

Original Article

# Pre-germination treatments of melon seeds for the production of seedlings irrigated with biosaline water

Tratamentos pré-germinativos de sementes de melão para a produção de mudas irrigadas com água biossalina

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#### **Abstract**

Melon production in the Brazilian semi-arid region is subject to the use of marginal waters with high salinity. However, the use of regulators and bioactivators in seed treatment can mitigate the harmful effects of salts in irrigation water. In this context, the objective was to evaluate the effect of pre-germination treatments with plant regulators and bioactivator in melon seeds for the production of seedlings irrigated with biosaline water from fish farming effluent. For this, two trials with the Goldex and Grand Prix hybrids were carried out separately. A completely randomized design was used in a  $4 \times 3$  factorial scheme (pre-germination treatments  $\times$  water dilutions). In addition to the control, the seeds were treated with salicylic and gibberellic acids and thiamethoxam. The waters used for irrigation were local-supply water, fish farming effluent (biosaline water) and these diluted to 50%. Physiological and biochemical analyses were performed for fourteen days. Biosaline water ( $5.0 \, \text{dS m}^{-1}$ ) did not affect the emergence of Goldex melon seedlings, but compromised the establishment of the Grand Prix cultivar. Seed pre-treatments with salicylic and gibberellic acids attenuate the effects of water salinity and promote growth modulations, resulting in more vigorous melon seedlings.

Keywords: Cucumis melo L., gibberellic acid, salicylic acid, biosaline water, thiamethoxam.

#### Resumo

A produção de meloeiro no semiárido brasileiro está sujeita a utilização de águas marginais com salinidade elevada. Entretanto, a utilização de reguladores e bioativadores no tratamento de sementes podem mitigar os efeitos nocivos dos sais na água de irrigação. Nesse sentido, objetivou-se avaliar o efeito de tratamentos pré-germinativos com fitorreguladores e bioativador em sementes de melão para a produção de mudas irrigadas com água biossalina de efluente de piscicultura. Para isso, dois ensaios com os híbridos Goldex e Grand Prix foram realizados separadamente. Utilizou-se delineamento inteiramente casualizado em esquema fatorial 4 × 3 (tratamentos pré-germinativos × diluições de água). Além do controle, as sementes foram tratadas com os ácidos salicílico e giberélico, e tiametoxam. As águas utilizadas para irrigação foram a de abastecimento local, efluente de piscicultura (água biossalina) e estas diluídas a 50%. Durante quatorze dias foram realizadas as análises fisiológicas e bioquímicas. A água biossalina (5,0 dS m-1) não afetou a emergência de plântulas de meloeiro Goldex, mas prejudicou o estabelecimento da cultivar Grand Prix. Os pré-tratamentos de sementes com os ácidos salicílico e giberélico atenuam os efeitos da salinidade da água e promovem modulações no crescimento, proporcionando mudas de meloeiro mais vigorosas.

Palavras-chave: Cucumis melo L., ácido giberélico, ácido salicílico, água biossalina, tiametoxam.

#### 1. Introduction

Melon (*Cucumis melo* L.) has an estimated world production of 31 million tons and a cultivated area of approximately 1.3 million hectares (FAO, 2018). In Brazil, the states of Bahia, Ceará and Rio Grande do Norte are the main producers, with 80% of the cultivated area of the latter

two destined for export (Kist et al., 2021). These producing states are located in the semi-arid region, whose crops are under high water demand due to the high temperatures and evapotranspiration rates, climatic characteristics that are inherent to this region (Bezerra et al., 2020).

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The availability of good quality water for agricultural production is considerably reduced in the Brazilian semiarid region. Thus, it is common to use low quality water, usually with high electrical conductivity, and wastewater from animal husbandry is often used for this purpose (Souza et al., 2019). However, one of the problems of this type of water is the large amount of salts, especially for those from fish farming, which cause damage to plant growth and development (Silva et al., 2015). Despite that, when properly managed, wastewater from fish farming (biosaline water) qualifies as an alternative source for the irrigation of agricultural crops, ensuring water savings in the production (Dantas et al., 2019).

