THERMAL AND MOISTURE CONTENT EFFECTS ON STORABILITY AND SEED DORMANCY RELEASING ON *Brachiaria brizantha* CULTIVARS [1]

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ABSTRACT **-** *Brachiaria* species normally show a double seed dormancy mechanism, mainly on fresh-harvested seeds, leading to germination percentages lower than those of viability detected by tetrazolium test (TZ) and causing problems as to storage, trading and seed inspection activities. The adoption of the methodology to detect the constants of the viability equation (high storage temperatures and fixed moisture contents) made feasible in this research to isolate the effects of 40, 50 and 65°C on *B. brizantha* cultivars Marandu, Mulato 1 and Mulato 2 seed dormancy releasing, after storage with moisture contents ranging from 1.9 and 17.8%. Seed samples presented high dormancy levels, detected by TZ and it was complete and partially released by chemical scarification and accelerated ageing test, respectively. No statistical differences were observed as to the speed of germination (T_{50}); however, differences among cultivars were detected as to number of seed per gram. Sorption and desorption isotherm curves were similar for the cultivars**.** Seed dormancy releasing was better achieved at 40 and 50°C with mc ranging from 7.6 to 10.8%. The temperature of 50°C appears to be adequate for seed dormancy releasing in all mc analyzed. No significant seed dormancy releasing result was observed at 65°C. The cultivar Marandu presented the highest storability throughout the experiment.

Index terms: activity of water, seed longevity, high storage temperatures.

EFEITOS TÉRMICOS E DE GRAUS DE UMIDADE NA ARMAZENABILIDADE E NA LIBERAÇÃO DA DORMÊNCIA DE SEMENTES EM CULTIVARES DE *Brachiaria brizantha*

RESUMO - As espécies do gênero *Brachiaria* apresentam mecanismo duplo de dormência de sementes, principalmente nas recém colhidas, resultando em porcentagens de germinação inferiores às de viabilidade detectadas pelo teste de tetrazólio (TZ), causando deste modo problemas com relação ao armazenamento, comercialização e fiscalização do comércio. A metodologia para determinar as constantes da equação de viabilidade (altas temperaturas e graus de umidade constantes) mostrou-se eficiente nesta pesquisa para isolar os efeitos de temperaturas de 40, 50 e 65°C na liberação da dormência de sementes de *B. brizantha* dos cultivares Marandu, Mulato 1 e Mulato 2, após armazenamento com graus de umidade variando de 1,9 a 17,8%. As amostras de sementes apresentaram altos níveis de dormência, que foram detectadas pelo TZ e completa ou parcialmente liberada através da escarificação química e do teste de envelhecimento acelerado, respectivamente. As isotermas de sorção e desorção foram similares para os cultivares. A liberação da dormência foi mais bem sucedida a 40 e 50°C com graus de umidade variando de 7,6 to 10,8%. A temperatura de 50°C parece ser a mais adequada para a liberação da dormência em todos os graus de umidade analisados. Não foi observada uma liberação da dormência das sementes significativa a 65°C. O cultivar Marandu apresentou a maior armazenabilidade durante todo o experimento.

Termos para indexação: atividade de água, longevidade de sementes, altas temperaturas de armazenamento.

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INTRODUCTION

Brachiaria species have a strong dormancy, mainly on fresh-harvested seeds, leading to germination percentages lower than those of viability detected by tetrazolium test (TZ) and causing problems as to storage, trading and seed inspection activities. *B. brizantha* was introduced in Brazil in 1983, mainly due its higher nutrition potential however requiring medium and high fertility soils (Usberti-Filho, 1993).

The demand for those seeds is highly competitive and requires the previous knowledge of seed lot viability and storability, mainly the seed lot agronomic value (physical purity x germination percentages).

A fast germination is highly desirable for successfully establishing forage grass pasture fields. However, seed dormancy could contribute for the setting and the persistence of the stand and avoiding the occurrence of the germination immediately after the harvest (Herrera, 1994).

According to Montório et al. (1997), a primary dormancy occurs during the seed development and a secondary one when the seeds fail to germinate due to unavailable heat, light, water and oxygen. The main causes of that dormancy are the impermeability of the tegument to O_2 as well as the embryo dormancy. Several methodologies have been tested as to those seeds dormancy releasing, mainly using H_2SO_4 scarification and some researches are relevant in *B. brizantha* (Castro et al., 1994; Montório et al., 1997; Lago and Martins, 1998; Martins and Silva, 2001), in *B. decumbens* (Jark-Filho, 1976; Atalla and Tosello, 1979; Whiteman and Mendra, 1982; Usberti, 1990; Herrera, 1994), in *B. ruziziensis* (Mclean and Grof, 1968) and also in *Panicum maximum* (Smith, 1971; Smith, 1979).

