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Viability *Mimosa bimucronata* (DC.) O. Kuntze. seeds by the tetrazolium test and oil content

Viabilidade de sementes de *Mimosa bimucronata* (DC.) O. Kuntze. pelo teste de tetrazólio e teor de óleo

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ABSTRACT: Maricá (*Mimosa bimucronata* (D.C.) O. Kuntze.) is a species that presents great importance in reforestation programs, in the recovery of permanent preservation areas, landscaping projects and is considered soil improver, being recommended for erosion and for planting on flooded land. The objective of the present study was to evaluate the viability of the seeds of *M. bimucronata* by the tetrazolium test and to determine the crude oil content and the percentage composition of fatty acids. The experiments were conducted at the Plant Propagation Laboratory of the Agricultural Sciences Center (CECA) of the Federal University of Alagoas (UFAL), located in the municipality of Rio Largo, Alagoas, Brazil. The obtained data submitted to the analysis of variance and the comparison between the means of viable seeds for each of the combinations in the tetrazolium test were performed by the Dunnett test at 5%. The concentration of 0.075% tetrazolium salt for two hours at 35 °C is an efficient combination for evaluating the viability of *M. bimucronata* seeds. The oil content was 2.75%, with unsaturated fatty acids prevailing (63.8%).
KEYWORDS: percentage of fatty acids, soil improver, viability of seeds.

RESUMO: Maricá (*Mimosa bimucronata* (D.C.) O. Kuntze.) é uma espécie que apresenta grande importância em programas de reflorestamento, na recuperação de áreas de preservação permanente, projetos paisagísticos e também é considerada melhoradora de solos, sendo recomendada para controle de processos erosivos e para plantio em terrenos sujeitos a inundações. Diante do exposto, o presente trabalho teve como objetivo avaliar a viabilidade das sementes de *M. bimucronata* pelo teste de tetrazólio e determinar o teor de óleo bruto e a composição percentual de ácidos graxos. Os ensaios foram conduzidos no Laboratório Propagação de Plantas do Centro de Ciências Agrárias (CECA) da Universidade Federal de Alagoas (UFAL), localizado no município de Rio Largo, Alagoas, Brasil. Os dados obtidos submetidos à análise de variância e a comparação entre as médias de sementes viáveis para cada uma das combinações no teste de tetrazólio foram realizadas pelo teste de Dunnett a 5%. A concentração de sal de tetrazólio a 0,075% por duas horas a 35 °C constitui eficiente combinação para avaliação da viabilidade de sementes de *M. bimucronata*. O conteúdo de óleo encontrado foi de 2,75%, prevalecendo os ácidos graxos insaturados (63,8%).
PALAVRAS-CHAVE: melhoradora de solos, percentual de ácidos graxos, viabilidade de sementes.

INTRODUCTION

Maricá (*Mimosa bimucronata* (DC.) O. Kuntze.) belonging to the family Mimosaceae (Leguminosae-Mimosoideae), is a medium-sized tree species that is distributed naturally in the Northeast, South and Southeast regions of Brazil, being particularly frequent in the states of Pernambuco, Alagoas and Paraná. It is a species that presents great importance in mixed reforestation programs, destined to the plantation for the recovery of areas of permanent preservation, management of forest fragments and landscaping projects, due to its rusticity and fast growth (CARVALHO, 2003). It is also considered a soil improver and is recommended for the control of erosive processes and for planting on land subject to periodic flooding (CARVALHO, 2004).

In the tetrazolium test, procedures, called preconditioning, are recommended to penetrate the solution in the tissues of interest to be evaluated. In seeds of forest species, several preconditioning treatments have been used as cutting, scarification and soaking in water (FERREIRA et al., 2001; MENDONÇA et al., 2001). In addition to preconditioning, the use of tetrazolium solution concentration, conditioning time and temperature, and proper seed color assessment are fundamental to obtain reliable results on viability.

Various concentrations of the tetrazolium solution may be applied in conducting the test, depending on the species evaluated, the seed preparation method and the permeability of the seed coat (OLIVEIRA et al., 2016), however the most used concentrations are 0.075; 0.1; 0.2; 0.5 and 1.0%.

