

# Physiological quality of *Urochloa brizantha* seeds submitted to priming with calcium salts<sup>1</sup>

Elizena Cristina Gomes Ribeiro<sup>2</sup>, Rodrigo de Góes Esperon Reis<sup>2</sup>,  
Cesar Crispim Vilar<sup>2</sup>, Flavia Carolina Moreira Vilar<sup>2</sup>

## ABSTRACT

Seed priming provides a variety of benefits, including seed invigoration and reduced dormancy. *Urochloa brizantha* seeds are typically primed with difficult-to-acquire potassium nitrate (KNO<sub>3</sub>). In this sense, calcium salts stand out as an alternative, but it is important to determine whether they provide the same benefits as potassium nitrate. This study aimed to evaluate the physiological quality of *U. brizantha* seeds after priming with calcium salts, and compare the results to those obtained with potassium nitrate. Seeds were submitted to priming with calcium nitrate [Ca(NO<sub>3</sub>)<sub>2</sub>] and calcium sulphate (CaSO<sub>4</sub>) at -0.55 MPa and -1.10 MPa, for 12 h and 24 h, and potassium nitrate (KNO<sub>3</sub>) at -1.10 MPa for 12 h, as well as to no priming (control). A completely randomized design, with ten treatments and four repetitions, was used. Priming promoted a higher germination percentage and lower seed dormancy, with a greater seedling emergence in a shorter time period. CaSO<sub>4</sub> did not differ from KNO<sub>3</sub> in shoot dry weight of seedlings, but reduced the electrical conductivity of seeds. Calcium nitrate and calcium sulfate show a promising potential for use in the osmotic priming of *Urochloa brizantha* (BRS Piatã cultivar) seeds, since they are as efficient as potassium nitrate, as well as less costly and easier to purchase. Calcium sulfate priming at -0.55 MPa for 12 h, at 25 °C, is recommended to replace the potassium nitrate solution.

**KEYWORDS:** Seed invigoration, calcium nitrate, calcium sulfate.

## RESUMO

Qualidade fisiológica de sementes de *Urochloa brizantha* submetidas a condicionamento com sais de cálcio

O condicionamento fisiológico proporciona vários benefícios, como melhoria no vigor das sementes e redução no grau de dormência. Em sementes de *Urochloa brizantha*, geralmente, se utiliza nitrato de potássio (KNO<sub>3</sub>), que é de difícil aquisição. Nesse sentido, os sais de cálcio se destacam, mas é necessário verificar se proporcionam os mesmos benefícios que o KNO<sub>3</sub>. Objetivou-se avaliar a qualidade fisiológica de sementes de *U. brizantha* após condicionamento com sais de cálcio, em comparação a KNO<sub>3</sub>. Utilizaram-se sementes submetidas a condicionamento fisiológico com nitrato de cálcio [Ca(NO<sub>3</sub>)<sub>2</sub>] e sulfato de cálcio (CaSO<sub>4</sub>) a -0,55 MPa e -1,10 MPa, por 12 h e 24 h, além de KNO<sub>3</sub> a -1,10 MPa, por 12 h, e sementes sem condicionamento (testemunha). Utilizou-se delineamento inteiramente casualizado, com dez tratamentos e quatro repetições. O condicionamento proporcionou menor porcentagem de sementes dormentes e maior porcentagem de germinação e de emergência de plântulas em menor tempo. O CaSO<sub>4</sub> não diferiu do KNO<sub>3</sub>, quanto à massa seca da parte aérea de plântulas, e reduziu a condutividade elétrica das sementes. Nitrato e sulfato de cálcio apresentam potencial promissor para serem utilizados no condicionamento osmótico de sementes de *U. brizantha* cv. BRS Piatã, visto que são tão eficientes quanto o KNO<sub>3</sub>, possuem menor custo e têm maior facilidade de aquisição. Recomenda-se o condicionamento em solução de sulfato de cálcio a -0,55 MPa por 12 h, a 25 °C, para substituir a solução de nitrato de potássio.

**PALAVRAS-CHAVE:** Envigoramento de sementes, nitrato de cálcio, sulfato de cálcio.

## INTRODUCTION

Grasses from the *Urochloa* (previously *Brachiaria*) genus have become increasingly important in the Brazilian livestock breeding due to their use as pasture (Valle et al. 2009), accounting for about 80 % of the pasture seed market (Costa et al. 2011). As such, Brazil has become the global leader

in *Urochloa* spp. seed production, consumption and exportation (Pereira et al. 2011).

