

# **Effect of salinity on germination of lettuce cultivars produced in Brazil**

## **ABSTRACT**

The challenge of recovering degraded soils due to salinity excess leads to the search for more effective strategies that can overcome this problem. Among these, one of the approaches is the use of resistant plant varieties in affected areas. This study aimed to evaluate the influence of different doses of salt on seed germination and seedling formation of two lettuce cultivars (Hanson and H121) and to verify the existence of tolerance among the cultivars. A completely randomized design was used in a 2x5x4 factorial scheme, where the cultivars were evaluated under five distinct salt doses (0, 25, 50, 75 and 100 mol.m<sup>-3</sup>, conductivities of 0.0, 2.8, 5.4, 8.0 and 10.6 dS.m<sup>-1</sup>, respectively), with four replicates. In this sense, the following variables were evaluated: germination rate (GR), germination speed index (GSI), seedling height (SH), root length (RL) and percentage of dry matter in relation to fresh matter (DM%). As a result, the Hanson cultivar presented better performance than the H121, under all the different salt doses, in all the studied variables. Also, the EC of 2.8 dS.m<sup>-1</sup> did not affect any of the studied variables, including both cultivars. However, EC above 2.8 dS.m<sup>-1</sup> had, significantly, reduced the development of the cultivars. The Hanson cultivar was influenced only in the variables SH, RL and DM%, where SH and DM% were influenced by EC values above 8.0 and 10.6 dS.m<sup>-1</sup>, respectively. The cultivar H121 was significantly influenced in all evaluated variables, which demonstrates its greater susceptibility to salinity.

*Keywords: Electrical conductivity, osmotic potential, water consumption, tolerance.*

## **1. INTRODUCTION**

Population growth, food security and the scarcity of nonrenewable natural resources are some of the challenging themes that are present in the main discussions about the transformations in contemporary agriculture [1,2]. In a more rational model, priority is given to respect for the environment, where there is a growing concern with the management of natural resources, and at the same time, with the economic viability of the agricultural sector for its professionals, in order to guarantee the sustainability of current and future generations [3,4].

The development of sustainable agriculture is intrinsically related to the possibility of adopting measures that overcome the main challenges of agricultural production, in a rational and environmentally friendly way [5]. Many are the adversities found in agricultural fields throughout the world, but in the past few years, one has been gaining prominence, the soil salinity [6]. Soil salinity can occur naturally due to the characteristics of the source material [7]. However, the most damaging salinity is that induced by modern agricultural

32 systems, where the irrational use of fertilizers and the use of brackish water for irrigation are  
33 the main agents that cause this adversity [8,9].

34 Soil salinity can make it unproductive, indirectly reducing the income of the farmers who  
35 cultivate the soil [10]. In Brazil, small farmers are the class that suffers the most from soil  
36 salinization, since the low income of these producers does not allow them to adopt  
37 technologies to reverse it. Thus, technologies that allow cultivation on lands with an excess  
38 of salts are indispensable for the success of these farmers in the field. Among the available  
39 technologies, there is the selection of cultivars that tolerate high salinity concentrations.

40 Among the crops produced by small farmers, which suffer from the elevation of salinity  
41 levels, there is the lettuce (*Lactuca sativa* L.) [12]. The lettuce stands out because of its low  
42 cost of production, market acceptability, and its cultivation requires small areas, which  
43 makes it suitable for small farmers [12,13]. Lettuce cultivation is strongly affected by salinity,  
44 and about 13% of the yield declines by each unit of electrical conductivity (EC) increased  
45 above  $1.3 \text{ dS m}^{-1}$  [13]. However, there is variation among cultivars, where some differ from  
46 the others regarding their tolerance to high levels of salinity [14,13].

47 The tolerance to salinity is a polygenic characteristic, which makes it difficult to identify  
48 tolerant genotypes, due to a large number of genes involved in the trait's control [15,16].  
49 Identifying tolerant materials among the cultivars on the market has become an important  
50 strategy for expansion of lettuce crops in places where salinity is a limiting factor. Since it  
51 reduces the time and cost to obtain superior lineages in breeding programs. Given the  
52 above, this study aimed to evaluate the influence of different salt concentrations on  
53 germination of two lettuce cultivars, as well as to verify the existence of tolerance to salinity  
54 in the cultivars.

