Physiological Quality of *Malpighia emarginata* D.C. seeds submitted to salt stress

**ABSTRACT**

**Aims:** To evaluate the effects of salt stress on the germination and initial growth of acerola seedlings.  
**Study design:** Completely randomized design.  
**Place and Duration of Study:** Federal Institute of Education, Science and Technology of Ceará (IFCE), between February and April 2018.  
**Methodology:** Seeds of the "Junko" cultivar were placed to germinate on germitest paper imbibed with different concentrations of NaCl, with osmotic potentials corresponding to 0, -0.3, -0.6; -0.9; and -1.2 MPa. The experimental design was completely randomized, with four replicates of 50 seeds. After sowing, the papers were rolled and stored in plastic bags, in order 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 the analysis of variance allowed verifying significant differences (P < .001) for the treatments in almost all the evaluated parameters, except for the shoot length (SL). The increase of the salinity level negatively compromised the germination and the initial growth. The most significant reductions occurred at levels with osmotic potentials lower than -0.6 MPa, being the development of the root system more affected than the 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 the 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].

Recognized for the high content of vitamin C, acerola is a natural source of excellence for this compound and other 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 of the soils of the Brazilian Northeast region can be attributed to the high temperatures, the water deficit and the 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 the ions concentration in the protoplasm [8].

The seed germination rate can be affected as a result of the restriction 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 affecting 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 growing conditions, strategies should be adopted to minimize salinity impacts on soil and crop yield, such as the use of salt leaching or the consortium with salinity tolerant species [11].

In the particular case of acerola, few studies have been made to investigate the tolerance levels of this species to salt stress, and the information on salinity effects on seed germination, growth and cultivation is scarce. In the absence of research results involving this crop, this work aimed to evaluate the effects of salinity on seed germination and initial growth of acerola seedlings (Malpighia emarginata D.C.).

2. MATERIAL AND METHODS

The experiment was carried out at the Federal Institute of Education, Science and Technology of Ceará (IFCE), campus Jaguaribe, Ceará 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, Ceará 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, in order to increase the chance of germination [13].

To evaluate the effect of salt stress, the seeds germinated in solutions of sodium chloride (NaCl) with the following osmotic potentials: 0; -0.3; -0.6; -0.9 and -1.2 MPa [14], being level zero equivalent to the control treatment, where distilled water was used. 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 was submitted to germination test in “germitest” papers imbibed in distilled water, in the control treatment, or 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, in order to decrease the rate of evapotranspiration, and they were kept at room temperature for 30 days, with a photoperiod of 12 hours. This time period 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 evaluated from the 10th day after sowing, where the first germination count was carried out to determine the initial germination percentage (GI), considering as germination criterion the radicle emission [19]. After the first count, new evaluations were performed every 4 days, in order to obtain the germination speed index (GSI) and the average germination time (AGT). Finally, at 30 days after sowing, the final germination percentage (FG) and percentage of normal and abnormal seedlings were evaluated according to Nassif and Cícero [17].

The germination speed index (GSI) was estimated according to Maguire [20], the average germination time (AGT) was obtained according to Laboriau and Valadares [21] and the percentage of germination (%G) was calculated from division of the number of germinated seeds by the total number of seeds sown, multiplied by 100, as determined by Lewandoski [22].
Morphology - Seedlings were evaluated after 30 days of sowing on shoot length (SL), root length (RL) and number of secondary roots (SR). The values of the SL and RL were obtained through measurements made with a graded ruler and the NR was counted visually and manually [22].

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

The data were submitted to analysis of variance and the means comparison was performed from the Tukey test (P = .05). Then, the treatment effects were unfolded by polynomial regression analysis, in order to verify the behavior of the variables as a function of the osmotic potential of the solution. The model of better fit of data and non-significant regression deviations was chosen. The statistical analyzes were performed in 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 the evaluated parameters, except for the shoot length (SL). The data in Table 2 indicate the averages obtained for each character in each saline concentration, as well as the means that differed between them, determined by the Tukey test.

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.

<table>
<thead>
<tr>
<th></th>
<th>IG%</th>
<th>FG%</th>
<th>NS%</th>
<th>GSI</th>
<th>AGT</th>
<th>SL</th>
<th>RL</th>
<th>SR</th>
</tr>
</thead>
<tbody>
<tr>
<td>SST</td>
<td>0.059</td>
<td>0.046</td>
<td>0.086</td>
<td>0.004</td>
<td>0.004</td>
<td>1,223</td>
<td>5,627</td>
<td>55,563</td>
</tr>
<tr>
<td>MS</td>
<td>0.002</td>
<td>0.002</td>
<td>0.0011</td>
<td>0.0001</td>
<td>0.0003</td>
<td>0.387</td>
<td>0.744</td>
<td>4,776</td>
</tr>
<tr>
<td>F</td>
<td>27.84**</td>
<td>24.15**</td>
<td>77.76**</td>
<td>28.26**</td>
<td>15,366**</td>
<td>3.16 NS</td>
<td>7.56**</td>
<td>11.63**</td>
</tr>
<tr>
<td>SA</td>
<td>0.982</td>
<td>0.979</td>
<td>0.994</td>
<td>0.98</td>
<td>0.967</td>
<td>0.827</td>
<td>0.931</td>
<td>0.956</td>
</tr>
</tbody>
</table>

