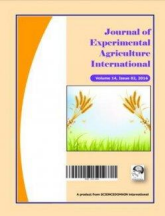

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Physiological Quality of *Malpighia emarginata* D.C. Seeds Submitted to Salt Stress

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aims: The present experiment aimed to study the germinability of *Malpighia emarginata* D.C. seeds and initial growth under different levels of salinity stress.
Study Design: Completely randomised design.
Place and Duration of Study: Federal Institute of Education, Science and Technology of Ceara (IFCE), between February and April 2018.
Methodology: Acerola seeds of "Junko" cultivar were sown on germination paper imbibed with different concentrations of NaCl solutions, with osmotic potentials corresponding to 0, -0.3, -0.6; -0.9; and -1.2 MPa. The experimental design was completely randomised, with four replicates of 50 seeds. After sowing, the papers were rolled and stored in plastic bags, to decrease the rate of evapotranspiration, and they were kept at room temperature for 30 days. The following parameters were evaluated: initial germination percentage (IG%), final germination percentage (FG%), percentage of normal and abnormal seedlings, germination speed index (GSI), average germination time (AGT), shoot length (SL), root length (RL) and number of secondary roots (SR).
Results: The results of analysis of variance allowed verifying significant differences ($P < .001$) for the treatments in almost all the evaluated parameters, except for shoot length (SL). The increase of

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the salinity level inhibited germination and initial growth. The most significant reductions were at osmotic potentials lower than -0.6 MPa, being the development of root system more affected than aerial part of the seedlings.
Conclusion: The results of the initial germination and growth test showed that the "Junko" cultivar of acerola can be considered moderately tolerant to salinity in germination and initial growth phases.

Keywords: Abiotic stress; salinity; acerola; germination of seeds.

1. INTRODUCTION

Acerola (*Malpighia emarginata* D.C.), also known as the Antilles cherry, is native to Central America and it has been cultivated in tropical and subtropical climates [1]. This species was known by the synonyms of *M. galbra* and *M. punicifolia*, but a more recent taxonomic work determined the nomenclature of *M. emarginata* for the species [2].

Recognised for its high content of vitamin C, acerola is a natural source of excellence for this compound and others important functional compounds, such as polyphenols and anthocyanins, whose biological properties are related to beneficial health effects [3]. According to Oliveira et al. [4], acerola presents great potential in the food industry and can be used as a nutritional supplement, or as an additive to increase the nutritional value of other products.

In Brazil, the Northeast region has excelled in the production of acerola, since the crop presents high tolerance to drought and low resistance to cold [5]. However, most soils in the semi-arid region of the Brazilian Northeast present a high salinity index and this salinity is potentially aggressive to the crop [6].

The excess salts in Brazilian Northeast region soils can be attributed to high temperatures, water deficit and low precipitation, being these limiting factors to the development of numerous plant species [7]. Thus, salinity can affect from germination to seed growth and production, by altering the osmotic balance of the plant, producing a physiological drought condition, and by exerting a toxic effect, resulting from ions concentration in the protoplasm [8].

The seed germination rate can be affected by a reduction in water uptake, which is essential for the initial metabolism and development of the embryo. Plants and seedlings may also undergo reduced growth and physiological disturbances caused by nutrient imbalance, as a function of high ionic concentration and inhibition of the absorption of other nutrients [9].

In addition to saline soils directly affect plant metabolism, the use of high saline water is becoming an alternative to global agricultural production, especially in regions of the country marked by freshwater shortage [10]. Thus, under such growth conditions, strategies should be adopted to minimise salinity impacts on soil and crop yield, such as the use of salt leaching or the consortium with salinity tolerant species [11].

Considering the nutritional and economic importance of acerola to Brazilian Northeast region and that few studies have been carried out to investigate the salinity effects on seed germination, growth and cultivation. The present experiment was conducted to study the germinability of *Malpighia emarginata* D.C. seeds and initial growth under different levels of salinity stress.