## 55 2. MATERIAL AND METHODS

56 The study was conducted in a completely randomized design in a  $2 \times 5 \times 4$  factorial scheme.  
57 The Hanson (cultivar 1) and H121 (cultivar 2) cultivars were used. To evaluate the tolerance  
58 to salinity, five distinct concentrations of sodium chloride (NaCl) were tested: 0, 25, 50, 75  
59 and  $100 \text{ mol.m}^{-3}$ . All concentrations of NaCl were diluted in distilled water, whose initial EC  
60 was zero. These concentrations corresponded to solutions with the following electrical  
61 conductivities: 0.0, 2.8, 5.4, 8.0 and  $10.6 \text{ dS.m}^{-1}$ , respectively.

62 To evaluate the germination, four replicates of 25 seeds were made in each treatment,  
63 wherein previous tests all the seed lots used in the study presented a germination rate (GR)  
64 higher than 90%. The test was performed in a  $10 \times 1.5 \text{ cm}$  petri dish with the use of  
65 germitest paper. The paper was accommodated in double sheets at the bottom of the petri  
66 dish, and the seeds were evenly distributed over it. The saline solution was added after the  
67 seed distribution at 2.5 times the dry paper weight.

68 In order to favor germination, the seeds were kept in a Biochemical Oxygen Demand  
69 incubator (BOD) for 7 days, allowing total control of lighting and temperature that was  
70 alternated to maintain 12 hours of light and 12 hours of dark at a temperature of  $25^\circ \text{C}$ .

71 The germination evaluation occurred on the seventh day with the seedling count as  
72 proposed by the authors [17]. Also, only seeds with a primary root greater than 2 mm, as  
73 established by the authors [18], were considered as germinated. Through the counting data,  
74 it was possible to determine the GR in each treatment.

75 Along with the germination test, daily germinated seedlings were counted from the beginning  
76 of the sowing until the seventh day, when the germination was stabilized. The germination  
77 speed index (GSI) was obtained using the equation (1) proposed by the author [19].

$$78 \quad GSI = \sum (n/t)(1)$$

79 Where:

80  $n$  = number of normal seedlings computed on the day of the count;

81  $t$  = number of days from sowing until the day of the count.

82 After the germination stabilization on the seventh day, the variables seedling height (SH),  
83 root length (RL) and percentage of dry matter in relation to fresh matter (DM%) were  
84 determined. To do so, ten normal seedlings of each replicate were selected. The  
85 determination of the SH and RL was performed using a graduated ruler (centimetres). SH  
86 was measured from the neck to the apical meristem, while the RL was measured from the  
87 neck to the root cap.

88 The DM% was obtained using the equation (2):

$$89 \quad DM\% = mf/mi \quad (2)$$

90 Where:

91 DM%: percentage of dry matter in relation to fresh matter;

92  $mf$ : Total dry mass of the seedling;

93  $mi$ : Total fresh mass of the seedling.

94 The seedlings had their fresh and dried masses measured by a precision scale. In order to  
95 obtain the dry mass values, the seedlings were left in an oven at 75° C until they reach a  
96 constant weight. Lastly, the data were submitted to the Kolmogorov Smirnov normality test,  
97 and then the variance analysis was performed. The means between treatments within the  
98 same cultivar were compared by the Tukey test, and the means of treatments between the  
99 cultivars were compared by the t-test. A simple linear regression analysis was used to infer  
100 about the behaviour of the different cultivars under the effect of different salt concentrations  
101 in the studied variables. All statistical analyzes were performed at a 5% probability level.

### 102 **3. RESULTS AND DISCUSSION**

103 The results presented in table 1 demonstrate that cultivar 1 obtained superior performance in  
104 relation to cultivar 2, for all the different EC tested. Cultivar 2 was significantly influenced (p-  
105 value <0.005) by the EC in all evaluated variables, while for cultivar 1, only SH, RL and  
106 DM% variables were influenced.

107 The EC of 2.8 dS.m<sup>-1</sup> did not affect any of the studied variables in both cultivars (Table 1).  
108 Similar results were found by the author [14] when studying salinity levels in the germination  
109 of the Elba cultivar. Conversely, EC higher than 2.8 dS.m<sup>-1</sup> negatively affected the root growth  
110 of both cultivars. The authors [20] emphasize the importance of root formation in this  
111 phenological phase of the vegetables. The low root development in this phase culminates in  
112 fragile seedlings that tend to suffer more from environmental and biological stresses.

