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# Ethanol and hormones in physiological conditioning on germination and seed dormancy of *Urochloa humidicola* cv. Llanero

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**ABSTRACT:** Seed conditioning improves germination uniformity, speed, and dormancy release. This work aimed the study the physiological conditioning of *Urochloa humidicola* cv. Llanero, using two lots of dormant seeds conditioned by imbibition with water, commercial bioregulator (auxin, gibberellin - 0.144 mM and cytokinin), gibberellin (0.144 and 1.44 mM) or 5% ethanol. Germination, dormancy, SOD activity, MDA and $H_2O_2$ content were evaluated. In both lots, germination percentage was positively influenced by the commercial bioregulator without affecting the viability. In the first, the germination, using a bioregulator or ethanol, was higher; in the second, the highest germination was from the treated seeds concerning the control and water treatment. The unconditioned seeds presented a higher $H_2O_2$ and protein content in both lots. The MDA content in the first lot was higher in the non-conditioned seeds and conditioned with ethanol. In the second lot, the water conditioned seeds had higher content. In both lots, the bioregulator conditioned seeds showed higher SOD activity. The $H_2O_2$ content was related to antioxidation activation. It was impossible to identify the oxidative window of germination in which $H_2O_2$ would be a signal, releasing germination. The conditioning of seeds with any product showed superior physiological quality than the unconditioned or conditioned with water.

Index terms: priming, forage, grass seed, growth regulator.

**RESUMO:** O condicionamento das sementes melhora a uniformidade da germinação, a velocidade e a liberação da dormência. Este trabalho teve como objetivo estudar o condicionamento fisiológico de *Urochloa humidicola* cv. Llanero, utilizando dois lotes de sementes dormentes condicionadas por embebição com água, biorregulador comercial (auxina, giberelina - 0,144 mM e citocinina), giberelina (0,144 e 1,44 mM) ou etanol 5%. A germinação, dormência, atividade de SOD e conteúdo de MDA e $H_2O_2$ foram avaliados. Em ambos os lotes, a porcentagem da germinação foi influenciada positivamente pelo biorregulador comercial, sem interferir na viabilidade. No primeiro, a germinação, com biorregulador ou etanol, foi maior. No segundo, as maiores germinações foram das sementes tratadas em relação ao controle e tratamento com água. Em ambos os lotes, as sementes não condicionadas apresentaram maior conteúdo de $H_2O_2$ e proteína. O conteúdo de MDA, no primeiro lote, foi maior nas sementes não condicionadas e condicionadas com etanol, já no segundo lote, as sementes condicionadas com água apresentaram maior conteúdo. Nos dois lotes, as sementes condicionadas com biorregulador apresentaram maior atividade de SOD. O conteúdo de $H_2O_2$ se relacionou com ativação de antioxidação. Não foi possível identificar a janela oxidativa da germinação onde $H_2O_2$ seria o sinal, liberando a germinação. O condicionamento das sementes com qualquer produto apresentou qualidade fisiológica superior às não condicionadas ou condicionadas com água.

Termos para indexação: condicionamento, forragem, semente de gramíneas, regulador de crescimento.

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## INTRODUCTION

The increase in beef production in Brazil is related to genetics, animal nutrition and pasture quality. Grasslands in Brazil are composed mainly of *Urochloa* (syn. *Brachiaria*) spp. They are increasing in the area because they are very adaptable to a broad range of climate and soil conditions (Cardoso et al., 2014). *Urochloa humidicola* cv. Llanero is mainly adapted to well-drained acidic and poor soils (Keller-Grein et al., 1996).

There are several definitions for seed dormancy, but this can generally be defined as a temporary failure of one seed to complete germination under favourable conditions, allowing dispersion in time (Baskin and Baskin, 2014).

