

# Priming and temperature limits for germination of dispersal units of *Urochloa brizantha* (Stapf) Webster cv. basilisk

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(With 2 figures)

## Abstract

The objective of this study was to evaluate the effect of priming treatments on the upper and lower thermal limits for germination of *Urochloa brizantha* cv. basilisk, and testing the hypothesis that pré-imbibition affect thermal parameters of the germination. Pre-imbibed seeds both in distilled water (0 MPa) and PEG 6000 solution (–0.5 MPa) were put to germinate in different temperatures. It is suggested that *U. brizantha* seeds have low response to priming when they were placed to germinate in medium where water is not limiting. The response of *U. brizantha* seeds to priming is dependent on the temperature and water potential conditions at which the seeds are pre-imbibed, as well as on the germination temperature. The optimum temperature for germination of *U. brizantha* shift toward warmer temperatures in primed seeds. Priming effect was more pronounced at temperatures closer to the upper and lower limit for germination, but probably that response cannot be accounted for changes in the thermal time constant ( $\theta T_{(g)}$ ) and ceiling temperature ( $T_{c(g)}$ ). Otherwise, a decrease in the base temperature ( $T_b$ ) was observed in primed seeds, suggesting that the  $T_b$  distribution in *U. brizantha* seeds is influenced by priming.

**Keywords:** palisade grass, osmoconditioning, osmotic potential, thermal time.

## Priming e temperaturas limites de germinação de unidades de dispersão de *Urochloa brizantha* (Stapf) Webster cv. basilisk

### Resumo

O objetivo deste estudo foi avaliar o efeito do tratamento pré-germinativo *priming* sobre os limites térmicos inferior e superior para a germinação de *Urochloa brizantha* cv. basilisk, e testar a hipótese de que a pré-embebição afeta os parâmetros térmicos da germinação. Sementes pré-embebidas, tanto em água destilada (0 MPa) quanto em solução de PEG 6000 (–0,5 MPa) foram colocadas para germinar em diferentes temperaturas. Os resultados sugerem que sementes de *U. brizantha* apresentam baixa resposta ao *priming* quando colocadas para germinar em meio onde a água não é limitante. A resposta de sementes de *U. brizantha* para o *priming* é dependente das condições de temperatura e potenciais hídricos em que as sementes são pré-embebidas, bem como para a temperatura de germinação. A temperatura ótima para germinação de sementes de *U. brizantha* altera-se para temperaturas mais altas em sementes pré-embebidas. O efeito de priming foi mais pronunciado em temperaturas mais próximas do limite superior e inferior para a germinação, mas, provavelmente essa resposta não foi responsável por mudanças na constante tempo térmico ( $\theta T_{(g)}$ ) e temperatura teto ( $T_{c(g)}$ ). Por outro lado, uma diminuição na temperatura base ( $T_b$ ) foi observada em sementes pré-embebidas, sugerindo que a distribuição  $T_b$  em sementes de *U. brizantha* é influenciada pelo *priming*.

**Palavras-chave:** grama paliçada, condicionamento osmótico, potencial osmótico, tempo térmico.

### 1. Introduction

In general, the seeds exhibit a minimum, an optimal and a maximum temperature for germination (the cardinal temperatures). A thermal time (or degrees-day) approach have been used to describe the distribution of the times to germination at different temperature regimes according to the models  $\theta T_{(g)} = (T - T_b)t_{(g)}$ , for suboptimal temperatures, and  $\theta T = (T_{c(g)} - T)t_{(g)}$ , for supraoptimal ones, where  $\theta T_{(g)}$  is

the thermal time required for (g) percent of the seeds germinate, T is the temperature,  $T_b$  is the minimum or base temperature,  $t_{(g)}$  is the time for (g) percent of the seeds germinate and  $T_{c(g)}$  is the maximum or ceiling temperature corresponding to a percentage fraction (g) (Garcia-Huidobro et al., 1982; Bradford, 1995). Once the model parameters  $T_b$ ,  $\theta T_{(50)}$  (median thermal time),

$\sigma_{gT}$  (standard deviation in thermal time),  $Tc_{(50)}$  (median  $Tc$ ) and  $\sigma_{Tc}$  (standard deviation in  $Tc_{(g)}$ ) are known, the germination time courses at different temperatures can be normalized on a common thermal time scale which allows the germination rate at any temperature regime can be predicted (Bradford, 1995).

