Revista Ciência Agronômica, v. 51, n. 1, e20196712, 2020
Centro de Ciências Agrárias - Universidade Federal do Ceará, Fortaleza, CE
www.ccarevista.ufc.br

Scientific Article
ISSN 1806-6690

Physiological quality evaluation of *Piptadenia stipulacea* (Benth.) Ducke seeds by tetrazolium test[1]

Avaliação da qualidade fisiológica de sementes de *Piptadenia stipulacea* (Benth.) Ducke pelo teste de tetrazólio

Kleane Targino Oliveira Pereira[2]*, Emanoela Pereira de Paiva[3], Maria Lilia de Souza Neta[2], Clarisse Pereira Benedito[4] and Salvador Barros Torres[5]

ABSTRACT - The tetrazolium test is one of the promising alternatives to determine the viability of dormant, recalcitrant and slow-germinating seeds. The objective of this study was to adapt the tetrazolium test methodology to estimate the viability and vigor of *Piptadenia stipulacea* (Benth.) Ducke seeds. For this, a sample of seeds was classified into three sublots using round sieves, corresponding to six, five and four millimeters in diameter, respectively. Moisture content, imbibition curve, germination, emergence, germination and emergence speed indices, mean germination time and seedling emergence were initially evaluated. The second stage was conducted using a completely randomized experimental design, in 3 x 9 factorial scheme, corresponding to three sublots and nine combinations between concentrations and time of immersion in tetrazolium salt (0.05%/2 h; 0.05%/4 h; 0.05%/6 h; 0.075%/2 h; 0.075%/4 h; 0.075%/6 h; 0.1%/2 h; 0.1%/4 h; 0.1%/6 h), totaling twenty-seven treatments with four replicates of 25 seeds, evaluated separately at 35 and 40 °C. For each treatment, the seeds were divided into four classes: viable and vigorous seeds (class I), viable and non-vigorous (class II), unviable (class III) and dead (class IV). The period of 8 h of pre-imbibition and 4 h of staining in tetrazolium solution at 0.075%, under 35 or 40 °C, is adequate to estimate the viability and vigor of *P. stipulacea* seeds.

Key words: Fabaceae. Biochemical test. Viability. Forestry species. Triphenyl formazan.

RESUMO - O teste de tetrazólio constitui uma das alternativas promissoras para determinar a viabilidade de sementes dormentes, recalcitrantes e de germinação lenta. Objetivou-se adequar a metodologia do teste de tetrazólio para estimar a viabilidade e vigor em sementes de *Piptadenia stipulacea* (Benth.) Ducke. Para isso, uma amostra de sementes foi classificada em três sublotes utilizando peneiras de crivo redondo, correspondendo a seis, cinco e quatro milímetros de diâmetro, respectivamente. Inicialmente, avaliou-se o teor de água, curva de embebição, germinação, emergência, índice de velocidade de germinação e emergência, tempo médio de germinação e emergência de plântulas. Na segunda etapa, utilizou-se o delineamento experimental inteiramente casualizado, em esquema fatorial 3 x 9, sendo três sublotes e nove combinações entre concentrações e tempo de imersão no sal de tetrazólio (0,05%/2 h; 0,05%/4 h; 0,05%/6 h; 0,075%/2 h; 0,075%/4 h; 0,075%/6 h; 0,1%/2 h; 0,1%/4 h; 0,1%/6 h), totalizando vinte e sete tratamentos com quatro repetições de 25 sementes, avaliados separadamente a 35 e 40 °C. Para cada tratamento, as sementes foram divididas em quatro classes: sementes viáveis e vigorosas (classe I), viáveis e não vigorosas (classe II), inviáveis (classe III) e mortas (classe IV). O período de 8 h de pré-embebição e 4 h de coloração na solução de tetrazólio a 0,075%, sob 35 ou 40 °C, é adequado para estimar a viabilidade e vigor de sementes de *P. stipulacea*.

Palavras-chave: Fabaceae. Teste bioquímico. Viabilidade. Espécie florestal. Trifenilforozan.

DOI: 10.5935/1806-6690.20200019
* Author for correspondence
Received for publication 29/04/2019; approved on 16/09/2019
[1]Parte da Dissertação de Mestrado do primeiro autor apresentada ao Programa de Pós-Graduação em Fitotecnia
[2]Programa de Pós-Graduação em Fitotecnia, Universidade Federal Rural do Semi-Árido/UFERSA, Av. Francisco Mota, 572 - Bairro Costa e Silva, Mossoró-RN, Brasil, 59.625-900, kleane_rn@hotmail.com (ORCID ID 0000-0002-3863-9606), lilia.agronomia@hotmail.com (ORCID ID 0000-0002-2108-7526)
[3]Bolsista de Pós-Doutorado Júnior/CNPq, Programa de Pós-Graduação em Fitotecnia/UFERSA, Mossoró-RN, Brasil, emanuelappaiva@hotmail.com (ORCID ID 0000-0003-4510-9205)
[4]Universidade Federal Rural do Semi-Árido/UFERSA, Mossoró-RN, Brasil, clarisse@ufersa.edu.br (ORCID ID 0000-0002-2846-1162)
[5]Departamento de Ciências Agronômicas e Florestais, Universidade Federal Rural do Semi-Árido/UFERSA, Mossoró-RN, Brasil, sbtorres@ufersa.edu.br (ORCID ID 0000-0003-0668-3327)

INTRODUCTION

Piptadenia stipulacea (Benth.) Ducke - Fabaceae is known in northeastern Brazil as 'Jurema-Branca' and occurs virtually throughout the Caatinga, from Piauí to Bahia. It is a species mainly used to recover erosion-degraded soils (MAIA, 2012). Its main form of multiplication is by seeds, whose quality evaluation is fundamental for the formation of seedlings in nursery.

