

Effect of salicylic acid on the physiological quality of salt-stressed *Cucumis melo* seeds

ABSTRACT

Aim: The aim of this study was to evaluate the physiological quality of seeds of two melon varieties (*Cucumis melo*) in response to saline stress and salicylic acid treatment.

Study design: The experimental design was a completely randomized design with five different water salinities and five doses of salicylic acid, with four replicates of 50 seeds.

Place and Duration of Study: The experiment was carried out in the Laboratory of Seed Analysis, located in the Center of Agricultural Sciences of the Federal University of Paraíba, Areia-PB, Brazil.

Methodology: The experiment was conducted with two varieties of melon (Cantalupensis and Eldorado 300). Creole melon seeds (cucumber melon - Cantalupensis) were extracted from completely mature fruits produced in a farmland located in Santa Luzia, PB, Brazil. The Eldorado 300 seeds were purchased in the local market of Areia, PB, Brazil. A completely randomized experimental design was used with five water salinities ($EC_w = 0.0, 1.74, 6.0, 10.26$ and 12.0 dS m^{-1}) and five salicylic acid doses ($SA = 0.0, 0.29, 1.0, 1.71$ and 2.0 mM), with four replicates of 50 seeds. The following variables were evaluated: percentage of germination, first germination count, percentage of abnormal seedlings, germination speed index, mean germination time, seedlings dry mass and the root, shoot and seedling length.

Results: Salicylic acid treatment was not effective in attenuating the harmful effects of saline stress on seed germination. Cantalupensis cultivar is more tolerant than the Eldorado 300 cultivar.

Conclusion: Saline stress has a negative effect on the germination and vigor of melon seeds. Salicylic acid was not effective in attenuate the deleterious effects promoted by water salinity.

Keywords: Melon; mitigation; salinity; seed vigor.

1. INTRODUCTION

Melon (*Cucumis melo* L.) is one of the main vegetable crops cultivated in the Brazilian northeast region. The species presents high economic value and adaptability to semi-arid conditions. Despite the melon's adaptability to grow in semi-arid conditions, the use of irrigation with saline water is a major threat to its cultivation [1]. In order to mitigate the harmful effects of soil salinity in lands irrigated with saline water, adequate water management and use of technologies that increase salt tolerance on plants are needed [2].

All stages of plant development are affected by salinity and most crops present greater sensitivity during germination [3]. In the literature there are several studies about the effect of salinity on melon's seed germination [4,5,6]. These authors verified that melon seeds are able to germinate under certain salinity level, but as the salinity increases there is a reduction on the germination rate and in the seedlings development.

Excessive soluble salts concentrations in irrigation water causes a reduction in osmotic potential of plants, leading to a lower water absorption capacity. Moreover, changes in physiological and metabolic processes are observed, due to functional disorders and damages caused by certain ions [7].

Therefore, many researches have been conducted with the purpose of seeking techniques that might mitigate the harmful effects of saline stress. Previous studies have shown that salicylic acid plays an important role in the plant's response to adverse environmental conditions such as saline stress [8,9,10]. Salicylic acid is a substance synthesized from L-phenylalanine, which, through the action of

phenylalanine ammonium lyase (PAL), originates trans-cinnamic acid that is converted to benzoic acid and finally converted to salicylic acid through the action of the enzyme benzoic acid 2-hydroxylase.

Salicylic acid is involved in several plant processes, including the defense responses to biotic and abiotic stresses [11]. Salicylic acid mediates the proteins expression that act on the photosynthetic process and in the plants defense mechanisms [12]. Moreover, its role as a signaling molecule in the genes expression that promotes reactive oxygen species accumulation in the apoplast [13], salicylic acid better be an effective alternative to mitigate the harmful saline stress effects. Therefore, the aim of this study was to evaluate the germination of two melon varieties (*Cucumis melo* L.) submitted to saline stress and salicylic acid treatment.

2. MATERIAL AND METHODS

Local and plant material

The experiment was carried out in the Laboratory of Seed Analysis (LSA), located in the Center of Agricultural Sciences of the Federal University of Paraíba (CCA/UFPB), Campus II, Areia, PB, Brazil.

