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## Viability of *Mimosa caesalpinifolia* Benth. seeds as a function of fruit maturity stage and storage temperature

### Viabilidade de sementes de *Mimosa caesalpinifolia* Benth. em função do estágio de maturação do fruto e temperatura de armazenamento

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#### Abstract

The maintenance of seed viability during storage is influenced by several factors related to the species and to the environment. This work aimed to determine the viability of *Mimosa caesalpinifolia* seeds from unripe and ripe fruits stored at different temperatures (ambient, 10 °C, 0 °C and -21 °C). For this, the germination performance of the seeds was evaluated at 0, 3, 6 and 12 months of storage. The experimental design was completely randomized, with four replicates of 25 seeds per treatment. The variables evaluated were: number of germinated seeds, number of hard seeds, number of unviable seeds and number of normal seedlings. The storage potential of *M. caesalpinifolia* seeds depends on the fruit maturity stage. Seeds from unripe fruits without drying do not tolerate storage. Seeds from unripe fruits with drying at 15% water content maintain viability, for 12 months, when stored at a temperature of 10 °C. Seeds from ripe fruits without drying maintain viability, for 12 months, when stored at temperatures of 0 °C and 10 °C.

**Additional keywords:** Fabaceae; germination; seed storage.

#### Resumo

A manutenção da viabilidade da semente durante o armazenamento é influenciada por diversos fatores relacionados à espécie e ao ambiente. Este trabalho teve por objetivo determinar a viabilidade de sementes de *Mimosa caesalpinifolia* provenientes de frutos verdes e maduros armazenadas em diferentes temperaturas (ambiente, 10 °C, 0 °C e -21 °C). Para isso, o desempenho germinativo das sementes foi avaliado nos períodos de 0, 3, 6 e 12 meses de armazenamento. O delineamento experimental foi o inteiramente casualizado, com quatro repetições de 25 sementes por tratamento. As variáveis avaliadas foram: número de sementes germinadas, número de sementes duras, número de sementes inviáveis e número de plântulas normais. O potencial de armazenamento das sementes de *M. caesalpinifolia* depende do estágio de maturação dos frutos. Sementes de frutos verdes sem secagem não toleram o armazenamento. Sementes de frutos verdes com secagem a 15% de teor de água mantêm a viabilidade, por 12 meses, quando armazenadas na temperatura de 10 °C. Sementes de frutos maduros sem secagem mantêm a viabilidade por 12 meses, quando armazenadas nas temperaturas de 0 °C e 10 °C.

**Palavras-chave adicionais:** armazenamento de sementes; Fabaceae; germinação.

#### Introduction

The lack of basic knowledge about the production and proper management of native seeds is one of the factors that can interfere in the availability of seeds for the market (Calvi, 2015), which is one of the main obstacles to meet the demands for the commercial production of forest products and for the recovery of ecosystems in Brazil.

Native species are not regular in terms of seed production period. Depending on the species, there may be a year of high production followed by one or two without production (Fowler & Martins, 2001). Therefore, storage is a necessary practice to ensure the supply of seeds and seedlings and, according to Kissmann et al. (2009), appropriate technology for each species should be employed in order to keep seeds viable and reduce the rate of deterioration during storage.

In order to define seed storage techniques, it is necessary to know *a priori* their physiological behavior in relation to storage. In general, seeds are divided into three categories: orthodox, which tolerate drying at water contents between 2 and 5% and storage at negative temperatures; intermediate, which tolerate drying at water contents between 7 and 11%, but are sensitive to negative temperatures; and recalcitrant, which do not tolerate drying at water content below 20% and storage at low temperatures (Hong & Ellis, 1996).

The maintenance of seed viability and vigor during storage is influenced by several factors related to the species and to the environment, including the water content of the seeds, the physiological potential at harvest and the temperature of the environment (Marcos Filho, 2015). Water content and temperature are the most important conditions that affect seed longevity during storage; high temperature and humidity accelerate seed deterioration, while low humidity and temperature reduce (Bewley et al., 2013). The stage of seed maturity at harvest is another aspect that can affect longevity during storage (Bewley et al., 2013; Colville, 2017). Seeds harvested very early or late tend to have low vigor and longevity (Colville, 2017).

