. In addition, one of the alternatives to solve this problem is the use of rapid tests, such as the tetrazolium, whose function is to optimize the prediction of seed viability (Paiva *et al.* 2017).

This test is based on the biochemical process of the seed, because once the seed is viable, the enzyme dehydrogenase, during the respiration process, reacts in contact with the 2,3,5-triphenyl-tetrazolium chloride, favoring the production of triphenyl-formazan, substance in living cells, which makes it possible to differentiate them from dead cells, which have milky white color (França-Neto & Krzyzanowski 2022).

The success in the use of the tetrazolium test is related to the concentration of the tetrazolium solution, time and temperature of immersion of the seed in the solution (Paiva *et al.* 2017, Paraíso *et al.* 2019). In addition, it is necessary to perform tests related to the need or not of seed preconditioning because, according to Lamarca & Barbedo (2014), seeds of *Eugenia brasiliensis*, *E. uniflora* and *E. pyriformis*, freshly removed from the cold chamber at 7 °C, show more evident staining of the tissues when immersed in water for 3 hours at 25 °C, followed by longitudinal cut and immediate immersion in the tetrazolium solution, so as to avoid tissue oxidation.

Regarding the factors related to the tetrazolium test, Cripa *et al.* (2014) observed that the tetrazolium test may be an alternative to determine the viability of *Eugenia* seeds more rapidly. Lamarca & Barbedo (2014) determined that the tetrazolium test was efficient in assessing viability when applied to *Eugenia brasiliensis* seeds at a concentration of 0.250% for 3 hours, to *E. uniflora* seeds at 0.125% for 3 hours and to seeds of *E. pyriformis* at 0.100% for 2 hours. Calvi *et al.* (2017) obtained positive viability responses of *E. stipitata* seeds, when subjected to 1% solution of 2,3,5-triphenyl-tetrazolium chloride.

However, it is necessary to carry out more detailed studies, as there is a lack of information in the literature on preconditioning procedures, which aim at penetration of the solution into the tissues of interest to be evaluated. According to the survey carried out, different preconditioning methods and variations in the concentrations of the tetrazolium solution, time and temperature of seed immersion in the different species of the genus *Eugenia* were observed.

In this context, although the tetrazolium test is applied in the areas of seed analysis and physiology, more information is needed to apply it in seeds of *Eugenia stipitata* ssp. *sororia*. Therefore, the objective was to adapt the methodology of the tetrazolium test, in relation to preconditioning, to determine the viability of araçá-boi (*Eugenia stipitata* McVaugh ssp. *sororia* McVaugh) seeds under different times of immersion in the solution and seed size.

Material and Methods

Ripe fruits of araçá-boi (*Eugenia stipitata* McVaugh ssp. *sororia* McVaugh, Myrtaceae) were harvested from trees maintained in the Araçá-boi (*Eugenia stipitata*) Germplasm Collection of the Federal University of Roraima (UFRR), at the Cauamé Campus, in Boa Vista, Roraima, located at coordinates 2°52'14.9"N and 60°42'47.9"W, (figure 1), altitude of 90 m and climate, according to the classification of Köppen & Geiger, type Aw, tropical rainy, average temperature of 27 °C and annual rainfall of 1600 mm, concentrated between the months of April to September (Araújo *et al. 2*001).

The experiments were conducted at the Seed Analysis Laboratory and Greenhouse of Embrapa Roraima. After collecting the fruits, they were pulped and the mucilage was completely removed by manual friction with fine sand, followed by washing in running water with the aid of a sieve. After obtaining the seeds (*E. stipitata* ssp. *sororia*), two experiments were conducted, both to adjust the methodology of the viability test (tetrazolium).

The first experiment was conducted to determine whether the efficiency of tetrazolium test with seeds (*E. stipitata* ssp. *sororia*) is influenced by their state, whole or sectioned, or by the presence or absence of seed coat. In the second experiment, the objective was to adapt the methodology of the tetrazolium test with different times of immersion in the tetrazolium salt solution and two classes of *E. stipitata* seeds in order to determine their viability.

Experiment I – Is the efficiency of the tetrazolium test with seeds of araçá-boi (*Eugenia stipitata* McVaugh ssp. *sororia* McVaugh, Myrtaceae) influenced by their state, whole or sectioned, or by the presence of absence of seed coat?

The water content of *E. stipitata* seeds was determined by the oven method at 105 ºC/24h, according to the Rules for Seed Analysis – RAS (Brasil 2009), with two subsamples of 5 g of seeds. The average water content of *E. stipitata* seeds was 50.4% (calculated in the wet basis).

The first experiment was conducted in a completely randomized design in a 2x2 factorial scheme, with 5 replicates of 5 seeds. The first factor consists of whole and sectioned seeds (figure 2) and the second factor is the presence and absence of seed coat (figure 2).

The seeds called sectioned were cut longitudinally, in half, with a stainless steel blade, and the seed coat was removed manually with a scalpel.

Subsequently, the seeds of each treatment were immersed for 30 hours in a solution of 2,3,5-triphenyl-tetrazolium chloride (pH 6.5 to 7.0), at a concentration of 1%, in a 50 mL plastic container. Then, the materials were placed in Biochemical Oxygen Demand (BOD) chamber, regulated at 26 ºC in the absence of light for both experiments.

