

SUITABILITY OF FORMS OF PREPARING SEEDS OF *Dalbergia miscolobium* BENTH FOR EXPOSURE TO THE TETRAZOLIUM TEST

ADEQUAÇÃO DAS FORMAS DE PREPARAÇÃO DE SEMENTES DE *Dalbergia miscolobium* BENTH PARA EXPOSIÇÃO AO TESTE DE TETRAZOLIUM

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ABSTRACT - *Dalbergia miscolobium* or Jacarandá do Cerrado is a species of legume in the Fabaceae family. It occurs in the *sensu stricto* Cerrado and in the dystrophic cerradão. It shows potential for landscaping and for recovering damaged areas. It is an endangered species and therefore is protected by the law that prevents cut in areas of the Federal District (Decree No. 14.783/93). The purpose of this study was to determine the best procedure to prepare seeds of *Dalbergia miscolobium* to assess viability in the tetrazolium test. We carried out the following treatments: i) hydration on filter paper at 25 ° C, ii) hydration on filter paper at 25 ° C followed by a cut in the tegument and iii) hydration on filter paper at 25 ° C followed by a complete removal of the tegument. The results were analyzed using analysis of variance and the Tukey range test. The analyzes showed that the best procedure to prepare seeds of *Dalbergia miscolobium* is the treatment in which there is a hydration followed by the complete removal of the integument. Where 78% of the seeds showed uniform staining, indicating that the seeds analyzed are of good quality. The other treatments, hydration and hydration followed by cutting, showed respectively 35% and 41% of viable seeds.

Keywords: Prepare. Tegument. Tetrazolium. Jacarandá do Cerrado. Fabaceae.

RESUMO - A *Dalbergia miscolobium* ou Jacarandá do Cerrado é uma espécie de leguminosa da família Fabaceae. Ocorre no sentido stricto Cerrado e no cerradão distrófico. Possui potencial para paisagismo e para recuperar áreas degradadas. É uma espécie ameaçada de extinção e, portanto, está protegida pela lei que previne o corte em áreas do Distrito Federal (Decreto 14.783 / 93). O objetivo deste estudo foi determinar o melhor procedimento de preparação das sementes de *Dalbergia miscolobium* para serem submetidas à análise de viabilidade pelo teste de tetrazólio. Foram realizados os seguintes tratamentos: i) hidratação em papel de filtro a 25 ° C, ii) hidratação em papel de filtro a 25 ° C seguida de um corte no tegumento e iii) hidratação em papel de filtro a 25 ° C seguido de remoção completa do tegumento. Os resultados foram analisados utilizando-se a análise de variância e o teste de médias de Tukey. As análises mostraram que o melhor procedimento para preparar sementes de *Dalbergia miscolobium* é o tratamento em que há uma hidratação seguida pela remoção completa do tegumento, onde 78% das sementes apresentaram coloração uniforme, indicando que as sementes analisadas são de boa qualidade. Os demais tratamentos, hidratação e hidratação seguida de corte, mostraram respectivamente 35% e 41% de sementes viáveis.

Palavras-chave: Preparação. Tegumento. Tetrazolio. Jacarandá do Cerrado. Fabaceae.

INTRODUCTION

The tetrazolium test also known as biochemical test for seed vitality is a technique used to estimate the viability and vigor of seeds (DESWAL & CHAND, 1997). It is a quick test used to determine the germination potential when there is need for an immediate sowing or when species have slow germination and for dormant seeds (ISTA, 2008).

The tetrazolium test is based on the activity of dehydrogenase enzymes, which are responsible for catalyzing the respiratory reactions in the mitochondria during glycolysis and the Krebs cycle (França Neto, 1999). The 2, 3, 5 triphenyl tetrazolium chloride or bromide solution is colorless and in contact with the seeds it spreads through the tissues and joins the hydrogen ions (H⁺), released from cells during the respiratory process. This hydrogenation of the tetrazolium solution is catalyzed by malic dehydrogenase producing triphenyl formazan. This compound is red and not diffusible thereby indicating respiratory activity in the tissue, which enables the identification of living tissues. Living tissues respond presenting the red color and dead tissues remain uncolored (Deminicis et al., 2009).

