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Pretreatment of seeds with plant regulators attenuates salt stress in pumpkin: effects on germination and initial seedling development[1]

O pré-tratamento de sementes com reguladores vegetais atenua o estresse salino em abóbora: efeitos na germinação e desenvolvimento inicial de plântulas

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ABSTRACT - Pretreatment of seeds results in faster emergence of seedlings and uniform stand, especially under stress. Thus, the objective was to evaluate the action of plant regulators as stress attenuators during germination and initial development of pumpkin seedlings, cv. 'Baiana Tropical', irrigated with saline water. For this, a completely randomized design was established, in a 4 x 3 factorial scheme, with four replications. Treatments consisted of four types of water (W_1 - 100% supply water; W_2 - 100% fish farming effluent; W_3 - 100% artesian well water; W_4 - mixture of 50% fish farming effluent + 50% artesian well water) and three seed treatments (control, salicylic acid and gibberellic acid). The variables measured were germination, first germination count, shoot and root length, shoot and root dry mass, total soluble sugars, total amino acids and proline. The data were subjected to analysis of variance and Scott-Knott test. Pumpkin seedlings performed osmotic adjustment under saline conditions of W_4 water. In addition, the treatment of pumpkin seeds with plant regulators (gibberellic and salicylic acids) favors the germination and initial development of seedlings under conditions of salt stress.

Key words: *Cucurbita moschata*. Cucurbitaceae. Gibberellic acid. Salicylic acid. Stress mitigation.

RESUMO - O pré-tratamento de sementes resulta em emergência de plântulas mais rápida e estande uniforme, principalmente, quando sob estresse. Sendo assim, objetivou-se avaliar a ação de reguladores vegetais como atenuantes de estresse durante a germinação e desenvolvimento inicial de plântulas de abóbora, cv. Baiana Tropical, irrigadas com água salina. Para isso, estabeleceu-se o delineamento inteiramente casualizado, em esquema fatorial 4 x 3, em quatro repetições. Os tratamentos foram constituídos de quatro tipos de água (A_1 – 100% água de abastecimento; A_2 – 100% efluente de piscicultura; A_3 – 100% água de poço artesiano; A_4 - mistura de 50% efluente de piscicultura + 50% água de poço artesiano) e três tratamentos de sementes (controle, ácidos salicílico e giberélico). As variáveis mensuradas foram germinação, primeira contagem de germinação, comprimento da parte aérea e raiz, massa seca da parte aérea e raiz, açúcares solúveis totais, aminoácidos totais e prolina. Os dados foram submetidos a análise de variância e teste de Scott-Knott. As plântulas de abóbora realizaram ajustamento osmótico em condições salinas da água A_4. Além disso, o tratamento de sementes de abóbora com reguladores vegetais (ácidos giberélico e salicílico) beneficia a germinação e o desenvolvimento inicial de plântulas sob condições de estresse salino.

Palavras-chave: *Cucurbita moschata*. Cucurbitaceae. Ácido giberélico. Ácido salicílico. Mitigação de estresse.

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INTRODUCTION

Pumpkin (*Cucurbita moschata* Duchesne) is an annual vegetable-fruit with great socioeconomic importance. This species is cultivated in virtually all countries, with China holding the world's highest production per year, 7.8 million tons (ATLASBIG, 2020). In Brazil, its cultivation and use in the food of the population are quite traditional, with production around 500,000 tons per year, especially in the Northeast region (EMATER-GO, 2019).

The Northeast region of Brazil is characterized by uneven rainfall regime and high evapotranspiration rates, causing the accumulation of solutes in the soil and in subsurface water sources (BEZERRA *et al*., 2020). Due to these characteristics, the availability and quality of water in this region are reduced (DINIZ *et al*., 2021). Consequently, the practice of irrigated agriculture becomes limited, as the negative effects of salinity affect from seed germination to production, which can cause death of plants due to the toxicity of ions (DOURADO *et al*., 2020). Salt stress of 2.5 dS m^{-1} causes negative effects on *Cucurbita pepo* L., characterized by low biomass accumulation in the initial development of the seedlings of this species (CANJÁ *et al.*, 2021).