The time essential for the development of coloration varies according to the species. During this time, the seeds should be kept in the dark submerged in the tetrazolium solution as the light may cause a change in the color of the solution and thus possible errors in the interpretation of the test. In the case of forest species, the seeds can remain in water in the refrigerator for up to 24 hours in the dark (PIÑA-RODRIGUES; VALENTINI, 1995).

Therefore, studies with seeds of forest species have been elaborated, seeking to define the best time, temperature and concentration of the tetrazolium salt solution to evaluate the viability of each species. Oliveira et al. (2016) recommended the staining of seeds of *Simira gardneriana* M.R. Barbosa & Peixoto in 0.075% tetrazolium solution for

6 hours at 35 °C. Fava and Albuquerque (2013) found that the temperature of 40 ° C and staining for 3 hours at the concentration of 0.5% of the tetrazolium solution were efficient for evaluating the viability of the seeds of *Palicourea rigida* Kunth. In seeds of *Piptadenia moniliformis* Benth., the concentration of the solution used is 0.075% and temperature of 35 °C, for 4 hours (AZERÊDO et al., 2011) For seeds of *Tabebuia serratifolia* Vahl Nich., the seeds are immersed in 0.5% solution for 12 hours at 30 °C (OLIVEIRA et al., 2005).

Vegetable oils are purchased from parts of plants through different extraction processes. Among the methods used to extract vegetable oils traditionally employed in Brazil and worldwide are extraction with organic solvent, distillation with organic solvent and water vapor and mechanical pressing (LEAL, 2008; MEHMOOD; WATSON, 2014). In the last 30 years, there has been an increase in the world production of vegetable oils from seeds. Because of the diverse applications of vegetable oils in the industry, many seed oils have been studied and characterized (CHIGOZIE et al., 2014). Vegetable oils extracted from seeds have in common their high content of polyunsaturated fatty acids (PUFAs), sources of essential fatty acids (AGEs). They have a ratio of linolenic acid to favorable linoleic acid compared with other vegetable oils; they also usually contain antioxidant substances, which suggests a great nutritional value (COELHO, 2015).

The objective of the present study was to evaluate the viability of the seeds of *M. bimucronata* by the tetrazolium test and to determine the crude oil content and the percentage composition of fatty acids.

METHODOLOGICAL PROCEDURE

Place of experiment

The experiments were conducted at the Plant Propagation Laboratory of the Agricultural Sciences Center (CECA) of the Federal University of Alagoas (UFAL), located in the municipality of Rio Largo, Alagoas, Brazil.

Harvesting and processing of seeds

The fruits of *M. bimucronata* were harvested using aerial scissors with extensor cable, from eight matrix trees belonging to forest fragments located in the municipality of Garanhuns, Pernambuco, Brazil at 08º 53' 25" S and 36º 29' 34" W, at an average altitude of 896 m. According to the climatic classification of Köppen, the climate is type As, tropical climate with rainy season. The collection took place between the months of March and May of 2017, having, on average, the following climatic conditions: average temperature 23.2 °C, while the maximum and minimum did not exceed 28.9 and 22.2 °C, respectively and the accumulated rainfall was 182.1 mm during that period.

Tetrazolium test

For the tetrazolium test, the seeds of *M. bimucronata* were pre-moistened for 24 hours and then, with the aid of a scalpel, were cut longitudinally and medianly, the portion of the cotyledon containing the embryo placed in plastic cups of 50 mL and submerged in a solution of 2,3,5-triphenyl tetrazolium chloride using four concentrations of 0.075; 0.1; 0.5 and 1.0%, for three periods of staining (2, 4 and 6 hours), in a chamber set at 30 and 35 ºC, in the absence of light.

After this time of staining, the solution was drained, the material washed in running water, the embryos were carefully extracted from the remaining part of the cotyledon and kept submerged in water in a refrigerated environment until the moment of the evaluation. The embryos were observed individually and evaluated for uniformity, color intensity, presence of milky white areas, tissue appearance and location of these stains in relation to the essential developmental regions (hypocotyl-radicle axis and vascular region).

