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Salt water irrigation in different cultivars of lima bean[1]

Irrigação com água salina em quatro cultivares de fava

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ABSTRACT - Lima bean (*Phaseolus lunatus* L.) is a crop with great economic and social importance for the Brazilian Northeast. The water crisis in recent years has affected crop production, especially in the semi-arid region. Thus, the use of lower quality waters (saline) becomes a necessary alternative, but it can affect the growth and yield of agricultural crops. The objective of this study was to evaluate the effect of salt stress on the initial growth and biomass accumulation of four lima bean cultivars. The experiment was carried out in a protected environment in a completely randomized experimental design in a 5 x 4 factorial scheme, with four replicates. The treatments consisted of 5 salinity levels in irrigation water with electrical conductivity (ECw) of 1.0, 2.0, 3.0, 4.0 and 5.0 dS m^{-1} and four cultivars: Branquinha (C1), Manteiguinha (C2), Espírito Santo (C3) and Orelha-de-vó (C4). The variables analyzed were plant height, number of leaves, leaf area, stem diameter, root length, shoot dry mass, root dry mass and total dry mass. At the end of the experiment, electrical conductivity of the soil saturation extract was evaluated. The cultivar Manteiguinha was more tolerant to salinity than the others, considering the variables number of leaves and plant height. However, it was not possible to identify variations in the tolerance of the four lima bean cultivars based on biomass production, indicating the need for further studies that can prove the existence of genetic variability in relation to the effects of salt stress on this species

Key words: Salt stress. *Phaseolus lunatus* L. Salinity tolerance.

RESUMO - A fava (*Phaseolus lunatus* L.) é uma cultura com grande importância econômica e social para o Nordeste brasileiro. A crise hídrica nos últimos anos tem afetado a produção das culturas, principalmente na região semiárida. Desse modo, o uso de águas de qualidade inferior (salinas) torna-se uma alternativa necessária, no entanto, pode afeta o crescimento e a produtividade das culturas agrícolas. Objetivou-se avaliar o estresse salino no crescimento inicial e no acúmulo de biomassa de quatro cultivares de fava. O experimento foi realizado em ambiente protegido em delineamento experimental inteiramente casualizado em esquema fatorial de 5 x 4, com quatro repetições. Os tratamentos foram constituídos de 5 níveis de salinidade na água de irrigação com condutividade elétrica (CEa) de 1,0; 2,0; 3,0; 4,0 e 5,0 dS m^{-1} e quatro cultivares: Branquinha (C1), Manteiguinha (C2), Espírito Santo (C3) e Orelha-de-Vó (C4). As variáveis analisadas foram: altura de planta, número de folhas, área foliar, diâmetro do caule, comprimento da raiz, massa seca da parte aérea, massa seca da raiz e massa seca total. Ao final do experimento foi avaliada condutividade elétrica do extrato de saturação do solo. A cultivar Manteiguinha se mostrou mais tolerante à salinidade que as demais, considerando-se as variáveis número de folhas e altura da planta. No entanto, não foi possível identificar variações na tolerância das quatro cultivares de fava com base na produção de biomassa, indicando a necessidade de mais estudos que possam comprovar a existência de variabilidade genética em relação aos efeitos do estresse salino nessa espécie.

Palavras-chave: Estresse salino. *Phaseolus lunatus* L. Tolerância à salinidade.

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INTRODUCTION

Phaseolus lunatus L. is a Fabaceae species known as lima bean, butter bean or sieva bean, cultivated in North and South America, Europe, East and West Africa, and Southeast Asia (OLIVEIRA; TORRES; BEBEDITO, 2011), playing an important role in food and nutrition security. It is produced in the Northeast region of Brazil, achieving average yields of 299 kg ha^{-1} in Ceará and 328 kg ha^{-1} in the country (IBGE, 2018). It is a crop adapted to the semi-arid climate, and its cultivation is carried out by small producers, who predominantly use heirloom varieties of indeterminate growth (CARMO *et al*., 2013).

