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CROP PRODUCTION

Seed priming improves *Salvia hispanica* L. seed performance under salt stress

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ABSTRACT. *Salvia hispanica* L. is an alternative crop cultivated by farmers who want to diversify their production. However, this species is sensitive to salinity, which affects its germination negatively. Seed priming with different attenuators is a technique with potential to mitigate the effects of salt stress. Thus, the objective of this study was to evaluate the effect of seed priming with the use of different attenuators on the germination, growth, and organic solute accumulation of *S. hispanica* seedlings under salt stress. The experimental design was completely randomized, with treatments distributed in a 4 × 5 factorial scheme, corresponding to four seed priming treatments and five osmotic potentials, with four replicates of 50 seeds in each treatment. The seed treatments consisted of presoaking seeds for 4h in salicylic acid, gibberellic acid, and distilled water and the control treatment, which did not involve soaking. These seeds were germinated at osmotic potentials of 0.0, -0.1, -0.2, -0.3, and -0.4 MPa, using NaCl as an osmotic agent to simulate the different salinity levels. Among all the treatments implemented, *S. hispanica* seed priming with salicylic acid was the most efficient in mitigating the salt stress effects.

Keywords: chia; salinity; osmotic potentials; salicylic acid; gibberellic acid.

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Introduction

Chia (*Salvia hispanica* L.) is an alternative crop cultivated by farmers who want to diversify their production (Busilacchi et al., 2013). This species can grow in arid environments and has been recommended as an alternative to traditional crops, as it is highly tolerant of abiotic factors (Migliavacca, Silva, Vasconcelos, Mourão Filho, & Baptistella, 2014). However, the species of the genus *Salvia*, including *S. hispanica*, are considered sensitive to salinity, which mainly affects the germination and chemical composition of the seeds, thereby altering their oil yield and fatty acid content (Rosa et al., 2015; Stefanello, Neves, Abbad, & Viana, 2015; Falco, Amato, & Lanzotti, 2017; Paiva et al., 2018).

Salinity has adverse effects on plant development, especially on germination and post-germinative growth (Taiz, Zeiger, Møller, & Murphy, 2015). The improvement of seed germination and emergence will improve agricultural production under salt stress conditions (Shu et al., 2017). One of the options to minimize the harmful effects of salts on plants is the use of substances, such as plant regulators, that reduce the intensity of these effects on the initial growth of seedlings with the use of saline water (Oliveira et al., 2015). Among these plant regulators, there are strong indications that salicylic acid participates in salt-stress signaling by regulating the antioxidant response of plants and providing resistance and protection against oxidative damage (Noriega et al., 2012). In addition, an exogenous supply of gibberellic acid can promote the germination process in seeds, by reconstituting the levels of this phytohormone, the biosynthesis of which is negatively regulated by salinity (Shu et al., 2017).

The hydropriming technique has also been employed as a physiological approach to help glycophytic species adapt to saline conditions (Matias, Ribeiro, Aragão, Araújo, & Dantas, 2015). This process results in controlled seed hydration, which triggers many of the physiological processes associated with the initial phase of germination and prepares the seed for radicle protrusion; this makes hydropriming an effective method in improving the emergence of seedlings, especially in adverse environmental conditions (Marcos Filho, 2015). Thus, seed priming with attenuating substances is an important method of induced resistance against abiotic stresses, thereby making seeds more tolerant to adverse environmental conditions (Agami, 2013; Hossain et al., 2015).

Attenuating agents usually play an active role in the cascade of signals involved in the expression of the stress response in plants and have gained much attention worldwide owing to their capacity to mitigate the adverse effects of abiotic stress (Tsegay & Andargie, 2018). However, the use of attenuators to mitigate salt stress in *S. hispanica* has not been researched adequately. Thus, the objective of this study was to evaluate the effect of seed priming with different attenuators on the germination, growth, and accumulation of organic solutes of *S. hispanica* seedlings under salt stress.

Material and methods

Location and experimental design

The experiment was carried out in the Seed Analysis Laboratory and Plant Physiology Laboratory of the Agrarian Sciences Center of the Federal Rural University of the Semi-Arid Region (UFERSA), Mossoró, RN, Brazil. In the experiment we used *S. hispanica* seeds obtained from a commercial production field, which was located in the municipality of Santana do Livramento, Rio Grande do Sul (30°53′27″ S, 55°31′58″ W), which is located at an altitude of 208 m. The seeds were processed manually, placed in a transparent plastic bag (0.15 mm thick), and stored in a cold and dry chamber (10 ± 2°C and 50% relative humidity) during the entire experimental period.

