

ARTICLE

## Development of a specific protocol for the tetrazolium test and evaluation of the physiological potential of *Cnidoscolus quercifolius* Pohl (Euphorbiaceae) seeds

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**ABSTRACT:** Rapid tests are essential for assessing the physiological quality of seeds, especially in native Caatinga species that remain little studied. *Cnidoscolus quercifolius* (Pohl) has ecological and socioeconomic importance but lacks protocols for determining viability. This study aimed to standardize the tetrazolium test (TZT) for evaluating *C. quercifolius* seeds. Three seed lots were used, from Massaroca (BA), Sento Sé (BA), and Petrolina (PE). The seeds were preconditioned for 16 h at 30 °C on moistened paper and then had the seed coat removed. The experiment followed a completely randomized design in a 7 × 4 factorial arrangement, with seven concentrations of 2,3,5 triphenyl tetrazolium chloride (0.025 to 0.75%) and four immersion times (1 to 4 h), with four replications of 25 seeds. Viability was assessed by the intensity and uniformity of carmine-red staining in the embryo and cotyledons. Low tetrazolium chloride concentrations ( $\leq 0.025\%$ ) underestimated viability, whereas high ones ( $\geq 0.5\%$ ) caused overstaining. Thus, the range of 0.05 to 0.075% for 3–4 h showed greater diagnostic efficiency, reflecting germination percentages. TZT distinguished viable seeds, with uniform staining and turgid tissues, from non-viable ones. It is concluded that TZT is rapid, reliable, and applicable to programs for conservation, seedling production, and ecological restoration in the Caatinga.

**Index terms:** biochemical test, Caatinga, native seeds, physiological quality.

**RESUMO:** Testes rápidos são essenciais para avaliar a qualidade fisiológica de sementes, especialmente em espécies nativas da Caatinga, ainda pouco estudadas. A faveleira (*Cnidoscolus quercifolius* Pohl) apresenta importância ecológica e socioeconômica, mas carece de protocolos para determinação da viabilidade. Este estudo objetivou padronizar o teste de tetrazólio (TTZ) para avaliação de sementes de *C. quercifolius*. Foram utilizados três lotes, Massaroca (BA), Sento Sé (BA) e Petrolina (PE). As sementes foram pré-condicionadas por 16 h a 30 °C em papel umedecido, submetidas à remoção do tegumento. O experimento seguiu delineamento inteiramente casualizado, em esquema fatorial 7 × 4, com sete concentrações de cloreto de 2,3,5-trifenil tetrazólio (0,025 a 0,75%) e quatro tempos de imersão (1 a 4 h), com quatro repetições de 25 sementes. A viabilidade foi avaliada pela intensidade e uniformidade da coloração vermelho-carmim em embrião e cotilédones. Concentrações baixas de cloreto de tetrazólio ( $\leq 0,025\%$ ) subestimaram a viabilidade, enquanto altas ( $\geq 0,5\%$ ) causaram sobrecoloração. A faixa de 0,05 a 0,075% por 3–4 h mostrou maior eficiência diagnóstica, refletindo os percentuais de germinação. O TTZ distinguiu sementes viáveis, com coloração uniforme e tecidos túrgidos, das não viáveis. Conclui-se que o TTZ é rápido, confiável e aplicável em programas de conservação, produção de mudas e restauração ecológica na Caatinga.

**Termos para indexação:** teste bioquímico, Caatinga, sementes nativas, qualidade fisiológica.

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## INTRODUCTION

*Cnidocolus quercifolius* (Pohl), Euphorbiaceae, is an endemic tree species of the Caatinga Domain, a seasonally dry tropical forest, which stands out for its rapid establishment and high resistance to drought, becoming strategic in programs for revegetation and restoration of degraded areas in arid and semi-Arid environments (Ribeiro and Brito, 2010). In addition, it contributes to essential ecosystem services, such as shading, organic matter input, nutrient cycling, and increased resilience of agroecosystems (Medeiros and Oliveira, 2020; Porto et al., 2024). The biogeographic classification of the Caatinga as a seasonally dry system (Pennington et al., 2001) helps to contextualize the ecophysiological adaptation of the species to water stress conditions.