The Urochloa seeds have no deep physiological dormancy, e.g. do not need extended time of stratification or gibberellic acid increases germination (Baskin and Baskin, 2004; 2014), and dormancy release occurs during storage. Some treatments may increase it, such as gibberellic acid application, acid scarification with sulphuric acid, or use of potassium nitrate 0.2% solution (Brasil, 2009; Silva et al., 2013; Sorigotti et al., 2016). However, the use of sulphuric acid may inspire some care as it could be harmful either for workers or for the environment (Alvarez et al., 2007) and to avoid seed damage (Usberti and Martins, 2007). Both substances, sulphuric acid, and potassium nitrate, are subject to regulation by federal agencies (Brasil, 2019 a, b). In the case of U. humidicola cv. Llanero, acid scarification may be the last resource; the seeds' storage might be preferred.

Some factors may trigger germination in dormant seeds, such as the hormone balance, scarification or lixiviation/solvents application. That changes in the embryo affect the activities of the enzyme the hormones balance, leading to an imbalance of reactive oxygen species (ROS), increasing germination.

Seed conditioning consists of soaking them in water, saline, osmotic solution, or moist substrates to activate the metabolism. However, the conditioning period must be restricted to phases I and II of the stages of imbibition for germination, when the repair of macromolecules and cellular structures occurs, and it is insufficient for the seeds to reach phase III, which is when the emergence of the primary root occurs (Bewley et al., 2013).

The efficiency of physiological conditioning was evaluated in seeds of different species and with varying techniques of hydration, which may influence the response to conditioning. In some species, it can cause improvements in germination, seed germination speed or initial seedling development as observed in *U. brizantha* (Batista et al., 2018; Pereira et al., 2018), or even minor morphological and cytochemical changes as seen in sorghum (Oliveira et al., 2011). However, there may be no increase in germination or initial seedling development in other species as millet (Peske and Novembre, 2010) or another cultivar of *U. brizantha* cv. MG5 (Cardoso et al., 2014).

Gibberellin stimulates germination. Its exogenous application is efficient in breaking seed dormancy. Cytokinins complement gibberellins, as they are responsible for cell division and promoting root growth; auxin promotes the formation of lateral and adventitious roots (Tuan et al., 2018; 2020). Therefore, using a bioregulator containing gibberellin and cytokinin can effectively break dormancy and seed germination. Some studies have associated seed conditioning with gibberellin or a bioregulator containing it. The results vary between species, as positive effects were observed in palisade grass (Batista et al., 2016) or with no difference between water and gibberellin solution (Ramos et al., 2015).

Gibberellins are soluble in ethanol, which can have a different effect depending on the concentration and the species studied. It could be able to improve or inhibit germination, promote changes in the morphology of the seed coating, as well as prolong germination, supporting the idea that germination inhibitors are leached from seeds immersed in ethanol (Keun et al., 2016) or water (Nakabayashi and Leubner-Metzger, 2021).

In the germination of seeds of 14 species of *Brachyscome* and two species of *Allittia*, 11 of these species were responsive to gibberellic acid ($GA_3$); however, the use of a low concentration of ethanol (0.5%) to dissolve $GA_3$ affected subsequent germination results, with four species responding positively and eight negatively to this solvent, masking the pure effect of $GA_3$ due to the presence of small concentrations of ethanol (Aleman et al., 2018) the methods used to dissolve $GA_3$ powder – particularly if using organic solvents – have the potential to affect germination outcomes. In this study we examined the influence of the solvent ethanol, used to dissolve $GA_3$, on the seed germination of 14 species

of *Brachyscome* and two species of *Allittia*. These species are important Australian native composites with potential for use in habitat restoration. Seeds of 11 of these species were found to be particularly responsive to $GA_3$. However, the use of a low concentration of ethanol (0.5% for *U. brizantha* cv. Piatã, the immersion of the seeds in alcohol 70% for 30 minutes), is harmful to the physiological quality of the seeds (Sbalcheiro et al., 2014).

In tomato seeds (*Lycopersicon esculentum* L.) soaked for 24 h in an aerated solution with 2 to 4% of ethanol, germination was improved (Afzal et al., 2013), as such a different sunflower genotypes (*Helianthus* sp.) submitted to ethanol 25% for 15 min, which was adequate to overcome the dormancy of these seeds, behind only to the acetone solution (Nasreen et al., 2015).

