

## LIMA BEAN (*Phaseolus lunatus* L.) SEEDS EXPOSED TO DIFFERENT SALT CONCENTRATIONS AND TEMPERATURES<sup>1</sup>

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**ABSTRACT** - Salinity problems are increasing in the semi-arid regions of northeastern Brazil. Thus, detailed studies of the region's cultured crops, primarily salt-tolerant species, are important. Therefore, the aim of the present study was to evaluate the salt stress tolerance of lima bean (*Phaseolus lunatus* L.) seeds at different temperatures. This study was conducted in the Seed Analysis Laboratory, Agricultural Sciences Center, Federal University of Paraíba (Areia, PB, Brazil) by using seeds of four lima bean cultivars (Branca, Orelha de Vó, Rosinha, and Roxinha) in a randomized experimental design. Sodium chloride (NaCl) was used as a solute for salt-stress simulation at the following concentrations: 0.0 (control), 1.5, 3.0, 4.5, 6.0, 7.5, and 9.0 dS m<sup>-1</sup>; temperatures were maintained constant at 25°C, 30°C, and 35°C. Germination and vigor tests were performed to evaluate the treatment effects (first count, germination rate index, seedling length, and dry matter). Germination rate of lima bean seeds increased in salt concentrations up to 9 dS m<sup>-1</sup>, and seeds of Roxinha cultivar exhibited the highest salinity tolerance. The development of lima bean cultivar seedlings was negatively affected at 35°C under high-salinity conditions.

**Keywords:** Lima bean. Germination. Vigor. Abiotic stress.

## SEMENTES DE *Phaseolus lunatus* L. SUBMETIDAS A CONCENTRAÇÕES SALINAS E DIFERENTES TEMPERATURAS

**RESUMO** - A problemática da salinidade está aumentando cada vez mais nas regiões semiáridas do nordeste brasileiro, por isso estudos aprofundados com culturas cultivadas nessas regiões principalmente que sejam tolerantes a níveis de salinidades são importantes. Nesse sentido, o objetivo com este trabalho foi avaliar a tolerância de sementes de cultivares de feijão-fava (*Phaseolus lunatus* L.) a condição de estresse salino em diferentes temperaturas. O trabalho foi realizado no Laboratório de Análise de Sementes do Centro de Ciências Agrárias da Universidade Federal da Paraíba, em Areia, PB, com sementes de quatro cultivares de feijão-fava (Branca, Orelha de Vó, Rosinha e Roxinha), em delineamento experimental inteiramente ao acaso. Para simulação do estresse salino, utilizou-se como soluto o cloreto de sódio (NaCl), nas concentrações de 0,0 (testemunha); 1,5; 3,0; 4,5; 6,0; 7,5 e 9,0 dS m<sup>-1</sup> submetidas a temperaturas constantes de 25, 30 e 35 °C. Na avaliação do efeito dos tratamentos, realizou-se teste de germinação e vigor (primeira contagem e índice de velocidade de germinação, comprimento e massa seca de plântulas). A germinação de sementes dos cultivares de feijão-fava (*Phaseolus lunatus* L.) é elevada quando submetidas a concentrações salinas de até 9 dS m<sup>-1</sup>, sendo as sementes do cultivar Roxinha as mais tolerantes à salinidade simulada com cloreto de sódio. A temperatura de 35 °C não favorece o desenvolvimento das plântulas em condições salinas para todos os cultivares avaliados.

**Palavras-chave:** Feijão-fava. Germinação. Vigor. Estresse abiótico.

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## INTRODUCTION

*Phaseolus lunatus* L. is commonly known as lima bean; however, it is referred to by different named depending on the cultivation regions in Brazil, e.g., feijoa, bongue, mangalô-amargo, fava-Belém, fava-terra, feijão-espadinho, feijão-farinha, feijão figado de galinha, or feijão-favona (LOPES et al., 2010). This species is economically and socially important, owing to its hardiness with extended harvests during the dry period (AZEVEDO; FRANCO; ARAÚJO, 2003). In northeastern Brazilian, the lima bean is an important alternative income and food source (GUIMARÃES et al., 2007), with a planted area of 24,581 ha producing 7,693-ton annually (IBGE, 2015).

*P. lunatus* is one of the main leguminous plants of the semi-arid regions of northeastern Brazil, including the states of Paraíba, Rio Grande do Norte, Ceará, Sergipe, Piauí, and Minas Gerais. Frequent soil salinization is noted at these locations owing to the elevated salt content of the irrigation water associated with high temperatures and intense evaporation (DJILIANOV et al., 2003).

Among external factors, stress frequently results in negative effects in plants (TAIZ; ZEIGER, 2013) and each stress component affects plant growth depending on various factors such as plant species, cultivar, phenological stage, environment salinity, and stress intensity and duration (CRAMER; ALBERICO; SCHMIDT, 1994). Most culture productivity is also affected by salt, osmotic, and temperature stresses, which has impaired agricultural expansion in various regions worldwide (SONG; LEI; TIAN, 2005).