Another methodology for seed dormancy releasing is the mechanical scarification (seed teguments removal), which presented positive results in *B. brizantha* (Montório et al., 1997; Vieira et al., 1998), in *B. decumbens* (Whiteman and Mendra, 1982) and in *Panicum maximum* (Smith, 1971). The accelerated ageing test conditions (43°C and 100% RH) also revealed positive effects in seed dormancy releasing in *B. brizantha* (Montório et al., 1997; Lago and Martins, 1998; Vieira et al., 1998), in *B. decumbens* (Usberti, 1990) and in *P. maximum* (Usberti, 1982).

The 40°C-pre-heating treatment, which is recommended for seed dormancy releasing (ISTA, 2004), was also adopted in Brazil for *B. brizantha* and *B. ramosa* (BRASIL, 1992) and its efficacy was also reported in *B. brizantha* (Lago and Martins, 1998).

The effect of high temperatures on *Brachiaria* spp. seed dormancy releasing have also been analyzed and presented positive results in *B. brizantha* (Vieira et al., 1998; Martins and Silva, 2001) and no effects in *B. decumbens* (Whiteman and Mendra, 1982). However, some doubts remain on the amount of heat necessary for different species seed dormancy releasing and, mainly, the isolated effect of those temperatures in seeds with constant moisture content (mc), which were not reported in those experiments.

Adopting the methodology for obtaining viability equation (Ellis and Roberts, 1980), using constant storage temperatures and mc, it would be feasible to verify the isolated effect of those temperatures in *B. brizantha* seed dormancy releasing. The main objectives of this work were: 1) Analyze the effects of high storage temperatures and mc on seed dormancy releasing and 2) Analyze seed performances under controlled deterioration.

MATERIAL AND METHODS

B. brizantha seed lots of cultivars Marandu (from Matsuda Seeds and Animal Nutrition Company) and interespecific hybrids Mulato 1 and Mulato 2 (from Papalotla Seed Company), were harvested in Sao Paulo State, Brazil and analyzed at 5 mc levels (ranging from 1.9 to 17.8%) and 3 storage temperatures (40, 50 and 65°C). This experiment has been carried out at Post-harvest Technology Laboratory, School of Agriculture Engineering, Campinas State University, Brazil.

Seed mc were adjusted at 25°C from initial values, either by rehydration above water (3 cm) in a closed recipient or by dehydration in desiccators with silica gel, in order to avoid seed damages due to fast drying. After 3-5 days for moisture homogenization, seed mc were estimated in 4 x 25 g pure-seed replicates using the high constant temperature oven method (130-133°C, 1 hour) (ISTA, 2004).

Seed sub samples (minimum of 200 seeds for each combination of storage temperature / mc) were sealed in laminated aluminum-foil packets. Seed samples were divided in 12 sub-sample blocks and stored at 40, 50 and 65°C. Seed samples with mc below the initial value were previously rehydrated above water at 25ºC during 2 days, aiming to avoid seed damages due to fast imbibition during the germination test (Ellis et al., 1988).

After the purity analysis, germination tests were performed using 4 x 50 intact or scarified seed replicates, which were placed in plastic square boxes (11 x 11 cm) over filter paper moistened with KNO_3 solution (0.2%) and kept in an incubator under 20-35°C alternating-diurnal-temperature regime (16 and 8 hours, white light at 35°C). Seedling counts were carried out at 7, 14 and 21 days after seeding. Accelerated ageing test (AAT) was carried out using 4 x 50 intact seeds during 48, 72 and 96 hours at 43°C and 100% RH (Usberti, 1982).

Chemical scarification was performed by immersion of 200 seeds in a beaker with sulphuric acid (96%, 36N) and stirring with a glass rod during 15 minutes; after draining the acid, seeds were soaked in water during one hour, followed by one minute-washing in running tap water and surface dried over blotter paper (Smith, 1979; ISTA, 2004). Viability tests were carried out in 2 x 50 pure seed replicates, using a 0.5% Tetrazolium solution during 2 hours at 40°C (BRASIL, 1992).

