



Priming of Pumpkin Seeds with Salicylic Acid in Response to Salt Stress

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Abstract

Salicylic acid is a phenolic hormone that influences the physiological and biochemical processes of plants, including resistance to biotic and abiotic stresses like salinity. The aim of this study was to evaluate the priming of pumpkin seeds with salicylic acid to mitigate salt stress during germination and early development. An entirely randomized design was used, in a 4×4 factorial scheme, using different doses of the phytohormone (100, 150, and 200 mg L⁻¹ and distilled water) and salinity levels (0, 2, 4, and 6 dS m⁻¹), with four replicates. Two genotypes, hybrid cv. Atlas and creole variety were used. The priming period of the seeds in salicylic acid was 8 hours. Then, the seeds were packed in germitest paper, soaked in saline solution, and placed in a germinating chamber. The initial germination count, germination percentage, and abnormal seedlings were evaluated. In a greenhouse, the emergence test was conducted with the seeds primed seeds. Saline solution was provided once a day. The percentage of emergence, emergence speed index, shoot and root length, and shoot and root dry mass were evaluated. The salt stress compromised seed germination and seedling development. The hybrid was sensitive to the salinity of 2 dS m⁻¹, while the creole variety tolerated salinity up to 2 dS m⁻¹. Priming of pumpkin seedlings with salicylic acid did not relieve salt stress during germination and early development.

Keywords: phytohormone, priming, plant regulator, salinity

Introduction

Cucurbita moschata is one of the most cultivated pumpkin species in Brazil, being classified as a fruit vegetable of socioeconomic and dietary importance (Resende et al., 2013). During the 2020/21 harvest, Brazilian production was around 385,000 tons *C. moschata* the northeastern region being the second largest producer (IBGE, 2020).

Cultivation of this vegetable occurs in two forms. First, is typical of family farming, with a predominance of native varieties with wide genetic variability (Lima et al., 2016). Second, is technified cultivation that used hybrids of high uniformity and productivity (Amaro et al., 2014).

The Northeast of Brazil has well-established climatic characteristics, such as irregular rainfall, high temperatures, and high evapotranspiration rates, processes that accentuate the accumulation of solutes in the soil (Bezerra et al., 2020). The use of low-quality

water, high concentration of salts, and inadequate management of fertilization and irrigation contribute to the salinization of the area (Nóbrega et al., 2018a).

Salt stress intensifies the absorption of specific ions (Na⁺ and Cl⁻), restricts nutrient absorption, and limits productivity (Dourado et al., 2020). Consequently, germination and early development of the seedlings are affected (Santos et al., 2020). Tolerance, however, occurs as an adaptation of plant species to salt concentration and soil and climate conditions (Pedrotti et al., 2015).

In view of this problem, strategies to optimize plant performance in saline environments need to be researched and adopted (Santos et al., 2016). A recurrent technique is physiological priming, which consists of controlled hydration of seeds, to promote the activation of the initial stages of germination without protrusion of the primary root (Thakur et al., 2022). This technique enables rapid and uniform emergence of batches under

unfavorable environmental conditions (Oliveira et al., 2016).

Salicylic acid (SA) is a phenolic hormone that participates in the processes of ion absorption and transport, seed germination, and the activation and signaling of the genes responsible for the defense mechanisms against biotic and abiotic stresses (Klessig et al., 2018; Silva et al., 2018).

Recently, research has been conducted on physiological priming with SA in cucurbit seeds, highlighting those developed with watermelon (Nóbrega et al., 2020), cucumber (Gastl Filho et al., 2017), melon (Nóbrega et al., 2018b), and pumpkin (Guirra et al., 2022). However, there is still a lack of information, as the results may vary according to genotype, environment, doses, and stress levels.

The aim of this study was to evaluate the priming of hybrid and creole pumpkin seeds with SA, to mitigate salt stress during germination and early development.

Material and Methods

The experiment was carried out at the Agricultural Laboratory and in a greenhouse of the Federal Institute of Piauí (IFPI)–Uruçuí Campus. A completely randomized design was used, in a 4×4 factorial scheme, with four replicates. The treatments consisted of doses of SA (100, 150, and 200 mg L⁻¹ and distilled water as control) and salinity levels corresponding to 0, 2, 4, and 6 dS m⁻¹ of electrical conductivity (EC).

Seeds of two pumpkin genotypes were used: hybrid cv. Atlas, American Butternut type (Sakata®) and a creole variety, of the Butternut Squash type. These were extracted from fruits from family farming and then washed and dried in the shade.

