

## Substrates for germination and physiological quality of storage seeds of *Parapiptadenia rigida* (Benth.) Brenan<sup>1</sup>

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**ABSTRACT** – “Angico-vermelho” [*Parapiptadenia rigida* (Benth.) Brenan] is an arboreal forestal species native from Brazil used for rural constructions, firewood, charcoal, and forest restoration programs; however it is little studied in regard to seed technology. Thus, the objective of this study was assessing different substrates for conducting tests of germination and physiological quality of seeds stored under different environments and packings for 420 days. Firstly, the most suitable substrate for the germination test was identified and subsequently the seeds were stored into three different types of packings (paper, plastic and glass) and three different environmental conditions (cold chamber, refrigerator and laboratory). Seed samples from each storage condition were removed at every 60 days for evaluating moisture content, germination and vigor (electrical conductivity test). A completely randomized experimental design was used with treatments arranged into a split-plot design for storage periods. It was found that the germination test can be conducted using between the sand as substrate, with the first count of emergence of seedlings performed at the fourth day and the final counting at 10 days after seeding. That seeds can be stored preferably in refrigerator into paper packings, although packings of plastic and/or glass are also suitable.

Index terms: angico-vermelho, seed technology, seed conservation, packings.

## Substratos para germinação e qualidade fisiológica de sementes de *Parapiptadenia rigida* (Benth.) Brenan durante o armazenamento

**RESUMO** – O angico-vermelho [*Parapiptadenia rigida* (Benth.) Brenan] é uma espécie arbórea nativa do Brasil, utilizada para construções rurais, lenha e carvão e também em programas de restauração florestal, porém pouco estudada quanto à tecnologia de sementes. Assim, o objetivo deste estudo foi avaliar diferentes substratos para condução do teste de germinação e a qualidade fisiológica das sementes armazenadas por 420 dias, em diferentes embalagens e ambientes. Primeiramente foi identificado o substrato mais adequado para condução do teste de germinação, e após procedeu-se o acondicionamento das sementes em três tipos de embalagens (papel, plástico e vidro) e o armazenamento em três ambientes (câmara fria, geladeira e laboratório). Amostras de sementes foram retiradas a cada 60 dias, e em cada condição de armazenamento, para avaliação do grau de umidade, germinação e vigor (teste de condutividade elétrica). Foi utilizado o delineamento experimental inteiramente casualizado, com tratamentos arranjados em esquema de parcelas subdivididas para períodos de armazenamento. Constatou-se que o teste de germinação pode ser conduzido na forma entre areia, com a primeira contagem realizada aos quatro e última aos 10 dias após a sementeira. As sementes podem ser armazenadas em geladeira, acondicionadas, preferencialmente, em embalagem de papel, porém embalagens de plástico e vidro também são adequadas.

Termos para indexação: angico-vermelho, tecnologia de sementes, conservação de sementes, embalagens.

### Introduction

The growing demand for seeds and seedlings of native forestal species for environmental and economic purposes has increased the studies about silviculture, chiefly aiming at developing new strategies for seed conservation in long term.

The storage of seeds is not needed when sowing is performed soon after harvest and processing, leading seeds to immediate germination; however, such procedure is rarely performed (Schmidt, 2000), and the seed availability is important to meet demand on seedling production along year. In addition, seasonality and spatial and temporal

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irregularities of the seed production are factors that interfere in marketing and conservation of forestal species, once seed production may be plentiful in a given year and deficient in others, making storage a need for ensuring annual supply of propagation material (Scremin-Dias et al., 2006).

The adequate storage of seeds requires knowledge of initial physiological quality of seeds assessed by germination test, which provides estimates of number of seeds that will produce normal seedlings (Karrfalt, 2008), determining their viability and germination capacity. However, seeds of each plant species present discriminated performance when subjected to different substrates what consists one of basic components of the test (Mondo et al., 2008). In choosing substrate, it is needed to be considered size and sensitivity of seeds to light, besides their capacity of water retention, easiness of handling, and assessment of results provided by the substrate (Brasil, 2009).

The tree *Parapiptadenia rigida* (Benth.) Brenan (Fabaceae), popularly known in Brazil as “angico-vermelho” is a species of relevant interest on environmental recovering and reforestation, notwithstanding, the shortage of studies on its silviculture aspects impairs its perpetuation and cultivation (Farias et al., 2005). In addition, it is one the arboreal species better known by population of Southern Region of Brazil, chiefly by being used for rural constructions, firewood (Backes and Irgang, 2009), and fence poles, besides other external uses, due to the natural resistance of the wood.