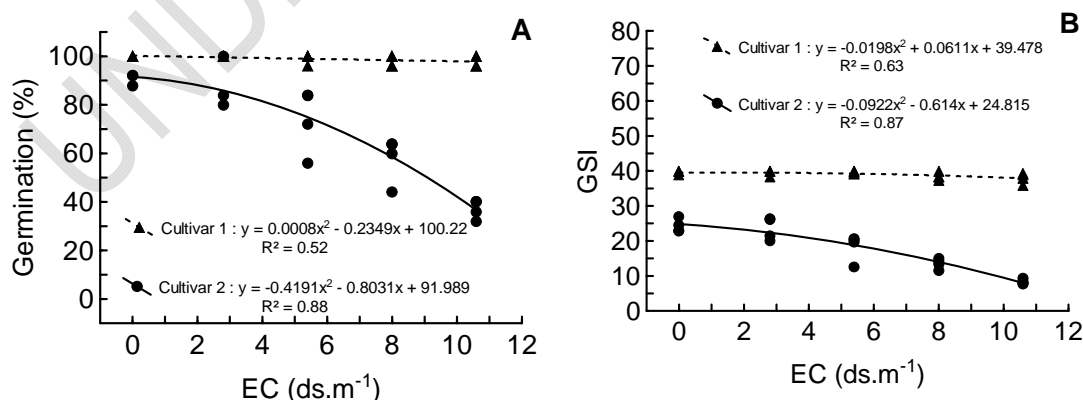
Table 1. Comparative test of averages for germination rate (GR), germination speed index (GSI), seedling height (SH), root length (RL) and percentage of dry matter in relation to fresh matter (DM%), for the cultivars 1 and 2 submitted to different electrical conductivities.

