Universidade Federal Rural do Semi-Árido
Pró-Reitoria de Pesquisa e Pós-Graduação
https://periodicos.ufersa.edu.br/index.php/caatinga

ISSN 0100-316X (impresso)
ISSN 1983-2125 (online)

PRIMING OF BRACHIARIA SEEDS WITH DIFFERENT SUGAR SOURCES AND CONCENTRATIONS[1]

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ABSTRACT - Seed priming is a practice for improving the expression of seed physiological potential. Such technique consists of synchronizing and reducing the time of seed germination by controlled hydration. The aim of this study was to evaluate the effect of seed-priming with different sugar sources and concentrations on the physiological quality *Urochloa brizantha* seeds and initial seedling performance. Before treating, seeds were scarified chemically with concentrated sulphuric acid (H_2SO_4) for 5 minutes to overcome physical dormancy. The experimental design was completely randomized in a 3 x 6 factorial scheme consisting of priming using three sugar sources (glucose, sucrose, and maltose) and six concentrations (zero [water control], 2%, 5%, 10%, 15%, and 20%), with four replicates. The seeds were primed by direct immersion for 2 hours at 25 ºC and, after hydration, they were dried for moisture equilibrium recovery. Seed germination, vigor, viability, and initial seedling growth were evaluated. The results showed that glucose was the source able to promote beneficial effects on the germination of *U. brizantha* cv. MG-5 seeds. Moreover, the supply of glucose at the concentrations of 2 and 5% for physiological conditioning increased seedling dry phytomass.

Keywords: *Urochloa brizantha*. Glucose. Sucrose. Maltose. Direct immersion.

CONDICIONAMENTO FISIOLÓGICO COM DIFERENTES FONTES E CONCENTRAÇÕES DE GLICÍDIOS EM SEMENTES DE BRAQUIÁRIA

RESUMO - O condicionamento em sementes é uma prática capaz de possibilitar maior expressão do potencial fisiológico das sementes. Esta técnica permite a sincronização e redução do tempo de germinação das sementes através da hidratação controlada. O objetivo foi avaliar o efeito do condicionamento fisiológico em sementes de *Urochloa brizantha* com diferentes fontes e concentrações de glicídios na qualidade fisiológica de sementes e desempenho inicial das plântulas. Anterior a aplicação do priming as sementes foram submetidas a escarificação química com ácido sulfúrico concentrado (H_2SO_4) por 5 minutos para remoção da dormência primária. O delineamento experimental foi o inteiramente casualizado, em esquema fatorial 3 x 6, constituído por condicionamento fisiológico utilizando três fontes de glicídios (glicose, sacarose e maltose) e seis concentrações (zero [controle em água], 2%, 5%, 10%, 15% e 20%), com quatro repetições. O condicionamento fisiológico utilizado foi via imersão direta por 2 horas a 25 ºC e, posteriormente a hidratação das sementes, foi realizada a secagem para a retomada da umidade de equilíbrio. Foi realizado teste de germinação, vigor, viabilidade das sementes e crescimento inicial de plântulas. A glicose como fonte de glicídio promoveu efeitos benéficos na germinação de sementes de *U. brizantha* cv. MG-5. O fornecimento de glicose nas concentrações de 2 e 5% pelo condicionamento fisiológico propiciaram incremento na fitomassa seca de plântulas.

Palavras-chave: *Urochloa brizantha*. Glicose. Sacarose. Maltose. Imersão direta.

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[1]Received for publication in 03/21/2017; accepted in 03/16/2018.
Extracted from the research project of the second author.
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INTRODUCTION

The use of *Urochloa* spp. in pasture areas have increased in Brazil, which stands out as the largest producer and exporter of tropical seeds worldwide (CARDOSO et al., 2014). Studies on this topic are of great agronomic importance since this species presents seed dormancy.

More recently, new studies have been developed to improve seed vigor such as by priming treatment. This technique consists of synchronizing and reducing the time of seed germination (MARCOS-FILHO, 2015) by controlled hydration, triggering the start of physiological processes (germination phases I and II), without radicle protrusion at phase III (LARA et al., 2014). These techniques aim at standardizing germination to establish an appropriate seedling stand.