Classified as viable and unviable according to the standards indicated by Moore (1972), Delouche (1976) and Grabe (1976), for various agricultural and forest species: 1) viable: embryos completely colored light pink or bright red; end of radicle without milky white/yellowish staining and 2) non-viable: embryos completely with crimson red/red-intense or milky white/yellowish; radicle end of discolored or red-intense. Results were expressed as percentage of viable seeds.

For the comparison of the results obtained in the tetrazolium test, the germination test was carried out in a germination chamber type B.O.D., at 30 °C, under constant white light, in four replicates of 25 seeds.

Extraction and determination of the crude oil content of the seed

The methodology of extraction and quantification of the oil chosen for this work was the solvent extraction method using the Soxhlet extractor system (OLIVEIRA et al., 2009). The determination of the percentage by mass of the crude oil of each sample was obtained by dividing the mass of the oil obtained by the mass of the sample, multiplied by 100. The average percentage of the oil content of the extractions was the arithmetic mean of the percentages of the three extractions.

Determination of the percentage composition of fatty acids

In order to determine and quantify the corresponding fatty acids of the oil, it underwent the transesterification process under special conditions to obtain 100% of these esterified acids (obtaining methyl esters) for analysis in gas chromatography (GC).

The transesterification reaction required 0.5 g of the crude oil, 10 mL of methanol and 0.5 mL of H_2SO_4 (catalyst), the three components were added in a 50 mL round flask. The flask was coupled to the condenser, heated to the temperature of 60 °C (maintained), and remained for one hour with moderate stirring (SILVA et al., 2010).

The method for the analysis was adapted from the method prescribed by the European standard EN 14103, it is necessary to dissolve 0.05 g of sample in 1 mL of hexane. Were realized three analysis (triplicates), at each analysis was injected 1 µL of the solution (sample in hexane) to CG-2010/Shimadzu instrument with an injection system split / spliteless operating at 250 °C, split ratio 100: 1, 1.0 µL sample volume and flame ionization detector (FID) operating at 250 °C. Was used, a nonpolar capillary column ZB-WAXplus with 30.0 mm length, 0.32 mm internal diameter, film 0.25 mm thick and hydrogen gas of high purity (99.95% LINDE) used as drag gas. The temperature programming was: initial temperature: 160 °C; heating from 160 °C to 225

°C at a rate of 15 °C/min; heating from 225 °C to 245 °C at a rate of 3 °C/min. The composition in fatty acids was calculated based on the identification and integration of the areas of the peaks by normalization (SILVA et al., 2010).

Statistical analysis

Statistical analyzes were performed by the SISVAR program, Federal University of Lavras (FERREIRA, 2011). The data obtained through analysis of variance (ANOVA) and the comparison between the means of viable seeds for each of the combinations in the tetrazolium test were performed by the Dunnett test at 5%.

RESULTS AND DISCUSSION

Tetrazolium

The results obtained for the percentage of viable seeds, the tetrazolium test at different concentrations and exposure times, and the standard germination test (control) to 30 °C are presented in Table 1.

It is observed that the mean of the viability of the seeds was higher in the staining period of 4 hours combined with the concentration of 0.075% of tetrazolium solution, differing statistically from the others. For the different concentrations, a decrease in the average of the viability of the seeds was observed as the concentration of the tetrazolium solution increased, with a significant effect for this factor. It was also verified that the average seed viability did not differ statistically from the results of the germination test (control – 99%), for the concentration of 0.075% and time of 4 hours tested at that temperature.

Table 1. Viable seeds of *M. bimucronata* obtained by tetrazolium test in different concentrations (0.075, 0.1, 0.5 and 1.0%) and staining periods (2, 4 and 6 hours) at 30 °C, in relation to the results of the germination test (control).

Coloring periods (hours)	Concentrations of tetrazolium solution (%)			
	0,075	0,1	0,5	1,0
2	52 bBy	56 aBy	34 cBy	34 cAy
4	98 aAz	88 bAy	64 cAy	32 dBy
6	42 aCy	33 bCy	18 cCy	6 dCy
	Germination = 99 z			
Value of "F" for periods (P)	1055.28**			
Value of "F" for concentrations (C)	515.04**			
Value of "F" for interaction (P x C)	55.62**			
Value of "F" for additional vs factorial	1276.16**			
Value of "F" for treatments	438.80**			
CV (%)	5.61			

Means followed by the same letter, upper case in column (A, B, C) and lowercase in line (a, b, c, d) do not differ significantly at a 5% probability by the Tukey test.