The *S. hispanica* seeds were soaked for 4h in the attenuator solutions, which were salicylic acid (1 mM L^{-1}), gibberellic acid (0.4 mM L^{-1}), and distilled water (hydropriming); in the control treatment the seeds were not soaked. Next, the seeds were sown under two sheets of blotting paper, placed in transparent plastic boxes (Gerbox®), and hydrated with sodium chloride (NaCl) solutions, in a volume equivalent to 2.5 times the dry weight of the paper. In order to determine the salt stress condition, NaCl solutions were used at the following concentrations: 0.0 MPa (control), -0.1, -0.2, -0.3, and -0.4 MPa (Paiva et al., 2018). The electrical conductivity of the solutions was checked with a conductivity meter and, at the level of 0.0 MPa, only distilled water was used to moisten the substrate.

Germination

The germination test was conducted in biochemical oxygen demand (BOD) germination chambers, regulated at a temperature of 25°C with an 8-h photoperiod (Paiva et al., 2016). The germinated seeds were counted daily until the eighth day after sowing; a seed was considered to have germinated when the primary root and aerial part of the seedling had grown.

The percentage of normal seedlings germinated in each treatment was calculated at the end of the experiment, eight days after sowing (Ministério da Agricultura, Pecuária e Abastecimento [MAPA], 2009).

The germination speed index (GSI) was calculated according to the equation proposed by Maguire (1962), by evaluating the seeds daily, from the beginning of germination until the eighth day after sowing.

$$GSI = \frac{G1}{N1} + \frac{G2}{N2} + \cdots \frac{Gn}{Nn} \quad (1)$$

where: GSI: germination speed index (GSI); G1, G2, and Gn: number of seeds germinated in the first, second, and last counts; N1, N2, and Nn: number of days after sowing after the first, second, and last counts.

Biometric parameters

Eight days after the germination test, the lengths of the shoots and primary root of the normal seedlings of each treatment were measured using a ruler graduated in centimeters. The shoot and primary root lengths were measured from the base of the collar to the apex of the seedling and from the base of the collar to the tip of the root, respectively (Paiva et al., 2018).

After these measurements, the seedlings were placed in paper bags, dried in a forced air circulation oven regulated at 65°C for 72h until a constant weight was achieved, and weighed on a precision weighing scale (0.001 g) to determine their total dry matter (Paiva et al., 2018).

Organic solutes

The samples for the biochemical analyses were obtained from the fresh matter of seedling shoots and roots, which were collected after the seedlings had been subjected to eight days of stress. Initially, the samples were extracted by weighing 0.2 g of fresh matter, which was placed in tubes and mixed with 1 mL of alcohol for analysis. Then, the material was macerated in an automatic macerator for 2 min. and then kept in a water bath at 60°C for 20 min. Subsequently, the material was centrifuged for 8 min. The supernatant was collected and the extraction process was repeated two more times. Finally, the resulting supernatant was collected to quantify the total soluble sugar, total free amino acid, and proline contents.

The total soluble sugar content was determined by measuring the absorbance at 620 nm using the anthrone method (Yemm & Willis, 1954) and glucose as the standard substance. The results were expressed in µmol GLU g^{-1} of fresh matter (FM).

The total free amino acid content was determined by measuring the absorbance at 570 nm using the acid ninhydrin method (Yemm, Coccking, & Ricketts, 1955) and glycine as the standard substance. The results were expressed in µmol GLY g^{-1} of FM.

The proline content was determined by following the methodology described by Bates, Waldren, and Teare (1973), which was based on a standard curve obtained from L-proline and by measuring the absorbance at 520 nm. The results were expressed in µmol PRO g^{-1} of FM.

Statistical analysis

The statistical design used was completely randomized, in a 4 × 5 factorial scheme, with four seed priming treatments (control, hydropriming, salicylic acid, and gibberellic acid) and five osmotic potentials (0.0, -0.1, -0.2, -0.3, and -0.4 MPa), with four replicates of 50 seeds in each treatment.