*Euphorbia heterophylla* L. seeds were placed to germinate on paper soaked in water or with ethanol solutions at concentrations ranging from 0.25 to 1.5%, causing a delay in germination, with inhibitory effect at concentrations above 0.75% in the evaluation periods (48, 72 and 96 hours) (Kern et al., 2009). In rice some genotypes can use ethanol and scavenge the ROS produced during germination (Miro et al., 2017).

Seed deteriorates during storage, a natural process that can be intensified independent of storage conditions. Therefore, breaking dormancy and decay are simultaneous events. The deterioration process can lead to excessive reactive oxygen species (ROS) production like superoxide radicals, hydrogen peroxide, hydroxyl radicals, and singlet oxygen. Excess ROS can activate the antioxidant defense pathway, composed of numerous enzymatic or non-enzymatic mechanisms against cellular oxidative damage (Gratão et al., 2012).

There is an ideal content of ROS that trigger germination (oxidative window of germination (Bailly et al., 2008; El-Maarouf-Bouteau and Bailly, 2008; Bailly, 2019; Bailly and Merendino, 2021). Below these ROS amounts, germination can be inhibited in dormant seeds; above that, the seeds start to suffer oxidative damage leading to deterioration and decreased germination. Therefore, the balance between the amount of ROS and the action of ROS removing enzymes are essential for the conclusion of germination (Bailly et al., 2008; El-Maarouf-Bouteau and Bailly, 2008; Kranner et al., 2010).

Some studies about ROS effects in seedlings from seeds germinated under stress conditions in several species were carried out. However, there is a lack of information regarding *Urochloa humidicola* seeds' responses to antioxidant enzymes such as SOD, lipid peroxidation (MDA content) and free radicals such as hydrogen peroxide - $H_2O_2$ in conditioned and unconditioned seeds to elucidate dormancy aspects. The physiological conditioning of seeds improves uniformity and better germination speed; gibberellic acid can break seed dormancy. So, it is essential to study the association of these two techniques in the treatment to break dormancy in forage seeds, as dormancy is a significant issue for seed companies.

The aim of this experiment was to verify the effects of physiological conditioning with gibberellin, including a commercial source (Stimulate®), and ethanol on seeds of *Urochloa humidicola* cv. Llanero on germination, dormancy, SOD activity, lipid peroxidation (MDA content) and $H_2O_2$ content.

## MATERIAL AND METHODS

Six lots of seeds were received and evaluated for germination and viability by tetrazolium test. From those lots, two with higher viability and dormancy were selected.

For the conditioning study, six treatments were established: i) unconditioned seeds (control); ii) water conditioned seeds; iii) 5% ethanol-conditioned seeds (5%); iv) conditioned with 0.144 mM $GA_3$; v) conditioned with 1.44 mM $GA_3$ and vi) conditioned with the commercial product Stimulate® (0.005% indole butyric acid, 0.009% cytokinin and 0.005% gibberellic acid equivalent to 0.144 mM, undiluted). The dilution of gibberellic acid was carried out in a 5% ethanol solution.

For physiological conditioning, five replications of 2 g of seeds were distributed for each treatment over two sheets of paper and covered with a sheet of germination paper, moistened with the solutions described for each treatment and kept in this condition for 24 hours in a germinator with a temperature of 25 °C for seed imbibition (Caseiro et al., 2004). The paper was soaked with 2.5 times its dry mass in each solution.

The conditioned seeds were dry at 20 °C for 24 hours. Later the moisture content was equilibrated in a mini chamber containing lithium chloride solution (36.4 g of LiCl per 100 mL of $H_2O$) for three days, which provides 50% relative humidity (RH) (Hay et al., 2008), a condition in which the seeds of *Urochloa humidicola* cv. Llanero has a water content on a dry basis of approximately 10%.

*Germination:* The germination test (G) was carried out in a germinator regulated to provide alternating temperature (15-35 °C) and a photoperiod of 8 hours, without applying methods to overcome dormancy, distributing the seeds equidistantly on two sheets of paper, in a transparent plastic box. The report was moistened with water as described above. The evaluations were carried out at 7, 14 and 21 days after sowing, the values expressed in percentage of normal seedlings, as indicated in the Rules for Seed Testing (Brasil, 2009). The germination speed index (GSI) was calculated based on germination results at 7, 14 and 21 days after sowing, dividing the number of germinated seeds by the number of days elapsed between sowing and germination count (modified from Maguire, 1962).

