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CONDITIONING WHITE OAT SEEDS IN SALICYLIC ACID TO MITIGATE SALT STRESS

CONDICIONAMENTO DE SEMENTES DE AVEIA BRANCA EM ÁCIDO SALICÍLICO PARA MITIGAÇÃO DO ESTRESSE SALINO

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ABSTRACT

Excess salt in the soil has negatively affected the cultivation of forage crops, such as white oats. Consequently, it is crucial to seek alternatives capable of inducing salt stress tolerance in these plants. The present study focuses on investigating the effects of salicylic acid in mitigating salt stress on seed germination and initial growth of white oats. The experiment was conducted in a completely randomized design, with five concentrations of salicylic acid (0; 0.5; 1; 1.5 and 2 mM) and three levels of salinity (0, 50 and 100 mM), with four replicates. The seeds were sown on germitest paper and stored in a Biochemical Oxygen Demand chamber set at 20 °C and a 12-hour photoperiod. Tests were conducted for germination, first count, length, and dry mass of seedlings. Excess salt in the medium resulted in reduced germination and initial growth of white oat seedlings, while salicylic acid was not harmful to seed germination up to 2 mM. Conditioning white oat seeds with salicylic acid can be used to mitigate the negative effects induced by salt stress, depending on the intensity of the stress and the concentrations used.

Keywords: *Avena sativa*; germination; salinity.

RESUMO

O excesso de sal no solo tem afetado negativamente o cultivo de culturas forrageiras, como a aveia branca. Consequentemente, é crucial buscar alternativas capazes de induzir tolerância ao estresse salino nessas plantas. O presente estudo se concentra em investigar os efeitos do ácido salicílico na mitigação do estresse salino na germinação de sementes e crescimento inicial de aveia branca. O experimento foi conduzido em delineamento inteiramente casualizado, sendo cinco concentrações de ácido salicílico (0; 0,5; 1; 1,5 e 2 mM) e três níveis de salinidade (0, 50 e 100 mM), com quatro repetições. As sementes foram semeadas em papel germitest e armazenadas em câmara de Demanda Bioquímica de Oxigênio regulada para 20 °C e fotoperíodo de 12 horas. Foram realizados testes de germinação, primeira contagem, comprimento e massa seca das plântulas. O excesso de sal no meio resultou em redução da germinação e do crescimento inicial das plântulas de aveia branca, enquanto o ácido salicílico não foi prejudicial para a germinação das sementes

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até 2 mM. O condicionamento das sementes de aveia branca em ácido salicílico pode ser utilizado para mitigar os efeitos negativos induzidos pelo estresse salino sendo dependente da intensidade do estresse e das concentrações utilizadas.

Palavras-chave*: Avena sativa; germinação; salinidade.*

INTRODUCTION

Oats (*Avena sativa* L.) is an important cereal crop belonging to the Poaceae family and is used as a multipurpose crop for fodder, food, feed and medicine. Its grains are rich in dietary fiber, vitamins, minerals and antioxidants (ALLWOOD *et al*., 2021). Due to its moderate tolerance and adaptability to saline environments, it is considered important for improving the productivity of other agricultural species in saline lands (WANG; DINGXUAN; SHI, 2021).

Most species currently cultivated for economic and agricultural purposes are sensitive to multiple environmental constraints (YOUSSEF *et al*., 2023). As a result of anthropogenic activities such as intensive agriculture, use of poor quality water, and continuous climate change, oats are exposed to abiotic stressors that cause soil degradation and a decline in crop productivity (BAILEY-SERRES *et al*., 2019; SINHA *et al*., 2021). One of the most serious problems is salinity stress caused by soil salinization, which consists of an increase in the concentration of dissolved salts in water and soil and can occur naturally or as a result of human activities. This high salinity can affect plant growth and development due to ionic pressure, and as the osmotic potential of the soil solution decreases, it affects the uptake of essential nutrients (DHIMAN *et al*., 2021).