Due to the scarcity of good quality water resources, the use of fish farming effluents is an alternative for the irrigation of agricultural crops with species relatively tolerant to salinity. These wastewaters are rich in minerals and organic matter; however, they may contain high salt contents (SIMÕES *et al*., 2016). Thus, the adoption of certain management techniques, such as water dilution, can enable the use of saline waters in crops subjected to restrictive environmental conditions (LEITE *et al*., 2017).

As techniques adopted to enhance the yield of species in stressful environments, plant regulators have been used (AMARO *et al*., 2020). Among the most used are the gibberellic acid (GA_3) and salicylic acid (SA). Gibberellic acid is a phytohormone considered a promoter of germination, acting on embryo growth and mobilization of energy reserves (PAIXÃO *et al*., 2021), while salicylic acid is a phenolic hormone fundamental to plant development, acting as a signaling molecule and mitigator of biotic and abiotic stresses (OLIVEIRA *et al*., 2016).

Even in an incipient way, research has been carried out on the exogenous application of gibberellic and salicylic acids in the germination performance of cucurbit seeds. Priming of *Citrullus lanatus* [(Thunb.) Matsum & Nakai)] seeds with gibberellic acid at 50 and 100 $\mu g\ g^{-1}$ (SILVA *et al*., 2014) and 1.0 mM salicylic acid (NÓBREGA *et al*., 2020) promoted higher germination and shoot length of seedlings, respectively. On the other hand, salicylic acid concentrations above 3 mM caused reduction in the germination and vigor of *Cucumis sativus* L. seeds (GASTL FILHO *et al*., 2017). Knowing the need for alternative techniques for the treatment of seeds and production of seedlings using wastewater, the objective was to evaluate the effects of gibberellic and salicylic acids as mitigators of salt stress during germination and initial development of pumpkin seedlings, cv. 'Baiana Tropical'.

MATERIAL AND METHODS

Experimental design

The experiment was conducted under controlled laboratory conditions with pumpkin seeds, cv. 'Baiana Tropical', sold by the company Topseed® and acquired in the market of the city of Mossoró, RN, Brazil, in October 2018. The experimental design was completely randomized, with four replicates of 50 seeds. The treatments were arranged in a 4 × 3 factorial scheme, with four types of water (W_1 - 100% supply water; W_2 - 100% fish farming effluent; W_3 - 100% artesian well water; W_4 - mixture of 50% fish farming effluent + 50% artesian well water) and three seed treatments (control, gibberellic acid and salty acid), according to methodology of Guirra *et al.* (2020).

Experimental conduction

The water used in the treatment of fish farming effluent (W_2) was collected in tilapia (Oreochomis spp.) production tanks. The other treatments [100% artesian well water (W_3) and 100% supply water (W_1)] were also obtained on the campus of the Federal Rural University of the Semi-Arid Region, Mossoró, RN. In addition to these, well water (50%) and fish farming effluent (50%) were mixed in the treatment W_4. The samples were collected in plastic pots and analyzed in the Water and Soil Laboratory of the same institution (Table 1).

The seeds were initially treated with a solution of gibberellic acid (GA_3) at the concentration of 50 mg L^{-1} and salicylic acid (SA) at the concentration of 30 mg L^{-1} of H_2O. For this, the seeds were placed to soak on paper towels (paper roll) previously moistened with the solutions of the acids in the proportion of twice their dry mass (BRASIL, 2009). Soon after, they were placed in moistened paper rolls and kept in the germination chamber at 25 °C for 24 hours (SILVA *et al*., 2019). After this period, the seeds were removed, dried on paper towels and subjected to germination test in four replicates of 50 seeds. For this, the paper towel was initially moistened with the water treatments in the proportion of twice its dry mass. Then, they were placed in paper rolls and packed in plastic bags, which were kept in a germination chamber, at 25 °C, with a photoperiod of 12 h (BRASIL, 2009).