The experiment was conducted with two melon varieties: a creole variety called "cucumber melon" (*Cucumis melo* var. *Cantalupensis* Naud) and a commercial cultivar named "Eldorado 300". The cucumber melon seeds were extracted from completely mature fruits produced in a farmland located in Santa Luzia, PB, Brazil. The seeds were washed in running water and dried on paper towel sheets at ambient temperature for seven days. The Eldorado 300 seeds were purchased in the local market of Areia, PB, Brazil.

Experimental design and treatments

The experiment was designed as completely randomized, with five different water salinities ($EC_w = 0.00, 1.74, 6.00, 10.26, 12.00 \text{ dS m}^{-1}$) and five salicylic acid doses ($SA = 0.0, 0.29, 1.0, 1.71, 2.0 \text{ mM}$), with four replicates of 50 seeds, adding up to 9 combinations generated through the Central Composite Design (CCD) suggested by Box and Wilson [14]. The experiment was conducted over a period of eight days.

Saline solutions were prepared using a mixture of the following salts: sodium chloride (NaCl), calcium chloride di-hydrate ($\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$) and magnesium chloride hexa-hydrate ($\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$) in a ratio of 7: 2: 1. The ratio used is equivalent to that predominant in the main sources of water available for irrigation in the Brazilian northeast region. The salts concentrations of the saline solution were measured with a portable conductivity meter.

The salicylic acid doses were prepared from the dilution in 200 mL of distilled water, which were then placed in plastic containers along with the seeds. After the eight-hour imbibition period, the seeds were washed with distilled water in order to remove the excess of acid.

Variables analyzed

The germination test were carried out in a BOD (Biochemical Oxygen Demand) type germination chamber, under the temperature of 25 °C and photoperiod of 12 hours. The seeds were sown on sheets of Germitest® paper moistened with distilled water in an amount equivalent to 2.5 times the weight of dry paper. The germination was evaluated daily from the 4th to the 8th day after sowing. Germination was counted when they presented the protrusion of the radicle and the two leaf primordia [15].

The first germination counting was performed on the 4th day after sowing, with the results expressed as percentage of germinated seedlings. The germination speed index was obtained by daily counts of the number of germinated seeds, calculating the results according to the formula proposed by Maguire [16]. The average germination time was evaluated by the daily counting of germinated seeds, and the results were calculated using the formula proposed by Labouriau [17].

After 8 days of germination test, the percentage of abnormal seedlings was evaluated, considering any of those that presented some abnormality. Aerial shoot, root and seedling growth were also evaluated using graded ruler, with the results expressed in centimeters. By the end of the experiment, the seedlings dry mass were obtained by the forced-air circulation oven drying at 65 °C until reaching the constant weight. The seedlings were then packed in Kraft paper bags and put to dry in a drying oven. Subsequently, the seedlings were weighed in a precision analytical balance, with the results expressed in g plant⁻¹.

Statistical analysis

Data were submitted to analysis of variance and regression analysis using the statistical program SAS University [18].

3. RESULTS AND DISCUSSION

Regarding the germination percentage, it was observed that both varieties presented quadratic behavior. Cucumber melon (*Cucumis melo* var. *Cantalupensis* Naud) presented the highest germination percentage in the lowest saline solution (0.0 dS m⁻¹), while the Eldorado cultivar showed best germination performance in the seeds submitted to the 4.4 dS m⁻¹ saline solution, switching to a severe reduction as the salt concentration increased (Figure 1A). However, when correlating the lowest results, reductions of 3 and 38.4% were observed between the seeds submitted to the lower and higher saline concentrated water for cucumber melon and Eldorado cultivar, respectively.