From a productive and socioeconomic perspective, the species has multiple uses. Its seeds have a high lipid content (30-35%), which qualifies them as a promising feedstock for biofuels, especially biodiesel, with the potential to strengthen the regional bioeconomy (Santos et al., 2017). After oil extraction, the residual cake can be used for animal feed, while the flour produced from its seeds has nutritional potential (Ribeiro et al., 2022). In addition, young leaves and branches are used as fodder in periods of drought, reinforcing the contribution of *C. quercifolius* to the food security of herds and the subsistence of rural populations (Oliveira et al., 2016; Nascimento et al., 2025).

Despite its recognized value, scientific advances in *C. quercifolius* seed technology are still incipient, especially with regard to the characterization of physiological viability, germination and storage for species from seasonally dry tropical forests, which limits domestication strategies, genetic improvement and large-scale seedling production programs (Ramos and Freire, 2019). Traditionally, the physiological quality of seeds is evaluated through the germination test, which is considered official (Brasil, 2025). However, it is a procedure that requires a relatively long time to obtain results, which delays decisions related to nurseries, germplasm banks and restoration programs (Lima et al., 2014; Pinheiro et al., 2024).

In this context, rapid alternatives have stood out, as is the case of the tetrazolium test (TZT), one of the most used methods to diagnose seed viability and vigor in different species. Its efficiency stems from the histochemical reaction promoted by dehydrogenase enzymes, which reduce 2,3,5-triphenyl tetrazolium chloride to a red compound (triphenylformazan) in living tissues (França-Neto and Krzyzanowski, 2019). In Brazil, several studies have consolidated the application of TZT, for both agricultural and forest species (Dantas et al., 2015; França-Neto and Krzyzanowski, 2018), highlighting its potential to provide rapid and reliable diagnoses.

However, the standardization of TZT depends on factors such as the preconditioning of seeds in water, types/positions of cutting to make the tissues evident for reaction with the solution, concentration of the solution, and time and temperature of exposure, variables that directly interfere in the clarity of the staining and in the interpretation of the results (Silva et al., 2023; Brasil, 2025). Thus, there is still no standardized protocol of the tetrazolium test for *C. quercifolius* seeds that allows quick and reliable viability estimates.

In view of this need, this study aimed to propose a protocol for conducting the tetrazolium test on *C. quercifolius* seeds, evaluating preconditioning conditions, type of cut, solution concentration, and staining time and temperature, in order to enable rapid and consistent diagnoses of viability and support actions aimed at conservation and sustainable use of the species.

## MATERIAL AND METHODS

**Seed collection and processing:** Ripe fruits of *C. quercifolius* (Figure 1) were harvested between August and September in three provenances: Massaroca (Juazeiro, BA; 2019), Sento Sé (BA; 2020) and Biological Collection of Embrapa Semi-Arid Region (Petrolina, PE; 2019). In each provenance, 10 parent trees were selected based on size, vigor and health, maintaining a minimum distance of 15 m between parent trees. Harvesting was carried out with a pruning hook and a tarpaulin, using gloves. After collection, the fruits were processed manually, with extraction and cleaning of the seeds, which were stored in cloth bags and kept in a cold chamber ( $10 \pm 3$  °C;  $60 \pm 4\%$  RH) until setting up the

trials. The lots from Massaroca and Embrapa Semi-Arid Region remained stored for 5 years, while the lot from Sento Sé remained stored for 4 years.

**Characterization of the collection areas:** The three collection areas are located in the Caatinga Domain, with xerophytic vegetation and BSh climate, according to Köppen (Alvares et al., 2013). This climate is characterized as hot semi-arid, with high temperatures throughout the year, high evapotranspiration, and prolonged seasonal water deficit, in addition to irregular rainfall and an average annual rainfall < 500 mm, concentrated between November and April (Silva et al., 2023). In general, there is a predominance of shallow, stony and low-fertility soils with low water retention.

**Water content (%):** Seed water content was obtained by the oven method at  $105 \pm 3$  °C for 24 h, with two subsamples of 25 seeds each, packed in aluminum capsules. The results were expressed as a percentage based on fresh mass (Brasil, 2025).

**Germination test (G%):** Conducted with four replications of 25 seeds, in Germitest® paper rolls moistened with a volume of distilled water equivalent to 2.5 times the mass of the dry paper (Brasil, 2025). The rolls were packed in transparent plastic bags and kept in a B.O.D.-type germinator at an alternating temperature of 20–30 °C and a photoperiod of 12 h (Silva and Aguiar, 2004). Germination was evaluated at 21 days, the period necessary for its stabilization. Seeds with radicle protrusion  $\geq 2$  mm were considered germinated.