The seeds of each genotype were characterized for water content (% WC) and weight of one thousand seeds (WTS) (BRASIL, 2009). The hybrid seeds presented values of 7.7% and 78.7 g, and creole seeds, 6.5% and 142.3 g for % WC and WTS, respectively.

Subsequently, two replicates of 50 seeds of each genotype were used to determine the seed imbibition curve. The samples were wrapped between three sheets of germitest paper, moistened with distilled water, to a volume equivalent to 2.5 times the weight of the dry substrate (BRASIL, 2009). The rolls were placed in a germination chamber at 25 °C, and the seed mass was measured every hour for ten hours; then at four-hour intervals, until root protrusion occurred in at least 50% of the seeds. The imbibition rate was calculated based on the methodology of Cromarty et al. (1985).

Based on the hydration curve (**Figure 1**), duration

of 8 hours was defined for physiological priming with SA. This time period was sufficient to significantly increase the water content, without causing sensitivity of the seeds to the drying process.

For the physiological priming of the seeds, SA was diluted in distilled water, according to the recommended concentrations. The seeds were distributed among three sheets of germitest paper, moistened with the respective dilutions of SA at a proportion of 2.5 times the weight of the dry paper. They were kept under these conditions for eight hours, at a temperature of $25^{\circ}\text{C} \pm 2^{\circ}\text{C}$; subsequently, 2 replicates of 25 seeds were used to determine the % WC. Drying took place in ambient conditions, until the humidity was close to the initial level. The saline solutions were prepared using sodium chloride (NaCl), following the methodology of Rhoades et al. (2000). The EC was measured by an AKSO portable conductivity meter (model AK51-V2) and the solutions were stored in properly closed containers.

The saline solution was applied simultaneously with the germination test. Four replicates of 25 seeds were used for each treatment, in a germination chamber, at a temperature of $25^{\circ}\text{C} \pm 2^{\circ}\text{C}$, and a photoperiod of 8 hours (BRASIL, 2009). The variables, first germination count (FGC) and germination (G) was evaluated 4 and 8 days after the test's initiation, respectively. The following evaluation criteria were used: good formation of the essential parts of the seedlings and release of at least 50% of the seed coat; seedlings with characteristics different from these were considered abnormal (BRASIL, 2009).

The emergence test (E) was conducted in a greenhouse, with a 50% shading screen. Sowing was carried out in plastic boxes, with a volume of 3 dm³ of medium-textured sand, using a depth of 3 cm. The seeds were watered with saline solution once a day, the first watering using a quantity equivalent to 80% of the field capacity (FC) and the rest at 10% of the FC. The temperature and relative humidity of the environment were measured daily using an AKSO thermohygrometer (model AK632), summarized in **Figure 2**.

Daily counts of the emerged seedlings were performed and, then the percentage of normal seedlings obtained after emergence stabilization, and the emergence speed index (ESI), were calculated (Maguire, 1962). At the time of emergence stabilization, 10 seedlings positioned in the central area of the plots were collected.

Then, the materials were taken to the Agricultural Laboratory for evaluations of shoot length (SL) and primary root length (RL), in cm, with the aid of a graduated ruler. For shoot dry matter (SDM) and root dry matter (RDM),

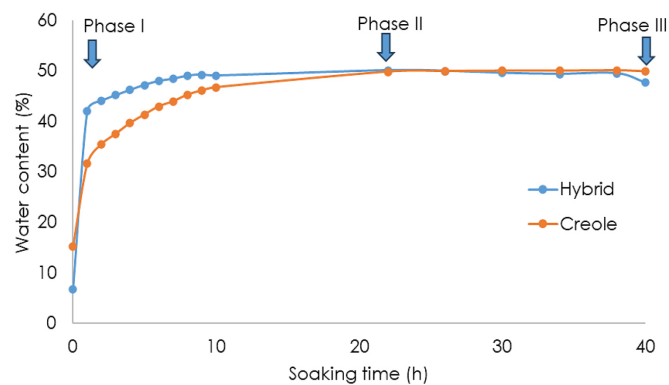


Figure 1. Soaking curve of hybrid and creole pumpkin seeds

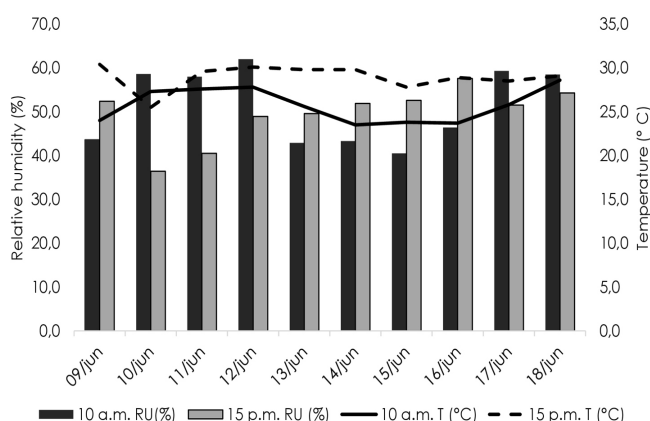


Figure 2. Relative humidity (%) and average temperature (°C) recorded at 10 am and 15 pm during the seedling emergence test in a greenhouse.