The demand for *Urochloa* spp. seeds has increased considerably, largely due to the use of straw in no-tillage areas, providing benefits such as higher biomass production, better soil coverage and slower decomposition, which suppress weeds and prevent re-infestation (Timossi et al. 2007, Torres et al. 2008).

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2. Universidade do Estado de Mato Grosso, Faculdade de Ciências Agrárias, Biológicas e Sociais Aplicadas, Nova Xavantina, MT, Brasil. E-mail/ORCID: elizenacgr@gmail.com/0000-0001-5955-7805, guidegoes@gmail.com/0000-0003-3656-664X, cesarcvilar@gmail.com/0000-0002-5415-6057, flacmoreira@gmail.com/0000-0001-9589-7436.

*Urochloa* spp. seeds do not germinate uniformly because of external structures that inhibit gas diffusion and/or lead to undeveloped embryos (Munhoz et al. 2009, Binotti et al. 2014), compromising the pasture implementation and contributing to its degradation (Lacerda et al. 2010).

Studies aimed at overcoming seed dormancy are indispensable in obtaining better quality seed lots with high germination and vigor. In this respect, pre-germination treatments such as priming may reduce the percentage of dormant seeds, accelerating germination and homogenizing the seedling establishment (Abdoli 2014, Binotti et al. 2014, Cardoso et al. 2015, Paparella et al. 2015, Gebreegziabher & Qufa 2017, Batista et al. 2018, Pereira et al. 2018).

Priming involves the partial moistening of seeds, what helps to unlock the metabolic processes that occur during the initial germination phases, preventing radicle protrusion and maintaining seed tolerance to desiccation (Paparella et al. 2015, Ibrahim 2016). As a result, the seeds prepare for germination by restructuring membranes and reorganizing metabolic systems, what favors a faster uniform germination when conditions become more favorable, or in adverse environments (Jisha et al. 2013, Ibrahim 2016, Batista et al. 2018). Osmotic conditioning, or osmopriming, is a priming technique whereby seeds are soaked in a solution with low hydric potential, and water absorption occurs until their internal hydric potential and that of the solution are the same (Jisha et al. 2013, Hussian et al. 2014).

According to the scientific literature on seed priming, potassium nitrate ( $\text{KNO}_3$ ) has shown promising results in improving the physiological quality of several species (Anosheh et al. 2011, Entesari et al. 2012), including *Urochloa brizantha* (Bonome et al. 2006, Binotti et al. 2014, Cardoso et al. 2015). In addition to osmotic priming, most studies use other substances to boost seed germination and seedling establishment in the field, including nutrients, growth regulators and biostimulants (Cardoso et al. 2015, Ramos et al. 2015, Pallaoro et al. 2016, Batista et al. 2018).

Calcium salts such as calcium nitrate [ $\text{Ca}(\text{NO}_3)_2$ ] and calcium sulfate ( $\text{CaSO}_4$ ) are an alternative to  $\text{KNO}_3$ , can be easily purchased and are less costly. Studies have found that priming using nitrates overcomes dormancy and increases germination, since they act as oxidants, converting

NAD(P)H to  $\text{NAD(P)}^+$  in the pentose-phosphate metabolic pathway (Hendricks & Taylorson 1974, Cardoso et al. 2015). In the case of calcium sulfate, studies have shown accelerated emergence and decreased mortality in seeds planted in acidic soil (Murata et al. 2008), likely due to the immobilization of  $\text{Al}^{3+}$ , the main problem in these soils.

This study aimed to evaluate the physiological quality of *Urochloa brizantha* (BRS Piatã cultivar) seeds after priming with calcium salts, and compare the results to those obtained with potassium nitrate priming.

## MATERIAL AND METHODS

The experiment was carried out at the Universidade do Estado de Mato Grosso, in Nova Xavantina, Mato Grosso state, Brazil, from October to December 2016. The *Urochloa brizantha* (BRS Piatã cultivar) seeds were harvested in the 2015/2016 season.