Three seed replicates for each mc were taken to detect the relation between seed mc and equilibrium relative humidity at 25±0.3°C, in a hygrometer using the chilled-mirror dew point technique (± 0.01 A_w); when A_w values of two readings were less than 0.001 apart, the measurement process stopped and the final water activity was recorded.

Complete seed survival curves were obtained for each combination of mc / storage temperature. Statistical analyses of storage temperature and mc combination effects on seed dormancy releasing were carried out using a complete randomized factorial design with portion subdivided in time, with the following factors: sampling times (minimum eight), five mc levels and three temperatures. For statistical analysis, the values of germination percentage were previously transformed into arcsine √ %/100. Tukey test was used for mean comparisons, at $p < 0.05$.

The variable germination has been analyzed according to Montgomery, 1991 and Santos and Gheyi, 2003, using three analysis phases for each cultivar, as follow: 1) analysis of temperature influence on germination percentages, after fixing mc, through an unbalanced statistical randomized design (for different repetitions inside each temperature) and statistical randomized block design (for same repetitions inside each temperature); 2) analysis of mc influence on germination results after fixing the temperatures, through an unbalanced statistical randomized block design; 3) comparisons among cultivars for the interaction between temperature and mc, through an statistical randomized block design.

RESULTS AND DISCUSSION

TZ viability results were higher than both standard germination and AAT ones (Table 1). Furthermore, seed dormancy was also detected by chemical scarification and AAT. The TZ test has already been considered reliable to detect seed viability in several forage grass species, as in *B. brizantha* (Martins and Lago, 1996) and *P. maximum* (Usberti et al., 2000). These results show clearly the presence of seed dormancy in the species and its releasing after chemical scarification have also been reported in *B. brizantha* (Castro et al., 1994; Montório et al., 1997; Lago and Martins, 1998; Martins and Silva, 2001).

TABLE 1. Seed germination percentages in *Brachiaria brizantha* cultivars Marandu, Mulato 1 e Mulato 2, obtained through standard germination, vigour test (AAT, accelerated ageing at 43°C), viability (Tetrazolium) and chemical scarification (H_2SO_4, 15 minutes).

Traits	Cultivars		
	Marandu	Mulato 1	Mulato 2
Standard germination	46.0 cd	42.5 b	38.0 c
AAT - 48h	61.0 bc	36.5 b	41.0 c
AAT - 72h	53.5 c	40.0 b	55.0 ab
AAT - 96h	44.5 cd	39.0 b	36.5 c
H_2SO_4 scarification, 15 minutes	74.0 b	82.0 a	50.0 b
TZ	89.0 a	78.0 a	62.0 a

Means followed by different letters show statistical difference at $p<0.05$.

AAT results show that 72 hours and 43°C appears to be an efficient method for partial seed dormancy releasing, which are similar to the results reported in another researches in forage grass species, as in *B. brizantha* (Montório et al., 1997; Lago and Martins, 1998; Vieira et al., 1998), *B. decumbens* (Usberti, 1990) and *P. maximum* (Usberti, 1982).

Figure 1 show that all *B. brizantha* cultivars revealed the same trend for seed drying over silica gel at 25°C. Seed mc have been reduced to around 4% in the first 10 days and the lowest values (around 2%) were achieved after 70 days.

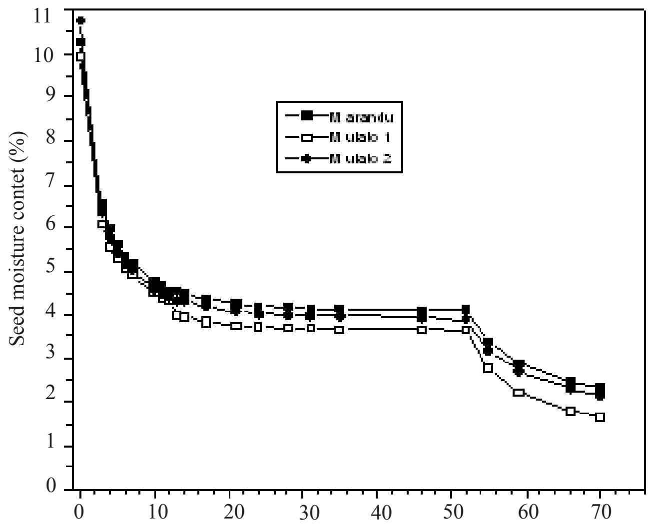


FIGURE 1. Seed drying over silica gel at 25ºC of *Brachiaria brizantha* cultivars Marandu, Mulato 1 and Mulato 2.