seedlings were placed in paper bags and packed in a ventilation oven at 65 °C for 72 hours. The mass was measured on an analytical balance (0.001 g), and the results were expressed in g seedling⁻¹.

The data were submitted to the normality test (Lillifors test) and then to analysis of variance by the F test, and the means were grouped by the Scott-Knott test, at 5% probability. The computer software SISVAR Version 5.6 (Ferreira, 2011) was used.

Results and Discussion

For cv. Atlas there was a significant interaction ($p < 0.05$) between SA $\times$ EC for the most variables, except for SL e RL. Conversely, for the creole variety, there was no interaction only for E, ESI, and SL.

Regarding the germination of cv. Atlas, the highest percentages were observed in the treatments with 0 dS m⁻¹, but without statistical difference between the doses of SA, while the lowest value occurred in the treatment 2 dS m⁻¹ $\times$ 150 mg L⁻¹ of SA (**Figure 3**). The combination of 200 mg L⁻¹ and EC from 4 dS m⁻¹ reduced the G (%), resulting in a percentage lower than the minimum (80%) for commercialization (BRASIL, 2009).

For the creole variety, the dose of 150 mg L⁻¹ attenuated the effects caused by ECs of 4 and 6 dS m⁻¹, with an increase of 13.4% and 11.2% in relation to 0 dS m⁻¹, respectively. Conversely, there was a reduction in G of seeds primed with 100 and 200 mg L⁻¹ SA and submitted to 2 dS m⁻¹ and 0 mg L⁻¹ $\times$ 4 dS m⁻¹ for the treatment.

Discordant results were observed in pumpkin seeds cv. Baiana Tropical, primed with 30 mg L⁻¹ of SA and submitted to EC of 5.97 dS m⁻¹, showing a 12.8% increase in germination compared to the control of 0.55 dS m⁻¹ (Guirra et al., 2022), and this difference in relation to the present study can be attributed to the cultivar or the dose used in seed priming.

The interaction between priming with SA and salt stress can culminate in increased germination as plant defense mechanisms activate stress resistance genes that influence the properties of the cell membranes and increasing the production of phenolic compounds, resulting in increased germination capacity (Silva et al., 2018).

For the hybrid, the highest percentages were observed in the first germination count in the absence of salt. Under these conditions, the application of SA had a negative effect, since the seeds primed with water showed higher FGC (**Figure 4**). This variable was reduced with the increase in EC, to the point that there was no attenuating effect of any of the SA concentrations.

The increase in salinity reduces the water potential, altering the absorption kinetics of the seed tissues and, as a consequence, there is a reduction in the percentage and speed of germination (Demontiêzo et al., 2016). In this assay, reductions in FGC, from 2 dS m⁻¹, indicate the lower ability of seeds to imbibe water under saline conditions, due to the alteration of the osmotic potential in the internal tissues.

In relation to the FGC of the creole variety, the mitigation of salinity for the treatment at 150 mg L⁻¹ from the saline concentration of 2 dS m⁻¹ was observed. Seeds treated with 0 and 200 mg L⁻¹ showed a reduction in germination percentage as EC increased, while priming with 100 mg L⁻¹ showed no significant difference, regardless of salinity.

The higher occurrence of abnormal cv. Atlas occurred in the 150 mg L⁻¹ $\times$ 2 dS m⁻¹ and 200 mg L⁻¹ treatments in EC4 and 6 dS m⁻¹ (**Figure 5**). Creole seeds primed with 0 mg L⁻¹ SA and managed at 4 dS m⁻¹, 100 mg L⁻¹ SA $\times$ 2 dS m⁻¹, 150 mg L⁻¹ $\times$ 0 dS m⁻¹, and 200 mg L⁻¹ $\times$ 6 dS m⁻¹ showed the highest abnormal seedlings percentages, showing that the higher concentrations of SA can promote the production of abnormal seedlings,