The germination test is officially the most used means for assessing seed viability in several species; however, in some cases, the duration of this test is long and leads to delay in decision making with respect to the purpose of seed lots. For *P. stipulacea*, the recommendation for germination test duration is 10 days after pre-germination treatment (BENEDITO *et al*., 2019) and 14 days for seedling emergence (FARIAS *et al*., 2013). For these reasons, it is important to develop rapid tests that consistently and reliably characterize seed quality.

In this context, the tetrazolium test is a promising alternative that enables rapid determination of the viability of dormant seeds, recalcitrant seeds and those that germinate slowly during routine tests (BRASIL, 2009). However, several factors interfere in the conduction of this test, such as solution concentration, temperature during tissue staining, time of seed exposure and seed preparation before staining (PAIVA *et al*., 2017).

Studies on adjusting the tetrazolium test methodology to assess the viability and vigor of *P. stipulacea* have not yet been conducted; however, studies have been conducted for some forest species, such as *Piptadenia moniliformis* Benth. (AZEREDO; PAULA; VALERI, 2011), *Tabebuia roseoalba* (Ridl.) Sandwith (ABBADE; TAKAKI, 2014), *Erythrina velutina* Willd. (CUNHA; GOMES, 2015), *Cassia grandis* L. F. (SANTOS *et al*., 2016) and *Libidibia ferrea* (Mart. ex Tul.) L.P. Queiroz var. ferrea (CARVALHO *et al*., 2017).

Given the lack of information on the conduction of the tetrazolium test to evaluate physiological quality of *P. stipulacea* seeds, this study aimed to adapt the tetrazolium test methodology to estimate viability and vigor.

MATERIAL AND METHODS

P. stipulacea seeds were harvested in 2011 from ripe fruits collected in twenty plants, ten located on the campus of the Federal Rural University of the Semi-Arid Region (UFERSA) (5º12'51'S and 37º18'44.7''W) and the others close to the Federal Institute of Rio Grande do Norte (IFRN) (5º12'58.8''S and 37°18'51.65''W), in the municipality of Mossoró, RN, Brazil. The seeds were extracted from the fruits, manually processed and dried in the shade. Then, they were placed in glass containers and stored in a controlled environment (16-18 °C and 40% relative humidity) until the experiment was conducted.

To obtain sublots, *P. stipulacea* seeds were classified as large, medium and small using sieves with round holes of 6, 5 and 4 mm in diameter, respectively. After forming the sublots, these were subjected to the following evaluations: a) seed moisture content - carried out by the oven method at 105±3 °C for 24 hours (BRASIL, 2009), with two replicates of 4.5±0.5 g, whose results were calculated based on wet mass and expressed as percentage; b) imbibition curve - two replicates of 50 seeds whose tips were removed were immersed in water and kept in B. O. D. (Biochemical Oxygen Demand) germination chambers at 25 ºC. The seeds were initially weighed on digital analytical scale (0.001 g) and after each predetermined time interval (every one hour, for eight hours of imbibition; every two hours, up to sixteen hours; every four hours, up to forty hours of imbibition; and lastly, every eight hours up to eighty-eight hours of imbibition) until at least 50% of the seeds produced radicles. Weight gain was calculated according to the formula proposed by Cromarty, Ellis and Roberts (1985) and expressed as a percentage; c) Germination - the tips of the seeds, in the region opposite to the micropyle, were previously removed with pliers, the substrate consisted of a roll of paper towel (Germitest®) moistened with distilled water in the amount corresponding to 2.5 times the dry paper weight (BRASIL, 2009), and the test was installed and conducted according to the methodology proposed by Benedito *et al*. (2019); d) seedling emergence - sowing was performed in expanded polystyrene trays of 128 cells, with coconut fiber initially moistened to 60% field capacity as substrate, kept in a greenhouse (screened) with 50% shading and irrigated once a day for 14 days until seedling emergence stabilized (fully exposed cotyledons) (FARIAS *et al*., 2013); e) germination and emergence speed indices - determined along with the germination and emergence tests, respectively, and the results were calculated according to the formula presented by Maguire (1962); f) mean germination and emergence times - obtained from the formula proposed by Labouriau (1983) through daily counts of the germinated seeds, and the results were expressed in days; g) tetrazolium test - based on preliminary tests, the tips of the seeds were removed and they were immersed in distilled water for 8 hours at 25 °C; after this period, the coat was removed to facilitate a more uniform staining of the tissues.

The experimental design used was the completely randomized design (CRD), in 3 x 9 factorial scheme, corresponding to three sublots and nine combinations of different concentrations of the tetrazolium solution and

periods of immersion in the salt (0.05%/2 h; 0.05%/4 h; 0.05%/6 h; 0.075%/2 h; 0.075%/4 h; 0.075%/6 h; 0.1%/2 h; 0.1%/4 h; 0.1%/6 h), totaling twenty-seven treatments, with four replicates of 25 seeds, conducted separately at temperatures of 35 and 40 °C in absence of light.

