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## PHYSIOLOGICAL QUALITY OF *Gliricidia sepium* (Jacq.) Steud. (LEGUMINOSAE - PAPILIONOIDEAE) SEEDS SUBJECTED TO DIFFERENT STORAGE CONDITIONS<sup>1</sup>

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**ABSTRACT** – Studies of seed physiological processes represent the starting point for the sustainable utilization of native or cultivated plant species from the *caatinga* biome for which germination studies are still scarce. In order to determine adequate methods for the conservation of *Gliricidia sepium* seeds, the physiological quality of propagules exposed to different conditions and times of storage were examined. Recently collected seeds of *G. sepium* were analyzed immediately, while the other sample was stored for three, six, nine, or twelve months in plastic or paper bags under refrigeration or at room temperature for further analysis. The germination rates of seeds from each storage regime were determined and these rates compared to their water content. It was observed that germination capacity was directly related to changes in seed humidity. Storing seeds in plastic bags yielded the best conservation, mainly when they were stored at low temperatures. However, even at temperatures near 25°C it was possible to prolong seed viability when their water content was controlled through the use of impermeable packing.

**Keywords:** Germination, *Gliricidia* e Physiological quality.

## QUALIDADE FISIOLÓGICA DE SEMENTES DE *Gliricidia sepium* (Jacq.) Steud. (LEGUMINOSAE – PAPILIONOIDEAE) SOB DIFERENTES CONDIÇÕES DE ARMAZENAMENTO

**RESUMO** – Estudos dos processos fisiológicos de sementes são efetivamente o ponto de partida para a utilização e exploração sustentável de espécies nativas ou cultivadas do bioma Caatinga, cujos trabalhos sobre germinação ainda são escassos. Visando contribuir para o estabelecimento de métodos de conservação de sementes de *Gliricidia sepium*, este trabalho foi realizado com o objetivo de avaliar a qualidade fisiológica das sementes, submetidas a diferentes condições e períodos de armazenamento. Sementes de *gliricidia* recém-coletadas foram imediatamente analisadas, sendo as demais armazenadas por três, seis, nove e 12 meses em sacos plásticos e de papel em geladeira e na temperatura ambiente, para análise posterior. Em cada ambiente e período de armazenamento testados foi analisada a taxa de germinação, relacionando-a com o teor de água das sementes. Observou-se que a capacidade germinativa de sementes de *gliricidia* está totalmente relacionada à tolerância da espécie ao ganho ou à perda de umidade das sementes. O armazenamento em saco plástico foi o que proporcionou melhores condições de conservação das sementes, principalmente quando acondicionadas em temperaturas baixas. No entanto, mesmo em temperaturas em torno de 25 °C é possível prolongar a viabilidade das sementes de *gliricidia*, quando se controla o seu teor de água inicial com a utilização de embalagens impermeáveis para o armazenamento.

**Palavras-chave:** Germinação, Teor de água, Qualidade fisiológica e *Gliricidia*.

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## 1. INTRODUCTION

*Gliricidia* [(*Gliricidia sepium* (Jacq.) Steud.) - (Leguminosae – Papilionoideae)] is a native species of Central and South America. It was introduced into northeastern Brazil in the 1980's and has since become the object of significant commercial and economic interest for the region, due to its multiple uses - which include its use as a forage species with high nutritive value for grazing animals and bees as well, and as a medicinal plant. Drought resistance is one of the most important characteristics of any plant that is to be cultivated in the *caatinga* region. *Gliricidia* stands out in this respect because of its ability to be easily propagated by seed and because of its rapid growth, high capacity for regeneration, resistance to drought, among other characteristics (DRUMOND and CARVALHO FILHO, 1999).

Studies of seed physiological processes, including germination studies, represent the starting point for the sustainable use of native or cultivated plant species from the *caatinga* region. The seeds of a majority of all cultivated species demonstrate orthodox characteristics, in which an increase in their water content during storage will result in a rapid loss of viability and a reduced germination percentage. The conservation potential of any seed depends directly on its physiological quality before the storage period. Desiccation tolerance is acquired progressively during seed development. Among orthodox seeds, desiccation tolerance occurs before the seeds go through any severe reduction in water content - but it is not yet possible to determine if this capacity is acquired beforehand, or if it is directly due to water loss (BEWLEY and BLACK, 1994).