** - significant at a probability level of 0.01% by the F test. NS - not significant by the F test

Table 2. 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 of secondary roots (SR) of roots (SR) of acerola (cultivar Junko) submitted to different osmotic potentials.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>C0</th>
<th>C3</th>
<th>C6</th>
<th>C9</th>
<th>C12</th>
</tr>
</thead>
<tbody>
<tr>
<td>IG%</td>
<td>13.5a</td>
<td>8b</td>
<td>1.5c</td>
<td>0d</td>
<td>0d</td>
</tr>
<tr>
<td>FG%</td>
<td>16a</td>
<td>15a</td>
<td>15.5a</td>
<td>7b</td>
<td>1c</td>
</tr>
<tr>
<td>NS%</td>
<td>12a</td>
<td>9.5a</td>
<td>11a</td>
<td>4.5b</td>
<td>1c</td>
</tr>
<tr>
<td>GSI</td>
<td>0.75a</td>
<td>0.62ab</td>
<td>0.51b</td>
<td>0.21c</td>
<td>0.03d</td>
</tr>
<tr>
<td>AGT</td>
<td>11.30b</td>
<td>13.03b</td>
<td>15.76a</td>
<td>17a</td>
<td>16a</td>
</tr>
<tr>
<td>SL</td>
<td>2.95a</td>
<td>2.80a</td>
<td>2.52a</td>
<td>1.74a</td>
<td>1.82a</td>
</tr>
<tr>
<td>RL</td>
<td>4.18a</td>
<td>4.03a</td>
<td>2.72ab</td>
<td>1.42b</td>
<td>2.05b</td>
</tr>
<tr>
<td>SR</td>
<td>9.08b</td>
<td>13.39a</td>
<td>9.05b</td>
<td>3.65c</td>
<td>4.83c</td>
</tr>
</tbody>
</table>

In the column, the averages followed by the same letter do not differ from each other to 5% by the Tukey test.

According to Figures 1a, 1b and 1c, percentages of IG, FG and NS were significantly influenced by the salt stress condition. The percentage data of FG and NS corresponded more adequately to the quadratic model (R2 = 0.98 and R2 = 0.96). It was possible to verify that, in the absence of NaCl, the seeds had, on average, 16% germination, 12% of normal seedlings and 4% of abnormal seedlings, with the highest decreases occurring at osmotic potentials below -0.6 MPa. In the potential -0.9 MPa, the reduction of these parameters was superior to 50% and, in the lower osmotic potential, -1.2 MPa,
the percentage of germination and the percentage of normal seedlings reached only 1% (Table 2, figure 1a, b and c).

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 fertilization [23].

A possible explanation for the other results in saline treatments is that they occur because as NaCl levels increase, salinity reduces the availability of water that the seed needs for imbibition, causing the entry of toxic ions and making it difficult to absorb K⁺, a cofactor of innumerable enzymes involved in photosynthesis and respiration, fundamental processes in providing the energy necessary for germination [15].
Figure 1 - Initial germination (1a), final germination and normal seedlings percentage (1b), germination speed index (1c) and average germination time (1d) submitted to different osmotic potentials.

Other authors also obtained low germination rates in acerola seeds, such as Ribeiro et al. [26], which 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 embibition time of the seeds. Considering only normal seedlings, Nassif and Cicero [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 varying 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.

The analyzes 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. Taking into account only the normal embryos, it is suggested that the germination rate obtained in this work would increase to approximately 34.3%. Nassif and Cicero [17] presented 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, the initial germination data (Table 2, figure 1a) were the most affected with the increase in osmotic potential. It was observed that the percentage of IG was higher in the control treatment and reduced gradually in the other concentrations, showing no germination in the osmotic potentials under -0.6 MPa. This result is
expected because the germination speed is the first variable to be affected by the reduction of water availability [28]. Furthermore, seeds that germinate more rapidly are more likely to survive the field, and therefore the IG% parameter is also important for determining the vigor index [29].

The data obtained in GSI and AGT (Table 2, figures 1c and 1d) corroborate this effect, being possible to verify that the reduction of the osmotic potential provided a delay in the seeds germination. For both factors, the greatest changes occurred in osmotic potentials lower than -0.6 MPa, indicating that it was necessary a longer 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 the germination speed was significantly affected as the electrical conductivity increased. However, the highest electrical conductivity evaluated by the authors was equivalent to the osmotic potential -0.5 MPa. Comparing with results of this study, it is possible to determine that acerola is able to tolerate higher osmotic stress, considering that the present work obtained more expressive reductions above the level evaluated by these authors.