After each staining period, the tetrazolium solution was drained and the seeds were washed in running water, longitudinally cut in the center of the embryonic axis and individually evaluated for staining uniformity and intensity in the tissues. With this, it was possible to classify the seeds into four levels of vigor through adaptations in the methodologies of França Neto, Kryzanowski and Costa (1998) and Masullo *et al*. (2017).

Class I (viable and vigorous) - Staining in uniform rosy shades; turgid tissues; presence of dead or deteriorating tissue in the peripheral region of the cotyledons or near the embryonic axis; damage to the surface, not affecting the inner region of the cotyledon.

Class II (viable and non-vigorous) - Presence of dead or deteriorating tissue in the opposite region to the embryonic axis, possibly occurring in the internal and external regions of the cotyledon periphery; in the middle region of the cotyledon, affecting both sides internally and externally; in the embryonic axis region externally and internally, not affecting the central cylinder or affecting it to a lesser extent (less than half its thickness); at the tip of the root, without affecting the central cylinder; close to the plumule region of the plumule, without affecting it; breaking of the cotyledon, dead or deteriorated tissue over an extension smaller than half of the total area of the cotyledons or of one of the cotyledons, leaving only the embryonic axis intact; breaking in the region near the point of attachment of the cotyledons with the embryo, but leaving the vascular region intact; embryonic axis is well defined.

Class III (unviable) - Presence of dead or deteriorating tissue in both cotyledons, blocking the vascular region on the embryonic axis, reaching the central cylinder at the attachment point of both cotyledons, reaching the vascular region; dead or deteriorating tissues in extension greater than half of the total surface of the cotyledons; breaking of the cotyledons with an area larger than the seed surface; deteriorating plumule.

Class IV (dead) - Whitish, greenish or necrotic color, sometimes with mixed dark rosy shades.

The results of viability and vigor were subjected to analysis of variance (ANOVA) by F test at 5% significance level. The data of vigor classes were transformed into an Arcsine $\sqrt{x}/100$ for normalization of their distribution. In case of significance, the means were compared by Scott-Knott test at 5% probability level using Sisvar software (FERREIRA, 2011).

RESULTS AND DISCUSSION

Analysis of the initial quality of the sublots showed that there was no significant effect on the variables evaluated, that is, the classification by size did not enable the three sublots to be stratified (Table 1). This was probably due to the small difference in size between the classes, which was 1 mm in diameter.

Studies demonstrate that seed size influences the physiological quality of the lot, as verified for *Myrciaria dubia* (Kunth) McVaugh, whose best results of emergence and germination speed index were obtained with large seeds (SOUZA *et al*., 2017). On the other hand, Mendonça *et al*. (2015) observed better results of germination in medium-sized seeds of *Fimbristylis dichotoma* Vahl (Kranz).

Obtaining results related to the initial quality of seeds is important for conducting the tetrazolium test. Thus, the aim is to obtain complementary responses to those provided by the germination and/or emergence tests, which enables the obtaining of consistent information

Table 1 - Summary of analysis of variance for germination (G), germination speed index (GSI), mean germination time (MGT), emergence (E), emergence speed index (ESI) and mean emergence time (MET) of sublots of *Piptadenia stipulacea* Benth. Ducke seeds

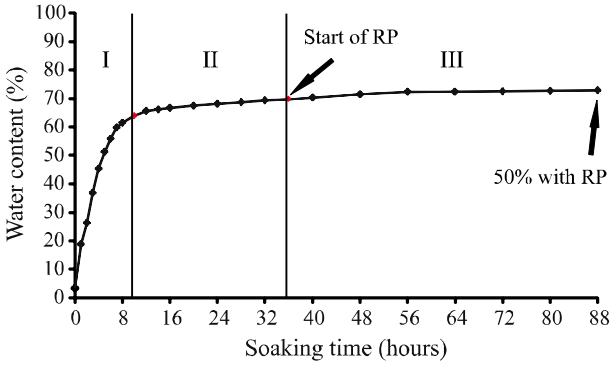
Analysis of variance							
Sources of Variation	Middle Squares						
	GL	G (%)	GSI	MGT	E (%)	ESI	MET
Sublots	2	33.33 n.s*	6.62 n.s	0.25 n.s	64.0 n.s	0.18 n.s	0.07 n.s
Error	9	34.67	2.19	0.19	21.78	0.08	0.09
Average		72.67	15.05	1.75	69.0	4.13	4.42
CV (%)		8.10	9.83	25.20	6.76	6.82	6.69

*n.s. - not significant; GL = degree of freedom; CV - coefficient of variation

that will help in choosing the best treatment to be used (OHLSON *et al*., 2010).

Regarding water gain, the seeds showed an average initial moisture content of 8.7% and, after eight hours of imbibition, they reached about 64.0% (Figure 1).