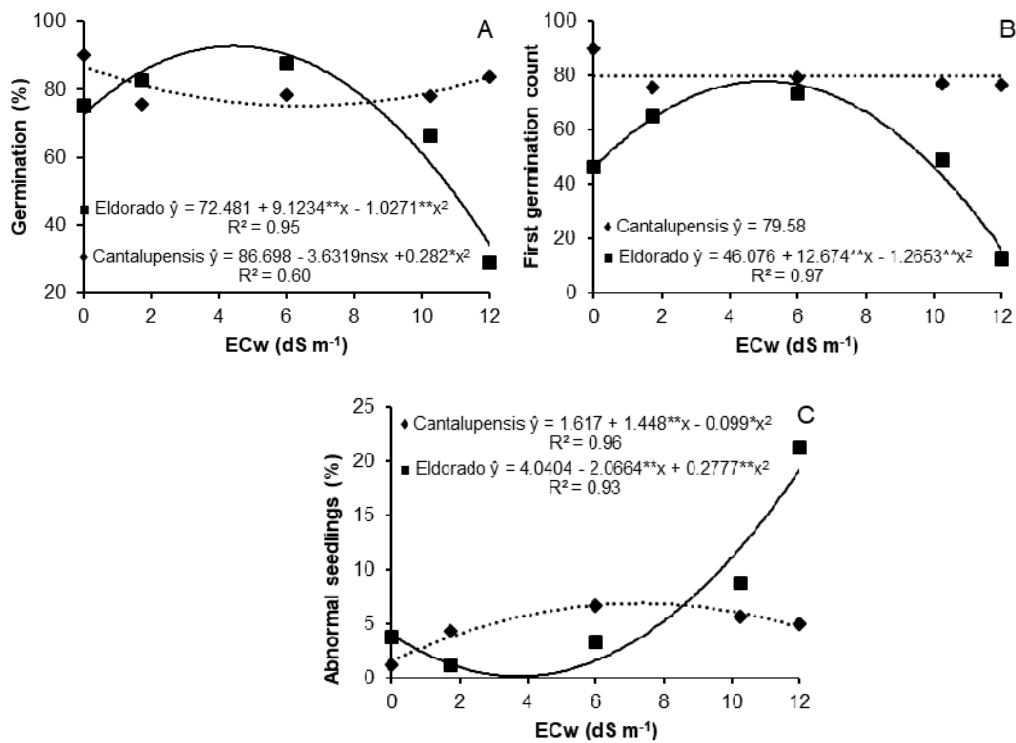


Figure 1. Percentage of germination (A), first germination count (B) and percentage of abnormal seedlings (C) of two varieties of melon (*Cucumis melo* L.) submitted to different salinity levels.

** $P < 0.01$; * $P < 0.05$; ^{ns} $P > 0.05$

Salinity tolerance varies greatly among plant species and within the same species, depending on factors such as cultivar, edaphoclimatic conditions, cultural management, irrigation, intensity and length of the saline stress [19,20,21]. Creole varieties, such as cucumber melon, have greater resistance to saline stress, due to its high genetic variability, which gives them better capacity to osmotically adjust to this condition [22].

Seed vigor evaluated by the first counting of the germination test showed that cucumber melon had a higher tolerance to salt stress. The creole variety exhibited a mean value of 78.5% of germinated seeds on the first day of the germination test (Figure 1B). The cultivar Eldorado showed a quadratic behavior in response to the different saline levels. It presented the highest value for seed vigor (77.8%) when submitted to the salt level of 5.0 dS m⁻¹. Severe reduction occurred as the salinity increased, reaching losses of 61.8% at the highest salt level.

This severe reduction condition is related to a lower water absorption capacity by the seeds, reflecting the number of seeds germinated on the first counting day. According to Munns and Tester [23], the aggressiveness of the salts is initially installed during the germination process, when the low water availability promoted by the reduced osmotic potential and the high salt concentrations can severely compromise the seeds germination capacity [24].

For the percentage of abnormal seedlings, the results obtained for both varieties were adjusted to the quadratic effect. Eldorado was most significantly affected from the concentration of 6.0 dS m⁻¹, reaching the largest number of abnormal seedlings (19.2%) at the highest salt level (Figure 1C). The harmful effects of the salts present in both soil and irrigation water can be manifested by reducing the osmotic potential and also by the toxicity of some elements, especially Na⁺ and Cl⁻, which provoke physiological disturbances on the plant [24].