Although not used on a large scale for major crops, seed priming has application in germplasm banks for small seed numbers (SILVA et al., 2016). Studies found in the literature have demonstrated that the priming of Brachiaria seeds increases the number of germinated seeds (CARDOSO et al., 2014), speed index (BINOTTI et al., 2014), and resistance to thermal stress (BATISTA et al., 2016a).

Sugars play an important role as energy supply during seed germination (MARCOS-FILHO, 2015) and in plant growth and development as both energy sources and signaling molecules (LEE et al., 2012). While studying the germination of *Arabidopsis* sp. seeds, Gibson, Laby and Kim (2001) observed that small concentrations of glucose and sucrose (nearly 0.0015% and 0.0185%, respectively) were able to increase the number of germinated seeds.

In contrast, controversial hypotheses about the effect of high concentrations of sugars on seed germination can also be verified, such as the reporting by Eveland and Jackson (2011), who suggested that an increase in sugar concentration, e.g. glucose, may raise the levels of abscisic acid (ABA) and that both components tend to act synergistically during embryonic growth, thus hindering germination.

Little is known about the performance of sugars in seed germination and plant growth, there being only controversial and poorly comprehensive information on this subject. Thus, studies on the influence of sugars on seed germination are still necessary to clarify their performance in plant metabolism. Based on this, this study aimed to verify whether the use of sugars in the priming of *Urochloa brizantha* seeds influences seed quality and seedling initial performance.

MATERIAL AND METHODS

The experiment was conducted in 2013 at the Seed Analysis Laboratory of the *Universidade Estadual de Mato Grosso do Sul* (State University of Mato Grosso do Sul), in Cassilândia, Mato Grosso do Sul state, Brazil, using *U. brizantha* cv. MG-5 seeds from the 2011/12 growing season. The initial physiological characteristics of the seeds were: water content = 11%; total germination = 51%; dormant seeds = 38%.

Prior to treatment, seeds were chemically scarified with concentrated sulphuric acid (H_2SO_4) for 5 minutes to overcome physical dormancy and, after that, they were washed and placed in deionized running water and naturally dried for 24 h (BATISTA et al., 2016b).

The experiment followed a completely randomized design (CRD) in a 3 x 6 factorial scheme, with four replicates for each treatment. The first factor consisted of seed priming with three sugar sources (glucose, sucrose, and maltose), while the second was six different concentrations of each sugar source [zero (water control), 2%, 5%, 10%, 15% and 20%].

Seeds were primed using hydration at 25 °C for 2 h. After priming, the seeds were dried at room temperature for 24 h (CARDOSO et al., 2014). Then, pure seeds were selected to perform physiological tests.

Four subsamples of 50 seeds were used for a germination test. The seeds were distributed into plastic germination boxes, using as substrate blotting paper moistened with 2.5 times its dry mass. The counts were performed on the 7th (first germination count) and on the 21st (total germination) days after sowing (DAS), as established by the Brazilian Rules for Seed Analysis (BRASIL, 2009).

Germination speed index was calculated by the sum of the number of germinated seeds at each day, divided by the number of days between sowing and germination adapted from (MAGUIRE, 1962).

After germination test, the remaining seeds (viable and unviable) were submitted to tetrazolium test, according to a standard method for *Urochloa* (*Brachiaria*) seeds (BRASIL, 2009). It consisted of counting the number of viable and remaining unviable seeds from the germination test.

An electrical conductivity test was also carried out using four subsamples of 50 seeds, weighed to at least two decimal places. The seeds were imbibed in 75 mL of deionized water and maintained at 25 ºC for 24 hours. Then, the soaking solution was measured for electrical conductivity (EC), with results expressed in $\mu S\ cm^{-1}\ g^{-1}$ (VIEIRA; KRYZANOWSKI, 1999).

The emerged plants were recorded until emergence stabilization, calculating the percentage for the 7th (first emergence count) and for the 28th (total emergence) DAS. The results were expressed in percentage of emerged seedlings.

