

EVALUATION OF VIABILITY OF *Tabebuia aurea* SEEDS THROUGH TETRAZOLIUM TEST¹

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ABSTRACT – The viability of *T. aurea* seeds is evaluated through the germination test, taking 21 days, which makes it difficult to obtain quick information about the viability of its seeds. In this context, using the tetrazolium test as an evaluation method would be appropriate because it provides faster and more reliable information for several species. In view of the above, the objective of this study was to adapt the tetrazolium test methodology to evaluate the viability of *T. aurea* seeds. Initially, the imbibition curve was constructed to determine the appropriate hydration period for the seeds and the germination test was conducted, both for evaluating the initial quality and for comparison with the tetrazolium test results. For the tetrazolium test, the seeds were hydrated for 24 hours, then their coats were removed and the seeds were immersed in tetrazolium solutions. The concentrations used were 0.05, 0.075 and 0.1% for three staining periods 2, 4 and 6 hours, at 35 and 40 °C, in the absence of light. The experimental design was completely randomized in a 3 x 3 + 1 factorial scheme (three concentrations of tetrazolium solution x three staining periods + one control = germination test) with four replicates of 25 seeds. Tetrazolium test was adequate to evaluate the viability of *T. aurea* seeds using the concentration of 0.05% for four hours at 40 °C.

Keywords: Bignoniaceae. 2,3,5 triphenyl tetrazolium chloride. Forest species.

AVALIAÇÃO DA VIABILIDADE DE SEMENTES DE *Tabebuia aurea* POR MEIO DO TESTE DE TETRAZÓLIO

RESUMO – A avaliação da viabilidade de sementes de *T. aurea* é realizada por meio do teste de germinação, demorando 21 dias, o que dificulta a obtenção de informações rápidas sobre a viabilidade de suas sementes. Nesse sentido, a utilização do teste de tetrazólio como método de avaliação seria adequado por proporcionar informações com maior agilidade e confiável para várias espécies. Diante do exposto, objetivou-se adequar a metodologia do teste de tetrazólio para avaliar a viabilidade de sementes de *T. aurea*. Inicialmente, realizou-se a curva de embebição para determinar o período adequado de hidratação das sementes e o teste de germinação, tanto para avaliar a qualidade inicial, quanto para fins comparativos com os resultados do teste de tetrazólio. Para a instalação deste, as sementes foram hidratadas por 24 horas, posteriormente, o tegumento foi removido e as sementes imersas em soluções de tetrazólio. As concentrações utilizadas foram de 0,05; 0,075 e 0,1% por três períodos de coloração 2, 4 e 6 horas, a 35 e 40 °C, em ausência de luz. O delineamento experimental foi o inteiramente casualizado em esquema fatorial 3 x 3 + 1 (três concentrações da solução de tetrazólio x três períodos de coloração + uma testemunha = teste de germinação), com quatro repetições de 25 sementes. O teste de tetrazólio mostrou-se adequado para avaliar a viabilidade de sementes de *T. aurea* utilizando a concentração de 0,05%, por quatro horas, a 40 °C.

Palavras-chave: Bignoniaceae. 2,3,5 trifenil cloreto de tetrazólio. Espécie florestal.

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INTRODUCTION

Tabebuia aurea (Silva Manso) Benth. & Hook. f ex S. Moore is a tree species popularly known in Portuguese as 'craibeira' or 'ipê-amarelo-do-cerrado', belonging to the Bignoniaceae family, common in the ecosystems of Caatinga, Cerrado, Amazon forest and Pantanal (LORENZI, 2014). The species can be used for reforestation, urban and rural afforestation, civil construction, paper production and carpentry, as it produces wood with high mechanical strength (GUEDES et al., 2012).

T. aurea is mainly multiplied by seeds, and it is essential to evaluate their viability. The germination test is officially used to evaluate viability; however, for *T. aurea*, it takes 21 days. This fact makes it difficult to make decisions about the choice and disposal of lots (BRASIL, 2013).

An alternative to this is the tetrazolium test, which has been widely used to evaluate the quality of seeds of various species for being considered effective, accurate and low cost, besides enabling the identification of causes of abnormality and providing information on physiological potential in less than 24 hours (DIAS; ALVES, 2008). In addition to evaluating the viability of seeds with slow germination and dormancy, this test makes it possible to obtain information about vigor and identifies several factors that affect their performance under field conditions (MARCOS-FILHO, 2015).