The germination speed index was also influenced by salinity. Cucumber melon was found to be more salt-tolerant than Eldorado, presenting the mean value of 5.26 (Figure 2A). Eldorado seeds were less vigorous, and its values adjusted to the quadratic model. From ECw 3,5 dS m⁻¹ on, as the salinity increased, the Eldorado germination speed decreased.

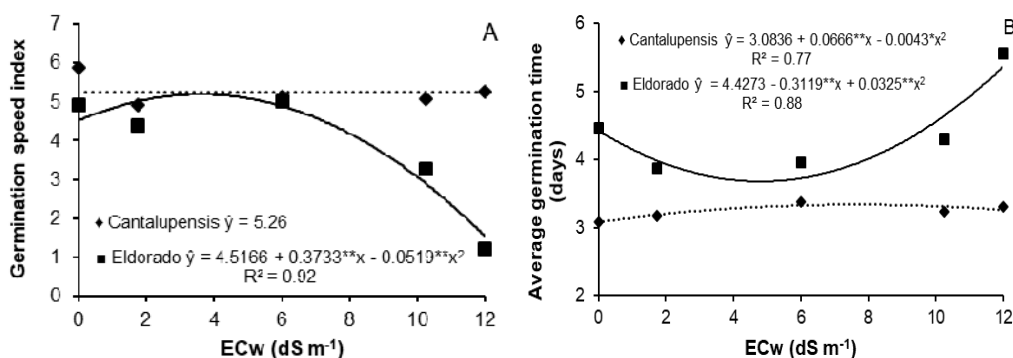


Figure 2.

Germination speed index (A) and average germination time (B) of two varieties of melon (*Cucumis melo* L.) submitted to different salinity levels.

** $P < 0.01$; * $P < 0.05$

This behavior can be explained by the osmotic potential external to the seed created by the high salt content, which prevents water uptake and therefore affects the germination process. The high salt content affects germination due to changes in the kinetics of the water absorption [25]; followed by a reduction in the plant reserves hydrolysis and in the ability to translocate them to the embryonic axis [26], resulting in slower seed germination.

The mean germination time for both varieties were adjusted to the quadratic effect. Cucumber melon presented a higher germination speed when compared to Eldorado, regardless of the saline level (Figure 2B). This behavior shows that the cucumber melon seeds were more vigorous, resulting in a faster germination process. During the germination process, the proteins within the cotyledons are almost entirely degraded and consumed, resulting in radicle protrusion [27]. Therefore, the greater the germination speed the greater the reserves supplying capacity for the embryonic axis, hence more vigorous the seedling [28].

Regarding the root, shoot and seedling lengths, salinity negatively affected the development of both melon varieties (Figure 3). Eldorado presented linear decrease for root length, ranging from 5.54 to 1.49 cm in the lowest and highest salinity level, respectively. The percentage of the decrease was of

73.1% between the lowest and the highest ECw. Cucumber melon values for root length were adjusted to the quadratic effect. Root length values varied between 6.67 and 0.77 cm in the salinity levels of 1.74 to 12.0 dS m⁻¹. The decrease between the highest and the lowest levels was of 88.5% (Figure 3A).

