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Priming *Urochloa ruziziensis* (R.Germ. & Evrard) seeds with signalling molecules improves germination

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ABSTRACT: As forage production increase, high-quality seeds demand follows. Priming have been reported as a technique for improve seed quality and stress tolerance. The aim of this study was to evaluate the priming agents effect on *Urochloa ruziziensis* seed germination and seedling establishment under water deficit and salt stress. A completely randomized experiment assessing six priming agents and three germination conditions were established. We evaluated: seed water content; germination percentage and speed index; viability of seeds remaining from the germination test; seedling length; antioxidant enzymatic activity; and malondialdehyde quantity. Seeds primed under water deficit and salt stress showed better physiological performance than dry seeds. There was a significant increase in seed germination percentage and speed and in seedling roots and shoots length. Sodium Nitroprusside shows significant potential for use in the physiological priming of *U. ruziziensis* seeds.

Index terms: NaCl, oxidative stress, PEG, physiological conditioning, *Urochloa ruziziensis.*

RESUMO: Com o crescimento da produção de forrageiras, a demanda por sementes de alta qualidade aumenta. O condicionamento fisiológico vem como técnica para produzir sementes de alta qualidade e tolerância à estresses. O objetivo deste trabalho foi avaliar o efeito de agentes condicionantes sobre a germinação de sementes e crescimento de plântulas de *Urochloa ruziziensis* em condições de estresse hídrico e salino. Foi estabelecido um experimento em delineamento inteiramente casualizado testando o efeito de seisagentes condicionantes e três condições de germinação. Foram avaliados conteúdo de água, porcentagem e velocidade de germinação, viabilidade das sementes remanescentes do teste de germinação, comprimento da plântula, atividade do sistema antioxidante e a quantidade do malonaldeído. Melhor performance foi observada em sementes condicionadas submetidas ao déficit hídrico e salino do que aquelas não condicionadas. Um aumento significante foi observado na percentagem e velocidade de germinação de sementes, bem como no tamanho de raízes e caule. O nitroprussiato de sódio tem maior potencial para uso no condicionamento fisiológico de sementes de *U. ruziziensis*.

Termos para indexação: NaCl, estresse oxidativo, PEG, condicionamento fisiológico, *Urochloa ruziziensis.*

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INTRODUCTION

Seeds are the main way for seedling production for most of agronomical crops. Rapid germination and seedling establishment is of essential importance on crop production, especially considering stressful conditions due climate changes (Bhanuprakash and Yogeesha, 2016), being stress tolerance an essential characteristic for crop production (Rahimi, 2013). Salinity and water deficit are the most limiting factors for agronomic crops worldwide (Bhanuprakash and Yogeesha, 2016), resulting in changes on plant morphological and physiological characteristics, prejudices in germination, seedling establishment, and plant growth (Saberali and Moradi, 2019). Seed priming is reported as the most promising for improve seed and seedling vigour (Cardoso et al*.,* 2015; Pires et al*.,* 2016; Faraji and Sepehri, 2018; Silva et al*.*, 2019). Besides, the potential improvement in plant stress tolerance as effect of priming have already been reported for some species as cumin (*Cuminum syminum*) (Rahimi, 2013) and carrot (*Daucus carota*) (Reza et al*.*, 2011).

Priming consists in controlled seed imbibition, at enough amounts to activate the metabolic events, but insufficient for radicle protrusion (Bhanuprakash and Yogeesha, 2016; Silva et al*.*, 2019). As consequence, seed will pass through the events of seed imbibition phases 1 and 2 (Bewley et al*.,* 2013), and interrupted before phase 3 starts. As result, there are improvements on germination uniformity and growth, even under unfavourable conditions (Bhanuprakash and Yogeesha, 2016; Zheng et al*.,* 2016; Ribeiro et al*.*, 2019). Molecules as polyethylene glycol (PEG), can be used to control seed water absorption, by reducing water potential, resulting on slow imbibition (Rahimi, 2013) and induction of stress tolerance. The use of other molecules was also successfully applied in priming as spermidine, gibberellic acid, potassium nitrate, hydrogen peroxide, and sodium nitroprusside (Lara et al., 2014; Ruttanaruangboworn et al., 2017; Habib et al., 2021; Roychoudhury et al., 2021). As results of those molecules usage, there are changes on plant metabolism and improvements on seed germination, seedling establishment, and growth are reported.

