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MITIGATING SALT STRESS WITH SALICYLIC ACID ON GERMINATION AND INITIAL GROWTH OF *Vachellia caven* (Molina) Seigler & Ebinger[1]

MITIGAÇÃO DO ESTRESSE SALINO COM ÁCIDO SALICÍLICO NA GERMINAÇÃO E CRESCIMENTO INICIAL DE Vachellia caven (Molina) Seigler & Ebinger

Marciéli Quatrin[2], Julia Savegnago Binotto[3], Gabriela Neves Righi Corrêa[4], Raquel Stefanello[5] e Silvane Vestena[6]

ABSTRACT

The objective of this study was to evaluate the effect of salicylic acid on the germination and initial growth of *Vachellia caven* (Molina) Seigler & Ebinger seeds exposed to salt stress. The experiment was conducted in an entirely randomized design with four replicates of 50 seeds each. Initially, the seeds were chemically scarified with concentrated sulfuric acid for two hours and then soaked in distilled water for three hours. The treatments consisted of different concentrations of salicylic acid (SA) (0.5, 1.0, 1.5, and 2.0 mM) with sodium chloride (NaCl) (100 mM), using distilled water as the control treatment. The results were subjected to analysis of variance, and the means were compared by using the Scott-Knott test at 5% probability when they showed a significant difference ($p<0.05$). The results indicated a reduction in germination and in the length of the aerial and root part of the plant under high concentrations of NaCl. There was also an attenuating effect of SA, in the presence of salt during the first count and in the germination of the seeds (up to 1.0 mM), on the length and dry mass of the shoot (up to 1.5 mM) and on the length of the root (0.5 mM) of the *V. caven* seedlings. Therefore, salicylic acid had a mitigating effect when subjected to salt stress, especially on germination and length of shoot of *V. caven* seedlings.

Keywords: Salinity; Seedling; Seeds; Sodium chloride.

RESUMO

O objetivo deste trabalho foi avaliar o efeito do ácido salicílico na germinação de sementes e crescimento inicial de Vachellia caven (Molina) Seigler & Ebinger expostas ao estresse salino. O experimento foi conduzido em delineamento inteiramente casualizado, com quatro repetições de 50 sementes. Inicialmente, foi realizada a escarificação química das sementes com ácido sulfúrico concentrado, por duas horas e, posteriormente, embebidas por três horas em água destilada. Os tratamentos consistiram em diferentes concentrações de

1 Trabalho de Iniciação Científica.

2 Bióloga, Mestranda em Microbiologia e Parasitologia pela Universidade Federal de Pelotas - UFPel, RS. E-mail: marcieliquatrin03@gmail.com. ORCID: https://orcid.org/0009-0007-3160-5810

3 Bióloga, Universidade Federal de Santa Maria - UFSM, RS. E-mail: binottojulia5@gmail.com. ORCID: https://orcid.org/0009-0001-8469-1368

4 Bióloga, Mestranda em Agrobiologia pela Universidade Federal de Santa Maria - UFSM, RS. E-mail: gabrielaneves-righi@gmail.com. ORCID: https://orcid.org/0009-0001-3443-4443

5 Doutora, Bióloga na Universidade Federal de Santa Maria - UFSM, RS. E-mail: raquelstefanello@yahoo.com.br. ORCID: https://orcid.org/0000-0003-3079-2099

6 Doutora, Bióloga, Docente em exercício provisório na Universidade Federal de Santa Maria - UFSM, RS. E-mail: silvanevestena@gmail.com. ORCID: https://orcid.org/0000-0002-9797-1211

ácido salicílico (AS) (0,5; 1,0; 1,5 e 2,0 mM) com cloreto de sódio (NaCl) (100 mM), sendo a água destilada como tratamento controle. Os resultados foram submetidos à análise de variância, com comparação das médias pelo teste Scott-Knott a 5% de probabilidade, quando apresentaram diferença significativa (p<0,05). Os resultados indicaram redução na germinação e no comprimento de parte aérea e radicular sob alta concentração de NaCl. Também, observou-se efeito atenuante do AS, na presença de sal, na primeira contagem e na germinação das sementes (até 1.0 mM), no comprimento e na massa seca da parte aérea (até 1.5 mM) e no comprimento da raiz (0,5 mM) das plântulas de V. caven. Portanto, o ácido salicílico apresentou efeito mitigador do estresse salino, principalmente em relação à germinação e ao comprimento da parte aérea das plântulas de V. caven.