2. MATERIALS AND METHODS

The experiment was carried out at the Federal Institute of Education, Science and Technology of Ceara (IFCE), campus Jaguaribe, Ceara State, Brazil, during the months of February, March and April of 2018. Seeds of acerola (*Malpighia emarginata* D.C.), belonging to the "Junko" cultivar, were supplied by EMBRAPA Tropical Agroindustry (Fortaleza, Ceara State, Brazil) and underwent a disinfestation process with sodium hypochlorite at a concentration of 2.0% for 5 minutes [12]. In the next step, they were soaked in water at room temperature for 48 hours, to increase the chance of germination [13].

Salt stress was evaluated putting seeds to germinate in solutions of sodium chloride (NaCl) and distilled water with the following osmotic potentials: 0, -0.3, -0.6, -0.9 and -1.2 MPa [14]. The level zero is equivalent to control treatment. The amount of NaCl to obtain the osmotic potentials was determined from the Van't Hoff equation, quoted by Taiz and Zeiger [15].

Four replicates of 50 seeds were sown on germination test papers imbibed in sodium chloride (NaCl) solutions in a proportion of 2.5 times the weight of the paper [16], according to a totally randomized design. After sowing, the papers were rolled and stored in plastic bags to avoid moisture loss. Seeds were kept at room temperature for 30 days in a 12-hour photoperiod induced by fluorescent lamps. This time was determined by preliminary tests and data from the authors Nassif and Cícero [17] since there are no records of germination tests on acerola seeds in the Rules of Seed Analysis [16]. The mean values of temperature and relative humidity were, respectively, 26.7°C and 74.4% during the day [18].

The following evaluations were performed:

Germination test - The seeds were evacuated from the 10th day after sowing, in this time the first germination count was performed to determine initial germination percentage (IG), considering as germination criterion the radicle emission [19]. New evaluations were performed every 4 days, to obtain germination speed index (GSI) and average germination time (AGT). Finally, at 30 days after sowing, final germination percentage (FG) and percentage of normal and abnormal seedlings were evaluated according to Nassif and Cícero [17]. Germination speed index (GSI) was estimated according to Maguire [20], average germination time (AGT) was obtained according to Laboriau and Valadares [21] and percentage of germination (%G) was estimated considering number of total germinated seeds/total number of seeds tested x 100, according to Lewandoski [22].

Morphology - Seedlings were evaluated after 30 days of sowing on shoot length (SL), root length (RL) and a number of secondary roots (SR). The values of SL and RL were obtained through measurements made with a graded ruler, and SR was counted visually and manually [22].

Due to low germination rate in acerola [13, 17], 1000 seeds sample was separated, and each seed was submitted to longitudinal cuts in the opposite region to the radicle emission, to verify the number of seeds with normal embryos, with abnormal embryos, and without embryos [23].

Germination data were transformed to arcsine before statistical analysis, when necessary. Data were subjected to analysis of variance and the treatment effects were unfolded by polynomial regression analysis. The best fit model of data and non-significant regression deviations was chosen. Statistical analyses were performed using the GENES software [24] and, as a measure of experimental precision, the selective accuracy (SA) was estimated according to Resende and Duarte [25].

3. RESULTS AND DISCUSSION

The results of the analysis of variance (Table 1) show significant differences ($P < .001$) for the treatments in almost all evaluated parameters, except for shoot length (SL).

According to Figs. 1a, 1b and 1c, percentages of IG, FG and NS were significantly influenced by salinity concentration. FG and NS data corresponded more adequately to the quadratic model ($R2 = 0.98$ and $R2 = 0.96$). It was possible to verify that, in the control, seeds had a maximum rate of initial germination, on average, 13,5%, 12% of normal seedlings and 4% of abnormal seedlings, with the highest decreases occurring at osmotic potentials below -0.6 MPa. Maximum final germination was estimated at 16.27% for an osmotic potential of -0.18.

