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Physiological quality of *Anadenanthera colubrina* var. cebil (Griseb.) Altschul seeds by the tetrazolium test

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ABSTRACT: The tetrazolium test quickly assesses seed viability, the efficiency of which depends on methodological adjustments for each species. Therefore, the objective was to evaluate the viability of *Anadenanthera colubrina* seeds using this test. The seeds were aged for 12 h and 24 h to obtain differences in physiological quality. Initial quality was assessed by the following tests: germination, emergence, GSI and ESI, and MGT and MET. For the tetrazolium test, a completely randomized experimental design was used in a 3 × 9 factorial arrangement consisting of three accelerated aging (AA) conditions (0, 12, and 24 h of AA) and nine combinations between concentrations of tetrazolium salt and immersion time in this salt (0.075% / 4 h, 0.075% / 6h, 0.075% / 8h, 0.1% / 4 h, 0.1% / 6 h, 0.1% / 8 h, 0.5% / 4 h, 0.5% / 6 h, and 0.5% / 8 h), with four replications of 25 seeds, evaluated at temperatures of 35 °C and 40 °C. The seeds were classified as viable and vigorous, viable and not vigorous, and inviable. The tetrazolium test was effective for estimating the viability of *A. colubrina* seeds. To make this estimation, the seeds should be chipped in the region opposite the micropyle, then pre-moistened in water for 10 h, followed by removal of the seed coat and immersion in a 0.1% tetrazolium solution for 4 h at 35 °C. Under these conditions, the tetrazolium test successfully identified differences in the physiological quality of unaged seeds and seeds aged for 12 h and 24 h.

Index terms: *angico*, seeds, viability, vigor.

RESUMO: O teste de tetrazólio avalia de forma rápida a viabilidade das sementes, cuja eficiência depende de ajustes metodológicos para cada espécie. Com isso, objetivou-se avaliar a viabilidade em sementes de *Anadenanthera colubrina* por meio desse teste. As sementes foram envelhecidas por 12 e 24 horas para obter diferenças na qualidade fisiológica. A qualidade inicial foi avaliada pelos testes de germinação; emergência; IVG e IVE; TMG e TME. Para o teste de tetrazólio utilizou-se o delineamento experimental inteiramente casualizado em esquema fatorial 3 x 9 e os tratamentos constaram: três condições de envelhecimento acelerado - EA (0, 12 e 24 h de EA) e nove combinações entre concentrações e tempo de imersão no sal de tetrazólio: 0,075%/4 h; 0,075%/6 h; 0,075%/8 h; 0,1%/4 h; 0,1%/6 h; 0,1%/8 h; 0,5%/4 h; 0,5%/6 h; 0,5%/8 h, com quatro repetições de 25 sementes, avaliados sob as temperaturas de 35 e 40 °C. As sementes foram classificadas em viáveis e vigorosas, viáveis e não vigorosas e inviáveis. O teste de tetrazólio foi eficiente para estimar a viabilidade de sementes de *A. colubrina*. Para isso, deve-se realizar o desponte na região oposta a micrópila, com pré-umedecimento em água por 10 h, seguido da remoção do tegumento e imersão na solução de tetrazólio a 0,1%, por 4 h, a 35 °C. Nessa condição, o teste de tetrazólio foi eficiente para identificar diferenças na qualidade fisiológica de sementes não envelhecidas e envelhecidas por 12 e 24 horas.

Termos para indexação: angico, sementes, viabilidade, vigor.

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INTRODUCTION

Anadenanthera colubrina var. cebil (Griseb.) Altschul, popularly known as *angico* or *angico-branco*, most frequently occurs in the Caatinga, Cerrado, and Atlantic Forest ecosystems (Bispo et al., 2017). As one of the timber species most traded in Brazil (SNIF, 2020), uncontrolled use may compromise its survival. Thus, studies related to evaluation of seed quality are essential for programs for preservation, management, and restoration of *A. colubrina* populations.

Plantlets of *A. colubrina* are basically produced by seeds, and successful production depends on this input being of high physical, physiological, sanitary, and genetic quality (Carvalho and Nakagawa, 2012). Therefore, quick and effective tests are necessary to assess these seed quality properties. Usually, physiological quality is evaluated by the germination test. This test is conducted under optimal conditions of temperature, light, and moisture, allowing the seed to express its maximum germination potential (Brasil, 2009). However, this test requires a relatively long period to obtain results, 10 days in the case of *A. colubrina* (Brasil, 2013; Bispo et al., 2017; Cruz et al., 2021), and the emergence test requires around 14 days (Bispo et al., 2017). Consequently, a methodology for quick and reliable viability tests must be devised for evaluation of the physiological quality of this forest species.