The results were subjected to analysis of variance ($p < 0.05$) and, in the case of significance, Tukey's test ($p < 0.05$) was performed for the attenuators and polynomial regression ($p < 0.05$) was performed for the osmotic potentials and their interaction, using the statistical program SISVAR® (Ferreira, 2011).

Results and discussion

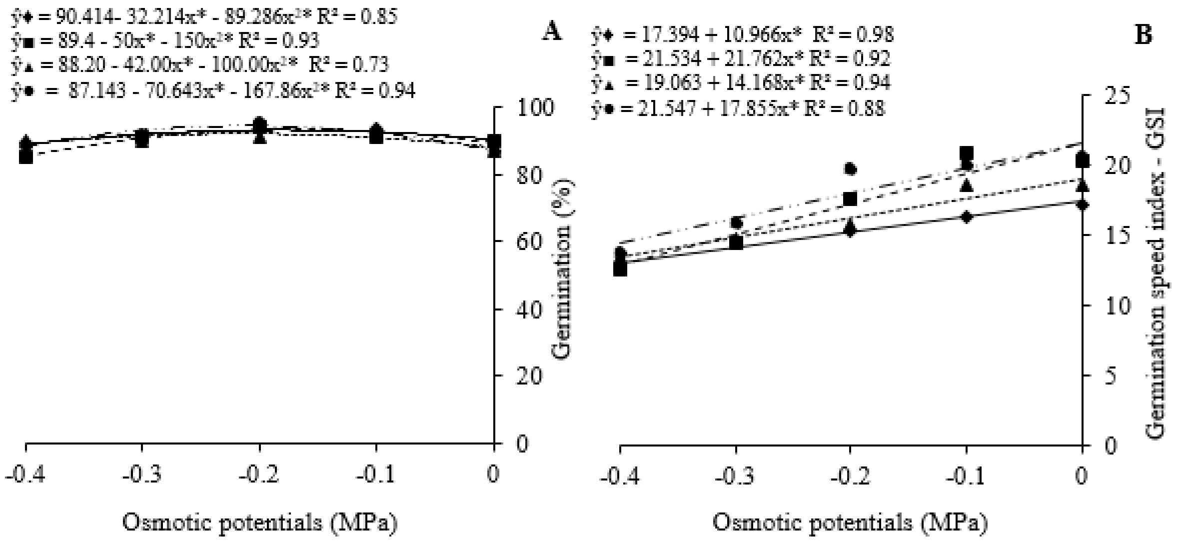
Germination

In order to obtain the germination percentage (Figure 1A), the quadratic behavior was observed in all treatments, with a reduction in the final germination percentage in all treatments at the highest levels of osmotic potential. In the control treatment, the highest germination rate obtained was 93% at the -0.18 MPa osmotic potential, while at the -0.4 MPa osmotic potential the germination rate reached 89%. From the -0.4 MPa to the 0.0 MPa osmotic potential, germination was reduced by 3, 6, and 8% owing to the effects of salicylic acid, gibberellic acid, and hydropriming, respectively.

The germination speed of *S. hispanica* seeds was also affected by the increase in substrate salinity. The germination speed index decreased linearly in all treatments tested (Figure 1B). The salt stress reduced the GSI in the control treatment from 17.39 at 0.0 MPa to 13.01 at -0.4 MPa, which represented a reduction of 1.09 units in the GSI per -0.1 reduction in the osmotic potential. The treatments with the attenuators resulted in higher GSI compared to the control treatment, up to an osmotic potential of -0.3 MPa. At the lowest osmotic potential studied, the gibberellic and salicylic acids led to the highest indices (14.40 and 13.39, respectively, at -0.4 MPa). Hydropriming caused the largest GSI reduction, which was equal to 2.17 per -0.1 reduction in the osmotic potential; these results were lower than those obtained from the control treatment at the lowest osmotic potential.

The best results for germination and the GSI of *S. hispanica* seeds in the present study were obtained by salicylic acid and gibberellic acid (Figure 1A and B). The deleterious action of salinity was reduced in seeds soaked in these attenuators, thereby leading to higher germination percentages in shorter periods of time.