Using biosaline water can creates obstacles in the initial stage of the crop, especially during germination and seedling establishment. This stage is considered critical to the crop cycle, and certain stresses, such as salt stress, can cause irreversible damage to plant growth (Zhang et al., 2017). However, the use of plant regulators such as gibberellin and salicylic acid in seed treatment can attenuate the negative effects of this stress because of their positive action on plant metabolism (Anaya et al., 2018; Ribeiro et al., 2020). Beneficial effects promoted by these regulators have been verified in the treatment of onion and pumpkin seeds under salinity conditions (Silva et al., 2019; Guirra et al., 2020).

Another product that has been used in the mitigation of abiotic stresses is thiamethoxam. It is a systemic insecticide that, when applied through seed treatment, stimulates germination and promotes maximum expression of seedling vigor (Almeida et al., 2014). The use of this product promoted a physiological stimulus in stored soybean seeds (Camilo and Lazaretti, 2020). Under water stress, thiamethoxam favored the germination of millet seeds up to -0.2 MPa (Cazarim et al., 2021).

In this context, the objective was to evaluate the effect of pre-germination treatments with plant regulators and bioactivator on melon seeds aiming at the production of seedlings irrigated with biosaline water from fish farming effluent.

## 2. Material and Methods

The study was conducted in two essays, separated by the melon hybrids Goldex (yellow) and Grand Prix (Piel de sapo), in greenhouse and laboratory environments at the Federal Rural University of the Semi-Arid Region (UFERSA), Mossoró, RN, Brazil. The average external temperature of these environments was 24.5 °C, with no occurrence of rainfall (INMET, 2019).

The design used was completely randomized, in a  $4\times3$  factorial scheme, corresponding to four pregermination treatments (control, salicylic acid, gibberellic acid and thiamethoxam) and three water dilutions (supply water, 50% dilution of biosaline water and biosaline water), with four replicates of 25 seeds, for each hybrid. Seeds with moisture contents of 8% (Goldex) and 8.5% (Grand Prix) remained stored in an air-conditioned environment (± 15 °C and 60% relative humidity) for six months until the beginning of the experiment.

The seeds were treated with salicylic acid (P.M. = 138.1 M) and gibberellic acid (ProGibb 400®), in addition to thiamethoxam (Cruiser 350® from Syngenta®). Preliminary tests were conducted to determine the appropriate dose for each product. Thus, the solutions of salicylic acid (50 μM) and gibberellic acid (50 mg L<sup>-1</sup>) to hydrate the paper towel substrate had the volume of twice the dry weight of the substrate. The seeds were arranged in a paper towel roll for a period of 20 h in a germination chamber, at 25 °C. This period was based on the information obtained during the seed imbibition curve. In the treatment of seeds with thiamethoxam, a dose of 1 mL of Cruiser 350® per kilogram of seed was used. For this, a mixture with 1 mL of the product (Cruiser 350®) diluted in 8 mL of distilled water was prepared. The amount of mixture used was 1 mL, sufficient to fully cover the seeds. This mixture was kept in contact with the seeds for 30 min in order to promote greater adhesion of the product to their surface, according to the recommendations of the manufacturer (Syngenta®). The seeds used in the control were not treated.

Sowing was performed in polystyrene trays with 200 cells (18 cm³) filled with the commercial substrate Carolina Soil®. Daily irrigation was fixed at 2.5 L of the waters evaluated per tray. These were randomly arranged in a greenhouse for 14 days, with average temperature and relative humidity of 28 °C and 55%, respectively.

After sowing, the trays were irrigated with urban supply water (W1), dilution of 50% supply water + 50% biosaline water (W2) and biosaline water (W3) – fish-farming effluent from tilapia (*Oreochromis* spp.) production tanks. Chemical analyses of the waters were carried out at the Soil and Water Laboratory of UFERSA (Table 1).

**Table 1.** Cation and anion contents, acidity, electrical conductivity, sodium adsorption ratio and classification of the waters used to irrigate melon (*Cucumis melo* L.) seedlings in a greenhouse.

Water	Ca <sup>2+</sup>	Mg <sup>2+</sup>	Na⁺	K+	CO <sub>3</sub> <sup>2-</sup>	HCO <sup>3</sup> .	Cl-	EC- 25 °C	nII.	SAR*	WOS**
dilutions				mmol <sub>c</sub>	dS m <sup>-1</sup>	- pH	SAK	WQS			
W1	0.9	0.4	3.4	0	0.6	3	3	0.55	8	4.2	C2S1
W2	10.8	10.9	1.3	0	1.2	3.6	5	3.18	8	0.4	C4S1
W3	15.7	20.1	30	1	0.6	3.4	42	5.97	8	7.3	C4S2

EC = electric conductivity. W1 = supply water. W2 = 50% supply water + 50% fish-farming effluent. W3 = fish-farming effluent. \*Sodium adsorption ratio; \*\*Water quality classification.