*Tetrazolium Test*: Seed viability It was evaluated by the tetrazolium test (2,3,5 triphenyl tetrazolium chloride; TZ) using four replications of 25 seeds. They were placed to soak submerged in water for 16 hours and manually cut lengthwise. The two halves of the seeds were identified and placed with the cut surface on two sheets of filter paper soaked with 4 mL of 0.1% TZ solution for 5 hours at 40 °C in the dark. After staining the embryos, the evaluation was carried out to identify and count seeds with the vital parts of the embryo coloured (viable) (adapted from Custódio et al., 2012). The results were expressed in the percentage of viable seeds.

At the end of the germination test, the seeds that did not germinate were submitted to the tetrazolium test to verify viable and dead seeds. Viable seeds that did not germinate at the end of the test were considered dormant, and the average number of dormant were calculated as $Dor = \frac{(DGT + (Tz - G))}{2}$; where *Dor* is Dormancy, *DGT* is Dormant seeds at the end of germination test, and the difference between the TZ (viability test) and G (germination test) (Abrantes et al., 2020).

At the end of this period, a portion of seeds from each treatment was removed and stored at -80 °C for further analysis of SOD activity, MDA and hydrogen peroxide ($H_2O_2$) content.

### *Quantification of malondialdehyde (MDA)*

*Quantitation of MDA*: was made using three fresh samples were used per treatment, in duplicate, of 100 mg of seeds macerated and homogenised in 3.25 mL of 80% ethanol (v/v) and centrifuged at 3000 rpm for 10 minutes. After centrifugation, 1 mL of the supernatant was transferred to a microcentrifuge tube with 1 mL of 0.65% thiobarbituric acid (TBA) (w/v) in 20% trichloroacetic acid (TCA) (w/v), it was incubated at 95 °C for 25 min, transferred to ice and centrifuged again. The reading was performed in a spectrophotometer at 532 and 600 nm absorbance. The MDA equivalents ($E_{MDA}$)were calculated using the following expression $E_{MDA} = \left(\frac{(A_{532} - A_{600})}{155000}\right) x10$, *w*here $A_{532}$ indicates the maximum absorbance of the MDA-TBA complex at 532 nm, $A_{600}$ is the absorbance at 600 nm, which corrects for non-specific interferent and *155000* is the molar extinction coefficient of MDA. The values were expressed in nanomol MDA per gram of fresh mass (nmol g.$MF^{-1}$) (Heath and Packer, 1968).

*Quantification of Hydrogen Peroxide ($H_2O_2$):* quantification was performed using three fresh samples of each treatment, in duplicate, of 1 g of seeds macerated in 5% TCA, centrifuged at 14000 rpm for 5 min, at 4 °C. After centrifugation, 800 µL of the supernatant was reserved and 200 µL of reading buffer I with 50% TCA was added, 200 µL of reading buffer II with 10 mM ferrous ammonium sulphate and 100 µL of reading buffer III with 2.5 mM potassium thiocyanate were used for reading in a spectrophotometer at 480 nm. The content of $H_2O_2$ was expressed in µmol.$g^{-1}$ fresh weight (Sagisaka, 1976).

*Superoxide Dismutase Activity (SOD, EC.1.15.11):* Three fresh samples of 100 mg of seeds per treatment were duplicated. The samples were macerated in a mortar with liquid nitrogen added 1 mL of 0.1 M sodium phosphate buffer (pH 7.8), containing 0.4 g of polyvinylpyrrolidone.$L^{-1}$, 2 mM dithiothreitol, 0.1 mM EDTA and 1.25 mM PEG 4000. The extracts were centrifuged at 12,000 *g* for 20 minutes at 4 °C, and a 50 µL aliquot was used for protein quantification (Bradford, 1976). The remaining supernatant was immediately analysed or stored at -80 °C to measure the SOD activity.

