Vol. 13(33), pp. 1689-1697, 16 August, 2018
DOI: 10.5897/AJAR2017.12292
Article Number: AE331A758110
ISSN: 1991-637X

http://www.academicjournals.org/AJAR

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African Journal of Agricultural Research

Full Length Research Paper

Physiological quality of seeds of shepherd's purse (*Zeyheria montana* M. Bignoniaceae) as a function of substrate temperature and storage

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Received 7 March, 2017; Accepted 2 January, 2018

The present study aimed to investigate the influence of different substrates, temperatures and storage conditions on shepherd's purse seed quality. It was evaluated in germination paper substrates Germitest®, vermiculite, sand and Plantmax®, and roller temperatures of 25, 30, 15-25 and 20-30°C. Longevity was measured by the temporal response to different packaging (paper, plastic and aluminum) and storage environments (laboratory and freezer). Germination percentage, germination rate index, percentage of normal, abnormal and dead seedlings were evaluated. The paper roll at 25°C provides an optimal condition for maximum germination potential and vigor. The storage for three months in freezer provides an increase in vigor in the seed without storing. The laboratory promoted reduction in germination potential from nine months of storage, with total loss of germination at 24 months. The combination of freezer and plastic packaging provide maintenance of seed longevity after two years of preparation.

Key words: Germination, vigor, longevity.

INTRODUCTION

Zeyheria montana Mart. (Bignoniaceae), commonly known as "shepherd's purse", is a plant species native to the Brazilian Cerrado. The extracts of leaves and roots of *Z. montana* are conventionally used in Brazilian popular medicine for the treatment of ulcers, cutaneous tumors and inflammatory diseases (Bertoni et al., 2007). Phytochemical analysis of *Z. montana* leaf extract showed the presence of terpenoids and flavonoids, whereas root

and stem extracts are particularly rich in lapachol (Machado et al., 2006).

Despite the medicinal and commercial importance of shepherd's purse seeds, there are few studies regarding their germination process (Dousseau et al., 2007). When the germination process is considered, it is necessary to take into account the factors that affect this process, being temperature and the substrate which are the basic environmental factors of the germination test (Carvalho and Nakagawa, 2012; Stockman et al., 2007).

Several authors report the importance of temperature in germination, controlling the intensity and velocity (Orzari et al., 2013), regulating imbibition rates, electrolyte release (Kader and Jutzi, 2002), reserve mobilization (Ataíde et al., 2013), affecting the growth and vigor of seedlings (Alves et al., 2013, Pacheco Junior et al., 2013) and regulating the transcription of genes associated with germination (Chiu et al., 2012), since the substrate exerts influence on the development of the root system and provides nutrients for plants (Nogueira et al., 2012). The substrate exerts a great influence on the development of the root system and nutrient supply to the plants (Nogueira et al., 2012). In addition, several materials can be used in the original composition of a substrate or its compounds (Delarmina et al., 2015), contributing to germinability (% G) and germination rate (IVG).

However, maximum values of germination and germination velocity index also depend on post-harvest conservation of seeds, adequate storage of seeds is a fundamental condition for maintaining their viability and longevity, regardless of whether they are orthodox or recalcitrant (Yamanishi et al., 2005).

Another factor that need to be highlighted is the type of packaging used, which directly interferes with the conservation of seed vigor when they are stored in packages through which gas exchange with the atmosphere occurs. Here, the seeds can gain or lose moisture which may influence its viability (Batista et al., 2011).

Seed Analysis Rules (Brazil, 2009) establish instructions for conducting the germination test, including; basically, the type of substrate and the requirements for the availability of water, light and temperature (Carvalho and Nakagawa, 2012). However, information on many species is scarce, even in the Instructions for Analysis of Seeds of Forest Species (Brazil, 2013), and it is necessary to expand the work in the area of seed propagation and production, mainly due to the growing demand for propagation and conservation of medicinal Cerrado species.

In this context, the objective was to evaluate the influence of different substrates and temperatures on germination of shepherd's bag seeds, as well as to evaluate the temporal response to packaging in different packaging and storage environments in longevity for a period of two years.