Palavras-chave: *Cloreto de sódio; Salinidade; Sementes; Plântulas.*

1 INTRODUCTION

The accumulation of soluble salts in the soil is characterized by salinization, and this phenomenon has a negative impact on agricultural production and biodiversity (Gopalakrishnan; Kumar, 2020). In addition, soil health is compromised as there is a reduction in fertility and changes in soil properties, leading to erosion and surface water runoff (Singh, 2021). The cause of these events includes both natural and anthropogenic causes, such as the excessive use of fertilizers and the use of saline water in irrigation (Cruz *et al*., 2017; Taiz *et al*., 2024). In arid and semi-arid regions, saline soils are predominant due to the lack of rainfall and high evapotranspiration, as well as in coastal regions flooded by seawater (Gopalakrishnan; Kumar, 2020). Worldwide, about 50% of irrigated agricultural land has salinity problems, and the percentage may increase due to low rainfall that prevents salt leaching, inadequate planting, high soil evaporation, and rock weathering (Shrivastava; Kumar, 2015).

Naturally, plants absorb mineral salts for their growth and development, but excessive accumulation within the plant leads to increased storage of ions in the vacuole, as this can cause toxicity to the plant's functioning. Excess leads to a reduction of the water potential in the roots, resulting in a deficiency of essential nutrients, since many of them cannot be absorbed without the presence of water (Shrivastava; Kumar, 2015; Maia Junior *et al*., 2020; Zahra *et al*., 2020).

There is a group of plants that present natural ways of resisting to salt stress, known as halophytes. On the other hand, salt-sensitive plants, called glycophytes, accumulate more solutes and suffer from more nutrient imbalances, resulting in a reduction in seed germination and plant growth (Biswas *et al*., 2020; Shahid *et al*., 2023).

The genus *Vachellia*, from the family Fabaceae, is widely distributed in arid and semi-arid regions, including drought-tolerant plants. In addition, some species can promote greater soil fertility by fixing atmospheric nitrogen (Odirile *et al*., 2019). The species *Vachellia caven* (Molina) Seigler & Ebinger is native to the extratropical region of South America and is commonly found in central Chile, central-eastern Argentina, Uruguay, Paraguay, and the western region of the state of Rio Grande do Sul (RS) in the south of Brazil (Escobar *et al*., 2010; Velasco *et al*., 2023). In RS, this species is

predominant in Espinilho State Park, in the municipality of Quaraí, on the west side of the state, but it can also inhabit other regions of the state, playing a role in the recovery of degraded areas and the revegetation of sites altered by human activity. Furthermore, the presence of this species in the ecosystem is of extreme biological relevance and a priority for the conservation of many flora and fauna species that are important at regional, national, and global levels (Rambo, 2016).

Plants have evolved several processes to withstand and adapt to stresses, particularly during germination (Wani *et al*., 2023). Phytohormone signaling, including salicylic acid, has been shown to be an important defense strategy for plants against various abiotic stresses, including salt stress (Yu *et al*., 2020). Protecting against stress and balancing plant growth is key to enabling the development of species (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). Initially, salicylic acid was found in species of the genus *Salix* sp., hence the name of the natural product, and later an Italian chemist obtained it in species of another genus, leading to studies on the route of synthesis and the importance of this substance for plants (Sharma *et al*., 2020).

Salicylic acid is a phenolic phytohormone found in plants that is characterized by stimulating the activation of molecules that contribute to resilience in times of abiotic and biotic stress (Chen *et al*., 2023). The main molecules stimulated are antioxidants that act on free radicals produced during salt stress (Hassoon; Abduljabbar, 2019). In addition, SA affects factors involved in growth and development, such as ethylene biosynthesis, ion uptake, respiration, photosynthesis, membrane permeability and stability, enzyme activity, flowering, and secondary compound production (Aminifard *et al*., 2019; El-Ramady *et al*., 2024).

Based on the points presented above, this study aims to analyze the effect of salicylic acid in mitigating salt stress during seed germination and early development of *Vachellia caven*.