Seed storage involves a series of procedures designed to preserve germination quality. However, good results depend as much on the nature of the seeds themselves as on the storage conditions (SANTANA, 2007). The packaging used in storing seeds plays an important role in the maintenance of their initial vigor. Additionally, environmental humidity and storage temperature can also influence the maintenance of seeds viability (WARD and POWELL, 1983).

Information about the water content of seeds allows one to choose the most adequate procedures, from harvesting to storage that will preserve their physical, physiological and sanitary qualities. This information may also aid in the identification of procedural problems

that might arise, as well as in the adoption of adequate responses (MARCOS FILHO et al., 1987). Seed conservation is very important, and adequate storage will diminish the rates of their irreversible deterioration (DELOUCHE et al., 1973; MELO et al., 1979; CABRAL et al., 2003).

Seed deterioration occurs at molecular, genetic, cellular, tissue, and population levels (MATTHEWS, 1985). It is a process that cannot be avoided, only controlled, and this is the principal objective of all storage protocols. Seed storage represents one of the most utilized strategies of *ex situ* species conservation as it preserves the genetic characteristics of the seeds until they are sown (CARNEIRO, 1985; NODARI et al., 1998).

The present evaluated the conservation of viability of *gliricidia* seeds using low-technology storage conditions that would be available to poor rural farmers.

## 2. MATERIAL AND METHODS

### 2.1. Obtaining and storing seeds

Mature seeds were collected in 2006 from *Gliricidia sepium* trees on Fazenda Morrinhos Farm located in the municipality of Queimadas, Bahia State, Brazil (39° 40' W x 11° 12' S), which had been declared a 'Private Nature Reserve' under the Brazilian law. The seed were collected from newly opening pods and dried in the open air in the semiarid *caatinga* region of northeastern Brazil. Samples of the recently collected seeds were cleaned and analyzed for their water content and germination rate.

The remaining seeds were placed into either paper or plastic bags and stored at room temperature (25°C ± 3) or under refrigeration (8°C ± 2) for three, six, nine, or twelve months in the Germination Laboratory of the Horto Florestal Experimental Unit-UEFS – Feira de Santana, Bahia State, Brazil, for posterior evaluation.

### 2.2. Germination tests

After each experimental storage period, four sub-samples of 25 seeds were sown in Petri dishes containing two layers of germination paper moistened with a quantity of distilled water corresponding to 2.5 times the weight of the paper substrate. The seeds were maintained in germination chambers at 25°C with a

photoperiod of 14 h for ten days. The seeds were examined daily and were considered to have germinated when the radicle protruded at least 2 mm from the seed coat.

### 2.3. Water content

The seed water content was assessed after all storage periods by drying ovens at 105°C/24h (BRASIL, 1992), in four replicates of 25 seeds for each package type and storage temperature.

### 2.4. Statistical analyses

Data related to water content and germination was expressed as percentages, with standard errors. The total germination, average germination time, average germination rate, and germination rate data were transformed by the function  $(x + 0.5)^{0.5}$  and submitted to variance analysis using the Sisvar 4.3 software program (FERREIRA, 2000). Differences between the averages were compared using the Tukey test at a 5% probability level.

## 3. RESULTS

While germination percentages changed in response to storage time and the packaging material used, the average germination time remained essentially constant (Table 1) and independent of these two variables.

Based on percentage germination, plastic bags provided the best conditions for seeds stored under both cold and room temperatures. However, seeds stored at room temperature in plastic or paper bags demonstrated a significant decrease in germination potential starting at nine months (Table 2).

**Table 1** – Variance analysis of the germination percentage (G), average germination time (Tm), average germination speed (Vm), and germination speed index (IVG) of stored *Gliricidia sepium* seeds.