During the evaluation period, daily counts of emerged seedlings (exposed hypocotyl) were performed until 14 days after sowing. With this, the emergence speed index (Maguire, 1962) and the percentage of emerged seedlings were obtained.

Shoot length measurement was taken considering the region between the insertion of the root and that of the cotyledons, while root length was measured considering the main root. This evaluation was performed in 10 seedlings, per replicate, at 14 days after sowing, using a millimeter ruler.

The plant parts used to evaluate seedling length were placed in a paper bag and kept in a forced air circulation oven at 65 °C for 72 h to determine the dry mass on a precision scale (0.001g) (Krzyzanowski et al., 2020).

Total soluble sugars, total amino acids and proline were quantified in 0.2 g samples of fresh tissue from the shoots of 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 °C, and the supernatant was collected. Total soluble sugars were determined by the anthrone method (Yemm and Willis, 1954), with results expressed in mg g<sup>-1</sup> of fresh mass. Total amino acids were analyzed by the ninhydrin method (Yemm et al., 1955), and the results were expressed in µmol g<sup>-1</sup> of fresh mass. Proline was determined using the methodology proposed

by Bates et al. (1973), with the results expressed in  $\mu$ mol g<sup>-1</sup> of fresh mass.

The collected data were subjected to analysis of variance ( $p \le 0.05$ ), and, in case of significance, the means were subjected to the Scott-Knott test. The statistical program used was the System for Variance Analysis - SISVAR® (Ferreira, 2011).

### 3. Results and Discussion

The results of the Goldex hybrid showed effects of the interaction between the factors on all variables, except for total amino acids and proline, with difference only for the water dilution factor. For Grand Prix, there were differences caused by the single factors in seedling emergence and emergence speed index. The variables shoot dry mass and proline of this hybrid had differences caused only by the water dilutions, while for the others there were interactions between the factors. The contents of total soluble sugars did not differ between treatments for both hybrids (Table 2).

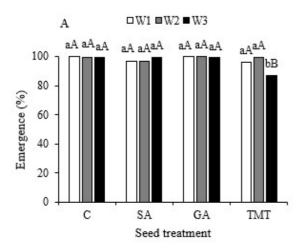
The emergence of Goldex hybrid seedlings, whose seeds were treated with thiamethoxam, was affected only by the biosaline water (W3), with higher salt concentration (87%) (Figure 1A). However, with the use of fish-farming effluent, the emergence speed index was compromised, regardless of the seed treatment, with a 25% reduction for seeds treated with thiamethoxam, in comparison to the

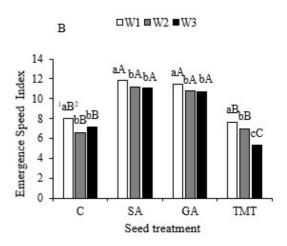
**Table 2.** Summary of the analysis of variance for the variables percentage of emergence (EP), emergence speed index (ESI), total shoot and root length (SL and RL), shoot and root dry mass (SDM and RDM), total soluble sugars (TSS), total amino acids (TAA) and proline (PRO) of melon seedlings (*Cucumis melo* L.) submitted to seed treatment with regulators and bioactivators (products) and irrigated with different water dilutions.