2 MATERIAL AND METHODS

The work was carried out at the Seed and Plant Tissue Culture Laboratory of the Federal University of Santa Maria (UFSM), in Santa Maria, RS. The fruits of *Vachellia caven* were collected from mother plants on the UFSM campus. They were manually processed to obtain the seeds, discarding the ones that were infected and dark. Finally, they were stored in paper packets in the refrigerator at 7 ºC until the experiment was carried out.

To break the dormancy of the seeds, a chemical scarification method was used with concentrated sulfuric acid (H_2SO_4). The seeds were left for 120 minutes (two hours) and then soaked in distilled water for three hours (Mendes *et al*., 2023).

V. caven seeds were immersed in salicylic acid (SA) at each concentration (0.5, 1.0, 1.5, and 2.0 mM) or distilled water (control) at room temperature for 24 hours (adapted from Nóbrega

et al., 2021 and Apon *et al*., 2023). The SA solution was heated in a microwave oven for 5 minutes to completely dilute the solute due to its low solubility in water (Puntel *et al*., 2024). After soaking, seeds were sown on germination paper moistened with distilled water (0 mM) or NaCl solution (100 mM), This NaCl concentration was established in previous studies conducted at the laboratory with cultivated and tree species. To adequately wet the paper, a ratio of 2.5 times the dry mass of the paper was used on which 50 *V. caven* seeds were placed, with 4 (four) replicates of each treatment in a completely randomized design.

The paper rolls were kept in a growth chamber with controlled temperature and controlled light exposure (25 ºC ± 2 ºC, 16 hours of light) (Brasil, 2025). The first count (%), length (cm) and dry mass (mg) of the shoot and the root system were analyzed 7 days after the start of the experiment. Germination (%) was evaluated on the 14th day (Brasil, 2025). Normal seedlings were considered as those with a primary root and hypocotyl according to the rules for seed analysis (Brasil, 2025). For length and dry mass, 10 seedlings were used per replicate of each treatment (Krzyzanowski *et al*., 2020). For dry mass, an oven with a temperature of approximately 60 ºC (48 hours) was used.

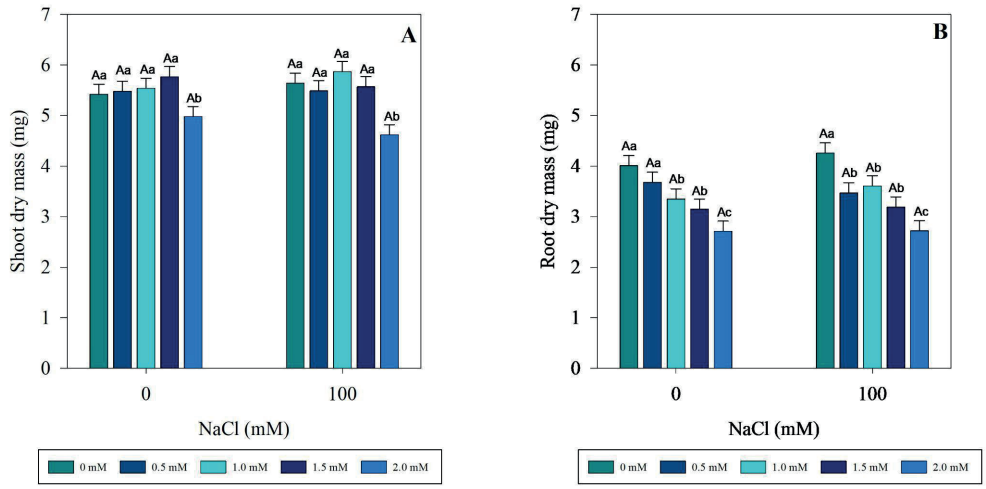
The results obtained were subjected to analysis of variance and when the treatments showed a significant difference ($p<0.05$), the means were compared using the Scott-Knott test at 5% probability using Sisvar software version 5.6.

3 RESULTS AND DISCUSSION

Data analysis indicated that salt stress had a significant effect ($p < 0.05$) on seed germination and the length of the shoot and root of *V. caven* seedlings (Figures 1 and 2).

Germination decreased when salt concentrations increased, from 91% in the control to 84% in 100 mM NaCl (Figure 1B). The percentage of normal seedlings and germination were not affected by the different concentrations of salicylic acid up to 1.0 mM (Figures 1A and 1B). In addition, the attenuating effect of salicylic acid on germination was observed when the seeds were conditioned in up to 1.0 mM (SA) and exposed to 100 mM NaCl (Figures 1A and 1B).