*Mimosa caesalpiniiifolia* Benth. (Fabaceae), popularly known in Portuguese as 'sabiá' or 'sansão do-campo', is a forest species native to the Northeast region of Brazil (Dutra et al., 2020), with multiple potentials of use, such as, recomposition of degraded areas (Alves et al., 2004), folk medicine (Albuquerque et

al., 2007), production of posts, stakes, lampposts, firewood and coal (Pinto et al., 2016; Avelino et al., 2018; Araujo & Paes, 2018; Araujo et al., 2020; Lins et al., 2020), landscaping and construction of living fences (Araujo & Paes, 2018).

Given the silvicultural importance of *M. caesalpiniiifolia*, the demand for its seeds is expected to increase in the near future (Araujo et al., 2020). Thus, the present study aimed to determine the viability of *Mimosa caesalpiniiifolia* seeds from unripe and ripe fruits stored at different temperatures (ambient, 10 °C, 0 °C and -21 °C).

### Material and methods

*Mimosa caesalpiniiifolia* fruits were collected from eleven plants located at the Experimental Farm of the Federal University of Recôncavo da Bahia (UFRB), Cruz das Almas campus, at the coordinates 12° 39' 19" S and 39° 04' 57" W, in September 2018. According to the Köppen-Geiger classification (Alvares et al., 2013), the climate of the region is Af type, with an average annual temperature of 24 °C, average annual rainfall of 1224 mm and relative humidity of 80%.

The fruits were separated based on the external color, green and brown, and characterized with the maturity stages unripe and ripe, respectively (Nogueira et al., 2013). The seeds were extracted from the fruits manually and grouped into two lots: seeds from unripe fruits and seeds from ripe fruits (Figure 1).



**Figure 1** - *Mimosa caesalpiniiifolia* Benth. seeds from unripe fruits (A) and ripe fruits (B).

To evaluate the physiological quality of the seeds prior to storage, a sample of each seed lot was subjected to water content determination and germination test. Another sample of seeds from unripe fruits was dried until it reached 15% water content (value close to the initial water content of seeds from ripe fruits), by the silica gel method, at 20 °C (Hong & Ellis, 1996). Then, a subsample of these seeds was subjected to the germination test.

The seeds without drying and with drying were placed in Falcon tubes and stored at different temperatures, composing 12 treatments: 1. seeds from ripe fruits without drying, in an environment without temperature control (average of 24 °C); 2. seeds from ripe fruits without drying, in B.O.D. germination chamber

at 10 °C; 3. seeds from ripe fruits without drying, in B.O.D. germination chamber at 0 °C; 4. Seeds from ripe fruits without drying, in freezer at -21 °C; 5. Seeds from unripe fruits without drying, in an environment without temperature control (average of 24 °C); 6. Seeds from unripe fruits without drying, in B.O.D. germination chamber at 10 °C; 7. Seeds from unripe fruits without drying, in B.O.D. germination chamber at 0 °C; 8. Seeds from unripe fruits without drying, in freezer at -21 °C; 9. Seeds from unripe fruits with drying, in an environment without temperature control (average of 24 °C); 10. Seeds from unripe fruits with drying in a B.O.D. germination chamber at 10 °C; 11. Seeds from unripe fruits with drying, in B.O.D. germination chamber at 0 °C; 12. Seeds from unripe fruits with drying, in freezer at

-21 °C. The storage periods evaluated were 3, 6 and 12 months.

In each storage period, part of the seeds of each treatment was subjected to water content determination and germination test. The tests to determine the water content of the seeds were performed by the oven method at  $105 \pm 3$  °C, for 24 hours (Brasil, 2009). Germination tests were conducted on Germitest® paper rolls, moistened with distilled water (2.5 times the mass (g) of the dry paper), packed in transparent polyethylene bags and kept in a B.O.D. germination chamber, with temperature of 30 °C and photoperiod of 12 hours.