As forage production have been expanding, demands on seed production follows, especially from species of *Urochloa* genus (ABRASEM, 2018). However, studies on *Urochloa* seed production are scarce, being satisfactory results are still to be achieved (Ribeiro et al*.,* 2019). Considering the importance of these species on pasture production, more information and techniques regarding *Urochloa* sp. seed and seedling production is essential. For *Urochloa brizantha*, Oliveira et al*.* (2021) reported interesting results achieved through priming. However more studies for other species are necessary. Considering the exposed, this research aimed to evaluate the effect of different priming agents over *Urochloa ruziziensis* seed germination and seedling under stress by water deficit or salinity.

MATERIALS AND METHODS

Urochloa ruziziensis seeds were obtained from material produced in a private commercial seed production field. Seeds were harvested on plants before pod shattering and removed from fruits, after that, were stored in cold chamber for about 20 days, when the experiments were started. Priming consisted in five solutions: distilled water, 0.2% potassium nitrate (Cardoso et al*.,* 2015), 0.5 mmol.L^{-1} spermidine (SPE) (Hussain et al*.*, 2015; Lopes et al*.*, 2018), 50 mg.L^{-1} gibberellin (GA) (Batista et al*.*, 2015; Cardoso et al*.*, 2015), and 0.10 mmol.L^{-1} sodium nitroprusside (SNP) (Faraji and Sepehri, 2018). Treatments consisted in immersion of 40 g seeds in 400 mL of above-described solutions in Erlenmeyer, being the solution kept aerated with the support of an air compressor. Seeds were conditioned at BOD chamber at 25 °C without light for 42 h (Pereira et al*.*, 2012). As control, non-primed seeds were used.

After priming, the seeds were washed in running water, dried in oven with forced air flow for 24 h at 25 °C and temperature was raised to 35 °C and kept for 72 h. Seed water content was measured immediately after priming (before drying) and after drying. For this purpose, four replications of 200 seeds were removed from each treatment and dried in an oven at 105 °C for 24 h according to Brasil (2009). Primed and non-primed seeds were subjected to different germination conditions: distilled water, salinity (NaCl solution), and water deficit (polyethylene glycol (PEG) 6000). PEG and NaCl solutions were prepared at -0.4 MPa (Pereira et al*.*, 2012). For the germination tests, four replications

of 50 seeds were used. Sowing was performed over two germination paper (substrate) in germination boxes (Gerbox). Substrate was moistened with a volume of solution equivalent to 2.5 times of the paper dry mass. Tests were incubated in BOD chambers with alternating temperatures of 20-35 °C and photoperiods of 8 h. First count was performed 7 days after sowing, and the final at 21 (Brasil, 2009). Germination speed index was determined concurrently with the germination test (Maguire, 1962). Normal seedlings were considered those that had broken through the coleoptile and whose primary leaf (above the coleoptile) length was the same size as the coleoptile, that is, when 50% of the shoot was surrounded by the coleoptile and 50% grew above it.

After the final count, remaining seeds (that had not germinated and were not deteriorated) were taken to the tetrazolium test (Brasil, 2009). Seeds were cut longitudinally with the aid of tweezers and scalpel. After cutting, they were placed in a 0.1% solution of 2,3,5-triphenyl-tetrazolium chloride in a dark container and kept in a BOD germination chamber at a constant temperature of 40 °C for 3 h. Seeds were evaluated as to location and intensity of colour of their parts, allowing the identification of viable seeds (Brasil, 2009). Results were expressed as percentages. For seedling image analysis, four replications of 25 seeds were used. Sowing was performed over two Germitest® paper and covered with a third one. Papers were moistened with a volume of the solutions described above equivalent to 2.5 times of the paper dry mass. Seeds were placed in BOD chambers with alternating temperatures of 20-35 °C and photoperiods of 8 h. Rolls were placed in plastic bags to avoid moisture loss. Seedling images were obtained 7 days after the test was established, equivalent to the first germination count period (Brasil, 2009).