MATERIALS AND METHODS

Fruits were collected from 30 plants located in the municipality of Ijaci - MG (21º 10 '12 "S latitude, 44º 55' 31" W longitude and 832 m altitude), in a private Cerrado reserve during the September / November harvest of 2006, at the time of dispersion. After collection, the fruits remained for 12 h, under laboratory conditions, for complete dehiscence and seed collection, after which the seeds were manually carved; the winged and disinfested expansion were removed by immersion for 5 min in 0.1% (w/v) Benomyl® solution (Dousseau et al., 2007). All experiments were conducted in germination chambers of type B.O.D., with photoperiod of 12 h and 58% relative humidity.

Three different experiments were conducted. In the first one, the influence of different substrates in the germination percentage (% G) and on the germination velocity index (IVG) of the shepherd's bag seeds was evaluated at 25°C; in the second, the response to different temperatures in the germination of germinated seeds in the best substrate chosen from the previous experiment and, in the third, storage conditions for maintenance of longevity under the predefined conditions of substrate and temperature.

The substrates were paper roll Germitest®, vermiculite, organic substrate Plantmax® and washed sand media. The roller system was conducted according to Dousseau et al. (2007). The other substrates were autoclaved and distributed in each Gerbox® polyethylene box. The sowing was done 1 cm deep, covering them with the respective substrate, the temperatures tested were the constants of 25 and 30°C and alternates of 15-25 and 20-30°C, in the roll of Germitest® paper.

For the storage study, the seeds with the winged expansion were treated with Captan ® 2% and packaged in 3 individual packages, made of materials of different permeabilities: paper bags, transparent plastic and aluminum. They were maintained in laboratory and cold chamber environments. The cold chamber was maintained at 10°C and 50% relative humidity, while in the laboratory the temperature ranged from 20 to 30°C. Stored seeds were evaluated for 0 (control), 3, 6, 9, 12 and 24 months. The analysis of the storage experiment was carried out in a 3 x 2 x 5 factorial scheme, corresponding respectively to the 3 types of packages, 2 storage environments and 5 storage periods.

In all trials, a completely randomized experimental design was used, with 4 replicates of 25 seeds per treatment. Germination, IVG, percentage of normal seedlings (% Pn), percentage of abnormal seedlings (% Pa) and percentage of dead seeds (% M) were evaluated. The percent G and IVG were calculated as described in Dousseau et al. (2007), where% G was obtained by calculating the germination percentage and IVG, according to Maguire (1962); after data collection was performed statistical analysis by means of the statistical program SISVAR (Ferreira, 2014) was done. The variance analysis was performed and the averages for germination in the substrates and at the temperatures were compared by the Tukey test ($p < 0.05$), while the storage averages were compared to the standard error of the mean,