**Tabela 1** – Análise de variância da porcentagem de germinação (G), tempo médio de germinação (Tm), velocidade média de germinação (Vm) e índice de velocidade de germinação (IVG) de sementes de *Gliricidia sepium* armazenadas.

Variable	G %	Tm, days	Vm, days <sup>-1</sup>	IVG
Storage time	74.01*	1.143 <sup>ns</sup>	41.336*	125.77*
Environments	132.97*	0.643 <sup>ns</sup>	75.075*	216.67*
Time* Environ.	52.46*	0.337 <sup>ns</sup>	4.569*	36.37*
CV%	7.85	24.26	3.12	5.67

ns = (not significant); \* = significant to a 5% level.

Changes in water content of *Gliricidia sepium* seeds over time during storage depended on the type of packaging used. The initial seed water content was 6.4%, and this percentage was maintained for the entire period in which the seeds were stored in the refrigerator in plastic bags. High germination rates were observed (94%) even after 12 months of storage under these conditions (Figure 1A).

The germination capacity of any species is very dependent on its tolerance to gaining or losing seed humidity. Figure 1B shows that germination decreased when the water content of *G. sepium* seeds attained lower than initial levels. Seeds stored in paper bags at room temperature demonstrated gains in water content after six months of storage (Figure 1D), and a concomitant loss of viability. According to Ribeiro (2008), this loss of viability points to alterations in seed carbohydrate reserves resulting from increasing water content.

Under low temperature conditions (refrigeration), gliricidia seeds stored in impermeable (plastic) bags (Figure 1A) and permeable (paper) bags (Figure 1B) demonstrated high germination rates and high average germination speed up to one year after initial storage (Table 2). Seeds stored at room temperature demonstrated a decrease in their average germination speed during the year, independent of the packaging used, as well as a decrease in the germination percentage (Table 2). Significant reductions in all of these parameters were observed starting in the sixth month of storage.

Significant differences in the seeds germination rate index were observed when the seeds were stored at room temperature in paper bags - but only after six months of storage (Table 1). This reduction in germination rate index can be attributed to a loss of vigor in seeds kept under uncontrolled environmental conditions, in comparison to storage in plastic bags at cold temperatures.

These results suggest that storing gliricidia seeds at low temperatures and in impermeable packages is much more suitable than holding them at room temperature and in permeable packaging, with seeds subjected to the latter conditions demonstrating much higher losses of viability.

**Table 2** – Germination percentage (G), average time (Tm), average speed (Vm), and germination speed index (IVG) of recently collected *Gliricidia sepium* seeds (RC) stored in a refrigerator in plastic bags (RPB) or in paper bags (RPPB); or stored at room temperature in plastic bags (TRPB) or in paper bags (TRPPB).

**Tabela 2** – Porcentagem de germinação (G), tempo médio (Tm), velocidade média (Vm) e índice de velocidade de germinação (IVG) de sementes de *Gliricidia sepium* recém-coletadas (RC) e armazenadas em geladeira em saco plástico (GSPL) e saco de papel (GSPP) e em temperatura ambiente em saco plástico (ASPL) e em saco de papel (ASPP).

Environments	Months					
	0	3	6	9	12	Average
G (%)						
RC	100aA	---	---	---	---	100A
RPB	---	96.00aA	98.00aA	89.00aAB	94.00aA	94.25AB
RPPB	---	99.00aA	98.00aA	93.00aA	91.00aA	95.25AB
TRPB	---	98.00aA	96.00aA	78.00bB	87.00abA	89.75B
TRPPB	---	96.00aA	86.00aA	3.00bC	1.33bB	49.60C
Average	100a	97.25a	94.50a	65.75b	72.80b	
Tm, (days)						
RC	1.490aA	---	---	---	---	1.490A
RPB	---	2.094aA	2.294aA	2.260aA	2.167aA	2.204A
RPPB	---	2.121aA	2.037aA	2.663aA	1.964aA	2.196A
TRPB	---	2.104aA	2.463aA	2.837aA	2.587aA	2.497A
TRPPB	---	2.127aA	3.570aA	4.500aA	2.000aA	3.112A
Average	1.490a	2.112a	2.590a	3.065a	2.191a	
Vm, (days <sup>-1</sup> )						
RC	0.672aA	---	---	---	---	0.672A
RPB	---	0.478aA	0.437aA	0.443aA	0.478aAB	0.459B
RPPB	---	0.472abA	0.508aA	0.378bA	0.513aA	0.467B
TRPB	---	0.477aA	0.408abA	0.361bA	0.390abB	0.409B
TRPPB	---	0.471aA	0.281bB	0.056cB	0.055cC	0.227C
Average	0.672a	0.475b	0.409bc	0.309d	0.380c	
IVG						
RC	19.71aA	---	---	---	---	19.71A
RPB	---	11.84abA	13.120aA	10.48bA	12.62abA	12.01B
RPPB	---	12.09abA	12.910aA	10.09bA	14.47aA	12.39B
TRPB	---	12.13aA	11.02aAB	7.83bB	10.01abB	10.25C
TRPPB	---	11.90aA	8.80bB	0.087cC	0.055cC	5.554D
Average	19.71a	11.99b	11.46b	9.904c	7.124d	