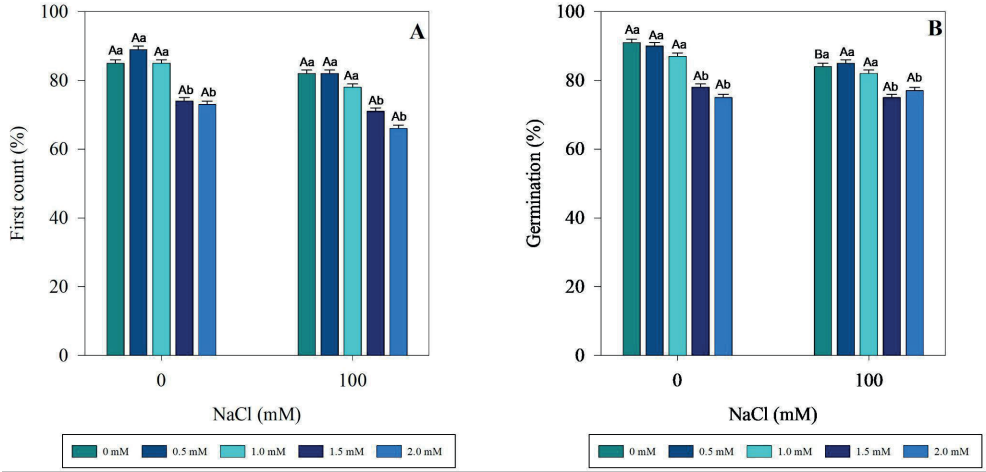
Figure 1 - (A) First count and (B) germination of *Vachellia caven* (Molina) Seigler & Ebinger seeds, conditioned with salicylic acid and subjected to salt stress (100 mM NaCl)



Uppercase letters indicate significant differences between salt concentrations within the same dose of salicylic acid and lowercase letters indicate significant differences between doses of salicylic acid within each salt concentration.
Source: Authors (2026).

In addition to negatively affecting seed germination, the increase in salt concentration had a detrimental effect on the growth of the shoot and root of *V. caven* seedlings seven days after sowing, with values varying from 3.60 to 2.51 cm (Figure 2A) and from 8.49 to 7.84 cm (Figure 2B), respectively. Root length did not differ from the control when exposed to 0.5 mM of SA (Figure 2B). Furthermore, under 100 mM salt stress, the attenuating effect of salicylic acid was seen in shoot growth up to 1.5 mM (Figure 2A) and root length up to 0.5 mM (Figure 2B).

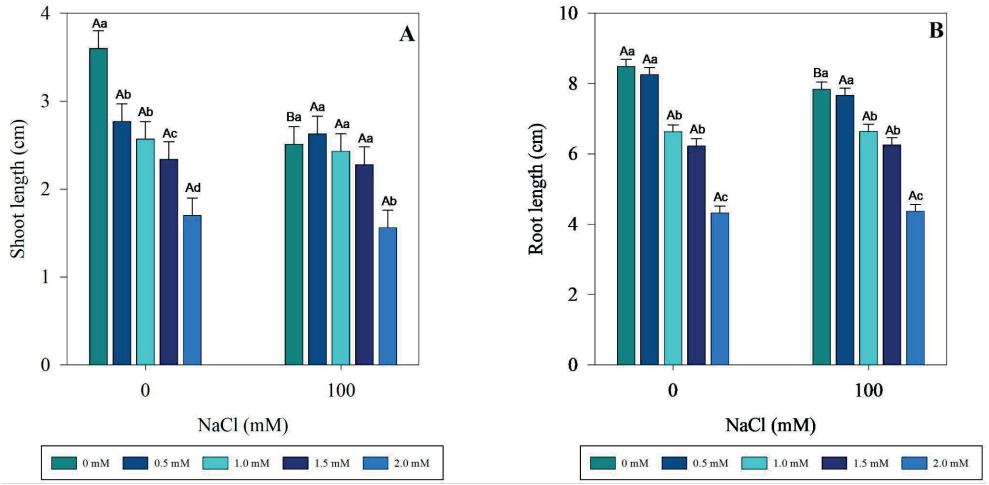
Figure 2 - (A) Shoot length and (B) root length of *Vachellia caven* (Molina) Seigler & Ebinger, conditioned with salicylic acid and subjected to salt stress (100 mM NaCl)



Uppercase letters indicate significant differences between salt concentrations within the same dose of salicylic acid and lowercase letters indicate significant differences between doses of salicylic acid within each salt concentration.
Source: Authors (2026).