Each treatment consisted of 20 g of seeds submitted to priming at 25 °C, in solutions of calcium nitrate [ $\text{Ca}(\text{NO}_3)_2$ ] and calcium sulfate ( $\text{CaSO}_4$ ), at -0.55 MPa and -1.10 MPa, for 12 h and 24 h, and a potassium nitrate ( $\text{KNO}_3$ ) solution at -1.10 MPa for 12 h (Bonome et al. 2006), as well as no priming (control). Each solution was prepared using the concentrations presented in Table 1, in order to obtain the desired osmotic potential.

The concentration of all solutions containing calcium salts and potassium nitrate were calculated using the Van't Hoff's equation (Hillel 1971):  $\Psi_{os} = -iRTC$ , where  $\Psi_{os}$  is the osmotic potential of the solution (MPa),  $i$  the isotonic coefficient,  $R$  the gas constant ( $0.0083 \text{ L MPa K}^{-1} \text{ mol}^{-1}$ ),  $T$  the temperature (K) and  $C$  the concentration ( $\text{mol L}^{-1}$ ).

The following equation was used to calculate the isotonic coefficient ( $i$ ):  $i = 1 + \alpha(q - 1)$ , where  $\alpha$  is the degree of ionization (decimal) and  $q$  the total

Table 1. Solutes, osmotic potential and concentrations of solutions used for osmotic priming of *Urochloa brizantha* (BRS Piatã cultivar) seeds.

Solute	Osmotic potential at 25 °C (MPa)	Concentration ( $\text{g L}^{-1}$ )
$\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$	-0.55	17.5568
	-1.10	35.1276
$\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$	-0.55	19.1816
	-1.10	38.3910
$\text{KNO}_3$	-1.10	22.5308

number of ions released during the ionization of a compound (mol).

Seeds from each treatment (20 g) were placed in 500 mL plastic containers to which 150 mL of priming solution were added in order to cover all the seeds. The control seeds did not receive any pre-treatment.

In order to prevent seed anoxia, all the solutions were aerated using silicone hoses connected to an aquarium compressor (MINJINAG, NS 750). After priming, the seeds were washed under running water for 5 min and dried at room temperature ( $\approx 25\text{ }^{\circ}\text{C}$ ) for 48 h, with a final moisture similar to the initial moisture content of 9 % (wet basis - wb).

The following tests were used to evaluate the seed physiological quality:

*Seed moisture* - measured after priming and drying, in triplicate, with 0.5 g of seeds per treatment placed in aluminum capsules and dried in an oven (ODONTOBRÁS, EL-1.4) at  $105\text{ }^{\circ}\text{C}$ , for 24 h. The moisture content was expressed in percentage (wb) (Brasil 2009);

*Germination* - quadruplicates of 50 seeds were planted in plastic germination boxes (gerbox) containing two sheets of blotting paper moistened with 14 mL of distilled water, and kept in a BOD incubator (ELETROlab, EL202/4) with temperatures alternating between  $20\text{ }^{\circ}\text{C}$  and  $35\text{ }^{\circ}\text{C}$  and photoperiod of 8 h. The germinated seeds were counted at 7, 14 and 21 days after planting (DAP), and germination percentages calculated after the first (7 DAP) and last (21 DAP) countings (Brasil 2009). Next, the non-germinated seeds were submitted to a tetrazolium test (0.1 %, at  $37\text{ }^{\circ}\text{C}$ , for 4 h) to check the percentage of dormant seeds (Brasil 2009);

*Emergence* - quadruplicates of 50 seeds were planted in sand beds, with daily irrigation and counting of emerged seedlings until 21 DAP, when counting stabilized. The emergence percentage and average emergence time were calculated based on the values obtained at counting (Labouriau 1983). Next, the emerged seedlings were cut at the ground level, placed in paper bags and dried in a ventilated oven (SOLAB, SL-102) at  $65\text{ }^{\circ}\text{C}$ , until constant weight, when the shoot dry weight was calculated. This result was then divided by 50 (initial number of seeds) to estimate the seedling vigor [ $\text{mg (50 seeds)}^{-1}$ ] for each replicate;

*Electrical conductivity* - quadruplicates of 0.5 g of seeds were immersed in 25 mL of distilled water and submitted to a temperature of  $25\text{ }^{\circ}\text{C}$ , for 24 h. Next, the electrical conductivity ( $\mu\text{S cm}^{-1}\text{ g}^{-1}$ ) of the solution was measured using a conductivity meter (DIGIMED, DM 3).

