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Priming and stress under high humidity and temperature on the physiological quality of *Brachiaria brizantha* cv. MG-5 seeds

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ABSTRACT. Palisade grass is a forage plant that is widely used in pasture cropping in the Brazilian savannah. The aim of this experiment was to evaluate palisade grass (*Brachiaria brizantha* cv. MG–5) seeds subjected to priming and stress at high humidity and temperature (before and after conditioning). The experimental design was completely randomized in a 2x5 factorial arrangement. Seeds were exposed to stress under high humidity and temperature (before and after conditioning) and five priming treatments [Water (Control), potassium nitrate (KNO_3) at 0.2%, calcium nitrate $Ca(NO_3)_2$ at 0.2%, gibberellin (GA_3) at 0.2% and glucose at 10%] with four replications. Two experiments were performed: Experiment I - seed with chemical scarification using H_2SO_4 and Experiment II - without scarification. The stress on the seed was applied using artificial aging at 41°C for 96 hours. Seed priming was accomplished by immersion at 25°C for 2 hours. Thereafter, the seeds were oven-dried at 35°C until they regained hygroscopic equilibrium. Seed germination and vigor were evaluated. Priming using KNO_3 and $Ca(NO_3)_2$ produced seeds with high tolerance to stress under high temperature, and this process is efficient to overcome dormancy.

Keywords: palisade grass, seed deterioration, expression of vigor, nitrate.

Condicionamento fisiológico e stress sob alta umidade e temperatura na qualidade fisiológica de sementes de *Brachiaria brizantha* cv. MG-5

RESUMO. Nas pastagens do cerrado brasileiro a braquiária é uma forrageira amplamente utilizada. O objetivo da presente pesquisa foi avaliar o desempenho de sementes de *Brachiaria brizantha* cv. MG–5 mediante condicionamento fisiológico e stress a alta umidade e temperatura (antes e após o condicionamento). O delineamento experimental utilizado foi o inteiramente casualizado em esquema fatorial 2x5, constituído por exposição das sementes ao stress a alta umidade e temperatura (anterior e posterior ao condicionamento) e cinco tratamentos com condicionamento fisiológico [água, nitrato de potássio (KNO_3) 0,2%, nitrato de cálcio $Ca(NO_3)_2$ 0,2%, giberelina (GA_3) 0,2% e glicose 10%], com quatro repetições. Foram realizados dois experimentos (Experimento I – em sementes com presença de escarificação química com H_2SO_4 e Experimento II – ausência de escarificação). O stress nas sementes foi proporcionado através do envelhecimento artificial (96h a 41ºC). O condicionamento foi realizado através da hidratação das sementes por imersão direta a 25ºC (2h). Posteriormente as sementes foram secas em estufas a 35ºC até retomada da umidade de equilíbrio higroscópico. Avaliaram-se a germinação e vigor das sementes. O condicionamento fisiológico com KNO_3 e $Ca(NO_3)_2$ propiciam sementes com maior tolerância ao stress a alta umidade e temperatura, sendo eficiente na superação de dormência.

Palavras-chave: braquiária, deterioração de sementes, expressão de vigor, nitrato.

Introduction

Plants of the *Brachiaria* genus are widely used in pasture establishment in the Brazilian savannah. Because pasture implantation occurs through seed, it is imperative that high-quality seed is sown.

Seed dormancy occurs in *B. brizantha*, which is a limiting factor to crop establishment. Dormancy in this species is linked to several factors, especially physical attributes that are related to seed coat restrictions imposed by the glumes (lemma and palea), pericarp and tegument (Binotti, Sueda Junior, Cardoso, Haga, & Nogueira, 2014). Under laboratory conditions (Ministério da Agricultura, Pecuária e Abastecimento [Mapa], 2009), seed technologists recommend chemical scarification using sulfuric acid to overcome dormancy. In addition, supplying the germination substratum

with potassium nitrate (KNO_3) and oven-dry thermal treatment can be used for dormancy release. Munhoz, Zonetti and Roman (2009) found that five minutes of chemical scarification is effective to overcome the dormancy of *Brachia brizantha* cv. MG–5. Meschede, Sales, Braccini, Scapim and Schuab (2004) reported that dormancy can be overcome by artificial aging, but the results depend on the physiological quality of the seed lot. However, this practice can expose the seeds to conditions that reduce their physiological potential (Cardoso et al., 2014).