The experimental design was completely randomized, with four replicates of 25 seeds, per treatment. Germination performance was evaluated daily until the stabilization of germination (seven days without occurrence of the event), by counting the number of germinated seeds (2-mm-long radicle), hard seeds and dead seeds, and the numbers of normal and abnormal seedlings (Brasil, 2009). At the end of each germination test, the seeds that remained hard were subjected to the tetrazolium test at 0.5% for 2 hours at a temperature of 30 °C (Marcos Filho, 2015), for evaluation of viability.

The variables (random component) number of germinated seeds, number of hard seeds, number of unviable seeds and number of normal seedlings, obtained in the treatments used (systematic component), were analyzed by generalized linear models, with binomial distribution and logit link function, and the inferences of the deviance analysis were based on the chi-square statistics, as recommended by Carvalho et al. (2018). The amplitudes of differences between treatments were verified by Tukey's multiple means comparison test, with confidence interval correction by the Sidák method. These analyses were performed using the packages Emmeans (Lenth et al.,

2020) and MultcompView (Graves et al., 2019) in the R Core Team 3.5.3 program (R Development Core Team, 2019).

## Results and discussion

The average initial water content of the newly harvested seeds of *M. caesalpinifolia* was 52.76% for those from unripe fruits, with Confidence Interval- $CI_{95\%}$  of  $52.76\% \pm 0.73\%$ , and 16.35% for those from ripe fruits, with a confidence interval- $CI_{95\%}$  of  $16.35\% \pm 1.67\%$ . A similar result was obtained by Nogueira et al. (2013), who evaluated *M. caesalpinifolia* seeds in five maturity stages and found that, as the seeds matured, there was a reduction in their water content (45.2%, 36.4%, 32.9%, 17.1%, 11.0%). Orthodox seeds, at the maturity stage, lose most of their water, a characteristic that favors their survival after dispersion, until environmental conditions are adequate for germination and plant establishment (Buitink et al., 2006). This characteristic is also important for *ex situ* conservation, because the low water content restricts deterioration and preserves the physiological potential of seeds for longer periods (Marcos Filho, 2015).

The result of the analysis for seed germination before storage is shown in table 1. The highest percentage of germination was observed in seeds from unripe fruits without drying (84%) and the highest percentage of normal seedlings in seeds from unripe fruits with drying (70%). However, there was no significant difference for these variables between seeds from ripe fruits without drying, seeds from unripe fruits without drying and seeds from unripe fruits with drying. These results indicate that seeds from unripe and ripe fruits have the same germination potential and that seeds from unripe fruits have drought tolerance.

**Table 1** - Percentage of germinated, unviable and hard seeds and formation of normal seedlings of *Mimosa caesalpinifolia* Benth., before storage.

Treatments	% Germinated	% Normal seedlings	% Hard	% Unviable
RWoD	75.0±10.3 a	64.0±11.5 a	24.0±10.2 a	12.0±7.8 b
UWoD	84.0±8.8. a	57.0±11.8 a	16.0±8.8 a	27.0±10.6 a
UWiD	80.0±9.6 a	70.0±10.9 a	0.0±0.0 b	30.0±10.9 a

Means followed by the same letter in the column do not differ by Tukey test ( $p > 0.05$ ). Seeds from ripe fruits without drying (RWoD), seeds from unripe fruits without drying (UWoD) and seeds from unripe fruits with drying at 15% water content (UWiD).

For hard and unviable seeds, differences were detected between treatments (Table 1). The percentage of unviable seeds, consisting of the sum of dead seeds and abnormal seedlings, was higher in those from unripe fruits, indicating that, although these seeds have acquired the ability to germinate, they have probably not yet reached physiological maturity, which corresponds to the stage at which they have the maximum germination potential and vigor (Marcos Filho, 2015). In

a study on the maturation of *Caesalpinia echinata*, Borges et al. (2005) verified that seeds harvested before physiological maturity are capable of germinating, but have low vigor.

