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Effect of physiological conditioning techniques on the germination of soybean seeds under saline stress

Paulo Roberto de Moura Souza Filho[1], Vanessa Silva Romanoski[1], Danielle Cristinne Mourão Guedes[1], Valéria Nogueira de Souza[1], Christiane de Fatima Martins França[2], Adérico Júnior Badaró Pimentel[1]

[1] Universidade Federal do Oeste da Bahia, câmpus Barra, Barra, Bahia, Brasil. E-mail: paulo.souza@ufob.edu.br, danielle.mourao15@gmail.com, nogueiravaleria555@gmail.com, aderico.pimentel@ufob.edu.br, vanessaromanoskiagronomia@gmail.com
[2] Universidade Federal de São Carlos, Araras, São Paulo, Brasil. E-mail: christiane@ufscar.br

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ABSTRACT

Physiological conditioning can improve seed performance by reducing the germination and emergence period and enhancing stress tolerance. The objective was to evaluate the effect of different physiological conditioning techniques, both with and without subsequent dehydration, on the germination performance of seeds of the BRS 7482 RR soybean cultivar under conditions of salt stress. The conditioning treatments included: hydropriming, soaking in distilled water; haloconditioning, soaking in NaCl solution; hormonal priming, in salicylic acid solution and osmopriming in PEG-6000 solution. In the first experiment, seeds were conditioned in distilled water or NaCl (150, 300 mM). In the second, seeds were treated with salicylic acid (0.075, 0.15, 0.30 g L^{-1}). In the third, seeds were exposed to PEG-6000 solutions (0, -0.4, -0.8 MPa) for 6 hours. The parameters assessed were germination and the length of the hypocotyl root axis. The physiological conditioning techniques demonstrated effects on the salinity stress tolerance of soybean BRS 7482 RR. Halopriming with 150 mM NaCl and hormonal conditioning with salicylic acid (0.15 g L^{-1}) improved germination and seedling growth under moderate stress. Osmopriming showed potential under moderate salinity stress. However, higher NaCl concentrations and subsequent dehydration reduced seed performance. The dehydration process should be further studied to determine its effectiveness in seed conditioning.

Keywords: Salicylic acid, Sodium chloride, Seed dehydration, Priming and abiotic stress.

Efeito das técnicas de condicionamento fisiológico na germinação de sementes de soja sob estresse salino

RESUMO

O condicionamento fisiológico pode melhorar o desempenho das sementes, reduzindo o período de germinação e emergência e aumentando a tolerância ao estresse. O objetivo foi avaliar o efeito de diferentes técnicas de condicionamento fisiológico, com e sem desidratação subsequente, no desempenho de germinação de sementes da cultivar de soja BRS 7482 RR em condições de estresse salino. Os tratamentos de condicionamento incluíram: hidrocondicionamento, imersão em água destilada; halocondicionamento, imersão em solução de NaCl; condicionamento hormonal, em solução de ácido salicílico e osmoacondicionamento em solução de PEG-6000. No primeiro experimento, as sementes foram condicionadas em água destilada ou NaCl (150, 300 mM). No segundo, as sementes foram tratadas com ácido salicílico (0,075, 0,15, 0,30 g. L^{-1}). No terceiro, as sementes foram expostas a soluções de PEG-6000 (0, -0,4, -0,8 MPa) por 6 horas. Os parâmetros avaliados foram a germinação das sementes e o comprimento do eixo hipocótilo-radícula. As técnicas de condicionamento fisiológico demonstraram efeitos na tolerância ao estresse salino da soja BRS 7482 RR. O halocondicionamento com NaCl a 150 mM e o condicionamento hormonal com ácido salicílico (0,15 g. L^{-1}) melhoraram a germinação e o crescimento das plântulas sob estresse moderado. O osmoacondicionamento mostrou potencial sob estresse salino moderado. No entanto, maiores concentrações de NaCl e a desidratação subsequente reduziram o desempenho das sementes. O processo de desidratação deve ser mais estudado para determinar sua eficácia no condicionamento de sementes.

Palavras-chave: Ácido salicílico, Cloreto de sódio, Desidratação de sementes, Condicionamento e estresse.

1. Introduction

Soybean (*Glycine max* L.) stands out as one of the most important species in the legume group, driven by high demand in agricultural markets for vegetable oil and protein (Watson et al., 2017; Hou et al., 2024), as well as its application in biofuel production (Heimann et al., 2024). In the 2022/2023 harvest, production reached 154,603.4 tons in a cultivated area of 44,072.9 ha, resulting in an average productivity of 3,508 kg ha^{-1} (Conab, 2023). However, one of the key issues that still requires significant improvement is the seedling emergence. Under field conditions, soybean seeds are susceptible to both biotic and abiotic stresses, such as saline stress, which can severely compromise the yield of the crop (Stania et al., 2023).