Priming is an alternative method to stress processes (Paparella, Araújo, Rossi, Wijayasinghe, Carbonera and Balestrazzi (2015). Seeds are often subjected to priming, as reported by Oliveira and Gomes Filho (2010) for sorghum seeds. These authors reported that priming partially reversed the negative effects of aging. Priming can improve the seed quality of *Brachiaria* and other grasses, e.g., Bonome, Guimarães, Oliveira, Andrade and Cabral (2006) reported the effects of osmoconditioning on the uniformity of germination after applying KNO_3 to the seeds of *B. brizantha* cv. Marandu for 12 hours, and Aragão et al. (2003) reported increased germination and expression of vigor when super-sweet sweet corn seeds were subjected to pre-soaking using gibberellic acid. Among the techniques used for priming, Soltani & Soltani (2015) highlights that hidropriming (direct immersion) is the most likely application.

The objective of this experiment was to evaluate the physiological quality of *Brachiaria brizantha* cv. MG–5 using seed priming and stress under high humidity and temperature conditions. This objective was based on the abovementioned factors, the need to overcome seed dormancy of *Brachiaria*, the benefits of priming on the quality of the seed lot and the limited number of studies on the effects of these practices on *Brachiaria* seeds.

Material and methods

The experiment was carried out from November 2013 to February 2014 using *Brachiaria brizantha* cv. MG-5 seeds from the 2013/14 growing season.

The experimental design was completely randomized using a 2 x 5 factorial arrangement that corresponded to seeds exposed to stress under high humidity and temperature (before and after conditioning) and five priming treatments [Water (Control), potassium nitrate (KNO_3) at 0.2%, calcium nitrate $Ca(NO_3)_2$ at 0.2%, gibberellin (GA_3) at 0.2% and glucose at 10%] with four replications. Two experiments were performed: Experiment I - seed with chemical scarification using H_2SO_4 and Experiment II - without scarification.

In Experiment I, the seeds were subjected to chemical scarification using concentrated sulfuric acid for 5 min. Thereafter, they were rinsed using running deionized water and placed on paper towels to dry.

Stress at high humidity and temperature was induced at 41ºC for 96 hours using the Gerbox method for artificial seed aging (adapted Marcos Filho, 1999). Seeds were primed using hydration at 25°C for 2 hours. The conditioning products were diluted in deionized water by preparing 100 mL of the solutions in plastic recipients placed in germination BOD chamber. For every treatment, we evaluated 9 g of *B. brizantha* seeds (subsamples were collected from each lot). Thereafter, the seeds were oven-dried at 35°C until they reached the same hygroscopic equilibrium as that before the priming application.

After treating the seeds, the following evaluations were conducted using pure seeds: 'Germination test' – This evaluation was based on 4 subsamples of 50 seeds per treatment that were distributed in plastic boxes (11.0 x 1.0 x 3.5 cm). Counting of normal seedlings occurred on the 7th ('First count of percent seed germination') and 21st days after sowing, based on the Rules for Seed Testing (Mapa, 2009). These results were expressed as a percentage; 'Dormant seeds' – The presence of seed dormancy was evaluated using the tetrazolium test described by the Rules for Seed Testing (Mapa, 2009) for *Brachiaria* seeds and was calculated using the number of remaining viable seeds from the germination test; 'Electrical conductivity' – The electrical conductivity of the seed soaking solution was measured using the 'Bulk Conductivity' test that was 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 25ºC for 24 hours. Then, the electrical conductivity of the soaking solution was measured and the results are expressed as $\mu S\ cm^{-1}\ g^{-1}$; 'Seedling Emergence test' – Seedling emergence was evaluated under greenhouse conditions using 4 subsamples of 50 seeds per treatment sown at a depth of 1 cm in perforated trays to allow water drainage from the vermiculite. The percentage of seedling emergence was evaluated within 28 days of sowing; emerged seedlings were those with a shoot length longer than 20 mm.