Lima bean is one of the four species of the genus *Phaseolus* commercially exploited and classified as moderately sensitive to salinity, with salinity threshold of 1.6 dS m^{-1}, considering the electrical conductivity of the soil saturation extract (MAAS; HOFFMAN, 1977). Compared to common bean (*Phaselous vulgaris*), lima bean varieties have greater diversity, adaptability and rusticity (AZEVEDO; FRANCO; ARAÚJO, 2003).

Water scarcity around the world leads to increasing interest in the use of lower quality waters and in multiple use of water sources, i.e., water demand is increasing due to unequal distribution and rapid socioeconomic development, particularly in arid and semi-arid regions of the world (COSTA; MEDEIROS, 2017). However, the soluble salts and sodium present in water reduce the growth and development of crops (GUIMARÃES *et al*., 2019; PEREIRA FILHO *et al*., 2017a).

Salinity in water or soil acts in the reduction of osmotic potential, hampering water uptake by the plant, causing changes in the non-selective absorption of nutrients, thus affecting growth and gas exchange (SANTOS *et al*., 2017; SILVA *et al*., 2019). When evaluating the initial growth of lima bean under different levels of irrigation water salinity, Granja *et al.* (2019), found that the increase in salts present in water inhibited the number of leaves and height of plants. In this same species, Souza *et al.* (2019), observed that salt stress affected photosynthesis, transpiration and stomatal conductance.

Salinity tolerance of crops is an important search on the part of researchers for production in arid, semi-arid and other areas, and the ability to tolerate stress varies according to genetic factors, stages of development, also involving the plant's ability to control selective absorption, preferential transport, removal of salt in the xylem, translocation in the phloem and excretion of salts (DIAS *et al*., 2016; SÁ *et al*., 2019).

Therefore, the objective was to evaluate the effect of salt stress on the initial growth and biomass accumulation of four lima bean cultivars.

MATERIAL AND METHODS

The experiment was conducted at the Auroras Seedling Production Unit (*Unidade de Produção de Mudas Auroras* - UPMA) of the University for International Integration of the Afro-Brazilian Lusophony (*Universidade da Integração Internacional da Lusofonia Afro-Brasileira* - UNILAB), Auroras Campus, Redenção-CE, Brazil, located at the following coordinates: 4°13'35'' S latitude, 38°43'53'' W longitude. The average annual temperature of the region is 26 °C and the annual rainfall is 1086 mm.

The greenhouse where the experiment was conducted is arch-type, 8 m by 15 m, covered with 150-micron agricultural diffusing plastic, with galvanized steel structure. The sides and front were covered with Sombrite® 50% shade net, with width of 16 m and length of 15 m, ceiling height of 3 m, masonry half wall, and floor filled with type 3 crushed stone.

The material used as substrate was an *Argissolo Vermelho Amarelo* (Ultisol) (EMPRESA BRASILEIRA DE PESQUISA AGROPECUÁRIA, 2018), collected in the 0-20 cm layer near the experimental area (Table 1), where several single samples were collected to form only one composite sample, which was taken to the Soil and Water Laboratory of the Department of Soil Sciences/UFC for chemical analysis, according to the recommendations of Teixeira *et al*. (2017).

Table 1 - Soil chemical characteristics

Characteristics								
BD (g cm^{-3})	pH	OM (g kg^{-1})	Ca	Mg	Na	K	V (%)	ECse (dS m^{-1})
			(cmol$_c$ kg^{-1})					
1.4	6.1	10.06	0.8	1.8	0.17	2.09	80	0.75

BD = soil bulk density; OM =Organic matter. V = Base saturation; ECse - Electrical conductivity of saturation extract