Low seedling emergence under saline stress conditions pose a serious challenge to the productivity of soybean crops. Efficient strategies to enhance seed germination and early seedling establishment in such stressful environments are needed to mitigate these adverse effects and ensure high productivity. In this context, the use of high-quality seeds is critical to ensure the emergence of vigorous plants capable of tolerating these stresses, particularly during the initial growth phase (Pinheiro et al., 2019). Fast seed germination and stand establishment are crucial factors influencing production under stress conditions. Therefore, pre-seeding treatments emerge as a promising strategy to promote faster and more uniform germination, ensuring efficient field emergence (Debta et al., 2023)

Physiological conditioning, also known as priming, consists of a set of techniques designed to improve the seed performance (Paparella et al., 2015). These techniques involve exposing seeds to different solutions for a predetermined period, allowing them to partially absorb water and specific solutions without initiating radicle emergence. This partial absorption is sufficient to initiate pre-germinative metabolism, which occurs in the early phase of the germination process (Ibrahim, 2016), aiding in the repair or reorganization of cellular membranes and benefiting the uniformity of the germination process (Santos et al., 2019).

Various physiological conditioning techniques, such as hydropriming, halopriming, hormonal, matric, chemical, and others, have been used to improve productivity in many crops under different types of abiotic stresses (Paparella et al., 2015). Positive effects of physiological conditioning have been reported in several crops, including triticale (Yamur and Kaydan, 2008), wheat (Sumbal et al., 2023), rice (Kharb et al., 2023), and beans (Junaid et al., 2023), among others.

Halopriming, also known as salt priming, involves controlling seed imbibition using a solution with water-soluble inorganic salts, such as sodium chloride (NaCl), potassium nitrate (KNO_3), or magnesium sulfate ($MgSO_4$), within tolerable limits for the crop. It is a simple and inexpensive technique recommended for growers aiming to improve the synchronization of emergence and stand establishment under different environmental conditions (Sedghi et al., 2010; Camargo et al., 2020). Plants can develop a "stress memory" or "stress imprinting" after initial exposure to a stress agent, which enhances their resistance to subsequent stresses (Santos et al., 2019).

The exogenous application or stimulation of endogenous salicylic acid synthesis can act as an inducer of protein tolerance to various stresses. Salicylic acid can thus be used as a hormonal conditioning method. In studies involving seed treatments, salicylic acid has promoted positive responses in germination percentage, germination rates, and induction of systemic resistance in marigold (Carvalho, 2007).

Osmopriming is another method widely used to simulate water stress conditions by controlling seed imbibition with different osmotic potentials (Aydinoglu et al., 2019). This can be achieved using polyethylene glycol (PEG), an inert and non-toxic chemical compound. Osmopriming of soybean seeds has shown that the treatment can improve plant growth and production (Arif et al., 2008; Muhammad et al., 2014); however, there is limited evaluation of its effects under stressful environments.

For field applicability, after physiological conditioning pre-treatments in specific solutions, seeds can be dehydrated to achieve a mass and size close to that at the beginning of the treatment (Adhikary and Mandal, 2019). Dehydration, also known as desiccation, should be performed to avoid seed damage and is typically carried out slowly (Hasanuzzaman et al., 2020). However, doubts remain regarding the effect of dehydration on physiologically conditioned seeds.

Due to the increasing susceptibility of soils to salinization and the rising rates of salt-affected areas (Cai et al., 2021; Singh, 2018), it is critical to develop specific treatments that accelerate and standardize seed germination under saline stress conditions to ensure productivity.

While seed preconditioning has been studied in various crops to accelerate and standardize germination, there are few studies investigating the effects of hydropriming, halopriming, osmopriming, and hormonal priming in soybean, particularly in response to abiotic stresses like salinity. Addressing this knowledge gap is crucial for improving crop resilience and productivity.

Thus, the objective of this study was to evaluate the effect of physiological conditioning techniques, with and without dehydration, on the germination performance of soybean seeds under saline stress conditions.

2. Material and Methods

The experiments were conducted at the Seed Laboratory of the Federal University of Western Bahia - Campus de Barra, Bahia. In these experiments, a batch of soybean seeds from the cultivar BRS 7482 RR, produced in the 2018/19 crop season in Luís Eduardo Magalhães, Bahia, was used. Initially, the batch of seeds underwent a germination test to assess batch viability and seed water content (Brasil, 2009). Subsequently, the batch was placed in waterproof packaging and stored in a refrigerator until the date of the experiments.