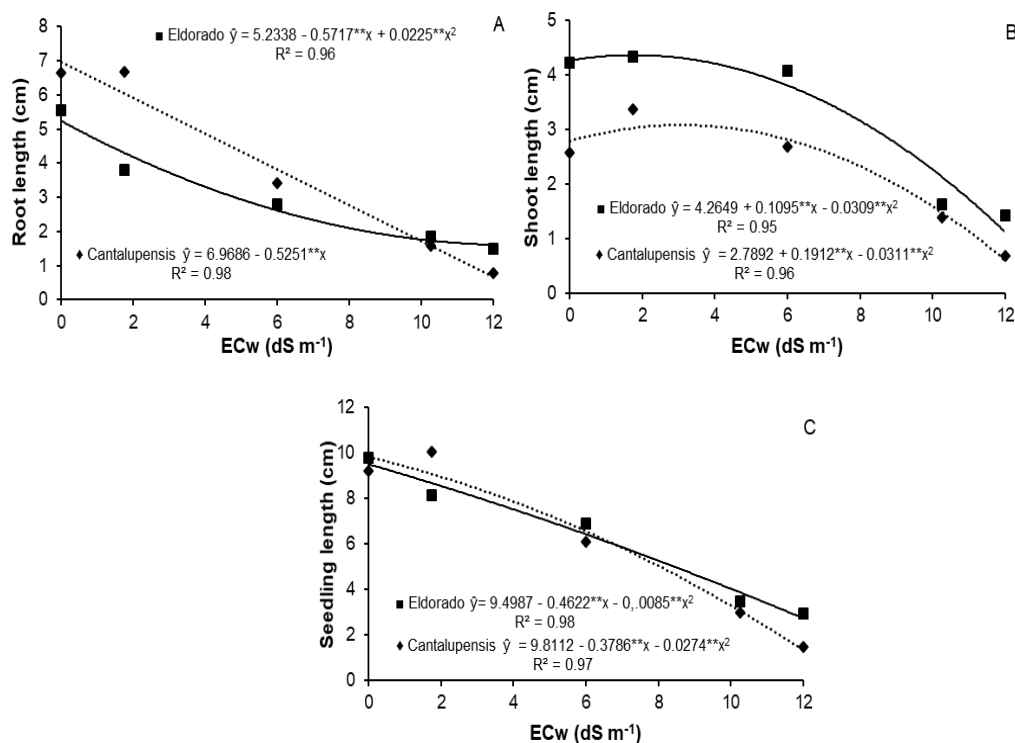


Figure 3. Root (A), shoot (B) and seedling (C) lengths of two varieties of melon (*Cucumis melo* L.), submitted to different salinity levels.

**** $P < 0.01$**

For the shoot length, there was a quadratic effect for the different salinity levels in both melon varieties (Figure 3B). The Eldorado shoot length values ranged from 4.34 to 1.43 cm in the salinities levels of 1.74 to 12.0 dS m⁻¹, with a reduction of 67.1% between the highest and the lowest level (Figure 3B). Cucumber melon values ranged from 3.37 to 0.69 cm at salinity levels of 1.74 to 12.0 dS m⁻¹, with a total decrease of 79.6% between the lowest and the highest level of water salinity (Figure 3B).

The root and shoot length were inhibited by the high salt concentration in the water. A greater reduction of the aerial part was observed as the salinity increased. Fageria et al. [29] emphasized that in short and long-term experiments the aerial part is more sensitive to salinity than the root system.

The quadratic effect was observed for seedling length in both melon varieties for the different salinity levels (Figure 3C). Cucumber melon values ranged from 10.05 to 1.47 at salinity levels of 1.74 to 12.0 dS m⁻¹, presenting a reduction of 85.3% between the highest and the lowest ECw levels (Figure 3C). Eldorado values ranged from 9.20 to 2.92 cm at salinity levels of 0.0 to 12.0 dS m⁻¹, with a total reduction of 68.3% between the lowest and the highest salinity level (Figure 3C).

When plants are submitted to saline stress, some ions reach toxic concentrations and may interfere in cellular metabolic processes, therefore reducing or hindering plant growth [30]. In some studies, high salt concentrations resulted in reduction of seedling vigor, inhibiting initial growth [4,5].

The treatment with salicylic acid showed no significant differences for the above-mentioned variables and its mean values are shown in Table 1.

Table 1. Germination (G), first germination count (FGC), germination speed index (GSI), mean germination time (AGT) and abnormal seedlings (AS) of two varieties of melon (*Cucumis melo* L.) submitted to different doses of salicylic acid.