Table 2 - Total water depth applied in each treatment according to the cultivars

Treatments	Cultivars			
	Branquinha	Manteiguinha	Espirito Santo	Orelha-de-Vó
	Total levels applied (L)			
1.0 (dS m^{-1})	14.8	26.5	25.2	25.8
2.0 (dS m^{-1})	14.8	26.5	25.2	25.8
3.0 (dS m^{-1})	14.8	26.5	25.2	25.8
4.0 (dS m^{-1})	14.8	26.5	25.2	25.8
5.0 (dS m^{-1})	14.8	26.5	25.2	25.8

The genetic material used in the experiment was lima bean (*Phaseolus lunatus*, L.). Planting was carried out in March 2018 in plastic pots with volume of 11 liters, height of 0.24 m and diameter of 0.27 m, sowing five seeds per pot. At 10 days after sowing (DAS), thinning was performed leaving only two plants per pot.

The experimental design was used completely randomized in a 5 x 4 factorial scheme, corresponding to five levels of electrical conductivity (ECw) in irrigation water (1.0 dS m^{-1}; 2.0 dS m^{-1}; 3.0 dS m^{-1}; 4.0 dS m^{-1} and 5.0 dS m^{-1}) and four lima bean cultivars (Branquinha, Manteiguinha, Espírito Santo and Orelha-de-vó), coming from the UNILAB accession bank, in four replicates and three pots per experimental unit.

According to Rhoades, Kandiah and Mashali (2000), the formulation of saline water was based on the following salts: NaCl, $CaCl_2.2H_2O$ and $MgCl_26H_2O$, in the ratio of 7:2:1 between Na, Ca and Mg, following the relationship between ECw and concentration ($mmol_c$ L^{-1} = EC x 10). Irrigation started after thinning (10 DAS) with a daily frequency, according to the drainage lysimeter principle (BERNARDO *et al.*, 2019) and keeping the soil at field capacity. The volume applied in each irrigation event was estimated in one lysimeter (pot), by treatment, through the difference between volume applied and the volume drained, and the leaching fraction was applied after the beginning of the differentiation of treatments with the different levels of water electrical conductivity, to try to remove salts from the root environment (CARVALHO *et al.*, 2016), following a fixed fraction of 15% (0.15) according to Ayers and Westcot (1999).

The total irrigation depths applied during the experiment are presented in Table 2.

At 35 DAS, plants were evaluated for: plant height - PH (measured with a ruler graduated in centimeters), number of leaves - NL (fully expanded leaves were counted), leaf area - LA (Scanner method - an image digitizer (Scanner) was coupled to a microcomputer and the image was analyzed by Sigmascan® software to calculate the area), stem diameter - SD (measured with a digital caliper, expressed in cm) and root length - RL (measured with a ruler graduated in centimeters).

Subsequently, the material was collected, placed in paper bags and then dried in a forced air circulation oven at 60 °C for 72 hours. Root dry mass (RDM) and shoot dry mass (SDM) were weighed on an AG200 analytical scale, with results expressed in grams. Total dry mass (TDM) corresponded to the sum of SDM+RDM. After plant collection, the soil of each pot was homogenized and samples were used to determine the electrical conductivity of the saturation extract (ECse), following the methodology contained in Richards (1954).

Kolmogorov-Smirnov test ($p<0.05$) was applied to evaluate normality of the following variables: plant height (PH), number of leaves (NL), leaf area (LA), stem diameter (SD), root length (RL), shoot dry mass (SDM), root dry mass (RDM) and total dry mass (TDM) and electrical conductivity of soil saturation extract (ECse), evaluating normal distribution ($p<0.05$). The data were then subjected to analysis of variance and, after verifying significance, Tukey means comparison test ($p<0.05$) and regression test were performed using the program ASSISTAT 7.7 BETA (SILVA; AZEVEDO, 2016).

RESULT AND DISCUSSION

The analysis of variance presented in Table 3 reveals significant interaction between salinity x cultivar (S x C), while the leaf area variable (LA) showed significance for the individual factor cultivar (C).