**Tetrazolium test (TZT):** Conducted in a completely randomized design, in a  $7 \times 4$  factorial arrangement, with seven concentrations of 2,3,5-triphenyl tetrazolium chloride (0.025, 0.05, 0.075, 0.1, 0.25, 0.5 and 0.75%) and four staining periods (1, 2, 3 and 4 h), with four replications of 25 seeds per treatment. The test was performed separately for each provenance/lot (Massaroca, Sento Sé and Biological Collection). The choice of concentrations and times was based on protocols for related species and/or species from the Caatinga (Araújo et al., 2019; Walter et al., 2020; Silva et al., 2023; Silva et al., 2025), aiming to identify the combination that would provide clear and uniform tissue staining.

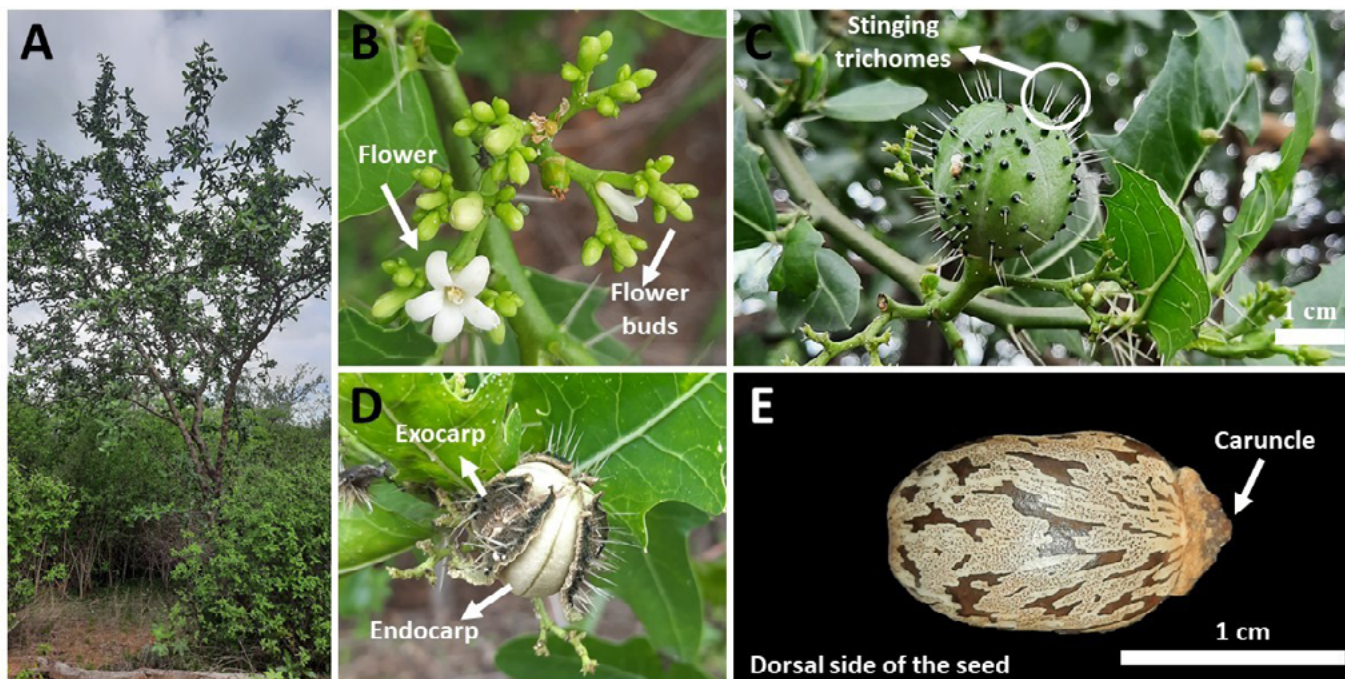


Figure 1. General view, flowers, fruits and seeds of *Cnidoscolus quercifolius* Pohl (Euphorbiaceae). A – General view of *C. quercifolius* in the Caatinga; B – Flower and Flower buds; C – Immature fruit with the presence of stinging trichomes; D – Ripe fruit showing the exocarp and endocarp; E – External view of the seed, with detail of the proximal region showing the caruncle.