Table 1 - Result of water analysis with cation and anion concentrations, acidity (pH) and electrical conductivity (EC) found in the waters used in the experiment with pumpkin seeds, cultivar 'Baiana Tropical'

Waters	pH	EC (dS m^{-1})	Cátions ($mmol_c$ L^{-1})				Ânions ($mmol_c$ L^{-1})			*SAR	**Class
			Ca^{2+}	Mg^{2+}	Na^{+}	K^{+}	CO_3^{2-}	HCO_3^{-}	Cl^{-}		
W1	8.4	0.55	0.9	0.4	3.36	0.24	0.6	3.0	2.8	4.2	C2S1
W2	7.9	5.97	15.7	20.1	30.99	1.13	0.6	3.4	42.0	7.3	C4S1
W3	8.1	5.33	18.5	20.6	21.95	0.77	6.4	5.2	6.4	5.0	C4S1
W4	8.0	5.59	17.0	19.8	36.59	0.98	1.2	4.1	34.0	8.5	C4S1

W_1 - 100% Supply water; W_2 - 100% Fish farming effluent; W_3 - 100% Artesian well water; W_4 - Mixture of 50% fish farming effluent + 50% artesian well water; *Sodium adsorption ratio; **Classification of water for irrigation (RICHARDS, 1954)

Physiological variables

Germination (G): evaluations of normal seedlings were performed at four and eight days, and the values were expressed as a percentage of normal seedlings (BRASIL, 2009).

First germination count (FGC): evaluated together with the germination test, in which normal seedlings were counted at four days after sowing according to the Rules for Seed Analysis (BRASIL, 2009), and the values were expressed as a percentage.

Shoot length (SL) and root length (RL): at the end of the germination test, ten normal seedlings of each repetition were randomly measured using a ruler graduated in millimeters, and the results were expressed in centimeters (cm).

Shoot dry mass (SDM) and root dry mass (RDM): normal seedlings were sectioned into shoots and roots, placed in paper bags and kept in a forced ventilation oven at 65 ºC for 72 h. The dried material was weighed on a precision analytical scale (0.0001 g) and the results were expressed in milligrams (mg).

Determination of organic solutes

Total soluble sugars (TSS), total amino acids (TAA) and proline (PRL) were quantified in 0.2 g samples of fresh tissue from whole normal seedlings. These were automatically macerated in hermetically sealed tubes containing 3 mL of 80% ethanol. Subsequently, the tubes were kept in a water bath at 60 °C for 20 min. They were then centrifuged at 10,000 rpm for 8 min at 4 ºC, and the supernatant was collected.

Total soluble sugars (TSS): determined by the anthrone method (YEMM; WILLIS, 1954), with results expressed in mg of TSS g^{-1} of fresh mass (FM).

Total amino acids (TAA): determined by the ninhydrin method (YEMM; COCKING, 1955), with results expressed in μmol TAA g^{-1} of FM.

Proline (PRL): determined by the methodology proposed by Bates, Waldren and Teare (1973), with results expressed in μmol PRL g^{-1} of FM.

Statistical analysis

The data were subjected to analysis of variance by the F test ($p \leq 0.05$) and, in case of significance, subjected to the Scott-Knott test ($p \leq 0.05$), using the statistical program System for Variance Analysis - SISVAR® (FERREIRA, 2011).