The preliminary test of seed tolerance to saline stress was conducted to identify working concentrations based on germination parameters, resulting in the determination of two concentrations: moderate stress and severe stress. In the assays, increasing concentrations of sodium chloride (NaCl) were used: 25, 50, 100, 150, 200, 300, 400 and 500 mM, while distilled water was used in the control. The determined working concentrations for moderate and severe stresses were 150 and 300 mM of NaCl, respectively. At the concentration of 150 mM, normal seedlings were found, while concentrations above 300 mM caused a significant reduction in germination percentage, affecting seed viability.

The initial germination test was conducted using four hundred seeds, randomly distributed on neutral pH Germitest® papers moistened with distilled water at a ratio of 2.5 times the weight of the paper, in eight replicates of fifty seeds each. The seeds were placed on two papers with perforated plates and covered with a third layer, then rolled up and placed in transparent plastic bags. These bags were organized on supports so that the rolls were in a vertical position. The rolls were placed in a B.O.D. germination chamber at a constant temperature of 25 °C in the dark. At the end of eight days, seeds with a radicle protrusion of at least 3 mm were considered as germinated and normal seedlings were evaluated (Brasil, 2009).

Three experiments were conducted to evaluate different physiological conditioning techniques for the seeds. In the first experiment, saline conditioning, seeds were conditioned in distilled water (hydropriming) and NaCl solutions of 150 and 300 mM. In the second experiment, hormonal priming, seeds were subjected to solutions of salicylic acid at concentrations of 0.075, 0.15 and 0.30 g L^{-1}. In the third experiment, involving osmopriming, seeds were exposed to PEG-6000 solutions with potentials of 0, -0.4 and -0.8 MPa for a period of 6 hours. In each experiment, a completely randomized design was used, with a factorial scheme of 3 (saline, hormonal or osmotic conditioning treatments) x 2 (post-conditioning treatments, with and without dehydration) x 3 (solutions of the germination test under saline stress), with four replicates of 200 seeds each. Germination percentage and hypocotyl/radicle axis length were evaluated in the germination tests using image scanning and subsequent analysis with ImageJ software.

In the conditioning treatments, the seeds were immersed in specific solutions in disposable 500 mL plastic cups for 6 hours, in the germination chamber at a temperature of 25±1 °C, in the dark. The proportion of solution or distilled water used was approximately 200 mL for each batch of 200 seeds. After each treatment period, the seeds were washed with running water to remove residues, rinsed with distilled water and superficially dried with paper towels. Part of the seeds was immediately taken for the germination test.

The other part of the pre-treated seeds was placed in shallow trays lined with paper towels to dehydrate under laboratory conditions, under ambient temperature (approx. 27.4±5.1) and humidity (approx. 55.2±19.9). The seeds were measured daily for the weight of the fresh mass. When the weight stabilized, close to the initial measurements, the seeds were used for a new germination test, and these seeds were considered as dehydrated and suitable for use in the field.

The germination test was conducted as described above, but with solutions of different NaCl concentrations: 0, 150 (moderate stress) and 300 (severe stress) mM, equivalent to electrical conductivity of 0, 11.55 and 23.1 $dS.m^{-1}$, respectively. These salinity levels were obtained from preliminary tests with the seed lot.

The data analyses were performed separately for each experiment using R software (R Core Team, 2019). For the length of the hypocotyl/root axis, analysis of variance (ANOVA) was conducted. To meet the prerequisites of ANOVA, the length of the hypocotyl/root axis data underwent logarithmic transformation. When there was significance in the factors, multiple mean comparison tests were performed using Tukey´s test (‘multcomp’ and ‘lsmeans’ packages).

For germination percentage, Generalized Linear Models (GzLM or GLM) with binomial distribution were employed (Carvalho et al., 2018). After selecting the best model using the Akaike Information Criterion (AIC) and confirming the significant effect of each factor in the model, estimated values were analyzed through multiple comparisons with Sidak adjustment for p-value (‘lme4’, ‘multcomp’ and ‘lsmeans’ packages).

3. Results and Discussion

The germination percentages and initial water content of cultivar BRS 7482 RR were 98% and 7%, respectively. Therefore, the seed batch can be considered as having good physiological quality for conducting the experiments.