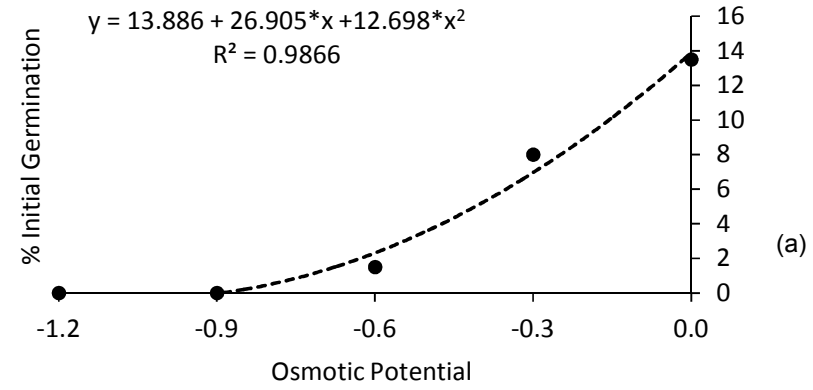
Table 1. Analysis of variance for initial germination percentage (IG%), final germination percentage (FG%), normal seedlings percentage (NS%), germination speed index (GSI), average germination time, (AGT), shoot length (SL), root length (RL) and number secondary roots (SR) of acerola (cultivar Junko) submitted to different osmotic potentials

IG%	FG%		NS%	GSI	AGT	SL	RL	SR
MS trait	0,0585	0,0461	0,0862	0,0039	0,0043	1,223	5,627	55,563
MS error	0,0021	0,0021	0,0011	0,00013	0,00028	0,387	0,744	4,776
F	27,84**	24,15**	77.76**	28,26**	15,366**	$3,16^{NS}$	7,56**	11,63**
SA	0,982	0,979	0,994	0,98	0,967	0,827	0,931	0,956

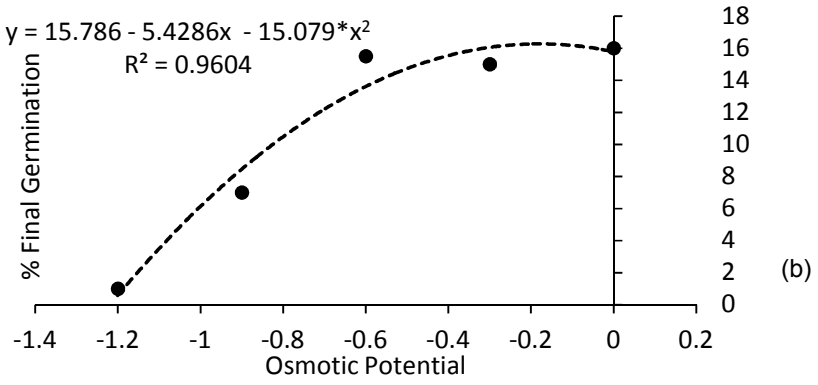
*** - significant at a probability level of 0.01% by the F test, ^{NS} - not significant by the F test, MS = mean square, SA - Selective Accuracy*

In osmotic potential -0.9 MPa, the reduction of these parameters was higher than 50% and, in the lowest osmotic potential tested, -1.2 MPa, final germination and normal seedlings reached only 1% (Fig. 1a, b and c). From the regression equation obtained for initial germination, it is estimated that at an osmotic potential of -0.88, the germination rate is zero.

The low percentage of germinated seeds in the absence of saline stress can be perfectly explained, since acerola seeds naturally present low germination rates, being common the occurrence of non-viable seeds due to factors such as malformation, degeneration of the embryo sac and absence of fertilisation [23].