The efficiency of the tetrazolium test depends on the adoption of procedures that favor agility in seed preparation and clarity for evaluation (ARAÚJO; REIS; NOVOBRE, 2016). In this context, the tetrazolium test methodology has been studied for several forest species, such as *Simira gardneriana* (M.R. Barbosa & Peixoto) (OLIVEIRA et al., 2016), *Libidibia ferrea* (Mart. ex Tul.) L.P. Queiroz var *L. ferrea* (CARVALHO et al., 2017), *Albizia hasslerii* (DUBOC; SILVEIRA, NASCENTES, 2018), *Genipa americana* L. (VIRGENS; CONCEIÇÃO; BARBOSA, 2019), and *Piptadenia stipulacea* (Benth.) Ducke (PEREIRA et al., 2020).

In view of the above, the objective of this study was to adapt the tetrazolium test methodology to evaluate the viability of *T. aurea* seeds.

MATERIAL AND METHODS

The experiment was carried out at the Seed Analysis Laboratory of the Department of Agronomic and Forestry Sciences (DCAF) of the Center for Agrarian Sciences (CCA) of the Federal Rural University of the Semi-Arid Region (UFERSA), Mossoró, RN, Brazil. The seeds were obtained from closed ripe fruits, collected from

approximately 25 native trees existing in the municipality of Mossoró (05° 12' 15" S; 37° 19' 31" W and 22 m altitude). After collection, the fruits were dried in the shade in order to standardize their moisture content and facilitate seed extraction. After extraction, seeds that were malformed, attacked by fungi or insects were eliminated. The processed seeds were placed in plastic bag packages and stored in a controlled environment (17 °C and 50% relative humidity) until the beginning of the experimental phase.

The experimental design was completely randomized in a 3 x 3 + 1 factorial scheme, three concentrations of tetrazolium solution (0.050, 0.075 and 0.1%) x three staining periods (2, 4 and 6 hours) and the control (germination test), at temperatures of 35 and 40 °C. Initially, the moisture content of the seeds was determined by the oven method at 105 °C ± 3 °C for 24 hours (BRASIL, 2009). Then, the imbibition curve was constructed using four subsamples of 50 seeds. For this, the seeds were weighed and subsequently placed on three sheets of paper towel (*Germitest*®), moistened with distilled water in an amount equivalent to 2.5 times the dry paper weight. The paper rolls with the seeds were placed in a plastic bag to maintain their moisture and put to soak in a Biochemical Oxygen Demand (B.O.D) germination chamber, at 25 °C. The seeds were weighed on a precision scale (0.001 g) every 60 minutes until the primary root was produced in 50% of the seeds.

To obtain a standard of comparison for the tetrazolium test results, the germination test was installed with four replicates of 25 seeds, on two sheets of paper towel (*Germitest*®) and covered by a third sheet, previously moistened with distilled water in an amount equivalent to 2.5 times the dry paper weight and placed to germinate in the form of rolls. These rolls were placed in plastic bags and taken to B.O.D. germination chambers at 25 °C. The evaluation was performed twenty-one days after sowing, and the data were expressed as a percentage of normal seedlings (BRASIL, 2013).

To conduct the tetrazolium test, the seeds were pre-moistened by direct immersion in distilled water for 24 hours. After moistening, the coat was completely removed and the seeds were immersed in a solution of 2,3,5 triphenyl tetrazolium chloride (50 mL), at three concentrations (0.05, 0.075 and 0.1%) for three staining periods (2, 4 and 6 hours), at 35 and 40 °C in the absence of light. For each combination, four subsamples of 25 seeds were used. After each staining period, the tetrazolium solution was drained, the seeds were washed in running water and longitudinally cut in the center of the embryonic axis. The evaluation was performed with a tabletop magnifying glass, and the seeds were classified as viable or non-viable according to the color pattern indicated by Nogueira, Torres and Freitas (2014).

Viable seeds showed bright light pink color, tissues with normal and firm appearance, embryonic axis with intense red color, but without reaching the central cylinder, and less than 50% of the unstained cotyledons, cotyledons with necrotic regions, but without affecting the region of attachment to the embryonic axis. Non-viable seeds were those with more than 50% of the unstained cotyledons, with intense red color or necrotic, embryonic axis with unstained, intense red and/or necrotic regions, reaching the central cylinder. The results were expressed in percentage of viable seeds.