Goldex									
Degrees of freedom	Pr>Fc								
	ESI	EP	SL	RL	SDM	RDM	TSS	TAA	PRO
2	0.000**	0.052ns	0.000**	0.000**	0.000**	0.000**	0.395ns	0.004**	0.008**
3	0.000**	0.005**	0.000**	0.036*	0.000**	0.029*	0.380 <sup>ns</sup>	0.346ns	0.641 ns
6	0.000**	0.002**	0.004**	0.004**	0.036*	0.000**	0.445ns	0.824ns	0.600ns
36									
	4.21	3.26	5.08	7.14	8.33	20.98	4.15	15.77	6.23
	of freedom 2 3 6	of freedom ESI  2 0.000**  3 0.000**  6 0.000**  36	of freedom         ESI         EP           2         0.000**         0.052ns           3         0.000**         0.005**           6         0.000**         0.002**           36	Degrees of freedom         ESI         EP         SL           2         0.000**         0.052**         0.000**           3         0.000**         0.005**         0.000**           6         0.000**         0.002**         0.004**           36	Degrees of freedom         ESI         EP         SL         RL           2         0.000**         0.052ns         0.000**         0.000**           3         0.000**         0.005**         0.000**         0.036*           6         0.000**         0.002**         0.004**         0.004**           36	Degrees of freedom         ESI         EP         SL         RL         SDM           2         0.000**         0.052ns         0.000**         0.000**         0.000**           3         0.000**         0.005**         0.000**         0.036*         0.000**           6         0.000**         0.002**         0.004**         0.004**         0.036*         0.036*           36	Degrees of freedom         ESI         EP         SL         RL         SDM         RDM           2         0.000**         0.052ns         0.000**         0.000**         0.000**         0.000**         0.000**           3         0.000**         0.005**         0.000**         0.036*         0.000**         0.029*           6         0.000**         0.002**         0.004**         0.004**         0.036*         0.036*         0.000**           36	Degrees of freedom         ESI         EP         SL         RL         SDM         RDM         TSS           2         0.000**         0.052ns         0.000**         0.000**         0.000**         0.000**         0.395ns           3         0.000**         0.005**         0.000**         0.036*         0.000**         0.029*         0.380ns           6         0.000**         0.002**         0.004**         0.004**         0.036*         0.000**         0.445ns           36	Degrees of freedom         ESI         EP         SL         RL         SDM         RDM         TSS         TAA           2         0.000**         0.052ns         0.000**         0.000**         0.000**         0.000**         0.395ns         0.004**           3         0.000**         0.005**         0.000**         0.000**         0.029*         0.380ns         0.346ns           6         0.000**         0.002**         0.004**         0.004**         0.036*         0.000**         0.445ns         0.824ns           36

Grand Prix											
Sources of variation	Degrees of freedom	Pr>Fc									
		ESI	EP	SL	RL	SDM	RDM	TSS	TAA	PRO	
Water (W)	2	0.003**	0.229ns	0.000**	0.000**	0.000**	0.000**	0.135 <sup>ns</sup>	0.002**	0.039*	
Product (P)	3	0.000**	0.048*	0.117 <sup>ns</sup>	0.000**	0.035*	0.008**	0.502ns	0.001**	0.380ns	
$W \times P$	6	0.847 <sup>ns</sup>	0.693ns	0.007**	0.000**	0.175 <sup>ns</sup>	0.001**	0.406 <sup>ns</sup>	0.015*	0.574 <sup>ns</sup>	
Error	36										
Coeficient of variation %		9.68	7.79	7.19	9.27	17.09	7.94	14.2	22.34	9.8	

<sup>\*</sup>Significant at 5%. \*\*Significant at 1%.





**Figure 1.** Emergence (A) and emergence speed index (B) of melon seedlings. Goldex hybrid. grown from seeds treated and irrigated with different dilutions of biosaline water. C = control; SA = salicylic acid; GA = gibberellic acid; TMT = thiamethoxam. C = control; CA = salicylic acid; CA = gibberellic acid;  $CA = \text{gibberellic a$ 

other waters. Gibberellic and salicylic acids were beneficial to melon seedlings compared to the other treatments (10.7 and 11.04 respectively), with a 40% increase in the emergence speed index, compared to the control, regardless of the water used (Figure 1B).

The emergence of Grand Prix melon seedlings grown from seeds treated with gibberellin and salicylic acids was superior to those of the other treatments, with values above 96%, regardless of the water used (Figures 2A and B). Biosaline water reduced the emergence speed index of the seedlings by 8% compared to supply water (Figure 2C). However, treatment of seeds with gibberellic and salicylic acids resulted in a higher emergence speed index (9,5 and 10 respectively), which was 35% higher than those of the control and of seeds treated with thiamethoxam (Figure 2D).

Biosaline water (W3) of high electrical conductivity (Table 1) did not affect the emergence of melon seedlings for both hybrids (Figures 1 and 2). Possibly, it was due to the organic load present in this type of water from fish waste, in addition to the contents of  $Ca^{2+}$  and  $Mg^{2+}$ , important elements for plant nutrition (Souza et al., 2019). Results similar to those of this study have been verified in watermelon (Silva et al., 2015) and pumpkin (Guirra et al., 2020), using biosaline water from fish farming with approximately 5.0 dS m<sup>-1</sup>.