The tetrazolium test is a promising alternative for evaluating physiological quality because of the speed in obtaining results of seed viability and vigor, and it may be used to complement the germination test (Silva et al., 2016; Brito et al., 2020; Pereira et al., 2020). Compared to the germination test, the tetrazolium test proves to be very advantageous in saving time, and it facilitates decision making regarding the choice of seed lots with greater viability, and it has already been used for agricultural crops. That is because it not only provides information on viability and vigor, but is also able to diagnose problems caused by insects, mechanical damage, deterioration in the field, and problems due to drying and inadequate storage (França-Neto and Krzyzanowski, 2020).

Nevertheless, knowledge about seed morphology, solution concentration, temperature, and period of incubation are essential for carrying out the test and obtaining reliable results. Studies seeking to adjust the methodology of this test on seeds from forest species have already been performed by Nogueira et al. (2014) on *Enterolobium contortisiliquum* (Vell.), by Oliveira et al. (2016) on *Simira gardneriana* M.R. Barbosa & Peixoto, by Souza et al. (2017) on *Poincianella pyramidalis* (Tul.) L. P. Queiroz, by Brito et al. (2020) on *Tabebuia aurea* (Silva Manso) Benth. & Hook. f ex S. Moore, by Pereira et al. (2020) on *Piptadenia stipulacea* (Benth.) Ducke, and by Silva et al. (2021) on *Campomanesia phaea* O. Berg. Landrum.

In light of the above, the aim was to determine the manner of preparing the seeds, as well as the concentration and time of staining, to evaluate the viability of *A. colubrina* seeds using the tetrazolium test.

MATERIAL AND METHODS

The *A. colubrina* seeds were made available by the Ecology and Environmental Monitoring Center (*Núcleo de Ecologia e Monitoramento Ambiental* – NEMA) of the *Universidade Federal do Vale do São Francisco* (UNIVASF) and by the São Francisco Integration Project (*Projeto de Integração do São Francisco – PISF*). The seeds were collected in July 2016 from 10 mother plants from different locations in the Caatinga ecosystem in Lagoa Grande - PE (8°38'45.23" S, 40°14'07.32" W). After the seeds were gathered, they were manually processed, placed in plastic bags, and stored in a climate-controlled environment (16-18 °C and 40% relative humidity) throughout the time of the experiment (August 2017 to January 2018).

One part of the *A. colubrina* seeds underwent traditional accelerated aging, conducted in transparent acrylic boxes (11 × 11 × 3.5 cm) with 40 mL of distilled water added to the bottom (Marcos-Filho, 2020). The seeds were arranged on a screen and kept in a Biochemical Oxygen Demand (BOD) germination chamber at 41 °C for the periods of 12 and 24 h in the dark, obtaining aged seeds, which underwent testing along with non-aged seeds. Soon after the aging, part of the seeds were placed to dry on plastic trays at ambient temperature (±32 °C; 45% RH) until obtaining a moisture content less than or equal to 13.0%. After that, the following evaluations were performed on the seeds:

Seed moisture content: this was performed by the laboratory oven method at 105±3 °C for 24 h (Brasil, 2009) with two replications of 4.5 ± 0.5 g of whole seeds, expressed in percentage (wet basis) before and after accelerated aging and after drying (Table 1).

Germination: this test was set up immediately after 12-h and 24-h aging of the seeds and with non-aged seeds using the roll of paper tower substrate previously moistened, with distilled water in the amount of 2.5 times the weight of the dry substrate (Brasil, 2009). After that, the seeds were incubated in a germination chamber at 25 °C with an 8-h photoperiod for 10 days (Brasil, 2013).

Seedling emergence: seeds were sown on plastic trays (28 × 20 × 7 cm) containing sand substrate initially moistened to 60% field capacity. The trays were kept in a greenhouse for 14 days with daily irrigation (Bispo et al., 2017).

Germination speed index and emergence index: these were obtained together with the germination test and emergence test, respectively, counting the seedlings daily (Krzyzanowski et al., 2020), calculated according to the formula presented by Maguire (1962):

$$IVG = \frac{G1}{N1} + \frac{G2}{N2} + \cdots + \frac{Gn}{Nn}$$

where GSI = germination speed index; G1, G2, and Gn = number of normal seedlings, obtained in the first, second, and last count; and N1, N2, Nn = number of days from sowing to the first, second, and last count.

Mean germination time and emergence time: these times were obtained from the formula recommended by Labouriau (1983) through daily counts of germinated seeds and emerged seedlings, respectively, and results were expressed in days.