The seed germination is also strongly sensitive to the water potential ( $\Psi$ ) of its environment, and the germination responses to  $\Psi$  have been analyzed in a manner similar to thermal time (Gummerson, 1986; Bradford, 1995). Accordingly, the germination time of a given percentage ( $t_g$ ) is inversely proportional to the difference between  $\Psi$  and  $\Psi b_{(g)}$  (the base or threshold  $\Psi$  capable of preventing a percentage ( $g$ ) to germinate), and the variation in germination rates among seeds in the population can be accounted by shifts in  $\Psi b_{(g)}$  distributions (Bradford and Still, 2004; Finch-Savage, 2004). Alvarado and Bradford (2002) proposed that the distribution of  $\Psi b_{(g)}$  among the seeds accounts for the distribution of  $Tc_{(g)}$  since above  $T$  optimum the accumulation of thermal time would stop and the temperature effects on the germination would be primarily due to changes in  $\Psi b_{(g)}$ .

No progress toward germination occurs when  $\Psi < \Psi b_{(g)}$ , but this does not encompass the phenomenon known as seed priming (Bradford, 2002), in which the seeds are placed in a solution containing a solute that reduces the kinetic energy of water molecules. In general, the polyethylene glycol has been widely used for not interfering in the germination process. Prehydration or priming treatments, followed by drying improves the germination rate when seeds are imbibed again, suggesting that metabolic events which cause progress toward germination occur at  $\Psi < \Psi b_{(g)}$  and are retained during subsequent drying and rehydration. The priming effects can be related to changes in the germination responses to temperature. According to Hardegree and Van Vactor (2000), priming increased low-temperature germination rate for many crop species and significantly lowered thermal time requirements and  $Tb$  for germination of grass seeds. Ellis and Butcher (1988) and Dahal et al. (1990) also observed that priming decreased thermal-time requirements for germination although they did not show whether priming reduced base temperature thresholds. Priming effects on germination rate are also known to be reduced at supra optimal temperatures, thus minimizing the relative benefit of priming at that range (Hardegree and Van Vactor, 2000). The temperature in which priming occurs also affects the germination response. For example, *Cucurbita pepo* submitted to priming at relatively high temperatures presented lower thermal requirements for germination than seeds primed at lower temperatures (Zehtab-Salmasi, 2006).

*Urochloa spp* are largely utilized as pasture grasses in Brazil. The species *U. brizantha* was introduced in Brazil in the 1960s as fodder for cattle, becoming invasive and changing the landscape and species interactions and composition of native flora (Barbosa et al., 2008). The germination is indifferent to light conditions (Lima and Cardoso, 1996) and non dormant seeds of *U. brizantha* are

capable of germinating in temperature intervals ranging from 7 – 10 °C to 40 – 45 °C (Horibe and Cardoso, not published). The germination rate of the *U. brizantha* cultivars ‘marandu’ and ‘xaraes’ is favored by imbibing the seeds previously in water potentials of 0 and/or –0.5 MPa at 25 °C during 48 h (Lima, 2007), and Bonome et al. (2006) reported that priming at  $\Psi < -1$  MPa for 12 h improve both the germinability and germination rate of the cv. marandu. The aiming of this study was to describe the effect of priming treatments on the temperature dependence on the germination of *Urochloa brizantha* cv. basilisk through the use of the thermal time model. Our working hypothesis was that pre-imbibition can widen the “thermal window” for germination by reducing  $Tb$  and increasing  $Tc_{(g)}$ .

## 2. Material and methods

### 2.1. Plant material

*Urochloa brizantha* cv. basilisk dispersal units (referred to as seeds) were obtained in March, 2010 from ProSementes Company, from Araçatuba, SP, Brazil). The seeds were harvested in April, 2009 and stored at 24 °C and 60% of air relative humidity. After being purchased the seeds were stored during 15 months at  $9 \pm 3$  °C and maximum germinability was 70%.