Regarding the beneficial effect of seed treatment with salicylic acid, the highest values of germination and germination speed index were observed in sesame seeds (10  $\mu$ M), even under water stress condition (-0.4 MPa) (Silva et al., 2017). Similarly, gibberellic acid resulted in positive effects for beet seeds subjected to salinity (Kandil et al., 2014). These results demonstrate the beneficial action of these attenuators in reducing the negative effects of abiotic stresses during seedling emergence. This fact may be related to the mode of action

of gibberellic acid, which acts as initiator of germination and mobilization of reserves (Tsegay and Andargie, 2018). In addition, gibberellins in the seeds may interact with salicylic acid, which is involved in the plant's defense system, both against herbivory and abiotic stresses (Nóbrega et al., 2020).

The shoot lengths of Goldex and Grand Prix melon seedlings were negatively affected by salinity. The use of biosaline water led to a reduction in the results of this variable in Goldex, regardless of seed treatment (Figure 3A). However, the seeds of this hybrid, treated with salicylic acid and thiamethoxam and irrigated with biosaline water (7.14 and 7.21 cm respectively), resulted in greater shoot length compared to those under the other treatments for the same water.

The treatment of Goldex seeds with salicylic acid and irrigation with 50% urban supply water plus 50% fish-farming effluent (9.41 cm) promoted a shoot length 15% higher than that obtained in the control (Figure 3A). The treatment of seeds with salicylic acid proved to be even more beneficial for shoot length in seedlings irrigated with supply water than the other regulators.

Regarding the root length of the Goldex hybrid, it was found that seedlings from the control treatment and those under salicylic acid, irrigated with dilution of the waters (W2), had shorter roots (10 and 9 cm respectively), compared to those under the other waters (Figure 3B). In the treatments with gibberellic acid and thiamethoxam, there were no differences in relation to the waters used. In addition, when water dilution was used, these treatments promoted higher root length compared to the others.

Regarding the Grand Prix hybrid, it was found that the treatment of seeds with salicylic acid and irrigation with W2 water (7.89 cm) resulted in shoot length similar to that obtained with supply water (Figure 3C). In the other treatments, there was a reduction in the shoots with

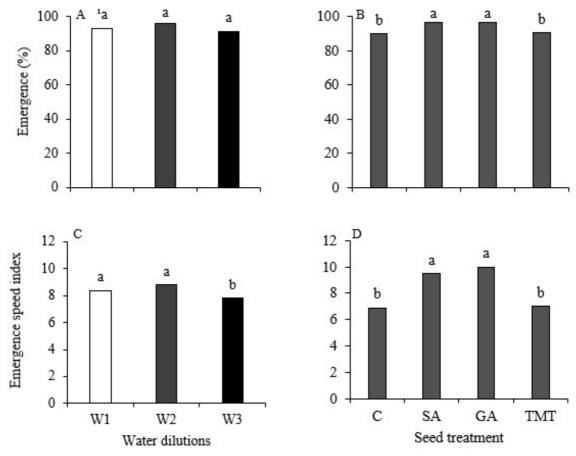


Figure 2. Emergence (A and B) and emergence speed index (C and D) of melon seedlings. Grand Prix hybrid. grown from seeds treated and irrigated with different water dilutions. C = control; SA = salicylic acid; GA = gibberellic acid; TMT = thiamethoxam. W1 = urban supply water; W2 = 50% urban supply water + 50% fish-farming effluent; W3 = fish-farming effluent.  $^{1}$ Means followed by the same letter do not differ by the Scott-Knott test (p  $\leq$  0.05).

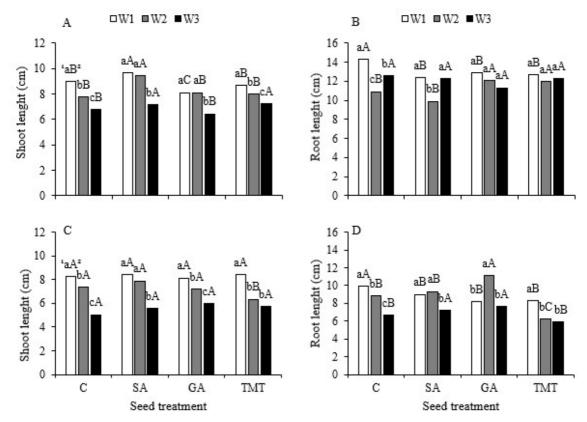
increased concentration of effluent water. Root length was also affected by water salinity in seedlings of the Grand Prix hybrid. Nevertheless, the treatment with gibberellic acid, irrigated with dilution of the waters (W2), promoted a better result compared to the other products (11.12 cm), with an increase of 20% in comparison to the control treatment, for the same water. In addition, it led to the highest root lengths among the other waters (Figure 3D).