After the period of exposure of the seeds in solution, they were drained and washed in running water, and then kept submerged in water in a refrigerated environment at temperature of 22 ºC, until the time of staining evaluation. To assist in the visualization of all details of the seeds, a benchtop magnifying glass with a fluorescent lamp of six magnifications (6x) was used in both experiments.

Tissue color differentiation was observed according to categories established for the tetrazolium test (Cripa *et al.* 2014). Category 1 (viable): embryo with pink color and tissues with normal and firm appearance, Category 2 (viable): less than 50% of endosperm is discolored, embryo is pink and intact, and the other tissues are firm, Category 3 (unviable): seed with tissues with white, yellowish or cream color, characteristic of dead tissues, Category 4 (unviable): seed with tissue with strong carmine red color, characteristic of decaying tissue (figure 3).

In order to verify the effect of the 2,3,5-triphenyl-tetrazolium chloride salt on seed germination and to elucidate the test, after completing the tetrazolium test, the seeds of each treatment were sown in trays containing fine sand as substrate. at a depth of 1.0 cm in plastic trays measuring 30 cm x 40 cm x 10 cm in a greenhouse with an average temperature of 25 ± 5 °C during the experiment period and relative air humidity of 60% to 70%.

To perform the germination test, the design was completely randomized, in a 2 x 2 factorial scheme (whole and sectioned seeds, with and without seed coat), with 10 replicates of 10 seeds each.

At 180 days after sowing (DAS), the percentage of seed germination was determined, considering seeds that showed radicle protrusion of at least one centimeter as germinated (figure 5). With the obtained data it was possible to determine and validate the viability, germination, efficiency of the tetrazolium test, and dead seeds expressed as percentage. It is worth noting that the best results obtained in experiment I were used in experiment II.

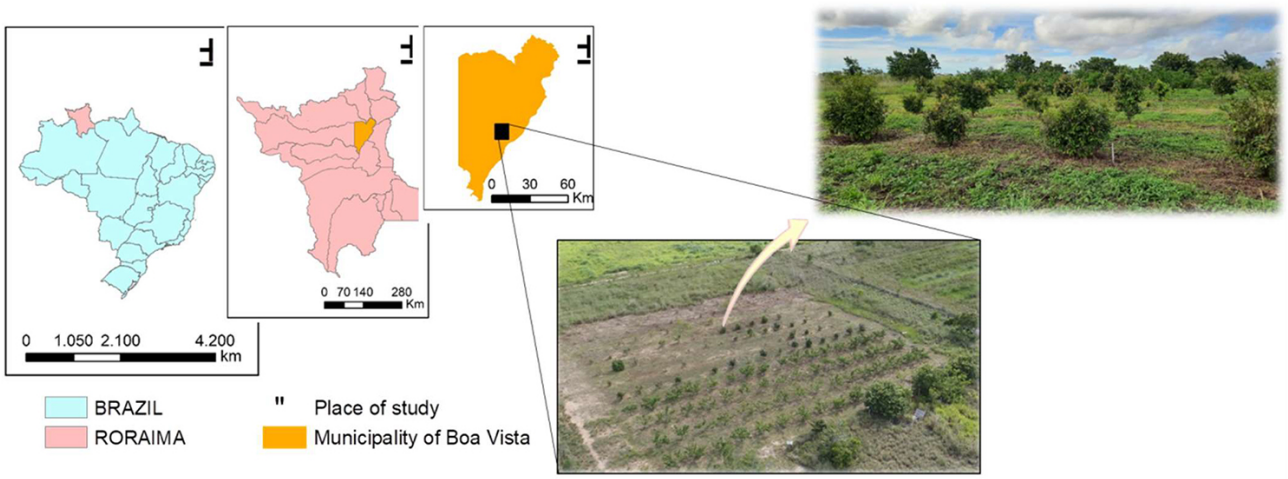

Figure 1. Distribution of the trees maintained in the araçá-boi (*Eugenia stipitata* McVaugh ssp. *sororia* McVaugh) Germplasm Collection in the Municipality of Boa Vista, Roraima State, Brazil. Image: Authors.

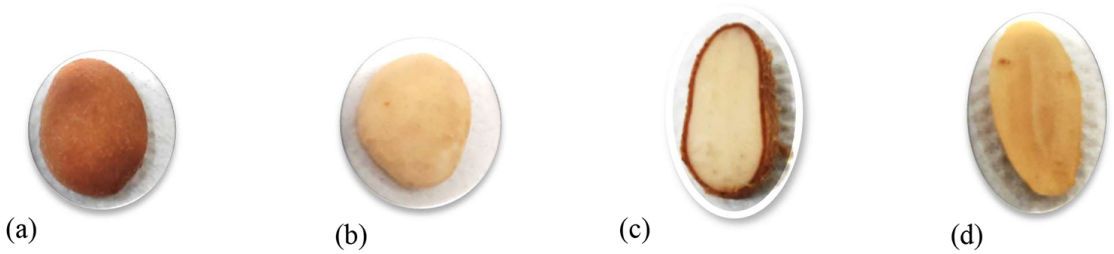

Figure 2. Whole seeds of *Eugenia stipitata* McVaugh ssp. *sororia* McVaugh. seeds with (a) and without (b) seed coat and sectioned seeds with (c) and without (d) seed coat, Municipality of Boa Vista, Roraima State, Brazil. 1 cm scale.