Imbibition curve and tetrazolium test: to estimate the seed pre-moistening time, the imbibition curve was first determined with two replications of 50 non-aged, chipped seeds (seed coat cut in the region opposite the micropyle) (Correia et al., 2017). The seeds were immersed in water and kept in germination chambers at 25 °C. The seeds were weighed on a digital electronic balance (0.001 g) to obtain initial weight, and were then weighed after each pre-determined interval (every hour over eight hours of imbibition, then every two hours up to sixteen hours of imbibition, and finally every four hours up to twenty-eight hours of imbibition) until radicle emergence in at least 50% of the seeds. Weight gain was calculated according to the formula proposed by Cromarty et al. (1985) and expressed as a percentage:

$$Wf = Wi\frac{(100 - Mi)}{(100 - Mf)}$$

where Wf = weight of the sample (g) after drying; Wi = weight of the sample (g) before drying; Mi = moisture content (%) before drying; Mf = moisture content (%) desired after drying.

After obtaining the results of the imbibition curve, the non-aged seeds and seeds aged for 12 h and 24 h, followed by drying, were chipped and then pre-moistened in distilled water for 10 h at 25 °C. After that, the seed coat was removed to facilitate uniform staining of the tissues, and the seeds were placed under different concentrations of tetrazolium salt and periods of imbibition (0.075% / 4 h, 0.075% / 6 h, 0.075% / 8 h, 0.1% / 4 h, 0.1% / 6 h, 0.1% / 8 h, 0.5% / 4 h, 0.5% / 6 h, 0.5% / 8 h) at the temperatures of 35 °C and 40 °C in the dark.

Table 1. Moisture content of *Anadenanthera colubrina* seeds not aged and after accelerated aging for 12 h and 24 h, followed by drying.

Aging (h)	Moisture content (%)	
0	8.9	
	After aging	After drying
12	21.1	10.4
24	35.7	13.0

After each staining period, the tetrazolium solution was drained and the seeds were washed in running water, sectioned longitudinally in the center of the embryonic axis, and evaluated individually in regard to uniformity and intensity of color shown by the tissues. That way, it was possible to classify the seeds through adaptations of the methodologies of França-Neto and Krzyzanowski (2020) and Masullo et al. (2017):

a) *viable and vigorous seeds:* with uniform pinkish color tones, turgid tissues, the presence of dead or deteriorating tissue at the peripheral region of the cotyledons or near the embryonic axis, surface damage not affecting the internal region of the cotyledon;

b) *viable and not vigorous:* the presence of dead or deteriorating tissue in the region opposite the embryonic axis, appearing in the peripheral region of the cotyledon internally and externally; in the middle region of the cotyledon, affecting both sides internally and externally; in the region of the embryonic axis externally and internally, not affecting the central cylinder or affecting it to a lesser extent (less than half of its thickness); at the tip of the radicle, without affecting the central cylinder; near the region of the plumule, without affecting it; fracture of the cotyledon, dead or deteriorating tissue extending over less than half of the total area of the cotyledons or of one of the cotyledons, leaving only the embryonic axis intact; fracture in the region near the point of connection of the cotyledons to the embryo, but leaving the vascular region intact; and a well-defined embryonic axis;

c) *nviable:* presence of dead or deteriorating tissue in both cotyledons, blocking the vascular region in the embryonic axis, reaching the central cylinder at the point of connection of both cotyledons, or reaching the vascular region; dead or deteriorating tissues extending over more than half of the total surface of the cotyledons; fracture of the cotyledons with area greater than the seed surface; plumule in deterioration; whitish, greenish, or necrotic color, at times mixed with dark pink tones.

Statistical analysis: a completely randomized experimental design was used in a 3 × 9 factorial arrangement (three accelerated aging conditions and nine combinations of different concentrations and immersion periods in tetrazolium solution), for a total of twenty-seven treatments, in four replications of 25 seeds, conducted separately for each temperature.

Analysis of variance (ANOVA) was conducted on the seed viability results (the sum of viable and vigorous seeds, and viable and non-vigorous seeds) by the F test at the 5% significance level. The means were compared by the Scott-Knott test at 5% probability using the Sisvar software (Ferreira, 2019).

RESULTS AND DISCUSSION

Through analysis of initial seed quality, a significant effect was found for germination, GSI, MGT, emergence, and MET ($p < 0.01$). The results show that accelerated aging (AA) of the seeds was effective in obtaining seed lots with different levels of physiological quality (Table 2).