In Table 1, the germination percentage attributes, revealed that concentrations of hydropriming and haloconditioning from 0 to 150 mM were similar, showing no significant difference. However, the pre-treatment of 300 mM was detrimental to soybean seeds, resulting in reduced germination. Across different pre-treatments, the effect of salt as preconditioning showed no significant positive effect on salinity stress tolerance. However, haloconditioning with 300 mM NaCl with dehydration resulted in a lower germination percentage compared to other treatments.

The dehydration technique performed after imbibition in the hydropriming and haloconditioning treatments directly affected seed germination, with a notable impact on the germination percentage of the 300 mM haloconditioning treatment.

Analyzing the length of the hypocotyl/radicle axis (Figure 1), it is observed that this attribute is more sensitive to the effect of salt stress. Under salt stress of 150 and 300 mM NaCl, all treatments showed growth retardation, as they directly affect seed development. No significant effect was detected among the pre-treatments employed when seeds are exposed to salt stress; thus, haloconditioning did not prove to be effective.

When comparing the three pre-treatments with 150 mM NaCl without dehydration, it is observed that the germination percentage of the treatments are similar. However, haloconditioning at 300 mM NaCl demonstrated that this concentration reduces the germinative power and growth of the seedlings. Additionally, exposure to dehydration further exacerbated the damage observed in the seeds (Table 1 and Figure 1A).

Dehydration in seeds accentuates the reduction in hypocotyl/radicle axis length, directly reflecting in the decrease in the vigor of the seed lots. Most treatments showed a significant reduction after dehydration compared to seeds that were not dehydrated. However, there was an exception with haloconditioning at 150 mM NaCl, where, when exposed to the absence of salt stress, it showed a higher length hypocotyl/radicle axis, indicating greater stress tolerance.

Haloconditioning at 150 mM NaCl showed significant difference compared to all other treatments with dehydration, presenting a longer axis when compared to the others. Therefore, haloconditioning at 150 mM provided the formation of hypocotyl/radicle axis with higher lengths (Figure 1), indicating greater tolerance to stress conditions. It is noteworthy that haloconditioning at 300 mM with dehydration showed lower performance in root size. However, the treatment without dehydration has high performance in root length, regressing according to the increase in NaCl concentrations used in haloconditioning.

Table 1. Germination percentage of soybean seeds under the effect of hydropriming and haloconditioning pre-treatments with 150 and 300 mM NaCl, without and with subsequent dehydration, and subsequently subjected to germination under saline stress levels (0, 150 and 300 mM)

	Salt stress levels		
Without dehydration	**0**	**150 mM**	**300 mM**
Hydropriming	88 a	82 ab	75 c
Haloconditioning (150 mM)	82 abc	85 ab	78 bc
Haloconditioning (300 mM)	85 ab	82 a	48 de
With subsequent dehydration			
Hydropriming	85 ab	80 abc	42 e
Haloconditioning (150 mM)	85 ab	80 abc	30 e
Haloconditioning (300 mM)	80 abc	73 cd	13 f

Lowercase letters stand for significant differences ($p<0.05$) between germinated seed proportions by multiple comparisons of regressions with Sidak adjustment.

The results of hydropriming and haloconditioning (150 and 300 mM) with and without subsequent dehydration (Figure 1) indicated that the dehydration process reduces the length of the hypocotyl/radicle axis. Additionally, treatments with dehydration resulted in shorter root length.

The increase in salt concentration resulted in a decrease in the length of the hypocotyl/radicle axis. Similar results were reported by El-Sanatawy et al. (2021), where haloconditioning reduced the length of the hypocotyl/radicle axis at a concentration of 130 mM NaCl. Kumar and Rajalekshmi (2021) observed that haloconditioning reduced the germination rate at a concentration of 125 mM NaCl and the length of the hypocotyl/radicle axis at a concentration of 250 mM NaCl.