For image capture, GroundEye® system (version S800) was used; the system consists of a capture module that has an acrylic tray, a high-resolution camera and integrated software for evaluation. Seedlings were removed from paper roll and placed in the capture module tray to obtain the images. In analysis configuration step, CIELab background colour calibration method was used: luminance index from 0 to 100, “a” dimension from -17.5 to 42.5 and “b” dimension from -57.0 to -28.9. After background colour calibration, images were analysed. Root and shoot length values were extracted.

Lipid peroxidation was determined by malondialdehyde (MDA) quantification, which is produced by the reaction of thiobarbituric acid (TBA) as described by Dhindsa et al. (1981). For this analysis, samples of 200 mg of seed fresh matter were macerated in liquid nitrogen and homogenized in 1.25 mL of trichloroacetic acid (TCA) (0.1%) and sodium duodecyl sulfate (SDS) (1%). Homogenate was centrifuged at 12.000 g for 15 min. For a 300 µL aliquot of the supernatant, 1 mL of 20% trichloroacetic acid (TCA) was added to a tube containing 0.5% thiobarbituric acid (TBA). The mixture was heated to 95 °C for 30 minutes and then quickly cooled in ice bath. Subsequently, absorbance reading was taken at 532 nm and the MDA concentration was calculated using the extinction coefficient of 155 $mM^{-1}.cm^{-1}$ (Baryla et al., 2000).

Enzymatic extracts were obtained following the protocol of Biemelt et al. (1998), where 200 mg of seedlings were macerated in liquid nitrogen supplemented with insoluble polyvinylpolypyrrolidone (PVPP), to which 1.5 mL of an extraction buffer composed of 400 mM potassium phosphate (pH 7.8), 10 mM EDTA and 200 mM ascorbic acid was added. Homogenate was centrifuged at 13,000 *g* for 10 minutes at 4 °C, and the collected supernatant was used to quantify enzyme activity. Superoxide dismutase (SOD) activity was estimated by the enzymes’ ability to inhibit photoreduction of nitro tetrazolium blue (NBT) (Giannopolits and Ries, 1977). Readings were performed at 560 nm. One unit of SOD corresponds to the amount of enzyme capable of inhibiting the photoreduction of NBT by 50% under the test conditions. Catalase (CAT) activity was determined according to Havir and McHale (1987). Activity of this enzyme was determined by the decrease in absorbance at 240 nm every 15 seconds for 3 minutes, monitored by the consumption of hydrogen peroxide. Molar extinction coefficient used was 36 $mM^{-1}.cm^{-1}$, as described by Azevedo et al. (1998). Ascorbate peroxidase (APX) activity was determined according to Nakano and Asada (1981) by monitoring the oxidation rate of ascorbate at 290 nm, and the molar extinction coefficient used was 2.8 $mM^{-1}.cm^{-1}$.

Experimental design was completely randomized with four replications in a 3x6 factorial arrangement with three germination conditions and six treatments that constituted the five priming solutions plus a control (non-primed seeds). The means were subjected to analysis of variance, and when significant (p = 0,05) the Tukey test was applied.

RESULTS AND DISCUSSION

Higher values on water content were found in primed seeds than in the non-primed (control) seeds (Figure 1). On this process, moisture levels must be enough for metabolism to be activated, but not for complete germination itself (Ribeiro et al., 2019). Seeds showed differences in imbibition among the priming solutions (Figure 1). Water absorption is limited by solution osmotic potential and thus, seed vigor can be homogenized, allowing less vigorous ones to reach the same physiological stage of more vigorous ones during phase 2, making germination faster and uniform when conditions become favourable (Bhanuprakash and Yogeesha, 2016; Ribeiro et al., 2019).

Priming with SNP and SPE led to the highest water contents (Figure 1). The water content obtained with SNP was seven percentage points higher than that obtained with potassium nitrate (KNO_3), which yielded the lowest content among the primed treatments. Both SNP and KNO_3 are saline compounds that are nitric oxide donors, and this difference in imbibition can be explained by the difference in osmotic potential among the solutions (Silva et al., 2019).