Sorption and desorption isotherms were similar for the cultivars; however the cultivar Mulato 1 revealed different A_w values on the extreme mc (Figure 2). The isotherms displayed consist of three regions, located at low, intermediate and high mc levels (Vertucci and Leopold, 1987).

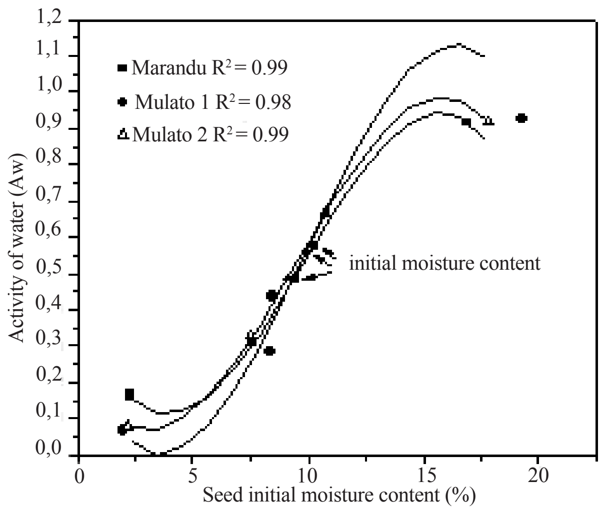


FIGURE 2. Sorption and desorption isotherms at 25ºC, in seeds of *Brachiaria brizantha* cultivars Marandu, Mulato 1 and Mulato 2.

Seeds of the cultivars with mc around 9% and stored at 40°C, presented increases in germination percentages until 25 days; from that point on, occurred generalized germination reductions (Figure 3). For mc around 10%, seed dormancy releasing was detected at 10, 20 and 30 days, however the cultivar Mulato 1 presented additionally a second dormancy releasing point at 50 days. For the highest mc values (around 17%), no seed dormancy releasing was detected throughout the storage period. Similar results were reported by Lago and Martins (1998) in *B. brizantha* seeds, after a 40°C pre-heating treatment during seven days.