Figure 1 - Imbibition curve of *Piptadenia stipulacea* Benth. Ducke seeds, at 25 °C, immersed in distilled water. RP = root protrusion



Root protrusion began after thirty-six hours of imbibition, with 69.7% of water, and the imbibition curve was completed after eighty-eight hours, when about 50% of the seeds produced radicles (Figure 1). These results demonstrated that this species did not show a clear three-phase pattern. Phase I stood out for rapid water absorption and increased respiratory rate, and phase II for the activation of seed metabolism and active transport of substances for embryonic growth to continue (CARVALHO; NAKAGAWA, 2012). Although phase III is characterized by the resumption of water absorption, this water gain was not observed for *P. stipulacea*; on the other hand, the primary root was produced, which is also attributed to this phase (MARCOS-FILHO, 2015). Similar results were observed for seeds of *Simira gardneriana* M.R. Barbosa & Peixoto (OLIVEIRA *et al*., 2016) and *L. ferrea* (CARVALHO *et al*., 2017), for which there was no resumption of water gain in phase III. In addition, for *S. gardneriana* 144 hours of hydration were necessary for the softening and proper staining of the seed tissues, although the species does not have dormancy, while *L. ferrea* required 42 hours after scarification, because it has seed coat dormancy.

For *P. stipulacea*, the time of 8 hours proved to be efficient to properly hydrate the tissues of the seeds when their tips were removed and, with subsequent removal of the coat and immersion in tetrazolium salt at 0.075% concentration for four hours (obtained through preliminary tests), it was possible to stain the tissues, enabling the differentiation of living tissues. On the other hand, when the seeds were immersed with the coat in the tetrazolium salt solution they were not stained, making it impossible to distinguish viable seeds from non-viable seeds, the main objective of this test (BRASIL, 2009).

Seed coat removal is indicated for species with impermeable coat, since it facilitates the absorption of tetrazolium salt and tissue staining, as in the case of seeds of *C. grandis* (SANTOS *et al*., 2016), *Poincianella pyramidalis* (Tul.) L. P. Queiroz (SOUSA *et al*., 2017) and *L. ferrea* (CARVALHO *et al*., 2017). In addition to seed coat removal, the efficiency of the test in evaluating seed viability and vigor depends on the adequate selection of the tetrazolium concentration, as well as on the period of time in which the seeds will be incubated in the solution, whose steps are decisive for obtaining reliable results (CERVI; MENDONÇA, 2009). Thus, for the viability of *P. stipulacea* seeds, between combinations of treatment and sublots, significant interaction ($p<0.01$) was found for the temperatures of 35 and 40 °C.

The temperature of 35 °C for the concentrations 0.05%/4 and 6 h and 0.1%/6 h led to high results of viability in sublot 1, higher than those obtained in the other sublots (Table 2). For this sublot, the results were overestimated, with values above those obtained for initial quality through germination and emergence tests. For sublot 2, the best results were caused by the concentrations 0.075%/6 h and 0.1%/2 h. Sublot 3 had lower results for the lowest concentration and staining period (0.05%/2 h) and for the highest concentration, 0.1%/4 h (Table 2).

Although these results were satisfactory, they did not rank the sublots according to the initial quality. Only the concentration 0.075%/2 and 4 h promoted the ranking, and the second combination (0.075%/4 h) led to results similar to those of germination, with a margin of 5% difference (Table 2), according to Pinho *et al.* (2011). In addition, the seed staining pattern varied according to the concentration of the solution and incubation time, and the tissues with lighter shades were found in the shortest time of immersion and the bright light pink color was associated with the concentration 0.075% for four hours (Figure 2B). This result evidences that tissue staining must provide the best results in seed viability analysis and the combination 0.075%/4 h is already indicated for *P. moniliformis* seeds (AZEREDO; PAULA; VALERI, 2011). A time of three hours is recommended for *E. velutina* seeds (CUNHA; GOMES, 2015) and a time of 90 minutes is recommended for *P. pyramidalis* seeds (SOUSA *et al*., 2017) using the same concentration.

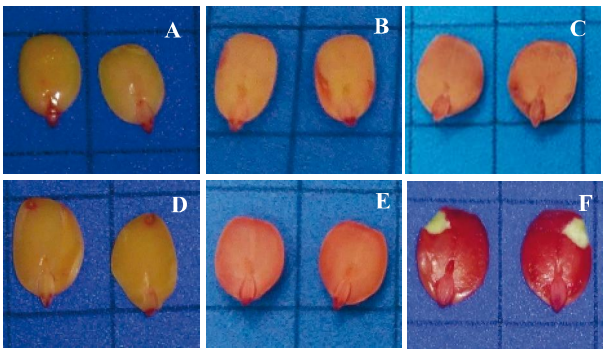
The tetrazolium test differs between species since, for *Senna macranthera* seeds (DC. ex Collad.) H.S. Irwin & Barneby, the best combination was 0.1%/3 h (PIVETA *et al*., 2018), while the concentration of 0.05%/3 h was

Table 2 - Percentage of viable seeds of three sublots of *Piptadenia stipulacea* Benth. Ducke seeds subjected to different combinations between periods and concentrations of tetrazolium salt at 35 and 40 °C

Combinations (Tetrazolium Solution x Staining Period)	Sublots		
	1	2	3
	35 °C		
C1 (0.05%/2 h)	63 bC	72 aB	45 cC
C2 (0.05%/4 h)	80 aA	73 bB	64 cA
C3 (0.05%/6 h)	80 aA	65 bC	68 bA
C4 (0.075%/2 h)	61 aC	65 aC	68 aA
C5 (0.075%/4 h)	75 aB	70 aB	71 aA
C6 (0.075%/6 h)	77 aB	78 aA	67 bA
C7 (0.1%/2 h)	51 cD	81 aA	65 bA
C8 (0.1%/4 h)	72 aC	71 aB	58 bB
C9 (0.1%/6 h)	82 aA	66 bC	68 bA
	40 °C		
C1 (0.05%/2 h)	75 aB	70 bA	67 bB
C2 (0.05%/4 h)	81 aA	67 bA	62 bB
C3 (0.05%/6 h)	80 aA	66 bA	75 aA
C4 (0.075%/2 h)	72 aB	59 bB	67 aB
C5 (0.075%/4 h)	73 bB	70 bA	78 aA
C6 (0.075%/6 h)	71 aB	57 bB	75 aA
C7 (0.1%/2 h)	72 aB	56 cB	64 bB
C8 (0.1%/4 h)	82 aA	56 cB	65 bB
C9 (0.1%/6 h)	68 aB	41 bC	67 aB
Germination (%)	71	71	76
Emergency (%)	69	73	65