The seeds with 0 h AA had an initial moisture content of 8.9%, but after 12 and 24 h AA, they reached 21.1 and 35.7%, respectively (Table 1). The increase in moisture content occurred due to the high moisture and temperature that the *A. colubrina* seeds were subjected to during accelerated aging. These conditions led to the increase in water absorption by the seeds and, consequently, in their weight (Table 2). That fact can interfere in interpretation of the data from the accelerated aging test in relation to the data of the non-aged seeds (Marcos-Filho, 2015). That is because an increase in the germination speed index and reduction in the mean germination time of the aged seeds can be observed as a response to the increase in moisture content in the seeds; in that case, the seed reserves had already begun their translocation to other growth areas of the seedlings (Table 2). On the other hand, this combination affected the germination potential of the *A. colubrina* seeds. When the seeds were aged for 12 and 24 h, the germination results were lower than those obtained for the non-aged seeds.

A result similar to that of germination was found for seedling emergence and mean germination time in *A. colubrina* (Table 2). Although the seeds with 0 h AA had better performance, those aged for 12 and 24 h did not differ

Table 2. Means testing for germination (G), germination speed index (GSI), mean germination time (MGT), emergence (E), emergence speed index (ESI), and mean emergence time (MET) for characterization of the initial quality of *Anadenanthera colubrina* seeds not aged and after accelerated aging (AA) for 12 and 24 hours.

	Means test					
Accelerated aging	G (%)	GSI	MGT	E (%)	ESI	MET
0 h AA	85 a	9.5 b	2.92 a	79 a	4.15 a	5.5 a
12 h AA	72 b	14.5 a	1.13 b	59 b	3.98 a	4.2 b
24 h AA	60 c	14.6 a	1.43 b	60 b	3.75 a	4.58 b
CV (%)	7.08	12.5	12.15	8.92	11.07	7.7
DMS	10.12	3.18	0.44	11.63	0.87	0.72

**significant at 1% probability.
Mean values followed by the same letter do not differ from each other by the Scott-Knott test at 5% probability.

statistically. Loss of germination potential is a characteristic of seeds with lower physiological quality (Silva et al., 2016); and when seed are exposed to these conditions (AA for 12 and 24 h), they deteriorate rapidly, thus interfering to reduce the germination rate, as well as to increase the number of abnormal seedlings (Marcos-Filho, 2015). These results reflect the initial physiological quality of the seeds, which in the face of unfavorable conditions, showed an increase in deterioration rate when under high temperature and moisture conditions. The difference in the initial quality of the *A. colubrina* seeds is important for comparison with the results obtained by the tetrazolium test because it allows confirmation of the effectiveness of that test when the seeds are placed under stress conditions.

As there is no methodology of the tetrazolium test for determining the viability of *A. colubrina* seeds, it is necessary to determine the hydration period of the seeds before immersing them in the tetrazolium salt (Figure 1). To determine the hydration period, it was necessary to chip the *A. colubrina* seeds in the region opposite the micropyle, due to the presence of the seed coat, which impedes water absorption (Correia et al., 2017).

Cutting in the region opposite the embryo or scarification is necessary for diverse species that have seed-coat-imposed dormancy. For example, seeds of the pacara earpod tree (*E. contortisiliquum*) were scarified with sandpaper in the region opposite the embryo for immersion in water for 24 h (Nogueira et al., 2014). Seeds of Brazilian ironwood (*Libidibia ferrea* (Mart. ex Tul.) L.P. Queiroz var. ferrea) also required scarification with no. 80 sandpaper, followed by pre-moistening for 42 h at 25 °C in paper rolls (Carvalho et al., 2017).

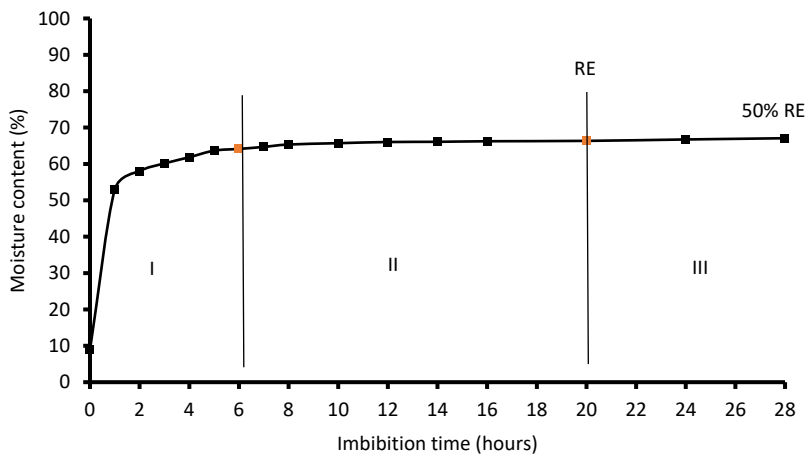

Figure 1. Imbibition curve of *Anadenanthera colubrina* var. cebil (Griseb.) Altshul seeds at 25 °C, immersed in distilled water. RE = radicle emergence; 50% RE = radicle emergence in 50% of the seeds.