Concerning “angico-vermelho” seeds storage, the scarce studies available indicate that variable conditions of temperature and relative humidity may be prejudicial by causing deterioration and consequently reduction of their viability (Fowler and Carpanezzi, 1998).

Therefore, control of storage environment is essential to increase viability period of seeds, mainly by metabolism reduction and consequently conservation of the nutritional reserves and embryo integrity; besides preventing infection by microorganisms (Bonner, 2008). The storage conditions can also be handled by the type of packaging used, which may be partial or completely hermetic, in a manner to delay atmospheric moisture reabsorption after seed drying process (Oliveira, 2007).

Packagings can be sorted in following categories: porous or permeable, which do not prevent the exchanges between the seeds and the environment, as screens of cotton and cardboard; semi-permeable, i.e., resistant to circulation of water vapor and of exterior air, as bags of multi-layered Kraft paper type and polyethylene; and hermetic or impermeable, which do not allow for water vapor exchanges, as metal and glass containers (Marcos-Filho, 2005).

In face of the foregoing and aiming at defining methods

for elaborating rules for seed testing of forestal species, this study had the objective of evaluating germination of *Parapiptadenia rigida* seeds on different substrates for germination test conduction; as well to assess physiological quality of seed stored during 420 days under different packagings and environments.

## Material and Methods

*Fruit harvesting and seed processing:* fruit harvesting was performed before natural dehiscence in 28 trees of “angico-vermelho”, which are located in a forestal segment of a stationary deciduous forest in municipality of Santa Maria, state of Rio Grande do Sul, Brazil, in September 2009. Soon after harvesting, collected material was transported to the Forestry Nursery of the Department of Forestal Sciences of the Federal University of Santa Maria for the manual opening of pods and extraction and processing of seeds.

*Initial physiological quality:* after processing, seeds were manually homogenized forming a single lot. These seeds were then packaged into semi-permeable plastic bags (90  $\mu$  thick) and stored in cold chamber, at 8 °C; and 86% RH, during 15 days. After such period, a sample was removed for characterize seed lot, by assessing 1,000 seeds weight (WTS) and moisture content (MC) through oven method, at 105 °C, for 24 h (Brasil, 2009); however, four replications of 25 seeds each were used for determining moisture content.

Additionally, electrical conductivity (EC) of seeds was determined by the method of mass, using four replications of 25 seeds each, which had been previously weighed with a 0.001 g precision electronic balance and then submerged in 75 mL distilled water. The containers with water were involved with aluminum foil and kept in a Mangelsdorf type germination chamber, at constant temperature of 25±2 °C, during 24 h (Vieira, 1994). Afterwards, readings of leachates of each sample was performed with aid of a condutivimeter (brand Quimis®, Q405M); and results were expressed in mS.cm<sup>-1</sup>.g<sup>-1</sup>.

*Germination test:* firstly, an experiment was performed aiming at identifying the most adequate substrate for conducting such test. For this, seeds were disinfested by immersion into a 2% sodium hypochlorite solution during 5 min., followed by three rinses in distilled water. The following treatments (substrates) were assessed: I – on top of blotter paper (OBP) – using two sheets of Gemitest® paper, moistened with distilled water, in a volume equivalent to 2.5 times the mass of such dry substrate, on which seeds were evenly distributed into plastic germination boxes (11 cm x 11 cm x 3.5 cm) (gerbox); II – on top of sand (OS) – for this test 200 g of fine sand, sieved through 0.84 mm mesh sieve,

was placed at bottom of the gerbox where seeds were evenly distributed; and between the sand (BS), where after the first procedure previously described, the seeds were covered with 100 g of this same substrate and then moistened with 45 mL of distilled water; III – on top of vermiculite (OV) – 30 g of such substrate with fine granulometry, the same way were placed at bottom of gerbox where the seeds were placed; and between vermiculite substrate (BV), where after the first procedure, the seeds were covered with 10 g of vermiculite and then moistened with 83 mL of distilled water; and IV – paper roll (PR) – for this test three sheets of Germitest® paper were used, where the seeds were evenly distributed, covered with another sheet of the same paper, moistened with distilled water, in a volume equivalent to 2.5 times the mass of such dry substrate, made into rolls, and the set (paper + seeds) were then placed into plastic bags.