The results of seedling length show that melon is more sensitive to water salinity than other cucurbits. In pumpkin and watermelon, even with electrical conductivity levels similar to those of this study (approximately 5.0 dS m<sup>-1</sup>), shoot length was not hampered, and results were similar to those of seedlings irrigated with supply water (0.5 dS m<sup>-1</sup>) (Silva et al., 2015; Guirra et al., 2020).

Even under salinity conditions, the use of salicylic and gibberellic acids promoted greater seedling length. Salicylic acid was responsible for the 25% increase of shoots in Goldex seedlings compared to the control. When the gibberellin-based regulator was used, it promoted an 18% increase of root length in the Grand Prix seedlings, compared to the control. These results can be explained

by the mode of action of gibberellins in cell elongation. In addition, salicylic acid may interact with gibberellins, stimulating the synthesis of this hormone even in stress situations (Nóbrega et al., 2020), which suggests a compartmentalization of defense mechanisms, that is, roots are able to activate specific mechanisms in response to the action of attenuators (Chuberre et al., 2018). These can also cause transcriptional changes and promote modulation of responses in the roots, because with the increase of peroxide there is an imbalance of NDH oxidase and, consequently, increments of H<sub>2</sub>O<sub>2</sub>, stiffening the roots and promoting negative effects on plant development. Thus, the direct action of the elicitor in the root system has specific immunological responses, such as the activation of non-host resistance (Jones and Dangl, 2006; Bigeard et al., 2015; Chuberre et al., 2018).

The action of salicylic acid, as stress attenuator, was also verified in barley seedlings subjected to water stress (Habibi, 2012). Similarly, the action of gibberellic acid in rice seedlings under salinity resulted in 25% root growth compared to the control (Chunthaburee et al., 2014). These results confirm the attenuating action of these



**Figure 3.** Shoot length (A and C) and root length (B and D) of melon seedlings. Goldex hybrid (A and B) and Grand Prix hybrid (C and D). grown from seeds treated and irrigated with different water. dilutions. C = control; SA = salicylic acid; GA = gibberellic acid; TMT = thiamethoxam. W1 = urban supply water; W2 = 50% urban supply water + 50% fish-farming effluent; W3 = fish-farming effluent. <sup>1</sup>Means followed by the same lowercase letter do not differ for water dilutions by the Scott-Knott test ( $p \le 0.05$ ). <sup>2</sup>Means followed by the same uppercase letter do not differ for seed treatments by the Scott-Knott test ( $p \le 0.05$ ).

regulators under salt stress for this study. Pumpkin root length, regardless of the use of plant regulators, was higher under salinity conditions, highlighting that this species performs morphological changes for survival in saline medium (Guirra et al., 2020). For melon, this morphological adaptation was observed in the present study, induced by the gibberellic acid when the crop was irrigated with the dilution of the waters (Figure 3D).

In the results of shoot dry mass of Goldex melon seedlings, a similar trend was verified for the shoot length of this same hybrid, and salicylic acid promoted better results (100 mg) (Figure 4A). The use of biosaline water (W3) was harmful for root dry mass accumulation in all treatments.

The dilution of the waters (W2) resulted in root dry mass similar to those obtained in seedlings irrigated with supply water, resulting from the control and the treatments salicylic acid and gibberellic acid. This same water and seeds treated with salicylic acid and thiamethoxam (35.4 and 41.5 mg respectively) promoted a 33% increase of root dry mass compared to the control (Figure 4B). The dry mass accumulation response may be related to the increase of secondary roots to the detriment of the elongation of the main root, allowing the expansion of

the root absorption area of the seedling. This response of alteration of root architecture is due to stress signaling, generating modifications to increase root absorption (Silva and Delatorre, 2009).

Salinity significantly affected the shoot dry mass of Grand Prix melon seedlings irrigated with biosaline water (71.7 mg), showing a reduction of 50% compared to the control treatment (Figure 4C). The highest root dry mass accumulation was obtained for seedlings grown from seeds treated with salicylic acid and irrigated with W2 water (74.3 mg). This result was superior to those obtained among the waters, as well as for all other treatments (Figure 4D).