Osmotic stress, which is responsible for affecting GSI and AGT, is one of the side effects of salt stress, which acts to inhibit water assimilation [31]. Plants normally absorb water under conditions where the root tissue soaking forces are higher than the water retention forces in the substrate, and, in the case of saline substrates, the retention forces are higher, causing osmotic stress and physiological drought [32].

Several authors have observed significant changes in the GSI and AGT of other fruit species submitted to salt stress, such as Souza et al. [28] who evaluated Jatropha seeds and identified greater changes for both parameters from the electrical conductivity equivalent to the osmotic potential -0.6 MPa.Already, Pinheiro et al. [14] observed higher tolerance in pigeon pea 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 the electrical conductivity, corroborating with the data obtained in this work.

In relation to the morphological data obtained through seedling measurements (Table 1 and 2), although there was a linear tendency to reduce shoot length with increasing salt concentrations (Figure 2a), it was not possible to observe significant statistically decreases. The non-significance of salinity on SL differs from the assumption by Oliveira et al. [1], which states that the aerial part is one of the structures most affected by salt stress, since the reduction of the leaf area is a mechanism of tolerance that the plant uses to reduce the transpirant surface.

However, in other crops, no significant changes were observed in shoot development, such as in cashew seedlings [28] and sunflower [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 the length of the acerola seedling root (Figure 2b), it was possible to observe higher averages in the control treatment and in the treatment with osmotic potential -0.3MPa. The highest declines began to occur from the level of stress with osmotic potential -0.6 MPa, the largest decrease being obtained in the saline treatment with osmotic potential -0.9MPa, where an average value of 1.41cm is observed. However, in the treatment with a higher level of salinity (-1.2 MPa), this value was bigger, 2.05 cm, although there was no significant difference to that observed in the osmotic potential -0.9 MPa (Table 2).

Souza et al. [28] had similar implications in ascertaining that the length of Jatropha roots was most affected from the stress level with electrical conductivity 6 dS.m-1, which is equivalent to -0.6 MPa. According to Gordin et al. [36] the root area of the plant is one of the main structures affected by salt stress, due to ionic imbalance and toxicity resulting from excess salts and low water potential.
Figure 2. Shoot length (2a), root length (2b) and number of secondary roots (2c) of acerola (cultivar Junko) seedlings submitted to different osmotic potentials.

Gurgel et al. [30] analyzed 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 the 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%, respectively. Thus, the roots that spent more time under the influence of salt stress and,
therefore, exposed to a longer period of salinity, were less affected. These results differ from those observed for the 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 (Table 2, figure 2).

Regarding the number of secondary roots (SR), the results corresponded to the third order polynomial model (Figure 2c), being the only model with deviations equal to zero (R² = 1). According to the data, the highest number of secondary roots occurred at the concentration of osmotic potential -0.3MPa, with no statistically significant differences between the control treatment and treatment with osmotic potential -0.6MPa. However, larger decreases occurred in the osmotic potential -0.9 MPa, which presented mean values similar to the values of the osmotic potential -1.2MPa.

Similar results were observed by Cruz et al. [24], who found that in addition to the length of the main root of lemon "clove" was affected by salt stress, the appearance of secondary roots was also inhibited. According to Daniel et al. (2011), in cotton, the root was the structure most compromised by the increase of saline concentrations that, besides reducing the size of the roots, also affected its morphology, causing the reduction of the number of secondary and tertiary roots.

In general, vegetables have several mechanisms that allow them to survive and develop in the environments where they live, responding to environmental changes with direct changes in their physiological and morphological aspects. In this sense, the aspects most affected by salt stress in the "Junko" cultivar were IG%, FG%, GSI and RL, where the reductions were more significant as the osmotic potential reduced. These results are in agreement with those obtained by Gurgel et al. (2007) in a grafting experiment with the cultivars BV1 and BV7 of acerola, where the authors also verified that the root system was more affected by salt stress than the 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 of Soil Science recommended increasing the limit to -0.2 MPa, which represents a considerable reduction in the osmotic potential of soil water [39]. Thus, the cultivar Junko of acerola showed a moderate resistance to salinity in the germination and initial growth phases, since the most significant reductions occurred in saline concentrations with osmotic potentials lower than -0.6 MPa.

4. CONCLUSION

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

REFERENCES


DOI: 10.2135/cropsci1962.0011183X000200020033x


DOI: 10.1104/pp.001164


DOI: 10.18406/2316-1817v7n22015620


DOI: 10.1093/aob/mcg058