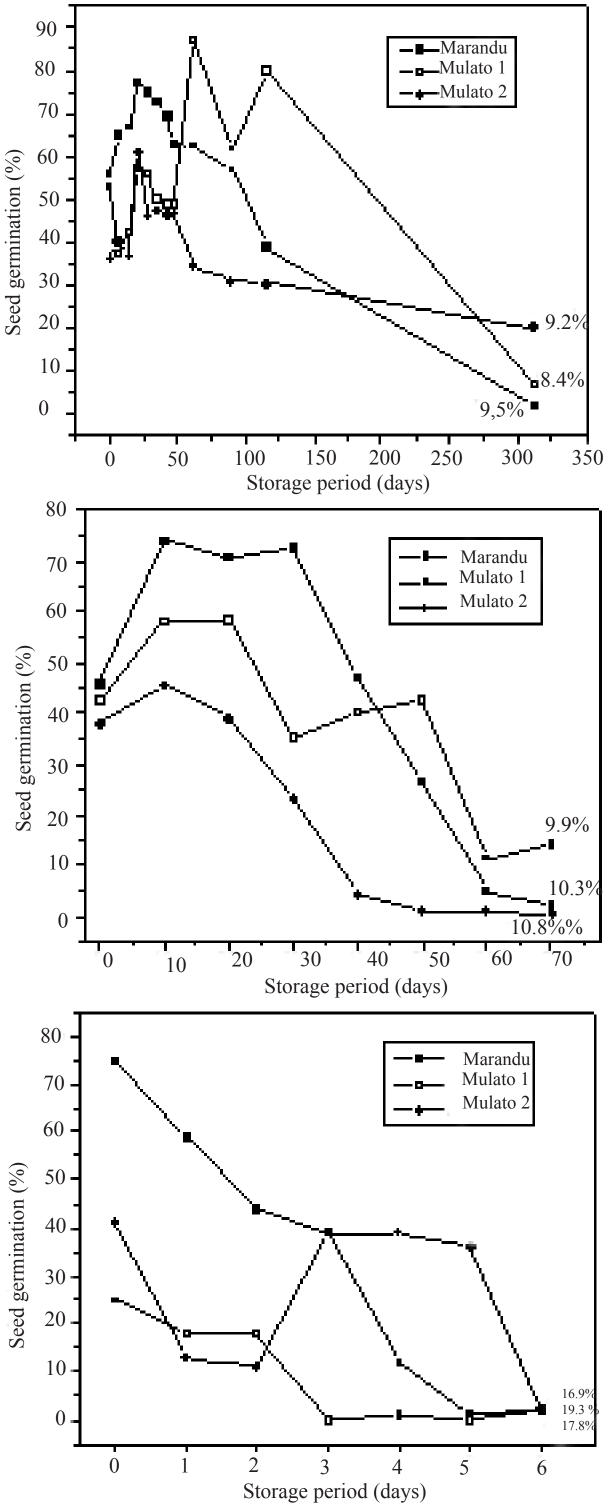


FIGURE 3. Seed germination of *Brachiaria brizantha* cultivars Marandu, Mulato 1 and Mulato 2, stored at 40ºC with several moisture contents.

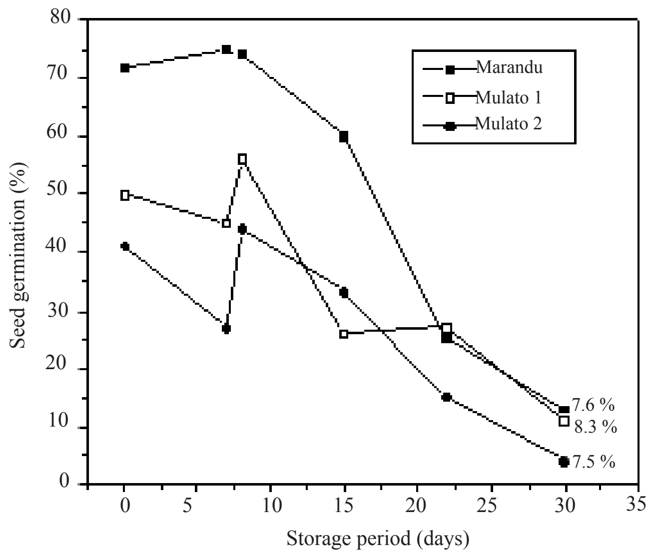
Cultivar Marandu presented the highest seed storability during storage at 40, 50 and 65°C (Table 2). The temperature has a strong effect on speed and germination percentage, mainly on water sorption to the seeds as well as in the biochemical reactions that regulate the metabolism involved in the process (Bewley and Black, 1994).

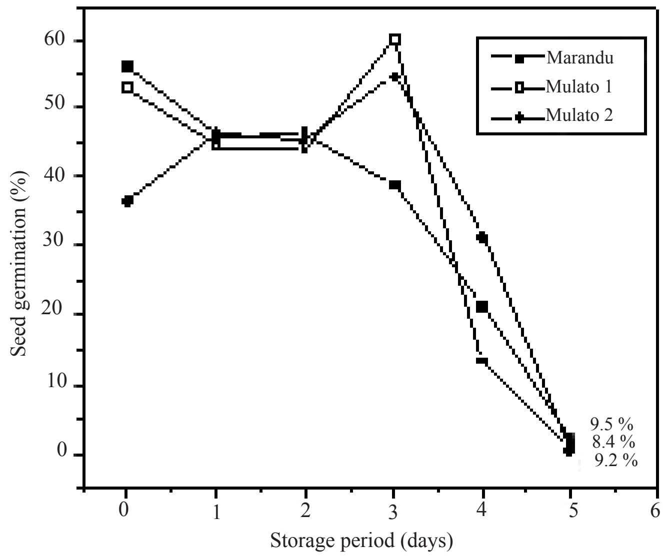
TABLE 2. Statistical analyses showing the effects of storage temperatures on seed germination of *Brachiaria brizantha* cultivars Marandu, Mulato 1 e Mulato 2.

Cultivar	40°C	50°C	65°C
Marandu	50.6 a	57.9 a	39.4 a
Mulato 1	39.4 b	35.2 b	27.5 b
Mulato 2	30.6 b	30.9 b	31.8 b

Means followed by different letters show statistical difference at $p<0.05$.