The results show that salinity affects the development of plant tissues due to the toxic and osmotic effects caused by the ions present in the solution. Thus, cell division and elongation are impaired, preventing growth and greater accumulations of plant biomass. A similar effect was also observed in watermelon, whose seedling dry mass was reduced with increasing salinity of irrigation waters (Silva et al., 2015; Nóbrega et al., 2020). On the other hand, the mode of action of salicylic acid stands out, because it is related to secondary plant metabolism and the production of metabolites that help in mitigating the osmotic effects caused by the stresses. This regulator

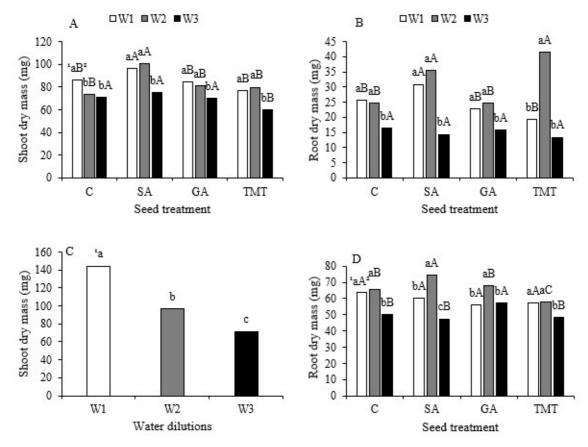


Figure 4. Shoot dry mass (A and C) and root dry mass (B and D) of melon seedlings. Goldex hybrid (A and B) and Grand Prix hybrid (C and D). grown from seeds treated and irrigated with different water dilutions. C = control; SA = salicylic acid; GA = gibberellic acid

promoted a greater amount of dry mass in barley and pepper seedlings under water stress conditions (Habibi, 2012; Prabha and Negi, 2014).

Regarding biochemical evaluations, the melon hybrids Goldex and Grand Prix showed different metabolic responses to stress, evidencing important intraspecific variations in mechanisms of defense against the effects of salinity. For the maintenance of the osmotic balance, plants develop protective mechanisms for osmoregulation through the accumulation of osmolytes, such as proline, soluble sugars and amino acids, when subjected to stress (Silva et al., 2019).

For the analysis of metabolites in Goldex melon seedlings, it was found that there was no interaction between the factors. However, the waters used resulted in significant differences for total amino acids and proline in seedlings of this hybrid. The use of biosaline water (W3) promoted greater accumulation of total amino acids (0.38  $\mu$ mol g<sup>-1</sup> FM) in comparison to that obtained with the other waters (Figure 5A), while the highest proline accumulation occurred in seedlings irrigated with supply water (0.06  $\mu$ mol g<sup>-1</sup> FM) (Figure 5B).

The results show that other amino acids involved in osmotic adjustment were more accumulated for maintaining the water potential in leaf tissue rather than proline in Goldex hybrid seedlings. Although proline is considered the main metabolite involved in osmotic adjustment, under conditions of salt and water stresses, there are others responsible, such as glycine, betaine, mannitol, trehalose sugar and, for some cucurbits, citrulline (Kusvuran et al., 2013; Song et al., 2020). Moreover, the increase in proline concentration may not be directly related to tolerance, but to the product of metabolic disorders caused by stress, whose synthesis still depends on the gene expression of the material (Silveira et al., 2016). Other factors that may be involved in the synthesis of this metabolite are glutamate pathways. Toxic ammonia content in irrigation water can interfere with proline synthesis, increasing free amino acids and decreasing proline levels (Nelson and Cox, 2019).