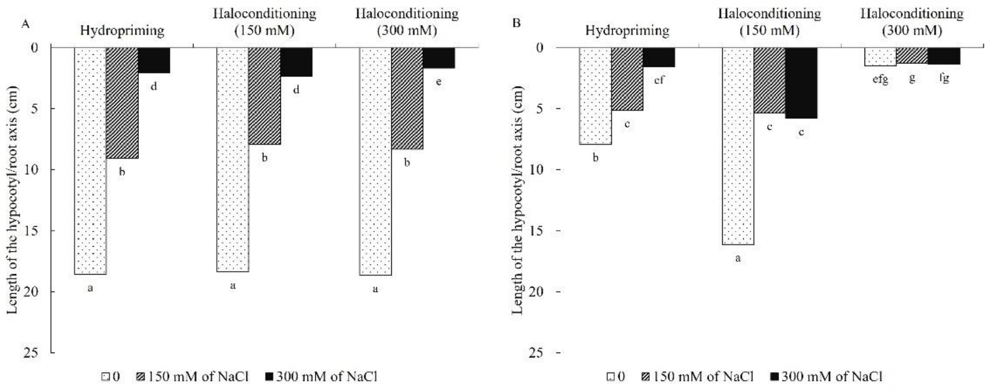

Figure 1: Length of the hypocotyl/radicle axis of soybean under the effect of hydropriming and haloconditioning pre-treatments, without dehydration (A) and with subsequent dehydration (B), and subsequently subjected to germination in a saline gradient (0, 150 and 300 mM). Lowercase letters stand for significant differences (p<0.05) in length of hypocotyl/radicle axis by multiple regression comparisons.

Due to the sharp drop in germination in the treatment using 300 mM NaCl, this concentration was considered as severe stress for the soybean cultivar, making it difficult to assess the effectiveness of physiological conditioning treatments. The results are in line with those obtained by Soares et al. (2015), which showed that there was a reduction in soybean seed germination with decreasing osmotic potential.

Salt stress promotes the synthesis of reactive oxygen species (ROS), disrupts hormonal functions and metabolic pathways, limiting root growth (Khan et al., 2017). The length of the hypocotyl/radicle axis is a suitable variable for assessing salt stress tolerance. The reduction in axis growth is indicative of the toxic effects of ions and osmotic stress.

The excess of salts in the soil affects plants in three main ways: reducing the root water potential leading to water deficit; toxic effect of Na^+ and Cl^- ions; and nutritional imbalance caused by the reduction of water absorption and/or transport by the seedling (Khan et al., 2017). Haloconditioning induces regulation of physiological and biochemical processes, considered as a stress memory, thus promoting rapid germination and tolerance to abiotic stress events (Gamir et al., 2014). In the present study, soybean seeds that underwent haloconditioning did not exhibit stress memory. This result may be a possible effect of genetic variability among soybean cultivars, demonstrating the importance of studies like this in investigating different physiological conditioning methods.

The imbibition with salicylic acid (SA) at different concentrations, followed by exposure to salt stress, had a positive impact on the proportion of germinated seeds, especially under moderate stress, where no significant reduction was observed (Table 2). However, it is important to highlight that the length of the hypocotyl/radicle axis of SA conditioning differs statistically among all treatments (Figure 2A). Additionally, when comparing the assessments conducted immediately after imbibition and after dehydration, a significant decrease in germination due to the dehydration process of the seeds was noted.

Hormonal priming reduced the harmful effects of NaCl on the growth of the hypocotyl/radicle axis. The length of the hypocotyl/radicle axis is relatively greater in the pre-treatments of 0.15 and 0.30 $g.L^{-1}$ of SA, showing a positive effect on the treatments with 150 mM NaCl when compared to hydropriming (Figure 2).

Table 2. Germination percentage of soybean seeds under the effect of Hydropriming and Hormonal Priming pre-treatments with Salicylic Acid (0.075, 0.15 and 0.3 g L^{-1}), without dehydration and with subsequent dehydration, and subsequently subjected to germination under saline stress levels (0, 150 and 300 mM)

	Salt stress levels		
Without dehydration	**0**	**150 mM**	**300 mM**
Hydropriming	89 ab	85 ab	77 b
Hormonal Priming (0.075 g L^{-1})	88 ab	89 ab	86 ab
Hormonal Priming (0.15 g L^{-1})	89 ab	91 ab	83 b
Hormonal Priming (0.30 g L^{-1})	89 ab	87 ab	76 b
With subsequent dehydration			
Hydropriming	80 b	75 b	26 c
Hormonal Priming (0.075 g L^{-1})	85 ab	72 b	26 c
Hormonal Priming (0.15 g L^{-1})	96 a	83 ab	35 c
Hormonal Priming (0.30 g L^{-1})	92 ab	76 b	27 c

Lowercase letters stand for significant differences (p<0.05) between germinated seed proportions by multiple comparisons of regressions with Sidak adjustment.