In a study performed with *jurema-branca* (*P. stipulacea*) seeds, Pereira et al. (2020) found that in addition to chipping the region opposite the embryo, 8 h of pre-moistening in water at a temperature of 25 °C was necessary. After that, the seed coat was removed before immersion in the tetrazolium salt for adequate staining of the tissues and evaluation of viability and vigor. For *A. colubrina*, removal of the seed coat before placing the seeds in the tetrazolium solution was also necessary for more uniform staining of the live tissues in that study. Presence of the seed coat impeded diffusion of the tetrazolium salt in the cells and thus hindered staining of the tissues, and as such, viability could not be evaluated.

During the imbibition curve, there was rapid absorption of water by the seeds in the first 6 h of hydration, reaching 64.0% moisture, and thus concluding Phase I of the imbibition curve (Figure 1). Between the period of 7 and 19 h of imbibition, absorption remained constant, characterizing Phase II of the germination process, when preparation and activation of seed metabolism for embryo growth occurs. After 28 h of hydration, radicle emergence was found in 50% of the seeds, with moisture content of 67.1%, without water gain occurring in this Phase III (Figure 1).

The duration of each phase of imbibition depends on factors inherent to the seed, such as the permeability of the seed coat and its size. In addition, external conditions during the imbibition process, such as temperature, affect the absorption rate, germination uniformity and speed, and biochemical reactions throughout the process; and consequently, these conditions affect germination percentage (Carvalho and Nakagawa, 2012).

Water gain by seeds is important for conducting the tetrazolium test. Pre-moistening favors removal of the seed coat and exposure of the embryo to contact with the solution used. The increase in moisture content in plant cells allows the beginning of respiratory activity and the softening of tissues and assists in penetration of the tetrazolium salt (Cunha and Gomes, 2014; Oliveira et al., 2016; Pereira et al., 2020; Brito et al., 2020). In *A. colubrina* seeds, the hydration process was more successful after cutting the seed coat in the region opposite the embryo.

Moisture content higher than 50% can lead to cell division, germination, and growth (Bewley et al., 2013). However, this response is also different for each species. For *A. colubrina* , Phase II of the imbibition curve remained at around 60% moisture content for several hours before the radicle emerged at the beginning of Phase III. This result is similar to that obtained by Brito et al. (2020) in *T. aurea* seeds, in which moisture content of around 60% was necessary for staining the live tissues. In contrast, seeds of scarlet morning glory (*Ipomoea hederifolia* L.) seeds showed primary radicle emergence when the moisture content reached 21.8% for non-AA seeds and 34.9% for seeds that underwent AA for 12 h, after 9 h of hydration (Araújo et al., 2016). Variation in the physiological response and in radicle emergence reinforces the importance of determining the hydration curve for each species. Thus, for conducting the tetrazolium test in *A. colubrina*, the seeds were chipped and then hydrated in water for 10 h in a germination chamber at 25 °C, followed by removal of the seed coat before immersion in the tetrazolium solution at different combinations and temperatures.

After the seeds were treated in the combinations (concentrations and hydration periods) at each temperature (35 and 40 °C), significant interaction was found for seed viability at the level of 1% probability ($p < 0.01$) (Table 3).

The *A. colubrina* seeds with 0 h AA treated with the 0.075% tetrazolium salt solution for 4 h at the temperature of 35 °C resulted in a higher percentage of viability and did not differ from the results obtained for the concentration of 0.1% / 4 h, and the values were similar to those of the germination and emergence tests, at 85% and 79%, respectively (Table 2). Identification of the percentage of viability in these combinations was possible due to the uniform pink color, indicating living tissue (Figure 2). The lowest result for the temperature of 35 °C in the non-aged seeds was obtained at the highest concentration of the solution and the longest hydration period, corresponding to 33% (Table 3).

The combination 0.1% / 4 h at 35 °C brought about the highest percentage of viable seeds (75%) in the seeds with 12 h AA, not differing from the non-aged seeds (Table 5). This result was made possible because of the clear color of the embryos, which allowed determination of the viability of *A. colubrina* seeds in an effective manner (Figure 2D). In addition, it facilitated analysis and interpretation of the results for most of the seeds, even after 12 h AA, with results similar to those obtained in the germination test, 72% (Table 2).