SOD activity was measured using the method described by Lei et al. (2005) modified by Moriya et al. (2015). One SOD unit was defined as the enzyme activity capable of inhibiting the photoreduction of NBT to blue formazan by 50% and was expressed in SOD units (mg.protein$^{-1}$). SOD data were normalised by the soluble protein content, determined by Bradford's (1976) method.

*Statistical analysis:* The experiment was carried out in a completely randomised design, using four replications per treatment in the germination and TZ and three in the biochemical analysis, data submitted to analysis of variance (ANOVA). When the F test was significant, the means of the treatments were compared by the Tukey HSD test ($p \leq 0.05$). All analyses were carried out with the aim of the SISVAR software 5.9 build 91 (Ferreira, 2011)

## RESULTS AND DISCUSSION

In the first lot, the germination of seeds conditioned with the commercial bioregulator was higher, and dormancy was low concerning the control and other treatments. There was no statistical difference in germination between the commercial bioregulator and the ethanol solution (Table 1). The germination speed index (GSI) of seeds not conditioned or conditioned with water was lower than the treatments in which the seeds were conditioned with alcohol, gibberellic acid or commercial compound (Table 1). In the second lot, the highest germination and the lowest percentages of dormancy were found in the conditioned seeds compared with the control, except for water conditioned ones. Furthermore, for the GSI, the highest indices and vigour were observed in those primed with ethanol or gibberellic acid (Table 1).

Ethanol is a suitable solvent, reinforcing the idea that germination inhibitors can leach from seeds immersed in ethanol, which requires further experiments to investigate the impact of leachate on germination of seeds that do not show dormancy (Keun et al., 2016). However, in *U. brizantha* cv. Piatã, the immersion of seeds in high concentrations of ethanol (70%) reduced the germination percentage (Sbalcheiro et al., 2014). Nevertheless, the low concentration of ethanol (5%) in this work did not reduce seed viability, as seen in the results obtained in the tetrazolium test (Tables 1 and 2), showing that at 5%, a germination-promoting effect can occur. Similarly, in seeds of tomato cvs. Roma and Nagina conditioned with water and ethanol solutions at 2, 4, and 6% for 24 h, the effect of dormancy breaking was dose-dependent as an increase in germination, seedling vigour and higher antioxidant activity to eliminate peroxide excess was found at concentrations of 2% and 4% of ethanol (Afzal et al., 2013).

This study showed a marked effect of conditioning with ethanol in breaking dormancy in the lots studied. The effect of ethanol is related to the activity of the cytosolic alcohol dehydrogenase, removing the alcohol from the aqueous phase. So, alcohol dehydrogenase activity establishes a narrow window of alcohol dormancy breaking. The optimal concentration for breaking is a balance between the promoting and toxic effects of alcohol (Hallett and Bewley, 2002).

The most common method to break dormancy in *Urochloa* seeds is the chemical scarification with sulphuric acid, which turns seeds more prompt to germinate and form more homogeneous pastures. However, acid scarification is an environmental risk and also to human health. According to Cardoso et al. (2014), this scarification impairs the seed vigour by increasing damage and leaving them more vulnerable to the deterioration processes. On the other hand, physiological conditioning contributes to reorganising cell membrane systems. Therefore, physiological conditioning with solutions containing gibberellin is an alternative to breaking dormancy and increasing germination without harming the physiological quality of the seed, as could be demonstrated in both lots of *U. humidicola* cv. Llanero is used here.

The biochemical analyses (Table 2) of lots 1 and 2, respectively, found that the unconditioned seeds had a higher content of $H_2O_2$ and protein in both lots. The MDA content in both lots was more elevated in seeds conditioned with

water and ethanol, in lot 1, and lower in seeds conditioned with the bioregulator. In lot 2, the MDA was lower in seeds conditioned with the bioregulator and non-conditioned seeds; the other treatments were intermediate. SOD activity, which is vital in the antioxidant defense in cells exposed to oxygen, was measured in both lots. The seeds, of both lots, conditioned with bioregulator showed the higher activity of this enzyme.