* Means followed by same letter, upper and lower column on the line, do not differ by Scott-Knott test at 5% probability

Figure 2 - Staining pattern of viable seeds of *Piptadenia stipulacea* Benth. Ducke for the combinations 0.075%/2, 4 and 6 h - A, B and C, at 35 °C, and D, E and F, at 40 °C



efficient to evaluate the viability of *L. ferrea* seeds (CARVALHO *et al*., 2017). Thus, the period, temperature and concentration of the solution should be determined from studies that compare their results with other tests that assess seed viability, such as germination and seedling emergence (GARLET; SOUZA; DELAZERI, 2015).

In the present study, the results were similar when the temperature of 40 °C was used. At this temperature, the concentration 0.075%/4 h resulted in a percentage of viable seeds similar to the values of germination and emergence for the three sublots (Table 2). In addition, it promoted adequate staining for the reliable evaluation of tissues, enabling clear visualization of the embryonic axis, central cylinder and plumule (Figure 2E).

The lowest concentration (0.05%) associated with periods of 4 and 6 h and the highest concentration (0.1%) for 4 h led to increases in seed viability estimates, mainly for sublot 1. However, the combination 0.1%/6 h resulted in a low percentage of viable seeds for sublot 2 (Table 2). This is related to the dark Carmine red staining pattern, which made it difficult to distinguish viable seeds from those in deterioration. These results show that evaluating the seeds in shorter and longer periods of incubation and, at the highest concentration, requires more attention, knowledge and experience on the part of the analyst, to differentiate viable tissues.

The concentration of 0.075%/4 h promoted satisfactory results for both temperatures regarding the percentage of viable seeds with higher quality. Thus, understanding the different shades observed on the seeds after incubation in the tetrazolium solution is the main characteristic that should be considered in the interpretation of the results (GASPAR-OLIVEIRA; MARTINS; NAKAGAWA, 2009).

Besides contributing to detecting the viability of seeds that have dormancy, the tetrazolium test is efficient to detect vigor and distinguishes possible damage, assisting in seed quality control (PIVETA *et al*., 2018). Therefore, it can be used as a complementary vigor test when the seeds are subjected to different storage conditions (ABBADE; TAKAKI, 2014; VICENTE *et al*., 2016), after accelerated aging (PEREIRA *et al*., 2017), to evaluate lots of different origins (NORONHA *et al*., 2018) and after dormancy breaking methods (PIVETA *et al*., 2018), which emphasizes the importance of adequate procedures for conducting the test.

The results of the vigor classes showed significant interaction between the combinations (concentrations and staining periods in tetrazolium salt) and the sublots, for temperatures of 35 and 40 °C. In all treatments, it was possible to establish the four classes previously proposed to better distinguish the physiological quality of the seeds.

The highest percentage of viable and vigorous seeds (class I), at 35 °C, was observed with the combinations 0.05 and 0.075% for 4 and 6 h in sublot 1. Only at the concentration of 0.075%/6 h, there was no difference between the sublots. Similar behavior was observed for the combination C9 (0.1%/6 h), but the percentage of viable and vigorous seeds was lower. In turn, sublot 2 required higher concentration of the solution (0.1%) for 2 h to obtain results of high percentage of seeds in this class, unlike sublot 3, which obtained similar results, except for the concentrations of 0.05%/2 h and 0.1% for 2 and 6 h, which led to lower values. In these combinations, it was more difficult to differentiate seed tissues by the staining pattern adopted, especially at the lowest concentration, at which the values did not differ from those of sublot 1, the one with the largest seed size (Table 3).

The concentration 0.1%/6 h at 35 °C led to higher percentage of viable and non-vigorous seeds (class II) for sublot 1, which differed from sublots 2 and 3. The concentrations 0.05% and 0.075% for 6 h resulted in higher values for sublot 2 and no difference in relation to sublot 1. In turn, the lowest concentration (0.05%) for two hours indicated lower results of viable and non-vigorous seeds for sublots 2 and 3 (Table 3). Also at 35 °C, it was possible to identify low percentage of unviable seeds (Class III) for sublot 1 under concentrations of 0.05% for 4 and 6 h and 0.1%/6 h. This result is justified by the high percentage of seeds classified in the previous classes. Conversely, these combinations promoted higher means of unviable seeds for sublots 2 and 3.

In relation to dead seeds at 35 °C, the combination C1 (0.05%/2 h) resulted in a higher percentage for sublot 1, but this combination led to a higher result for sublot 3. 36% (Table 3). Therefore, it was observed that the seeds with smaller diameter, at the lowest concentration and shortest staining period, showed poor staining, making it difficult to distinguish live, viable and vigorous seeds from those without staining (dead).