Data were submitted to analysis of variance (Anova), according to a completely randomized design, with 10 treatments and four repetitions. When the F-test was significant ( $p \leq 0.05$ ) for the treatment effects, the means were compared by the Scott-Knott test ( $p = 0.05$ ).

## RESULTS AND DISCUSSION

The physiologically primed *U. brizantha* seeds reached between 26.5 % and 31.6 % of moisture, considering all priming treatments, while the control seeds exhibited 8.7 % of moisture (Table 2).

During the physiological priming treatments, it is vital that seeds achieve moisture levels capable of initiating the preliminary germination, while

Table 2. Moisture of *Urochloa brizantha* (BRS Piatã cultivar) seeds submitted to osmotic priming with different salts, at two osmotic potentials for two time periods, and control (without priming), shortly after priming and after 48 h of drying.

Salt	Osmotic potential (MPa)	Time period (h)	Moisture (%)	
			Shortly after priming	After 48 h of drying
$\text{Ca}(\text{NO}_3)_2$	-0.55	12	28.9	8.9
		24	29.3	8.9
	-1.10	12	26.5	9.0
		24	29.3	8.7
$\text{CaSO}_4$	-0.55	12	27.3	9.3
		24	31.6	8.9
	-1.10	12	28.2	8.9
		24	30.9	8.9
$\text{KNO}_3$	-1.10	12	29.1	8.4
Control			8.7	8.7

avoiding radicle protrusion. The membrane repair, initial digestion of reserves and translocation to the embryonic axis occur during this period (Santos et al. 2008, Varier et al. 2010). However, since water absorption is limited by the osmotic potential of the solution, the vigor of the lot can be homogenized to allow less vigorous seeds to catch up with their more vigorous counterparts during the phase 2 of germination, making germination faster and more uniform when conditions become favorable.

Seeds returned to their initial moisture content after drying, varying between 8.4 % and 9.3 % (Table 2). Thus, the water absorbed during priming was extracted and did not influence the results of the subsequent tests, leaving only the effects of treatments, which were determined next.

All the priming treatments exhibited germination percentages significantly higher than that of the control (Table 3), which obtained germination below 40 %. Although there was no difference between the priming treatments, their superiority to the control illustrates one of the advantages of priming, namely an increase in the germination percentage.

Different results have been reported for other *U. brizantha* cultivars. MG-5 did not differ from the control when submitted to priming with  $\text{KNO}_3$  and  $\text{Ca}(\text{NO}_3)_2$ , displaying germination percentages between 83 % and 87 % (Batista et al. 2015). With respect to the priming period for 'Marandu' seeds, germination declined, but treatments did not differ significantly from the control, even after 12 h (Bonome et al. 2006).

The seeds that did not germinate were submitted to the tetrazolium test to assess their

viability within each treatment. Based on the results, it can be inferred that primed seeds exhibited a higher germination percentage due to the lower percentage of dormant seeds, with controls displaying around 16 % of dormant seeds, if compared to an average of 7 % in the other treatments (Table 3). Similar results were presented by Batista et al. (2016), especially for priming with  $\text{KNO}_3$  and  $\text{Ca}(\text{NO}_3)_2$ .

Some studies have reported that the use of nitrates in priming overcomes dormancy and increases germination because they act as oxidants, converting NAD(P)H to NAD(P)<sup>+</sup> in the pentose-phosphate metabolic pathway (Hendricks & Taylorson 1974, Cardoso et al. 2015).

The treatments showed a significant effect for emergence percentage (Table 3), where priming with  $\text{Ca}(\text{NO}_3)_2$  at -0.55 MPa for both periods (12 h and 24 h), as well as  $\text{CaSO}_4$  at -0.55 MPa for 12 h and -1.10 MPa in both periods did not differ, albeit with higher means than the other treatments, including  $\text{KNO}_3$  and the control.

Similar results were observed for MG-5 seeds primed with  $\text{Ca}(\text{NO}_3)_2$  and  $\text{KNO}_3$ , which exhibited a higher emergence percentage (Batista et al. 2016). It is noteworthy that seed vigor is related to the ability to germinate and/or emerge under adverse conditions, resulting in a fast uniform seedling establishment in the field, where the strongest effects of priming are observed.