Salt stress, owing to high sodium or other cation concentrations in solution, affects element availability and thus indirectly affects plant development (ZANANDREA et al., 2006). Germination of seeds exposed to different salt concentrations was used as an indicator of species tolerance to salinity; thus, germination and vigor tests are useful to determine the physiological responses of species under salt stress (DANTAS; RIBEIRO; ARAGÃO, 2005).

Some studies have shown that germination and initial seedling growth were negatively affected by salt stress in some species, e.g., *P. vulgaris* L. (COELHO et al., 2010; DEUNER et al., 2011) and *Albizia lebeck* (L.) Benth. (LIMA et al., 2015).

Therefore, the aim of the present study was to evaluate the salt tolerance of lima bean seeds exposed to salt stress at different temperatures.

## MATERIAL AND METHODS

This study was conducted in the Seed Analysis Laboratory of the Agricultural Sciences

Center of the Federal University of Paraíba, Areia, PB, Brazil, using seeds of four lima bean cultivars (Branca, Orelha de Vó, Rosinha, and Roxinha), obtained from the 2014 yield of Queimadas, PB, Brazil. Seeds were obtained and then taken to the laboratory, where they were homogenized, packed in plastic bags, and refrigerated for 45 days.

Sodium chloride (NaCl) was used as the solute for salt-stress simulation at the following concentrations: 0.0 (control), 1.5, 3.0, 4.5, 6.0, 7.5, and 9.0 dS m<sup>-1</sup>. Dilution was performed in distilled and deionized water, and the electrical conductivity of the solutions was measured using a conductivity meter. Only distilled and deionized water were used for substrate moistening in the control treatment, and the electrical conductivity values of the NaCl solutions were obtained using Richards' equation (1954):  $SC = 0.001 (EC_s - EC_{an}) EW / 0.097$ , where: SC = salt concentration (g L<sup>-1</sup>); EC<sub>s</sub> = electrical conductivity of the mixture at 25°C (dS m<sup>-1</sup>); EC<sub>an</sub> = electrical conductivity of the water (dS m<sup>-1</sup>); EW = equivalent weight of the salt, and 0.97 = estimated purity (%) of sodium chloride.

For the germination test of each treatment, 200 seeds treated with Captan® fungicide were used (240 g fungicide 100 kg<sup>-1</sup> seeds), and were divided into 4 × 50 seed groups and distributed over two paper towel sheets, covered with a third sheet, and wrapped in rolls. The towel paper was moistened using the previously mentioned NaCl solutions at three times the dry paper towel mass, with no additional moistening. The control treatment was moistened with distilled and deionized water at the same amount. Rolls were packed in 0.04 mm thick transparent plastic bags to avoid evaporation.

Germination was performed using a *Biological Oxygen Demand* (B.O.D.) germinator calibrated for 25°C, 30°C, and 35°C constant temperatures, and an 8-h photoperiod using daylight type fluorescent bulbs (4 × 20W). Evaluations were performed daily after test establishment for 9 days, when the experiment was finished and the following criteria were obtained: number of seeds that emitted primary root and aerial parts (normal seedlings), and percentage of abnormal seedlings (PAS).

The germination rate index (GRI) was calculated during the germination test and daily normal seedling counts were carried out at the same time of the day every day from day 5 to 9. The index was calculated through Maguire's equation (1962):  $GRI = G_1/N_1 + G_2/N_2 + \dots + G_n/N_n$ ; where: GRI = germination rate index, G<sub>1</sub>, G<sub>2</sub>, and G<sub>n</sub> = number of germinated seeds on the first, second, and last counts, N<sub>1</sub>, N<sub>2</sub>, and N<sub>n</sub> = number of days from sowing to the first, second, and last counts.

At the end of the experiment, the seedling length was measured for each treatment. Then, they were weighed and packed in Kraft paper bags, and dried using an oven with air circulation and

exchange at 65°C until a constant weight was obtained (72 h) for dry mass measurement. After drying, samples were weighed using an analytical balance with 0.001 g accuracy and results were expressed as grams (g).

A randomized experimental design was adopted, and treatments were distributed in a  $7 \times 3 \times 4$  factorial arrangement (osmotic potentials temperatures and cultivars) with four repetitions. Obtained data was submitted to variance analysis and converted into  $\sqrt{x+0.5}$  through F-Test at 5% probability for the following variables: germination rate index, dry mass of root and aerial parts, and abnormal plants. Qualitative data was submitted to

means tests, whereas quantitative data was evaluated using a polynomial regression analysis through SISVAR® software (FERREIRA, 2007).

## RESULTS AND DISCUSSION

Among lima bean seeds, the Roxinha cultivar presented the highest physiological potential, with better germination and vigor results in the first count. The lowest physiological quality was observed in the Branca cultivar, which presented the lowest value for all evaluated characteristics, except for the germination rate index (Table 1).

**Table 1.** Initial seed quality of four lima bean (*Phaseolus lunatus* L.) cultivars characterized by germination (G), first germination count (FGC), germination rate index (GRI), aerial part length (APL), primary root length (RL), aerial part dry mass (APDM), root dry mass (RDM), and abnormal seedlings (PAS).