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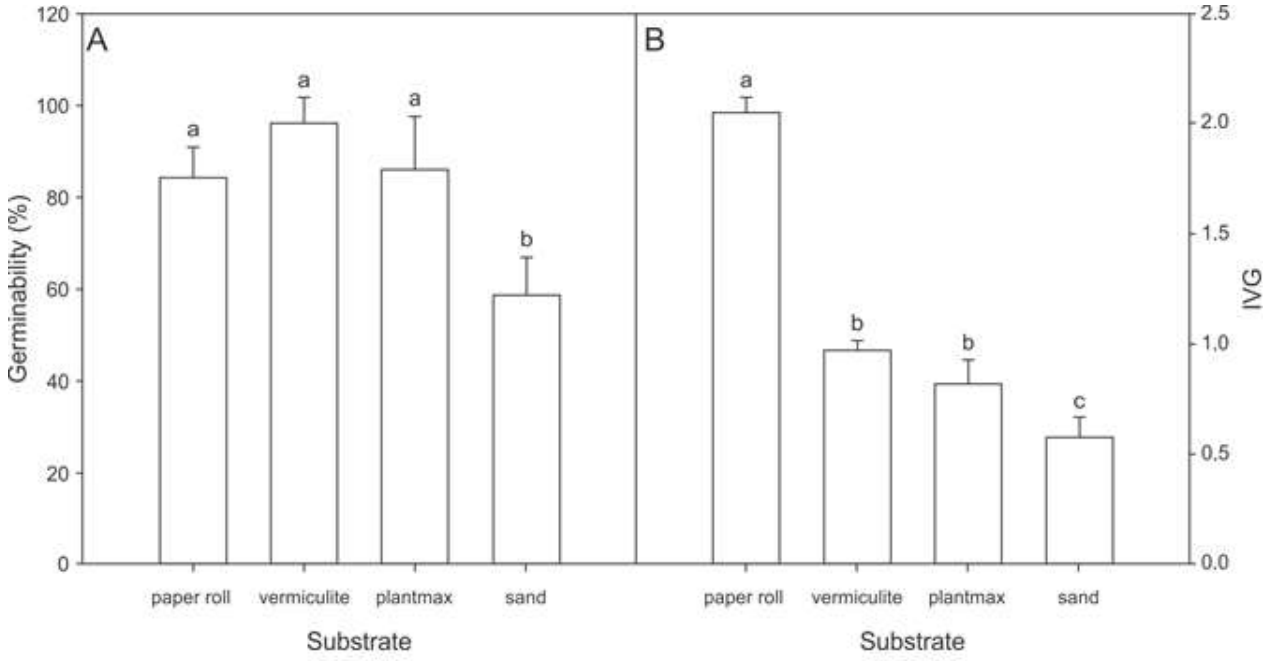

Figure 1. Germination of shepherd's bag seeds as a function of different substrates. A, Germinability (%); B, germination velocity index (IVG). Means followed by the same letter do not differ by Tukey test at 5% probability.

represented by the bars of error.

RESULTS AND DISCUSSION

The analysis of the best substrate for the germination of shepherd's pouch revealed that there was no statistical difference in the % G when compared with the paper roll, vermiculite and Plantmax® substrates, while the sand provided lower % G (Figure 1A). However, there were differences in the IVG for these substrates, where the paper roll provided higher IVG values when compared to vermiculite, Plantmax® and sand, respectively (Figure 1B).

Seeds germination of other species of Bignoniaceae showed that the response to the substrate is very variable, with no pattern. The substrate paper blotter is the most recommended condition for the germination tests of *Handroanthus serratifolius* seeds (Leão et al., 2015). The paper and sand substrate was more adequate for the evaluation of the physiological quality of the seeds of *Tabebuia aurea* (Silva Manso) Benth. & Hook f. S. Moore (Pacheco et al., 2008). For *Tabebuia roseo-alba* (Ridl.), sand was the ideal substrate, both on paper and paper roll, and between vermiculite, the lowest germination and vigor was observed (Stockman et al., 2007).

The effect of different temperatures on seeds germination was evaluated by the paper roll, which allowed the highest values of IVG and high germinability. The results show that % G values at temperatures of 25, 30 and 20-30°C did not differ from each other, while the temperature of 15-25°C provided lower % G (Figure 2A). However, when the IVG is analyzed, it is observed that the highest index occur at 25°C, followed by temperatures of 30, 20-30 and 15-25°C (Figure 2B). Both for the evaluation of the best temperature and for the best substrate in the germination process, all germinated seeds developed normal seedlings and no dead seeds were observed.

For some species, seed germination performance is favored by constant temperatures (Machado et al., 2016), by temperature alternation (Pereira et al., 2013) and by indifference to the temperature regime used (Martins et al., 2008).

Tests carried out with seeds from other forest species recorded that the thermal range suitable for seed germination of these species are between 20 and 30°C (Pascuali et al., 2012). However, variations can occur even among populations of the same species due to environmental conditions and adaptive and ecological characteristics (Mattana et al., 2012); for example, for other species of the family, Bignoniaceae at 35°C is the most recommended for germination of *T. aurea* (Pacheco et al., 2008) and *Pyrostegia venusta* (Ker Gawl.) Miers (Rossatto and Kolb, 2010).