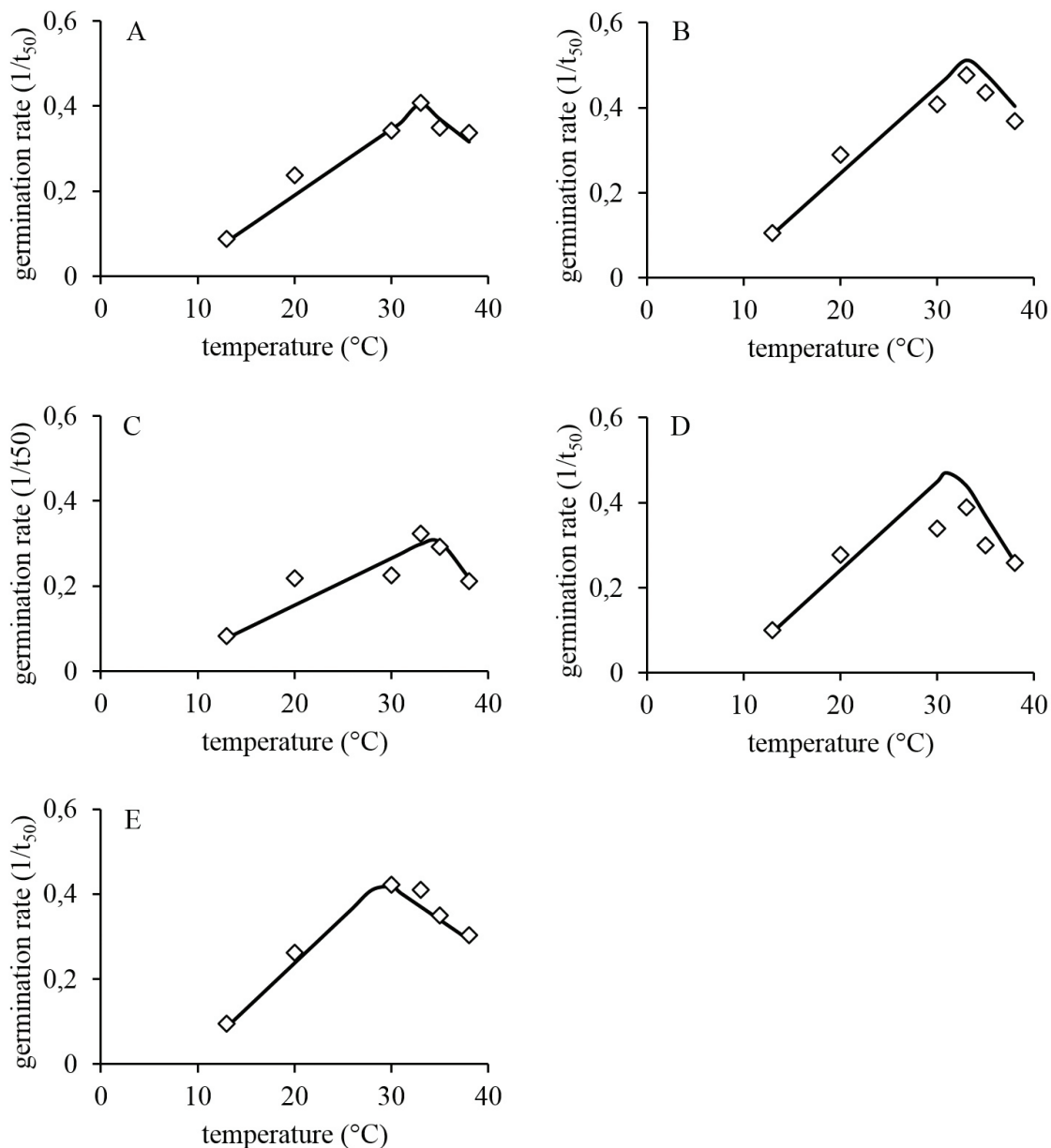
### 2.2. Germination assays

Two assays were performed: in the assay I we tested the priming effect at optimum, minimum and maximum temperature for germination as determined from previous experiments. Seed germination (primary root protrusion) was recorded on five repetitions of 50 seeds each in 18 combinations involving three priming temperatures (8 °C, 32 °C and 39.5 °C) and two osmotic potentials (0.0 MPa and –0.5 MPa), and three germination temperatures (8 °C, 32 °C and 39.5 °C). The  $\Psi$ s of –0.5 MPa was maintained using a polyethylene glycol (PEG 6000) solution prepared according to Michel and Kaufmann (1973). Seeds were fully immersed during 20 h in aerated PEG solution or distilled water (Lima, 2007) and after priming the seeds were quickly rinsed with water and dried during 72 h at 25 °C under forced air flow. The moisture levels from seed samples were both determined before and after the drying by oven drying at  $105 \pm 3$  °C (Brasil, 2009). For germination assays, seeds were put in plastic boxes (gerbox) on two layer of filter paper kept saturated with distilled water and maintained in germination chambers under continuous white light ( $\cong 33 \mu\text{mol.m}^{-2}.\text{s}^{-1}$  at seed level) at the constant temperatures of 8 °C, 32 °C and 39.5 °C, close respectively to minimum, optimum and maximum temperatures for germination. Non primed seeds (control) were germinated at the same temperatures. The number of germinated seeds (radicle protrusion) was recorded daily up to it ceased. The germinability (G) or germination capacity was the maximum accumulated germination expressed as percentage.