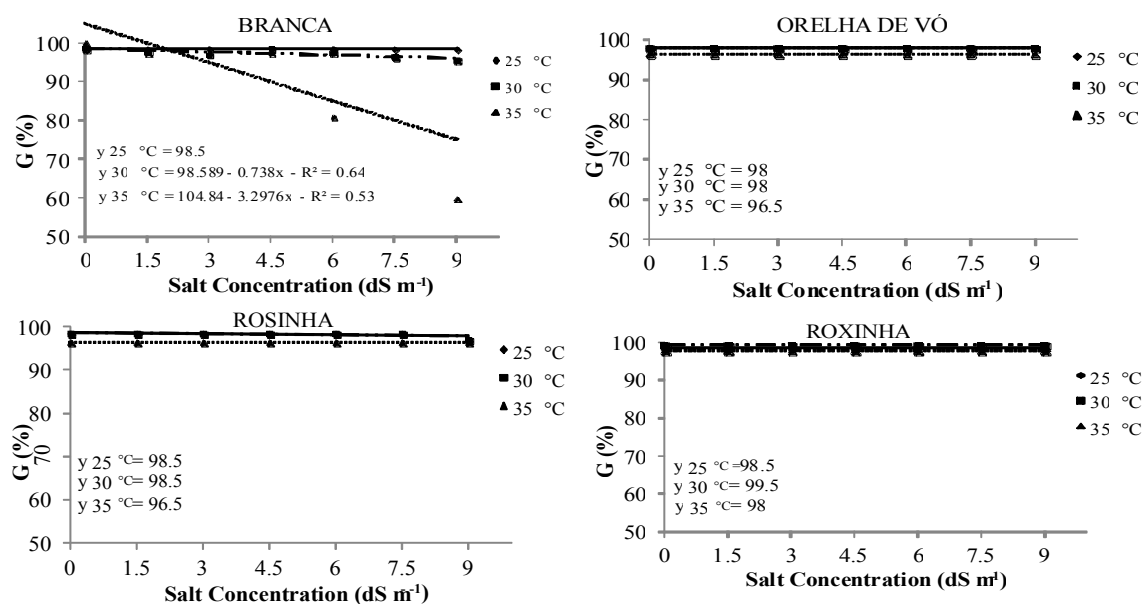
Cultivar	G	FGC	GRI	APL	RL	APDM	RDM	PAS (%)
	%			cm	g			
Branca	95 c	95 c	12.40 a	1.15 c	1.57 c	0.721 c	0.715 c	1.81 a
Orelha de Vó	97 b	97 b	12.61 a	1.18 c	1.88 b	0.827 b	0.834 b	1.71a
Rosinha	97 b	96 b	12.66 a	1.68 a	2.23 a	1.098 a	1.121 a	1.61 a
Roxinha	99 a	98 a	10.57 a	1.59 b	2.22 a	0.732 c	0.717 c	1.27 b
CV (%)	4.1	4.52	18.17	15.92	20.23	19.17	20.45	47.22

\*Means followed by the same letter in columns do not differ by Scott-Knott's test at 5% probability.

Seed germination percentage (Figure 1) of the majority of lima bean cultivars was >96% at all evaluated temperatures and salt concentrations; however, this value began to decrease for Branca cultivar seeds at 35°C and 1.5 dS m<sup>-1</sup>. Germination percentage did not change among different concentrations at 25°C and 30°C. These results demonstrate that Branca cultivar seeds are more

salt-stress sensitive when exposed to higher temperatures, since the salt accumulation in their tissues possibly increases the abscisic acid (ABA) activity, which reduces seed germination.

Yu et al. (2016) evaluated the association of ABA with salinity in *Arabidopsis thaliana* seeds and observed that ABA negatively affects seeds germination.



**Figure 1.** Germination of lima bean (*Phaseolus lunatus* L.) cultivar seeds under different salt concentrations at different temperatures.

Plants growing in saline environments withstand low osmotic potential values (solutes), which significantly reduce the water potential of cells and enable an excessive salt entry (TAIZ; ZEIGER, 2013). In addition, metabolite repair or accumulation may occur along the osmotic adjustment, improving germination during stress, e.g., saline and dry periods, and along the entire treatment (HAGHPANAH; YOUNESI; MORADI, 2009).