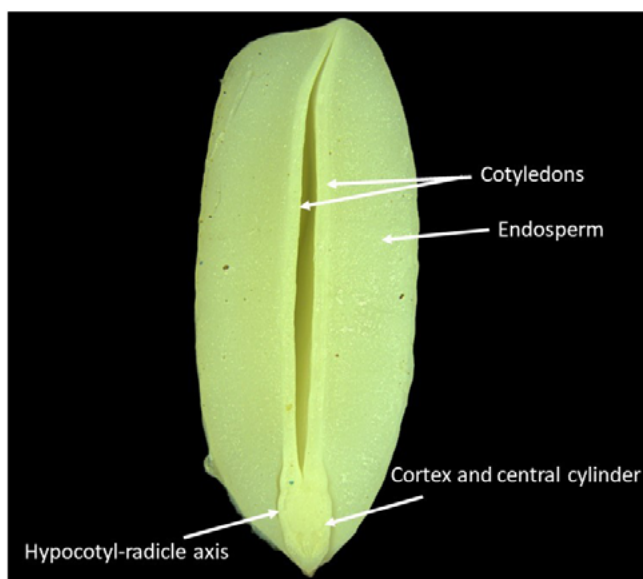


Figure 2. Median longitudinal section of a *Cnidoscolus quercifolius* Pohl (Euphorbiaceae) seed showing its internal morphology: Cotyledons, Endosperm and Hypocotyl-radicle axis.

Prior to staining, the seeds were preconditioned for 16 h between sheets of paper moistened with distilled water at 30 °C. Next, the seed coat was removed and the seeds were sectioned longitudinally, crossing cotyledons and embryonic axis (Figure 2). Staining was carried out using the half that contained the embryonic axis, which was immersed in the tetrazolium solution and kept at 30 °C, in the absence of light (Santos et al., 2006). At the end of each period, the halves were washed under running water, dried on paper towels and evaluated for tissue staining and integrity/turgidity.

Seeds were classified as viable when they showed uniform carmine-red staining in essential tissues (especially the embryonic axis), without extensive whitish areas, and as non-viable when they showed absence of staining (milky-white tissue), very irregular staining and/or severe damage in regions essential to development. Evaluations were performed with the naked eye and under a Leica M125 C stereomicroscope (Leica Microsystems GmbH, Wetzlar, Germany), with photographic records.

*Statistical analysis:* Normality of residuals was evaluated by the Shapiro-Wilk test (Shapiro and Wilk, 1965) and homogeneity of variances by the Bartlett test (Bartlett, 1937). As the assumptions of normality and homogeneity of variances were not met, the viability data estimated by the tetrazolium test (TZT) were analyzed by Generalized Linear Models (GLM). The significance of the effects was assessed by analysis of deviance. Multiple comparisons were performed based on estimated marginal means, with interaction decomposition and Tukey's adjustment ( $\alpha = 0.05$ ).

Comparison between the results of viability and germination percentage was performed using Dunnett's test (Dunnett, 1955). All statistical analyses were conducted using R software (R Core Team, 2023).

## RESULTS AND DISCUSSION

The Shapiro-Wilk and Bartlett tests indicated violation of the assumptions of normality of residuals and homogeneity of variances ( $p < 0.05$ ) in all lots evaluated (Table 1). Thus, the viability data obtained by the TZT were analyzed by Generalized Linear Models (GLM). Analysis of deviance showed that both factors, solution concentration and immersion time, contributed to the variability of the estimated viability, with a greater reduction in deviance associated with concentration and an additional effect of time. A significant interaction between concentration and time was detected in all lots, indicating that the TZT response results from the combination of these factors. The observed behavior shows

that, although concentration and time influence the test, it is the appropriate adjustment of the immersion period that ensures homogeneous tissue staining and more accurate viability estimates.

The coefficients of variation found indicate different behaviors between the lots. The lot harvested in the Biological Collection of Embrapa had the lowest CV (17.83%), suggesting greater uniformity of response among the seeds, while Sento Sé recorded the highest value (28.59%), reflecting greater heterogeneity. The lot from Massaroca showed an intermediate pattern (21.84%) (Table 1).