The results of water content of the seeds during the storage period are presented in Table 2, which shows that the water content varied little throughout storage in most treatments.

**Table 2** - Confidence intervals ( $\alpha=0.05$ ) for water content of *Mimosa caesalpinifolia* Benth. seeds from ripe fruits without drying (RVoD), green fruit seeds without drying (UWoD) and green fruit seeds with drying at 15% water content (UWiD), as a function of storage time (ST).

ST (months)	Temperature (°C)	RVoD	UWoD	UWiD
3	WoC	12.08±1.18	60.5±7.5	15.59±3.45
3	10	12.02±1.39	58.6±3.7	13.86±1.90
3	0	11.76±3.18	51.0±2.7	14.53±3.75
3	-21	12.25±1.74	50.9±3.6	13.40±2.96
6	WoC	11.59±1.82	51.4±7.3	16.47±*
6	10	12.50±1.08	57.5±15.3	14.31±1.05
6	0	9.74±2.31	53.7±3.2	11.11±1.65
6	-21	19.62±5.81	34.1±10.0	13.70±5.01
12	WoC	15.07±0.16	48.1±14.0	15.65±2.03
12	10	15.97±1.99	59.52±2.3	17.75±1.13
12	0	16.76±*	56.7±3.1	15.96±2.26
12	-21	15.93±2.01	61.1±1.9	18.39±0.62

\*Loss of samples made it impossible to obtain the confidence interval. WoC = without temperature control (laboratory room temperature)

Seeds from unripe fruits without drying did not tolerate storage at any of the temperatures tested. Germination was zero in all periods and the tetrazolium test made it possible to confirm that all seeds were dead. The loss of viability observed may be related to the combination of two factors: high water content of the seeds and storage temperatures. Water and temperature are the main factors that influence seed viability during storage (Bewley et al., 2013; Colville, 2017). Seeds stored with high water content and exposed to higher temperatures exhibit intense respiratory activity, which favors the accumulation of reactive oxygen species (ROS) (Ebene et al., 2019; Zhang et al., 2021). ROS cause oxidative damage to lipids, proteins and nucleic acids and accelerate the process of aging and loss of seed viability (Zhang et al.,

2021). In seeds stored with high water content and exposed to freezing temperatures, the loss of viability may be associated with cellular damage caused by the formation of ice crystals (Hellmann et al., 2006; Marcos Filho, 2015).

At three months, germination and formation of normal seedlings were above 85%, regardless of seed maturity stage and storage temperature, except for green seeds with drying, stored in an environment without temperature control (Table 3). Although the germination performance was lower in this treatment, the percentages obtained for the variables analyzed were close to those of the newly collected seeds. These results show that, at three months of storage, the physiological quality of the seeds was maintained in all treatments.

**Table 3** - Percentage of germinated seeds, normal seedlings and unviable seeds of *Mimosa caesalpinifolia* Benth., after three months of storage at different temperatures.

Temp. (°C)	Germinated (%)		Normal Seedlings (%)		Unviable (%)	
	RVoD	UWiD	RVoD	UWiD	RVoD	UWiD
WoC	96.0±4.5 Aa	79.0±10.1 Bb	93.0±6.4 Aa	78.0±10.3 Ab	7.0±6.4 Ab	22.0±10.3 Aa
10	91.0±7.1 Aa	88.0±8.1 ABa	89.0±7.8 Aa	86.0±8.6 Aa	11.0±7.8 Aa	14.0±8.6 Aa
0	89.0±7.8 Aa	94.0±5.9 Aa	88.0±8.1 Aa	89.0±7.8 Aa	12.0±8.1 Aa	11.0±7.8 Aa
-21	87.0±8.4 Aa	83.0±9.4 ABa	86.0±8.6 Aa	80.0±10.0 Aa	14.0±8.6 Aa	20.0±10.0 Aa

WoC = without temperature control (laboratory room temperature). Means followed by the same uppercase letter in the column and lowercase letter in the row do not differ by Tukey test ( $p > 0.05$ ). Seeds from ripe fruits without drying (RVoD), seeds from unripe fruits without drying (UWoD) and seeds from unripe fruits with drying at 15% water content (UWiD).