Cultivars stored at 50°C with mc around 8% presented seed dormancy releasing until 7 days; from that point on occurred germination percentage reductions throughout the storage period (Figure 4). Seed mc around 9% presented similar germination percentages between 1 and 2 days storage period; however, the cultivars Mulato 1 and Mulato 2 showed seed dormancy releasing at 3 days. Cultivar Marandu seeds with mc around 10% revealed three dormancy releasing points until 3.5 days, with germination percentages above 70%; however, the cultivars Mulato 2 e Mulato 1 have also presented dormancy releasing points at 0.5 and 1.5 days, respectively.





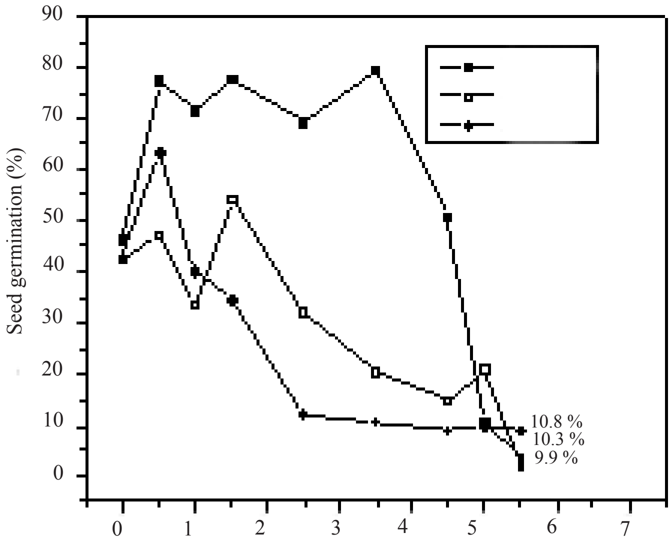
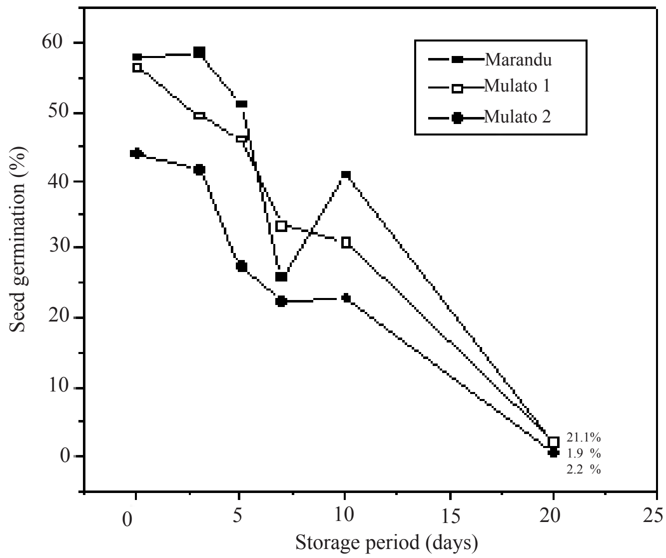
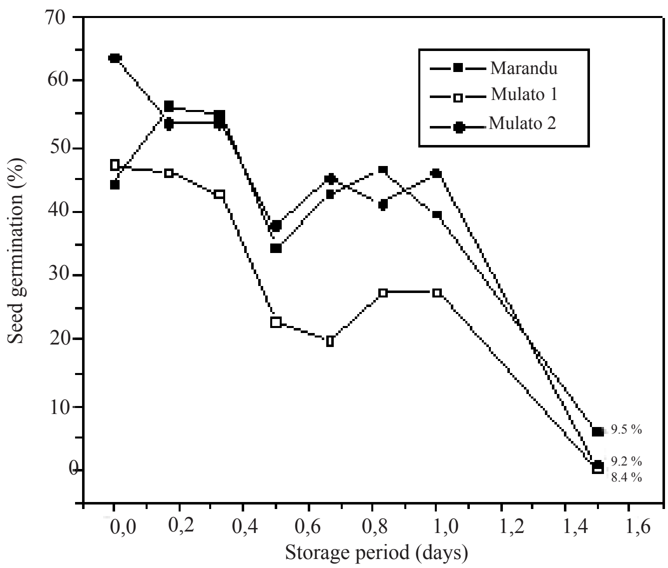


FIGURE 4. Seed germination of *Brachiaria brizantha* cultivars Marandu, Mulato 1 and Mulato 2, stored at 50ºC with several moisture contents.