Depending on the species under study, the tetrazolium test has shown higher efficiency with the use of lower concentrations, and this can be verified in the studies conducted by Abbade and Takaki (2014) with *T. roseoalba* and Carvalho *et al*. (2017) with *L. ferrea*. For these species, the concentration 0.05% promoted gentle tissue staining and enabled higher accuracy in the evaluation, ensuring the identification of damage to the embryonic axis and cotyledons.

For the temperature of 40 °C, there was statistical difference in the percentage of viable and vigorous seeds in sublot 1 only for combinations 0.05%/2 h and 0.1% for 2 and 6 h. The best result was observed in sublot 2, promoted by the concentration 0.05% for 2 h; while it increased the concentration and staining period, it reduced the percentage of viable and vigorous seeds. For sublot 3, higher results were observed at the concentrations of 0.05 and 0.075%/4 and 6 h (Table 3).

For class II, at 40 °C, there was a high percentage of viable and vigorous seeds in sublot 1 at the concentrations 0.05%/2 h and 0.1%/4 h, at which the values differed from those of sublots 2 and 3. The combination C6 (0.075%/6 h) resulted in a higher percentage of viable and non-vigorous seeds for sublot 2, higher than that obtained for the other sublots. For sublot 3, the lowest combination (0.05%/2 h) led to the best

Table 3 - Vigor classes of *Piptadenia stipulacea* Benth. Ducke seeds subjected to different concentrations of tetrazolium salt and periods of exposure at temperatures of 35 and 40 °C

Combinations (Tetrazolium Solution x Staining Period)	35 °C			40 °C		
	Sublots			Sublots		
	1	2	3	1	2	3
Class I - Viable and vigorous						
C1 (0.05%/2 h)	38 bC	61 Ab	40 bB	44 bB	53 aA	43 bB
C2 (0.05%/4 h)	60 aA	51 Bc	50 bA	55 aA	42 bC	56 aA
C3 (0.05%/6 h)	56 aA	37 Cd	49 bA	55 aA	40 bC	62 aA
C4 (0.075%/2 h)	38 bC	49 aC	47 aA	50 aA	41 bC	50 aB
C5 (0.075%/4 h)	55 aA	48 bC	50 bA	54 aA	45 bB	61 aA
C6 (0.075%/6 h)	52 aA	49 aC	49 aA	51 aA	26 bD	56 aA
C7 (0.1%/2 h)	40 bC	68 aA	43 bB	47 aB	34 bC	51 aB
C8 (0.1%/4 h)	46 aB	39 bD	46 aA	50 aA	36 bC	50 aB
C9 (0.1%/6 h)	42 aB	42 aD	44 aB	45 aB	16 bE	46 aB
Class II - Viable and non-vigorous						
C1 (0.05%/2 h)	25 aB	11 bC	5 cC	31 aA	17 cC	24 bA
C2 (0.05%/4 h)	20 aC	22 aB	14 bB	26 aB	25 aB	6 bD
C3 (0.05%/6 h)	24 aB	28 aA	19 bA	25 aB	26 aB	13 bC
C4 (0.075%/2 h)	23 aB	16 bC	21 aA	22 aC	18 aC	17 aB
C5 (0.075%/4 h)	20 aC	22 aB	21 aA	19 bC	25 aB	17 bB
C6 (0.075%/6 h)	25 aB	29 aA	18 bA	20 bC	31 aA	19 bB
C7 (0.1%/2 h)	11 bD	13 bC	22 aA	25 aB	22 aC	14 bC
C8 (0.1%/4 h)	26 bB	32 aA	12 cB	32 aA	20 bC	15 bC
C9 (0.1%/6 h)	40 aA	24 bB	24 bA	23 aB	25 aB	20 aB
Class III - Unviable						
C1 (0.05%/2 h)	16 aB	13 aB	19 aA	12 aA	15 aB	15 aB
C2 (0.05%/4 h)	7 bD	8 bC	14 aB	5 bB	17 aB	17 aA
C3 (0.05%/6 h)	5 bD	21 aA	23 aA	13 aA	17 aB	14 aB
C4 (0.075%/2 h)	32 aA	17 bA	14 bB	12 bA	19 aB	17 aA
C5 (0.075%/4 h)	16 aB	14 aB	15 aB	11 bA	17 aB	13 bB
C6 (0.075%/6 h)	12 aC	9 bC	15 aB	15 aA	17 aB	18 aA
C7 (0.1%/2 h)	34 aA	9 bC	12 bB	14 bA	21 aB	17 bA
C8 (0.1%/4 h)	11 aC	14 aB	16 aB	11 bA	21 aB	20 aA
C9 (0.1%/6 h)	5 bD	22 aA	23 aA	14 cA	46 aA	19 bA
Class IV - dead						
C1 (0.05%/2 h)	21 bA	15 cA	36 aA	13 aA	15 aB	18 aA
C2 (0.05%/4 h)	13 bC	19 aA	22 aB	14 bA	16 bB	21 aA
C3 (0.05%/6 h)	15 aB	14 aB	9 bD	7 bB	17 aB	11 bB
C4 (0.075%/2 h)	7 bC	18 aA	18 aC	16 bA	22 aA	16 bA
C5 (0.075%/4 h)	9 bC	16 aA	14 aC	16 aA	13 aB	9 bB
C6 (0.075%/6 h)	11 bC	13 bB	18 aC	14 bA	26 aA	7 cB
C7 (0.1%/2 h)	15 bB	10 bB	23 aB	14 bA	23 aA	18 bA
C8 (0.1%/4 h)	17 bB	15 bA	26 aB	7 cB	23 aA	15 bA
C9 (0.1%/6 h)	13 aC	12 aB	9 aD	18 aA	13 aB	15 aA