(a)



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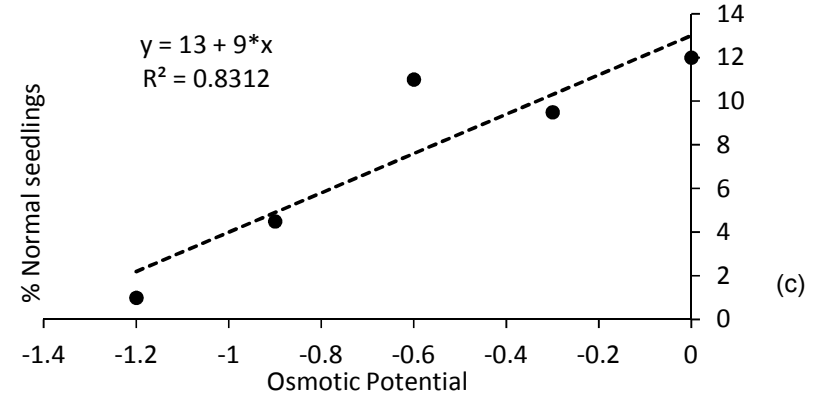

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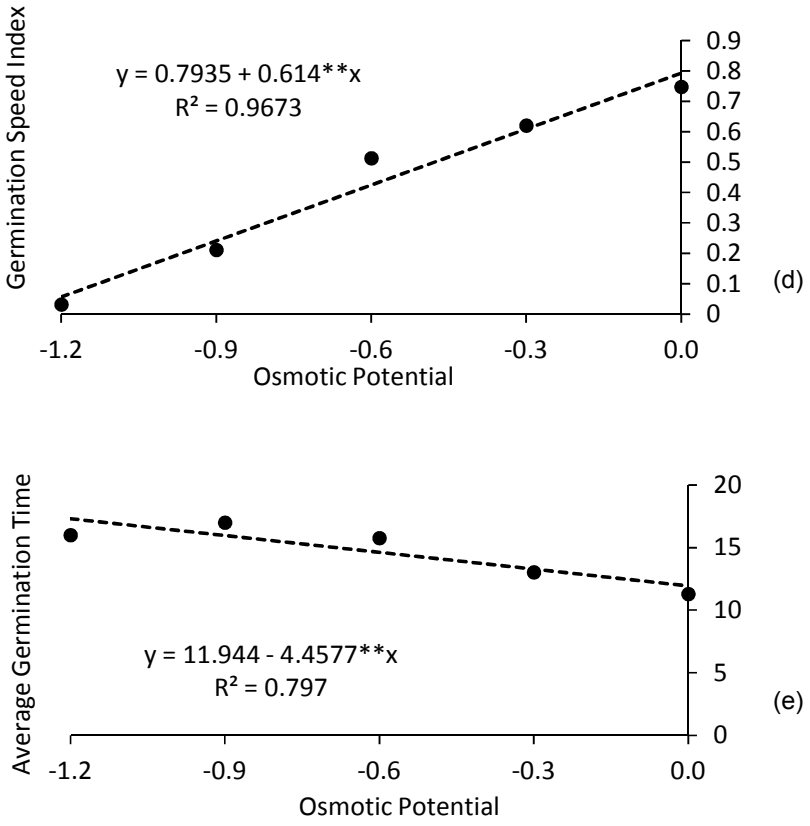

Fig. 1. Initial germination - IG (1a), final germination- FG (1b), normal seedlings NS (1c), germination speed index – GSI (1d) and average germination time- AGT (1e) submitted to different osmotic potentials

A possible explanation for the results in saline treatments is high NaCl levels. Salinity reduces the availability of water that seeds need to imbibe and causes the entry of toxic ions, which makes it difficult to absorb K^+, a cofactor of innumerable enzymes involved in photosynthesis and respiration, fundamental processes in providing the necessary energy for germination [15].