Alternating temperatures also reduced seed germination and vigor of *T. serratifolia* and *T. impetiginosa* (Oliveira et al., 2005). According to Brancalion et al. (2010), alternation of temperatures is more common in pioneer

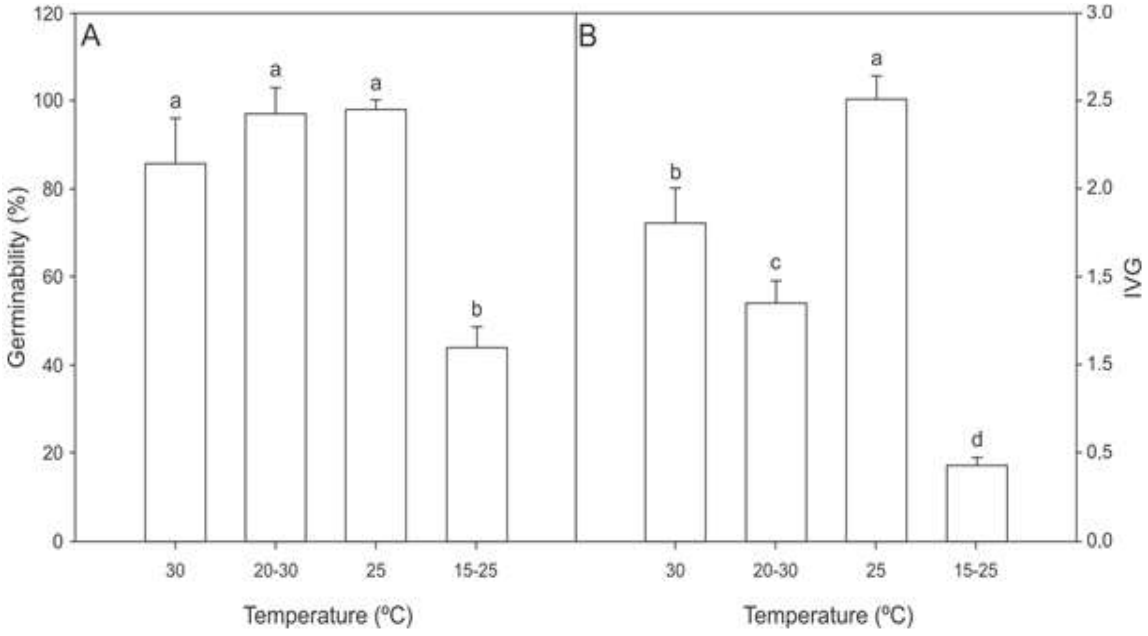

Figure 2. Germination of shepherd's bag seeds according to different temperatures. A. Germinability (%). B. Germination velocity index (IVG). Means followed by the same letter do not differ by Tukey test at 5% probability.

species, where alternating temperature regimes may be essential for overcoming dormancy, both physical and physiological, although the seeds of later species of succession may also benefit.

Shepherd's purse seeds were able to germinate under all the evaluated thermal regimes (Figure 2), showing the importance of this genus, which can be characterized by its ability to change its physiology according to environmental conditions, a feature that is a priority for the reforestation projects, mainly in the recovery of degraded areas and areas of permanent preservation (Martins et al., 2012, Sampaio et al., 2012).

For the storage experiments, the seeds that were placed to germinate in paper roll conditions at 25 °C provided the best IVG and% G, showing that there was no significant difference in seed moisture content of 7 to 8% in the laboratory environment (Figure 3A) and 7 to 10% in the cold chamber (Figure 4A).

However, for the other variables, there was a triple interaction between the studied factors (packaging, environment and storage time) (Table 1).

The longevity of shepherd's purse seeds differed from laboratory and cold storage. In the laboratory environment, a reduction was observed in the % G (Figure 3B) and in the normal seedlings (% Pl) (Figure 3C) from the 3 months of storage in aluminum, not differing in the other packages. At 9 months of storage, it was observed that expressive decrease in the % G and % Pn in all packages with the maximum value of dead seeds (Figure 3 E). The Pa appeared after 6 months of storage (Figure 3D). The vigor, expressed by the IVG value, was higher than 3 months of storage, then decreased (Figure 3F).