There were no significant differences to the dry mass of the shoot and root compared to the control when subjected to salt stress (Figure 3). The dry mass did not differ from the control up to 1.5 mM SA (shoot) and up to 0.5 mM (root). The mitigating effect of salicylic acid was only observed in the dry mass of the shoot when the seeds were conditioned up to 1.5 mM (Figure 3A), while for root dry mass, no mitigating effect of SA on salt stress was found (Figure 3B).

Figure 3 - (A) Shoot dry mass and (B) root dry mass of *Vachellia caven* (Molina) Seigler & Ebinger, conditioned with salicylic acid and subjected to salt stress (100 mM NaCl)



Uppercase letters indicate significant differences between salt concentrations within the same dose of salicylic acid, and lowercase letters indicate significant differences between doses of salicylic acid within each salt concentration.
Source: Authors (2026).

The results of this study indicate that *V. caven* seedlings germinated in a high salt concentration exhibited the typical effects of salt stress. This abiotic condition reduced both the average germination and growth parameters of the seedlings compared to those grown from seeds germinated under optimal conditions (Figures 1 and 2). In this case, toxic ions and the restriction on seed imbibition may have limited the mobilization of nutrient reserves essential for embryo survival and the initiation of the germination process. These findings corroborate previous studies on seed *Enterolobium contortisiliquum* (Vell.) Morong., *Peltophorum dubium* (Sprengel) Taubert, *Erythrina velutina* Willd., *Psidium guajava* L., *Avena sativa* L., *Lactuca sativa* L., *Stevia rebaudiana* (Bertoni) Bertoni and *Hordeum vulgare* L. e *H. maritimum* O.F. Müll., where increasing salt concentrations resulted in a decrease in parameters related to germination and seedling growth (Dutra *et al*., 2017; Nóbrega *et al*., 2021; Lacerda *et al*., 2023; Puntel *et al*., 2024; Ben Youssef *et al*., 2025; Cavalcante *et al*., 2025; Janah *et al*., 2025).

Among these studies, those conducted by Dutra *et al.* (2017) stand out, as they found that the germination and initial growth of monkfish and canafistula seedlings were influenced by an increase in the salinity of the medium. In addition, there was a prolongation of the average germination time and a reduction in the average germination rate and, an increase in root length, with the lowest values

recorded in the treatment -1.8 MPa (NaCl). Similarly, guava seedlings irrigated with saline water (0.6 and 3.2 dS m^{-1} NaCl) showed a reduction in growth, lower chlorophyll levels (a and b) and rate of CO_2 assimilation compared to those that received a lower salt concentration (Lacerda *et al*., 2023).

When plants are exposed to salinity, they produce reactive oxygen species (ROS) that alter the structure of the plasma membrane through lipid peroxidation, affecting the permeability and fluidity of this structure (Taïbi *et al*., 2016). Excessive Na^+ absorption limits potassium assimilation, resulting in a deficiency of this essential mineral nutrient and the toxic effects of sodium. Excess sodium in the cytoplasm causes an imbalance in ion homeostasis, which impairs water movement and plant growth (Talaat; Hanafy, 2022). In addition, excess salt can cause osmotic problems by reducing leaf water potential, turgor pressure, and osmotic potential, which influences stomatal opening. Ionic disturbances also occur due to the high uptake of Na^+ and Cl^- ions, which reduces the uptake of essential nutrients such as K^+ and Ca^{2+}, increases the production of reactive oxygen species, degrades chlorophyll, and impairs RuBisCO function (Albuquerque *et al*., 2024).