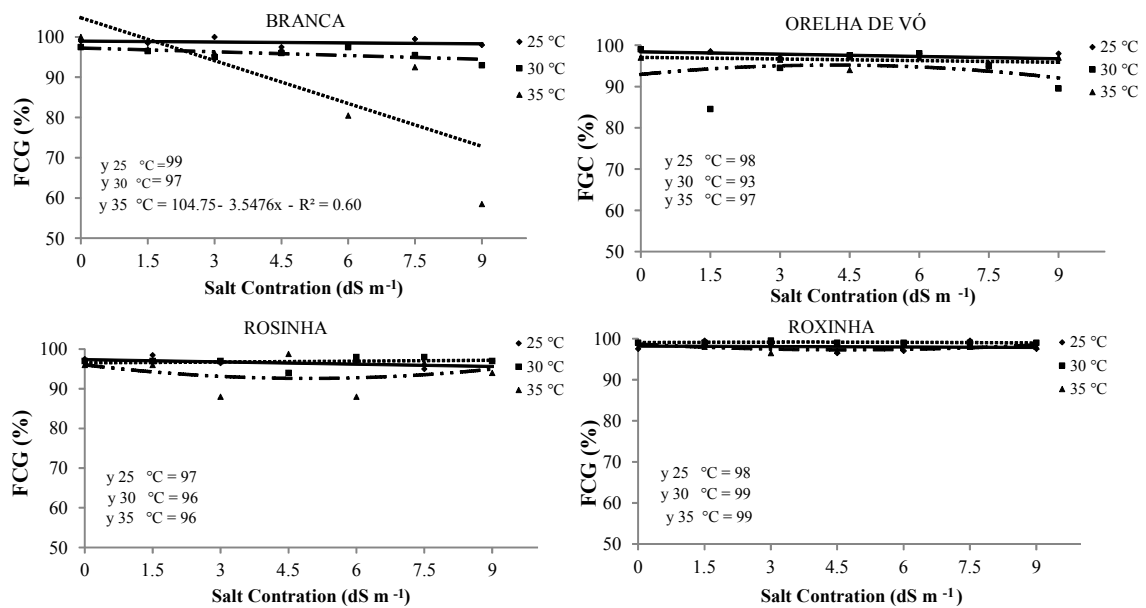
Another aspect that may be associated with this process is cross-tolerance, which releases stress responses to a specific or various stresses, when the seed is exposed to a specific stress (RIZHSKY; LIANG; MITTLER, 2002). Thus, seed and seedling performance is positively affected by environmental stresses, which can induce cross tolerance and favor the development and performance of mature plants (SCHUCH et al., 2013).

Only the percentage of seeds that germinated was negatively affected in the Branca cultivar at 35°C, corroborating a study conducted by Larcher (2000), who observed germination delays or a decrease in the number of germinated seeds depending on the salt concentration and species'

tolerance. Flamboyant seeds (*Delonix regia* (Bojer ex Hook.) Raf.) presented a maximum emergence percentage of 74% at 0.5 dS m<sup>-1</sup> with a progressive decrease at 1.5 dS m<sup>-1</sup> and above; however, differences occurred only at 6.0 dS m<sup>-1</sup> (NOGUEIRA et al., 2012). Arugula seeds (*Eruca sativa* Mill.) germinated under saline conditions up to -0.4 MPa, showed a significant germination reduction at higher concentrations (ARAÚJO et al., 2014).

The first germination count data from Branca cultivar seeds only fitted the linear regression model when exposed to 35°C, with 100% germination at 0.0 dS m<sup>-1</sup>; however, there was a decrease in germination at salinities above 1.5 dS m<sup>-1</sup>. An average 97%+ germination was obtained for 25°C and 30°C, thus not fitting any regression models and presenting the highest percentages at 25°C (Figure 2). This seeds of cultivars are possibly unable to present satisfactory development under saline conditions and high temperatures.

The first germination count of Orelha de Vó, Rosinha, and Roxinha cultivars seeds exposed to salt concentrations did not fit polynomial regression models and presented averages varying from 93% to 99% in each temperature (Figure 2).



**Figure 2.** First germination count of lima bean (*Phaseolus lunatus* L.) cultivar seeds under different salt concentrations at different temperatures.

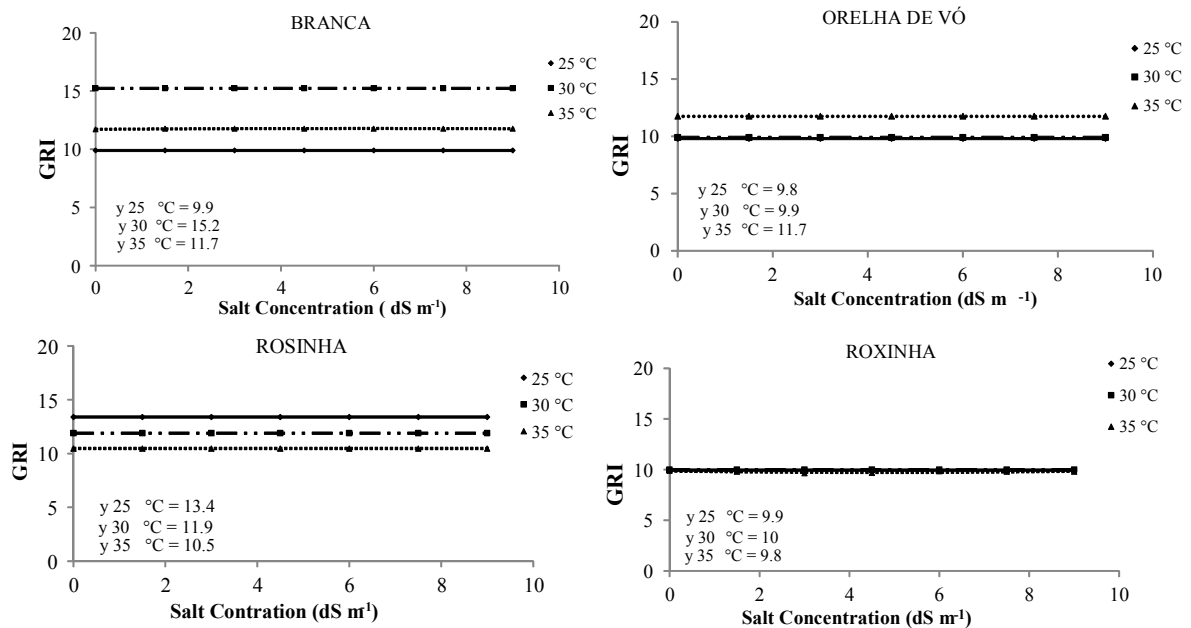
Most studies involving salinity reported that the presence of salt affects the water potential of the soil and/or substrates, resulting in a decreased soil-seed surface potential gradient, and restraining water absorption (LOPES; MACEDO, 2008). Excessive diluted salt cause salinity and toxicity problems, impair water absorption, and result in cellular and molecular damage in the majority of glycophytes (TAIZ; ZEIGER, 2013). Thus, it is important to understand the survival ability of these plants under saline conditions. One of the most used