In order to make the contact between the tetrazolium solution and the tissues of the seeds happen, allowing the evaluation of the physiological quality, a number of specific determinations are necessary for each species of interest. Seeds have different characteristics especially regarding the permeability of the integument. These determinations are related to the preconditioning of the seeds aiming the hydration, which leads to the reactivation of the enzyme activities as well as the penetration of the solution into the tissues (Deminicis et al., 2009). The hydration also leads to the determination of the concentration of the solution, the temperature, the time for conditioning, and the correct interpretation of the staining obtained in seeds (Oliveira et al., 2005; BRAZIL, 2009).

Methodologies for the test requiring previous soaking in water and subsequent staining are described by Brazil (2009) and other authors. However, intrinsic characteristics of many species demand distinctive exposure times, temperatures and concentrations requiring specific methodologies (SILVA et al, 2012).

Forest tree species due to the impermeability of the majority of the teguments demand, besides the metabolic reactivation, the prior preparation of the seeds so that the tegument is not an obstacle in the contact between the tissue and the tetrazolium solution. Cutting the seed coat, seed coat removal, scarification using sandpaper, scraping with hot water and soaking in water are among the preparations prior to the test (BRAZIL, 2009).

Other factors such as the solution concentration or exposure time to the solution can affect the efficiency of the test in the evaluation of seed quality. The time required for the color to develop appropriately, according to the Rules for Seed Analysis (BRAZIL, 2009), varies according to each species

and can be between 30 and 240 minutes.

The tetrazolium test has been widely used in seeds of various species due to the speed and efficiency in the characterization of viability and vigor of seeds, and the possibility of distinguishing damages, assisting in the process of quality control since the harvesting and storage step (GREY et al, 2007).

The objective of this study was to determine the best way to prepare seeds of *Dalbergia miscolobium* Benth for the tetrazolium test.

MATERIALS AND METHODS

A) GATHERING AND PREPARATION OF SEEDS

The seeds of *Dalbergia miscolobium* Benth were collected according to Figliolia and Aguiar (1993). They were taken from 10 arrays in the Olympic Area at the Darcy Ribeiro Campus – UnB, in the Setor de Clubes Norte and in the mediations of the Fazenda Água Limpa in Brasília-DF, Brazil.

Seeds were extracted from fruits and by hand mixed in order to homogenize the batch. Then they were disinfected by immersion bath in hypochlorite 1% and washed several times in running tap water for 5 minutes. After this process, the seeds were dried on towel papers followed by drying in open trays for another 24 hours, inside the lab, to only then be performed experimental procedures. The analyses were performed in the facilities of the Laboratory of Seeds and Incubators of the Department of Forestry at UnB.

B) APPLICATION OF TECHNICAL PROCEDURES AND TETRAZOLIUM TEST

The seeds were divided into 3 sub-samples that were analyzed by the following treatments: i) hydration on filter paper at 25°C; ii) hydration on filter paper at 25°C followed by cutting the tegument; and iii) hydration on filter paper at 25°C followed by complete removal of the tegument. After this procedure, seeds were divided in recipients by repetition and covered with a tetrazolium 1% solution for 24 hours at constant temperature in a calibrated chamber for 25°C. After this exposure period, the seeds were washed and divided into 2 classes: 1) Viable: equally colored seeds; and 2) Unviable: uncolored seeds. To each treatment, 10 repetitions were made. The data found were submitted to analysis of variance and to the Tukey test. All analysis were performed using SAEG (Statistical Program version 9.1) (2007).

RESULTS AND DISCUSSION

Among the results for the different treatments were observed the presence of injured seeds, dead seeds, seeds in the process of deterioration and completely viable seeds (Figure 1). However, for statistical effect, only two categories were created: 1) viable seeds, and 2) unviable seeds – which gathered injured seeds, dead seeds and seeds in the process of deterioration.