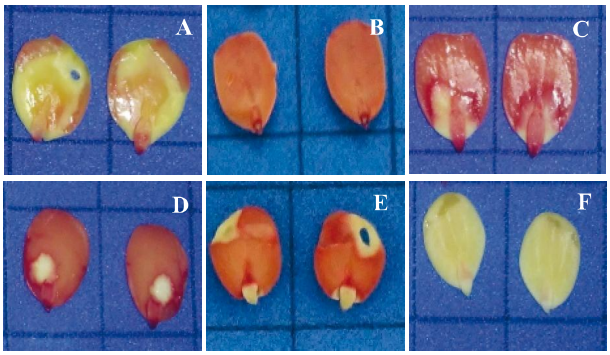
* Means followed by same letter, upper and lower column on the line, do not differ by Scott-Knott test at 5% probability

result, but with only 24% of viable and vigorous seeds (Table 3). On the other hand, for class III (unviable) at the same temperature, the lowest results were obtained in sublot 1, at the concentration of 0.05% for 4 h. For this combination, there was a high percentage of viable and vigorous seeds (above 50%), which explains the reduction of unviable seeds. For sublot 3, the lowest results of unviable seeds were observed at the concentrations 0.05%/2 and 6 h and 0.075%/4 h (Table 3).

The highest combination (0.1%/6 h) promoted 46% of unviable seeds for sublot 2 (Table 3). This result differs from those found for sublots 1 (14%) and 3 (19%). This concentration hampered the interpretation of the vigor classes due to the formation of a dark Carmine red color throughout the seed, so evaluation became more difficult because it did not enable the differentiation of healthy, dead and deteriorating tissues (CUNHA; GOMES, 2015).

A high percentage of dead seeds was identified at the concentration 0.075%/2 and 6 h, not differing from the results obtained at the concentration 0.1%/ 2 and 4 h for sublot 2, whose results were higher than those found in sublots 1 and 3. In this class, the seeds showed tissue with no color (Figure 3F) or mixed color, milky white with dark Carmine red.

Figure 3 - Staining pattern of the unviable and dead seeds of *Piptadenia stipulacea* Benth. Ducke. Seed with compromised cotyledons, vascular region and plumule - A, C and D; disruption of central cylinder - B; dead embryonic axis - E; dead seed - F



In addition to identifying dead tissue, the tetrazolium test enabled the visualization of structures and damage in all treatments for both temperatures. In Figure 3, it is possible to identify damage to critical regions, such as: plumule, vascular region, embryonic axis and dead seeds. Besides identifying one of the causes of the damage, such as that caused by insect with piercing-sucking mouthparts (Figure 3D), it allows the producer to discard or not the lot of seeds, since these areas are essential for normal plantlet development and ensure the development and quality of the seedlings that will be produced.

Evaluating the quality of *P. stipulacea* seeds by using the tetrazolium test is possible in a shorter period, 12 hours, whereas the emergence test requires 21 days, and without pre-germination treatment the percentage of viable seeds is around 50%. After removing the tip in the opposite region to the micropyle, the period necessary for germination to occur is 10 days, with results of viability above 80% (BENEDITO *et al*., 2019).

CONCLUSIONS

The period of 8 h of pre-imbibition and 4 h of staining in the tetrazolium solution at 0.075%, under 35 or 40 °C, is adequate to estimate the viability and vigor of *P. stipulacea* seeds.

REFERENCES

ABBADE, L. C.; TAKAKI, M. Teste de tetrazólio para avaliação da qualidade de sementes de *Tabebuia roseoalba* (Ridl.) Sandwith - Bignoniaceae, submetidas ao armazenamento. **Revista Árvore**, v. 38, n. 2, p. 233-240, 2014.

AZEREDO, G. A.; PAULA, R. C.; VALERI, S. V. Viabilidade de sementes de *Piptadenia moniliformis* Benth. pelo teste de tetrazólio. **Revista Brasileira de Sementes**, v. 33, n. 1, p. 61-68, 2011.

BENEDITO, C. P. *et al*. Dormancy overcoming and germination test in *Piptadenia stipulaceae* (Benth.) Ducke seeds. **Revista Ciência Agronômica**, v. 50, n. 2, p. 338-344, 2019.

BRASIL. Ministério da Agricultura, Pecuária e Abastecimento. **Regras para análise de sementes**. Brasília, DF: Mapa/ACS, 2009. 395 p.

CARVALHO, S. M. C. *et al*. Viability of *Libidibia ferrea* (Mart. ex Tul.) L.P. Queiroz var. *ferrea*) seeds by tetrazolium test. **Journal of Seed Science**, v. 39, n. 1, p. 7-12, 2017.

CARVALHO, N. M.; NAKAGAWA, J. **Sementes**: ciência, tecnologia e produção. 5. ed. Jaboticabal: FUNEP, 2012. 590 p.

CERVI, F. MENDONÇA, E. A. F. Adequação do teste de tetrazólio para sementes de algodoeiro. **Revista Brasileira de Sementes**, v. 31, n. 1, p. 177-186, 2009.