The imbibition allows the water to displace ROS inside the seeds, or at least the long-lived molecule such as hydrogen peroxide, which can reach locations far from the place of production. Thus, ROS produced in the dry state would be efficient as a cellular messenger only when the seeds are rehydrated. So, conditioning activates the antioxidant systems and later returns to the dry state, contributing to lower peroxide levels in hydrated seeds (Bailly et al., 2008). It seems that ethanol effectively reduces the amount of hydrogen peroxide (Table 2), which increases germination in both lots (Table 1).

ROS, such as hydrogen peroxide ($H_2O_2$), are strongly reactive and oxidative molecules that damage, affect function, and produce cascading reactions that amplify oxidative stress in cells, leading to cell death. The accumulation of these molecules can be promoted by environmental stress, resulting in crop productivity losses (Gill et al., 2012). According to Bailly et al. (2008), depending on the amount of $H_2O_2$ accumulated in the embryo cells, it can overcome dormancy or cause seed deterioration. The authors proposed an "oxidative window" that establishes a minimum and a critical level of ROS, which prevents or promotes germination; that is, the seed cannot germinate if the ROS level is below the minimum or above the maximum. Below the minimum, the impediment would be due to seed dormancy and above the maximum to seed ages or deteriorate.

The amount of hydrogen peroxide produced during imbibition of non-dormant sunflower seeds was higher than the amount produced in dormant seeds, suggesting a regulation between the production and mechanisms of elimination of ROS between non-dormant seeds and dormant seeds (Bailly et al., 2008). In this work, a decrease of $H_2O_2$ was observed when seeds were imbibed with any solution and germination, and SOD activity was increased, not allowing the formation of new $H_2O_2$ molecules.

The release of ROS accompanies the germination process. Still, the accumulation of ROS is inhibited by ABA. At the same time, GA cancels this inhibition, showing that the action of ROS is related to the hormonal balance between ABA and GA because, in the dormant state, the ABA pathway is active and blocking germination. A high concentration of ABA can keep ROS removal enzymes high, resulting in low ROS accumulation during dormant seed imbibition (El-Maarouf-Bouteau and Bailly, 2008).

Additional evidence suggested that GA induces germination of dormant oat caryopsis (*Avena fatua*), regulating ABA level and ROS antioxidant status. The induction of germination by karrikinolide, a component of smoke, and $GA_3$ was related to increasing content of $H_2O_2$, $O_2^-$ and SOD and CAT activities in embryos. Therefore, ROS homeostasis was probably necessary for the germination of dormant caryopsis (Cembrowska-Lech et al., 2015).

ROS may loosen the cell wall and release endosperm rupture in mutant seeds of *Arabidopsis thaliana* (Lariguet et al., 2013). Endogenous ROS levels after GA and ABA application suggested that ABA inhibits germination by repressing ROS accumulation and that, conversely, GA triggers germination by promoting an increase in ROS levels. Therefore, the balance between ABA and GA is essential to trigger seed germination (Topham et al., 2017).

Li et al. (2018), working with tobacco seeds conditioned with iodonium diphenylene chloride (DPI), uniconazole (UNI), $H_2O_2$ and inhibitor of GA synthesis, found that $H_2O_2$ and GA completely reversed the inhibition caused by DPI or UNI. GA or $H_2O_2$ increased the GA and reduced ABA content through corresponding gene expressions involving homeostasis and signal transduction. In addition, activation of storage reserve mobilisation, an increase in soluble sugar content and isocitrate-lyase (ICL) activity were also induced by GA or $H_2O_2$, which suggests that $H_2O_2$ and GA were essential for the germination of tobacco seeds and, by decreasing the ABA/GA ratio and inducing the mobilisation of the reserve composition, they mutually promoted seed germination.

On dormant barley seeds, Bahin et al. (2011) concluded that $H_2O_2$ might be involved in relieving dormancy through activation of GA signalling and synthesis rather than repression of ABA signalling. According to Bailly (2019) and Kranner et al. (2010), when the accumulated amount of ROS is above the limit, lipid peroxidation occurs; this process is the most serious for the life of organisms because during this process there is the formation of MDA, which is a hydrocarbon fragment of polyunsaturated precursors and serves as an indicator of lipid peroxidation.