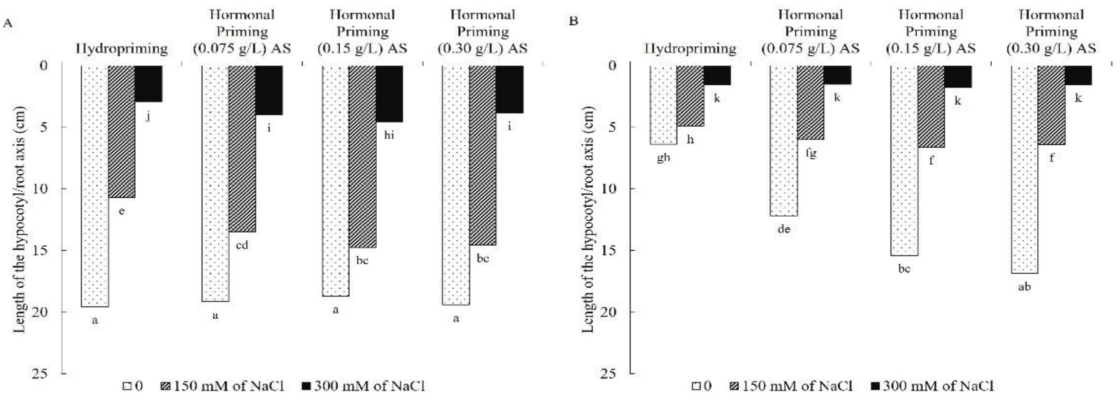

Figure 2: Length of the hypocotyl/radicle axis of soybean under the effect of hydropriming and hormonal priming pre-treatments with Salicylic Acid (0.75, 0.15, and 0.3 g L^{-1}), without dehydration (A) and with subsequent dehydration (B), and subsequently subjected to germination in a saline gradient (0, 150, and 300 mM). Lowercase letters stand for significant differences ($p<0.05$) in germinated seed proportion by multiple regression comparisons.

Without dehydration, pre-treatments with water and salicylic acid showed little difference, except at concentrations of 300 mM NaCl.

All pre-treatments, in the treatment with dehydration at 300 mM NaCl, do not show statistical difference (Figure 2B), as it also demonstrated in the graph that they present a shorter length hypocotyl/radicle axis compared to the other treatments, reinforcing the hypothesis that both concentrations of 300 mM NaCl and dehydration directly affect the germination percentage and the hypocotyl/radicle axis.

The results of the hypocotyl/radicle axis growth attributes indicated that the most effective concentrations of hormonal priming are in the range of 0.15 g L^{-1} of SA, demonstrating better seedling performance under saline stress conditions. Therefore, hormonal priming with salicylic acid proves to be more effective compared to haloconditioning treatment with NaCl. However, it was observed that dehydration treatment, although important for sowing processes, has a negative effect on seedling root length, both in pre-conditioning with SA and in pre-conditioning with NaCl. Thus, the dehydration procedure should be further studied to determine its effectiveness in seed conditioning.

Hormonal priming reduced the detrimental effects of NaCl on the hypocotyl/radicle axis growth. Tolerance to water stress was observed by applying SA pre-treatment in *Brassica napus* seeds (Muchlas et al., 2023). Hormonal priming induced by SA reduced the detrimental effects of NaCl on growth (Ellouzi et al., 2023). The accumulation of H_2O_2 due to oxidative stress was reduced with SA pre-treatment, resulting in decreased lipid peroxidation of the plasma membrane (Muchlas et al., 2023).

The osmotic pre-treatments with -0.4 and -0.8 MPa did not result in increased salinity tolerance (Table 3). Additionally, when comparing the performance of seeds subjected to different osmotic treatments, no significant differences were observed, especially regarding the germination percentage under saline stress conditions.

When soybean seeds were subjected to osmopriming and subsequently dried, the results indicate that the hydration-dehydration process did not influence the performance of the seeds (Table 3). In Table 3, a difference between the osmopriming treatments is observed when subjected to a saline gradient of 150 mM NaCl, with a higher germination percentage in the -0.8 MPa treatment compared to the 0 MPa treatment. This suggests that osmopriming contributes to greater seed tolerance to salt stress, possibly by enhancing osmotic adjustment mechanisms and the early activation of enzymes related to cellular protection. These mechanisms may have allowed treated seeds to maintain water balance and structural integrity under adverse conditions, such as NaCl-induced stress, promoting more efficient germination. In contrast, the control treatment (0 MPa), without the osmopriming preconditioning, likely failed to activate these defense mechanisms efficiently, resulting in lower germination performance under salt stress.

It was observed that regardless of the seed pre-treatment, its development was affected by salinity, becoming more evident in the length of the hypocotyl/radicle axis, which reduced by approximately 50 and 80% in the treatment with saline stress of 150 and 300 mM NaCl, respectively (Figure 3).

The reduction in osmotic potential had a negative impact on the length of the hypocotyl/radicle axis, as indicated by Figure 3. This reduction resulted in a lower growth of this structure, showing a direct correlation between the decrease in osmotic potential and the inhibition of hypocotyl/radicle axis growth.