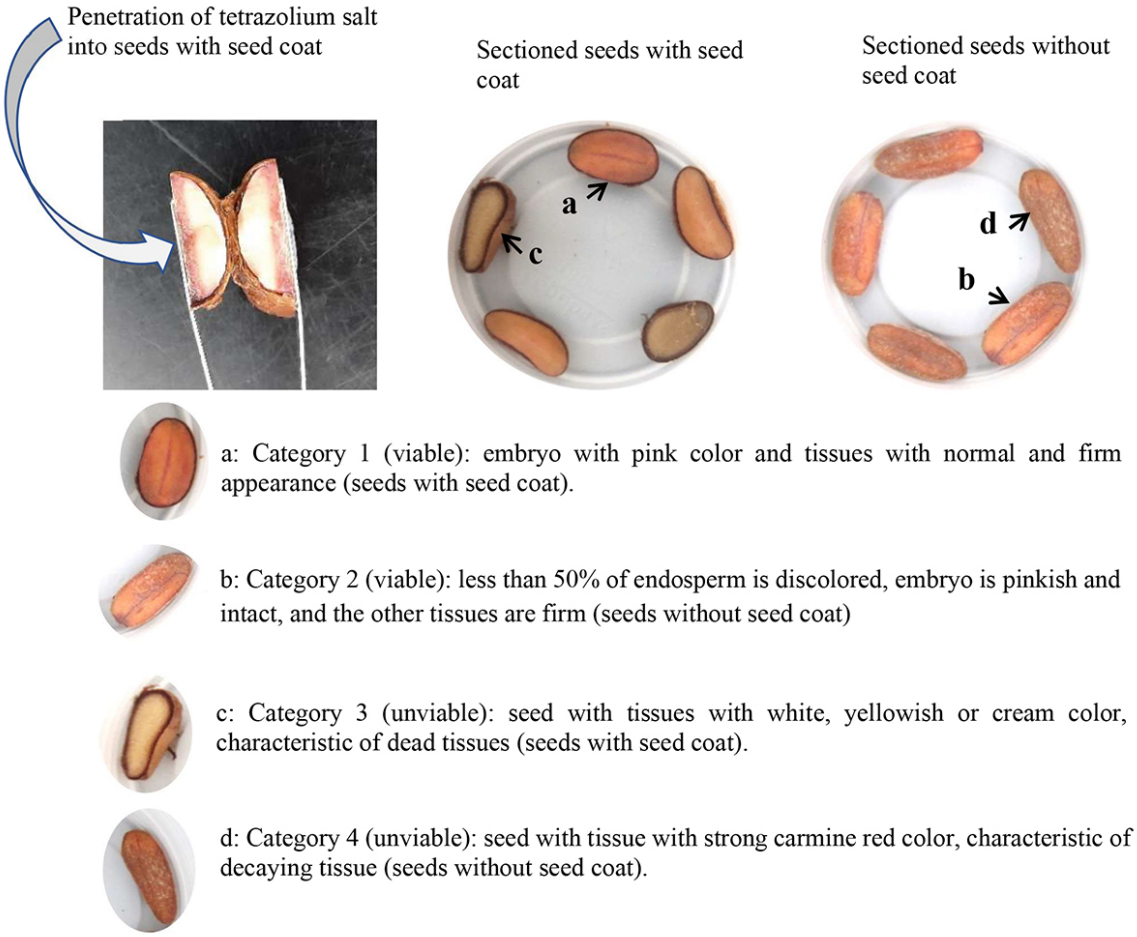

Figure 3. Staining of sectioned seeds of *Eugenia stipitata* McVaugh ssp. *sororia* McVaugh with and without seed coat subjected to the tetrazolium test (1% at 26 °C), Municipality of Boa Vista, Roraima, State, Brazil. 1 cm scale.

Experiment II – Adaptation of the methodology of the tetrazolium test under different times of immersion in tetrazolium salt solution with two seed classes to determine viability.

First, the seeds of *E. stipitata* ssp. *sororia* that had intact external appearance were separated into two mass classes, small and large seeds, based on their individual fresh mass, obtained on a precision scale (0.001 g). Small seeds were those with mass between 0.80 and 1.20 g and large seeds were those with mass from 1.40 to 2.50 g.

The water content of the seeds was determined for each seed mass class, in an oven (105 ± 3 ºC) for 24 hours, according to Brasil (2009), with two subsamples of 5 g of seeds. The average water content for small and large seeds was 49.02% and 51.82%, respectively.

The design was completely randomized, in a 2x3 factorial scheme, with 4 replicates of 5 seeds. The first factor consisted of two classes of seeds (small and large) and the second factor consisted of three times of immersion (26, 30 and 34 hours), kept in the dark, at a concentration of 1% of the tetrazolium salt.