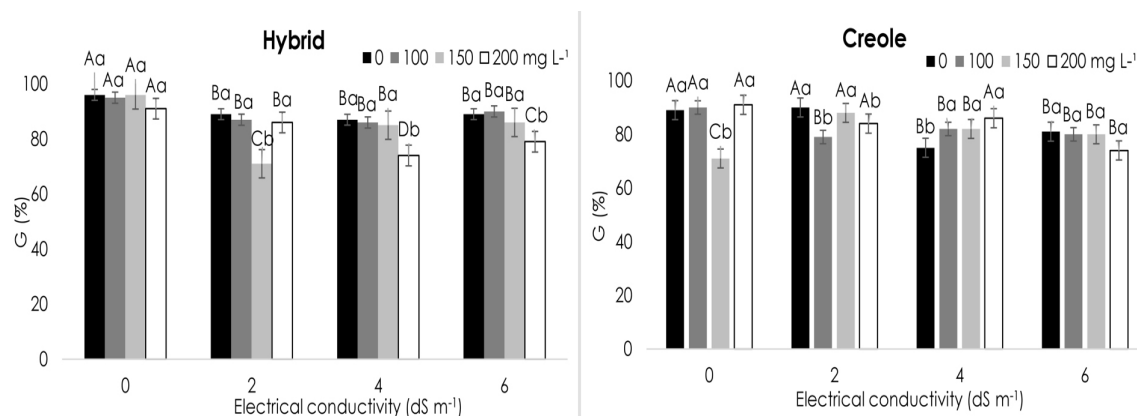


Figure 3. Germination (G) of hybrid pumpkin and creole seeds conditioned at different concentrations of salicylic acid (SA) and subjected to levels of electrical conductivity (EC). Means followed by different lowercase letters for AS concentrations and uppercase for CE levels differ by the Scott-Knott test ($p < 0.05$).

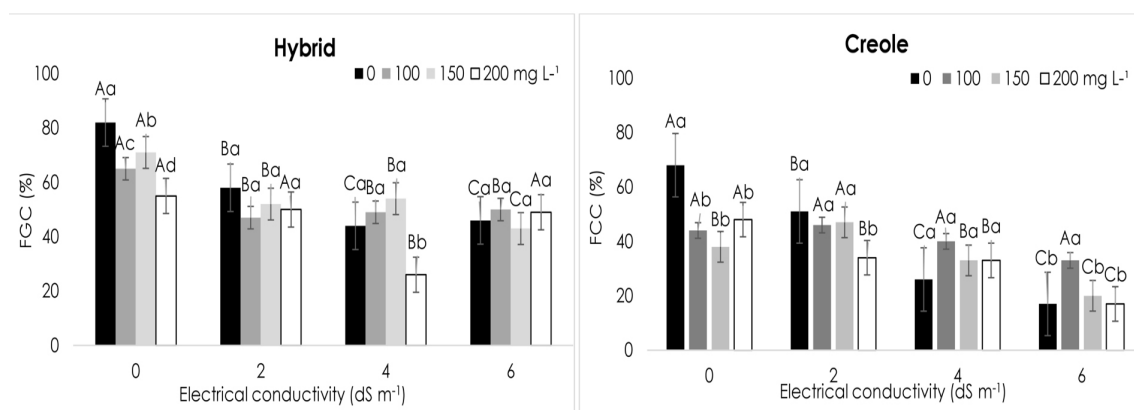


Figure 4. First germination count (FGC) of hybrid pumpkin and creole seeds conditioned at different concentrations of salicylic acid (SA) and subjected to levels of electrical conductivity (EC).

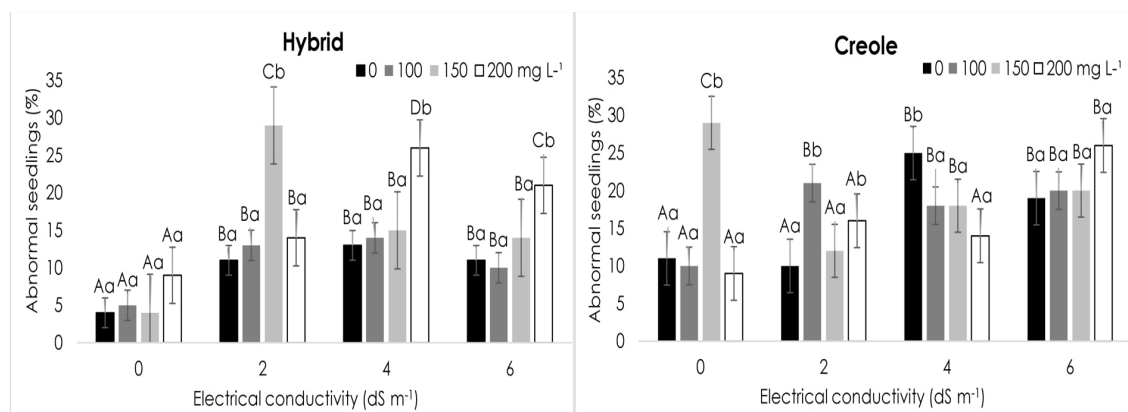


Figure 5. Percentage of abnormal seedlings (AP) of hybrid pumpkin and creole seeds conditioned at different concentrations of salicylic acid (SA) and subjected to levels of electrical conductivity (EC).

similar to unprimed seeds submitted to salinity conditions from 4 dS.m⁻¹.