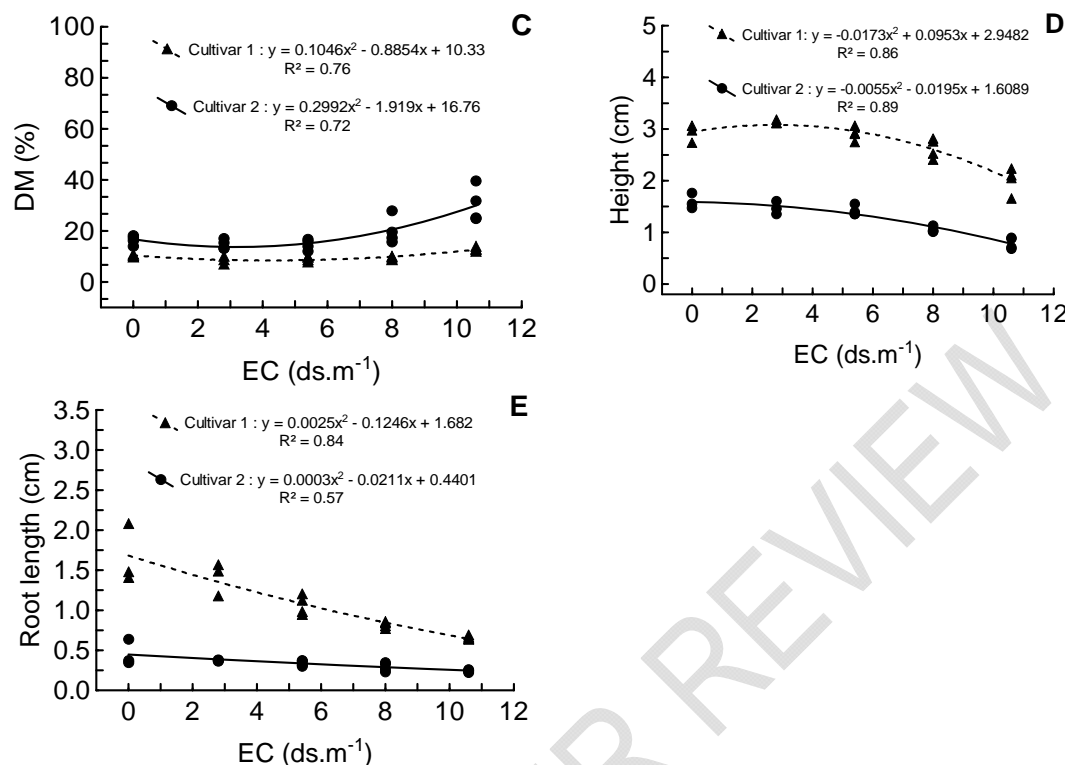
	EC (dS.m <sup>-1</sup> )				
	0	2.8	5.4	8	10.6
<b>Cultivar</b>	<b>GR (%)</b>				
<b>Cultivar 1</b>	99.67±0.33 Aa	99.67±0.33 Aa	99.0±1 Aa	98.0±1.15 Aa	98.0±1.15 Aa
<b>Cultivar 2</b>	91.0±1 Ab	89.0±4.43 Aa	74.0±6.63 ABb	58.0±4.76 Bb	37.0±1.91 Cb
	<b>GSI</b>				
<b>Cultivar 1</b>	39.49±0.24 Aa	39.34±0.34 Aa	39.54±0.17 Aa	38.41±0.51 Aa	38.0±0.74 Aa
<b>Cultivar 2</b>	24.34±0.94 Ab	23.54±1.62 ABb	18.29±1.94 BCb	13.49±0.71 CDb	8.3±0.39 Db
	<b>SH (cm)</b>				
<b>Cultivar 1</b>	2.97±0.08 Aba	3.18±0.04 Aa	2.91±0.06 ABa	2.63±0.1 Ba	2.02±0.12 Ca
<b>Cultivar 2</b>	1.62±0.07 Ab	1.46±0.05 Ab	1.42±0.05 Ab	1.06±0.03 Bb	0.79±0.06 Cb
	<b>RL (cm)</b>				
<b>Cultivar 1</b>	1.64±0.15 Aa	1.40±0.08 ABa	1.07±0.06 BCa	0.82±0.02 CDa	0.66±0.01 Da
<b>Cultivar 2</b>	0.45±0.07 Ab	0.37±0.01 ABb	0.34±0.02 ABb	0.3±0.03 Bb	0.24±0.01 Bb
	<b>DM (%)</b>				
<b>Cultivar 1</b>	9.93±0.4 Aa	8.28±0.7 Aa	9.05±0.51 Aa	9.39±0.32 Aa	12.91±0.41 Ba
<b>Cultivar 2</b>	16.37±0.92 Ab	14.68±0.96 Ab	14.7±1.04 Ab	20.13±2.70 Ab	30.32±3.48 Bb

Means followed by the same capital letter, in the same line, do not differ by the Tukey test ( $P < 0.05$ ) and means followed by the same lowercase letter, in the same column, do not differ from each other by the  $t$ -test ( $P < 0.05$ ).

Figure 1A illustrates the influence of EC on the GR of seedlings from both cultivars. Cultivar 1 did not suffer influence in the number of germinated seedlings, unlike cultivar 2, which can also be observed in table 1. In cultivar 2, the germination decreased from 91%, in the control group, to 37% for the highest EC tested, demonstrating its sensitivity to high levels of salinity. Studies conducted by the authors [21] demonstrate that may exist different levels of salinity tolerance during the germination process among commercial lettuce cultivars.

The absence of influence on the seedlings GR of cultivar 1 may indicate that it tolerates high saline concentrations in this phenological phase. Salinity tolerance in the germination process is associated with the activation of mechanisms that lead to changes in cellular metabolism. Among these mechanisms, there is the ability to accumulate organic ions and low molecular weight solutes in cell vacuoles, to maintain water absorption, even in unfavourable osmotic conditions [22].