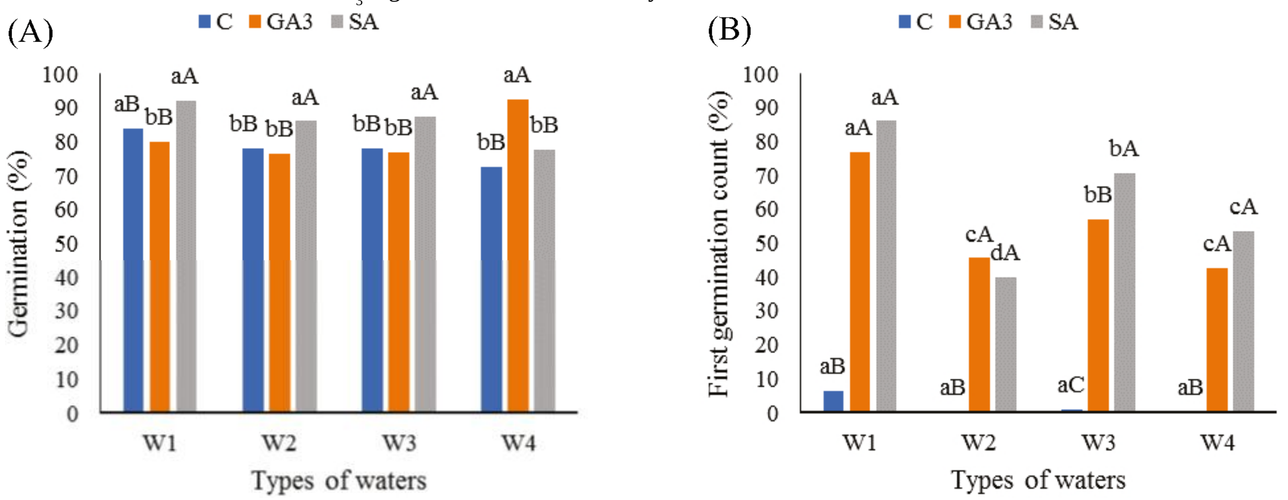
RESULTS AND DISCUSSION

The treatments of pumpkin seeds with plant regulators and different types of water had significant interaction ($p \leq 0.05$) for all variables, except for shoot length and root length.

Water salinity compromised seed germination; however, plant regulators, mainly salicylic acid (Figure 1A), favored germination in fish farming effluent (W_2) and artesian well water (W_3). In addition, in the supply water (W_1), this plant regulator promoted higher germination than the other treatments, control and gibberellic acid. Seeds treated with gibberellic acid and subjected to the mixture of types of water (W_4) had statistically superior results compared to those treated with salicylic acid for this same water. It was also verified that the seeds of the treatment without plant regulators under supply water had germination of 92%, which is approximately 9.5% higher than the values found in the other treatments.

The presence of salts in the solution can cause ionic toxicity, leading to delays in germination and mobilization of reserves, besides reducing the viability of seeds (NÓBREGA *et al*., 2020). Nevertheless, the treatment of seeds with salicylic acid increased germination by 10.2% in fish farming effluent and 12.8% in artesian well water when compared to the control (supply water). Similarly, salicylic acid (0.5 mM) promoted a 40% increase in the germination of *Vicia faba* L. seeds under salinity conditions (90 mM of NaCl) (ANAYA *et al*., 2018). Thus, the interaction between salicylic acid and salinity can induce the activation of stress resistance genes and promote greater germination (JINI; JOSEPH, 2017).

Figure 1 - Germination (A) and first germination count (B) of pumpkin seeds, cultivar ‘Baiana Tropical’, under salt stress. W_1 = 100% supply water; W_2 = 100% fish farming effluent; W_3 = 100% artesian well water; W_4 = mixture of 50% fish farming effluent + 50% artesian well water; C = control; GA_3 = gibberellic acid; SA = salicylic acid



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Gibberellic acid promoted a 27.6% increase in germination in diluted water (W_4). The present study also demonstrates that gibberellic acid has stress attenuating action, with benefits to seed germination, as emphasized by Khan *et al*. (2015).

The results of first germination count were better in seeds treated with plant regulators, regardless of the water used (Figure 1B). When the seeds were exposed to saline waters, there was a reduction in this variable; however, the treatment of seeds with gibberellic and salicylic acids resulted in statistically higher values compared to untreated ones. In fish farming effluent (W_2), the results were above 45.5% of normal seedlings with salicylic acid and 40% with gibberellic acid, compared to the treatment without plant regulator. Similar behavior was observed in artesian well water (W_3) and in the dilution of these two waters (W_4).