Regarding the effects of osmopriming, the present study differs from others found in the literature, which indicate that conditioning with PEG-6000 is partially effective in overcoming salt stress, increasing the proportion of seed germination when exposed to saline environments (Jeller et al., 2003).

Table 3. Germination percentage of soybean seeds under the effect of osmotic pre-treatments with PEG-6000 (0, -0.4 and -0.8 MPa), without dehydration and with subsequent dehydration, and subsequently subjected to germination in saline gradient (0, 150 and 300 mM).

	Salt stress levels		
Without dehydration	**0**	**150 mM**	**300 mM**
Hydropriming	72 bc	60 c	23 d
Osmopriming (-0.4 MPa)	79 abc	79 abc	21 d
Osmopriming (-0.8 MPa)	88 ab	86 ab	30 d
With subsequent dehydration			
Hydropriming	82 abc	70 c	35 d
Osmopriming (-0.4 MPa)	76 bc	85 ab	28 d
Osmopriming (-0.8 MPa)	84 abc	79 abc	30 d

Lowercase letters stand for significant differences (p<0.05) between germinated seed proportions by multiple comparisons of regressions with Sidak adjustment.

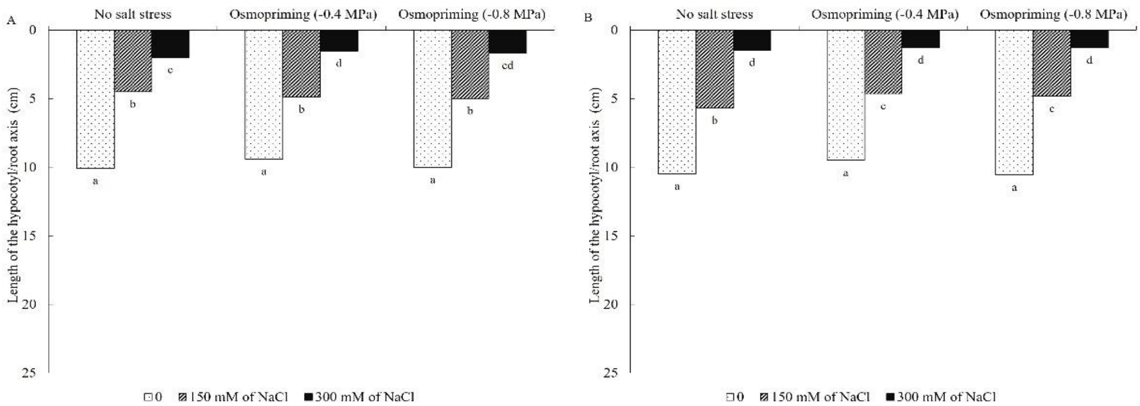

Figure 3: Length of the hypocotyl/radicle axis of soybean under the effect of osmotic pre-treatments with PEG-6000 (0, -0.4 and -0.8 MPa), without dehydration (A) and with subsequent dehydration (B), and subsequently subjected to germination in a saline gradient (0, 150 and 300 mM). Lowercase letters stand for significant differences (p<0.05) in germinated seed proportion by multiple regression comparisons with Sidak adjustment.

Soybean seed germination, shoot and primary root lengths are negatively affected by the reduction in osmotic potential caused by salt stress promoted by NaCl (Soares et al., 2015). PEG-6000 concentrations and the pretreatment period can be detrimental to the growth of the hypocotyl/radicle axis by limiting the availability of oxygen to cells (Debta et al., 2023).

Although the cultivar shows sensitivity to salt stress, it is important to emphasize that the level of salinity used in the experiments is higher than the minimum value used in the saline soil classification scale. Saline soils are defined as those that have an electrical conductivity (EC) ≥ 4.0 $dS.m^{-1}$. The concentrations of 150 and 300 mM used in the present study were based on the response of the seeds under laboratory conditions, and not in the field, and are equivalent to 11.55 and 23.1 $dS.m^{-1}$, respectively. Therefore, the concentrations applied may have caused a very severe stress in the seeds, preventing them from showing full development.

Hydropriming is a low-cost, simple and environmentally friendly method to minimize the effects of osmotic and saline stress (Madani et al., 2023). Hydropriming can increase the expression of aquaporin genes, regulate antioxidant and DNA repair genes and improve water absorption by the seeds (Matsunami et al., 2022). However, this treatment was not effective in this study, which may be related to intrinsic characteristics of the cultivar.