The interaction between salinity and salicylic acid possibly induced the genes that encode the tolerance to salinity to act on germination, by increasing the physiological activity and mobilization of the reserve material required for embryonic growth (Jini & Joseph, 2017). According to Anaya, Fghire, Wahbi and Loutfi (2018), the metabolic activity in seeds treated with salicylic acid begins much earlier than the appearance of the radicle and plumule. Therefore, treated seeds germinate earlier than untreated seeds. In addition, Anaya et al. (2018) pointed out that low concentrations of salicylic acid induce and maximize germination, possibly because they stimulate seed germination through the biosynthesis of gibberellic acid.



♦ Control; ■ Hydro-conditioning; ▲ Salicylic acid; ● Gibberellic acid

* = significant at 5% probability ($p < 0.05$).

Figure 1. Germination (A) and germination speed index (GSI) (B) of Salvia hispanica L. seeds subjected to seed priming with different attenuators and osmotic potentials.

A study conducted by Turkyilmaz (2012) on wheat seeds subjected to salt stress, showed that treatments with salicylic acid at concentrations of 200 ppm and 400 ppm increased the germination rate by 3.19 and 4.44%, respectively, compared to the control treatment. Fardus, Matinm, Hasanuzzamanm, and Hossain (2018), who also worked with wheat seeds under salinity conditions, observed that pretreatment with 1 mM of salicylic acid resulted in a higher germination percentage. Anaya et al. (2018) found that treating fava beans with salicylic acid was beneficial, as it led to an increase in germination under various stress levels and a reduction in the inhibitory effects of salt stress, especially at a concentration of 0.25 mM.

Although the hydropriming technique is effective in promoting metabolic activities in the seeds (Pinedo & Ferraz, 2008), in this study it was not efficient at osmotic potentials lower than -0.3 MPa (Figure 1B).

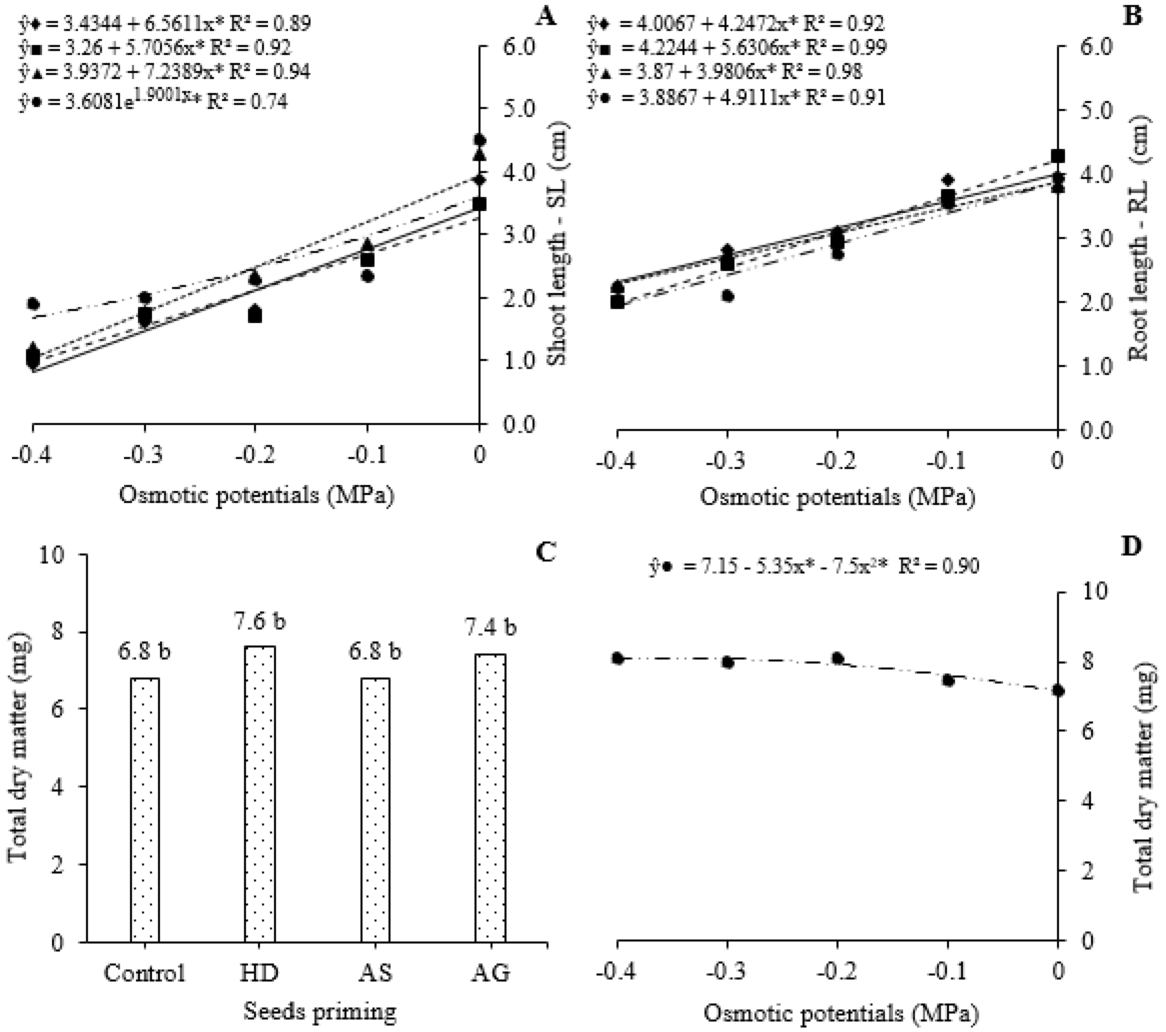
The improvement in the germination rate of *S. hispanica* seeds caused by gibberellic acid could be explained by the capacity of this phytohormone to act as a chemical signal that activates genes that encode hydrolytic enzymes. Enzymes such as amylase, protease and, in some cases, lipase, play vital roles in the initial growth and development of the embryo (Tsegay & Andargie, 2018). Any increase in the activity of these enzymes may result in vigorous and early germination (Taiz et al., 2015). Tian et al. (2014) studied corn seeds pretreated with gibberellic acid and found that the GSI increased significantly when the seeds were conditioned in 10 mg L^{-1} of gibberellic acid. A similar effect was observed by Tsegay and Andargie (2018) when corn seeds were subjected to salt stress. According to these authors, seed priming with gibberellic acid at 0.2 g L^{-1} reduced the time required for germination.