Considering the average emergence time of seedlings (Table 3), the control plants needed more time to reach a maximum emergence, with an average of 5.2 days. However, they did not differ significantly from the two calcium priming solutions

Table 3. Percentage of germination (G), dormant seeds (D) and emergence (E), average emergence time (AET), shoot dry weight (SDW) and seed electrical conductivity (EC) of *Urochloa brizantha* (BRS Piatã cultivar) seeds submitted to osmotic priming with different salts, at two osmotic potentials, for two time periods, and control (without priming).

Salt	Osmotic potential	Time period	G	D	E	AET	SDW	EC
	MPa	h	%		days	mg 50 seeds <sup>-1</sup>	$\mu\text{S cm}^{-1} \text{g}^{-1}$	
$\text{Ca}(\text{NO}_3)_2$	-0.55	12	52 a*	8 b	66 a	4.2 b	7.0 b	65.15 e
		24	50 a	8 b	63 a	4.4 b	7.2 b	79.05 d
	-1.10	12	48 a	6 b	61 b	4.6 b	6.3 b	101.64 c
		24	53 a	5 b	58 b	4.8 a	8.8 a	108.92 b
$\text{CaSO}_4$	-0.55	12	48 a	8 b	65 a	4.4 b	11.4 a	56.96 e
		24	46 a	8 b	57 b	4.4 b	10.2 a	105.74 b
	-1.10	12	53 a	9 b	70 a	4.4 b	10.1 a	60.55 e
		24	50 a	6 b	68 a	4.8 a	8.6 a	64.20 e
$\text{KNO}_3$	-1.10	12	53 a	5 b	59 b	4.3 b	9.5 a	121.46 a
Control			36 b	16 a	55 b	5.2 a	7.3 b	89.16 c

\* Means followed by the same letter, in the columns, do not differ according to the Scott-Knott test at 5 % of significance.

with -1.10 MPa applied for 24 h, with an average emergence time of 4.8 days. Subsequent priming treatments with calcium salts achieved a maximum emergence in less time, with average emergence times between 4.2 and 4.6 days. Although the differences were small, it is important to underscore that seed vigor is more significant and evident under adverse conditions.

With respect to shoot dry weight (Table 3), all the  $\text{CaSO}_4$  treatments displayed higher means and did not differ from the treatments with  $\text{KNO}_3$  and  $\text{Ca}(\text{NO}_3)_2$  at -1.10 MPa for 24 h. The remaining treatments, including the control, did not differ and produced seedlings with less dry biomass.

In electrical conductivity measurements (Table 3), seeds primed with  $\text{KNO}_3$  obtained a higher mean value ( $121.46 \mu\text{S cm}^{-1} \text{g}^{-1}$ ), when compared to the other treatments. Controls exhibited intermediate values, with an average of  $89.16 \mu\text{S cm}^{-1} \text{g}^{-1}$ . The decreased osmotic potential of the solution (higher salt concentration), when the priming period increased from 12 h to 24 h, was followed by a rise in the electrical conductivity of seeds primed with  $\text{Ca}(\text{NO}_3)_2$ . Lower mean values were observed when  $\text{CaSO}_4$  was used, except for an osmotic potential of -0.55 MPa applied for 24 h. The highest mean values were recorded for  $\text{KNO}_3$ . This can be attributed to the salt residue remaining on and inside the seeds after washing, which may have raised the electrical conductivity.

In light of these results, priming using a  $\text{CaSO}_4$  solution at -0.55 MPa for 12 h can be recommended as a replacement for  $\text{KNO}_3$ , since germination and dormant seed percentages were similar to those produced by  $\text{KNO}_3$ , while the shoot dry weight and electrical conductivity exhibited better results. Additionally, the  $\text{CaSO}_4$  solution at -0.55 MPa for 12 h also resulted in shorter germination times and required less material.

## CONCLUSIONS

1. Calcium nitrate and calcium sulfate show a significant potential for use in the osmotic priming of *Urochloa brizantha* (BRS Piatã cultivar) seeds, since they are as efficient as potassium nitrate, less costly and easier to purchase;
2. Priming using a calcium sulfate solution at -0.55 MPa for 12 h, at 25 °C, is recommended as a replacement for potassium nitrate.

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