The morphological attributes of seven soybean cultivars under the effect of hydropriming reduced the number of pods per plant, as well as the number and weight of seeds per plant (Lewandowska et al., 2020). Similar results were found in Niger seed (*Guizotia abyssinica* Cass.), which showed a negative effect of hydropriming, resulting in reduced germination. Additionally, increasing levels of salt stress contributed to the decrease in seed vigor (Badalzadeh e Shahraki, 2021).

Seed dehydration also proved to be harmful to soybean seeds. The subsequent dehydration of the seeds initially aims at making practical use of the seeds in the field, as sowing occurs in a mechanized way and soaked seeds could cause problems in the process (Shaw et al., 2020). Therefore, dehydration was thought of as an alternative to enable conditioning for large-scale producers. However, treatments with subsequent dehydration showed a negative effect on germination, especially for seeds subjected to germination under saline stress conditions.

The dehydration process resulted in a reduction in the length of the hypocotyl/radicle axis in the hydropriming and haloconditioning treatments (150 and 300 mM), with and without dehydration, possibly due to the disruption of cell membranes during dehydration. Treatments involving the dehydration process exhibited a shorter root length. This occurs because cells subjected to dehydration undergo significant changes in turgor pressure, leading to cell shrinkage and mechanical stress due to water loss and changes in the cell wall and membranes (Dekkers et al., 2015).

For orthodox seeds, dehydration/rehydration cycles are reversible, without causing a loss of germination due to their ability to tolerate desiccation (Leprince et al., 2017). However, the cultivar did not exhibit this characteristic of desiccation tolerance. Seeds that tolerate drying have some protective mechanisms capable of maintaining cell membrane systems, macromolecular structures and reserve substances in conditions to regain their physiological functions when the seeds are rehydrated (Wang et al., 2021). However, no single mechanism is responsible for this tolerance. Each component is equally critical, acting synergistically and genetically controlled. These techniques offer a range of benefits, such as reducing injuries during the imbibition phase, overcoming seed dormancy in some species, increasing seedling emergence speed and uniformity, and even seed germination (Rehman et al., 2011).

The physiological conditioning techniques applied to the soybean cultivar BRS 7482 RR revealed that hydropriming and halopriming with 150 mM NaCl, without dehydration, contributed to maintaining a higher germination percentage and growth of the hypocotyl/radicle axis under moderate salinity stress. However, halopriming with 300 mM NaCl, especially when combined with dehydration, resulted in a significant reduction in germination percentage and seedling growth, characterizing it as a severe stress level for this cultivar.

Hormonal priming with salicylic acid, particularly at concentrations of 0.15 g L^{-1}, was also effective in mitigating the adverse effects of moderate salinity stress, promoting the growth of the hypocotyl/radicle axis and thus improving seed vigor. However, the subsequent dehydration process negatively affected seed performance in all treatments, highlighting the need for further studies to optimize this practice.

Osmopriming at -0.8 MPa showed promising results in terms of germination under moderate salinity stress, suggesting effective osmotic adjustment and activation of defense mechanisms. However, osmopriming treatments under high salinity conditions were not effective, reinforcing the cultivar's sensitivity to more severe stresses. Thus, osmopriming may offer benefits under moderate salinity stress conditions.

This study utilized a wide range of physiological treatments on soybean seeds, highlighting advancements in seed physiology research. By testing treatments such as halopriming, hormonal priming, and osmopriming, this research provides a deeper understanding of stress tolerance mechanisms, particularly under saline conditions. These findings represent a significant scientific breakthrough, offering a solid foundation for future applications in managing soybean seeds in adverse environments.

4. Conclusions

The physiological conditioning techniques demonstrated effects on the salinity stress tolerance of soybean BRS 7482 RR. Halopriming (150 mM NaCl) and hormonal priming with salicylic acid (0.15 $g.L^{-1}$) improved germination and seedling growth under moderate stress. Osmopriming showed potential under moderate salinity stress. However, higher NaCl concentrations and subsequent dehydration reduced seed performance. These results indicate that the appropriate selection of techniques and concentrations is crucial for optimizing soybean seed resilience in saline environments. The dehydration process should be further studied to determine its effectiveness in seed conditioning.

Authors' Contribution

Paulo Roberto de Moura Souza Filho: conceptualization, methodology, formal analysis, resources, writing. Vanessa Silva Romanoski: formal analysis, writing, translation. Danielle Cristinne Mourão: data curation, investigation, writing. Valéria Nogueira de Souza: data curation, investigation, writing. Christiane de Fatima Martins França: conceptualization, methodology, writing. Adérico Júnior Badaró Pimentel: conceptualization, methodology, resources, writing.

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