methods for determining high salt tolerance is the first germination count percentage (OLIVEIRA et al., 2007). Metabolite repair or accumulation could occur along an osmotic adjustment with enhanced germination under stress, e.g., salinity and dry periods, during the entire treatment (HAGHPANAH; YOUNESI; MORADI, 2009).

Salt concentrations that impair lima bean growth vary widely among varieties depending on salt type, exposure period, and development stage (DEUNER et al., 2011). The germination rate index

of the cultivar seeds did not fit polynomial regression models when exposed to salt concentrations; however, Branca, Orelha de Vó, and

Rosinha cultivar seeds presented the highest values at the following temperatures 30 (15.2); 35 (11.7), and 25°C (13.4) (Figure 3).



**Figure 3.** Germination rate index of lima bean (*Phaseolus lunatus* L.) cultivar seeds under different salt concentrations at different temperatures.

The germination rate index is proportional to seed vigor and these plants are the most salinity-sensitive; thus, even the lowest salt concentration may result in a decreased in the production quantity and quality of seeds (LOPES et al., 2014). Temperature may also cause stress and lead to differences in germination and vigor, determined by first count and germination rate index, and depending on the seed's physiological quality, i.e., its physiological maturity degree during harvest, or membrane deterioration level (MARINI et al., 2012).

Seed vigor, evaluated using the germination rate index, of various species exposed to salt stress was negatively affected, e.g., fava d'anta (*Dimorphandra mollis* Benth.) (MASETTO et al., 2014), niger (*Guizotia abyssinica* (L.f.) Cass.) (GORDIN et al., 2012), and cucumber (*Cucumis sativus* L.) (MATIAS et al., 2015).

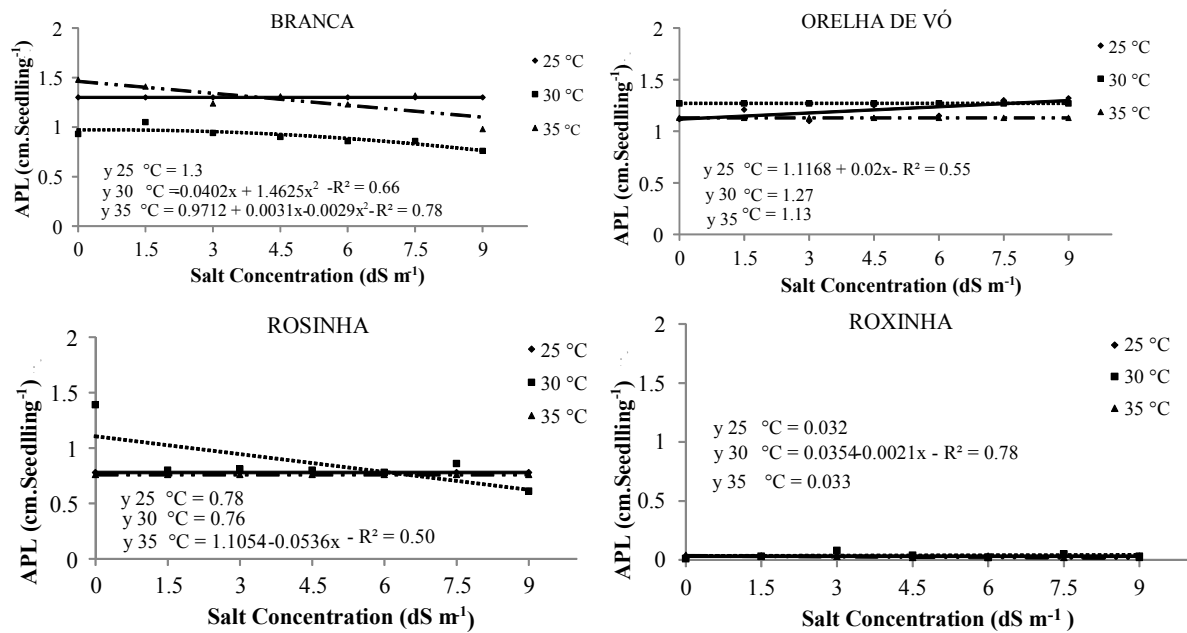
The initial aerial length of Branca cultivar seedlings (Figure 4), of which seeds were exposed to 30°C in accordance to the derivative of function, was 1.44 cm; there was a 1.87 cm increase at 0.0 concentration and 35°C with a posterior decrease, whereas length was uniform at all salt concentrations at 25°C with 1.3 cm average. Orelha de Vó cultivar presented average values of 1.27 cm at 30°C, 1.13 cm at 35°C and 1.11 cm at 25°C, and small aerial part length increases associated with salt concentration increases; Rosinha and Roxinha cultivars presented a short length (<1.1 cm).