A series of metabolic events occur during germination that culminate in embryonic growth and development and consequently in radicle protrusion, and this process is resumed by imbibition (Marcos-Filho, 2015). Therefore, water absorption by seed is considered a limiting factor for the occurrence of physical and physiological processes inside the seed. Salinity is also described as a limiting factor because it hinders the kinetics of water and ion absorption at toxic concentrations (Braccini et al., 1996). Thus, when osmotic potential in solution is lower than that in embryo, there is a reduction in germination speed and percentage (Marcos-Filho, 2015).

After drying, seeds returned to initial moisture content, and there was no difference among treatments (Figure 1). Thus, water absorbed during the process was removed and seeds were able to tolerate this process, without permanent damage, while moisture levels considered proper for storage and commercialization were reached (Ribeiro et al., 2019).

There was a significant interaction among the factors priming solution and germination conditions for the total germination, first count, germination speed index, and viable seeds remaining from the germination test (Tables 1 and 2). In germination process in water (no stress), seeds primed with SNP showed higher germination than control and KNO_3 treatments but did not differ from water, SPE and GA treatments (Table 1). Bonome et al. (2006) reported that due to its low molecular weight, KNO_3 can penetrate seed tissues, causing phytotoxicity, which tends to be more severe with higher seed exposure to the solution. Ataíde et al. (2015) saw a reduction in the germinative power of *Dalbergia nigra* seeds pre-primed in KNO_3 with an increase in concentration from 0.1 to 1 and 10 $mmol.L^{-1}$.

Kaiser et al. (2016) evaluated cabbage seeds primed with different concentrations of KNO_3 and observed that seeds subjected to salt stress conditions had significantly lower germination values (36%) than non-primed seeds (66%). These authors associated this result with the reduction in water potential, which resulted in less water absorption, affecting the germination ability and seedling length.

Treatments with priming under salinity (NaCl) had higher germination percentages than the control, and among priming solutions, SNP supplied higher germination percentage (Table 1). Under water deficit (PEG), lower germination percentage was observed for spermidine, while higher on SNP, for this condition, no statistical differences were observed among the remaining treatments and control.

Higher germination percentage obtained with SNP under salt stress and water deficit can be explained by SNP being nitric oxide donor. Nitric oxide promotes plant tolerance to salt stress by reducing the transport of Na^+ and Cl^- to leaves and by enabling plant to compartmentalize these ions in vacuoles to prevent their accumulation in cytoplasm or cell walls and thus avoid salt toxicity (Pires et al., 2016; Faraji and Sepehri, 2018; Silva et al., 2019).

In evaluation of first count, seeds subjected to priming showed higher germination percentage than under all germination conditions (Table 1). The salt stress condition showed the greatest differences between control and primed seeds. For all treatments subjected to priming, water deficit condition reduced germination more than salt stress. Seeds primed with SNP showed higher germination at first count than all other treatments under stress conditions.