'Statistical Analysis': The data were evaluated using analysis of variance (F test; $p \leq 0.05$). When significant, we used the Tukey test to compare

treatments. These analyses were performed using the Sanest program.

Results and discussion

Priming and stress interactions at high humidity and temperature were evaluated for the first count of germination, total germination, and percent dormancy of chemically scarified seeds (Table 1).

Table 1. The effects of priming and stress under high humidity and temperature on the first germination count, total germination, and percent dormancy of scarified *B. brizantha* MG–5 seeds.

Stress under high humidity	Priming				
and temperature	Water	KNO_3	$Ca(NO_3)_2$	GA_3	Glucose
			Total Germination (%)		
Before	[M]64 aCD	85 bA	73 bBC	80 aAB	61 aD
After	52 bB	94 aA	94 aA	52 bB	59 aB
			C.V. = 8.09		
			First germination count (%)		
Before	61 aBC	79 bA	68 bAB	76 aA	52 aC
After	47 bB	93 aA	93 aA	51 bB	58 aB
			C.V. = 8.54		
			Dormant seeds (%)		
Before	22 bAB	5 aC	12 aBC	12 bBC	26 aA
After	39 aA	0 aB	1 bB	38 aA	32 aA
			C.V. = 28.28		
			Seedling Emergence (%)		
Before	77 aB	74 bB	75 bB	89 aA	76 bB
After	84 aAB	94 aA	93 aAB	82 aB	91 aAB
			C.V. = 6.60		

[M]Means followed by different lowercase letters within columns and uppercase letters within rows for priming and for stress at high humidity and temperature, respectively, vary at 5% probability based on the F test and the Tukey test; C.V., coefficient of variation.

The results showed the highest germination percentage (94%) at 21 days was obtained with priming using KNO_3 or $Ca(NO_3)_2$ followed by stress. These treatments represented a significant gain of 42% compared with the germination (52%) obtained in response to water and gibberellin (priming followed by stress). Priming using KNO_3 or $Ca(NO_3)_2$ followed by stress resulted in rapid germination based on the first germination count (7 days). The *B. brizantha* seeds conditioned with KNO_3 or $Ca(NO_3)_2$ showed higher tolerance to stress at high humidity and temperature, indicating that the expression of their physiological potential with the use of these agents was not affected even after stress (Table 1).

These results are similar to those presented by Binotti, Sueda Junior, Cardoso, Haga and Nogueira (2014), who found an improvement in the germination rate of *B. brizantha* cv. MG-5 seeds resulting from a pre-germinative treatment using KNO_3 at 0.2%. Reis, Silva, Neves and Guimarães (2013) also confirmed a positive effect on the physiological quality of gherkin seeds in response to KNO_3. Pereira, Borges, Oliveira, Leite and Gonçalves (2010) verified that soaking *P. reticulate* seeds in nitric oxide reduced the germination inhibition caused by accelerated aging.

We found that priming using KNO_3 and $Ca(NO_3)_2$, even after the stress process, was able to break dormancy in *B. brizantha* cv. MG-5 seeds. In the treatments with these agents, the percentage of dormant seeds was 0 and 1% for KNO_3 and $Ca(NO_3)_2$, respectively, compared with 39 (water), 38 (gibberellin) and 32% (glucose). Priming using water, $Ca(NO_3)_2$ or gibberellin after stress promoted lower number of seeds dormancy with 22% of water and 12% of $Ca(NO_3)_2$ and gibberellin against 39 (water), 1% $Ca(NO_3)_2$ and 38% (gibberellin) before stress (Table 1). We concluded that after overcoming dormancy, the entrance of water and diffusion of gases occurred, caused by the chemical removal of the seed coat through scarification, and the secondary dormancy experienced by the *B. brizantha* seeds, which was probably associated with physiological processes, was released with the use of KNO_3 and $Ca(NO_3)_2$.