The data were subjected to tests of normality and homogeneity of variances, showing normal distribution. The means of viable seeds obtained by the tetrazolium test were compared by Tukey test ($p \leq 0.05$) and the means of viable seeds obtained by the germination test were compared by Dunnett test ($p \leq 0.05$), following recommendations of Banzatto and Kronka (2006). Statistical analyses were performed using the program ASSISTAT 7.7 beta (SILVA; AZEVEDO, 2002).

RESULTS AND DISCUSSION

The initial moisture content of the seeds was 6.5%, with Phase I lasting 14 hours (Figure 1). Such rapid water absorption occurs as a result of the difference in matric potential between the dry seeds and the moist substrate, regardless of whether the seed is dormant or the coat is more impermeable to water (BEWLEY *et al.*, 2012). This phase is important because the seeds change from inert to the activation of all their metabolic activities, including respiratory activities, which are necessary to conduct the tetrazolium test. However, the duration of this phase varies according to the species.

For *Adenanthera pavonina* L. seeds (MANTOAN *et al.*, 2012), the duration of Phase I was three hours; for *Copernicia hospita* Martius it lasted only two hours (OLIVEIRA; BOSCO, 2013), while for *Jatropha curcas* L. the duration was 32 hours (SMIDERLE; LIMA; PAULINO, 2013) and for *S. gardneriana* seeds this phase was completed after 33 hours of immersion in water (OLIVEIRA *et al.*, 2016).

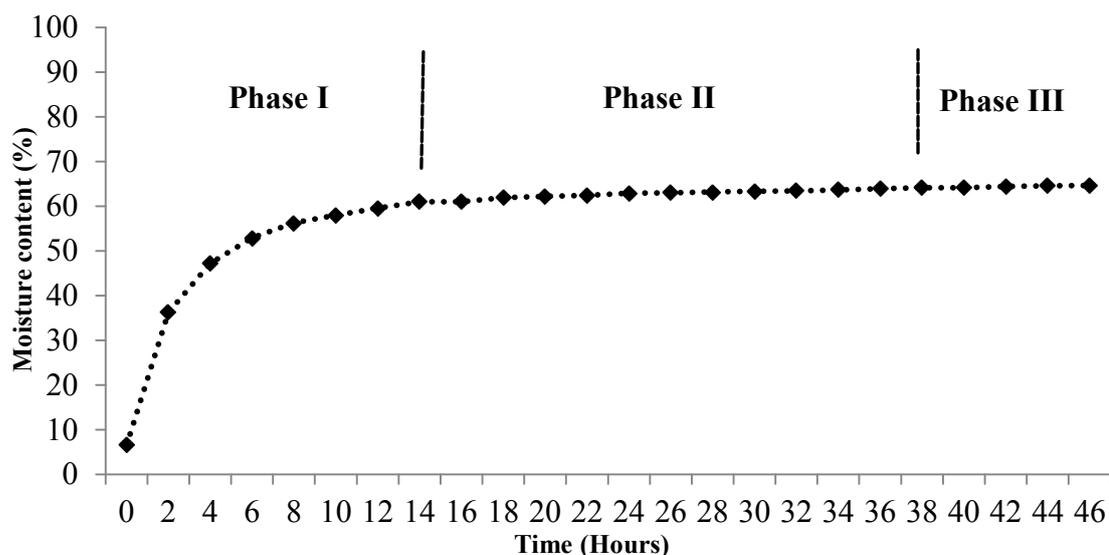


Figure 1. Three-phase hydration pattern in *Tabebuia aurea* seeds.

In Phase II, water absorption stabilized around 60% between 12 and 36 hours, because the cells within the seeds had already reached the maximum expansion limit and achieved a balance between the water potential of the seed and the surrounding environment (CASTRO; BRADFORD, HILHORST, 2004). At this stage, water absorption by the seeds was quite slow because at this stage the activities are limited to the recovery of cellular integrity, mitochondria and DNA repair and intensification of respiratory activity, with no intense digestion or significant protein synthesis (MARCOS-FILHO, 2015). Identifying this phase is important to define the hydration period of the seeds before

immersion in tetrazolium solution, mainly because it is in this phase that the increase in respiratory activity occurs, favoring the reduction reaction with tetrazolium salt.