The water content of the seeds, determined prior to the tests, was 4.91%, 4.53% and 4.47% for the lots from Massaroca, Biological Collection and Sento Sé, respectively. The proximity of these values indicates that the lots were evaluated under similar water conditions. When comparing different lots of the same species, it is important that they have similar water contents, ensuring that the other evaluations reflect real physiological differences, since variations in moisture can interfere with the standardization and interpretation of the tests (Marcos-Filho, 2016).

In the germination test, the lots from Massaroca, Sento Sé and the Biological Collection showed percentages of 62%, 36% and 87%, respectively (Table 2), pointing to differences in physiological quality between the lots. As the seeds were stored under similar conditions ( $10 \pm 3$  °C;  $60 \pm 4\%$  RH), these variations suggest a predominant effect of the initial quality, defined in the field and in the post-harvest management.

Table 1. Analysis of deviance (ANODEV) for the viability of *Cnidocolus quercifolius* Pohl seeds (Euphorbiaceae) as a function of different tetrazolium concentrations and immersion periods, for three lots.

Massaroca/2019					
Source of variation	DF	Residual DF	Deviance residual	$\Delta$ Deviance	p-value
Null		111	880.25		
Concentration (C)	6	105	261.36	618.89	< 0.001
Time (T)	3	102	204.41	56.95	< 0.001
C x T	18	84	47.70	156.71	< 0.001
CV (%) = 21.84	p(Shapiro-Wilk) = 0.0049		p(Bartlett) $\leq$ 0.001		
Sento Sé/2020					
Null		111	1143.03		
Concentration (C)	6	105	480.59	662.44	< 0.001
Time (T)	3	102	238.75	241.84	< 0.001
C x T	18	84	28.56	210.19	< 0.001
CV (%) = 28.59	p(Shapiro-Wilk) = 0.0401		p(Bartlett) $\leq$ 0.001		
Biological collection/2019					
Null		111	880.25		
Concentration (C)	6	105	261.35	618.90	< 0.001
Time (T)	3	102	204.41	56.94	< 0.001
C x T	18	84	47.70	156.71	< 0.001
CV (%) = 17.83	p(Shapiro-Wilk) = 0.001		p(Bartlett) $\leq$ 0.001		

Notes: DF: degrees of freedom; C: solution concentration; T: Immersion time; CV: experimental coefficient of variation; p(Shapiro-Wilk): probability of the error normality test; p(Bartlett): probability of the variance homogeneity test.

Table 2. Viability (%) of *Cnidocolus quercifolius* Pohl (Euphorbiaceae) seeds, estimated by the tetrazolium test (TZT), as a function of TZT concentration (%) and immersion time (h), for three seed lots (Massaroca/2019, Sento Sé/2020 and Biological Collection/2019).

Concentration (%)	Time (hours)			
	1	2	3	4
Massaroca/2019				
G (%) = 62				
0.025	0* cB	35* bA	45 bA	45 bA
0.05	0* cC	40 bB	70 aA	55 bB
0.075	0* cB	65 aA	65 aA	70 aA
0.1	50 bB	75 aA	85 aA	60 bB
0.25	45 bB	75 aA	70 aA	75 aA
0.5	85 aA	90* aA	75 aA	80 aA
0.75	75 aA	85 aA	85 aA	90* aA
Sento Sé/2020				
G (%) = 36				
0.025	0* dA	0* dA	15* cA	10* cA
0.05	5* dB	25* cA	35 bA	35 bA
0.075	0* dB	40 bA	35 bA	40 bA
0.1	0* dC	40 bB	75* aA	80 aA
0.25	25* cB	20* cB	70 aA	65 aA
0.5	55 bB	70 aA	80* aA	85* aA
0.75	75 aA	85* aA	85* aA	80* aA
Biological Collection/2019				
G (%) = 87				
0.025	0* cB	35* bA	45* bA	45* bA
0.05	0* cC	40* bB	70 aA	55* bB
0.075	0* cB	65 aA	65 aA	80 aA
0.1	50* bB	75 aA	85 aA	60 bB
0.25	45* bB	75 aA	70 bA	75 aA
0.5	85 aA	90 aA	75 aA	80 aA
0.75	75 aA	85 aA	85 aA	90 aA

Notes: G (%) = germination percentage of the respective lot. Values followed by an asterisk (\*) differ from G (%) by Dunnett's test, at 5% probability level. In each column, means followed by the same lowercase letter do not differ from each other; in each row, means followed by the same uppercase letter do not differ from each other by Tukey test at 5% probability level. n = 4 replications of 25 seeds per treatment.