The sensitivity of a plant to excess salt varies with the species or cultivar, irrigation, the presence of ions or cations in the water, the intensity of the stress, the type of soil, and the climatic conditions of the growing site (PINHEIRO *et al*., 2022). In addition, seed germination and seedling establishment are the two critical stages of plant growth that are affected by adverse environmental conditions, such as high salinity (ZHANG *et al*., 2019).

Protecting against stress and balancing plant growth is key to enabling the development of crop fields (HU *et al*., 2022). One of the strategies used to mitigate the effects of salt stress on plants is seed conditioning with salicylic acid (SA). SA plays an important role in seed germination, cell membrane permeability, ion absorption, stomatal regulation, and increased antioxidant activity (EL-TAHER *et al*., 2022). In addition, it acts as a signaling molecule that induces plant tolerance to stress by regulating physiological processes and providing protection against biotic and abiotic stresses, such as those caused by excess salt in the substrate (SILVA *et al*., 2020). In this regard, several authors have reported that the exogenous application of salicylic acid increases plant tolerance to salt stress, as observed for example in rice (FARHANGJOO *et al*., 2020; RAFIQ *et al*., 2021), pepper (KUMAR *et al*., 2022), wheat (ABDI *et al*., 2023), sugarcane (APON *et al*., 2023), eggplant (MADY *et al*., 2023), and barley (YOUSSEF *et al*., 2023).

Within this context, the global problem of excess salts in the soil, the possible positive role of salicylic acid in mitigating this negative impact on plants and the environment, and the beneficial properties of oats for human and animal health, make it necessary to conduct research on these issues. Thus, the present study focuses on investigating the effects of salicylic acid in mitigating salt stress on seed germination and initial growth of white oats.

MATERIAL AND METHODS

The experimental work was carried out with untreated white oat (*Avena sativa* L.) seeds from the 2022-2023 harvest, purchased from a traditional seed trading company.

The treatments consisted of diluting salicylic acid ($C_7H_6O_3$) in distilled water at concentrations of 0.0900 g L^{-1} (0.5 mM), 0.1801 g L^{-1} (1 mM), 0.2704 g L^{-1} (1.5 mM), and 0.3603 g L^{-1} (2 mM), according to the methodology adapted from Apon *et al*. (2023). In the control treatment, only distilled water was used (0 mM). Due to the low water solubility of SA, the solution was heated in a microwave oven for 5 min to achieve complete dissolution. The seeds were soaked in the different treatments for 24 h at room temperature ± 25 ºC (FERNANDES *et al*., 2019) and after conditioning, they were spread on germitest paper moistened with distilled water or saline solution. Salt stress was applied using sodium chloride (NaCl) at concentrations of 0 (control), 50 mM (2.22 g L^{-1}), and 100 mM (5.844 g L^{-1}) according to the methodology of Farhangjoo *et al*. (2020).

After sowing, the paper rolls (four replicates of 50 seeds each) were kept in a germination chamber (Biochemical Oxygen Demand - BOD), at a temperature of 20 °C, with 12 hours of light, and counts were made on the 5th (first count) and 10th day, when the test was completed (BRASIL, 2009). To evaluate the length and dry mass of the seedlings, the methodology of Krzyzanowski *et al*. (2020) was used.