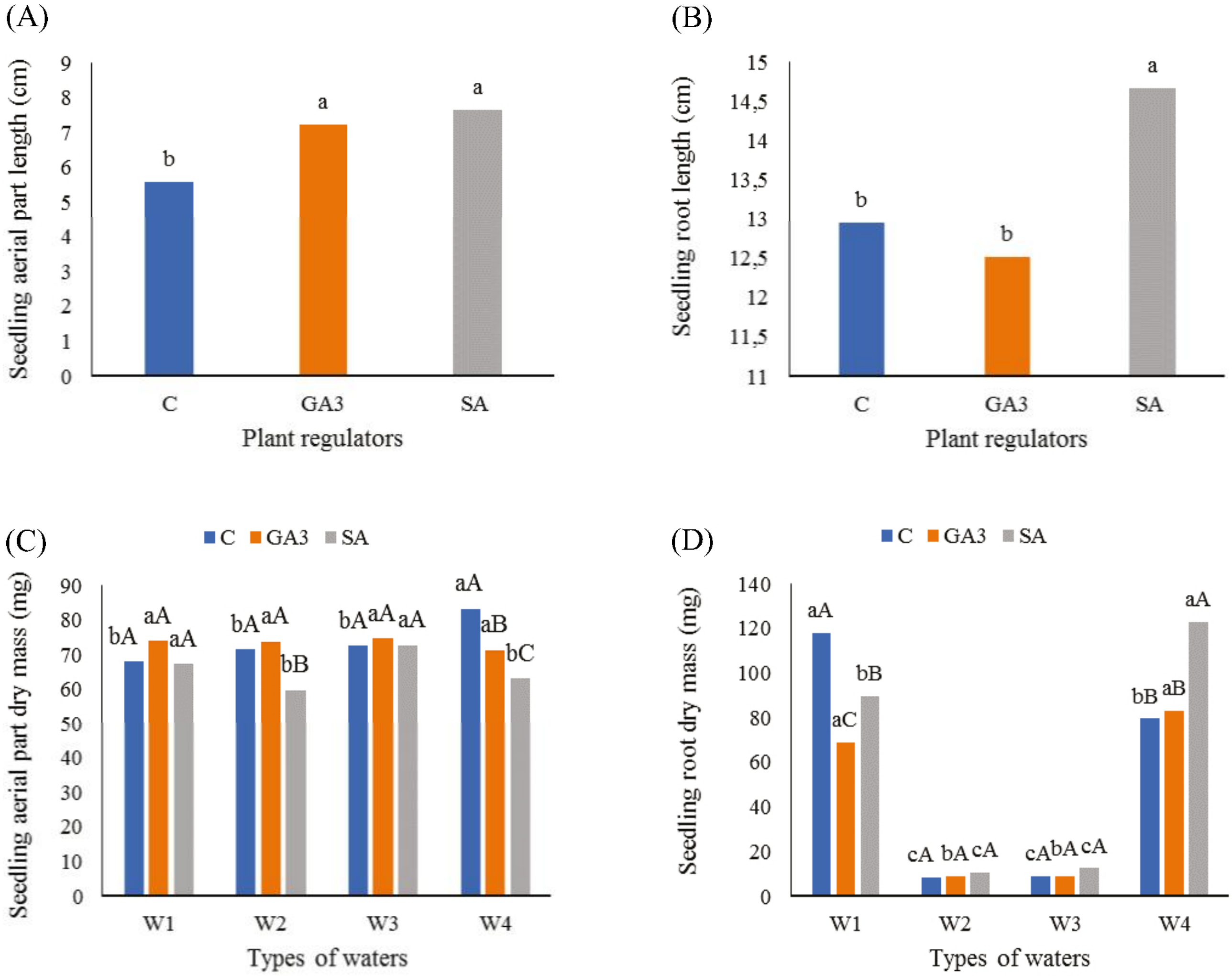
The results were quite significant when the seedlings were irrigated with supply water (W_1). In the treatments with plant regulators, there were 77 and 86% of normal seedlings, respectively. These results can be explained by the cell elongation and division functions of the regulators used, which stimulate the growth and establishment of seedlings (TSEGAY; ANDARGIE, 2018). Similarly, the treatment of seeds with regulators mitigated the effects of water salinity, promoting the occurrence of a greater number of normal seedlings in the first count.

For the variables of length in both shoots and roots, there was no significant interaction. However, there were single effects of the regulators on these variables. The treatment of seeds with gibberellic and salicylic acids promoted 28.9% and 36.6% higher shoot length compared to the control, respectively (Figure 2A). The beneficial effects of regulators were also verified in *Oryza sativa* L. seedlings under saline conditions, whose shoot length was higher with the application of gibberellin (CHUNTHABUREE *et al*., 2014). On the other hand, salicylic acid, for being involved in the stress defense mechanism, can influence the synthesis of gibberellins, hence leading to longer seedling lengths (TAIZ *et al*., 2017).

Salicylic acid proved to have greater action on root length, whose value was above 14 cm, resulting in growth up to 13.2% higher than that of the control (Figure 2B). On the other hand, in zucchini plants irrigated with saline water of 2.5 dS m^{-1}, this stress caused a negative effect on plant development, with a decrease of approximately 14% in root length (CANJÁ *et al*., 2021). Plant metabolism is more stimulated to overcome the condition of stressful environment (LICHTENTHALER *et al*., 2021). This behavior was verified in the present study, in which plant regulators acted together with the situation of salt stress because, as a response to adverse conditions, seedlings developed more their root system to find an appropriate environment for plant development.