Salicylic acid (mM)	G	FGC	GSI	AGT	AS
	----- (%) -----			(days)	B
<i>Cucumis melo</i> cv. Eldorado 300					
0.00	78.75	61.25	4.36	4.33	6.25
0.29	81.25	68.75	4.31	3.83	4.38
1.00	66.25	46.25	3.90	4.54	8.33
1.71	67.50	45.00	3.38	4.34	5.00
2.00	88.75	78.75	5.08	3.94	3.75
<i>Cucumis melo</i> var. Cantalupensis					
0.00	90.00	90.00	5.88	3.08	1.25
0.29	75.63	75.63	4.90	3.17	4.38
1.00	78.33	79.17	5.15	3.37	6.67
1.71	78.13	76.88	5.09	3.23	5.63
2.00	83.75	76.25	5.27	3.31	5.00

Eldorado presented the highest average germination (88.75%), first germination count (78.75%) and germination speed index (5,08) when submitted to 2.0 mM of salicylic acid. Opposite behavior occurred with cucumber melon, that had its highest germination, first germination count and germination speed index when submitted to the control treatment of salicylic acid (0 mM). Germination time, number of abnormal seedlings and dry matter of seedlings, presented highest mean values for both cultivars when submitted to 1 mM of salicylic acid, and reducing occurred as the salicylic acid dose increased.

Therefore, salicylic acid doses above 1.0 mM can negatively affect the above mentioned variables. Salicylic acid treatment does not always diminish the harmful effects of salinity. The effectiveness of salicylic acid vary according to the species, the application method, and other factors [31]. This may explain the fact that the acid did not foment a significant effect on the above mentioned variables.

The salicylic acid treatment did not significantly affect root length for both studied melon varieties (Figure 4). However, aerial and seedling length values for cucumber melon decreased until the dose of 1.0 mM of salicylic acid and then increased from this dose on. Eldorado cultivar did not show significant difference for these last two variables when treated with salicylic acid.

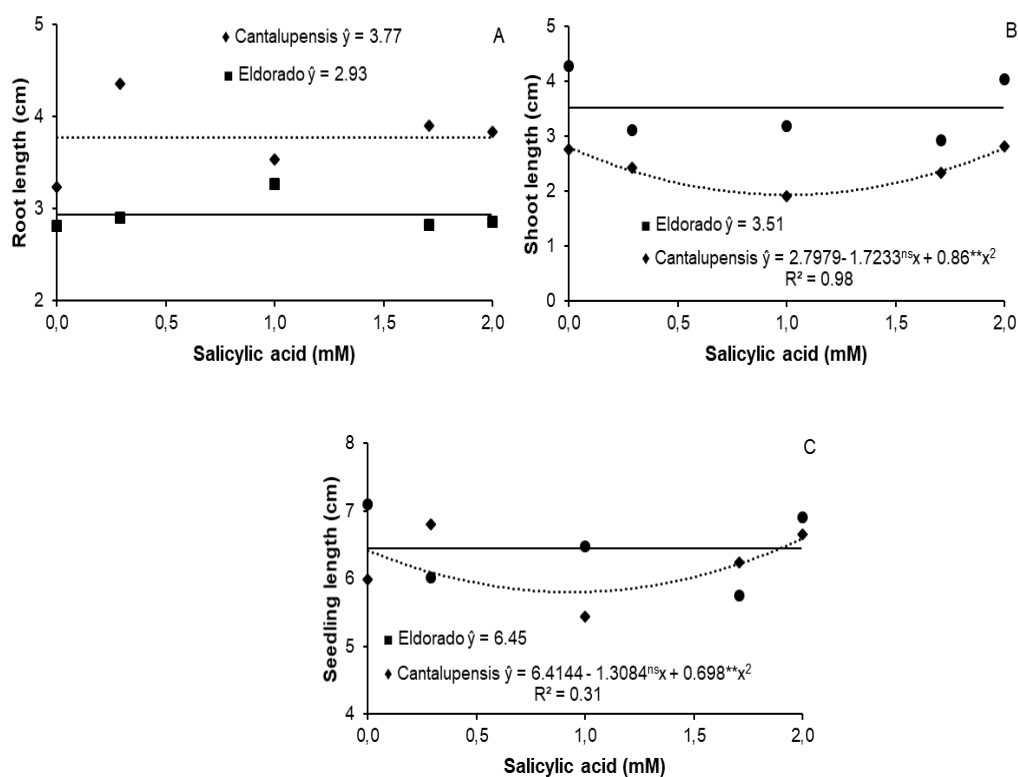


Figure 4. Root (A), shoot (B) and seedlings (C) lengths of two varieties of melon (*Cucumis melo* L.) submitted to different doses of salicylic acid.