In addition to damaging the physico-chemical

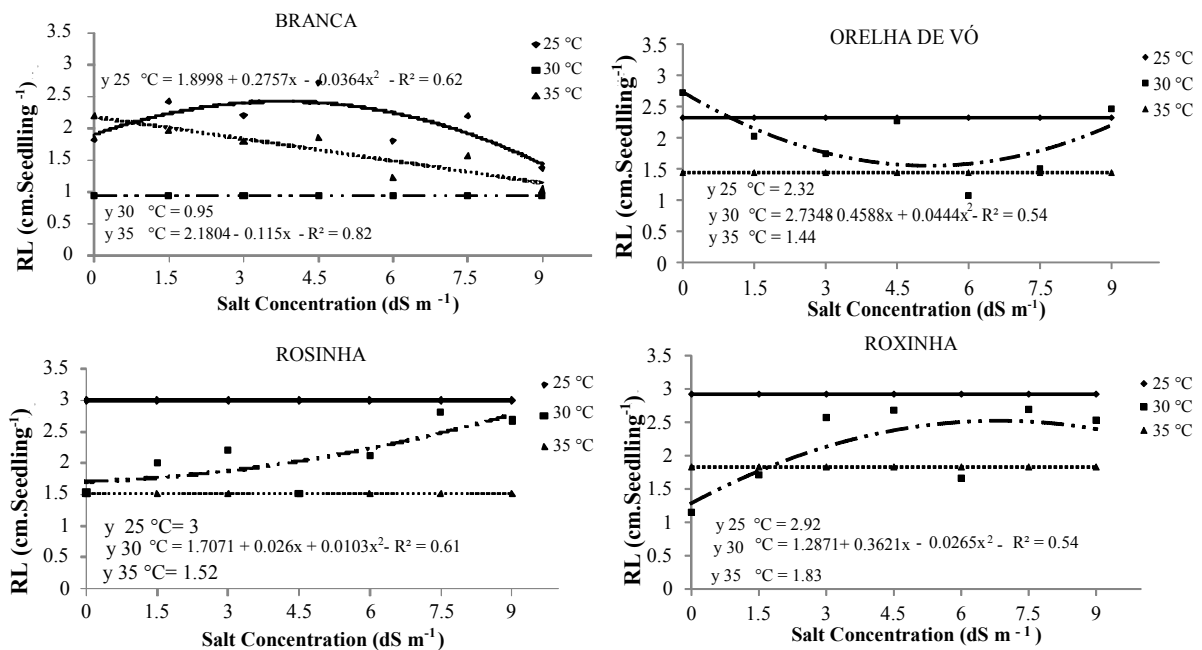
properties of soil, sodium salt excess leads to a generalized reduction of plant growth that causes serious losses in agriculture (CAVALCANTE et al., 2010). Furthermore, it may lead to the inhibition of growth in most species (TOBE; XIAOMING; OMASA, 2000) owing to the osmotic effect, causing physiological drought and toxicity, resulting from protoplasm's ion concentration.

These results are observed in most species owing to a salt excess in the rhizosphere of seedlings, which resulted in lower water availability and water absorption by roots; thus, osmotic pressure increases and inhibits cell expansion and division (MAHAJAN; TUTEJA, 2005).

The root length of Branca cultivar seedlings was determined through the derivative of the function: 2.25 cm at 3.13 dS m<sup>-1</sup> and 25°C. A decrease was observed at 30°C and higher concentrations with a 0.95 cm average, while length reached 2.18 cm at 35°C (Figure 5). Orelha de Vó cultivar's length remained constant among all concentrations at 25°C and 35°C and 2.32 and 1.44 cm, respectively. A minimum of 0.063 cm was observed at 30°C and 0.084 dS m<sup>-1</sup>. Regarding Rosinha and Roxinha cultivars, uniformity was observed at 25 and 35°C (3 and 1.52 cm; and 2.92 and 1.82 cm, respectively); however, differences were observed between both cultivars at 30°C, reaching the minimum length value at 1.26 dS m<sup>-1</sup> (Rosinha) and the maximum of 2.52 cm at 6.83 dS m<sup>-1</sup> (Roxinha).