Emergence speed index (ESI) was also

estimated by the sum of the number of emerged seeds at each day, divided by the number of days between sowing and emergence adapted from (MAGUIRE, 1962), until the number of emerged seedlings stabilized, within a 28-day period after sowing.

At the end of emergence test, twenty normal seedlings from each repetition were randomly removed, maintaining the root system intact, and then shoot and root lengths were measured.

After washed and dried in a forced-air oven at 65°C for 72 h, the dry phytomass of these seedlings was randomly evaluated using an analytical scale, with values expressed in mg seedling $^{-1}$.

All the data were analyzed through analysis of variance (ANOVA) using the F test. In case of significant differences among treatments, the Tukey's comparison test ($p<0.05$) was applied for sugar sources and a regression adjustment for the sugar concentrations. For the remaining unviable seeds, the data were analyzed after normalization by transforming them to square root arcsine (x/100).

RESULTS AND DISCUSSION

An interaction among the studied factors was verified for total germination and remaining unviable seeds (Figure 1 and 2). By comparing priming with glucose and maltose at concentrations of 2 and 10%, germination percentage increased by 84 and 87%, respectively. And, when compared with sucrose at 15%, this increase was 85%. Moreover, a positive linear equation was found to fit the glucose data, indicating that this source promoted a linear increase in the germination of *U. brizantha* cv. MG -5 seeds (Figure 1).

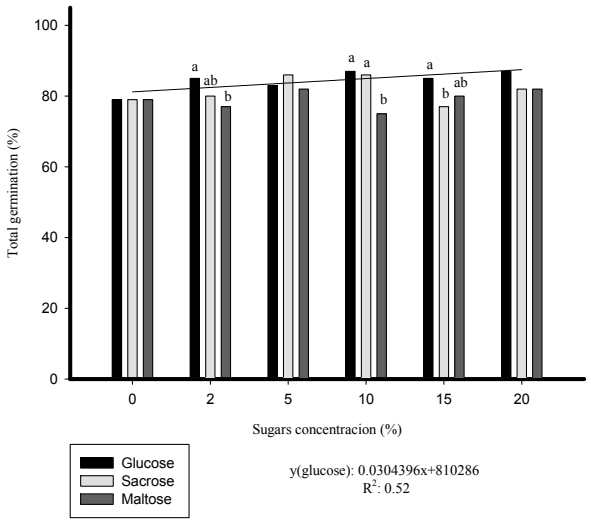

Figure 1. Effect of priming with different sugar sources and concentrations on total germination of *U. brizantha* seeds. Means followed by different letters in the column differ statistically from each other by the Tukey's test at 5% probability.

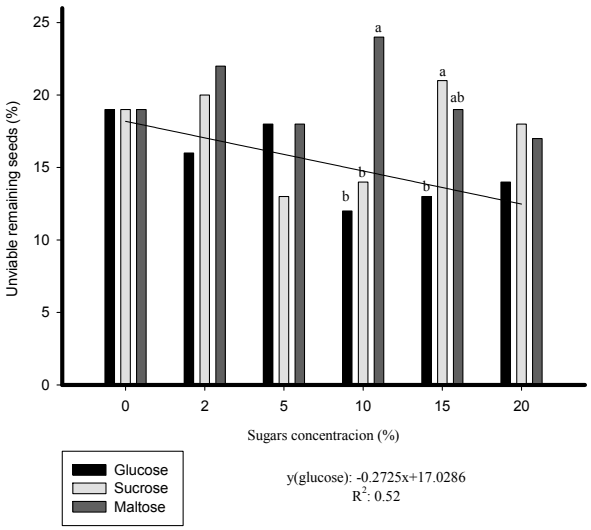

Figure 2. Effect of priming with different sugar sources and concentrations on remaining unviable seeds of *U. brizantha*. Means followed by different letters in the column differ statistically from each other by the Tukey's test at 5% probability.