Grand Prix melon seedlings grown from seeds treated with thiamethoxam and irrigated with saline waters (W2 and W3) had higher concentrations of total amino acids compared to those of the other treatments. The highest

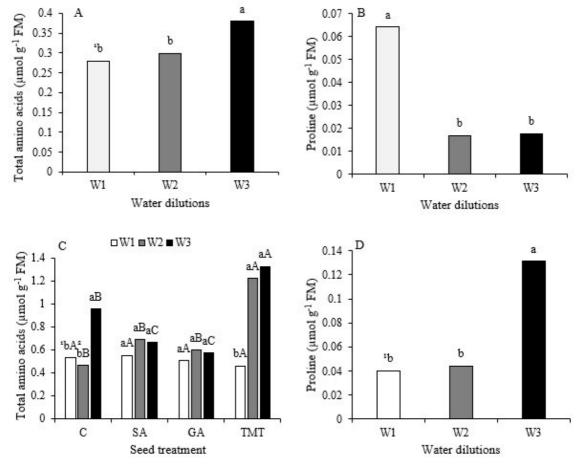


Figure 5. Total amino acids (A and C) and proline (B and D) of melon seedlings. Goldex hybrid (A and B) and Grand Prix hybrid (C and D), grown from seeds treated and irrigated with different water dilutions. C = control; SA = salicylic acid; GA = gibberellic acid; TMT = thiamethoxam. W1 = urban supply water; W2 = 50% urban supply water + 50% fish-farming effluent; W3 = fish-farming effluent. W3 = fish-farming effluent when W3 = fish-farming effluent is W3 = fish-farming effluent. W3 = fish-farming effluent is W3 = fish-farming effluent. W3 = fish-farming effluent is W3 = fish-farming effluent. W3 = fish-farming effluent is W3 = fish-farming effluent. W3 = fish-farming effluent is W3 = fish-farming effluent. W3 = fish-farming effluent is W3 = fish-farming effluent. W3 = fish-farming effluent is W3 = fish-farming effluent. W3 = fish-farming effluent is W3 = fish-farming effluent. W3 = fish-farming effluent is W3 = fish-farming effluent. W3 = fish-farming effluent is W3 = fish-farming effluent. W3 = fish-farming effluent is W3 = fish-farming effluent. W3 = fish-farming effluent is W3 = fish-farming effluent. W3 = fish-farming effluent is W3 = fish-farming effluent. W3 = fish-farming effluent is W3 = fish-farming effluent. W3 = fish-farming effluent is W3 = fish-farming effluent.

accumulation of total amino acids was obtained in seedlings irrigated with biosaline water (1.32 µmol g<sup>-1</sup> FM) (Figure 5C). For proline concentration, there was a difference only for the water factor, with higher accumulation in seedlings irrigated with biosaline water (0.13  $\mu$ mol g<sup>-1</sup> FM) (Figure 5D). In this context, the increase in amino acid content in cucumber seedlings irrigated with biosaline water was indicative of osmotic adjustment (Matias et al., 2015). Total amino acids and proline act directly in the osmotic adjustment process, mainly under salinity conditions. Thus, there was an increase in the accumulation of total amino acids and proline in pumpkin seedlings (Guirra et al., 2020), under conditions similar to those of the present study. Thus, it is evident that the quality of irrigation water influences the production and vegetative development of seedlings, determining the agricultural activity.

Thiamethoxam promoted higher levels of total amino acids in Grand Prix seedlings, but was not efficient in obtaining vigorous melon seedlings. However, it is understood that the use of plant regulators such as salicylic

and gibberellic acids in the treatment of melon seeds may promote greater tolerance to salinity in the initial stage. In addition, when fish-farming effluent is used as an alternative source of irrigation, the morphological responses of melon seedlings were promising.

This research study suggests a continuous discussion about the use of biosaline water and its possible uses in plant production. As noted, electrical conductivity of up to 3.5 dS m<sup>-1</sup> does not compromise the initial growth of Goldex and Grand Prix hybrid melon seedlings. In addition, seed treatments with salicylic and gibberellic acids promote the attenuation of salinity effects on melon seedlings of both cultivars.

The use of different waters satisfactorily responds to sustainable production and environmental preservation, because they lead to their reuse in irrigation through the use of fish-farming effluent, which is currently disposed of inadequately and without any utilization. Thus, adequate planning and management of the use of non-potable sources of water can be a low-cost alternative for the

production of melon seedlings from seeds treated with these plant regulators.

So it is concluded that fish-farming water (5.0 dS m<sup>-1</sup>) does not affect the emergence of Goldex melon seedlings, but compromises the establishment of seedlings of the Grand Prix cultivar. Pre-treatments of seeds with salicylic and gibberellic acids attenuate the effects of salinity of biosaline water and promote growth modulations, resulting in more vigorous melon seedlings. Treatment of seeds with thiamethoxam results in the accumulation of osmoprotectant metabolites, but is not able to mitigate the effects of salinity on melon seedlings of the Goldex and Grand Prix hybrids

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