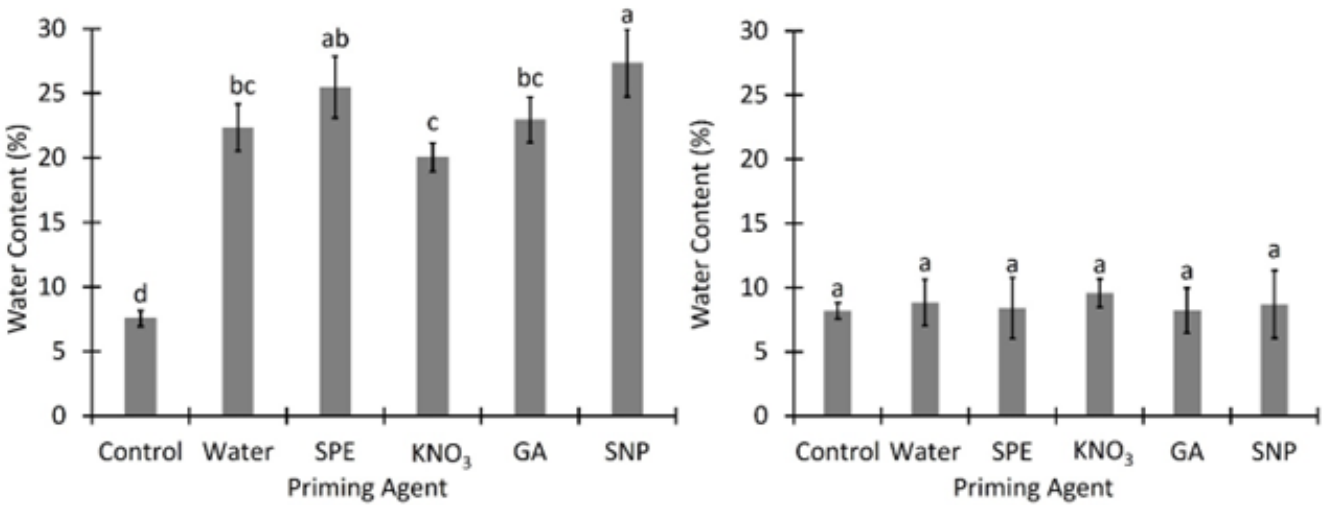

Figure 1. Water content of *Urochloa ruziziensis* seeds before (A) and after drying (B) without subjected to physiological priming (control) and subjected to priming in water, spermidine (SPE), potassium nitrate (KNO_3), gibberellin (GA), and sodium nitroprusside (SNP). Bars are means ± standard error (n=4). Same letters do not differ from each other on Tukey's test set at 5% of probability.

Table 1. Total germination and first germination count from of conditioned *Urochloa ruziziensis* seeds on conditions of no stress, salinity (NaCl), and water deficit (PEG).

Priming solutions	Total Germination			First Count		
	No Stress	NaCl	PEG	No Stress	NaCl	PEG
Contol	68.5 Ba	35.0 Cc	56.0 Bb	37.5 Da	5.0 Cb	3.0 Bb
Water	74.5 ABa	55.5 Bb	45.5 BCb	56.5 BCa	12.0 BCb	5.0 Bc
SPE	78.0 ABa	59.0 Bb	36.0 Cc	57.5 BCa	20.0 Bb	2.0 Bc
KNO_3	68.0 Ba	57.5 Bab	50.0 BCb	55.5 Ca	16.5 BCb	5.5 Bc
GA	78.0 ABa	54.0 Bb	46.5 BCb	67.5 ABa	15.5 BCb	5.5 Bc
SNP	84.0 Aa	72.5 Aa	64.5 Ab	76.5 Aa	37.5 Ab	24.5 Ac

Means followed by the same uppercase letter among priming solutions (within each condition) and by the same lowercase letter among germination conditions does not differ. Without subjected to physiological priming (control) and subjected to priming in water, spermidine (SPE), potassium nitrate (KNO_3), gibberellin (GA), and sodium nitroprusside (SNP).

Pereira et al. (2012) evaluated the effects of water deficit and salt stress on germination of *U. ruziziensis* seeds and concluded that water stress causes a greater reduction in vigor, germination speed and final germination than salt stress. These authors observed that for final germination, there was a reduction of 74% and 61% compared to control in the treatments with PEG and NaCl, respectively, at a potential of -0.4 MPa. Higher values on germination speed index were observed for seeds subjected to priming with SNP, independently of the germination condition (no stress, salinity, or water deficit), with lower values observed for control (Table 2). Under the condition of germination in water (no stress), both SNP and GA were superior to the other treatments.

GA increases transcription levels of the gene encoding alpha-amylase (Li et al., 2019). In Poaceae seeds, embryo produced GA accelerates endosperm nutrient reserves digestion because it stimulates production of hydrolytic enzymes, which degrade starch and other macromolecules to smaller molecules that can be absorbed by the embryo (Batista et al., 2015; Li et al., 2019). Seeds within each treatment remaining from germination test and that were not

dead were subjected to the tetrazolium test to evaluate their viability (Table 2). Based on the results of this test, groups subjected to priming had a lower percentage of remaining viable seeds. Comparable results were reported by Batista et al. (2016) and Ribeiro et al. (2019).