Tissue color differentiation was observed according to the categories established for the tetrazolium test proposed by Cripa *et al.* (2014). Category 1 (viable): embryo with pink color and tissues with normal and firm appearance. Category 2 (unviable): seed with tissues with white, yellowish or cream color, characteristic of dead tissues (figure 4).

In order to verify the effect of the 2,3,5-triphenyl-tetrazolium chloride solution on seed germination and to elucidate the test, after completing the tetrazolium test, the seeds were sown in trays containing fine sand at 1.0 cm depth in plastic trays of 30 cm x 40 cm x 10 cm in a greenhouse with average temperature in the experimental period of 25 ± 5 °C and relative humidity from 60% to 70%.

To perform the germination test, the design was completely randomized, in a 3 x 2 factorial scheme, corresponding to three immersion times (26, 30 and 34 hours) and two classes of seeds, with 8 replicates of 10 seeds.

At the end of the germination test (at 180 days after sowing), the percentage of seed germination was determined, considering seeds that showed radicle protrusion and emergence of the primary root with at least one centimeter as germinated (figure 5).

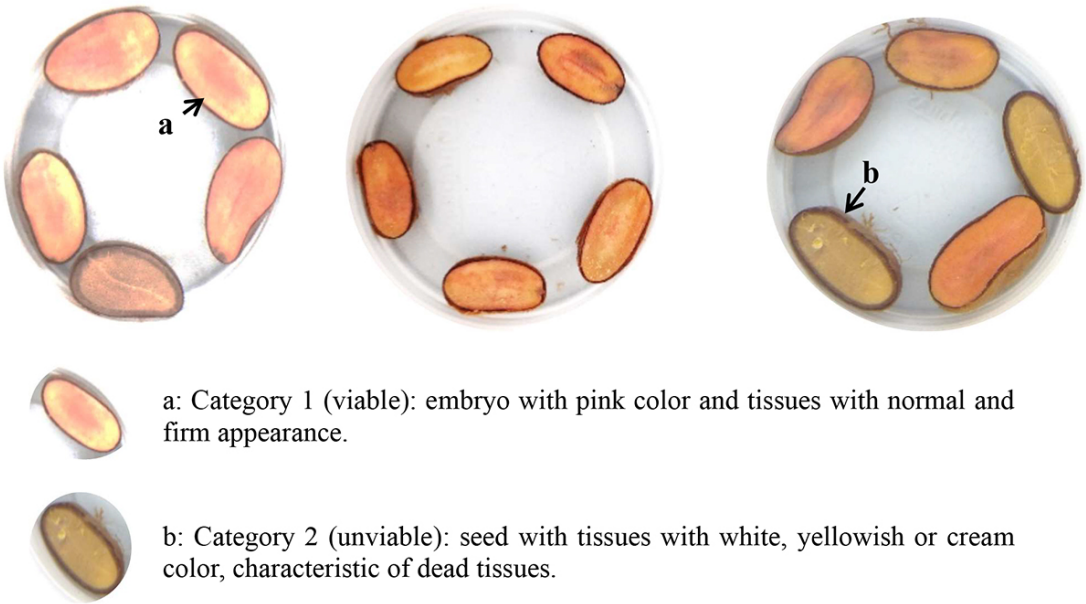

Figure 4. Staining of *E. stipitata* ssp. *sororia* seeds subjected to the tetrazolium test (1% at 26 °C). Municipality of Boa Vista, Roraima, Brazil. 1 cm scale.

Figure 5. Visualization of germinated and dead seeds of *Eugenia stipitata* McVaugh ssp. *sororia* McVaugh at 180 days after sowing, Municipality of Boa Vista, Roraima State, Brazil. 1 cm scale.

With these data it was possible to determine and validate the percentage of viability (%), percentage of germination (%), efficiency of the tetrazolium test (%), and dead seeds.

The germination percentage was calculated using the formula G = (N/A).100, where G is the germination percentage, N is the number of germinated seeds and A is the total number of seeds set to germinate.

The efficiency of the tetrazolium test was obtained according to the formula proposed by Carvalho *et al.* (2017): Efficiency TZn=[1-(|G-TZn|)/G]100, where G is the percentage of normal seedlings obtained in the germination test and TZn is the percentage of viable seeds obtained in the tetrazolium tests. For dead seeds, at the end of the germination test, seeds with softened tissue were counted, as well as those attacked by microorganisms and those that did not show the beginning of germination.

Data were subjected to analysis of variance and means compared by Tukey's test at 5% probability, with the help of the ExpDes.pt© package by R Core Team (2018). Seeking to determine the correlation between the treatments and the variables analyzed, multivariate analysis of principal components was applied using the statistical package Infostat (Di-Rienzo 2008).