Another assay (assay II) was designed to find the thermal time parameters. Seeds were primed in 0.0 and

–0.5 MPa solutions at temperatures of 20 and 30 °C and placed to germinate at different isothermal conditions. Priming and germination conditions were as described above (assay I). Primed and non-primed seeds were assayed at the temperatures of 13 °C, 20 °C, 30 °C, 33 °C, 35 °C and 38 °C, and the resulting germination time courses were fitted by Weibull function (Dumur et al., 1990). The time (days) to 50% of the seeds to germinate ( $t_{50\%}$ ) were estimated from each curve and the germination rate (GR) was calculated as the reciprocal of  $t_{50\%}$ , or  $t_{5\%}$  if the germinability was low. The GR values were regressed against

suboptimal temperatures, and the x-intercept from each of these regressions was the estimated base temperature ( $T_b$ ) (Steinmaus et al., 2000), whereas the thermal times ( $\theta_{\text{infra}}$ ) to germination were equal to the inverse of the slopes of the lines (Bradford, 2002). Similarly, the values of  $T_c$  and  $\theta_{\text{supra}}$  were estimated from the regression line of GR against supra-optimum temperatures. The expected germination rates ( $1/t_{50}$ ) at different temperatures (Figure 1) were calculated according to the equations:  $1/t_{50} = (T - T_b) / 10^{(\text{probit}(50) - a) \cdot b}$ , for infra optimum temperatures; and  $1/t_{50} = [10^{(\text{probit}(50) - a) \cdot b} - T] / \theta$ , for supra optimum temperatures, where  $a$  and  $b$  are,



**Figure 1.** Temperature dependence on the germination rate ( $1/t_{50\%}$ ) *Urochloa brizantha* seeds primed in PEG 6000 –0.5 MPa (A, C) and distilled water (B, D) at the temperatures of 20 °C (A, B) and 30 °C (C, D). The germination rate of non-primed seeds is also presented (E). Germination under continuous white light. Lines were fitted according to the thermal time model (see Material and Methods for details).

respectively, the intercept and the slope of the regression line of the cumulative germination percentages transformed to probit on  $\log \theta_{(g)}$ , for infra-optimum T, or on  $\log Tc_{(g)}$ , for supra-optimum T (Ellis et al., 1987).  $\text{Probit}_{(50)}$  is the median probit corresponding to 50% germination.

A completely randomized experimental design was used in the assays (Vieira, 1999). The germinabilities and average germination rate were tested for homogeneity of the variances (Bartlett test) and submitted to Anova if the variances were homogeneous. The Tukey's test ( $\alpha = 0.05$ ) was applied to the significant differences.

### 3. Results

#### 3.1. Assay 1

When the seed water content was measured just after priming, lower water content was observed in seeds pre imbibed at 8 °C relative to that imbibed at 32 °C and 39.5 °C (Table 1). The water uptake during priming was improved by imbibition in distilled water (DW) as compared to polyethylene glycol 6000 (PEG), except at 8 °C in which no difference occurred between DW and PEG treated seeds. After drying the differences in seed water content among the pre-imbibitions treatments disappeared and the seed moisture was approximately 13% (Table 1).

The germinability of *Urochloa brizantha* at 8 °C did not exceed 10% and attained the highest values in seeds primed at 32 °C in PEG (32-PEG), which germination was significantly higher than in not primed seeds (Figure 2A).

**Table 1.** Water contents of *Urochloa brizantha* cv. Basilisk seeds primed at the temperatures of 8 °C (8), 20 °C (20), 30 °C (30), 32 °C (32) and 39.5 °C (39.5) in distilled water (DW) and -0.5 MPa polyethylene glycol solution (PEG). Data were taken either immediately after the seeds were removed from priming treatments (not dried) or after the primed seeds were dried 72h at 25 °C (dried).

priming treatment (assay 1)	percentage of water (fresh weight basis)	
	not dried	dried
8-DW	25.5 ± 0.00 <sup>c</sup>	13.0 ± 0.19 <sup>a</sup>
8-PEG	25.3 ± 0.03 <sup>c</sup>	13.2 ± 0.20 <sup>a</sup>
32-DW	33.5 ± 0.05 <sup>a</sup>	12.9 ± 0.25 <sup>a</sup>
32-PEG	30.1 ± 0.05 <sup>b</sup>	13.1 ± 0.20 <sup>a</sup>
39.5-DW	33.4 ± 0.05 <sup>a</sup>	13.0 ± 0.17 <sup>a</sup>
39.5-PEG	30.1 ± 0.02 <sup>b</sup>	13.3 ± 0.21 <sup>a</sup>
priming treatment (assay 2)		
20-DW	29.5 ± 0.35 <sup>c</sup>	9.6 ± 0.25 <sup>a</sup>
20-PEG	29.0 ± 0.09 <sup>c</sup>	9.7 ± 0.16 <sup>a</sup>
30-DW	34.2 ± 0.23 <sup>a</sup>	9.3 ± 0.16 <sup>a</sup>
30-PEG	30.8 ± 0.48 <sup>b</sup>	9.4 ± 0.08 <sup>a</sup>