Phase III began after 38 hours and occurred at the moment when the radicle breaks the coat and is released to the external environment, being called the primary root. This occurs due to successive mitoses of the meristematic tissue, which cause the growth of the embryonic axis, resulting from the activation of the enzymatic system during Phase II. In this phase, the presence of water is essential, because the seed is in an intense process of mitotic division important for embryo elongation and shoot

growth, and this event is extremely dependent on water (MARCOS-FILHO, 2015).

Based on the imbibition curve, the 24-hour period of hydration preceding the tetrazolium test is sufficient for tissue hydration. In this period, the seeds reached a moisture content around 63% and did not reach Phase III. According to preliminary tests, it is in this period of hydration that there is better penetration of tetrazolium salt into the tissues, promoting uniform staining (BRASIL, 2009), also facilitating the softening of tissues for cutting and

removing the coat, improving the quality and evaluation of the test (AOSA, 2009).

After the hydration period was determined, the seeds were exposed to the treatments. The results show that there was significant interaction for viability at 1% probability level between periods and concentrations, at both temperatures. The same result can be verified between the seed viability estimates obtained by the tetrazolium test and the results of the germination test (Table 1).

Table 1. Summary of the analysis of variance for viable seeds of *Tabebuia aurea* obtained by the tetrazolium test at different concentrations and staining periods, at 35 and 40 °C, compared to the results of the germination test (control).

Sources of variation	Mean square	
	35 °C	40 °C
Period (P)	248.44**	4917.44**
Concentration (C)	1501.78**	1337.44**
Int P x C	73.78**	833.11**
Factorial x Control	6622.04**	7525.88**
Treatments	1157.51**	2596.46**
Error	6.40	27.43
Mean	33.40	30.85
CV (%)	7.57	16.98

**Significant at 1% probability level.

There was low viability of *T. aurea* seeds at all concentrations and exposure periods at 35 °C, compared to the results of the germination test (Table 2). At this temperature, the highest mean viability was obtained at the lowest concentration

(0.05%) with 4 hours of immersion in tetrazolium salt, while the lowest results were obtained at the highest concentration (1%), not differing between the periods of 2 and 6 hours (Table 2).

Table 2. Means of viability of *Tabebuia aurea* seeds from the tetrazolium test conducted at different concentrations and staining periods, at 35 °C.

Periods	Concentrations (%)		
	0.05*	0.075	0.1
2	34 cAz	30 aAz	17 aBz
4	48 aAz	34 aBz	21 aCz
6	40 bAz	21 bBz	17 aBz
Germination (%)	72x		

*Means followed by the same uppercase letter (A, B, C) in the row and lowercase letter (a, b, c) in the column do not differ significantly by Tukey test at 5% probability level. Means followed by the same letter (x, z), between germination (control) and viability obtained in the tetrazolium test, do not differ significantly by Dunnett test at 5% probability level.

The temperature of 35 °C led to problems for embryo staining, especially at the highest concentration in all periods. This fact occurred in response to the excess salt, which caused points with more intense color in the seeds, making it difficult to evaluate viable seeds. At the lowest concentrations, the tetrazolium solution did not spread evenly, which made it difficult to distinguish viable and deteriorating tissues.

Studies conducted by Oliveira et al. (2016)

with 'pereiro-vermelho' (*S. gardnerian* M.R. Barbosa & Peixoto) and Carvalho et al. (2017) with 'juçá' (*L. ferrea*) found that the temperature of 35 °C was insufficient to evaluate the viability of these seeds, similar to the results obtained in this study. Thus, the seeds that did not develop the ideal color in their tissues may have been classified as non-viable when the color was not adequate, resulting from the treatments tested (temperature, combined with different concentrations and periods).

However, the temperature of 40 °C at a concentration of 0.05% for 4 hours promoted viability results similar to those obtained in the germination test (Table 3). This treatment showed clear staining of the embryos (Figure 2A), as well as damage due to necrosis, dead seed, deteriorating

cotyledons and compromised embryonic axis (Figure 2B, 2C, 2D and 2E), which facilitated the analysis and interpretation of the results, demonstrating the importance of identifying appropriate periods and concentrations.