There was a reduction in the number of leaves with the increase in salt concentration, and a decreasing linear model was fitted for all cultivars (Figure 1); for each unit increase in ECw, there were reductions of 7.2, 5.98, 12.65 and 8.33% for the cultivars Branquinha, Manteiguinha, Espírito

Table 3 - Summary of the joint analysis of variance (F values) for plant height (PH), leaf area (LA), number of leaves (NL) and stem diameter (SD) of four lima bean cultivars as a function of different levels of salinity

SV	DF	F			
		PH	LA	NL	SD
Treatments	19	0.10**	1901.88^{ns}	23.12**	0.73**
Salinity (S)	4	0.11^{ns}	1423.71^{ns}	75.89^{ns}	1.69^{ns}
Cultivars (C)	3	0.17**	5301.42**	12.55**	0.95**
SxC	12	0.08**	1211.39^{ns}	8.17**	0.35**
Resíduo	60	0.02	1.089.41	2.13	0.06
Total	79	-	-	-	-
Overall mean	-	1.12	117.00	9.01	3.24
CV%	-	13.95	28.21	16.19	7.77

SV= source of variation, CV= coefficient of variation, DF= degrees of freedom, **Significant at 1% probability level (P<0.01). *Significant at 5% probability level (P=<0.05). ns = not significant

Santo and Orelha-de-vó, respectively. For the values of lowest (1.0 dS m^{-1}) and highest (5.0 dS m^{-1}) salinity, there were reductions in the number of leaves of 41.10%, 27.28%, 57.91% and 36.31% for the cultivars Branquinha, Manteiguinha, Espírito Santo and Orelha-de-vó, respectively.

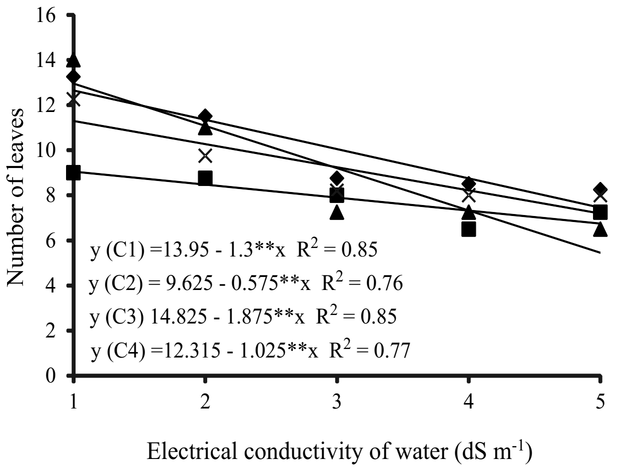
This result reveals superiority of the cultivar Manteiguinha, that is, evidencing greater tolerance to the decrease in cell elongation rate and low water absorption caused by excess soluble salts (SILVA *et al*., 2019).

Corroborating the results obtained, Granja *et al*. (2019) worked with the lima bean crop (Raio-de-sol and Rajada varieties) irrigated with saline water and found that the number of leaves decreased linearly as a function of the increase in salt stress. Similarly, Pereira Filho *et al.* (2017a), evaluating the initial growth of cowpea plants subjected to salt stress, verified a linear reduction in the number of leaves.

The increase in irrigation water electrical conductivity caused a linear reduction in plant height (Figure 2). The unit increase in ECw caused reductions of 6.2, 2.03, 6.11 and 8.05%, for the cultivars Branquinha, Manteiguinha, Espírito Santo and Orelha-de-vó, respectively. The values from the lowest (1.0 dS m^{-1}) to the highest (5.0 dS m^{-1}) salinity showed reductions in plant height of 25%, 8.34%, 25.64% and 34.64% for the cultivars Branquinha, Manteiguinha, Espírito Santo and Orelha-de-vó, respectively.

The cultivar Manteiguinha was the most tolerant for this variable, that is, possibly it adapted better with the decrease in the osmotic potential of the soil solution, resulting from salinity, causing at some point the plant to not lose its capacity to absorb water, preventing it from undergoing plasmolysis (DIAS *et al*., 2016).