In the cold chamber environment, the % G (Figure 4B), % Pn (Figure 4C) and IVG (Figure 4F) also increased at 3 months, however, it was maintained until 12 months, with reduction only at 2 years. Pa remained at low values throughout storage (Figure 4D). The dead seeds were expressive only in the last evaluation (Figure 4E).

Seed longevity varies according to the genotype, but the period of conservation of the physiological potential depends to a large extent on the degree of moisture and storage environment conditions (Marcos Filho, 2005). Shepherd's purse seeds can be considered orthodox, similar to most species of Bignoniaceae (Carvalho et al., 2006) and storage at low temperatures is essential for the conservation of this class of seeds, once they guarantee the reduction of metabolism (Carvalho et al., 2006). Thus, since the values of water content did not reach harmful limits to the physiological quality of the seeds, the loss of viability when kept in the laboratory is related to the temperatures recorded in this environment, which varied from 20 to 30°C. High temperatures accelerate the degeneration processes of biological systems, where intense respiration occurs, consuming their reserve material, so that under these conditions the seeds lose their vigor and ability to germinate (Delouche and Potts, 1974). Seeds of *T. chrysotricha* stored at a water content of 21.1 and 15.9% at 20°C underwent an intense deterioration process, whereas the temperature and

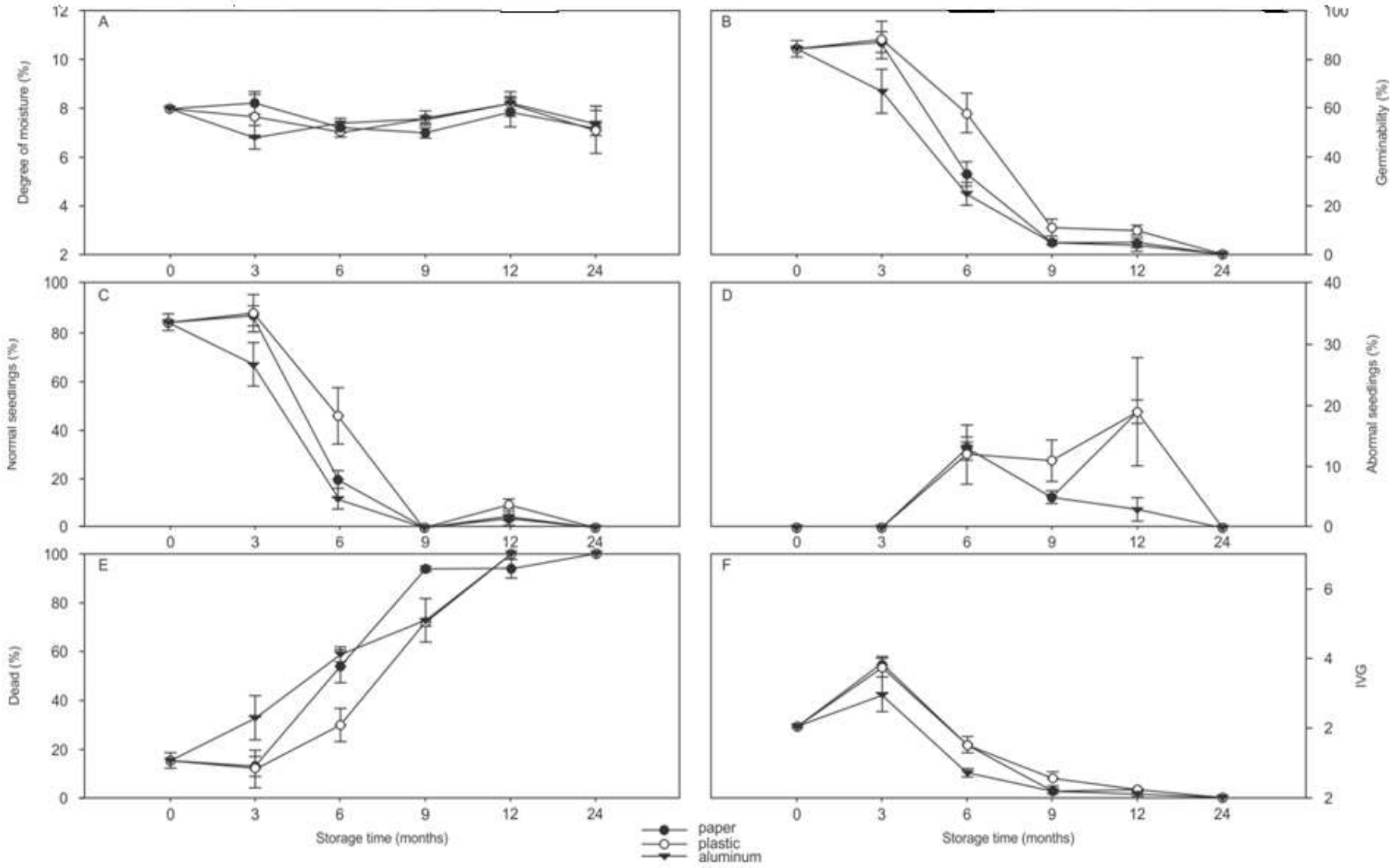

Figure 3. Time response of germination due to packaging in different packages under laboratory conditions. A. Degree of seed moisture; B. % of germination, considering root protrusion (% G); C. % of normal seedlings (% Pn); D. % of abnormal seedlings (% Pa); E. % of dead seeds (% M); F. germination velocity index (IVG).