Other authors also reported low germination rates in acerola seeds, such as Ribeiro et al. [26], who presented values ranging from 12% to 18%, depending on the substrate used. Paiva, Alves and Barros [27] identified 13.9% germination in commercial cultivation, while Azerêdo et al. [13] obtained values between 15% and 21%, in evaluations on imbibition time of seeds. Considering only normal seedlings, Nassif and Cícero [17] observed germination rates between 6 and 18%, according to the cultivar used.

Azerêdo et al. [19] obtained higher rates of FG% in acerola seeds, with values ranging from 17% to 54%, depending on the substrate and temperature used. However, the authors selected only seeds with morphologically normal embryos for the experiment, increasing the chances of germination.

Analyses carried out using a longitudinal cut made in 1000 seeds of the Junko cultivar allowed to identify 46.6% of seeds with morphologically normal embryos, 44.5% of seeds with morphologically abnormal embryos, and 8.9% of seeds without embryos. Considering only normal embryos, it is suggested that the germination rate obtained in this work would increase to about 34.3%. Nassif and Cícero [17] reported similar results when evaluating different acerola cultivars by x-ray, identifying between 30% and 40% of morphologically viable embryos.

When the three parameters (IG%, FG% and NS%) were compared, initial germination data (Fig. 1a) were the most affected by the osmotic potential reduction. It was observed that IG was higher in the control treatment and reduced gradually in the other concentrations, showing no germination in osmotic potentials lower than -0.6 MPa. This result is expected because germination speed is the first variable to be affected by the reduction of water availability [28]. Furthermore, seeds that germinate quickly are more likely to survive the field, and therefore IG% parameter is also important for determining vigour index [29].

Data obtained in GSI and AGT (Figs. 1d and 1e) corroborate this effect, being possible to verify that reduction of osmotic potential provided a delay in seeds germination. For both factors, the greatest changes occurred in osmotic potentials lower than -0.6 MPa, indicating it is necessary a long time for the seeds to be able to initiate germination in higher saline concentrations.

Gurgel et al. [30] observed similar effects in acerola, noting that germination speed was significantly affected as electrical conductivity increased. However, the highest electrical conductivity evaluated by the authors was equivalent to osmotic potential -0.5 MPa. Compared to the results of this study, it is possible to determine that acerola can tolerate higher osmotic stress, considering the present work obtained more expressive reductions above the stress level evaluated by these authors.

Osmotic stress is responsible for affecting GSI and AGT once it inhibits water assimilation [31]. Plants usually absorb water under conditions where the root tissue soaking forces are higher than water retention forces in the substrate. In saline substrates, water retention forces are higher, causing osmotic stress and physiological drought [32].

Several authors have observed significant changes in GSI and AGT of other fruit species submitted to salt stress, such as Souza et al. [28] who evaluated jatropha (*Jatropha curcas* L.) seeds. They identified greater changes for both parameters from electrical conductivity equivalent to osmotic potential - 0.6 MPa. Already, Pinheiro et al. [14] observed higher tolerance in pigeon pea (*Cajanus cajan*) seeds, since GSI showed strong reductions only in osmotic potentials lower than -0.9 MPa. Souza, Bezerra and Farias [33] found that cashew seeds presented a linear increase for GSI, with 4.4% for each unit increase in electrical conductivity, corroborating with results obtained in this work.

In relation to morphological data obtained through seedling measurements, although there was a linear tendency to reduce shoot length with increasing salt concentrations (Fig. 2a), it was not possible to observe significant statistically decreases (Table 1). Non-significance of salinity effect on SL differs from results obtained by Oliveira et al. [1], who state that aerial part is one of the most affected by salt stress since the reduction of the leaf area is a mechanism of tolerance that plants use to reduce transpiration surface.

However, in other crops, no significant changes were observed in shoot development, such as in cashew (*Anacardium occidentale*) seedlings [28] and sunflower (*Helianthus annuus*) [34], which may have occurred as a result of multiple factors, such as: type of cultivar, phenological stage, types of salts, intensity and duration of salt stress, cultural and irrigation management, and soil and climatic conditions [35].