The higher germination of the lot from the Biological Collection of Embrapa (87%) may be associated with the collection carried out in a monitored area, with greater control of the maturity point and more standardized processing, reducing damage and post-maturity losses. In contrast, the low performance of the lot from Sento Sé (36%), harvested in 2020, may reflect differences in the maturity stage at harvest and interannual and edaphoclimatic variations during seed formation. In addition, because it remained in storage for a shorter time (4 years) than the others (5 years), the difference is hardly explained only by deterioration during storage (Marcos-Filho, 2016; Nascimento et al., 2021; Gomes et al., 2023).

Proper execution of each phase of the tetrazolium test (TZT) is essential to ensure reliable results in seed viability and vigor evaluation. The preconditioning applied to *C. quercifolius* seeds (blotting paper moistened with distilled water for 16 h at 30 °C) promoted uniform imbibition, activating the initial metabolism and favoring the reaction of the tetrazolium salt with the dehydrogenase enzymes of the living tissues, which resulted in clear and homogeneous staining and facilitated the interpretation (França-Neto and Krzyzanowski, 2019). Similar results regarding the importance of preconditioning, removal of structures, and concentration/time adjustment have also been reported for native species of the Caatinga, such as *Handroanthus spongiosus*, *Anadenanthera colubrina*, and *Commiphora leptophloeos* (Silva et al., 2023; Pereira et al., 2024; Silva et al., 2025).

However, even between related species, TZT parameters are not directly transferable. In the Euphorbiaceae family, for example, 0.25% for 4 h at 40 °C is recommended for *Jatropha mollissima* (Walter et al., 2020), while in *C. quercifolius* the highest efficiency was obtained with lower concentrations (0.05-0.075%) and 3-4 h of staining at 30 °C. This contrast reinforces the need for a species-specific procedure, highlighting the originality and relevance of the proposed standardization.

When comparing the germination percentages with the results of the tetrazolium test (TZT), it was found that the concentration of 0.075%, associated with immersion periods of 3 and 4 hours, promoted consistent viability estimates, with no statistical differences relative to germination in the three lots, according to Dunnett's test (Table 2). The concentration of 0.05% for 3 hours also produced results equivalent to germination in all lots, while for 4 hours, there was a slight underestimation in the lot with the highest quality (Biological Collection).

The ideal combination of concentration and time of exposure to tetrazolium salt can vary between species, depending on the intrinsic characteristics of the seeds. Although it belongs to the same family as *C. quercifolius*, the species *Jatropha mollissima* (Pohl) Baill. has a different protocol, with recommendation of a 0.25% solution for 4 hours at 40 °C (Walter et al., 2020).

Depending on the species, very low concentrations ( $\leq 0.025\%$ ) may underestimate viability, as observed in *Crateva tapia* L. (Rodrigues et al., 2025), while high concentrations ( $\geq 0.5\%$ ) may result in excessively intense staining, as reported for *Cicer arietinum* L. seeds (Paraíso et al., 2019), making it difficult to differentiate between viable and deteriorated tissues and leading to overestimated results. This effect was particularly evident in the lot with the lowest quality, from Sento Sé/2020, in which the combinations with 0.5% and 0.75% overestimated viability relative to germination (Table 2). Thus, developing an appropriate methodology for tetrazolium testing becomes essential, especially in the evaluation of lots with different levels of physiological quality.

Appropriate combination between solution concentration, immersion time, and preconditioning should allow accurate identification of differences in viability and, consequently, vigor between lots, ensuring consistent results that are representative of the physiological condition of the seeds (Pereira et al., 2024). In *C. quercifolius* seeds, the tetrazolium test made it possible to clearly distinguish viable and non-viable seeds. Figure 3 shows viable seeds after TZT staining, characterized by continuous pink to carmine-red hues in the vital tissues of the embryo (embryonic axis and cotyledons), which are intact and turgid.

The continuous staining in the regions of cotyledon attachment and embryonic axis, associated with the turgidity of the tissues, confirms the integrity of the vital structures, typical of metabolically active and physiologically viable seeds. This staining pattern reflects adequate penetration and reaction of the tetrazolium salt, indicating good membrane permeability and absence of mechanical damage or cell deterioration (Silva et al., 2023; Tronco et al., 2025). Such characteristics, homogeneous carmine-red staining and intact and turgid tissues, are widely used criteria for interpreting the tetrazolium test (França-Neto et al., 2020).