We also verified higher seedling emergence (21 days) with the application of gibberellin after stress, i.e., 89 versus 77, 74, 75 and 75% for water, KNO_3, $Ca(NO_3)_2$ and glucose, respectively. The application of gibberellin followed by stress resulted in lower (82%) seedling emergence than KNO_3 (94%). The application of KNO_3, $Ca(NO_3)_2$ and glucose resulted in higher seedling emergence when applied before stress, i.e., 94, 93 and 91%, respectively, versus 74 (KNO_3), 75 $Ca(NO_3)_2$ and 76% (glucose) when applied after stress (Table 1).

Gibberellin is a hormone that acts directly in germination and dormancy breaking (Cardoso, 2013). When applied after stress, gibberellin was able to improve seedling emergence; however, when applied after stress, this effect did not occur even within the germination and dormancy breaking patterns, pointing to the sensitivity of this hormone to deterioration processes (high humidity and temperature). Câmara and Stacciarini-Seraphin (2002) reported that gibberellin applied to *B. brizantha* cv. Marandu showed reduced action under application conditions used for the seeds and during subsequent storage.

Priming using $Ca(NO_3)_2$ or KNO_3 resulted in a lower electrical conductivity (30.14 $\mu S\ cm^{-1}\ g^{-1}$) than that obtained using glucose (38.34 $\mu S\ cm^{-1}\ g^{-1}$) but did not differ from the other treatments. Stress after priming resulted in a lower rate of 31.94 versus 34.61 $\mu S\ cm^{-1}\ g^{-1}$ for stress before priming (Table 2). Thus, we concluded that primed seeds under conditions that lead to deterioration tend to have less damage caused to their cell membranes, which results in less loss of cellular components.

Table 2. Electrical conductivity in *B. brizantha* MG–5 in response to priming and stress under high humidity and temperature.

Electrical conductivity (μS cm^{-1} g^{-1})			
Priming			
Water	[M]33.14 ab	Stress under high humidity and temperature	
KNO_3	32.31 b		
$Ca(NO_3)_2$	30.14 b	Before	34.61 a
GA_3	34.95 ab	After	31.94 b
Glucose	38.34 a		
F	4.49**	7.92**	
C.V. = 12.17			

[M]Means followed by different letters within columns differ significantly at 5% probability based on the Tukey test and the F test; **significant at 1% probability; C.V., coefficient of variation.

The effects of priming and stress at high humidity and temperature were determined based on the first germination counts, total germination, percent dormant seeds and electrical conductivity of non-scarified seeds (Table 3).

Table 3. The effects of priming and stress under high humidity and temperature on the first germination count, total germination, and percent dormancy of non-scarified *B. brizantha* MG–5 seeds.

Stress under high humidity	Priming				
and temperature	Water	KNO_3	$Ca(NO_3)_2$	GA_3	Glucose
			Total Germination (%)		
Before	[M]45 aB	65 bA	50 bAB	42 aB	42 aB
After	38 aB	80 aA	80 aA	38 aB	41 aB
C.V. = 15.56					
			First germination count (%)		
Before	32 aB	55 bA	37 bB	26 aB	31 aB
After	26 aB	75 aA	63 aA	30 aB	32 aB
C.V. = 21.06					
			Dormant seeds (%)		
Before	42 aA	23 aB	36 aAB	41 aA	44 aA
After	44 aA	4 bB	8 bB	44 aA	37 aA
C.V. = 20.50					
			Electrical conductivity (μS cm^{-1} g^{-1})		
Before	8.78 bC	12.45 bB	10.71 aBC	9.86 bBC	20.04 bA
After	11.82 aC	19.90 aA	12.62 aBC	15.18 aB	12.17 aBC
C.V. = 11.88					
			Seedling Emergence (%)		
Before	62 aAB	72 aA	63 bAB	56 aB	63 aAB
After	46 bC	70 aAB	74 aA	57 aBC	68 aAB
C.V. = 11.54					