Vieira et al. (1998) stored *B. brizantha* seeds in paper bags with 12% mc at 50ºC during 3 and 4 days and reported that the treatment was efficient in seed dormancy releasing. Those results are similar to the values obtained in this experiment although in that experiment seed mc was not controlled. Martins and Silva (2001), working with the same species, verified that 55°C during 10 hours did not promote increases in the germination. Moreover, Usberti (2007), working with several seed lots of *B. brizantha*, reported that the best seed dormancy releasing results were achieved at 50 and 40°C for intact and scarified seeds, respectively, not taking into account the seed mc.

Cultivar Marandu seeds stored at 65°C with mc around 2% had an increase on germination percentage at 2.5 days, which was not observed in the other cultivars (Figure 5). At mc values around 9%, the cultivar Marandu presented seed dormancy releasing points at 0.2 and 0.8 day, also observed at 0.7 and 1.0 day for cultivar Mulato 2. For seed mc around 10%, the cultivars Marandu and Mulato 2 presented increases on germination until 0.2 day.





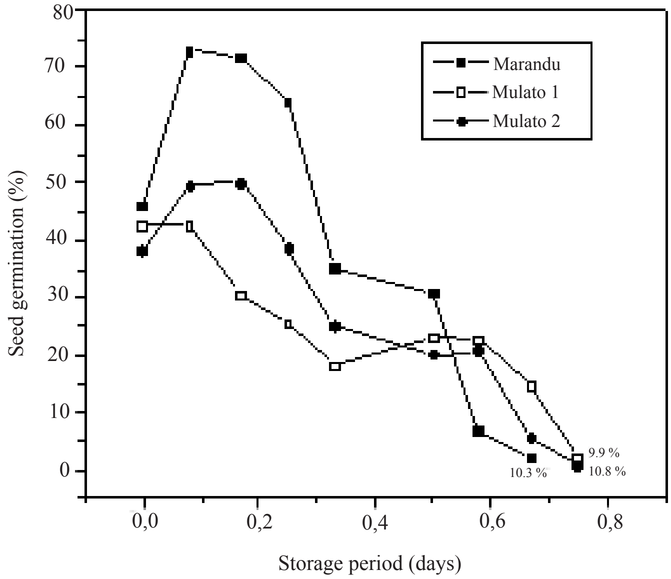


FIGURE 5. Seed germination of *Brachiaria brizantha* cultivars Marandu, Mulato 1 and Mulato 2, stored at 65ºC with several moisture contents.

The temperature of 65°C is considered excessive for most of seed technologists, but had a positive effect as to seed dormancy releasing in several mc for the cultivars analyzed. Vieira et al. (1998) stored *B. brizantha* seeds at 70°C during 10 and 15 hours and recorded a seed dormancy releasing, without generating latent physiological deterioration, which however occurred at 85°C. Martins and Silva (2001) reported that 70°C during 72 hours was also effective in promoting the germination of most of the dormant seeds of the species.

Cultivar Marandu seeds revealed the best results for dormancy releasing at 40 and 50°C with 9.5% and 7.6 / 10.3% mc, respectively (Table 3); however, at 65°C no seed dormancy releasing was observed. The best dormancy releasing results for cultivar Mulato 1 were detected at 40°C with 8.5% mc; no statistical differences on germination percentages were observed at 50 and 65°C. The cultivar Mulato 2 has shown the best seed dormancy releasing results at 40°C and 9.2% mc.

Considering the interaction between storage temperature / seed mc, Table 4 shows that at 40°C the cultivar Marandu presented the highest seed dormancy releasing at 9.5 and 10.3% mc. Comparing the results of H_2SO_4 scarification and viability (Tables 1 and 4), it is possible to conclude that, at 40°C, seed dormancy releasing for the cultivars only occurred with mc around 9%.

TABLE 3. Unbalanced statistical randomized design of *Brachiaria brizantha* cultivars seed germination, using fixed storage temperatures (T) and moisture contents (MC).