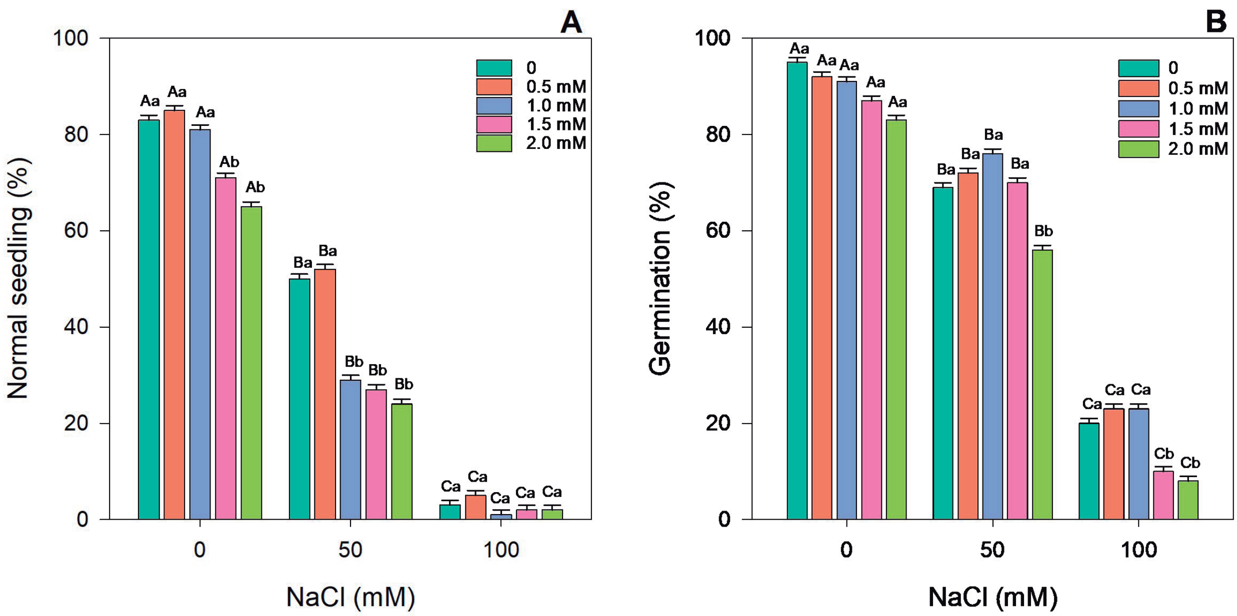
The treatments were arranged in a factorial experiment (5 x 3), in a completely randomized design, with 4 replications, consisting of different concentrations of salicylic acid (5) and salt (3). The data were analyzed using Sisvar software (version 5.6). The significance level was set at $p < 0.05$.

RESULTS AND DISCUSSION

Data analysis showed that NaCl treatment had a significant effect ($p < 005$) on the percentage of normal seedlings assessed by the first count (Figure 1A) and seed germination (Figure 1B) tests. The first count, assessed five days after sowing, showed a decrease in the percentage of normal seedlings from 83% (control, no NaCl) to 50% at 50 mM and to 3% at the highest salt concentration used (100 mM NaCl) (Figure 1A). Following the same trend, germination also decreased with increasing salt concentrations, from 95% in the control to 69% in 50 mM and 20% in 100 mM NaCl (Figure 1B).

In the absence of salt, the percentage of normal seedlings was reduced from 1.5 mM SA (Figure 1A), while germination was not affected by the different concentrations of salicylic acid up to 2 mM (Figure 1B). Furthermore, under salt stress, the attenuating effect of salicylic acid on germination was more pronounced when seeds were conditioned in 0.5, 1 and 1.5 mM (SA) and exposed to 50 mM NaCl and 0.5 and 1 mM (SA) in 100 mM salt (Figure 1B).

Figure 1 - Effect of conditioning white oat seeds with salicylic acid on first count (A) and germination (B) under salt stress (50 and 100 mM NaCl) or in the absence of salt (0 mM NaCl)