When comparing the use of glucose and sucrose at 10% to maltose at 24%, the number of remaining unviable seeds decreased in 11% and 14%, respectively. In addition, the use of 15% glucose provided a lower number of dead seeds (13%) if compared to 21% sucrose, but not differing from maltose. For this variable, a negative linear equation was found to fit glucose data, showing that increasing concentrations of this source decrease the number of dead seeds (Figure 2).

The benefits of priming by direct immersion for *U. brizantha* seeds have already been reported in the literature, both with water or other chemical agents (BATISTA et al., 2016a; BATISTA et al., 2016b). In this study, there was an additional benefit to the effect of priming using glucose during conditioning by direct immersion under germination.

In studies conducted with *Arabidopsis* spp. seeds, Arenas-Huertero et al. (2000) observed the influence of glucose on plant development and seed germination, and Price et al. (2003) pointed out that this sugar source reduces the effect of ABA, accelerating germination. Such reduction of ABA is essential for germination to occur since the high concentration of this hormone inhibits embryo development (OLIVEIRA JÚNIOR et al., 2009). These findings corroborate the results obtained in the present study for *U. brizantha* seeds, in which increasing glucose concentrations improved the percentage of germinated seeds and, if compared to the other sugar sources, this effect may be even higher for certain concentrations. In addition, since the primary dormancy of *U. brizantha* seeds had already been overcome by chemical scarification, glucose was responsible for promoting the germination process and preventing a secondary dormancy mechanism, thus leading to greater germination when supplied during the priming.

Increases in germination rate and speed were observed at 7 days when glucose and sucrose were used as a sugar source in conditioning if compared to the use of maltose (Table 1).

Table 1. Effect of priming with different sugar sources and concentrations on the first germination count (FGC), germination speed index (GSI), remaining viable seeds after germination test, and electrical conductivity for *U. brizantha* seeds.

Sugar sources	Germination		Remaining viable seeds	Electrical conductivity
	FGC	GSI		
	-----(%)-----		-----(%)-----	--(μS cm^{-1} g^{-1})--
Glucose	80 a	5.87 a	1	30.66 ab
Sucrose	79 a	5.74 a	0	33.33 a
Maltose	76 b	5.52 b	0	29.61 b
Sugar concentrations (%)				
0	77	5.52	2	41.90
2	78	5.65	0	26.59
5	80	5.85	0	27.28
10	81	5.83	0	30.74
15	77	5.60	1	28.78
20	80	5.80	0	31.90
F Source	7.51**	8.21**	0.94^{ns}	4.69**
Regression adjustment	ns	ns	Ns	ns
CV (%)	5.68	5.33	7.29	17.03

Means followed by different letters in the column differ statistically from each other by the Tukey's test at 5% probability; ** Significant at 1% probability; ^{ns} non-significant; CV – coefficient of variation.

But unlike these results, when studying the control of plant development and gene expression by using sugar, Gibson (2005) reported that increasing levels delayed the germination of *Arabidopsis* seeds. Interestingly, the results of this study showed a contrary effect on *U. brizantha* seeds since sugar addition during priming had no negative effect on germination speed.

By the EC test, the use of maltose showed a decrease in the content of leachates in the imbibition solution compared to sucrose use, but not differing from glucose (Table 1). Such a reduction indicates a greater integrity of cell membranes and has a direct influence on seed vigor. Therefore, the EC test was inefficient in predicting the vigor of seeds conditioned in glucose, as the first germination count and GSI test showed increases when using glucose and sucrose.

The studied factors had no influence on the variables first emergence count, total emergence, and ESI (Table 2). However, shoot length was higher using sucrose compared to the use of maltose, but

not differing from glucose. Yet the root length data were adjusted to a quadratic equation in response to the used concentrations, with a maximum value when applying 11.58% of the tested sugars (Table 2).

Corroborating the results of this study, Lee et al. (2012) found no inhibition of root growth for *Arabidopsis* mutants when in the presence of 6% glucose. However, at 1% glucose, the results suggested a reduction in the cell division of the root meristem, which results in a delay in root growth. Therefore, higher sugar concentrations could benefit plant growth, the longest roots were found at a sugar concentration of 11.58%.