[M]Means followed by different lowercase letters within columns and uppercase letters within rows for priming and for stress at high humidity and temperature, respectively, vary at 5% probability based on the F test and the Tukey test; C.V., coefficient of variation.

A higher total germination percentage (80% at 21 days) was observed through the application of KNO_3 and $Ca(NO_3)_2$ before stress in contrast to 38% (water and gibberellin) and 41% (glucose), i.e., a significant gain of 42 and 39%, respectively. A higher first germination count (7 days) was also observed with the use of KNO_3 (of the 84% available seeds, 75% had already germinated) and $Ca(NO_3)_2$ (of the 88% available seeds, 63% had already germinated). Similar responses were observed for the scarified seeds, which confirm the effectiveness of KNO_3 and $Ca(NO_3)_2$ application before stress at high humidity and temperature (Table 3).

There was a decrease in the number of dormant seeds with the use of KNO_3 and $Ca(NO_3)_2$ before stress, i.e., 4 and 8%, respectively, in contrast to 44 (water and gibberellin) and 37% (glucose). KNO_3 application after stress resulted in a lower number of dormant seeds, i.e., 23% compared with 42 (water), 41 (gibberellin) and 44% (glucose) (Table 3). This result is different from that obtained by Cardoso et al. (2014) who did not observe dormancy breaking using priming with 0.2% KNO_3 in non-scarified *B. brizantha* cv. MG – 5 seeds that went through artificial aging (stress at high humidity and temperature) before priming.

However, Cardoso et al. (2014) observed that increasing periods of aging (stress at high humidity and temperature) positively influenced dormancy breaking, which was also verified by Meschede et al. (2004). However, these authors also highlighted that this effect depends on the physiological quality of the initial lot because aging (stress at high humidity and temperature) can break physical dormancy in non-scarified *B. brizantha* seeds.

When applied before stress, these treatments reduced the number of dormant seeds, e.g., the application of KNO_3 before stress resulted in 4% dormancy compared with 23% after stress. However, the results obtained with the application of $Ca(NO_3)_2$ (8% before stress and 36% after stress) showed a significant decrease in the number of secondary dormant seeds, i.e., 19 and 28%, respectively, when applied before stress (Table 3). According to Cardoso (2013), nitrate stimulates seed germination by reducing seed dormancy. Seshu and Dadlani (1991) observed that dormancy in rice seed is controlled by nonanoic acid and abscisic acid in the hull and pericarp. The presence of these compounds have negative effects on the activity of amylase, which is an enzyme related to starch break down. Thus, the percentage of seed germination is improved, and pre-soaking in KNO_3 reduces the seed dormancy imposed by the nonanoic acid.

There was better organization in the membrane system (after stress) in response to the application of water, KNO_3 and gibberellin, based on the average values of 8.78, 12.75 and 9.86 μS cm^{-1} g^{-1}, respectively, compared with glucose (20.04 μS cm^{-1} g^{-1}). The application of $Ca(NO_3)_2$ before stress promoted higher seedling emergence (74%), which indicates a significant gain of 28 and 17% over the 46 and 57% seedling emergence measured with the use of water and gibberellin, respectively (Table 3).

Conclusion

Seeds conditioned using KNO_3 and $Ca(NO_3)_2$ show higher tolerance under adverse conditions such as high humidity and temperature.

Priming using KNO_3 and $Ca(NO_3)_2$ followed by artificial aging treatments is efficient in breaking dormancy.

Gibberellin application through priming provides beneficial effects to seeds that are not exposed to deterioration processes.

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