Salt stress and water deficit, especially water deficit, increased the percentage of remaining viable seeds in all treatments except for SNP priming (Table 2). SNP treatment, in addition to not exhibiting differences among the germination conditions, showed the lowest percentage of remaining viable seeds among the priming solutions. Some studies report that the use of SNP breaks dormancy and increases germination because nitric oxide acts as an oxidizer and accelerates metabolic flow through the phosphate pentose pathway, indirectly increasing NADPH oxidation (Pires et al., 2016; Faraji and Sepehri, 2018; Silva et al., 2019).

SNP has the potential to promote seed germination recovery under salt stress (Silva et al., 2019), providing increased germination, vigor, and seedling growth (Pires et al., 2016). In addition, nitric oxide donors increase the antioxidant enzymes activity (peroxidase, superoxide dismutase, and catalase) that minimize the oxidative stress caused by salinity. Nitric oxide is a messenger molecule, and in most cases, the stress response is the interaction with phytohormones (Du et al., 2015).

As can be observed on Table 3, independently of the treatment, under no stress condition seedlings developed higher values on shoot length. As stress condition is applied, except for seeds treated with SNP under salinity, shoot length tends to decrease. For control, water, and giberellin, lower shoot length is observed on salinity, while no statistical differences are observed between salinity and water deficit for espermidin and KNO_3 (Table 3). Differently, for SNP, no statistical differences were observed between no stress and water deficit, with lower values on salinity (Table 3). Regarding root length, for no stress and salinity, higher values were observed for SNP, with lower values on control (without stress) and gibberellin (under salinity) (Table 3). Under water deficit, no statistical differences were observed between control and SNP, both with the higher values comparing with other treatments. Lower root length for this stress was observed for gibberellin (Table 3). Du et al. (2015) observed that salt stress (200 mM NaCl) treatment of *Spinacia oleracea* plants resulted in elevated levels of toxic substances, such as malondialdehyde and hydrogen peroxide. In germination of *Senna macranthera* seeds subjected to salt stress at -0.3 and -0.4 MPa NaCl, there was a reduction in root protrusion, radicle protrusion speed index, normal seedling percentage, shoot and root length, shoot and root dry weight (Silva et al., 2019).

Salt stress, in addition to hindering water absorption due to the reduction in osmotic potential of medium, allows the entry of toxic amounts of Na^+ and Cl^- ions into seeds during imbibition (Silva et al., 2019). This toxicity resulting from excess ions causes an increase in production of reactive oxygen species (ROS), which can lead to oxidative damage in various cellular components, such as proteins, lipids, and DNA, interrupting the vital cellular functions of plants (Gupta and Huang, 2014). It is important to note that in addition to reducing root length, it was observed during the evaluations that the roots grown under salt stress were thicker than those grown under other conditions.

In general, no clear pattern was observed for superoxide dismutase, catalase, and ascorbate peroxidase activity in response to treatments applied (Tables 4 and 5). For superoxide dismutase activity (Table 4), at no stress condition, higher activity was found for control, while lower values for water, KNO_3, and GA. Under salinity, higher values were observed for control, SPE, and SNP. Under water deficit, control, SPE, and KNO_3 resulted on higher activity, while lower was observed for SNP (Table 4). By comparing the three germination conditions (no stress, salinity, or water deficit) for each priming solution, higher values o SOD activity was observed always under salinity (Table 4). For catalase activity, no statistical differences were observed among priming agents at no stress condition (Table 4). Under salinity, higher values were observed on control and lower for KNO_3. Under water deficit higher CAT activity was also observed for control, with lower values for SPE, GA, and SNP (Table 4). For ascorbate peroxidase, we observed higher values for water under no stress condition, control under salinity, and KNO_3 under water deficit (Table 5). The higher ascorbate peroxidase activity in response to germination condition for each priming agent vary depending on the molecule used.