Values are followed by ±SE; small letters represent Tukey's test for means comparisons ( $\alpha = 0.05$ ). Control group (seeds without priming) represented 12.8%±0.02 of moisture content (fresh weight basis).

The germination rate at 8 °C was significantly promoted by priming at 32 °C both in distilled water (32-DW) and PEG 6000 solution (32-PEG) as compared to not primed seeds (control) and other priming treatments (Figure 2B). At the germination temperature of 32 °C the germinabilities did not differ statistically amongst the priming treatments and between primed and not primed seeds, with exception of the seeds primed in PEG at 39.5 °C (39.5-PEG), which germination was slightly lower although it did not differ from control (Figure 2C). Priming in distilled water at 8 °C (8-DW) and 32-DW improved significantly ( $P < 0.05$ ) the germination rate in comparison to not primed seeds (Figure 2D). At 39.5 °C, the highest germination (around 30%) was obtained for seeds primed at 32 °C in polyethylene glycol solution, which germinability was higher than control (Figure 2E). The germination rate at that supra-optimal temperature was promoted by priming in 32-DW, as compared to control (Figure 2F).

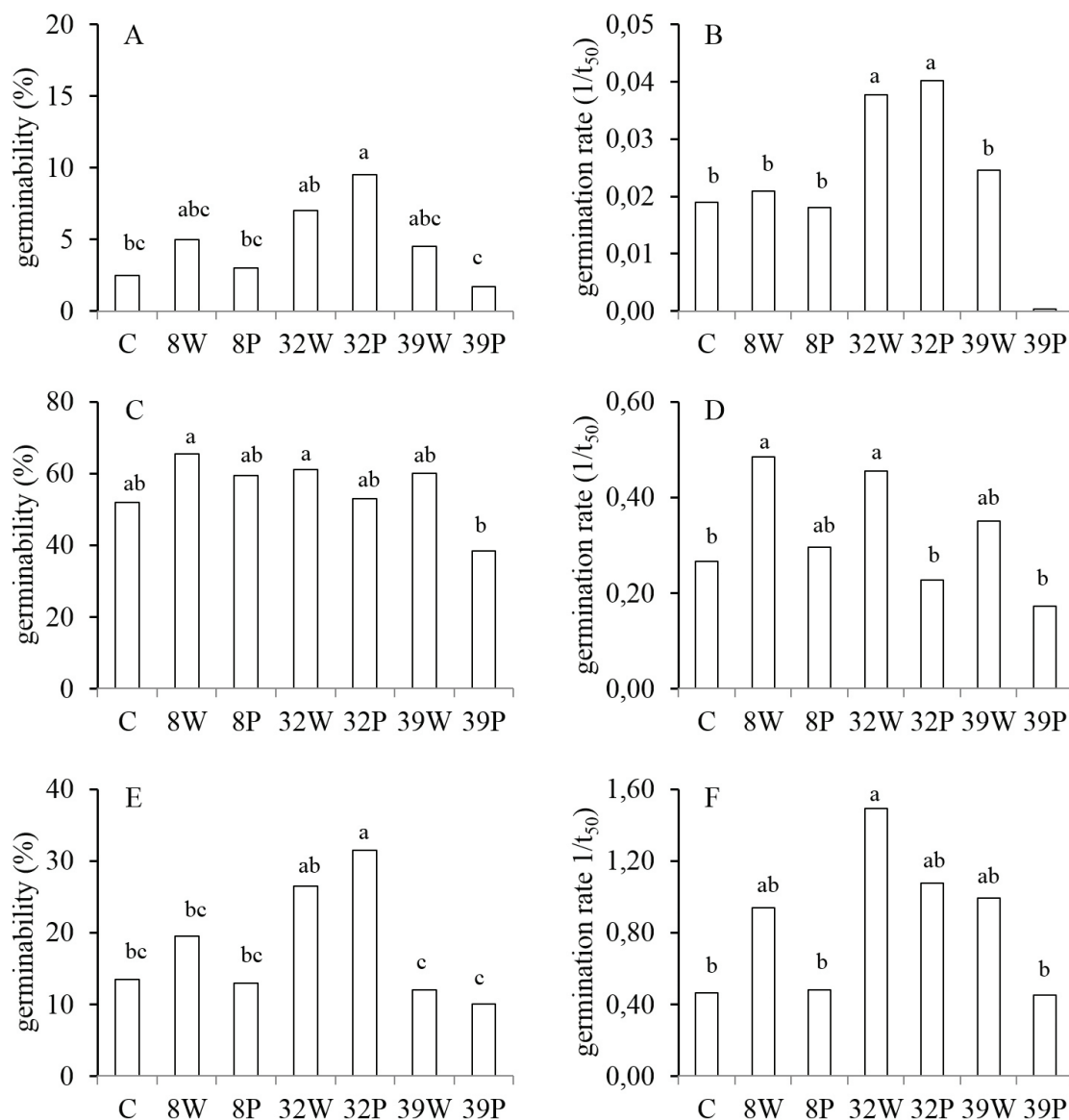
#### 3.2. Assay 2

The water content measured immediately after priming was lower in seeds primed in PEG solution than in DW at the temperature of 30 °C, whereas no difference between the priming media occurred at 20 °C (Table 1). After drying the seed water content was similar ( $\approx 9.5\%$ ) among pre-imbibitions treatments (Table 1).

The germinability at the temperatures of 13 °C (infra-optimal), 30 °C (optimal) and 38 °C (supra-optimal) was not affected by priming treatments whereas the germination rate ( $1/t_{50\%}$ ) both at 13 °C and 38 °C was promoted by priming in distilled water at 20 °C (Table 2). The germination rate (GR) of seeds put to germinate at 30 °C was not increased by priming the seeds both in -0.5 MPa PEG solution and distilled water (Table 2). Priming in PEG at 30 °C (30-PEG) inhibited the GR of seeds placed to germinate either at 13 °C, 30 °C or 38 °C (Table 2). Otherwise, when compared to not primed seeds, the pre-treatment 30-PEG caused a decrease in the base temperature ( $T_b$ ) as well as an increase of thermal time requirement for 50% ( $\theta_{50}$ ) of the seeds to germinate at the infra-optimum temperature range (Table 2). Pre-treatment with distilled water at 30 °C (30-DW) also resulted in a significant ( $\alpha = 0.05$ ) decrease in  $T_b$ , although it did not affect  $\theta_{50}$  (Table 2). Both the ceiling temperature ( $T_{c50}$ ) and  $\theta_{50}$  for supra-optimal temperatures were not influenced by priming treatments (Table 2). The analysis of the temperature dependence on the germination rate of *Urochloa brizantha* based on the predicted curves according to the thermal time model suggests a displacement of the optimum temperature for germination ( $T_o$ ) toward higher values in response priming (Figure 1). The model in general matched actual germination rate, as can be seen in Figure 1 which compares actual (symbols) and predicted (smooth line) values.

### 4. Discussion

The water content of *Urochloa brizantha* seeds soaked for 24h in PEG and DW was affected both by temperature and priming medium. Relatively low priming temperatures



**Figure 2.** Germinability (A, C, E) and germination rate (B, D, F) of *Urochloa brizantha* seeds primed in distilled water (W) and polyethylene glycol  $-0.5\text{MPa}$  solution (P) at 8 °C (8), 32 °C (32) and 39.5 °C (39), and placed to germinate at 8 °C (A, B), 32 °C (C, D) and 39.5 °C (E, F) under continuous white light. C = not primed seeds. Small letters (Tukey test,  $\alpha = 0.05$ ) compare treatments within each germination temperature.

caused lower water content (WC), whereas imbibition in PEG ( $-0.5\text{MPa}$ ) tended to reduce WC as compared to distilled water, although no effect of the osmotic medium was observed at relatively low (8 °C and 20 °C) priming temperatures. That response was expected since earlier reports show that temperature affects the rates of water uptake primarily by changing the water viscosity, although the increase in water uptake with temperature can be discontinuous from 5 °C to 35 °C as reported for pine embryos in which the slope of the regression line of the water uptake on temperature was significantly steeper above 20 °C (Murphy and Noland, 1982). Once the previously imbibed *U. brizantha* seeds were dried

for 72h at 25 °C the seed water content was found to be similar among priming treatments within each assay, ensuring that WC was similar among the seeds before use in the germination tests. Otherwise, the seeds used in the assay I and II differed in water content probably due to the assays were performed in different times, and the storage environment conditions must have influenced the results.