After storage for six months, the percentages of germination and normal seedlings for seeds from ripe fruits without drying reduced only at -21 °C (Table 4). In this treatment, there was a low percentage of unviable seeds, and the reduction in germination performance observed was due to a higher amount of hard seeds. These results were very close to that of the newly

collected seeds and, therefore, it can be inferred that there was no loss of viability and/or vigor in this treatment. The obtained percentage of hard seeds may have resulted from the presence of dormancy, since no pre-germinative treatment was used in the present study. *Mimosa caesalpinifolia* seeds have dormancy characterized by seed coat impermeability to water,

which is established at the end of the maturation stage, when the seeds have already lost most of their water content (Nogueira et al., 2013).

**Table 4** - Percentages of germinated seeds, normal seedlings, unviable seeds and hard seeds of *Mimosa caesalpinifolia* Benth., after six months of storage at different temperatures.

Temperature (°C)	Germinated (%)	
	RWoD	UWiD
WoC	92.0 ± 6.8 Aa	67.0 ± 11.7 Cb
10	95.0 ± 5.4 Aa	95.0 ± 5.4 Aa
0	93.0 ± 6.4 Aa	87.0 ± 8.4 ABa
-21	76.0 ± 10.6 Ba	81.0 ± 9.8 BCa
Temperature (°C)	Normal seedlings (%)	
	RWoD	UWiD
WoC	85.0 ± 8.9 ABa	56.0 ± 12.4 Bb
10	87.0 ± 8.4 ABa	90.0 ± 7.5 Aa
0	93.0 ± 6.4 Aa	73.0 ± 9.8 Bb
-21	75.0 ± 10.8 Ba	69.0 ± 11.5 Ba
Temperature (°C)	Unviable (%)	
	RWoD	UWiD
WoC	15.0 ± 8.9 Ab	44.0 ± 12.4 Aa
10	13.0 ± 8.4 ABa	10.0 ± 7.5 Ca
0	4.0 ± 4.9 Bb	27.0 ± 11.1 ABa
-21	4.0 ± 4.9 Bb	21.0 ± 10.1 BCa
Temperature (°C)	Hard (%)	
	RWoD	UWiD
WoC	0.0 ± 0.0 Ca	0.0 ± 0.0 Ba
10	0.0 ± 0.0 Ca	0.0 ± 0.0 Ba
0	7.0 ± 6.4 Ba	0.0 ± 0.0 Bb
-21	21.0 ± 10.1 Aa	10.0 ± 8.6 Ab

WoC = without temperature control (laboratory room temperature). Means followed by the same uppercase letter in the column and lowercase letter in the row do not differ by Tukey test ( $p > 0.05$ ). Seeds from ripe fruits without drying (RWoD), seeds from unripe fruits without drying (UWoD) and seeds from unripe fruits with drying at 15% water content (UWiD).

The seeds from unripe fruits with drying stored in an environment without temperature control showed the lowest percentages of germination and normal seedlings and the highest percentage of unviable seeds (Table 4). Most of the unviable seeds consisted of dead seeds, indicating that storage at room temperature negatively influenced the viability of these seeds. For seeds from unripe fruits with drying stored at 0 °C and -21 °C, there were also a significant reduction in the percentage of normal seedlings and an increase in the percentage of unviable seeds (Table 4). In these treatments, the number of unviable seeds consisted mainly of abnormal seedlings, suggesting that there was a reduction in vigor.