Figure 1 - Number of leaves of different lima bean cultivars, (Branquinha (C1♦), Manteiguinha (C2■), Espírito Santo (C3▲) e Orelha-de-vó (C4×)) irrigated with saline water



Pereira Filho *et al*. (2017a), evaluated the effect of salinity on cowpea crop and also verified a reduction in plant height with the increase in the electrical conductivity of irrigation water. Silva *et al.* (2019), studied cotton crop irrigated with saline water and observed that salt stress inhibited plant height.

Figure 3 represents the variation in stem diameter of the plants under the different salt concentrations, showing the fit of a quadratic polynomial model for the cultivars Branquinha, Manteiguinha and Orelha-de-vó, with minimum values of 3.25, 2.66 and 2.91 cm at ECw levels of 2.72, 2.83 and 3.24 dS m^{-1}, respectively. The data of Espírito Santo were described by a decreasing linear model, where the unit increase in ECw caused reduction of 8.85%. There was a reduction of 41.10% between the extreme values of salinity (1.0 to 5.0 dS m^{-1})

The reduction in diameter as a function of the salt concentration can be justified by the fact that the plant activated its mechanism of survival in the surrounding medium, since the presence of soluble salts in the soil solution reduces the osmotic potential, increasing the water retention forces and causing the reduction in water and nutrient absorption by the plant, which results in low vegetative growth (GUIMARÃES *et al*., 2019; SANTOS *et al*., 2011). Similarly, Granja *et al.* (2019), also observed reduction in stem diameter when studying the effect of salt stress on lima bean (cultivar Espírito Santo).

Figure 2 - Plant height of different lima bean cultivars, (Branquinha (C1♦), Manteiguinha (C2■), Espírito Santo (C3▲) e Orelha-de-vó (C4×)) irrigated with saline water

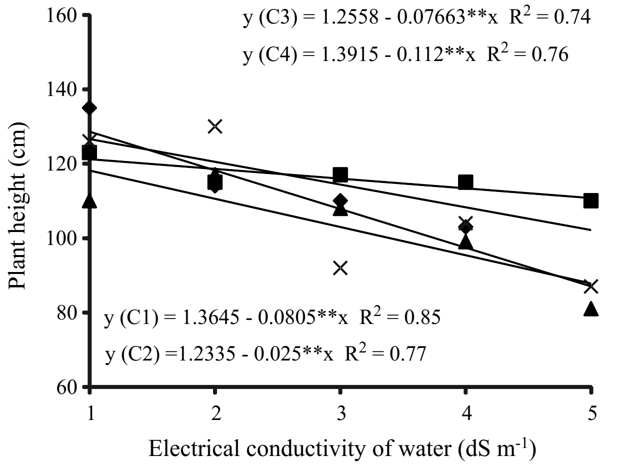
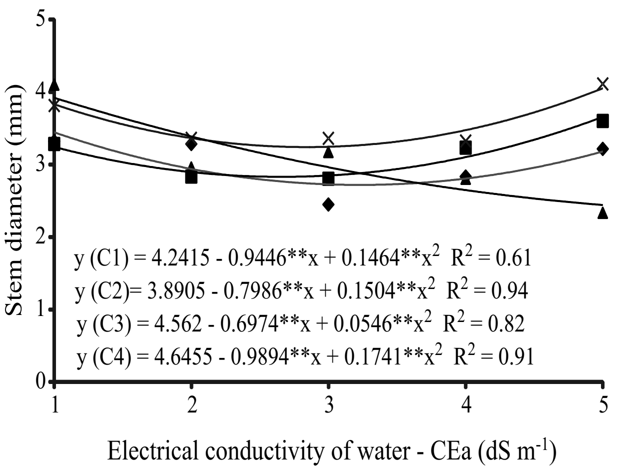


Figure 3 - Stem diameter of different lima bean cultivars, (Branquinha (C1♦), Manteiguinha (C2■), Espírito Santo (C3▲) e Orelha-de-vó (C4×)) irrigated with saline water



Regarding the cultivar Espírito Santo, it is worth mentioning that, despite the negative effects caused by salts on plants, the crops or even the varieties of the same species respond differently, some being more tolerant than the others, that is, they are influenced by the differences in the phenological stages within the same species.