Results and Discussion

Experiment I – Whole seeds in the presence and absence of seed coat did not react when exposed to 2,3,5-triphenyl-tetrazolium chloride solution. However, there was staining of the tissues when the seeds were submitted to the fractionation process, in the presence or absence of tegument (figure 6). Thus, it was observed that the methodology used for the

tetrazolium test did not provide viability estimates (table 1) for *E. stipitata* ssp. entire seroria in the presence and absence of tegument, being confirmed in the germination test (table 1). This absence of staining can be attributed to two aspects: presence of seed coat or formation of gelatinous substance during seed coat removal, preventing or hindering the uniform penetration of the tetrazolium solution into the seed.

Thus, it is suggested that physical limitations, imposed by the seed coat of *E. stipitata* ssp. *sororia*, contributed decisively to low germination of whole seeds with seed coat, as confirmed in Table 1. These results were similar to those reported by Pinedo Panduro *et al.* (1981), who found that the highest level of dormancy in *E. stipitata* ssp. *sororia* seeds was mainly imposed by the seed coat.

In turn, seed coat removal requires labor and represents an extra cost for the nursery owner; however, this management is not justified when carried out with sectioned seeds of *E. stipitata* ssp. *sororia* with seed coat, since their germination did not differ from that of whole seeds without seed coat (table 1).

In general, seeds of *E. stipitata* ssp. *sororia* fractionated in the presence of tegument, showed an increase in germination of 66.6% in relation to fractionated seeds without tegument (table 1).

Studies indicate values between 50 and 60% for the initial water content in *E. stipitata* seeds (Calvi *et al.* 2017, Mendes & Mendonça 2012, Anjos & Ferraz 1999, Gentil & Ferreira 1999). Thus, the water content in *E. stipitata* ssp. *sororia* seeds presented here (50.4%) is within the range indicated by the aforementioned authors. This pattern demonstrates that the initial water content did not interfere in the percentages of germination between the treatments of the present study.

Table 1. Mean values of viability (%), germination (%), efficiency of the tetrazolium test (%) and dead seeds (%) of whole or sectioned seeds of *Eugenia stipitata* McVaugh ssp. *sororia* McVaugh with and without seed coat.

Seed coat	Viability (%)		Germination (%)		Efficiency (%)		Dead (%)	
	Whl	Sec	Whl	Sec	Whl	Sec	Whl	Sec
With	0 aB	60 aA	40 bB	60 aA	0 aB	100 aA	60 bA	40 bB
Without	0 aB	60 aA	60 aA	40 bB	0 aB	66 bA	40 aB	60 aA
CV %	6.94	5.29	4.59	4.62				

Means followed by the same letters, lowercase in the column and uppercase in the row, do not differ from each other by Tukey test at 5% probability level. Whl: Whole seeds; Sec: Sectioned seeds; With: With seed coat; Without: Without seed coat.

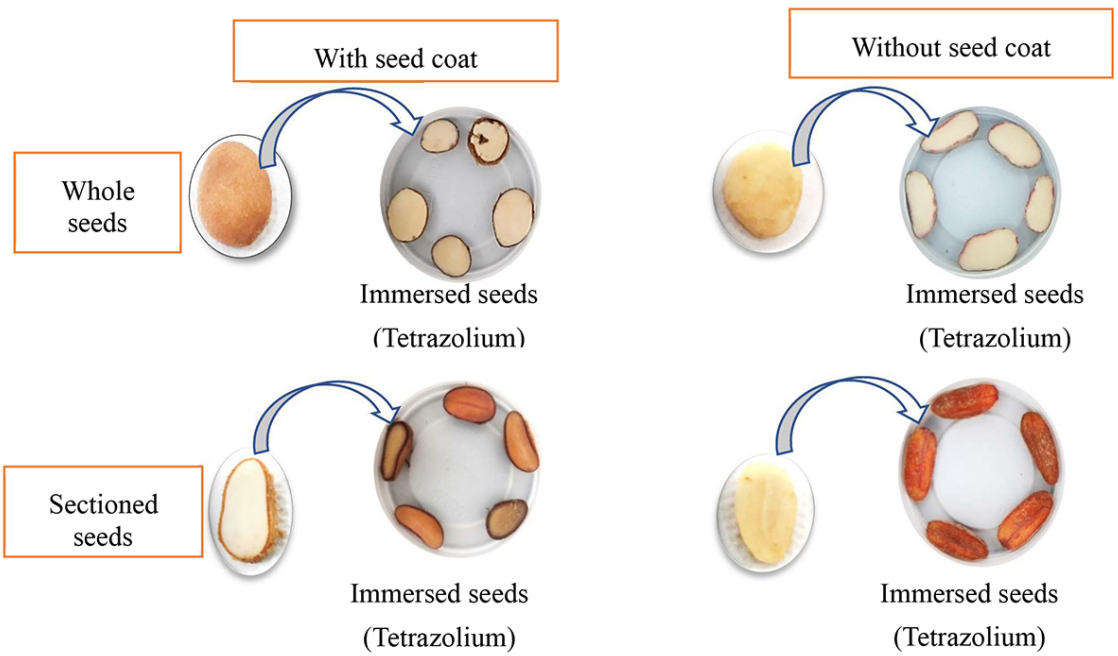

Figure 6. Visualization of the staining of whole and sectioned seeds of *Eugenia stipitata* McVaugh ssp. *sororia* McVaugh subjected to the tetrazolium test for 30 hours at 26 ºC, 1%. Municipality of Boa Vista, Roraima State, Brazil. 1 cm scale.