Marandu				Mulato 1				Mulato 2			
T	Mean	MC	Mean	T	Mean	MC	Mean	T	Mean	MC	Mean
7.6%		40ºC		8.3%		40ºC		7.5%		40ºC	
50°C	53.1	9.5%	58.7 a	50º C	35.8	8.5%	52.5 a	50º C	25.6	9.2%	39.7 a
65º C	33.8	10.3%	42.9 ab	65º C	25.6	9.9%	37.7 b	65º C	25.0	10.8%	19.0 b
		16.9%	33.5 b			19.3%	10.5 c			17.8%	25.8 b
9.5%		50ºC		8.4%		50ºC		9.2%		50ºC	
40°C	58.7 a	7.6%	53.1 a	40°C	52.5 a	8.3%	35.8	40º C	39.7	7.5%	25.6
50°C	35.1 b	9.5%	35.1 b	50°C	37.5 b	8.5%	37.5	50º C	35.8	9.2%	35.8
65°C	42.0 b	10.3%	52.7 a	65°C	29.9 b	9.9%	29.7	65º C	39.2	10.8%	37.6
10.3%		65ºC		10.3%		65ºC		10.8%		65ºC	
40°C	42.9	2.2%	39.4	40°C	37.7 a	1.9%	36.4	40º C	19.0 b	2.2%	26.5 b
50°C	52.7	7.6%	33.8	50°C	29.7 ab	8.3%	25.6	50º C	37.6 a	7.5%	25.0 b
65°C	35.7	9.5%	42.0	65°C	24.5 b	8.5%	29.9	65º C	27.5 ab	9.2%	39.2 a
		10.3%	35.7			9.9%	24.5			10.8%	27.5 b

Means followed by different letters show statistical difference at ***p***<0.05.

At 50°C and 7.6% mc, the cultivar Marandu presented a remarkable seed dormancy releasing. For mc around 9.0%, no dormancy releasing was observed; however, for mc of 10.3%, the cultivar Marandu had the highest germination percentage again due to seed dormancy releasing.

At 65°C no seed dormancy releasing was observed. Considering that mc around 2.0% are in equilibrium with A_w around 0.15 (Figure 2), it was expected a different performance of the cultivars. A possible hypothesis should be that seed dormancy releasing is counterbalanced by the high deterioration due to that temperature, despite the low mc values.

TABLE 4. Unbalanced statistical randomized design of *Brachiaria brizantha* cultivars seed germination, using fixed storage temperatures (T) and variable moisture contents (MC).

40°C			50°C			65°C		
Cultivar	MC	Mean	Cultivar	MC	Mean	Cultivar	MC	Mean
Marandu	9.5%	63.4 a	Marandu	7.6%	74.7 a	Marandu	2.2%	46.0 a
Mulato 1	8.4%	57.4 b	Mulato 1	8.3%	50.3 b	Mulato 1	1.9%	38.5 b
Mulato 2	9.2%	41.1 c	Mulato 2	7.5%	34.0 c	Mulato 2	2.2%	25.2 c
Marandu	10.3 %	42.5 a	Marandu	9.5%	38.7	Marandu	7.6%	39.5 a
Mulato 1	9.9 %	37.1 ab	Mulato 1	8.4%	33.2	Mulato 1	8.3%	28.2 b
Mulato 2	10.8 %	16.3 b	Mulato 2	9.2%	33.9	Mulato 2	7.5%	34.0 ab
Marandu	16.9%	38.8 a	Marandu	10.3%	55.9 a	Marandu	9.5%	40.1 a
Mulato 1	19.3%	12.0 c	Mulato 1	9.9%	28.2 bc	Mulato 1	8.4%	27.7 b
Mulato 2	17.8%	29.8 b	Mulato 2	10.8%	34.2 b	Mulato 2	9.2%	35.7 ab
						Marandu	10.3%	35.9 a
						Mulato 1	9.9%	22.2 c
						Mulato 2	10.8%	27.9 b

Means followed by different letters show statistical difference at *p*<0.05.

This hypothesis could be supported by the values obtained at 8 and 9% mc, where germination percentages stayed practically constant. A more sensitive reduction in germination percentages was evident with the results for 10.3% mc, where the cultivar Marandu has shown again the highest performance.

After analyses of the deterioration curves (Figures 3, 4 and 5) and the statistical analysis (Tables 3 and 4), it is evident that the adoption of constant storage temperatures and mc allowed to identify and isolate the effects of those factors on seed dormancy releasing, however this methodology has not been used in similar researches (Vieira et al., 1998; Martins and Silva, 2001).

CONCLUSIONS

- Cultivars analyzed presented high seed dormancy values, detected mainly by H_2SO_4 scarification and TZ test;
- Seed dormancy releasing was better achieved at 40 and 50°C with mc ranging from 7.6 to 10.8%;
- No significant seed dormancy releasing was observed at 65°C;
- The temperature of 50°C appears to be adequate for seed dormancy releasing in all mc analyzed;
- Cultivar Marandu presented the highest seed storability throughout the experiment.

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