Table 2. Germination Speed Index and viable seeds from germination tests of conditioned *Urochloa ruziziensis* seeds on conditions of no stress, salinity (NaCl), and water deficit (PEG).

Priming solutions	Germination Speed Index			Viable Seeds		
	No Stress	NaCl	PEG	No Stress	NaCl	PEG
Contol	4.99 Ca	1.67 Cc	2.00 Bb	6.00 Ab	21.00 Aa	14.00 ABb
Water	6.43 Ba	2.94 Bb	2.18 Bb	5.00 Ab	11.00 Ba	10.00 Bab
SPE	6.18 Ba	3.47 Bb	1.65 Bc	3.00 Ac	8.50 Bb	18.00 Aa
KNO_3	6.69 Ba	3.19 Bb	2.40 Bb	3.50 Ab	6.00 Bb	15.00 ABa
GA	8.00 Aa	3.06 Bb	2.33 Bb	3.50 Ab	11.00 Ba	15.50 ABa
SNP	9.01 Aa	5.03 Ab	4.17 Ab	2.50 Aa	5.00 Ba	5.00 Ca

Means followed by the same uppercase letter among priming solutions (within each condition) and by the same lowercase letter among germination conditions does not differ. Without subjected to physiological priming (control) and subjected to priming in water, spermidine (SPE), potassium nitrate (KNO_3), gibberellin (GA), and sodium nitroprusside (SNP).

Table 3. Root and shoot length from seedlings developed from of conditioned *Urochloa ruziziensis* seeds on conditions of no stress, salinity (NaCl), and water deficit (PEG).

Priming solutions	Root Lenght (cm)			Shoot Lenght (cm)		
	No Stress	NaCl	PEG	No Stress	NaCl	PEG
Contol	8.70 Cb	2.83 BCc	10.53 Aa	4.33 Ba	1.44 Bc	2.78 BCb
Water	9.09 BCa	3.36 ABCb	10.13 Aba	6.08 Aa	2.22 ABc	3.48 Bb
SPE	10.95 ABa	3.61 ABCc	8.19 BCb	5.87 Aa	2.39 ABb	2.61 BCb
KNO_3	9.02 BCa	4.87 ABb	5.82 Db	4.62 Ba	2.04 ABb	2.39 Cb
GA	9.44 ABCa	2.29 Cc	7.44 CDb	4.71 Ba	1.84 ABc	3.34 BCb
SNP	11.33 Aa	5.41 Ab	11.12 Aa	5.75 Aa	2.61 Ab	5.17 Aa

Means followed by the same uppercase letter among priming solutions (within each condition) and by the same lowercase letter among germination conditions does not differ. Without subjected to physiological priming (control) and subjected to priming in water, spermidine (SPE), potassium nitrate (KNO_3), gibberellin (GA), and sodium nitroprusside (SNP).

Table 4. Superoxide dismutase and catalase enzyme activity on seedlings developed from of conditioned *Urochloa ruziziensis* seeds on conditions of no stress, salinity (NaCl), and water deficit (PEG).

Priming solutions	Superoxide Dismutase (U SOD $min^{-1}.g^{-1}$ FW)			Catalase (nmol H_2O_2 $min^{-1}.g^{-1}$ FW)		
	No Stress	NaCl	PEG	No Stress	NaCl	PEG
Contol	246.42 Ab	323.68 Aa	115.95 Ac	0.022 Ab	0.051 Aa	0.055 Aa
Water	136.39 Cb	291.73 ABa	73.07 ABc	0.017 Ab	0.026 BCb	0.053 ABa
SPE	158.82 BCb	254.49 Ba	116.7 Ab	0.022 Aa	0.028 BCa	0.026 Ca
KNO_3	140.58 Cb	316.71 Aa	102.06 Ab	0.021 Aab	0.019 Cb	0.035 BCa
GA	142.55 Cb	191.35 Ca	40.31 BCc	0.021 Aa	0.025 BCa	0.030 Ca
SNP	207.96 ABb	320.62 Aa	2.09 Cc	0.030 Aa	0.041 ABa	0.028 Ca

Means followed by the same uppercase letter among priming solutions (within each condition) and by the same lowercase letter among germination conditions does not differ. Without subjected to physiological priming (control) and subjected to priming in water, spermidine (SPE), potassium nitrate (KNO_3), gibberellin (GA), and sodium nitroprusside (SNP).