It is worth pointing out that the mean values for percentage of viability of sectioned seeds with and without seed coat were similar (table 1), showing ideal staining condition for tissue differentiation, demonstrating with validation the categories of viable, deteriorated and dead seeds (figure 6).

In addition to that, seeds fractionated in the presence and absence of tegument, obtained the results of the germination test proportional to the mean values obtained for the efficiency of the tetrazolium test (table 1).

It is important to emphasize that the superiority in the germination of sectioned seeds or without seed coat, in relation to the whole seed with seed coat in the present research allows to evidence that the physical and physiological dormancy is present and needs to be overcome with removal of the seed coat or cuts (fractionated).

This shows the polarity of sectioned *E. stipitata* ssp. *sororia* seeds is probably based on the migration of the hormones auxin and cytokinin. This means that the *E. stipitata* ssp. *sororia* seed, when cut, behaves like a stem and appears to have some hormonal gradient that converges from the distal region to the meristematic zone (França-Neto & Krzyzanowski 2022). Indeed, *E. stipitata* ssp. *sororia* seeds proved to be highly regenerative, maintaining a similar polarity, and are very resistant to physical damage.

Thus, for whole seeds, as described in experiment I, the absence of germination may be due to the lack of stimulus to regeneration or a balance favorable to inhibition. However, in the fractionated seeds, it was evidenced that the stimulus to regeneration occurs and that, when germination starts (figure 7, table 1 and table 2), the processes of regeneration of new roots also begin (figure 7).

Experiment II – Based on the best technique (whole seeds x sectioned seeds) of the results obtained in the first experiment, sectioned seeds were considered more appropriate, but the time of immersion in solution (26. 30 and 34 hours) and two classes of seed mass (small and large) were adopted.

According to the results obtained in Figure 7, small and large seeds of *E. stipitata* ssp. *sororia*, submitted to different times of immersion in the tetrazolium solution (1% at 26 °C), exhibited different categories of seed color (figure 4). Thus, the use of the tetrazolium test helps to identify the viability of *E. stipitata* ssp. *sororia*.

Small seeds of *E. stipitata* ssp. *sororia* immersed for 26 hours showed 100% pink color in the reserve tissue, exhibiting ideal staining for differentiation and determination of seed viability, as well as the efficiency of the tetrazolium test. When these seeds were immersed in tetrazolium solution for 30 hours, 60% of them showed white or cream-colored tissues, characteristic of dead tissues. When small seeds were