Physiological conditioning with water in four rice cultivars reduced MDA total soluble sugar content and promoted glucose metabolism. It increases the range of proline and soluble protein, increasing phenylalanine ammonia-lyase (PAL), SOD, peroxides (POD) and catalase (CAT) activities, which increase during seed germination under water stress (Yuan-Yuan et al., 2010). In this work, conditioning with all solutions but water improved germination and germination speed index but decreased dormancy.

The physiological conditioning of sunflower seeds was positively related to antioxidant enzymes, except SOD. Priming resulted in an increase in germination rate and improved seedling development, and osmopriming of aged seeds almost wholly restored the initial rate of germination and seedling growth and increased the activity of catalase (CAT) and glutathione reductase (GR) enzymes during germination and seedling development, except for SOD (Bailly et al., 2000).

However, in sunflower seed conditioning, malondialdehyde (MDA) and superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR) activities and osmotic treatment resulted in a substantial increase in SOD and CAT activities. Still, they did not significantly affect MDA and GR activity (Kibinza et al., 2011).

Some antioxidant enzymes act to combat the toxic effects of these oxidative molecules. The enzyme SOD is the first and most effective enzymatic antioxidant that fights the toxic action of ROS and promotes greater tolerance of plants to environmental stresses (Moriya et al., 2015; Mittler, 2017)

The content of molecules indicative of oxidant activity ($H_2O_2$) was related to the activation of antioxidation. Still, it was impossible to identify the oxidative window of germination in which $H_2O_2$ would be a signal, releasing germination.

Table 1. Tetrazolium (TZ), Germination (G) and Dormancy (DOR) tests and Germination Speed Index (GSI) results in unconditioned and conditioned seeds.

| Treatment | TZ | G | DOR | GSI |
|---|---|---|---|---|
| Conditioning [§] | | % | | |
| | | Lot 1 | | |
| Control | 89.8 ± 2.48 [£] a[*] | 33.7 ± 4.25 c | 51.5 ± 4.28 a | 3.7 ± 0.39 b |
| Water | 90.0 ± 1.15 a | 25.2 ± 4.50 c | 51.5 ± 1.79 a | 3.3 ± 0.52 b |
| Ethanol | 91.0 ± 1.00 a | 58.7 ± 2.53 ab | 39.8 ± 0.85 b | 7.4 ± 0.65 a |
| $GA_3$ (0.144 mM) | 88.0 ± 3.65 a | 55.2 ± 3.40 b | 37.7 ± 1.45 b | 7.2 ± 0.40 a |
| $GA_3$ (1.44 mM) | 92.0 ± 1.63 a | 52.5 ± 1.94 b | 39.3 ± 0.69 b | 6.6 ± 0.54 a |
| Commercial compound (0.144 mM) | 87.0 ± 2.52 a | 73.5 ± 2.50 a | 27.8 ± 1.77 c | 6.1 ± 0.35 a |
| CV (%) | 5.0 | 13.3 | 10.4 | 16.9 |
| | | Lot 2 | | |
| Control | 88.0 ± 1.63 a | 30.5 ± 0.87 b | 48.7 ± 1.30 a | 3.7 ± 0.04 bc |
| Water | 93.0 ± 1.91 a | 24.5 ± 0.87 b | 56.7 ± 2.05 a | 3.0 ± 0.36 c |
| Ethanol | 83.0 ± 3.42 a | 59.5 ± 2.47 a | 19.5 ± 1.24 b | 7.9 ± 0.23 a |
| $GA_3$ (0.144 mM) | 84.0 ± 3.27 a | 52.0 ± 0.96 a | 21.8 ± 3.05 b | 7.1 ± 0.25 a |
| $GA_3$ (1.44 mM) | 86.0 ± 3.46 a | 61.2 ± 3.08 a | 15.8 ± 0.66 b | 8.3 ± 0.37 a |
| Commercial compound (0.144 mM) | 82.0 ± 2.58 a | 61.5 ± 1.89 a | 14.2 ± 2.33 b | 5.3 ± 0.63 b |
| CV (%) | 6.5 | 10.3 | 13.1 | 12.3 |

[§] Control = unconditioned seeds; Water = water conditioning; Ethanol = conditioning with 5% ethanol solution; $GA_3$ = conditioning with 0.144 mM gibberellin; $GA_3$ = conditioning with 1.44 mM gibberellin; Commercial compound = conditioning with commercial compound containing 0.144 mM gibberellin. [£] Mean ± Standard Error.[*] Means followed by the same letters in the columns do not differ by the Tukey test ($p > 0.05$). CV: coefficient of variation.