Table 3. Percentage of viable seeds of *Anadenanthera colubrina* var. cebil (Griseb.) Altshul. not aged and after accelerated aging (AA) for 12 h and for 24 h treated with different combinations of tetrazolium salt concentrations and staining periods at 35 °C and 40 °C.

Combinations (tetrazolium solution × staining period)	35 °C			40 °C		
	AA (h)			AA (h)		
	0	12	24	0	12	24
0.075%/ 4 h	81 aA	51 cC	61 bA	52 bB	59 bA	68 aA
0.075%/ 6 h	69 aB	55 bC	27 cC	61 aA	58 aA	48 bB
0.075%/ 8 h	49 bC	62 aB	44 bB	50 bB	58 aA	48 bB
0.1%/ 4 h	80 aA	75 Aa	61 bA	67 aA	46 bB	44 bB
0.1%/ 6 h	64 aB	53 Bc	48 bB	45 bB	62 aA	63 aA
0.1%/ 8 h	65 aB	53 Bc	42 cB	37 cC	65 aA	52 bB
0.5%/ 4 h	53 aC	19 cE	31 bC	45 aB	26 bC	23 bC
0.5%/ 6 h	47 aC	29 Bd	16 cD	20 aD	10 bD	18 aC
0.5%/ 8 h	33 aD	23 Be	18 bD	19 aD	26 aC	0 bD

*Mean values followed by the same uppercase letter in the column and lowercase letter in the row do not differ from each other by the Scott-Knott test at 5% probability.

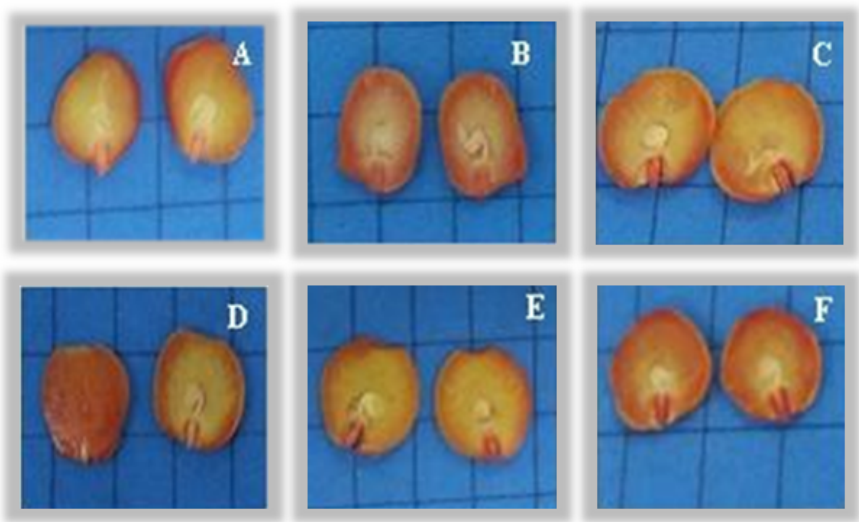

Figure 2. Staining pattern of the viable seeds of *Anadenanthera colubrina* var. cebil (Griseb.) Altshul. for the combinations 0.075% / 4 h, 6 h, and 8 h – A, B, and C; and 0.1% / 4 h, 6 h, and 8 h – D, E, and F) at 35 °C.

Seeds with 24 h AA and treated to the combinations 0.075% / 4 h and 0.1% / 6 h obtained 61% viable seeds for *A. colubrina*. In other words, the results of the tetrazolium test were very near those obtained by the germination and emergence tests, 60% (Table 3). However, at the temperature of 35 °C, the combinations of 0.5% / 6 h and 8 h reduced identification of viable *A. colubrina* seeds (Table 3).

The choice of the combination may be linked to the cost of tetrazolium salt for preparation of the solution. The use of a lower concentration is recommended to reduce costs (Oliveira et al., 2016; França-Neto and Krzyzanowski, 2020; Melo-Junior et al., 2022). However, the concentration of tetrazolium salt of 0.1% / 4 h brought about viability results very near those obtained by the germination and emergence tests. The shorter period of hydration in the solution is

also important for obtaining reliable results, provided that this combination allows visualization of modifications in color and allows the types of damage to be distinguished. That way viable and inviable tissue are more easily identified, and seed lots with distinct physiological qualities are able to be differentiated (Azeredo et al., 2011; Pereira et al., 2020).