At 12 months, storage in an environment without temperature control favored the reduction of viability and vigor of seeds from ripe fruits without drying

and the loss of viability of seeds from unripe fruits with drying (Table 5). Storage at temperatures of 10 °C, 0 °C and -21 °C promoted the maintenance of physiological quality for both seeds from ripe fruits without drying and seeds from unripe fruits with drying (Table 5).

The observed results indicate that storage for 12 months in an environment without temperature control (mean of 24 °C) enhances the process of deterioration of *M. caesalpinifolia* seeds. Storage at room temperature also promoted faster loss of viability of *Caesalpinia echinata* seeds, at three months (Hellman et al., 2006), *Tabebuia caraiba* seeds, at three months (Guedes et al., 2012b), *Myracrodruon urundeuva* seeds, at 150 days (Guedes et al., 2012a) and *Melanoxylon brauna* seeds, at 24 months (Freire et al., 2021), compared to colder temperatures.

**Table 5** - Percentages of germinated seeds, normal seedlings, unviable seeds and hard seeds of *Mimosa caesalpinifolia* Benth., after twelve months of storage at different temperatures.

Temperature (°C)	Germinated (%)	
	RWoD	UWiD
WoC	76.0 ± 10.6 Ba	0.0 ± 0.0 Bb
10	95.0 ± 9.9 Aa	92.0 ± 6.8 Aa
0	92.0 ± 6.8 Aa	88.0 ± 8.1 Aa
-21	89.0 ± 7.8 ABa	82.0 ± 9.6 Aa
Temperature (°C)	Normal seedlings (%)	
	RWoD	UWiD
WoC	58.0 ± 12.3 Ba	0.0 ± 10.0 Bb
10	88.0 ± 8.1 Aa	84.0 ± 9.1 Aa
0	85.0 ± 8.9 Aa	72.0 ± 11.2 Ab
-21	89.0 ± 7.8 Aa	77.0 ± 6.0 Ab
Temperature (°C)	Unviable (%)	
	RWoD	UWiD
WoC	40.0 ± 12.2 Ab	100.0 ± 0.5 Aa
10	11.0 ± 7.8 Ba	16.0 ± 9.1 Ba
0	12.0 ± 8.1 Bb	26.0 ± 10.9 Ba
-21	4.0 ± 4.9 Bb	21.0 ± 10.1 Ba
Temperature (°C)	Hard (%)	
	RWoD	UWiD
WoC	2.0 ± 3.5 Aa	0.0 ± 0.0 Aa
10	1.0 ± 2.5 Aa	0.0 ± 0.0 Aa
0	3.0 ± 4.3 Aa	2.0 ± 3.5 Aa
-21	0.0 ± 0.0 Aa	0.0 ± 0.0 Aa

WoC = without temperature control (laboratory room temperature). Means followed by the same uppercase letter in the column and lowercase letter in the row do not differ by Tukey test ( $p > 0.05$ ). Seeds from ripe fruits without drying (RWoD), seeds from unripe fruits without drying (UWoD) and seeds from unripe fruits with drying at 15% water content (UWiD).

Several factors influence the rate of deterioration of seeds, including their initial physiological quality and storage temperature (Marcos Filho, 2015). Seeds harvested with incomplete maturity and/or lower vigor show greater intensity of deterioration during storage (Marcos Filho, 2015), which may explain the fact that seeds from unripe fruits were more affected. Storage at high temperatures increases metabolic activity, consumption of reserves and oxidative stress in seeds, which intensifies the deterioration process (Ebene et al., 2019; Zhang et al., 2021).

## Conclusions

The degree of fruit maturity interferes with the storage potential of *M. caesalpinifolia* seeds.

*M. caesalpinifolia* seeds from unripe fruits and dried at 15% of water content can be stored for 12 months at 10 °C, without loss of viability and vigor.

*M. caesalpinifolia* seeds from ripe fruits and not subjected to drying maintain viability and vigor for 12 months, when stored at temperatures of 10 °C and 0 °C.

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