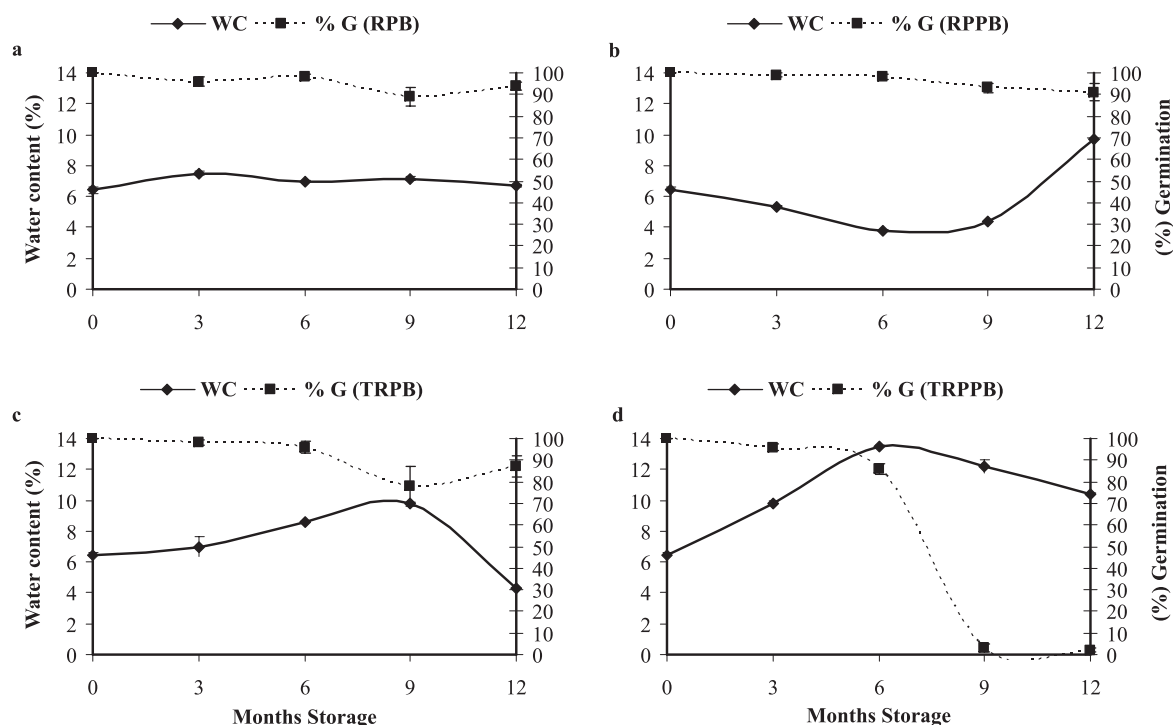
Averages followed by the same lowercase letter on the same line, or by the same uppercase letter in the same column, are not significantly different from each other by the Tukey test at a 5% probability level.