Means followed by the same letter (z, y), between germination (control - germination test) and viability obtained in the tetrazolium test, did not differ significantly at 5% probability by the Dunnett test.

In relation to the temperature of 35 ° C, the two-hour staining period provided the highest viability estimates in the 0.075 and 0.1% concentrations of the tetrazolium solution, not statistically different from each other (Table 2), with the highest values registered periods of coloring. The six-hour period at concentrations of 0.5 and 1.0% (0% of viable seeds) was not favorable for seed evaluation, providing statistically different estimates of the results of the germination test (99%). Regarding the other combinations of periods and concentrations, there was a significant difference between these and the control, due to the lack of adequate staining, and underestimating the viability of the seeds. Among the temperatures tested, that of 35 °C provided the highest viability values.

Table 2. Viable seeds of *M. bimucronata* obtained by the tetrazolium test in different concentrations (0.075, 0.1, 0.5 and 1.0%) and staining periods (2, 4 and 6 hours) at 35 °C, in relation to the results of the germination test (control).

Coloring periods (hours)	Concentrations of tetrazolium solution (%)			
	0.075	0.1	0.5	1.0
2	99 aAz	98 aAz	85 bAy	63 cAy
4	46 aBy	18 bBy	12 cBy	10 dBy
6	31 aCy	14 aCy	0 bCy	0 bCy
	Germination = 99 z			
Value of "F" for periods (P)	2761.53**			
Value of "F" for concentrations (C)	243.96**			
Value of "F" for interaction (P x C)	48.90**			
Value of "F" for additional vs. factorial	1579.17**			
Value of "F" for treatments	677.29**			
CV (%)	7.46			

Averages followed by the same letter, upper case in column (A, B, C) and lower case in line (a, b, c, d) do not differ significantly at 5% probability by Tukey's test.

Means followed by the same letter (z, y), between germination (control - germination test) and viability obtained in the tetrazolium test, did not differ significantly at 5% probability by the Dunnett test.

These results reinforce the claims Grabe (1976), that staining of the seeds in the tetrazolium test settles faster at higher temperatures. For this reason, Piña-Rodrigues and Valentini (1995) and Marcos Filho et al. (1987) recommended that the seeds immersed in the tetrazolium solution be placed in a regulated chamber at a temperature of 30 to 40 ºC. In the present research, the best combinations to evaluate the viability of *M. bimucronata* seeds were observed at 35 °C, while at 30 °C all combinations of concentrations and periods differed statistically from the germination test except 0.075% in the 4-hour period (Table 1).

The staining patterns observed in the embryos varied from dark red and bright (viable seeds), light pink (viable seeds) to white on dead seeds (Figure 1). The viable embryos presented uniform light pink color throughout, showing that the tissues are alive and vigorous. On the other hand, unviable embryos, when exposed to tetrazolium solution, exhibited a bright red-intense coloration (deteriorating tissue) or milky white (dead tissue) throughout or red-intense coloration only at the radicle end.