**** $P < 0.01$; $^{ns} P > 0.05$**

Salicylic acid plays an important role in several stages of plant growth, such as cell expansion, division and cell death. The application of salicylic acid induces the expression of genes that encode stress-related proteins [32], which enhance plant tolerance to stressful environments.

The dry mass of cucumber melon seedlings submitted to different salinity levels reached a maximum increase of $13.5 \text{ mg plant}^{-1}$ under the salt concentration of 6.0 dS m^{-1} . Reduction in the biomass accumulation was observed as the salinity increased (Figure 5). Dry mass decreasing might be associated to the lower seed vigor caused by the high salt concentration. The excess of salts may have affected the transfer of dry matter from the reserve tissues to the embryonic axis [3].

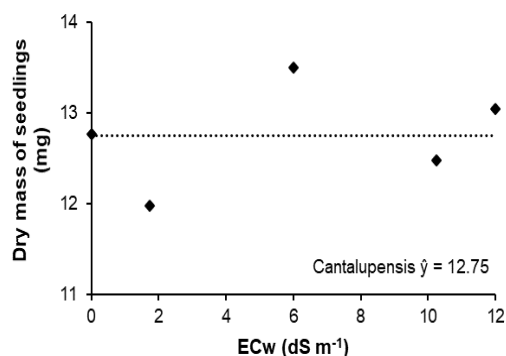


Figure 5. Dry mass of cucumber melon seedlings (*Cucumis melo* cv. Cantalupensis Naud), submitted to different salinity levels.

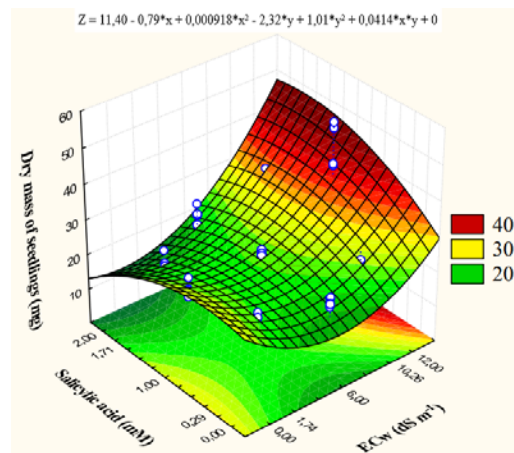


Figure 6. Dry mass of melon seedlings (*Cucumis melo* L. cv. Eldorado), submitted to different salinity levels and doses of salicylic acid.

Interaction between the two factors were observed for the Eldorado seedlings dry mass, with the best result being reached when the ECw was 12.0 and the salicylic acid dose was 1.0 mM. Salicylic acid plays an important role in several plant functions, such as seed germination, photosynthesis, glycolysis and cell expansion and division [33]. Salicylic acid can also enhance plant tolerance to saline stress [34]. The role of salicylic acid in alleviating the harmful effects of salinity may be associated to the biomass accumulation when both of the evaluated factors were increased.

4. CONCLUSION

Cucumis melo seeds quality were affected by saline stress, damaging its vigor and germinative capacity. However, salicylic acid application was not efficient in attenuating the harmful effects of saline stress in the seeds physiological quality. Greater losses were observed in the Eldorado seeds physiological quality, indicating that the commercial variety is more sensitive to saline stress. Cucumber melon (var. *Cantalupensis*) seeds were more vigorous, indicating that the Creole variety is more tolerant to the saline stress.

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