Determining the combination for conducting the tetrazolium test for viability testing is important because seeds with high viability will form strong and vigorous seedlings with faster emergence and better performance under different soil and climate conditions. These properties are normally desired by seedling producers (França-Neto et al., 2010).

The combinations that led to the best results for viability were 0.075% / 6 h and 0.1% / 4 h at 40 °C for the non-aged seeds, at 61% and 67%, respectively. However, the results of the combination 0.075% / 6 h were similar to those obtained in the germination and emergence tests. The concentration of 0.5% for 6 h and 8 h resulted in lower results for viability, 20% and 19%, respectively (Table 3).

The highest percentage of viability for seeds with 12 h AA occurred at the temperature of 40 °C and 0.075% concentration for the three periods tested, which did not differ from the results obtained from the combinations 0.1% for 6 h and for 8 h (Table 3). These results were also similar to the result of the emergence test (Table 3). The lowest viability result for this temperature occurred for the combination 0.5% / 6 h, with 10% viable seeds (Table 3).

The best result of viability by the tetrazolium test with *A. colubrina* seeds with 24 h AA was obtained for the combination 0.075% / 4 h at 40 °C, at 68%. Under this same condition of accelerated aging, the combination 0.1% / 6 h also allowed viability to be determined, with results similar to those obtained by the germination and emergence tests. In contrast, the combination 0.5% / 8 h did not allow identification of viable *A. colubrina* seeds (Table 3).

The results of this study are similar to those found by Azeredo et al. (2011) in *Pityrocarpa moniliformis* (Benth.) Luckow & R. W. Jobson seeds and by Pereira et al. (2020) in *P. stipulacea* seeds, species that belong to the same family (Fabaceae). For these species, the concentration of tetrazolium salt at 0.075% for four hours favored evaluation of seed viability. In addition, this concentration is recommended for evaluation of the physiological quality of other forest species, such as *S. gardneriana* (Oliveira et al., 2016) and *P. pyramidalis* (Souza et al., 2017) for different staining periods.

The tetrazolium test conducted under the combination 0.1% / 4h at 35 °C allowed morphological identification of the deterioration process of the three types of *A. colubrina* seeds, with results similar to those of the germination test (Table 2 and 3). This combination allowed identification of all the critical regions of the seed, such as the plumule, the radicle, the vascular region, and the embryonic axis, and it facilitated distinction of living tissues from those in deterioration (Figure 3).

As the tetrazolium salt concentrations and hydration periods increased, seed viability declined. Yet it is noteworthy that the lowest results were obtained for the seeds aged for 12 h and 24 h. In other words, the process of controlled seed deterioration, along with the high concentration, hydration period, and high temperature, made it impossible to distinguish viable seeds from inviable ones, due to intensification of the color to a strong carmine red tone. This problem was more intense in seeds aged for 24 h under the combination 0.5% / 8 h at 40 °C. In that case, it was not possible to identify viable *A. colubrina* seeds (Figure 4).

Higher temperatures, such as 40 °C, affect metabolic processes, including respiration. Consequently, reduction reactions occurred rapidly, indicated by intensification of color, as seen in this study with *A. colubrina*. The intensification of respiratory activity made analysis of seed viability difficult, which made it impossible to distinguish viable tissues from those in deterioration, and for that reason, they were classified as inviable.

The response to high respiratory activity was found by the intensification of intense red color in the seed tissues, especially in the embryonic region. That make it impossible to evaluate vital areas of the seeds (embryo, plumule, and vascular region) (Figure 4D). These regions cannot be compromised, otherwise there will be no formation of normal seedlings, nor guarantee of their establishment under adverse environmental conditions, especially if the amount of seed reserves is not enough for their development to proceed. Therefore, the temperature of 40 °C along with high concentration of and periods of prolonged immersion in the tetrazolium solution proved not to be suitable for evaluating differences in the physiological quality of *A. colubrina* seeds.