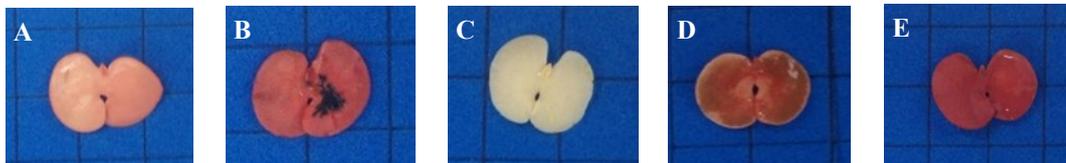


Figure 2. *Tabebuia aurea* seeds considered viable by the tetrazolium test: (A) Seeds with bright light pink color, tissues with normal and firm appearance; (B) Cotyledons with necrotic regions, but not affecting the region of attachment to the embryonic axis. *Tabebuia aurea* seeds considered non-viable by the tetrazolium test: (C) Seeds completely unstained; (D) With more than 50% of cotyledons with damaged tissues; (E) With intense red color in all parts, cotyledons and embryonic axis reaching the central cylinder.

This combination of period and concentration is recommended for evaluation of forest species, such as ‘ipê branco’ (*Tabebuia roseoalba* (Ridl.) Sandwith) (ABBADE; TAKAKI, 2014), ‘juçá’ (*L. ferrea*) (CARVALHO et al., 2017), for 24 hours at 36 °C and for 3 hours at 40 °C, respectively. On the other hand, it differs from the results obtained for ‘jurema-branca’ (*P. stipulacea* (Benth.) Ducke),

which showed greater viability at the tetrazolium salt concentration of 0.075% for 4 hours, at temperatures of 35 or 40 °C (PEREIRA et al., 2020), and for *Poincianella pyramidalis* (Tul.) L. P. Queiroz, for which the best results were obtained using the tetrazolium salt concentration of 0.075% for 91 minutes at 41 °C (SOUSA et al., 2017).

Table 3. Average viability of *Tabebuia aurea* seeds from the tetrazolium test conducted at different concentrations and staining periods at 40 °C.

Periods (hours)	Concentrations (%)		
	0.05*	0.075	0.1
2	7.5 cAz	8 bAz	15 bAz
4	72 aAx	50 aBz	25 aCz
6	35 bAz	10 bBz	14 bBz
Germination (%)	72x		

*Means followed by the same uppercase letter (A, B, C) in the row and lowercase letter (a, b, c) in the column do not differ significantly by Tukey test at 5% probability level. Means followed by the same letter (x, z), between germination and viability obtained in the tetrazolium test, do not differ significantly by Dunnett test at 5% probability level.

The results obtained in this study agree with Krzyzanowski, Vieira and França-Neto (1999), who state that the lower concentrations are indicated because they enable better visualization of unstaining disorders and identification of different types of lesions. In addition, when subjected to higher temperatures, they provide faster results (AOSA, 2009).

Studies have demonstrated efficiency in obtaining methodologies to evaluate the physiological quality of forest species through the tetrazolium test, and these studies demonstrate different results among the studied species. For example, in a study with ‘ipê branco’ (*T. roseoalba*),

the concentration of 0.05% for twenty-four hours at 36 °C is efficient to evaluate the physiological quality of seeds (ABBADE; TAKAKI, 2014). However, for ‘pereiro-vermelho’ (*S. gardnerian*) the concentration of 0.075% for six hours at 35 °C enables an efficient evaluation of the physiological quality of seeds (OLIVEIRA et al., 2016).

On the other hand, for ‘juçá’ (*L. ferrea*) a lower concentration, 0.05%, and shorter immersion period, three hours of staining, at temperatures of 35 or 40 °C, resulted in acceptable percentage of viability (CARVALHO et al., 2017); For ‘jenipapo’ (*G. americana*), it was found that the most efficient method to evaluate seed vigor and

viability is the immersion of seeds, not soaked initially, but immersed in a 0.10% solution for three hours (VIRGENS; CONCEIÇÃO; BARBOSA, 2019).

However, the period of immersion in tetrazolium salt should be evaluated with caution, because the concentration of 0.05% and the shortest staining period (2 h) at 40 °C underestimated the physiological quality of the seeds, but did not differ statistically from the other concentrations (Table 3). That is, the period of immersion in the tetrazolium salt of two hours was insufficient to evaluate the viability of the seeds, not allowing adequate staining. On the other hand, the time of six hours was excessive for staining the seeds, whose tissues were intensely stained, making it difficult to interpret the results and not allowing living tissues to be distinguished from dead or damaged tissues, especially at concentrations of 0.075 and 0.1% (Figure 2E).

CONCLUSION

The tetrazolium test is adequate for evaluating the viability of *T. aurea* seeds using a concentration of 0.05% for four hours at 40 °C.

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