Biometric parameters

The increased substrate salinity reduced the vigor of *S. hispanica* seedlings. The reduced shoot length of the seedlings accompanied the decrease in the osmotic potential of the substrate; the highest reduction was observed in the control treatment (76.38%), which led to a shoot length of 0.81 cm at the osmotic potential of -0.4 MPa (Figure 2A). In seedlings treated with attenuators, the greatest shoot length at the osmotic potential of -0.4 MPa was caused by gibberellic acid (1.68 cm); this was reduced by 53.16% compared to the shoot length caused by the lowest salinity level. The shoot length decreased by 69.94% with hydropriming and by 73.60% with salicylic acid (Figure 2A) from the -0.4 MPa to the 0.0 MPa osmotic potential.

The primary root length was also reduced with the increased salinity in all the treatments (Figure 2B). Salicylic acid led to the best results, with a reduction of only 41.08% in root length as the osmotic potential changed from 0.0 to -0.4 MPa. This reduction was equal to 42.39% in the control treatment, 50.64% in the gibberellic acid treatment, and 53.32% in the hydropriming treatment.

The different attenuators and osmotic potential levels (Figure 2C and D) had single effects on the total dry matter of the seedlings. For the attenuators, there was no statistical difference between them (Figure 2C). The osmotic potential levels caused an increasing linear behavior, with the highest dry matter accumulation (8.24 mg) at the -0.4 MPa osmotic potential level (Figure 2D).



♦ Control; ■ Hydro-conditioning; ▲ Salicylic acid; ● Gibberellic acid.
* = significant at 5% probability ($p < 0.05$).

Figure 2. (A) Shoot length (SL), (B) root length (RL), and (C and D) total dry matter of Salvia hispanica L. seedlings subjected to seed priming with different attenuators and osmotic potentials.

Gibberellic acid and salicylic acid attenuated the adverse effects of NaCl on *S. hispanica* seeds, by improving the growth performance of the shoots and roots of the seedlings, compared to the seedlings of the control treatment (Figure 2A, B). Gibberellic acid and salicylic acid acted more effectively on the growth of different parts of the seedlings; the former had a greater influence on shoot growth and the latter on root growth.