The treatments of seeds with gibberellic acid and salicylic acid did not promote increments in shoot dry mass. On the other hand, salicylic acid hampered the shoot dry mass accumulation of seedlings grown from seeds treated with fish farming effluent (W_2) (Figure 2C). In general, salinity compromised root dry mass. In supply water (W_1), untreated seedlings accumulated more root dry mass than treated

Figure 2 - Shoot length (A), root length (B), shoot dry mass (C) and root dry mass (D) of pumpkin seeds, cultivar 'Baiana Tropical', under salt stress. W_1 = 100% supply water; W_2 = 100% fish farming effluent; W_3 = 100% artesian well water; W_4 = mixture of 50% fish farming effluent + 50% artesian well water; C = control; GA_3 = gibberellic acid; SA = salicylic acid



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seedlings, while in W_4, salicylic acid promoted higher root dry mass accumulations (Figure 2D). The use of salicylic acid in a stress situation promoted a 54.4% increase in root dry mass when compared to the control treatment. Likewise, salicylic acid promoted higher dry mass of *Solanum lycopersicum* L., *Cucumis melo* L. and *Vicia faba* L. seedlings under salinity conditions (ANAYA *et al.*, 2018; LOPES *et al*., 2017). This occurs because this plant regulator acts on physiological (germination and growth) and biochemical (synthesis of metabolites in response to stresses) processes in plants and can be used to improve their development under salinity conditions (RAFIQUE *et al*., 2011).

To overcome the condition of abiotic stress, seedlings shifted their metabolism of shoot developmental to root growth. In seeds that were not treated, the salinity of the waters did not interfere in the accumulation of total soluble sugars. However, the treatments with gibberellic acid and salicylic acid, when subjected to dilution (W_4), were superior to the control under the same condition, with accumulation of 19.5 μg g^{-1} FM and 22.4 μg g^{-1} FM, hence representing increments of 35.4% and 55.8%, respectively (Figure 3A).