**Figure 4.** Aerial part length of lima bean (*Phaseolus lunatus* L.) cultivar seedlings obtained from seeds exposed to different salt concentrations at different temperatures.



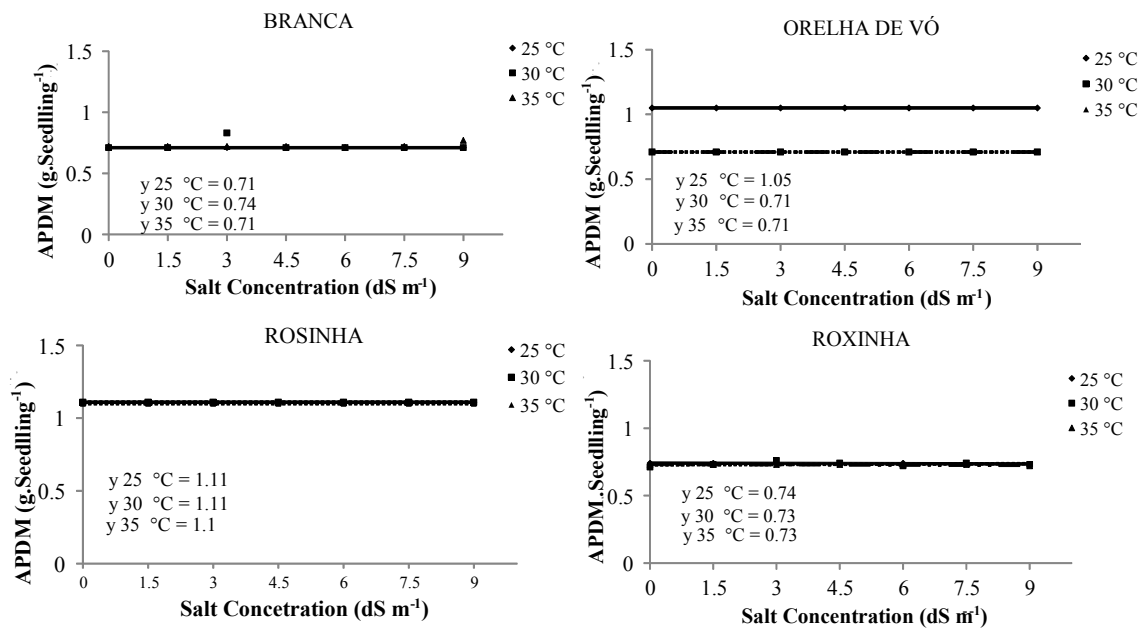
**Figure 5.** Primary root length of lima bean (*Phaseolus lunatus* L.) cultivar seedlings obtained from seeds exposed to different salt concentrations at different temperatures.

Studies evaluating watermelon (*Citrullus lanatus* (Thunb.) Matsum. and Nakai) seeds exposed to salt stress using NaCl reported longer seedling root lengths until the -8.0 MPa potential with a subsequent decrease, owing to salt becoming harmful for this species (SOUSA; SILVA; SIMON, 2009).

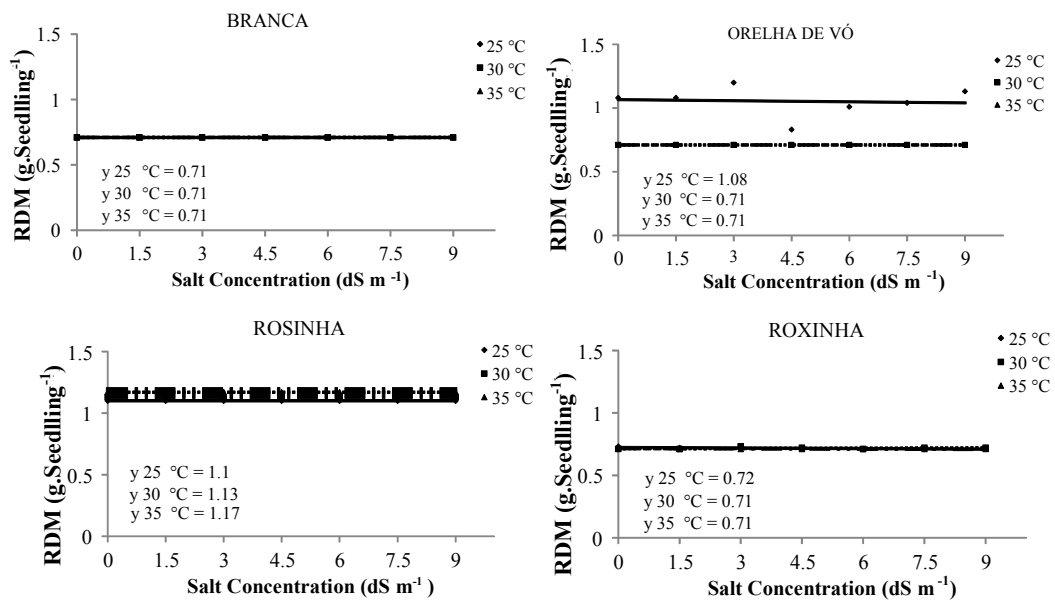
Aerial part and root dry mass of lima bean cultivar seedlings were not affected by increases of salt concentrations to which their seeds were exposed; however, temperatures caused a slight variation (0.71 and 1.11 g; and 0.71 and 1.17 g) in both mentioned varieties. Under these conditions, a

decrease in biomass was observed, because all cultivars presented dry seedlings weights below 3 g (Figures 6 and 7).