The values presented in Figure 4 show that there was statistical difference only between the cultivars Manteiguinha and Espírito Santo, with the lowest values of leaf area obtained for the cultivar Manteiguinha. The results found for the cultivars can be explained by the rusticity of the species, where the dynamics of the cultivars comes from the mixture of heirloom accession lines, which can result in large differences in genotypes, being one of the possible explanations for the differences between varieties with larger or smaller leaf area.

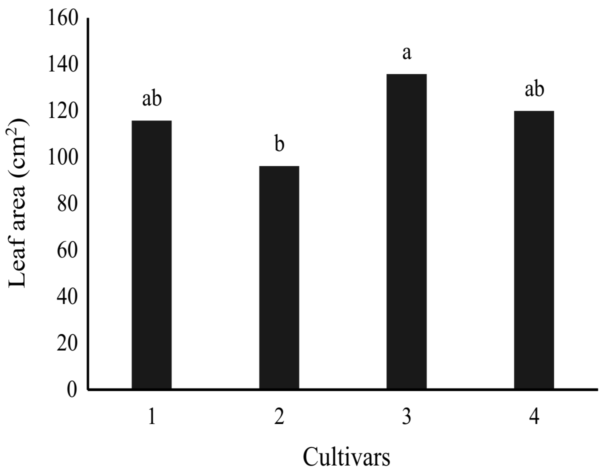
The results show that the cultivars Branquinha, Espírito Santo and Orelha-de-vó adapted better compared to Manteiguinha. This morphophysiological adaptation may result in greater plant efficiency in photosynthetic processes and in the transport of organic solutes in plant tissues (PEREIRA FILHO *et al*., 2019).

The analysis of variance presented in Table 4 reveals that there was significant interaction between salinity and cultivar for electrical conductivity of the soil saturation extract at 1 and 5% significance levels by the F test.

Figures 5A and 5B show that the cultivar Manteiguinha differed statistically from the others, having the lowest mean values for SDM and TDM accumulation. For RDM, the cultivar with the lowest result was Orelha-de-vó (Figure 5C).

The difference of the variables presented between the cultivars may have occurred due to the genetic variability among them, which come from a mixture of

Figure 4 - Leaf area of different lima bean cultivars (Branquinha (C1); Manteiguinha (C2), Espírito Santo (C3); Orelha-de-vó (C4)). Means followed by the same letter do not differ by Tukey test ($p<0.05$)



strains. These results may also have been influenced by the protected environment, corroborating Santos *et al.* (2011), who worked with cowpea and found that plants grown in protected environments tend to elongate longitudinally due to the abundant presence of the red-light spectrum.

Table 4 - Summary of the analysis of variance for root length (RL), shoot dry mass (SDM), root dry mass (RDM), total dry mass (TDM) and electrical conductivity of soil saturation extract (ECse) of four lima bean cultivars as a function of different levels of salinity

SV	DF	Mean square				
		RL	SDM	RDM	TDM	ECse
Treatments	19	43.40**	0.83**	0.10*	1.14**	1.44**
Salinity (S)	4	131.70^{ns}	2.43^{ns}	0.22^{ns}	3.82^{ns}	5.62^{ns}
Cultivars (C)	3	43.15**	0.95**	0.21**	0.97*	0.57**
SxC	12	14.02^{ns}	0.27^{ns}	0.03^{ns}	0.29^{ns}	0.26*
Resíduo	60	8.41	0.16	0.03	0.25	0.12
Total	79	-	-	-	-	-
Overall mean	-	16.95	1.44	0.68	2.08	1.05
CV%	-	17.11	28.00	24.64	23.79	32.79