In the present study, under no stress condition, higher MDA content was observed on KNO_3-primed seeds (Table 5). Under salinity, control was among treatments with higher values on this parameter (Table 5). However, different results were observed for each stress condition. Under salinity, higher values on MDA were observed for SPE, with no differences from control, and GA. Lower values on this condition were observed only when seeds were treated with water (Table 5). Under water deficit, higher values of MDA were observed when seeds were treated with SPE, and remaining treatments has no statistical differences among each other (Table 5). Regardless the little variations on our parameters results, our results show that priming of seeds, especially with SNP, has significant effects on the germination and development of brachiaria seedlings when subjected to water deficit and salt stress conditions.

Polyamines, such as SPE, are polycationic nitrogen compounds and compatible solutes that can be conjugated to insoluble anionic macromolecules such as DNA, proteins, and phospholipids, positively regulating seed germination and the synthesis of new molecules (Shi et al., 2010). It has been shown that SPE participates in plant and seed tolerance to abiotic stresses by enhancing the accumulation of diverse types of polyamines under these conditions (Pál et al., 2015), and this accumulation is linked to osmotic adjustment by increasing prolines and reducing sugars (Saruhan et al., 2006). Bioactive GA plays a key role during seed germination and the initial stages of seedling establishment. Use of GA in priming of seeds grown under salt stress and low water availability conditions triggers an improvement in the antioxidant system, reducing the overproduction of ROS and the activation of the enzymatic system (Iftikhar et al., 2020). A study with rice by Li et al. (2019) showed the significant role of GA in the regulation of seed germination under salt stress.

Under water and salt stress conditions, the balance between ROS generation and elimination is altered (Munns and Tester, 2008). Because these molecules are highly toxic at high concentrations, damage to macromolecules such as proteins, lipids and nucleic acids is commonly found, in addition to structural damage, such as to wall membranes (Manaa et al., 2011). Under these conditions, lipid peroxidation is a key factor to be measured because it inhibits seed germination (Yang et al., 2010). At low concentrations and with the equalization of antioxidant enzyme generation and activity, ROS play a significant role as signalling molecules and regulate physiological responses (Suzuki et al., 2014).

Table 5. Ascorbate peroxidase enzyme activity and MDA content on seedlings developed from of conditioned *Urochloa ruziziensis* seeds on conditions of no stress, salinity (NaCl), and water deficit (PEG).

Priming solutions	Ascorbate Peroxidase (µmol AsA.g^{-1} FW.min^{-1})			MDA (µmol.g^{-1} FW)		
	No Stress	NaCl	PEG	No Stress	NaCl	PEG
Contol	0.033 ABb	0.064 Aa	0.0244 BCc	14.66 Ca	7.87 ABb	2.83 Bc
Water	0.042 Aa	0.010 BCc	0.020 Cb	13.97 Ca	5.22 Cb	3.05 Bc
SPE	0.028 Ba	0.010 BCb	0.031 ABa	16.88 Ba	9.73 Ab	7.04 Ac
KNO_3	0.035 ABa	0.010 BCb	0.040 Aa	19.02 Aa	6.77 BCb	4.55 Bc
GA	0.016 Ca	0.003 Cb	0.019 Ca	7.84 Da	7.74 ABa	3.99 Bb
SNP	0.014 Cb	0.013 Bb	0.027 BCa	8.96 Da	7.33 BCa	3.75 Bb

Means followed by the same uppercase letter among priming solutions (within each condition) and by the same lowercase letter among germination conditions does not differ. without subjected to physiological priming (control) and subjected to priming in water, spermidine (SPE), potassium nitrate (KNO_3), gibberellin (GA), and sodium nitroprusside (SNP).

CONCLUSIONS

Physiological performance is better in seeds subjected to priming under water deficit and salt stress conditions. Sodium Nitroprusside shows significant potential for use in the physiological priming of *U. ruziziensis* seeds.

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