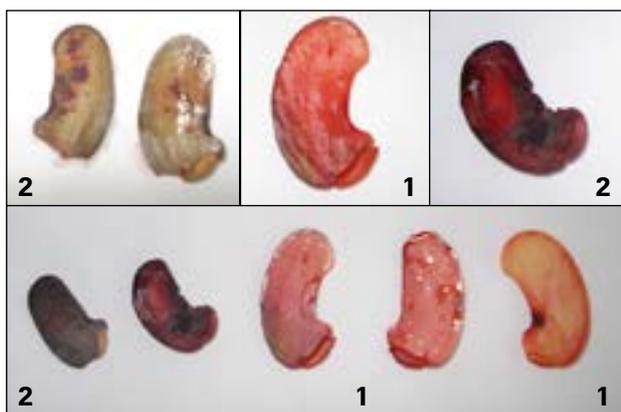


Figure 1. Seeds followed by the number 1 – Viable Seeds; Seeds followed by the number 2 – Unviable Seeds.

The obtained data by the three treatments were submitted to analysis of variance at a significance level of 1% of probability. The results of the analysis are presented in Table 1. The average obtained for the tetrazolium test was 51.33%. The variation coefficient was found to be 27.8%, which can be explained by two factors: i) the genetic variability and the physiological quality of the batch analyzed, and ii) the treatments themselves that may have favored the contact between the tissues of the seeds and the tetrazolium solution differently. Even with the interference of these factors, the variation coefficient showed a good experimental control. Garcia (1989) proposes a table to analyze this variable and classifies the value found as an acceptable one, what implies that there was a good experimental control.

Table 1. Results of analysis of variance applied to the data obtained from the three different treatments used to prepare seeds of *Dalbergia miscolobium* Benth for the tetrazolium test.

Data	Source of Variability	Degrees of Freedom	Mean Square	F	Sig.	Average	Standard Deviation	Coefficient of Variation
Percentage	Time	2	5423.33	26,62	26.6236**	51,33	27,80	27,80
	Residual	27	203.70	-	-	-	-	-

** Significant level of 1 percent ($p < .01$)

As showed in Table 1 above there is a significant difference between treatments. Therefore,

the Tukey test was applied. The results are shown in Table 2.

Table 2. Results of the Tukey test for the three different treatments used to prepare seeds of *Dalbergia miscolobium* Benth.

Treatment	Averages	Comparisons
3 – Hydration at 25°C and total removal of the tegument	78	A
2 – Hydration at 25°C and cut of tegument	41	B
1 – Hydration at 25°C	35	B

Averages followed by the same letters do not differ statistically. The Tukey test was used at 5% of probability

The treatments, hydration at 25°C and hydration at 25°C followed by cut in the tegument do not differ. In other hand, the treatment hydration at 25°C and complete removal of the seed coat differ from the other, as it presented a greater number of viable seeds.

Hydration at 25 ° C followed by removal of the seed coat was the treatment that showed the best performance of the tetrazolium test in the analysis of seeds of *Dalbergia miscolobium* Benth. Despite being a laborious work that requires many skills to be done in order not to cause any injuries to the seeds during the process, the results allow us to conclude that the contact between tissues and the tetrazolium solution happened with no doubt. This result is also explained by the resistant morphology of the integument being present in Fabaceae species, which is proved in studies described in Rolston (1978).

Silva (2012) studying the preparation procedures for sunflower seeds to perform the tetrazolium test, concluded that hydration was enough to prepare the seeds for the test. But the rules of analysis does not recommend only one treatment because of morphological characteristics as seed size, seed coat type and the position of the embryo in the seed, which may influence the test results (BRAZIL, 2009). These are even more evident in forest species because of the huge diversity and the many ecological strategies used for dispersal of species.

The results obtained from the tetrazolium test for seeds of *Dalbergia miscolobium* Benth showed that the batch has good vigor, as 78% of the seeds were identified as viable when prepared with the best and more efficient treatment. This way, it is recommended the hydration procedure on filter paper at 25 ° C for 24 hours followed by

complete removal of the seed coat to evaluate seeds of *Dalbergia miscolobium* Benth using the tetrazolium test.

CONCLUSION

Hydration on filter paper at 25 ° C for 24 hours followed by removal of the seed coat was the treatment that allowed the best performance of the tetrazolium test in the analysis of the seeds of *Dalbergia miscolobium* Benth.

The seeds studied by the tetrazolium test have good vigor.

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