All the substrates were sterilized by autoclaving (120 °C during 60 min.) and were used four replications of 25 seeds each, for each treatment. The substrates sand and vermiculite were moistened until 60% water retention capacity. Containers with seeds were kept into Mangelsdorf type germination chamber, at temperature of 25±2 °C and 8/16 h photoperiod (L/D). The counting of germinated seeds was daily performed, starting from the fourth day after seeding (first count) and ending at the thirteenth day, when majority of seeds had already germinated.

The percentage of normal seedlings was the assessment criterion used, i.e., all seedling presenting normal structures (primary root, hypocotyl and cotyledons) were considered, besides the percentage of dead seeds, according to definition of Rules for Seed Testing (Brasil, 2009); and the germination speed index (GSI) was also complementarily computed through Maguire (1962) equation.

*Seed storage:* for this assessment were used 55 g of seeds (approximately 2,000 seeds), which were packed into three types of packaging: dark Kraft paper bags; transparent polypropylene bags (90 µ thick), sealed in a heat sealing machine; and glass flasks (600 mL capacity) involved with aluminum foil. After packed, the seeds were stored during a period 420 days in cold chamber (8 °C; 86% RH), in refrigerator (3 °C; 48% RH); and under laboratory environment (24±3 °C; 83±10% RH), controlling only the temperature. At experiment beginning, as well as after each 60 storage days, moisture content and germination and vigor of seeds were also determined by electrical conductivity test.

The tests were installed following the same procedures previously described, however, for tests of moisture content and electrical conductivity, only two replications of 25 seeds each were used; and for germination test it was used the

substrate between of fine sand, which was considered the most adequate in the preliminary test, was performed with four replications of 25 seeds each, for each treatment.

*Experimental design and analyses of data:* a completely randomized experimental design, with treatments arranged into a split plot scheme within time was adopted. The main plot was constituted by a factorial arrangement 3 x 3 (3 storage environments x 3 packagings for storing the seeds) and the subplots were constituted by the 7 storage periods. Data were subjected to normality test of Shapiro-Wilk and to homogeneity test of Bartlett, and when these conditions were not met, the data transformation was performed for: MC, which was transformed by  $\arcsin(x/100)^{0.5}$ ; EC by  $1/x$ ; germination percentage by  $\arcsin(x/100)^{0.5}$ ; and GSI by  $(x + 0,5)^{0.5}$ . Unfolding of interactions, when needed, was performed by comparing the means by the tests of Scott-Knott and/or polynomial regression, at 5% probability. In case of significant effects of quadratic equations, critic point (CP) was determined. For all statistical analyses the software SISVAR (Ferreira, 2008) was used.

## Results and Discussion

*Initial physiological quality:* the seed lot used in this study presented initial values for weight of 1,000 seeds equal to 27.7 g what represents 36,062 seeds.kg<sup>-1</sup>, moisture content of 16.9%, and electric conductivity of 6.58 mS.cm<sup>-1</sup>.g<sup>-1</sup>. Fowler and Carpanezzi (1998) have verified that for the same species results were similar to those found within this study (MC 15.5%; WTS 26.6 g; e 37.565 seeds.kg<sup>-1</sup>). The variations on moisture content and weight of seeds may be attributed to several factors, which range from region and period of harvest until the processing method and environmental conditions during pre-drying process.

According to results achieved in the germination test performed in the different substrates it was observed that for the first count, performed in the fourth day after test beginning, the substrate BS has provided the highest germination percentage (65%), and that the maximum value for this parameter has occurred at the tenth day (Table 1; Figure 1). Still for the first count, the paper roll test has also provided statistically significant increase on germination rate (37%), while the remaining treatments have provided much reduced germination percentages; although not statistically differing between each other.

The assessment of first count of germination is based in the principle according to what the sample of seeds that presents high percentages of normal seedlings is the most vigorous, what is directly correlated to the germination speed

(Nakagawa, 1994). However, on considering that seeds are originated from the same seed lot, the result indicates that by using substrate BS it is possible concluding the test more rapidly (first counting at the fourth day and last counting at the tenth day), and thus prevent or reduce incidence of pathogens and commitment of results.

Table 1. Mean values of first count of germination (FC), normal (NS) and abnormal (AS) seedlings, dead seeds (DS), and germination speed index (GSI) of seeds of *Parapiptadenia rigida* obtained in the germination test performed in six different types of substrates.