The deleterious effects caused by salinity, such as the accumulation of Na⁺ and Cl⁻ ions in the tissues, cause physiological alteration in the seeds, culminating in a higher percentage of non-standard seedlings (Wiladino & Camara, 2010). Additionally, high concentrations of SA may favor the development of abnormal seedlings under salinity conditions, as highlighted in the study with melon

seeds (Moreira et al., 2014). A considerable increase in the percentage of abnormal seedlings was also observed in pumpkin seeds (*Cucurbita pepo* c.v. Caserta and Redonda, and *C. maxima* cv. Coroa) when subjected to EC >2 dS m⁻¹ (Dantas et al., 2019).

For the sand emergence of cv. Atlas, a statistical difference was observed only in the treatments with 150 and 200 mg L⁻¹ SA and 2 dS m⁻¹ in which there was a reduction of 34% and 25.5%, respectively, in relation to

the 0 mg L⁻¹ dose, under the same EC (**Figure 6**). Priming with SA did not demonstrate a significant influence on the emergence of native seeds. Moreover, they were disadvantaged when exposed to the two highest saline concentrations, presenting a drastic reduction in the percentage of emergence.

Hydroprimed seeds showed lower ESI when subjected to 4 and 6 dS m⁻¹ of salinity, as well as those treated with 150 mg L⁻¹ SA, and managed with EC from 2 dS m⁻¹ (**Figure 7**). Priming with 100 mg L⁻¹ SA maintained the ESI even with increased salt stress, while the use of 200 mg L⁻¹ SA under EC 4 dS m⁻¹ did not show significant difference in relation to the control.

The percentage of emergence and ESI of the seeds of the creole pumpkin showed only an isolated effect of salinity (Figures 6 and 7). For both variables, there was no statistical difference for ECs of 0 and 2 dS m⁻¹, while ECs of 6 dS m⁻¹ resulted in a reduction of 66.3% for emergence and 79.7% for ESI compared to treatments without salt stress.

According to Nóbrega et al. (2020), the emergence of watermelon seeds cv. Crimson Sweet primed with SA was not affected by salinity while ESI was impaired by increased EC. Conversely, the increase in

salinity reduced the ESI of the seeds of the pumpkin cvs. Caserta, Redonda, and Coroa in EC >2 dS m⁻¹ (Dantas et al., 2019), as in this study.

SA has the ability to regulate the absorption, translocation, and accumulation of Na⁺ from the root system to the shoot, reducing the negative effects caused by salinity (Methennl et al., 2018). In this sense, the capacity of water absorption and regulation of the defense mechanisms of the seeds, combined with the shorter time of exposure to salts, reduce the harmful effects (Nunes et al., 2016). The increase in salt levels alters the kinetics of water absorption through seed tissues, reducing the capacity of breakdown and translocation of reserves to the embryonic axis and limiting the speed of emergence (Nóbrega et al., 2020).

In both genotypes, there was only an isolated effect of salinity for SL (**Figure 8**). EC from 4 dS m⁻¹ negatively affected the aerial growth of cv. Atlas, while for the creole variety, the seedlings subjected to the EC of 4 and 6 dS m⁻¹ showed reductions of 24.1% and 52.6%, respectively, compared to those managed at 2 dS m⁻¹, which presented higher average length.