In the assay I the priming temperatures were the same used in the germination assays. Under the conditions described in the present assay the germination response of *U. brizantha* seeds to priming can be influenced by the temperature and priming medium as well as by the germination temperature. When the priming osmotic

**Table 2.** Thermal parameters from germination data (Assay II) of primed *Urochloa brizantha* seeds. The priming treatments were: distilled water, at 20°C (20-DW) and 30 °C (30-DW); and PEG 6000, at 20 °C (20-PEG) and 30 °C (30-PEG). Not primed seeds were used as control (C). Means  $\pm$  sd. Small letters compare treatments (Tukey test,  $\alpha = 0.05$ ).

	C	20-DW	20-PEG	30-DW	30-PEG
Tb (°C)	7.0 $\pm$ 0.8 <sup>a</sup>	5.4 $\pm$ 1.0 <sup>ab</sup>	6.0 $\pm$ 1.3 <sup>ab</sup>	4.2 $\pm$ 0.5 <sup>bc</sup>	1.6 $\pm$ 1.8 <sup>c</sup>
$\theta T_{(50)}$ (° day)	57.5 $\pm$ 5.6 <sup>b</sup>	61.6 $\pm$ 7.1 <sup>b</sup>	72.2 $\pm$ 5.4 <sup>b</sup>	77.2 $\pm$ 13.3 <sup>b</sup>	110.1 $\pm$ 11.2 <sup>a</sup>
Tc <sub>(50)</sub> (°C)	53.3 $\pm$ 4.0	55.8 $\pm$ 4.1	55.0 $\pm$ 6.3	48.9 $\pm$ 6.9	49.2 $\pm$ 3.7
$\theta_{(50)}$ (° day)	55.7 $\pm$ 10.9	56.9 $\pm$ 16.5	57.4 $\pm$ 21.0	50.2 $\pm$ 32.4	56.7 $\pm$ 20.8
G <sub>13°C</sub> (%)	51.0 $\pm$ 6.9	53.0 $\pm$ 1.1	51.0 $\pm$ 4.1	53.5 $\pm$ 4.9	50.5 $\pm$ 1.9
G <sub>30°C</sub> (%)	64.5 $\pm$ 2.5	57.5 $\pm$ 10.5	58.5 $\pm$ 7.4	58.5 $\pm$ 8.8	51.5 $\pm$ 6.3
G <sub>38°C</sub> (%)	52.5 $\pm$ 1.9	55.5 $\pm$ 7.6	51.5 $\pm$ 4.3	49.0 $\pm$ 5.2	44.5 $\pm$ 4.3
GR <sub>13°C</sub> (1/t <sub>50%</sub> )	0.09 $\pm$ 0.002 <sup>b</sup>	0.10 $\pm$ 0.004 <sup>a</sup>	0.08 $\pm$ 0.004 <sup>c</sup>	0.10 $\pm$ 0.007 <sup>ab</sup>	0.08 $\pm$ 0.004 <sup>c</sup>
GR <sub>30°C</sub> (1/t <sub>50%</sub> )	0.39 $\pm$ 0.029 <sup>a</sup>	0.36 $\pm$ 0.054 <sup>ab</sup>	0.32 $\pm$ 0.028 <sup>b</sup>	0.31 $\pm$ 0.027 <sup>b</sup>	0.22 $\pm$ 0.024 <sup>c</sup>
GR <sub>38°C</sub> (1/t <sub>50%</sub> )	0.28 $\pm$ 0.024 <sup>bc</sup>	0.35 $\pm$ 0.025 <sup>a</sup>	0.31 $\pm$ 0.009 <sup>ab</sup>	0.23 $\pm$ 0.018 <sup>cd</sup>	0.19 $\pm$ 0.025 <sup>d</sup>

Tb = base temperature;  $\theta T_{(50)}$  = thermal time for 50% germination (infra-optimum temperature range); Tc<sub>(50)</sub> = ceiling temperature for 50% germination;  $\theta_{(50)}$  = thermal time for 50% germination (supra-optimum temperature range); G<sub>c</sub> = germinability at 13 °C, 30 °C or 38 °C; GR °C = germination rate at 13 °C, 30 °C or 38 °C.