Substrates	FC	NS	AS	DS	GSI
	%				
OBP	6 c*	96 <sup>ns</sup>	0 <sup>ns</sup>	4 <sup>ns</sup>	3.69 c
OV	1 c	92	1	7	3.22 c
BV	13 c	97	1	2	3.94 c
OS	0 c	98	0	2	3.64 c
BS	65 a	95	0	5	5.38 a
PR	37 b	93	1	6	4.58 b
CV(%)	56.01	4.34	282.84	94.01	8.22

\*Means followed by the same letter in the column do not statistically differ between each other by the Scott-Knott test, at 5% probability; <sup>ns</sup> = non-significant by the F test; OBP = on top of blotter paper; OV = on top of vermiculite; BV = between two layers of vermiculite; OS = on top of sieved fine sand; BS = between two layers of sieved fine sand; and PR = into paper rolls.

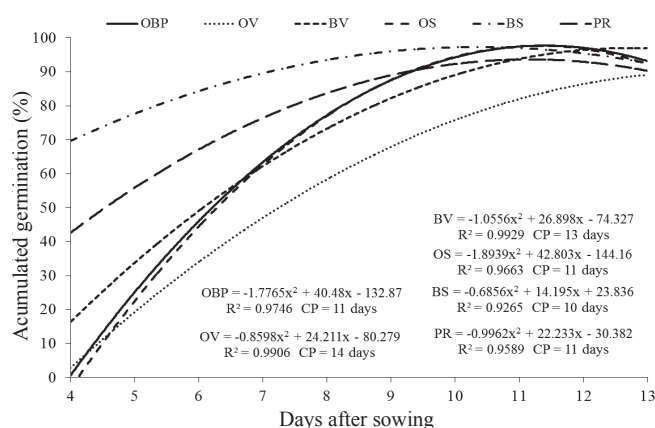


Figure 1. Percentage of accumulated germination of seeds of *Parapiptadenia rigida* assessed on six different types of substrates: OBP = on top of blotter paper; OV = on top of vermiculite; BV = between two layer of vermiculite; OS = on top of sieved fine sand; BS = between two layers of sieved fine sand; and PR = into paper roll.

In the substrate OS, the germination has been nil at the fourth day, not differing statistically from substrates OBP, OV, and BV; and in the substrate OV, however, the critical point

has been higher than the time assessed (14 days). Despite this, there has been no statistically significant difference among treatments for percentage of normal seedlings at ending of test; thus allowing for observing the high germination percentage (> 90%) in all substrates assessed (Table 1). The GSI confirmed that substrate BS, has provided the highest performance (5.38), and followed by the OBP (4.58). Probably such result was a consequence of higher contact area of substrate with the seeds, this way highlighting the substrates BS and RP, once that according to Oliveira-Jr. and Delistioianov (1996) when the contact area of substrate with seeds is small, the water absorption speed may be smaller than rate of loss by evaporation.

When values obtained in substrates OV, BV, and PR are observed, it is possible verifying that it has occurred a low percentage of abnormal seedling. Despite the apparently higher values on dead seeds rate in the substrates OV (7%) and PR (6%) that did not differ statistically from other substrates (Table 1). The main abnormalities found were the oxidation and/or lack of radicle, absence of hypocotyl and badly formation of the cotyledons. Such abnormalities may be derived from the substrate itself, for not providing adequate support to development of seedlings, or even in consequence of the dryness. In the substrate PR, it was observed the smallest seedling length as compared to remaining substrates, once in many cases; such substrate has caused breaks of radicle and cotyledons, probably as consequence of configuration of the roll that presses the tender seedling tissues in “folds”. For Figliolia et al. (1993), the vertical position of seedling within paper roll can cause excessive dehydration and loss of moisture in its center.

Mondo et al. (2008) have concluded that for the species *Parapiptadenia rigida* the germination test should be conducted at temperature of 25 °C using substrate BV, in presence or absence of light. However, for this same species Ramos et al. (1995) recommend the use of substrates of sand, vermiculite, blotter paper, and paper towel, at temperatures of 20 °C and 25 °C.

Wielewiczki et al. (2006) have proposed that, for seeds of “angico-vermelho”, the standard germination test should have duration of nine days, using the substrate PR; and also have proposed a minimum germination standard of 87% and moisture content of seeds of 17.5% for this species.

Although in the literature there are not reports on use of the substrate BV in germination test of “angico-vermelho” seeds; in this study this test was promising, allowing for the ending of test assessments in a shorter period, which was approximate to the period proposed by Wielewiczki et al. (2006). However, its main disadvantage is the difficulty during preparation, once in addition to being heavier than the other tested substrates, needs more care with the seeding



deepness, for not impairing germination.