The reduction in SL based on the increase in salinity is due to the metabolic and physiological changes that

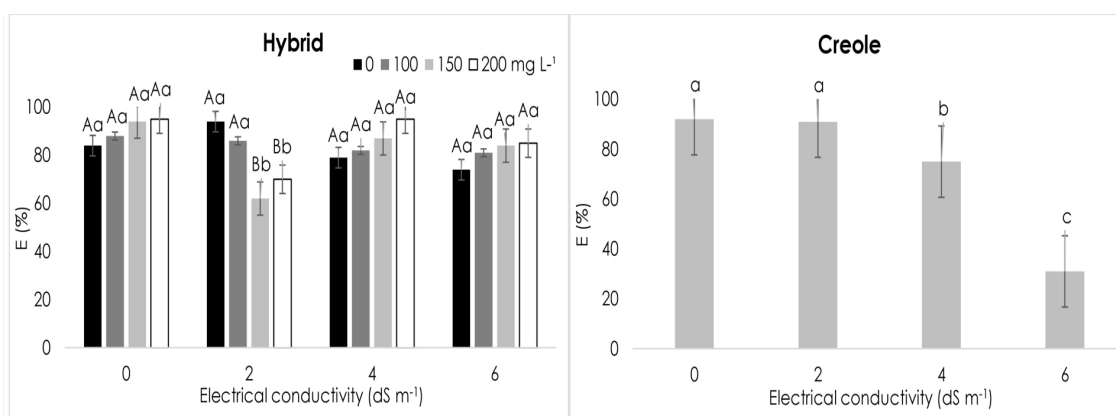


Figure 6. Emergence (E) of hybrid pumpkin and creole seeds conditioned at different concentrations of salicylic acid (SA) and subjected to levels of electrical conductivity (EC).

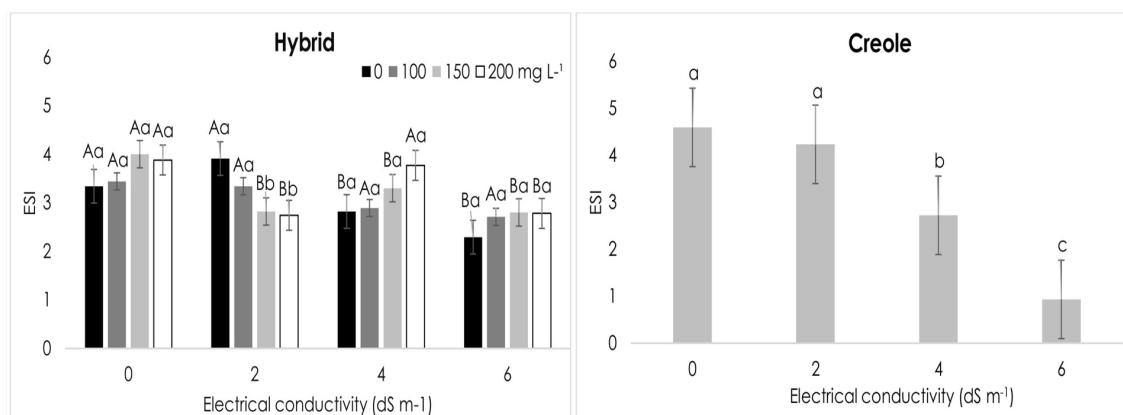


Figure 7. Emergence speed index (ESI) of pumpkin seeds conditioned in different concentrations of salicylic acid (SA) and subjected to levels of electrical conductivity (EC).

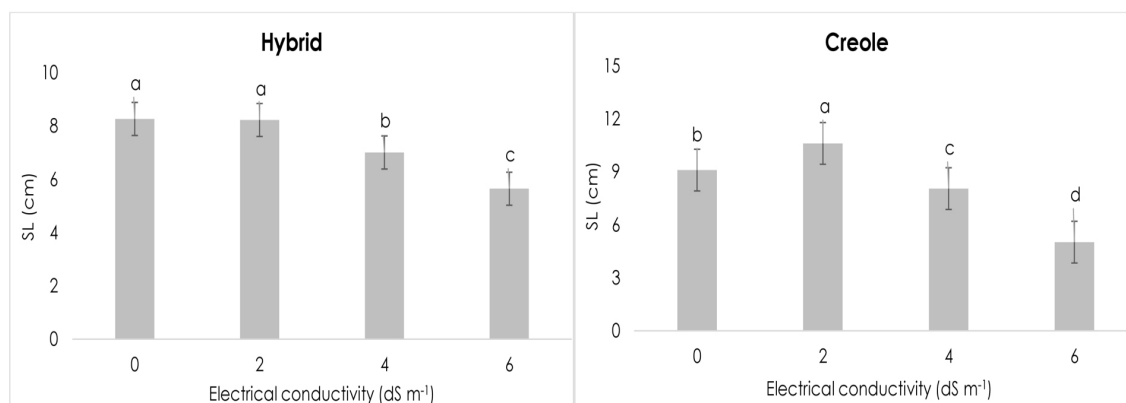


Figure 8. Shoot length (SL) of seedlings from hybrid and creole pumpkin seeds conditioned at different concentrations of salicylic acid (SA) and submitted to levels of electrical conductivity (EC).

affect, above all, the seedling phase (Nascimento et al., 2017). However, the pumpkin seeds were not responsive to SA priming, unlike the tomato seeds in which the increase in SA concentration provided an increase in SL in the absence of SA in a saline environment (Maia Júnior et al., 2020).