potentials of 0.0 MPa (DW) and -0.5 MPa (PEG) were compared to each other the former tended to be more effective than the latter as priming agent chiefly when the *U. brizantha* seeds were placed to germinate at 32 °C, an “optimum” temperature (Nakao, 2012). Otherwise, when the effect of the priming treatments were compared to not primed control, and taking into account the effect both on the germinability and germination rate, the priming effect on *U. brizantha* seeds appeared to be more pronounceable both nearby the lower and upper thermal limits reported for germination of the species (Nakao, 2012), what is partially in agreement with Hardegree and Van Vactor (2000) which reported that the germination rate of some grasses was enhanced by priming, and the effect was most notable in the cooler temperature treatments. Furthermore, assays with other species, such as *Capsicum annuum* L (Posse et al., 2001) indicate that the use of primed seeds is only viable when they are sowed at sub or supra-optimal temperatures. The results presented here also are in accordance with a previous report with *U. brizantha* cv. Marandú (Lima, 2007) which shows a dependence on the exposure time and concentration of the priming agent of the seed response to priming. The relative low effect of the priming temperature of 8 °C on the germination of *U. brizantha* as compared to 32 °C can be accounted for the lower water in the former, suggesting that the advancement of early physiological processes related to seed germination during priming can be limited by the seed water levels. Otherwise, the lack of response of *U. brizantha* seeds to a supra optimum priming temperature was not related to the seed moisture since the percentage of water was similar between the priming temperatures of 32 °C and 39.5 °C. More investigations are required in order to access for the lower and upper temperature limits to priming of *U. brizantha*, considering that some physiological and metabolic activities related to progress toward germination show a threshold-type at low  $\Psi$  and temperature (Bradford, 2002), although no

model has yet been proposed that uses parameters for ceiling temperatures.

In the assay II the seeds were primed in 0.0 and -0.5 MPa at the temperatures of 20 °C and 30 °C chosen based on Zehtab-Salmasi (2006), to access for a possible effect of the priming temperature on the T-dependence on the germination of *U. brizantha* as described through the thermal time model. The temperature dependence on the germinability of *U. brizantha* changed with priming since the optimum temperature for germination shifts toward warmer temperatures in primed seeds. Reinforcing the results obtained in the assay I, priming effects on the germination rate of *U. brizantha* seeds were observed only at the germination temperatures of 13 °C and 38 °C, close to the lower and upper thermal limits for germination, respectively (Nakao, 2012). Those results can neither be explained in terms of variation in the ceiling temperature (Tc<sub>(50)</sub>) or the amount of thermal time ( $\theta T_{(g)}$ ) required for germination, since Tc<sub>(50)</sub> values were similar among the treatments and  $\theta T_{(g)}$  values were not reduced by priming. Otherwise, a decrease in the base temperature (Tb) was observed in primed seeds, supporting the hypothesis that priming could improve the germination rate of *U. brizantha* by reducing Tb. However, the results presented here did not support our hypothesis that a priming effect on the germination of *U. brizantha* can be accounted for a decrease in  $\theta T_{(g)}$ , as reported by Hardegree and Van Vactor (2000) in some grasses, and an increase in Tc<sub>(g)</sub>, considering that the germination rate is directly proportional to Tc<sub>(g)</sub> and inversely proportional to Tb for a given T and  $\theta$  (Garcia-Huidobro et al., 1982). In onion (*Allium cepa* L.) seeds the major effect of priming was to reduce  $\theta_{(g)}$  at both sub- and supra-optimal temperatures (Ellis and Butcher, 1988), but this feature was not observed in the present study, suggesting such parameters may have a strong genotypic basis which is relatively unaffected by environmental factors prior the visible germination in *U. brizantha*. However, further assays testing more priming conditions are required

to ascertain that hypothesis. Based on the relationship between  $T_{c(g)}$  and base water potential ( $\Psi_{b(g)}$ ) (Alvarado and Bradford, 2002), the results presented here suggest that priming treatments did not affect the  $\Psi_{b(g)}$  distribution in the seed population of *Urochloa brizantha*, however further experiments must be conducted in a wide range of water potentials in order to determine a possible variation of the base water potential ( $\Psi_{b(g)}$ ) and, thus, evaluate the sensitivity of *U. brizantha* seeds to water deficit (Bradford and Somasco, 1994).

The results presented here show that the response of *U. brizantha* seeds to temperature can be affected by priming, depending on the temperature and water potential conditions at which the seeds are pre-imbibed. The general conclusions can be drawn from the two assays are that priming tend to improve the germination at temperatures closer to the upper and lower limit for germination and, with exceptions, it has no effect when the seeds are placed to germinate at temperatures close the optimum. The thermal time model described relatively well the isothermal germination rate seeds, although the variation in the germination responses to priming treatments was not consistently explained by the thermal-time and  $T_{c(g)}$  variation. However, the  $T_b$  distribution in *U. brizantha* seeds can be influenced by priming.

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