In relation to acerola seedling root length (RL) (Fig. 2b), it was possible to observe higher averages in the control treatment and in osmotic potential -0.3MPa. The highest decreases began to occur from osmotic potential -0.6 MPa and the lowest root length was obtained in osmotic potential -0.9MPa, with this stress a mean value of 1.41cm was observed. However, treatment with higher salinity (-1.2 MPa), this value was higher, 2.05 cm, although there was no significant difference in the mean value observed in osmotic potential -0.9 MPa.

Souza et al. [28] reported similar salinity effects in ascertaining that length of jatropha (*Jatropha curcas* L.) roots. They also noted that roots were more affected by electrical conductivity 6 dS.m-1, which is equivalent to -0.6 MPa. According to Gordin et al. [36] the root is one of the plant structures most affected by salt stress, due to ionic imbalance and toxicity, resulting from excess salts and low water potential.

Gurgel et al. [30] analysed the dry mass of acerola seedlings roots submitted to different levels of salinity in irrigation water during 50 and 90 days after germination and found that roots were less affected at 90 days. Comparing the decreases in dry mass at the highest water salinity level 5.5 dS m-1 (equivalent to -0.55

MPa), at 50 and 90 days after germination, the results were 89.80% and 79.57% from de control, respectively. Thus, the roots were kept more time under influence of salt stress and, therefore, exposed to a longer period of salinity, were less affected. These results differ from those observed for root length in this study, which, despite presenting a higher mean in the osmotic potential -1.2MPa, in relation to the potential -0.9, this difference was not significant (Fig. 2).