SV= source of variation, CV= coefficient of variation, DF= degrees of freedom, **Significant at 1% probability level (P<0.01). *Significant at 5% probability level (P=<0.05). ns = not significant

Figure 5 – Shoot dry mass (A), root dry mass (B) and total dry mass (C) of different lima bean cultivars (Branquinha (C1); Manteiguinha (C2), Espírito Santo (C3); Orelha-de-vó (C4)). The means followed by the same letter do not differ by the Tukey test (p<0.05)

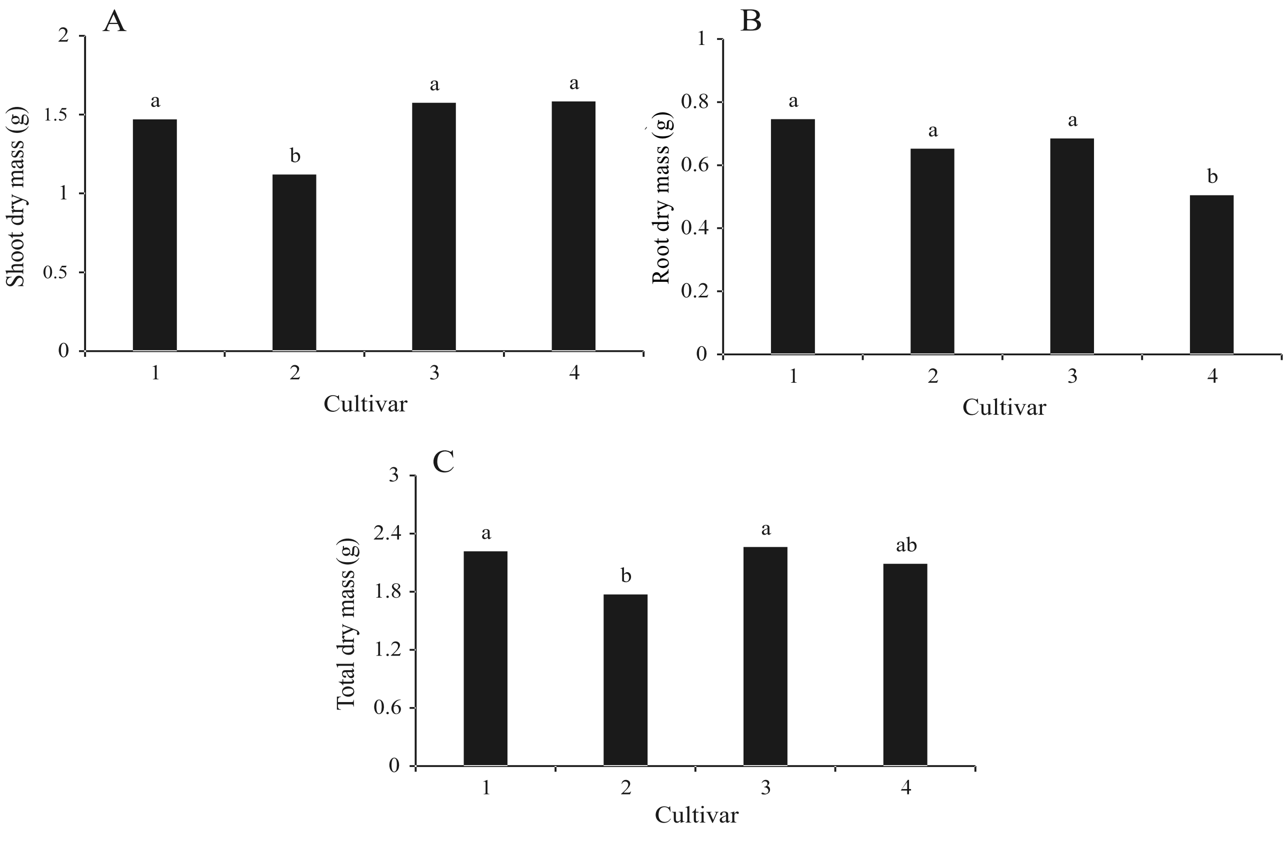


Figure 6 shows the mean values for root length. The cultivars Branquinha, Manteiguinha and Espírito Santo were significantly superior to Orelha-de-vó.