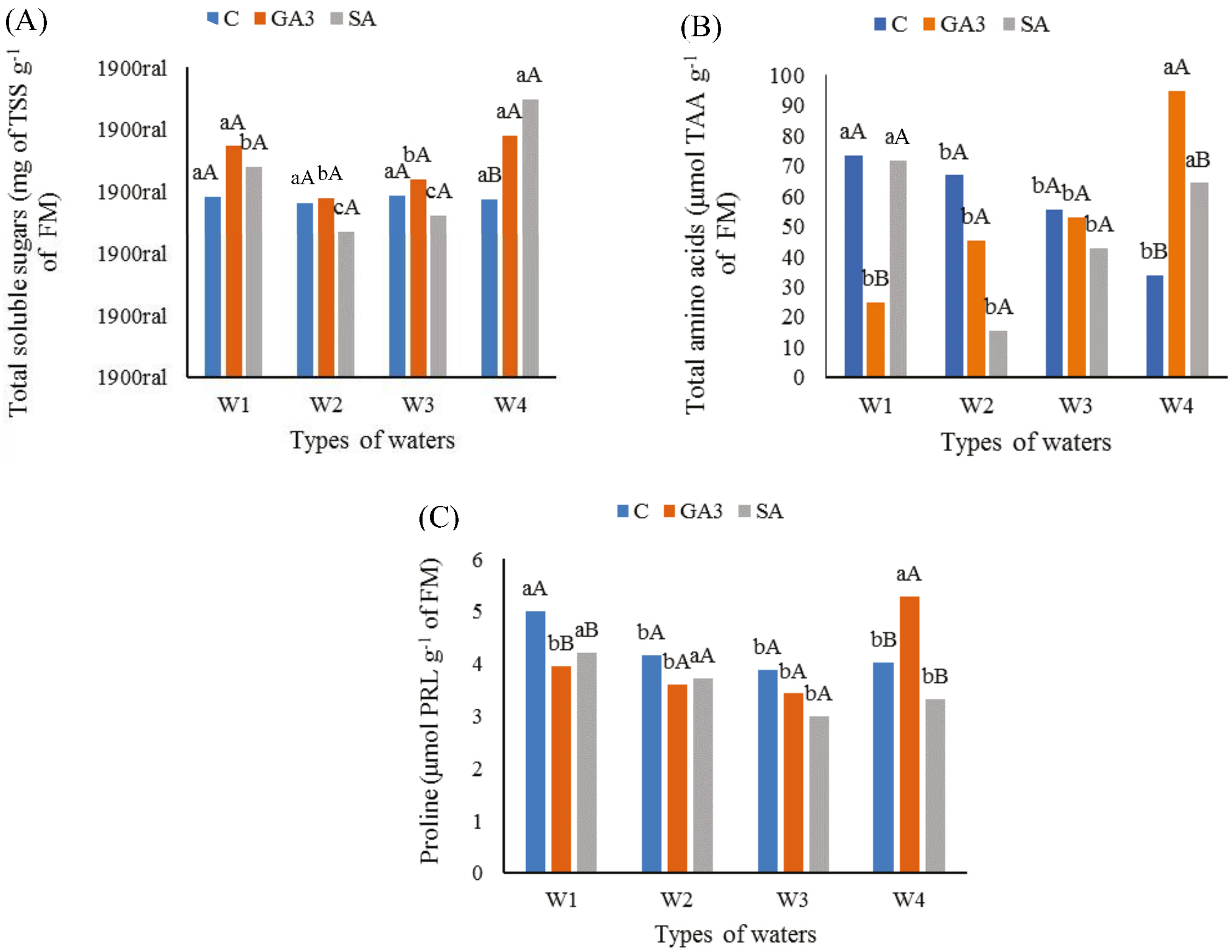
Regarding the total amino acid contents, the treatment of seeds with gibberellic acid promoted 2.8 times more accumulation in seedlings under diluted water (W_4) compared to the control (Figure 3B). In relation to the increase in the concentration of amino acids, Paiva *et al*. (2018) also found a similar response in *Salvia hispanica* L. seedlings under salinity conditions. However, in *Cucumis sativus* L. seedlings prepared from osmoprimed seeds, it was found that the amino acid content was increased when plants were subjected to fish farming effluent (EC = 5.5 dS m^{-1}), in which case osmotic adjustment is suggested (MATIAS *et al*., 2015). On the other hand, the treatment with fish farming effluent (W_2) of the present study showed no significant difference when compared to the control.

Thus, for the maintenance and performance of cellular functions, it can be understood that plants in general maintain balance through the accumulation of metabolites, allowing their development under conditions of abiotic stresses. This was not found partially in the present study, because pumpkin seedlings, cv. 'Baiana Tropical', were not able to perform osmotic adjustment in the waters from fish farming and artesian well. It is evident that in situations of restriction of water with more adequate quality for seedling production, one could use W_4 water, referring to the dilution of fish farming effluent and well water, combined with the treatment with plant regulators, without damage to germination and vigor.

In the evaluation of proline content, the gibberellic acid promoted 31% greater accumulation in seedlings maintained in fish farming effluent + artesian well water, when compared to the treatment without regulator for this same type of water (Figure 3C). The accumulation of this organic solute as osmoprotectant was also observed in seedlings of *Salvia hispanica* L. when exposed to salinity (PAIVA *et al*., 2018).

Faced with salt stress, plants develop adaptation strategies to maintain their growth metabolism. Among the strategies, the osmotic adjustment stands out, which consists of the accumulation of biomolecules such as

Figure 3 - Total soluble sugars (A), total amino acids (B) and proline (C) of pumpkin seeds, cultivar 'Baiana Tropical', under salt stress. W_1 = 100% supply water; W_2 = 100% fish farming effluent; W_3 = 100% artesian well water; W_4 = mixture of 50% fish farming effluent + 50% artesian well water; C = control; GA_3 = gibberellic acid; SA = salicylic acid



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sugars, proteins and amino acids in cells, to reduce the water potential inside them (NÓBREGA *et al*., 2020). From the results obtained and in view of plant metabolism in situations of abiotic stress, it can be verified that the use of gibberellic acid is efficient in mitigating salt stress in germination and that the use of salicylic acid is also beneficial to the initial development mainly of the root system under salt stress.

CONCLUSIONS

Treatment of pumpkin seeds, cv. 'Baiana Tropical', with plant regulators (gibberellic and salicylic acids) favors the germination and initial development of seedlings under conditions of salt stress.

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