For the RL of the hybrid genotype, the seedlings in the absence of salt showed higher root system development than those subjected to salt stress, with mean reductions of 19.2%, 25.7%, and 41.3% for ECs of 2, 4, and 6 dS m⁻¹, respectively (**Figure 9**).

Treatments 0 mg L⁻¹ of SA and EC of 4 and 6 dS m⁻¹, and 200 mg L⁻¹ under EC of 2 and 6 dS m⁻¹ reduced root growth of seedlings of the creole variety (Figure 9). For the EC of 2 dS m⁻¹, the doses of 150 and 200 mg L⁻¹ showed reductions of 16.6% and 12.7% in relation to the priming with distilled water. Conversely, there was no difference for the 100 mg L⁻¹ SA dose, regardless of EC. Priming with 0.5 mM of SA was able to mitigate the damage caused to RL by the action of salinity (50 and 150 mM NaCl), in ten tomato cultivars (Ma et al., 2019). The reduction in RL is directly linked to the direct contact of the pumpkin root system with the salts present in the medium (Guimarães et al., 2013).

In regards to SDM of cv., Atlas there was a statistical difference only for 2 dS m⁻¹ × 100 mg L⁻¹ (**Figure 10**). For the creole genotype, the seeds not primed with SA (0 mg L⁻¹) and maintained in the absence of salt, or in lower concentration of salt (2 dS m⁻¹), gave rise to seedlings with lower dry mass, but promoted an increase in mass in EC 4 and 6 dS m⁻¹, similar to the seeds treated with SA.

For the RDM of cv. Atlas, the treatments 100 mg L⁻¹ × 4 dS m⁻¹ and 150 mg L⁻¹ × 6 dS m⁻¹ showed an increase in dry matter accumulation, although they were harmful in the absence or at the lowest saline concentration (**Figure 11**). For the creole variety, however, the 0 mg L⁻¹ × 2 dS m⁻¹ treatment and the 150 mg L⁻¹ treatment of SA in 4 dS m⁻¹ EC mitigated the action of salinity, favoring the accumulation of dry matter. Similarly, seeds primed with 200 mg L⁻¹ of SA also produced seedlings with greater accumulation of root mass, in the absence of salt or under more severe salt stress (4 dS m⁻¹).

The mitigation of the effects of salinity may have resulted from the action of SA in reducing the damage caused to the internal tissues of seedlings under stress conditions (Nóbrega et al., 2021). The use of 30 mg L⁻¹ of

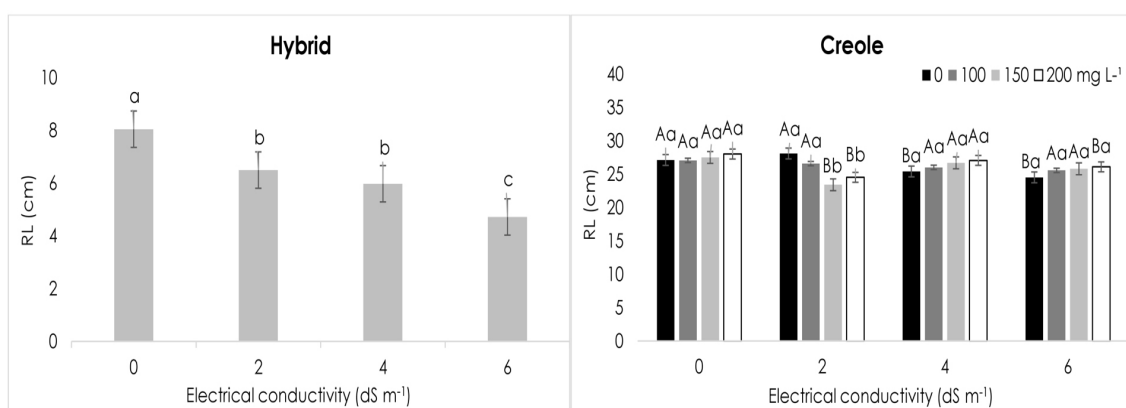


Figure 9. Root length (RL) of seedlings from hybrid and creole pumpkin seeds conditioned at different concentrations of salicylic acid (SA) and submitted to levels of electrical conductivity (EC).