Figure 4 shows non-viable seeds after the tetrazolium test (TZT), characterized by the absence of staining (milky-white appearance) or by the presence of intense and irregular red staining in the regions of the embryo, cotyledons and endosperm. Flaccid tissues and staining failures are observed, especially in the regions of cotyledon attachment, indicating loss of cellular integrity and physiological non-viability. These symptoms reflect flaws in the permeability

of membranes and impairment of the enzymatic activity necessary for the reduction of tetrazolium salt, typical of dead or severely deteriorated tissues (França-Neto and Krzyzanowski, 2018). Similarly, Silva et al. (2023) reported cell degeneration and absence of staining in non-viable seeds of native forest species, corroborating the pattern observed in this study. This morphological characterization, combined with quantitative results, reinforces the potential of TZT as a fast and reliable method for evaluating the physiological quality of *C. quercifolius* seeds, in agreement with previous findings in species from the Caatinga (Souza et al., 2017; Cunha et al., 2021).

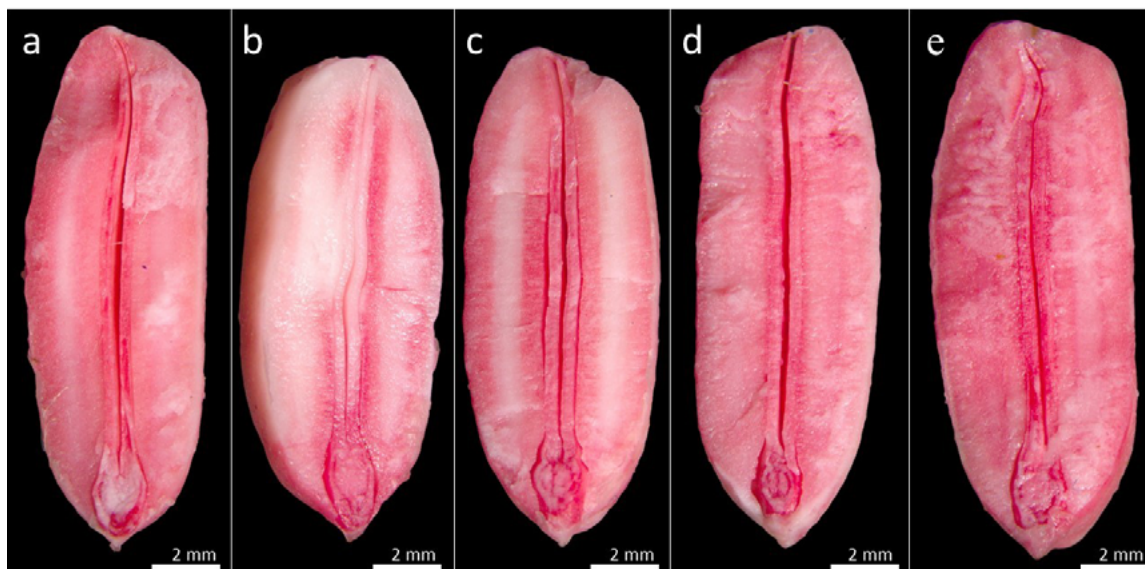


Figure 3. Viable seeds of *Cnidocolus quercifolius* Pohl (Euphorbiaceae) after the tetrazolium test (TZT), showing uniform pink to carmine-red staining in the tissues of the embryo and cotyledons, with a completely stained embryonic axis and tissues with a turgid appearance. (a–e) Examples of seeds classified as viable. Bar = 2 mm.