Table 2. Results of the quantification of hydrogen peroxide ($H_2O_2$), malondialdehyde (MDA), soluble protein (PROT) and superoxide dismutase (SOD).

| Treatment | $H_2O_2$ | MDA | PROT | SOD |
|---|---|---|---|---|
| Conditioning [§] | µmol.g FM$^{-1}$[#] | nmol.g FM$^{-1}$ | mg.prot.g$^{-1}$ FM$^{-1}$ | unid SOD.mg prot$^{-1}$ |
| | | | Lot 1 | |
| Control | 1.58 ± 0.05 [£] a[*] | 3.26 ± 0.42 ab | 10.27 ± 0.56 a | 0.0014 ± 0.0001 d |
| Water | 1.32 ± 0.03 bc | 4.70 ± 0.65 a | 7.67 ± 0.25 b | 0.0017 ± 0.0001 cd |
| Ethanol | 1.01 ± 0.04 c | 4.47 ± 0.14 a | 4.89 ± 0.39 c | 0.0026 ± 0.0002 b |
| $GA_3$ (0.144 mM) | 1.37 ± 0.14 abc | 3.03 ± 0.34 ab | 6.33 ± 0.21 ab | 0.0021 ± 0.0001 bc |
| $GA_3$ (1.44 mM) | 1.16 ± 0.07 bc | 3.10 ± 0.43 ab | 5.48 ± 0.34 c | 0.0023 ± 0.0001 bc |
| Commercial compound (0.144 mM) | 1.06 ± 0.05 bc | 2.38 ± 0.36 b | 7.80 ± 0.27 b | 0.0075 ± 0.0003 a |
| CV (%) | 14.07 | 29.45 | 12.4 | 13.47 |
| | | | Lot 2 | |
| Control | 1.78 ± 0.08 a | 1.35 ± 0.18 d | 9.60 ± 0.63 a | 0.0014 ± 0.0001 b |
| Water | 0.97 ± 0.06 b | 6.01 ± 0.60 a | 5.40 ± 0.18 bc | 0.0022 ± 0.0001 b |
| Ethanol | 0.93 ± 0.07 b | 5.57 ± 0.87 ab | 4.89 ± 0.38 c | 0.0023 ± 0.0001 b |
| $GA_3$ (0.144 mM) | 1.02 ± 0.08 b | 4.44 ± 0.65 abc | 4.16 ± 0.23 c | 0.0026 ± 0.0002 b |
| $GA_3$ (1.44 mM) | 1.21 ± 0.12 b | 2.99 ± 0.71 bcd | 4.01 ± 0.39 c | 0.0027 ± 0.0002 b |
| Commercial compound (0.144 mM) | 1.17 ± 0.07 b | 2.78 ± 0.66 cd | 6.73±0.24 b | 0.0071 ± 0.0009 a |
| CV (%) | 16.8 | 41.04 | 15.53 | 29.86 |

[§] Control = unconditioned seeds; Water = water conditioning; Ethanol = conditioning with 5% ethanol solution; $GA_3$ = conditioning with 0.144 mM gibberellin; $GA_3$ = conditioning with 1.44 mM gibberellin; Commercial compound = conditioning with commercial compound containing 0.144 mM gibberellin. [£] Mean ± Standard Error.[*] Means followed by the same letters in the columns do not differ by the Tukey test ($p > 0.05$).[#]FM=Fresh mass. CV: coefficient of variation.

## CONCLUSIONS

Seeds conditioned with any product showed superior physiological quality (higher germination and less dormancy) than those non-conditioned or conditioned with water. However, it was not possible to determine the oxidative window of germination.

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