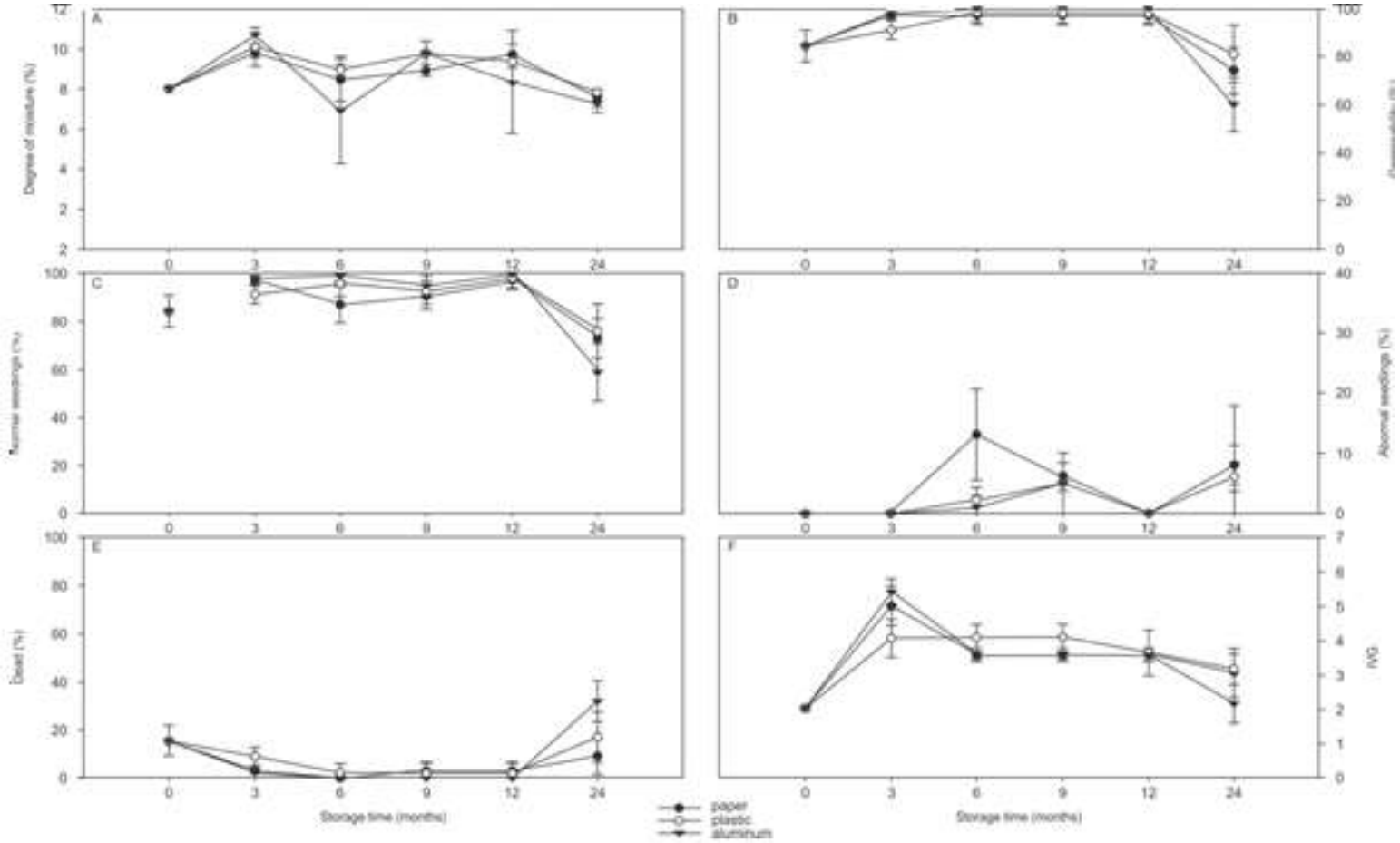

Figure 4. Time response of germination as a function of packaging in different packages under cold chamber conditions. A. Degree of seed moisture; B. % of germination, considering root protrusion (% G); C. % of normal seedlings (% Pn); D. % of abnormal seedlings (% Pa); E. % of dead seeds (% M); F. rate of germination (IVG).

water content combinations of 11.9% at 10°C; 11.9% at 12°C and 13.6% at -12°C favored seed conservation (Martins et al., 2009).

Analyzing the different packages, there were also differences between storage environments over time (Table 1). In the cold chamber, where % G and % Pn differed only at 24 months (Figure 5), the highest values of % G (Figure 5A, E) and % Pn were found in plastic and paper packages,

Table 1. Deployment of triple interaction between packing containers, environments (Laboratory and Cold Chamber) and months of storage of shepherd's bag seeds.

Packing	Time (months) and environments									
	3		6		9		12		24	
	LAB	CF	LAB	CF	LAB	CF	LAB	CF	LAB	CF