Gibberellins interact with other hormones to regulate various metabolic processes in plants, including growth. Hence, the exogenous application of gibberellic acid in seeds can neutralize the toxic effects of NaCl on seedling growth (Tsegay & Andargie, 2018). Gibberellic acid acts by increasing cell division in the apical meristems (Taiz et al., 2015); the effect of this phytohormone can be verified by assessing seedling elongation, especially in the shoots, in response to the exogenous application of this compound. Tsegay and Andargie (2018) reported that treating corn and pea seeds with gibberellic acid improved significantly the growth of seedlings of these species up to an 8 $dS \cdot m^{-1}$ potential.

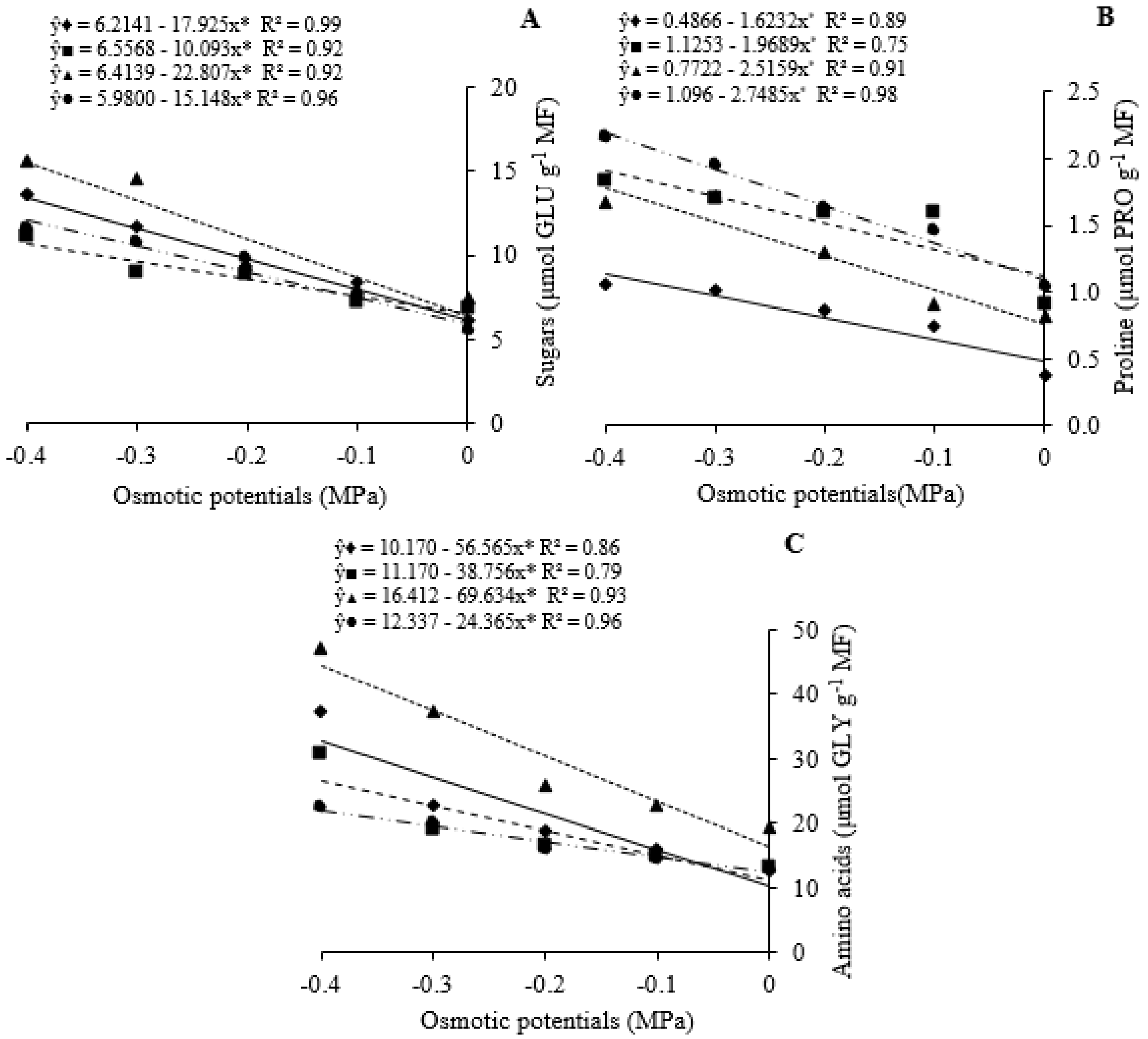
Furthermore, according to Shakirova, Sakhabutdinova, Bezrukova, Fatkhutdinova, and Fatkhutdinova (2003), salicylic acid reduces the indoleacetic acid and cytokinin levels in saline environments, thereby increasing cell division in the apical meristem of the seedling roots, in order to reduce the destructive effects of stress. This finding corroborates the results obtained in the present study with regard to the root length of *S. hispanica* seedlings (Figure 2B). Salicylic acid prevented drastic reductions in root length compared to the other treatments because of the preservation of the indoleacetic acid content, an auxin directly involved in root growth (Saini, Sharma, Kaur, & Pati, 2013). The same result was obtained by Turkyilmaz (2012) who studied the root growth of wheat seedlings treated with 400 ppm of salicylic acid and subjected to salt stress (100 mM NaCl). According to Turkyilmaz (2012), alterations in salicylic acid levels may be the first step in controlling the reduction of root growth under saline conditions.