Table 2. Effect of priming with different sugar sources and concentrations on the first emergence count (FEC), total emergence (TE), emergence speed index (ESI), and shoot and root length of *U. brizantha* seedlings.

Sugar sources	Emergence			Length	
	FEC	TE	ESI	Shoot	Root
	----------(%)----------			--(cm seedling^{-1})--	
Glucose	74	76	5.37	13.15 ab	15.84
Sucrose	75	78	5.53	13.76 a	15.38
Maltose	75	77	5.47	12.33 b	15.57
Sugar concentrations (%)					
0	74	77	5.42	12.12	14.32
2	77	80	5.63	13.74	15.83
5	74	76	5.39	13.50	15.87
10	72	73	5.26	12.80	16.06
15	77	79	5.58	12.74	15.88
20	75	78	5.45	13.58	15.61
F Source	0.45^{ns}	0.38^{ns}	0.56^{ns}	5.51**	0.80^{ns}
Regression adjustment	ns	Ns	Ns	ns	QR $^{(1)}$
CV (%)	9.58	9.82	9.69	11.40	8.19

Means followed by different letters in the column differ statistically from each other by the Tukey's test at 5% probability; ** Significant at 1% probability; ^{ns} non-significant; ^{QR} quadratic regression; $^{(1)}$ $y = -0.01054076x^2+0.2443329x+14.807487$ and $R^2 = 0.68$; C.V. – coefficient of variation.

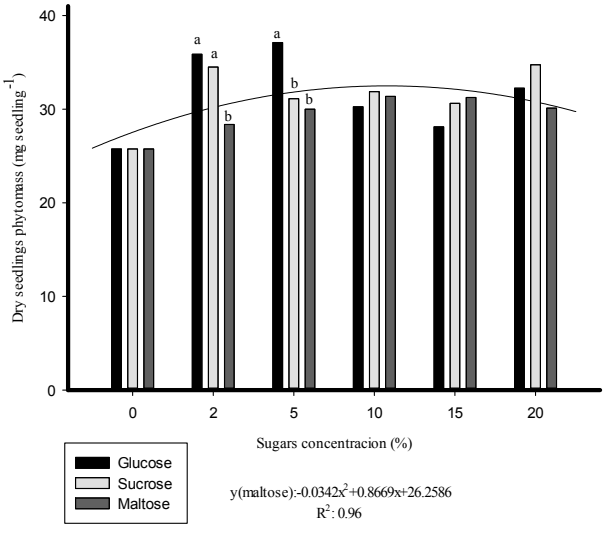

Figure 3. Effect of priming with different sugar sources and concentrations on the dry phytomass of *U. brizantha* seedlings. Means followed by different letters in the column differ statistically from each other by the Tukey's test at 5% probability.

Interactions among the studied factors were verified for seedling dry phytomass (Figure 3). At a concentration of 2%, glucose and sucrose showed higher accumulation of dry phytomass (35.87 and 34.50 mg seedling^{-1}, respectively) when compared to maltose (28.37 mg seedling^{-1}). For a concentration of 5%, the highest dry phytomass accumulation was verified when using glucose (37.12 mg seedling^{-1}) against the values of 31.12 mg seedling^{-1} and 30 mg seedling^{-1} by the use of sucrose and maltose, respectively. Data on maltose concentrations were adjusted to a quadratic equation with maximum dry phytomass reached when using a concentration of 12.68% (Figure 3).

As it acts on seed germination, glucose may favor seedling performance (PRICE et al., 2003). Eveland and Jackson (2011) found that carbohydrates (sugars) are essential in basic processes required for plant growth. The findings of this study reinforce these claims since there was an increase in the dry phytomass of *U. brizantha* seedlings conditioned with glucose.

The sugar sources used in the seed-priming technique promoted germination and initial development of *U. brizantha* cv. MG-5 plants, evidencing their potential to obtain seeds with a higher physiological quality.

CONCLUSIONS

The priming of seeds of *Urochloa brizantha* cv. MG-5 using glucose as a source of sugar promoted increases in germination rate, and the concentrations of 2 and 5% glucose provided increases in the dry biomass of its seedlings.

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