%G										
Paper	87 Ba	97 Aa	33Bb	97 Aa	5 Ba	97 Aa	4 Ba	97 Aa	0 Ba	74 Aa
Plastic	88 Aa	91 Aa	58 Ba	98 Aa	11 Ba	98 Aa	10 Ba	98 Aa	0 Ba	81 Aa
Aluminum	67 Bb	98 Aa	25Bb	100 Aa	5 Ba	100 Aa	5 Ba	100 Aa	0 Ba	60 Ab
IVG										
Paper	3.8 Ba	4.7Ab	1.5 Ba	4.1 Aa	0.6 Ba	4.1 Aa	0.2 Ba	3.6 Aa	0 Ba	3.2 Aa
Plastic	3.8 Ba	5.0 Aa	1.5 Ba	3.6 Aa	0.2 Ba	3.6 aa	0.2 Ba	3.6 Aa	0 Ba	3.1 Aa
Aluminum	2.9Bb	5.4 Aa	0.7 Bb	3.6 Aa	0.2 Ba	3.6 Aa	0.1 Ba	3.6 Aa	0 Ba	2.2 Ab
%PI										
Paper	87 Ba	97 Aa	20 Bb	87 Aa	0 Ba	91 Aa	4 Ba	97 Aa	0 Ba	73 Aa
Plastic	88 Aa	91 Aa	46 Ba	96 aa	0 ba	93Aa	10 Ba	98 Aa	0 Ba	76 Aa
Aluminum	67 Bb	98 Aa	12 Bb	99 Aa	0 Ba	95 Aa	5 Ba	100 Aa	0 Ba	59 Ab
%Pa										
Paper	0 Aa	0 Aa	13 Aa	13 Aa	5 Aa	6Aa	13 Ab	0 Ba	8 Aa	0 Ba
Plastic	0 Aa	0 Aa	12 Aa	2 Bb	11 aa	5 Aa	26 Aa	0 Ba	0 Aa	6 Aa
Aluminum	0 Aa	0 Aa	13 Aa	1 Bb	5 Aa	5 Aa	5 Ac	0 Aa	0 Ba	8 Aa
%M										
Paper	13 Ab	3 Aa	54 Aa	0 Ba	94 Aa	3 Ba	82 Aa	3 Ba	100 Aa	9 Bb
Plastic	12 Ab	9 Ba	30 Ab	2 Ba	89 Aa	2 Aa	64 Ab	2 Ba	100 aa	17 Bb
Aluminum	33 Aa	2 Ba	59 Aa	0 Ba	95 Aa	0 Ba	88 Aa	0 Ba	100 Aa	32 Ba