*Physiological quality of the seeds during storage:* according to ANOVA the triple interaction between environment and packaging x storage time was statistically significant for seed moisture content. Before being packed, the seeds of *Parapiptadenia rigida* were presenting moisture content of 16.9%. In cold chamber the seeds packed into packagings of paper, plastic, or glass have had a positive quadratic behavior; and in 265, 253, and 235 days, respectively, there has been the maximum increase on moisture content (Figure 2A).

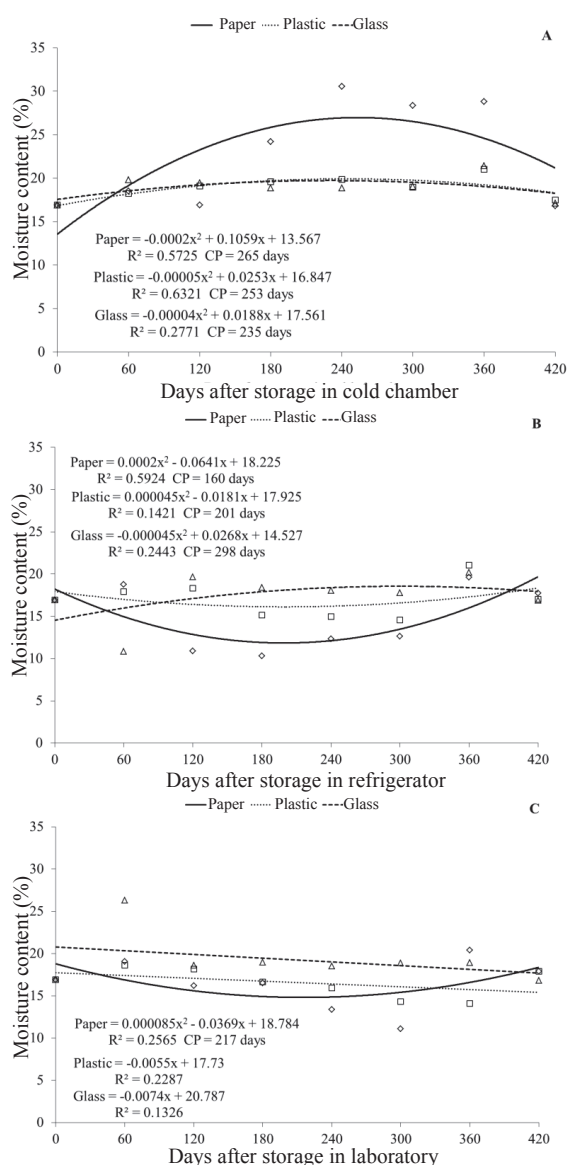


Figure 2. Percentage moisture content of seeds of *Parapiptadenia rigida* packaged in three different types of packagings and stored under three different environmental conditions: cold chamber (A); refrigerator (B); and laboratory (C) during 420 days.

Generally, it was verified that the MC was higher for seed stored in cold chamber (20.2%), surpassing values observed for seeds stored into refrigerator (16.45%) and in laboratory (17.4%); and the glass packaging allowed for higher MC (18.5%), if compared to packagings of paper (18%) and plastic (17.5%).

The packaging in impermeable packs into cold chamber may increase the MC of the seeds during storage for being an environment of high relative humidity. For occurrence of low variation in the MC of seeds stored in this type of environment the use of impermeable packagings, such as glass packagings, are most adequate (Villela and Peres, 2004); thus confirming results here in obtained.

At 60 days of storage under refrigerator environment there was a decrease in the MC of the seeds, if compared to initial value obtained for packagings in bags of paper and plastic. In these packagings, the critical points examined were 160 and 201 days, on which the lowest MC values were observed, respectively (Figure 2B). However, in the seeds packed in glass packaging, an inverse behavior was observed, i.e., the maximum MC values have occurred at 298 days. At end of storage, the seeds stored into refrigerator were presenting values of 14.9%, 17.0%, and 17.3%, respectively, for seeds packaged into bags of paper or plastic or into glass containers.

The seeds stored in refrigerator in impermeable packagings (glass) have presented increase in the MC, inside which it was expected that values were kept constant, as it was verified under conditions of cold chamber. However, for Bonner (1978) and Silva et al. (2011a), even when seeds are stored into impermeable packagings, the gases exchanges can occur as consequence of high rates of respiration, once restriction to entrance and exit of gases from the packages may increase deterioration rate of the seeds, and thus cause reduction on viability of seed lot.