CROMARTY, A. S.; ELLIS, R. H.; ROBERTS, E. H. **Design of seed storage facilities for genetic conservation**. Rome: Internacional Board of Plant Genetic Resources, 1985. 100 p.

CUNHA, M. C. L; GOMES, I. H. R. A. Viabilidade de sementes de *Erythrina velutina* Willd pelo teste de tetrazólio. **Nativa**, v. 3, n. 3, p. 196-200, 2015.

FARIAS, R. M. *et al*. Superação de dormência em sementes de jurema-branca (*Piptadenia stipulacea*). **Revista de Ciências Agrárias**, v. 56, n. 2, p. 160-165, 2013.

FERREIRA, D. F. Sisvar: a computer statistical analysis system. **Ciência e Agrotecnologia**, v. 35, n. 6, p. 1039-1042, 2011.

FRANÇA NETO, J. B. *et al*. **O teste de tetrazólio em sementes de soja**. Londrina: EMBRAPA-CNPSo, 1998. 72 p. (EMBRAPA-CNPSo. Documentos, 116).

GARLET, J.; SOUZA, G. F. S.; DELAZERI, P. Teste de tetrazólio de sementes de *Cassia leptophylla*. **Enciclopédia Biosfera**, v. 11, n. 21, p. 1800-1808, 2015.

GASPAR-OLIVEIRA, C. M.; MARTINS, C. C.; NAKAGAWA, J. Concentração da solução de tetrazólio e período de coloração do teste para sementes de mamoneira. **Revista Brasileira de Sementes**, v. 31, n. 3, p. 38-47, 2009.

LABOURIAU, L. G. **A germinação das sementes**. Washington: Secretaria Geral da Organização dos Estados Americanos, 1983. 174 p.

MAGUIRE, J. D. Speed of germination-aid in selection and evaluation for seedlig emergence and vigor. **Crop Science**, v. 2, n. 1, p. 176-177, 1962.

MAIA, G. N. **Caatinga**: árvores e arbustos e suas utilidades. Fortaleza: Printcolor Gráfica e Editora, 2012. 413 p.

MARCOS-FILHO, J. **Fisiologia de sementes de plantas cultivadas**. 2. ed., Londrina: ABRATES, 2015. 660 p.

MASULLO, L. S. *et al*. Optimization of tetrazolium tests to assess the quality of *Platymiscium floribundum*, *Lonchocarpus muehlbergianus* and *Acacia polyphylla* DC. Seeds. **Journal of Seed Science**, v. 39, n. 2, p. 189-197, 2017.

MENDONÇA, G. S. *et al*. Aspectos físicos e fisiológicos de sementes de *Fimbristylis dicothoma* relacionados à germinação e dormência. **Revista Ciência Agronômica**, v. 46, n. 3, p. 539-545, 2015.

NORONHA, F. C. C. *et al*. Germinação e crescimento inicial de plântulas de espécies de diferentes procedências do gênero *Schizolobium spp*. **Revista da Universidade Vale do Rio Verde**, v. 12, n. 2, p. 1-9, 2018.

OHLSON, O. C. *et al*. Teste de envelhecimento acelerado em sementes de trigo. **Revista Brasileira de Sementes**, v. 32, n. 4, p. 118-124, 2010.

OLIVEIRA, F. N. *et al*. Viability of *Simira gardneriana* M.R. Barbosa & Peixoto seeds by the tetrazolium test. **Journal of Seed Science**, v. 38, n. 1, p. 7-13, 2016.

PAIVA, E. P. *et al*. Teste de tetrazólio para avaliação da viabilidade de sementes de maxixe. **Revista Ciência Agronômica**, v. 48, n. 1, p. 118-124, 2017.

PEREIRA, D. S. *et al*. Physiological in hybrid brachiaria cv. Mulato II after accelerated aging overcome dormancy. **Journal of Seed Science**, v. 39, n. 3, p. 244-253, 2017.

PINHO, D. S. *et al*. Adequação da metodologia do teste de tetrazólio para avaliação da viabilidade de sementes de angico. **Pesquisa Florestal Brasileira**, v. 31, n. 67, p. 269-272, 2011.

PIVETA, G. *et al*. Qualidade sanitária e fisiológica de *Senna macrnathera* (DC ex. Collad.) H. S. Irwin & Barneby quando submetida a métodos de superação de dormência. **Ciência Florestal**, v. 28, n. 2, p. 836-844, 2018.

SANTOS, H. O. *et al*. Viability of canafistula (*Cassia grandis* L. F.) seeds by the tetrazolium test. **International Journal of Current Research**, v. 8, n. 3, p. 28031-28035, 2016.

SOUSA, D. M. M. *et al*. Viabilidade e vigor de sementes de *Poincianella pyramidalis* (Tul.) L. P. Queiroz pelo teste de tetrazólio. **Revista Ciência Agronômica**, v. 48, n. 2, p. 381-388, 2017.

SOUZA, A. G. *et al*. Optimization of germination and initial quality of seedlings of *Prunus persica* tree rootstocks. **Journal of Seed Science**, v. 39, n. 2. p. 286-292, 2017.

VICENTE, D. *et al*. Viabilidade de sementes de *Ocotea puberula* (Rich.) Ness ao longo do armazemento. **Floresta e Ambiente**, v. 23, n. 3, p. 418-426, 2016.