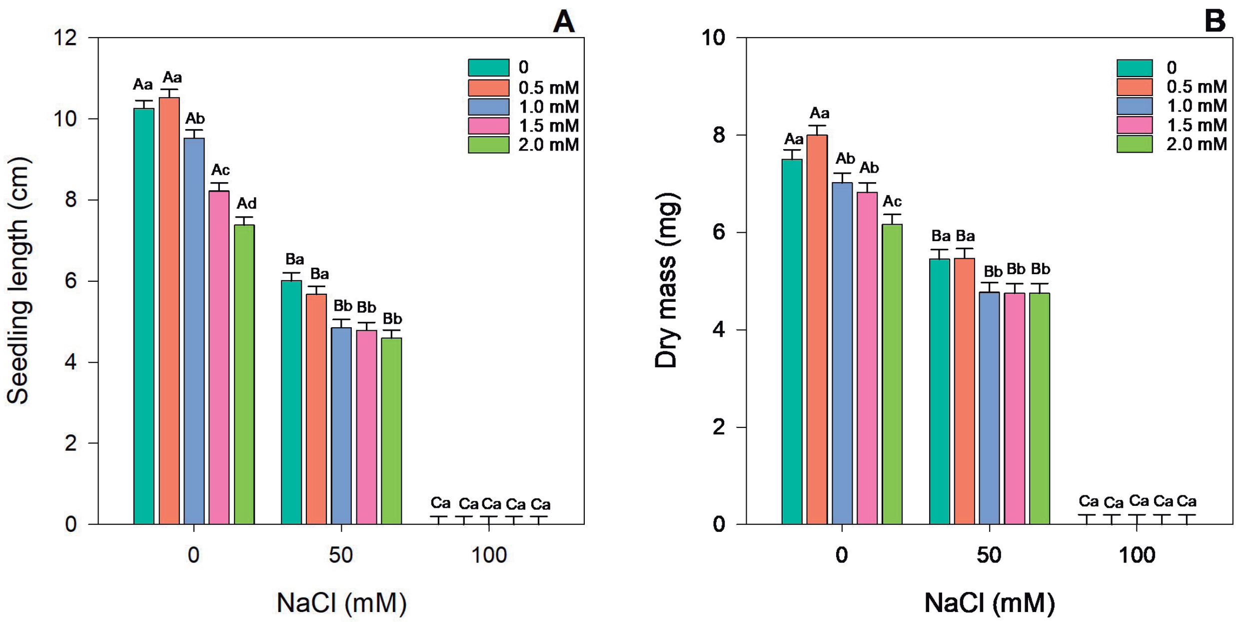


*Lowercase letters indicate significant differences between salicylic acid doses within each NaCl concentration and uppercase letters indicate significant differences between NaCl concentrations within the same salicylic acid dose.
Source: Research data.

In addition to the effect on seed germination, the increase in salt concentration had a detrimental effect on growth (Figure 2A) and seedling dry mass (Figure 2B) five days after sowing, especially at 100 mM NaCl, where the values were zero.

The initial growth and dry mass of oat seedlings at 0.5 mM SA were similar to the control. However, a significant reduction in both variables was observed after 1 mM SA (Figures 2A and 2B). Also, under 50 mM salt stress, the attenuating effect of salicylic acid on seedling growth and biomass was observed when seeds were conditioned with 0.5 mM (Figures 2A and 2B).

Figure 2 - Effect of conditioning white oat seeds with salicylic acid on the length (A) and dry mass (B) of seedlings under salt stress (50 and 100 mM NaCl) or without salt (0 mM NaCl)



*Lowercase letters indicate significant differences between doses of salicylic acid within each NaCl concentration and uppercase letters indicate significant differences between NaCl concentrations within the same dose of salicylic acid.
Source: Research data.

Taken together, our observations suggest that the high salt concentration reduced the germination and initial growth of white oat seedlings (Figures 1 and 2). These results are consistent with previous findings in seeds of quinoa (*Chenopodium quinoa* Willd), rice (*Oryza sativa* L.), chia (*Salvia hispanica* L.), rue (*Ruta graveolens* L.), pepper (*Capsicum annuum* L.), wheat (*Triticum aestivum* L.), sugarcane (*Saccharum officinarum* L.), canafistula (*Peltophorum dubium* (Spreng.) Taub.), and barley (*Hordeum vulgare* L.), where increasing salt concentration resulted in reduced seed germination and aerial and root growth (BARBIERI *et al*., 2019; FARHANGJOO *et al*., 2020; STEFANELLO *et al*., 2020; BARICHELLO *et al*., 2021; KUMAR *et al*., 2022; ABDI *et al*., 2023; APON *et al*., 2023; RAMOS *et al*., 2023; YOUSSEF *et al*., 2023).

The effect of salt stress was more drastic on seedling growth than on seed germination (Figures 1B and 2A). This high level of damage could have serious implications for crop yield, both in terms of quantity and quality. One of the main negative effects of NaCl in the substrate is the induction of osmotic stress, which reduces water uptake capacity, weakens roots, limits metabolic activity and seed germination, and negatively affects root and shoot length and biomass accumulation (SOFY *et al*., 2021; MALIK *et al*., 2022).