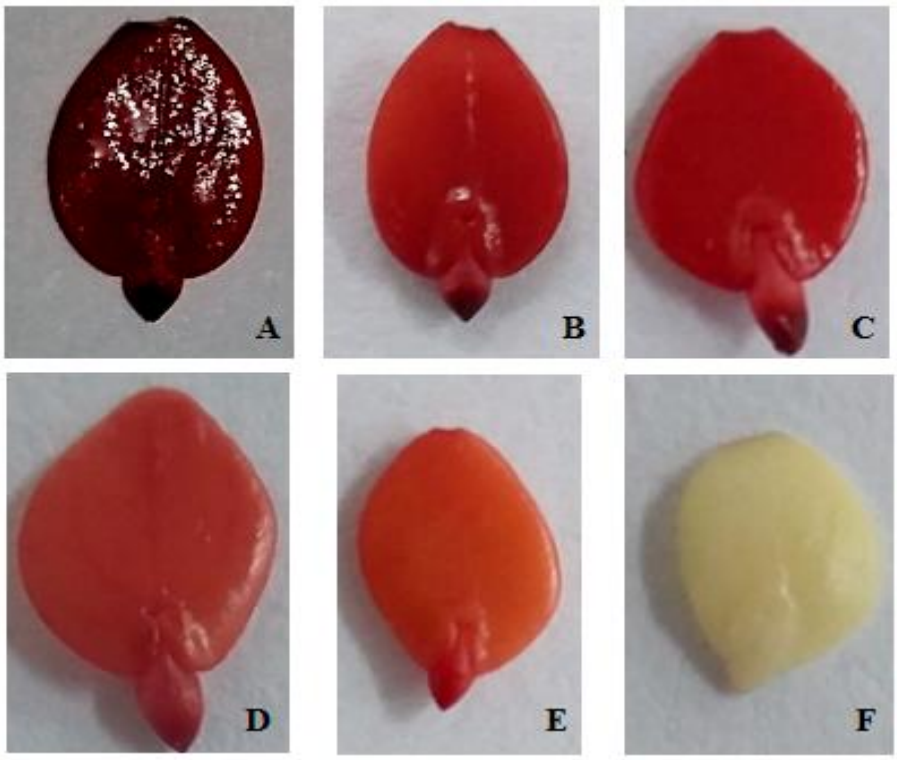

Figure 1. Non-viable seeds of *Mimosa bimucronata* (DC) O. Kuntze: embryo with intense bright red color (A); hypocotyl-radicular axis with intense red coloration in the cortex (B, C); embryo with discolored regions / milky white (F). Viable seeds of *Mimosa bimucronata* (DC) O. Kuntze: embryo with coloring ranging from pink to light pink throughout.

The choice of the appropriate method for the tetrazolium test should be based on the ease of identification of viable and non-viable tissues and on the ability to differentiate seed vigor (AZERÊDO et al., 2011). The color differences observed in the seeds after staining in the tetrazolium solution are the main characteristics that should be considered in interpreting the test results. The intensity of seed coloration in the tetrazolium test is variable among the species. For example, the pink color observed in viable *Leucaena leucocephala* (Lam.) de Wit. seeds (COSTA; Santos, 2010) is clearer than that found in *Brachiaria brizantha* (Hochst. Ex A. Rich) Stapf (DIAS; ALVES, 2008). In this species, the color that signals a viable fabric is red or intense pink. In *L. leucocephala* seeds, this intense pink color would mean deteriorating tissue. This is because the terminology used to name the colors observed in the seeds in the tetrazolium test is usually established by the authors, and therefore may vary among the works.

Composition and percentage of fatty acids

The profiles of methyl esters of fatty acids obtained from the seeds of *M. bimucronata* are present in Table 3. Predominated linoleic acid (C18:2), palmitic (C16:0) and oleic (C18:1), followed by lower levels of behenic acid (C22:0), stearic (C18:0), erucic (C22:1), eicosanoic (C20:0), gadoleic (C20:1) and palmitolytic (C20:1).

Table 3. Profiles of fatty acids obtained from the oil of the seeds of Mimosa bimucronata (DC.) O. Kuntze

Fatty acid	Representation	% Percentage
Palmitic	C16:0	18.8
Palmitoleic	C16:1	0.2
Stearic	C18:0	1.6
Oleic	C18:1	12.9
Linoleic	C18:2	49.4
Eicosanoic	C20:0	0.8
Gadoleic	C20:1	0.2
Beenic	C22:0	2.2
Erucic	C22:1	1.1
Others	-	11.4

Despite the low oil content found (2.75%), the higher amount of unsaturated fatty acids (63.8%) than saturated fatty acids (23.4%) is highlighted. This high oil unsaturation confers greater oxidative instability under normal conditions of temperature and luminosity (FERRARI, 2001). Among the unsaturated acids, linoleic acid predominates, important from the nutritional point of view, since it is considered essential fatty acid, therefore not produced by the animal metabolism, and should be administered through the diet (TURATTI, 2000).

CONCLUSIONS

The concentration of 0.075% tetrazolium salt for two hours at 35 ° C is an efficient combination for evaluating the viability of *M. bimucronata* seeds.

The oil content was 2.75%, with unsaturated fatty acids (63.8%) prevailing.

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