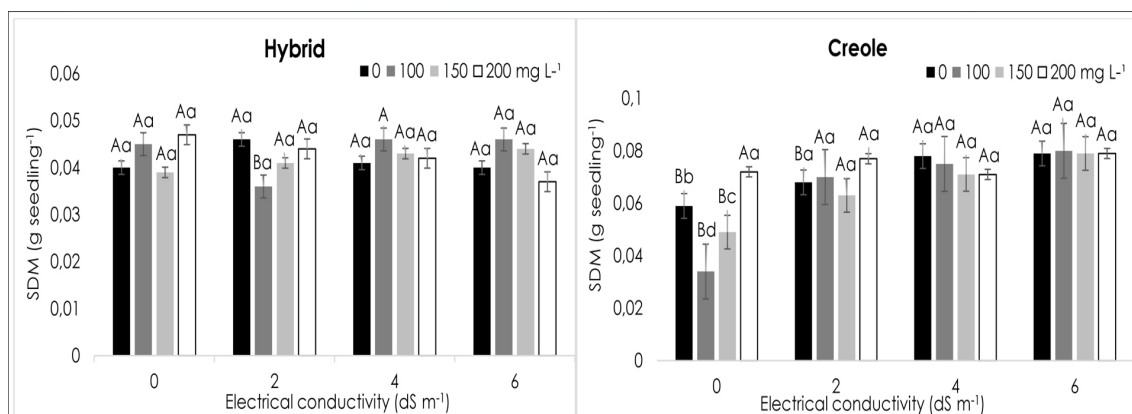


Figure 10. Shoot dry mass (SDM) of seedlings from hybrid and creole pumpkin seeds conditioned at different concentrations of salicylic acid (SA) and subjected to levels of electrical conductivity (EC).

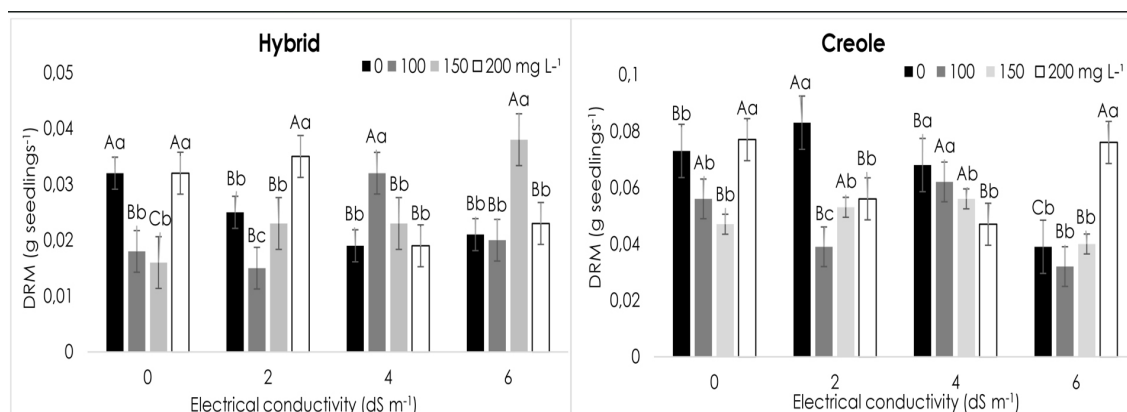


Figure 11. Root dry mass (DRM) of seedlings from hybrid and creole pumpkin seeds conditioned at different concentrations of salicylic acid (SA) and subjected to levels of electrical conductivity (EC).

SA in seeds of the pumpkin cv. Baiana Tropical, in EC 5.97 dS m⁻¹, promoted an increase of 54.4% in RDM compared to 0.55 dS m⁻¹ (Guirra, 2022). However, the results pointed out by Rafique et al. (2016), in treatments without salt stress, showed that the use of lower doses (15 and 30 mg L⁻¹ SA) promoted an increase in the accumulation of dry matter in pumpkin seeds.

Although pumpkin is considered a species with low sensitivity to salinity (Lima Júnior et al., 2010), the results of this study show the susceptibility of seeds and seedlings to salt stress, corroborating the data of Gabriel Filho et al. (2022) who found the harmful effect of saline water on the biometric characteristics of pumpkin plants, as well as the results presented by Oliveira et al. (2014), which can be attributed to the small difference between the genotypes regarding the stress caused by salinity.

Conclusions

Salt stress causes a reduction in the percentage and speed of germination of pumpkin seeds;

The seeds and seedlings of the pumpkin cv. Atlas are sensitive to salt stress of 2 dS m⁻¹, while those of the creole variety can tolerate salinity up to 2 dS m⁻¹;

The priming of pumpkin seeds with SA does not relieve salt stress during seed germination and early seedling development.

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