Through the respiration high rate, the seed releases its composition water to environment, this manner increasing relative humidity within packaging. With this, seeds will adjust themselves to the new RH and consequently will acquire more elevated MC than initial value (Carvalho and Nakagawa, 2000). Such fact also favors development of fungi and bacteria, causing losses on germination capacity as well as on vigor of the seed lot (Oliveira, 2007). The water formation may also be consequence of union of hydrogen and oxygen atoms released by decomposition of organic compounds (Marcos-Filho, 2005).

After 420 days under laboratory conditions, it was also found reduction of the MC of the seeds stored in the three different packagings studied, on which were found values of 17.9%, 17.8%, and 16.8%, respectively, in the seeds stored in packs of paper, plastic, and glass (Figure 2C). However, independently of environment the seeds packaged in the

glass container have presented higher value for MC (18.5%), although not presenting differences statistically significant between the remaining packagings.

The vigor of seeds of *Parapiptadenia rigida*, assessed by electrical conductivity test before packaging, was  $6.58 \mu\text{S}\cdot\text{cm}^{-1}\cdot\text{g}^{-1}$ . By the ANOVA only the interaction between environment and storage time was detected. In the last periods of storage (360 and 420 days) the cold chamber environment has provided higher EC in the seeds, although not differing from laboratory environmental conditions, on which there had been oscillation along time; besides, at 120 days the values for EC have increased expressively.

Along storage time, it was observed that there was an increasing linear behavior of EC for the seeds stored in cold chamber and quadratic for seed stored under laboratory conditions, with the maximum value of EC reached in the 274<sup>th</sup> day (Figure 3). It was also verified that, under the refrigerator environment, it have not occurred changes statistically significant among values, which presented a linear behavior, represented by a straight line along time.

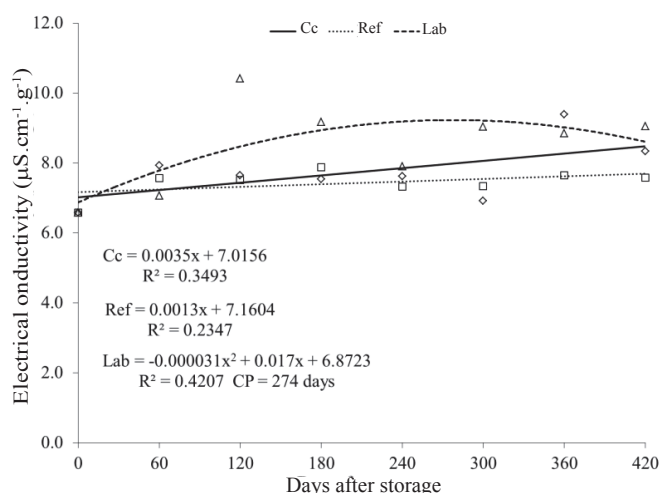


Figure 3. Electrical conductivity measured in leachates of seeds of *Parapiptadenia rigida* after storage in three different environmental conditions during 420 days, obtained with aid of a conductivimeter after the seeds were immersed in 75 mL distilled water and incubated at  $24 \pm 2$  °C, during 24 h.

Deteriorated or dead seeds release higher amounts of electrolytes than the seeds possessing high vigor, in a manner that the largest release of these compounds cause increase in water electrical conductivity (Karrfalt, 2008). Likewise, Copeland and McDonald (1995) have described that low vigor seeds have membranes with smaller integrity, as consequence of their deterioration or to mechanical damages occurring during storage.

Through EC test, it was possible observing that there were not statistically significant difference among packagings, which also have not presented interactions with environment or with storage time. Only the storage environment factor has influenced EC along time; and the best result has been achieved in the refrigerator environmental condition.

Silva et al. (2011a) have found that the EC test was not efficient in assessing physiological quality of seeds of *Psidium cattleianum* Sabine, once a distinct behavior among treatment were found in the germination test. The EC test was not also sensitive in detecting variations on vigor of seeds of *Tabebuia roseo-alba* (Ridl.) Sand. and seed of *Tabebuia impetiginosa* (Mart. ex DC.) Standl. (Borba-Filho and Perez, 2009). In this study it was detected that, due to the fact of EC test presents constant values along storage time as well as low conductivity, it is possible to identify the best storage environment after 360 days (Table 2; Figure 3) allowing for its description as an inefficient method for detecting variations of seed vigor along storage time. This manner, other factors such as: volume of water; amount of seeds, and period of seed imbibition should be investigated, aiming at suitability of EC test for obtaining more conclusive results.