This superiority for root length among these cultivars may be related to morphology and adaptation under pot conditions or to the utilization of soil fertility, as observed by Souza *et al*. (2019) in lima bean (cultivar Milagrosa).

The different levels of irrigation water electrical conductivity linearly increased the electrical conductivity of the soil saturation extract (Figure 7). The increase in electrical conductivity of the soil saturation extract (ECse) was caused by the accumulation of salts applied during irrigation. In other words, despite this increase in ECse, these values are lower than expected, which may have been evidenced by the leaching fraction applied and the time of application of the saline waters (25 DAS).

Figure 6 - Root length of different lima bean cultivars (Branquinha (C1); Manteiguinha (C2), Espírito Santo (C3); Orelha-de-vó (C4)). Means followed by the same letter do not differ by Tukey test (p<0.05)

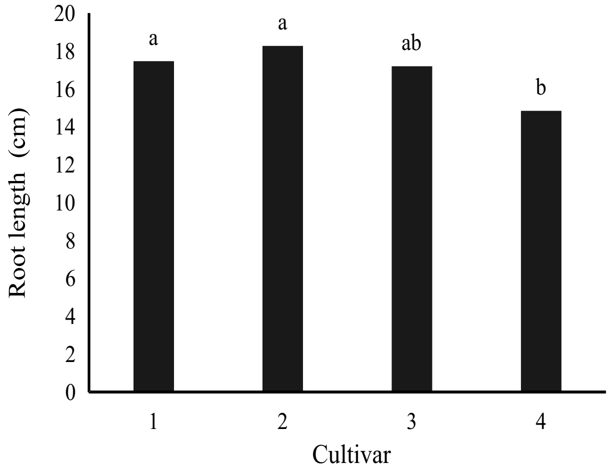
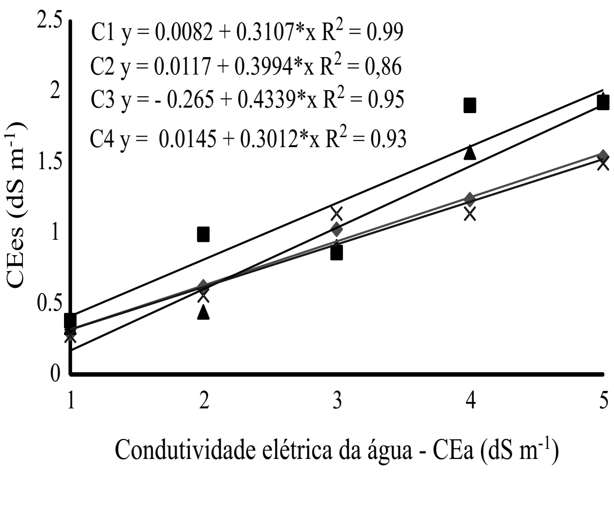


Figure 7 - Electrical conductivity of soil saturation extract for different lima bean cultivars (Branquinha (C1♦), Manteiguinha (C2■), Espírito Santo (C3▲) and Orelha-de-vó (C4×)) irrigated with saline water



Corroborating the present study, Fonseca *et al*. (2016), evaluated cowpea crop irrigated with saline water and found an increase in the electrical conductivity of the soil saturation extract. Similar behavior was observed by Pereira Filho *et al.* (2017b), in the same type of soil as that of the present study (*Argissolo Vermelho-Amarelo* - Ultisol), when irrigating the cowpea crop with water of increasing salinity.

CONCLUSIONS

The cultivar Manteiguinha was more tolerant to salinity than the others, considering the variables number of leaves and plant height. However, it was not possible to identify variations in the tolerance of the four lima bean cultivars based on biomass production, indicating the need for further studies that can prove the existence of genetic variability in relation to the effects of salt stress on this species.

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