On the other hand, the increase in the dry matter of *S. hispanica* seedlings caused by a reduction in the osmotic potential of the substrate up to -0.4 MPa (Figure 2C), may be related to the increase in seedling density owing to the low amount of water in the tissues (Sá et al., 2017). Osmotic adjustment is a fundamental mechanism for maintaining cell turgor under salt stress conditions, as it favors the increase of the water content of cells by increasing the number of osmolytes (Ashraf, Akram, Alqurainy, & Foolad, 2011; Marijuan & Bosch, 2013).

Thus, in this study, hydropriming and seed priming with salicylic acid and gibberellic acid did not stimulate an increase in the total dry matter of *S. hispanica* seedlings under different salinity levels because, in general, there was no significant interaction between the salinity factors and seed priming, only a single effect.

Organic solutes

The free sugar concentrations increased linearly with the reduction in the osmotic potential of the substrate in all the treatments that involved attenuators (Figure 3A). Salicylic acid led to the highest free sugar accumulation (15.54 μmol GLU g^{-1} FM) at the -0.4 MPa potential; this represented a 58.75% increase from the free sugar accumulation that resulted from the 0.0 MPa potential. In the control treatment, the free sugar accumulation was 13.38 μmol GLU g^{-1} FM at the -0.4 MPa potential, which was 53.75% higher than the free sugar accumulation that resulted from the 0.0 MPa potential. The other attenuators led to free sugar contents that were lower than those obtained from the control treatment; however, increases of 50.33 and 38.05% were obtained from the gibberellic acid and the hydropriming treatments, respectively, from the -0.4 MPa to the 0.0 MPa potentials.



♦ Control; ■ Hydro-conditioning; ▲ Salicylic acid; ● Gibberellic acid.

* = significant at 5% probability ($p < 0.05$).

Figure 3. Sugar (A), proline (B), and amino acid (C) contents of *Salvia hispanica* L. seedlings subjected to seed priming with different attenuators and osmotic potentials.

The free proline concentration in *S. hispanica* seedlings increased linearly in all the treatments that incorporated attenuators, as a function of the osmotic potential reduction (Figure 3B). The control treatment resulted in a 53.64% increase in the free proline concentration as the osmotic potential decreased from 0.0 to -0.4 MPa. The treatment of seeds with salicylic acid resulted in a 56.74% increase in the free proline concentration, from 0.77 to 1.78 μmol PRO g^{-1} FM, from 0.0 to -0.4 MPa. The free proline concentration increased by 50.23 and 41.36% from -0.4 to 0.0 MPa when *S. hispanica* seeds were subjected to gibberellic acid and the hydropriming technique, respectively.