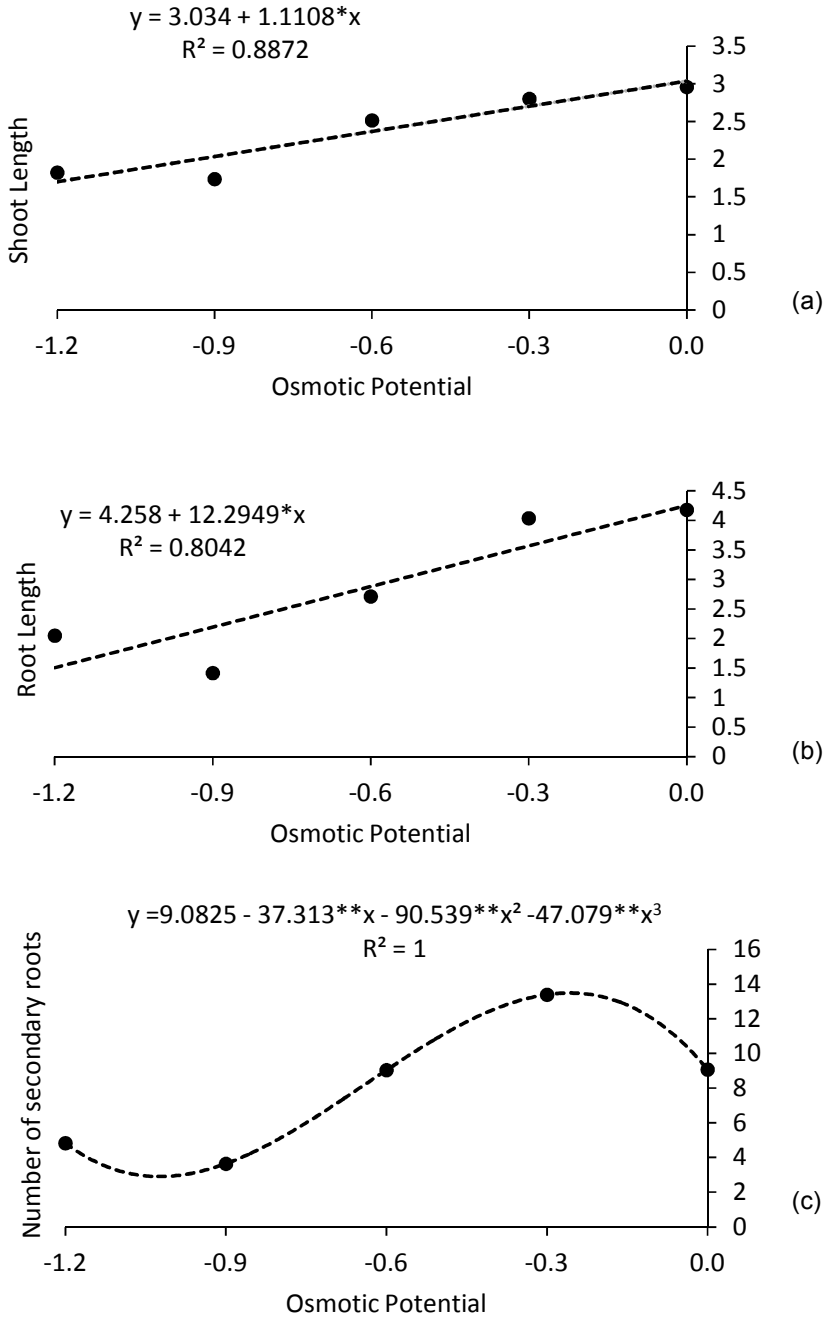

Fig. 2. Shoot length (2a), root length (2b) and a number of secondary roots (2c) of acerola (cultivar Junko) seedlings submitted to different osmotic potentials

Regarding a number of secondary roots (SR), data corresponded to the third order polynomial model (Fig. 2c), being the only model with deviations equal to zero ($R^2 = 1$). The highest number of secondary roots was 13.49, the estimated value for roots grown on osmotic potential -0.26 MPa. There were no statistically significant differences between the control treatment and treatment with osmotic potential -0.6MPa. The lowest number of secondary roots was estimated in 2.90, in osmotic potential -1.02 MPa, this mean is like that presented in osmotic potential -1.2MPa.

Similar results were observed by Cruz et al. [24], who found that length of the main root of lemon "clove" (*Citrus limonia* Osbeck) was affected by salt stress, in addition, the appearance of secondary roots was also inhibited. According to Daniel et al. [37], in cotton (*Gossypium hirsutum* L.), the root was the most compromised structure by the increase in saline concentrations that, besides reducing the size of the roots, also affected its morphology, causing a reduction in a number of secondary and tertiary roots.

In general, vegetables have several mechanisms that allow them to survive and develop in the environments in which they live, responding to environmental changes with direct in their physiological and morphological aspects. In this sense, the most affected aspects by salt stress in the "Junko" cultivar were IG%, FG%, GSI and RL, whose reductions were more significant as osmotic potential reduced. These results agree with those obtained by Gurgel et al. (2007) in a grafting experiment with acerola cultivars BV1 and BV7, they also verified that root system was more affected by salt stress than aerial part.

According to the United States Salinity Laboratory [38], the value established to classify soils as saline is 4 dS m-1, corresponding to the osmotic potential -0.4 MPa. However, the Terminology Committee of the American Society and Soil Science recommended increasing the limit to -0.2 MPa, which represents a considerable change in osmotic potential of soil water [39]. Thus, the cultivar Junko of acerola showed a moderate resistance to salinity in germination and initial growth phases, since the most significant reductions occurred in saline concentrations with osmotic potentials lower than -0.6 MPa.

4. CONCLUSION

Germination and initial growth of the “Junko” acerola cultivar are affected as salt stress intensifies, with more significant effects on salinity levels with osmotic potential lower than -0.6 MPa. In this work, root system was more affected by saline stress than aerial part of the seedlings in early stages of development. Finally, initial germination and growth test results showed that the "Junko" acerola cultivar can be considered moderately tolerant to salinity in germination and initial growth phases.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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