On evaluating germination percentage and germination speed index were found interactions between the following factors: environment x packing types, and environment x storage time. By data presented on Table 3, the combination of the factors, environment of refrigerator x packaging into Kraft paper bags, have induced the highest germination percentage (93%) and a GSI of 5.04. In this same environment, there was no statistically significant difference for the characteristics previously described for seeds packed into plastic packagings or into glass containers. The laboratory environmental condition was not adequate for keeping physiological quality of seeds, due to low values obtained in germination test.

On analysing the germination percentage and the GSI, after each storage period, it was found that the environment inside refrigerator has been more adequate for conservation of seeds, once such environment has allowed obtaining highest values for these two characteristics; while under the laboratory environmental conditions the viability loss of seeds was total in 180 days storage period (Figures 4A and 4B). This fact may be related to the wide oscillation of the relative humidity (between 57 and 92%) and to high mean temperature ( $\pm 24$  °C) that have occurred under the laboratory environmental conditions; once according to Marcos-Filho (2005), the increase on speed of chemical reactions, as well as on acceleration of respiratory activity of the seeds cause reduction on their physiological quality.

Table 2. Electrical conductivity ( $\mu\text{S}\cdot\text{cm}^{-1}\cdot\text{g}^{-1}$ ) measured in leachates of seeds of *Parapiptadenia rigida* after storage in three different environmental conditions during 420 days, obtained with aid of a conductivimeter after the seeds were immersed in 75 mL distilled water and incubated at  $24\pm 2$  °C, during 24 h.

Environmental conditions	Days after storage							
	0	60	120	180	240	300	360	420
Cc	6.58 a*	7.93 a*	7.65 a*	7.53 a*	7.61 a*	6.92 a*	9.39 b*	8.33 b*
Ref	6.58 a	7.57 a	7.50 a	7.87 a	7.32 a	7.34 a	7.64 a	7.58 a
Lab	6.58 a	7.06 a	10.41 b	9.17 b	7.90 a	9.04 b	8.84 b	9.04 b

\*Means followed by the same letter in the columns do not statistically differ between each other by Scott-Knott test, at 5% probability; Cc = cold chamber; Ref = refrigerator; and Lab = laboratory.

Table 3. Germination percentage and germination speed index (GSI) of seeds of *Parapiptadenia rigida* stored in three different types of packagings and three different environmental conditions during 420 days.

Environment/packaging	Germination (%)			GSI		
	Paper	Plastic	Glass	Paper	Plastic	Glass
Cc	69.6 Cb*	75.46 Bb*	80.5 Ab*	3.37 Cb*	3.67 Bb*	4.01 Ab*
Ref	93.4 Aa	89.7 Ba	89.4 Ba	5.04 Aa	4.66 Ba	4.67 Ba
Lab	17.4 Ac	13.9 Bc	13.6 Bc	0.78 Ac	0.62 Ac	0.69 Ac
C.V. (%)		14.68			6.62	

\*Means followed by the same upper case letter in the lines and lower case letter in the columns do not statistically differ between each other by the Scott-Knott test, at 5% de probability; Cc = cold chamber; Ref = refrigerator; and Lab = laboratory.

Several factors influence seed deterioration as relative humidity of environment and the temperature, which are considered the most important ones. The first factor directly influences seed moisture content, when the hygroscopic balance with surrounding atmosphere occurs; and the second factor determines the amount of water that seeds can contain, as well as the speed of decaying reactions when temperature increases (McDonald, 2004). According to a practical technique, known as “Harrington’s Rule”, the safe storage is duplicated for each 1% reduction on moisture content of seeds, or decrease of 5.5 °C in the environmental temperature. When observed simultaneously, these factors have additive effects on conservation of orthodox seeds (Harrington, 1963).

The morphological changes that have occurred on seeds packed into Kraft paper and stored into the cold chamber environment, as well as those seeds stored under laboratory environment, were detected by changes that have occurred on the color of tegument, which have varied from light-brown to dark-brown, or even black (non-presented data). Changes of seed tegument are frequently indicatives of seed decay, particularly on leguminous, and occur due to acceleration of oxidative reactions caused by high temperatures and relative humidity (Copeland and McDonald, 1995).

Borba-Filho and Perez (2009) reported that the values of moisture content have not reached damages levels able to affect physiological quality of *Tabebuia* sp. seeds stored under laboratory environmental conditions, correlating loss of seed viability with

the registered temperatures (21° C to 31° C). The minimization of metabolic rates can be achieved with low temperatures for orthodox and recalcitrant seeds (Bonner, 2008); however, the seeds of tropical species are susceptible to damages caused by cold, but generally are tolerant to temperatures varying from 5 °C until 20 °C (Schmidt, 2000).