The amino acid concentration in *S. hispanica* exhibited an increasing linear response to the reduction in the osmotic potential of the substrate in all treatments (Figure 3C). In the control treatment, a concentration of 32.80 μmol GLY g^{-1} FM was recorded at the -0.4 MPa potential. The salicylic acid treatment resulted in the highest amino acid concentration (44.27 μmol GLY g^{-1} FM) at the -0.4 MPa potential, which was 34.97% higher than that obtained from the control treatment. The hydropriming and gibberellic acid treatments led to free amino acid concentrations of 26.67 μmol GLY g^{-1} FM and 22.08 μmol GLY g^{-1} FM, respectively, at the -0.4 MPa potential; these values were 18.69 and 32.68% lower than those obtained from the control treatment, respectively.

Among all treatments tested, salicylic acid was the most effective in increasing the organic compound contents in *S. hispanica* seedlings. Seeds treated with salicylic acid exhibited the highest increases in the total soluble sugar, proline, and total free amino acid contents (Figure 3A, B, and C). In this respect, salicylic acid stood out from the other attenuators. According to Singh and Gautam (2013), salicylic acid can induce the balance of sugars in plants subjected to salt stress, thereby favoring their accumulation. In addition, according to these authors, sugars are inexpensive, easily accessible sources of energy that are accepted as osmolytes. Sugars such as glucose, fructose, sucrose, fructan, and starch can act on osmotic adjustment, serve as energy sources, store carbon, and assist in the elimination of free radicals under stress conditions.

Proline accumulation increases remarkably in the presence of salicylic acid; this represents an increase of this amino acid during the osmotic adjustment process in the plant, which aims to increase its hyperosmotic tolerance against the cell water content reduction induced by salt stress (Singh & Gautam, 2013). Under stress conditions, proline synthesis can be mediated by an alternative pathway, the pentose phosphate pathway. This pathway may be linked to the stimulation of proline metabolism in response to the application of salicylic acid, using glucose-6-phosphate-dehydrogenase as the precursor and promoting the concomitant increase in the content of this amino acid (Mccue, Zheng, Pinkham, & Shetty, 2000). Increases in the proline and sugar contents were also observed by Agami (2013) in corn seedlings subjected to salinity. According to the author, the immersion of seeds in salicylic acid (10^{-4} M) exceeded significantly the toxicity caused by NaCl stress.

High amino acid concentrations are accumulated under salt stress conditions in order to reduce the damage caused by the toxic and/or osmotic effects of salinity (Shahid et al., 2013). These compounds mainly act on osmotic adjustment and the protection of macromolecules and are commonly related to salinity tolerance (Esteves & Suzuki, 2008). However, salinity disturbs the hormonal balance of plants (Javid, Sorooshzadeh, Moradi, Sanavy, & Allahdadi, 2011). Hence, the attenuator concentrations used in the present study were not effective in restoring the hormonal balance required to increase the synthesis of amino acids in *S. hispanica* seedlings. Thus, it could be suggested that the salicylic acid concentration used may have been low, as it resulted in a low amino acid synthesis increase.

Our results showed that both the salicylic and the gibberellic acids were the most efficient in mitigating the effects of salt stress on the germination and growth of *S. hispanica*; however, salicylic acid outperformed gibberellic acid with respect to the synthesis of organic compounds.

Gibberellic acid led to the lowest accumulation of solutes among all the attenuators. Despite this, gibberellic acid was efficient in mitigating the effects of salt stress on *S. hispanica*, which indicates that its mitigating action is not related to the accumulation of solutes, but to its capacity to prevent the formation of reactive oxygen species (H_2O_2) and lipid peroxidation in the shoots (Grohs, Marchesan, Moraes, & Roso, 2016).

Although hydropriming activated several metabolic reactions in the seeds, it was not efficient in neutralizing the toxic effects of NaCl on the germination, vigor, and synthesis of osmoprotectants at the highest osmotic potentials tested, thereby leading to lower results than those obtained from the salicylic acid treatments.

Conclusion

Salt stress reduced the germination and growth of *S. hispanica*. However, both gibberellic acid and salicylic acid mitigated the effects of salt stress on *S. hispanica* up to the -0.4 MPa osmotic potential. Seed priming with salicylic acid was the most efficient method in attenuating the effects of salt stress on *S. hispanica* seeds; this treatment resulted in increased germination, vigor, and organic solute accumulation in *S. hispanica* seeds up to the -0.4 MPa osmotic potential. Hydropriming mitigated the salt stress effects on *S. hispanica* seeds up to the -0.3 MPa osmotic potential.

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