Previous studies have shown that the effects of salicylic acid treatments can vary among species or cultivar, depending on the developmental stage of the plant, the dose, and the application method (Poór *et al*., 2019; Neumann *et al*., 2023). When applied to *V. caven* seeds, salicylic acid had no adverse effects (Figures 1, 2, and 3). In *Trifolium resupinatum* L. seeds, salicylic acid up to 1.0 mM had a positive effect on seed germination (Stefanello *et al*., 2024), while doses of SA up to 2 mM improved the germination potential of maize seeds (Ferrazza *et al*., 2024). However, Lima *et al.* (2025), working with two *Capsicum chinense* Jacquin cultivars, evaluated the mitigating effect of different salicylic acid concentrations (0, 3.31, 5.71, 7.84, and 9.68 dS m^{-1}) under various sodium chloride treatments (0, 30, 60, 90, and 120 mM), reporting no mitigating effect of SA on the germination parameters or early seedling development of this species.

In plants exposed to salt stress, salicylic acid affects the efflux and influx of sodium atoms, resulting in a reduction in sodium accumulation within the cells. The application of exogenous SA can promote greater water movement in the plant, as the acid stimulates the synthesis of proline, carbohydrates, and proteins (Liu *et al*., 2022). Furthermore, SA is involved in increasing nitrogen metabolism, in the functioning of RuBisCO and enhances the action of the antioxidant system, mitigating the damage caused by ROS produced in saline conditions (Mir *et al*., 2021).

Other studies indicate that *Erythrina velutina* Willd seeds, subjected to salinity stress caused by different electrical conductivities of water, had a positive effect on seed germination when combined with salicylic acid (1.0 mM) (Nóbrega *et al*., 2021). Equally, in an experiment conducted with white oats, Puntel *et al.* (2024) observed detrimental effects when seeds were placed in a substrate with high salt concentrations. While 95% germination was achieved in the control treatment (0 mM), only 20% of the seeds germinated at 100 mM. Furthermore, they also found that salicylic acid (0.5 mM) had an attenuating effect on the dry mass and seedling growth under 50 mM NaCl.

In addition, Janah *et al.* (2025) found that conditioning *Stevia rebaudiana* seeds with SA (0.25 and 0.5 mM) under saline conditions (0 and 80 mM NaCl) significantly increased germination rates, reduced the average germination time, and improved salt tolerance index, including greater shoot and root lengths compared to untreated seeds. Cavalcante *et al.* (2025) analyzed different varieties of lettuce under salt stress or in combination with different hormones, and concluded that treatment with SA resulted in an increase in production of about 25% compared to the saline solution.

In barley seeds, excess salt reduced germination parameters and affected seedling growth by preventing the necessary mobilization of nutrients and causing a significant increase in electrolyte leakage and malondialdehyde. On the other hand, when seeds were preconditioned with salicylic acid and subjected to abiotic stress (100 or 200 mM NaCl), an increase in the germination rate, length, and dry mass of seedlings of both species was observed (Ben Youssef *et al*., 2025).

Among the main pathways in which salicylic acid alleviates salt stress include the regulation of ion balance and hormone levels, antioxidant action through stimulation of the enzymes SOD, CAT, and POD, and regulation of the expression of genes related to stress responses. As a result, plants can have similar growth and yield in the absence of saline conditions (Song *et al*., 2023).

Finally, considering the increase in extreme weather events, such as reduced rainfall leading to droughts, and the increasing use of irrigation to maintain monocultures, the soil will tend to have a high concentration of Na^+ and Cl^- ions (Sánchez-Bermúdez *et al*., 2022). Therefore, it is essential to find ways to mitigate the damage caused by salt stress to plants. The results of the present study provide consistent guidelines for the safe application of salicylic acid to the *V. caven* cultivation and highlight the importance of the phytohormone as a promising and cost-effective agent to mitigate the adverse effects caused by excess salt. Thus, seed priming technology offers the possibility of maintaining the life cycle of species under abiotic stress by ameliorating stress-induced changes, especially during the seed germination process, which is highly susceptible to damage (Liu *et al*., 2022).

4 CONCLUSIONS

Based on the results, it can be concluded that salt stress caused a reduction in seed germination, shoot length, and root length of *V. caven* seedlings.

Under salt stress, salicylic acid had a mitigating effect on first count and seed germination (up to 1.0 mM), shoot length and dry mass (up to 1.5 mM), and root length (0.5 mM) of *V. caven* seedlings.

Thus, salicylic acid proved to be partially effective in mitigating the negative effects of salt on *V. caven*. Further studies in this area are warranted and could contribute to the development of effective strategies to manage salinity and, avoiding the degradation of natural areas, especially in the southern region of Brazil, where this species has a wide distribution and is ecologically important.

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