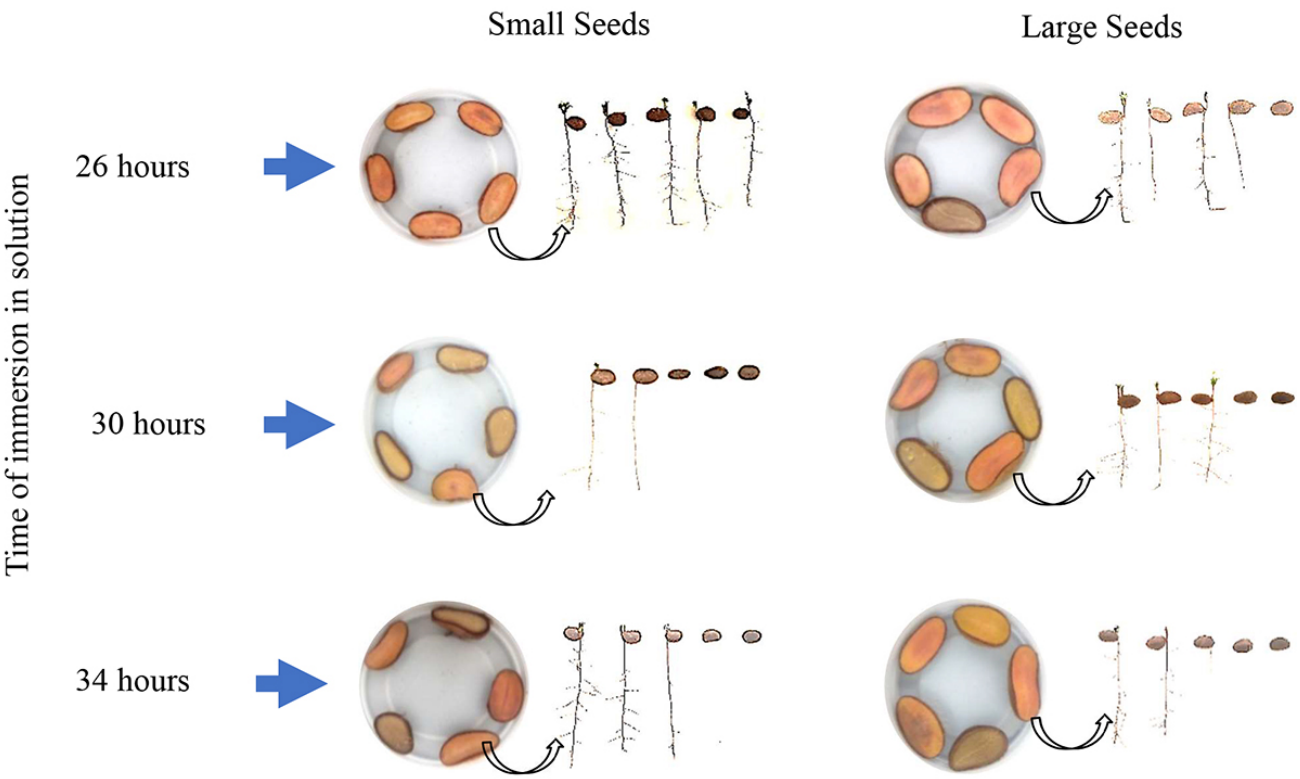

Figure 7. Color visualization of small and large seeds of *Eugenia stipitata* McVaugh ssp. *sororia* McVaugh submitted to three times of immersion (26, 30 and 34 hours) and two classes of seeds (small and large) in the tetrazolium solution (1% at 26 °C) and germination, Municipality of Boa Vista, Roraima State, Brazil. 1 cm scale.

immersed in solution for 34 hours, they showed variation in the color obtained, i.e., 40% in the unviable category and 60% in the viable category, as described in Figure 7.

In addition, large seeds of *E. stipitata* ssp. *sororia* immersed for 26 hours (figure 7) showed 80% of tissues with uniform pink or red color, typical of healthy tissue. On the other hand, they showed only 20% of dead tissues, i.e., white or yellowish color.

Among the evaluated seed classes and immersion time in tetrazolium solution, the treatments showed a significant difference between the analyzed variables (table 2), which demonstrates that the tetrazolium test methodology used efficiently estimated the viability of the analyzed seeds, with highlight for small seeds and large seeds immersed in a 1% tetrazolium solution for 26 hours at 26 °C.

As for the percentage of viability and germination of seeds, it is observed that the best results of immersion time were obtained in small seeds immersed for 26 hours in tetrazolium solution. Regarding seed classes, it was noted that small and large seeds showed the best viability and germination values, when exposed to a time of 26 hours of immersion in the tetrazolium solution (table 2).

The seed germination (table 2 and figure 7) probably result from a balance between several promoting and inhibiting factors, including gibberellin and abscisic acid (França-Neto & Krzyzanowski 2019). Therefore, one cannot rule out the possibility that sectioned seeds are under the influence of both processes, induction and inhibition, with balance that is sometimes favorable and sometimes unfavorable to the development of new roots and seedlings.

For principal component analysis, when selecting the components, it is important to choose those that have eigenvalues greater than 1.0 and concentrate the most significant variation of the data so that it is acceptable to perform a divergent grouping between the variables (Menegatti *et al.* 2022), which was demonstrated with the data of the present study.

Medina *et al.* (2011) describe that values of the first principal components above 70% are sufficient to explain the total variance between the variables, which can be observed in Figure 8 of the present study.

The first quadrant contains the variables viability and efficiency of the tetrazolium test, considered efficient and responsive (ER), which showed values above the average for the two Cartesian axes, with high efficiency of the tetrazolium test. The second quadrant contains the germination variable, classified as non-efficient and responsive (NER), that is, it has values below the average for the abscissa axis and above the average for the ordinate axis, with high germination efficiency and low utilization efficiency.

The knowledge of the correlations between traits of interest for selection is one of the initial stages for the determination of genetic diversity (Menegatti *et al.* 2021, Montenegro *et al.* 2022). The obtained values of Pearson's correlation (table 3) for the variables evaluated in the present study indicated that there was a significant and positive association between germination (G) and viability (V).

The efficiency of the test (EF) is positively correlated with germination (G). A positive and strong correlation was also observed between EF and V. The efficiency of the test (EF) is positively correlated with germination (G). A positive and strong correlation was also observed between EF and V. According to the criterion of Santos (2010), the correlation is considered strong when it has coefficient of variation of $0.8 \leq p < 1$.

Knowledge of the correlation between the variables viability, germination, efficiency of the tetrazolium test and dead seeds and their amplitude of variation assists in the selection process (Paraíso *et al.* 2019, Campos *et al.* 2017), because it allows defining the interference of the selection performed in one characteristic on another, as well as performing indirect selection for characteristics that are difficult to measure (Montenegro *et al.* 2022).

In summary, the seeds of *E. stipitata* ssp. *sororia* need to go through preconditioning, fractionation being an indispensable technique to be performed before immersion of the seed in the tetrazolium solution, as it allows the contact of the salt with the tissue to be analyzed, which,

Table 2. Mean values of viability (V%), germination (G%), efficiency of the tetrazolium test (EF%) and dead seeds (D%) of two classes of seeds (S: small and L: large) of *Eugenia stipitata* McVaugh ssp. *sororia* McVaugh subjected to immersion for 26, 30 and 34 hours in the tetrazolium solution (1% at 26 °C).