In addition to reducing the potential gradient between the seed surface and the surrounding environment, salts also act as stressors and induce membrane degradation through the accumulation of toxic ions in seedlings, compromising cell membranes (KHAN *et al*., 2019). In excess, they can reduce intercellular CO_2 concentration and induce stomatal closure, resulting in reduced biomass, delayed plant growth, and even death (ZHANG *et al*., 2022; YU *et al*., 2020). The undesirable effects of salt stress on plants occur in morphology (reduced seed germination and stunted growth), physiology (imbalance in nutrient uptake and inhibition of photosynthesis), and biochemical activities (disorganization of cell membranes, electrolyte leakage, and oxidative stress) (JI; TANG; ZHANG, 2022; HANNACHI *et al*., 2022).

Salicylic acid, when applied to seeds, had no deleterious effects on germination (Figure 1B). Recent research indicates that SA can regulate seed germination, seedling growth, and plant establishment (SONG *et al*., 2023). In addition, it affects plant functions in a dose-dependent manner, where induction or inhibition can be achieved with low or high concentrations of SA, respectively. However, this effect varies with species, concentration, application method, and growing environment (MADY *et al*., 2023).

Conditioning of seeds with salicylic acid attenuated the negative effects of salt stress on germination and growth of oat seedlings, depending on the intensity of the stress and the concentrations used (Figures 1 and 2). Some studies report that application of salicylic acid to pepper seeds alleviated the salinity-induced reduction in plant growth (KUMAR *et al*., 2022), and the use of 1.0 mM SA reduced the negative effects of salt on eggplant growth and increased yield (MADY *et al*., 2023). In sugarcane, the use of salicylic acid (0.5 to 2 mM) as a priming material to mitigate the negative effects of salt stress (0.5, 4, and 8 dS m^{-1}) indicated that both 1.5 mM and 2 mM significantly reduced deficiencies and thus showed better growth recovery under salinity treatments (APON *et al*., 2023).

In addition, salicylic acid (1.5 mM) minimized the deleterious effects of salt stress (100 mM and 200 mM) on wheat cultivars, which showed an increase of up to 50% in growth parameters such as shoot and root dry weight (ABDI *et al*., 2023). Furthermore, in *Hordeum marinum* and *H. vulgare*, seed conditioning with SA significantly improved aerial and root growth under different salt treatments (up to 200 mM), suggesting that the effectiveness of this approach may vary with the species and the intensity of stress applied (YOUSSEF *et al*., 2023). And treatment with salicylic acid may be one of the suitable alternatives to reduce the deleterious effects of salt stress in rice, where joint application improved its vegetative characteristics and showed beneficial effects on seed germination and seedling vigor index (FARHANGJOO *et al*., 2020; RAFIQ *et al*., 2021).

Salicylic acid is a small-molecule endogenous phenolic compound that has the property of attenuating abiotic effects detrimental to plant development by triggering the plant defense system by inducing genes that act as signal sensors to regulate the plant response and protect plant cells from the toxicity of ion accumulation and cell death (ARIF *et al*., 2020; PENG *et al*., 2021). Thus, seed priming technology offers the possibility of maintaining crop yield under abiotic stress by ameliorating stress-induced changes, especially during the seed germination process, which is highly susceptible to damage (CHAUHAN *et al*., 2023).

Based on the results, it can be concluded that low concentrations of salicylic acid combined with NaCl have more positive effects on oat growth than high concentrations. The results of the present study are consistent with other studies mentioned above, as they also show the positive effect of seed conditioning with SA on seed germination under salt stress. Further studies in this area are warranted and could contribute to the development of effective strategies to manage salinity and improve crop productivity.

CONCLUSIONS

Excess salt in the medium resulted in reduced germination and initial growth of white oat seedlings, while salicylic acid was not harmful to seed germination up to 2 mM.

Conditioning white oat seeds with salicylic acid can be used to mitigate the negative effects induced by salt stress, depending on the intensity of the stress and the concentrations used.

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