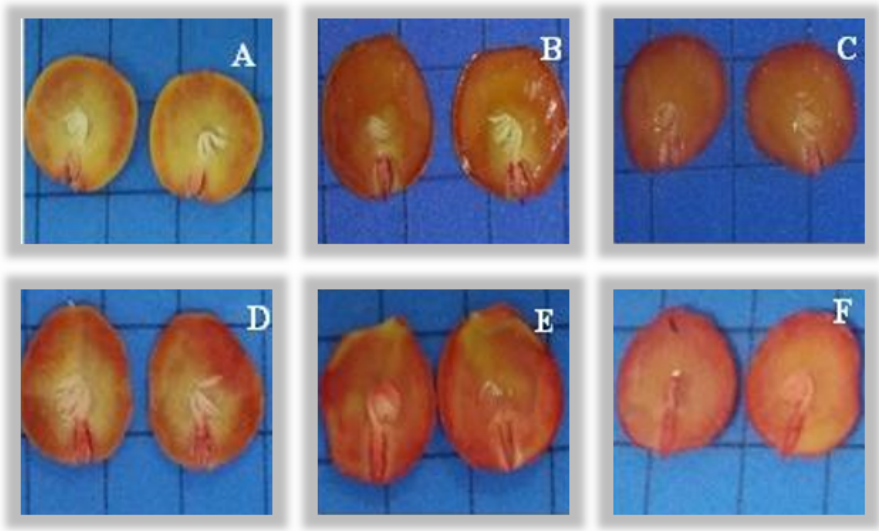

Figure 3. Staining pattern of the viable and vigorous seeds of *Anadenanthera colubrina* var. cebil (Griseb.) Altshul. for the combinations 0.075% / 4 h, 6 h, and 8 h – A, B, and C; and 0.1% / 4 h, 6 h, and 8 h – D, E, and F) at 40 °C.

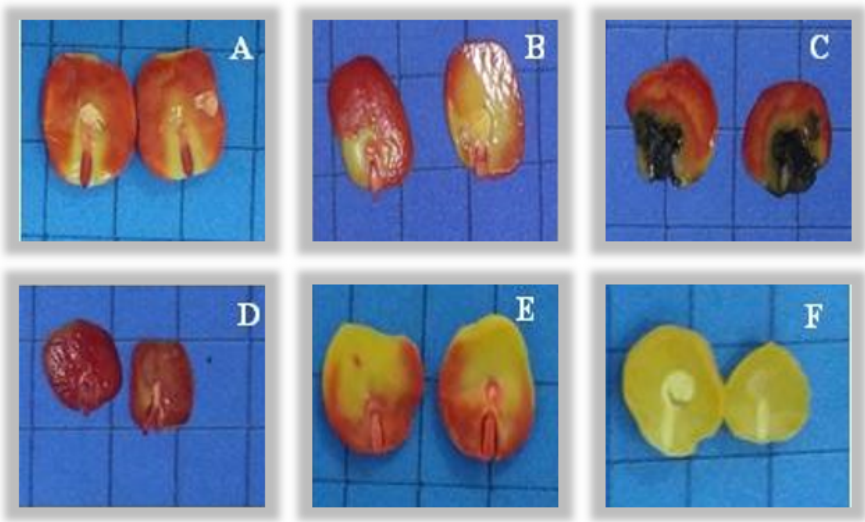

Figure 4. Staining pattern of inviable and dead seeds of *Anadenanthera colubrina* var. cebil (Griseb.) Altshul.; seeds with compromised cotyledons, vascular region, and plumule – A and B; rupture of the central cylinder – B; necrotic embryonic axis, vascular region, and plumule – C; seed in deterioration – D; seed with more than 50% dead tissue – E; and dead seed – F.

The lack of viable and non-vigorous seeds in the combination 0.5% / 8 h at 40 °C may be related to the accelerated aging for 24 h that the seeds underwent (Table 3). In that stress situation, the seeds absorbed water in a humid and hot environment, affecting the membrane system and the energy mechanism, resulting in reduced resistance to storage and tolerance to environmental stresses (Marcos-Filho, 2015; Marcos-Filho, 2020). As a consequence, there was reduction in seed viability, which corroborates the results of this study.

The results found in this study show the importance of adequately choosing the temperature, concentration, and periods of immersion in tetrazolium salt to evaluate seed physiological quality, especially under unfavorable conditions, such as those caused by accelerated aging. Identification of low-quality seeds before their use allows the producer to

discard seed lots and, consequently, reduce production costs that encumber the undertaking.

In light of the above, it is clear that the methodology suitable for conducting the tetrazolium test for different species must be individualized, especially because the response differs among the species, mainly under high temperature and relative humidity conditions. For *A. colubrina*, viability can be analyzed in at most 14 h, from preparation up to final evaluation of the seeds, whereas the results of the germination test take an average of 10 days.

CONCLUSIONS

The tetrazolium test effectively evaluates the viability of *A. colubrina* seeds. For that evaluation, the seeds should be chipped in the region opposite the micropyle, followed by pre-moistening in water for 10 h, and then removal of the seed coat and immersion in 0.1% tetrazolium solution at 35 °C for 4 h. Under these conditions, the tetrazolium test effectively identified differences in the physiological quality of non-aged seeds and seeds aged for 12 h and 24 h.

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