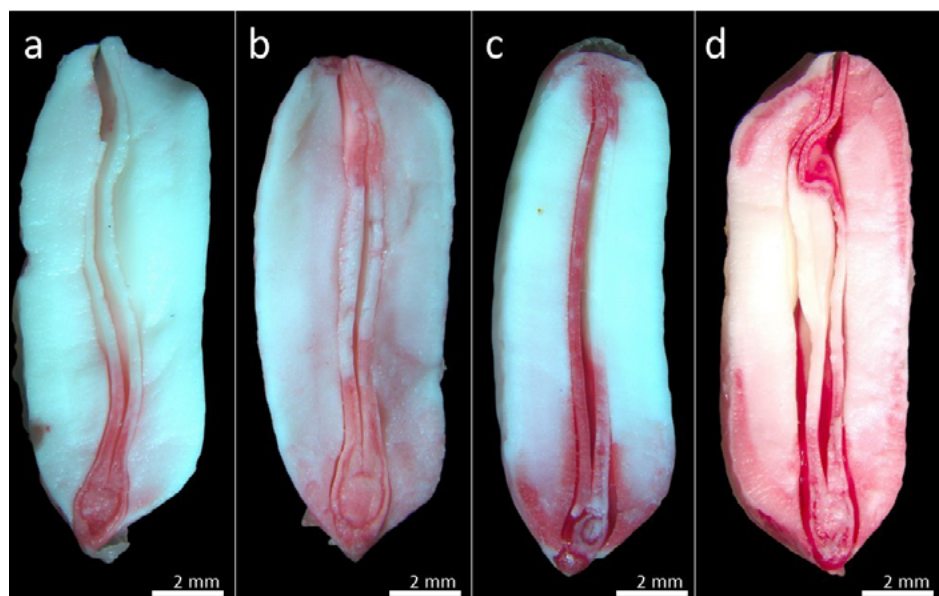


Figure 4. Non-viable seeds of *Cnidocolus quercifolius* Pohl (Euphorbiaceae) after the tetrazolium test (TZT), showing absence of staining (milky-white appearance) or localized intense red staining, especially in the embryonic axis, associated with flaccid tissues and staining failures in critical regions of the embryo and cotyledons. (a–d) Examples of non-viable seeds. Bar = 2 mm.

In relation to the genus *Cnidoscolus* Pohl, recent literature is scarce regarding detailed protocols for the tetrazolium test. From this perspective, the protocol proposed here for *C. quercifolius* represents an advance in standardization, by combining preconditioning, longitudinal cut and concentration/time ranges compatible with the general guidelines of the TZT (França-Neto et al., 2020; Alves et al., 2022) with the accumulated experience on species from the Caatinga (Dantas et al., 2015).

While TZT meets the demand for rapid diagnoses of seed viability, its integration with other vigor and quality tests, such as electrical conductivity (membrane integrity), accelerated aging, and X-ray analysis, allows for a more comprehensive assessment of physiological quality. This combined approach can increase the efficiency in controlling the quality of native species, especially for small and heterogeneous seeds, such as those from *C. quercifolius* (Marcos-Filho, 2016; Krzyzanowski and França-Neto, 2019; Krzyzanowski et al., 2020; ISTA, 2023).

## CONCLUSIONS

It is concluded that the tetrazolium test is efficient to estimate the viability of *Cnidoscolus quercifolius* Pohl seeds, providing fast and consistent results relative to the germination test. Preconditioning for 16 h on blotting paper moistened with distilled water at 30 °C favors uniformity of imbibition and differentiation of tissues. The combinations of 0.075% tetrazolium for 3 to 4 h of immersion and 0.05% for 3 h proved to be the most adequate, as they ensure clear staining and viability estimates statistically equivalent to germination in most lots. Under these conditions, TZT is consolidated as a practical and strategic tool for programs aimed at conservation and management of Caatinga species.

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## AUTHORS' CONTRIBUTION

Jailton de Jesus Silva contributed with: Conceptualization, Methodology, Investigation, Formal analysis, Visualization, Writing - original draft, Writing – review & editing, Jamille Cardeal da Silva contributed with: Methodology, Investigation, Formal analysis, Visualization, Writing - original draft, Raquel Araujo Gomes contributed with: Methodology, Investigation, Formal analysis, Visualization, Writing - original draft, Visêlido Ribeiro de Oliveira contributed with: Investigation, Writing - original draft, Writing – review & editing; Bárbara França Dantas contributed with: Conceptualization, Methodology, Investigation, Formal analysis, Visualization, Writing - original draft, Writing – review & editing.

## DATA AVAILABILITY

The datasets generated and analyzed during the current study are publicly available in the Redape repository: Dantas, B.F.; Silva, J.C.; Silva, J.J.; Gomes, R.A. *Tetrazolium test in seeds native to the Brazilian Caatinga*. Redape. <https://doi.org/10.48432/WVOSO9>, 2026.

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