Time	V%		G%		EF%		D%	
	S	L	S	L	S	L	S	L
26h	100 aA	80 aB	100 aA	80 aB	100 aA	100 aA	0 cB	20 cA
30h	40 cB	60 bA	20 cB	40 cA	50 bB	66 cA	80 aA	60 aB
34h	60 bA	40 cA	60 bA	60 bA	100 aA	67 bB	40 bA	40 bA
CV%	4.6	4.7	4.2	5.3	5.7	5.5	3.7	5.8

Means followed by the same letters, lowercase in the column and uppercase in the row, do not differ from each other by Tukey test at 5% probability level. S = Small Seeds, and L = Large Seeds.

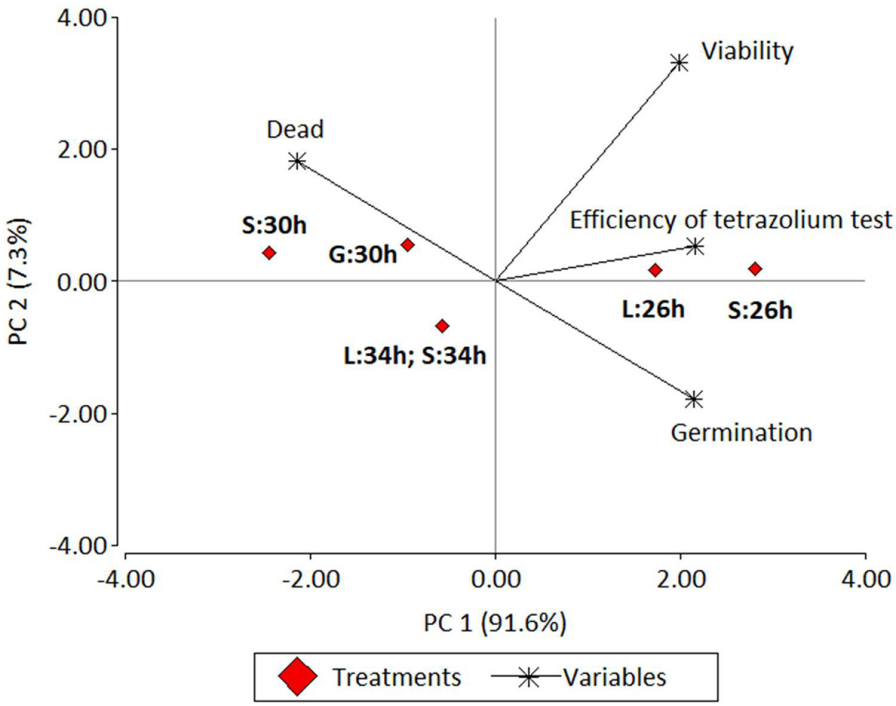

Figure 8. Principal component analysis of the variables as a function of two seed classes and three periods of immersion of *Eugenia stipitata* McVaugh ssp. *sororia* McVaugh seeds in tetrazolium solution at 1% at 26 °C, Municipality of Boa Vista, Roraima State, Brazil. 1 cm scale.

Table 3. Matrix of Pearson's correlation between the variables viability (V), germination (G), efficiency of the tetrazolium test (EF) and dead seeds (D) of two classes of seeds (small and large) of *Eugenia stipitata* McVaugh ssp. *sororia* McVaugh subjected to three times (26, 30 and 34 hours) of immersion in the tetrazolium solution (1% at 26 °C).

	V	G	EF
G	0.78*		
EF	0.90*	0.93*	
D	-0.78^{ns}	-1.00^{ns}	-0.93^{ns}

Significant at 5% probability level by Bartlett Shapiro-Wilk test. Source: Authors (2022).

when feasible, staining occurs (formation of the substance triphenyl-formazan). Regarding the immersion time in 1% solution, it is recommended to use a time of 26 hours at 26 °C.

This information is essential to outline conservation strategies for this species, which has potential to be explored by farmers. In addition to being a plant that composes naturally in the Amazon, which represents the appreciation of local ecology, with concern for maintenance, establishment and conservation for the stability of the ecosystem. Thus, ethnobotany can be one of the main ways to discover bioactive natural products.

Therefore, the results found here provide technical-scientific support regarding the use of the tetrazolium test to evaluate the viability of *E. stipitata* ssp. *sororia*.

Conclusions

The tetrazolium test, in seeds of *Eugenia stipitata* ssp. *sororia,* it is recommended as a pre-conditioning to fractionate the seeds in half longitudinally and maintain the seed coat.

The immersion time of 26 hours in the tetrazolium solution (1% at 26 °C) is recommended for determining the viability of fractionated seeds of *Eugenia stipitata* ssp. *sororia*, regardless of the class of seed used.

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Author Contributions

Sonicley da Silva Maia: Substantial contribution in the concept and design of the study; contribution to data collection; contribution to data analysis and interpretation.

Oscar José Smiderle: Substantial contribution in the concept and design of the study; contribution to critical revision, adding intellectual content.

Aline das Graças Souza: Substantial contribution in the concept and design of the study; substantial contribution to the production of figure boards; contribution to manuscript preparation, adding intellectual content.

Salvador Barros Torres: Contribution to critical revision, adding intellectual content.

Conflicts of interest

There is no conflict of interest.

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