In this study it was found that under conditions assessed the seed have not reached the “lethal moisture content”, that is a limit below which all seeds lose viability (Hong and Ellis 1996), once at 180 days into packagings of paper Kraft bags and stored under laboratory environmental conditions, the moisture content of seeds was 16.5% and with null germination; while, under the environmental conditions of refrigerator, in the same type of packaging, the moisture content was 10.3% and with germination of 98%. This manner, it was not possible to infer the moisture content limit for performing the drying of seeds, indicating that the species is tolerant to desiccation, i.e., presents intermediary or orthodox behavior to the storage.

Before storage, the seeds had 95% germination and GSI of 5.39. However, in the refrigerator environment the initial germination decreased from 94% to 87%; and under the cold chamber environment, the initial germination decreased from 71% to 60%, at 420 days, with results presenting decreasing linear behavior of germination along time, in both environments (Figure 4A). The GSI in the cold chamber environment has presented the same behavior of the germination percentage (Figure 4B), when decrease on final values were observed,

if compared to control sample (time zero; before storage). Under refrigerator environmental conditions, the GSI has had little oscillation, with results presenting decreasing quadratic behavior along time, and with the smallest value at 204 days.

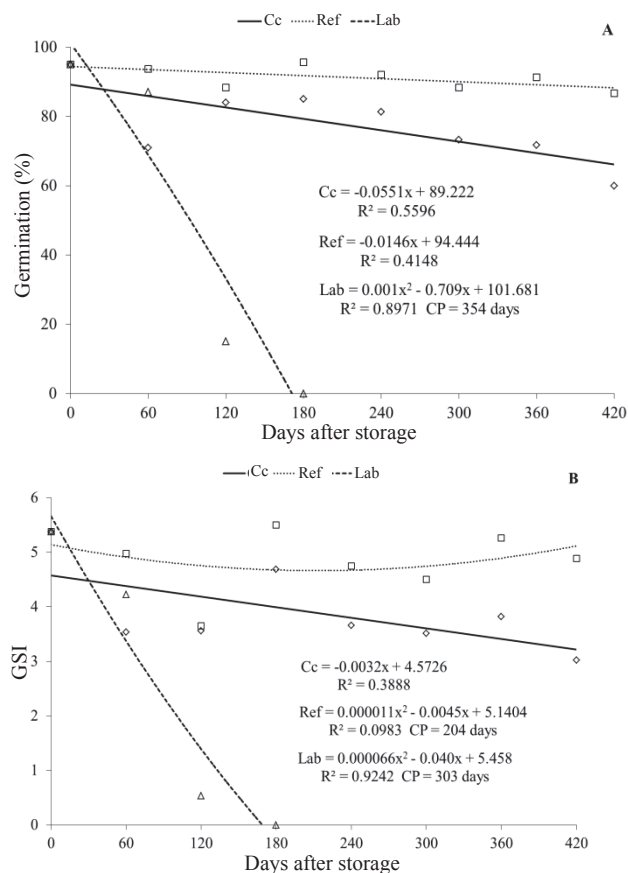


Figure 4. Germination percentage (A) and germination speed index (GSI; B) of seeds of *Parapiptadenia rigida* stored in three different environmental conditions during 420 days.

In a study carried out by Santos and Paula (2007) with seeds of *Sebastiania commersoniana* (Baillon) Smith & Downs it was verified that storage of these seeds under environmental conditions without control of temperature and relative humidity can be performed for a short time period ( $\pm 165$  days); while, for the cold chamber environmental conditions, the packagings of plastic and glass can be used during a period of 18 months ( $\pm 540$  days). However, for the seeds of *Erythrina velutina* Wild., the packing into paper, cloth, or glass are recommended for storage periods of until 225 days under environmental conditions of laboratory, refrigerator and/or cold chamber, without occurring significant losses on physiological quality of seeds (Silva et al., 2011b).

## Conclusions

The germination test of *Parapiptadenia rigida* seeds can be performed between layers of fine sand, in temperature of 25 °C; with the first count of germination performed in the fourth day and final counting performed at 10 days after the seeding.

The seeds of *Parapiptadenia rigida* can be stored into refrigerator, preferably in packagings of paper of Kraft type; however packagings of plastic or glass are also adequate.

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