#### 4. DISCUSSION

Variations in water content are of fundamental importance in seed storage, especially in terms of carbohydrate metabolism, and seed sugar levels are generally affected by storage temperatures. The quantity of sucrose found in seeds of the legume *Caesalpinia echinata* Lam. is related to its desiccation tolerance, and high sucrose levels have been reported in viable seeds stored for 18 months at 7°C (BARBEDO et al., 2002). Ribeiro (2008) observed that an increase in the water content of *Gliricidia sepium* seeds initiated a series of reactions that contributed to their deterioration by diminishing their sugar content.

The accumulation of soluble carbohydrates in seeds seems to be involved in desiccation tolerance and the longevity observed in this species.

Permeable wrapping that permit water vapor exchange between the seeds and the atmosphere are more adequate for use in dry environments and for short storage periods (FERREIRA and BORGHETTI, 2004). Freitas et al. (1992) reported that seeds maintained in highly permeable wrappers in humid environments are subject to relatively rapid deterioration. Cisneiros et al. (2003) observed that seeds of *Psidium guineense* stored in paper sacks increased their water content even when stored in a



**Figure 1** – Water content and germination percentage of the seeds of *G. sepium* stored in a refrigerator [in plastic bags (RPB) – a or in paper bags (RPPB) – b], or stored at room temperature [in plastic bags (TRPB) – c or in paper bags (TRPPB) – d], during 0, 3, 6, 9, or 12 months. Average of four trials  $\pm$  ASE.

**Figura 1** – Teor de água e porcentagem de germinação de sementes de *G. sepium* submetidas ao armazenamento em geladeira [saco plástico (GSPL)- a e papel (GSPP)- b] e no ambiente natural [sacos plástico (ASPL)- c e de papel (ASPP)- d] durante 0, 3 6 9 e 12 meses. Média de quatro repetições  $\pm$  EPM.

freezer, and their ability to germinate became greatly reduced over time. Maluf & Pisciotano-Ereio (2005) reported that the best condition for storing and conserving seeds of *Campomanesia phaea* was in plastic bags under controlled cold conditions. Matos et al. (2008) observed that seeds of *Apeiba tibourbou* stored in plastic bags in cold chambers demonstrated constant germination percentages over different periods of time. Plastic and glass containers (CISNEIROS et al., 2003) act as barriers to gas exchange between the seeds and their external environment, thus reducing their metabolism and retarding their deterioration; these beneficial effects become more accentuated at lower storage temperatures.

The present work established that the physiological quality of *gliricidia* seeds was affected by the permeability of the packing material used during storage. It is interesting to note, however, that these results are

different from those obtained with other species from the semi-arid region, such as *Anadenanthera macrocarpa*, *Pseudobombax simplicifolium*, *Myracrodruon urundeuva* (SOUZA et al., 1980), and *Tabebuia impetiginosa* (CABRAL et al., 2003) when stored at low temperatures in either cloth or polyethylene bags under low relative humidity. According to Carvalho and Nakagawa (2000), the type of packing used for seed storage determines their preservation, viability, and vigor, and will depend on the particular environmental conditions under which the seeds will be stored. Cold and dry environments are more favorable for storing orthodox seeds (VILLELA and PERES, 2004).

Germination rate index is one of the most traditional measures of seed vigor (VIEIRA and CARVALHO, 1994), and seed lots with similar germination percentages frequently demonstrate differences in germination rate



index - indicating differences in physiological vigor (KRZYZANOWSKI et al., 1999). The changes in germination speed rate observed here in gliricidia seeds stored at room temperature indicated decreasing overall physiological vigor. According to Drumond and Carvalho Filho (1999), gliricidia seeds lose their viability or enter into secondary dormancy after 12 months in storage. Successful conservation of gliricidia seeds is intimately related to their initial water content and the stability of that water content during storage. High viability (that is, a greater degree of tolerance to storage) was maintained under low temperature conditions.

The results obtained in the present study are promising in terms of the conservation of gliricidia seeds, for they indicate that the seeds can be conserved with high viability for reasonably long periods of time (up to 12 months), and even at temperatures near 25°C, if the initial low water content of the seeds is maintained through the use of impermeable storage containers.

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