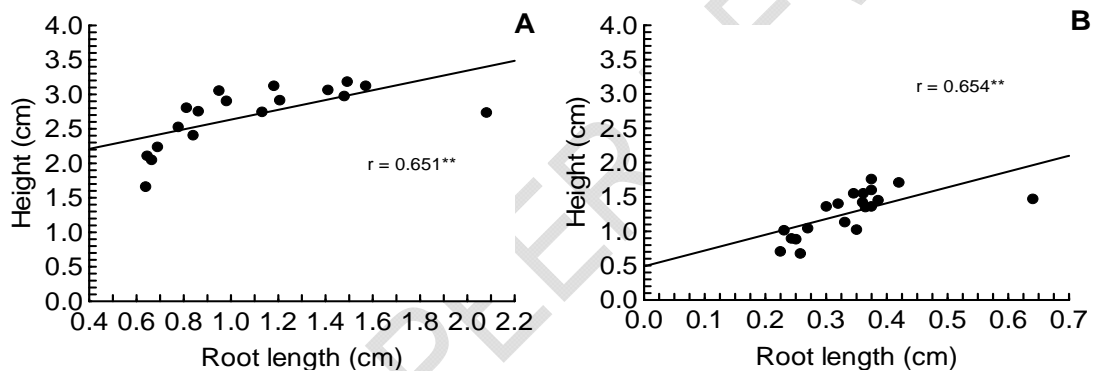
**Fig. 1. Germination (A), germination speed index(B), percentage of dry matter in relation to fresh matter (C), seedling height (D) and root length (E) in function of different levels of salinity.**

According to Figure 1B, GSI was different between the evaluated cultivars. For cultivar 1, it was practically constant, not differing between the treatments (Table 1). Conversely, a contrary effect can be observed for cultivar 2, where the decreasing curve in Figure 1B demonstrates how affected it was. These results reinforce the argument previously mentioned, regarding the cultivar 1 tolerance and the cultivar 2 susceptibility when submitted to saline environments. Studies carried out by the authors [23] confirm that the seeds tend to be vulnerable when subjected to the effects of salinity. The GSI of the seeds can be altered by a forced dormancy. The dormancy occurs due to the decrease in the water absorption by the seeds, which negatively affects the imbibition and, consequently, reduces the elongation and divisions of the cells, thus preventing the mobilization of indispensable reserves in the germination process [24,23].

The adjusted curves in Figure 1C show the behaviour of the DM%. This variable is completely associated with the water intake of the seedlings, since the higher dry mass/fresh mass ratio, the lower water content in plant tissues [25]. Results obtained by the authors [26] showed that the water consumption by lettuce plants is reduced linearly by the increase of the EC induced by the addition of salts, and it can be reduced by up to 45% when EC is raised to 10.4 dS m<sup>-1</sup>. In the present study, from the values of the DM% variable, it can be inferred that the water consumption of the cultivars was differently affected. The adjusted curves for the DM% variable show that the water consumption in cultivar 1 was not influenced by the EC increase, while in cultivar 2 it was reduced when the EC reached 5.0 dS m<sup>-1</sup>.

The growth of the seedlings' shoot and the root system was reduced with the progressive increase of the salinity (Figure 1D and 1E). Similar results were found by the authors [21] when studying the influence of salinity on two lettuce cultivars. Seedling development is affected by the decline of phytohormones levels, such as auxins, gibberellins, jasmonic acid and salicylic acid in plant tissues. This decrease is associated with the toxic effect of NaCl excess on plants [27,28]. The reduction of these plant hormones along with a decrease in the osmotic potential, caused by saline stress, results in a smaller number of cell divisions. Consequently, a lower vegetative growth of the seedlings occurs [29,27].

The linear correlation analysis (Figure 2A and 2B) showed that there is a positive correlation between root growth and shoot growth in both cultivars. It shows that, despite the negative influence of salinity on seedling development, the balance between these variables was not affected. Studies carried out by the authors [30,31, 32] in coffee, eucalyptus and potato cultivation, respectively, demonstrate the importance of good aerial and root system relationship for plants. Regarding lettuce cultivation, the relation of plant's root-shoot plays a fundamental role in the seedlings' formation. According to the authors [33], the increase in this ratio produces seedlings with superior quality, due to the better adhesion rates after transplanting.



**Fig. 2. Pearson's linear correlation between seedling height and root length for cultivar 1 (A) and cultivar 2 (B).** \*  $P < 0.05$ ; \*\*  $P < 0.001$ .

#### 4. CONCLUSION

Salinity affected root growth for both cultivars when EC was greater than  $2.8 \text{ ds m}^{-1}$ . For all the studied variables, cultivar 1 has shown better performance when compared to cultivar 2, allowing to affirm that it tolerates higher salinity rates in this phenological phase. The cultivar 2 was more susceptible to salinity since all the studied variables were statistically affected by different salt concentrations.

182 **COMPETING INTERESTS**

183 Authors have declared that no competing interest exist.

184 **AUTHORS' CONTRIBUTIONS**

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

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