Means followed by the same letter do not differ by Tukey test at 5% probability. On the line, uppercase letters compare environments in each packaging and month of storage, while in the column, lower case letters compare packages in each environment and month of storage. LAB, Laboratory environment; CF, cold environment.

which did not differ between them (Figure 5D), and aluminum packaging provided lower results. Regarding IVG, at 3 months, plastic and aluminum provided higher IVG in relation to paper, while in the other months no difference was observed between the packages, differing only in the last evaluation, with lower aluminum vigor. Abnormal seedlings (% Pa) appeared from the six months of evaluation (Figure 5C), while Pm at 24 months (Figure 5F).

In the laboratory environment, the values of % G, % Pn and IVG were higher on the paper and plastic packages compared to aluminum at 3 months of storage, not differing in the other times. However, in this environment, the germination capacity decreased at 6 months, with a drastic reduction at nine months of storage. Pa was obtained at 6 months of evaluation, as occurred in the cold chamber environment, but the presence of Pm was observed after 6 months (Figure 5B). The characteristics of the containers that store seeds influence the deterioration of seeds, depending on the greater or lesser ease of water vapor exchange between the seeds, the atmosphere and the environmental conditions in which the seeds are stored.

Paper, for example, is considered a porous packaging, which does not obstruct such exchanges. However, the plastic is resistant to the movement of water vapor between the seeds and the outside air while the aluminum packaging is considered impermeable because it does not allow the exchange of water vapor (Marcos Filho, 2005).

Thus, although no statistical difference was observed in the water content of the seeds in the tested different packages, it is speculated that the lower values of % G and IVG of the seeds conditioned in aluminum foil at 3 and 6 months storage in laboratory and At 24 months in cold chamber conditions, are due to the absence of water vapor exchange between the seeds and the media. According to Carvalho and Nakagawa (2000), changes in

Figure 5. Seed germination at 24 months storage in plastic packaging. A. Cold room environment; B. Laboratory environment. C. Detail of an abnormal seedling of a seed stored in a cold chamber; D. Detail of a normal seedling of a seed stored in a cold chamber; E. Radicular protrusion of a normal seed stored in a cold chamber; F. Dead seed stored in a cold room.

temperature and relative humidity of the air cause constant adjustments in the water content of the seeds stored in water vapor permeable packages until they reach an equilibrium moisture content. In the case of aluminum packaging, these variations in seed water content did not occur and provided the lowest values of % G and IVG in relation to paper and plastic.

Conclusion

The Germitest® roll paper substrate at a constant temperature of 25°C provided the highest values of germination and rate of germination. The period of three months in the cold chamber provides maintenance on the vigor in relation to the seeds without storing. The laboratory environment promotes reduction in germination potential from nine months of storage, with total germinability loss at 24 months. The combination of cold compartment and plastic packaging ensures the longevity of the seed after two years of packaging.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGMENTS

The authors thank the Federal University of Lavras, the Foundation for Research and Innovation of Espírito Santo (Fapes), the National Council for Scientific and Technological Development (CNPq) and the Capixaba Institute of Research from Espirito Santo, Technical Assistance and Rural Extension (INCAPER), by the physical structure for the development of the proposed project activities and by the financial support.

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