Melon cultivar (*Cucumis melo* L.) seedlings exposed to salt stress presented a reduction of wet and dry mass at salinity levels above 4 dS m<sup>-1</sup>; however, there was a lower reduction for Sancho and Gaúcho cultivars, which are more salt stress tolerant (ARAGÃO et al., 2009). The aerial part of the dry mass of the flamboyant (*Delonix regia* (Bojer ex Hook.) Raf.) decreased as salinity levels increased, and a greater decrease was observed at 10.8 dS m<sup>-1</sup> NaCl (CHAVES et al., 2013) and above.



**Figure 6.** Aerial part of the dry mass of lima bean (*Phaseolus lunatus* L.) cultivar seedlings obtained from seeds exposed to different salt concentrations at different temperatures.

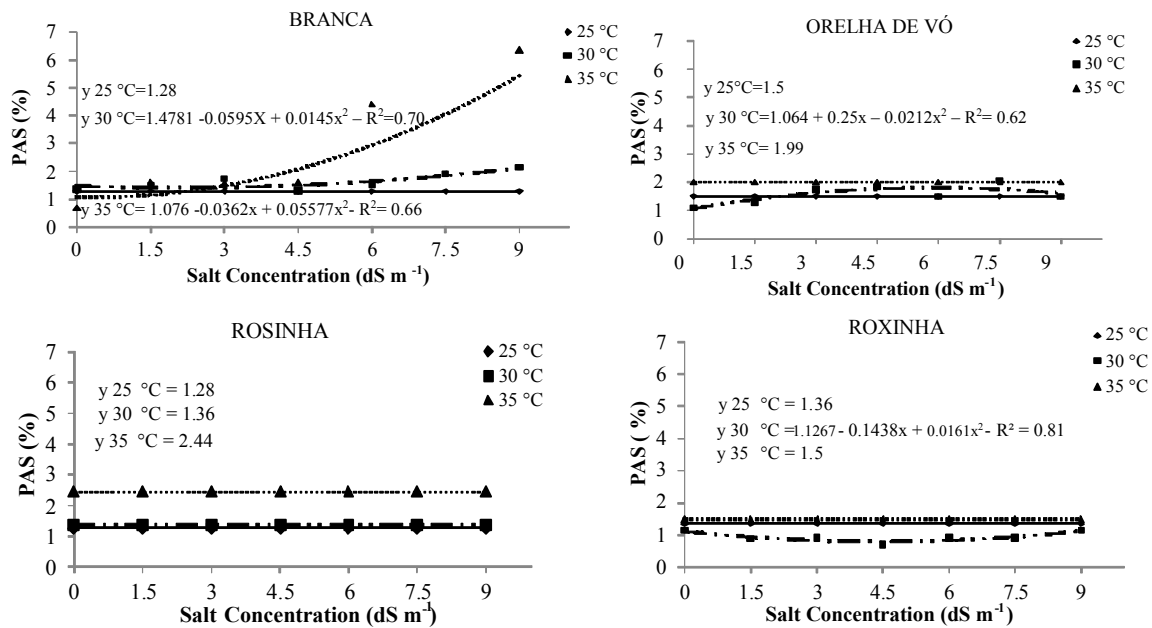


**Figure 7.** Root dry mass of lima bean (*Phaseolus lunatus* L.) cultivar seedlings obtained from seeds exposed to different salt concentrations at different temperatures.

The percentage of abnormal seedlings was 1% for the Branca cultivar at 30°C and 35°C and at least 2 and 1% abnormal seedlings were observed at 2.0 and 0.31 dS m<sup>-1</sup> (Figure 8). Respective 1.5 and 2% were obtained for Orelha de Vó cultivar at 25°C and 35°C, whereas a maximum of 1.80% was obtained at 30°C and 5.89 dS m<sup>-1</sup>. Uniformity was observed in the Rosinha cultivar under different salt concentrations at different temperatures: 1.28% at 25°C, 1.36% at 30°C, and 2.44% at 35°C. The minimum percentage of Roxinha cultivar was 1% at

30°C and 4.46 dS m<sup>-1</sup>; percentages were constant for both other temperatures at all salt concentrations: 1.36% at 25°C and 1.5% at 35°C.

The highest percentage of abnormal watermelon (*Citrullus lanatus* (thumb) Masnf.) plants was observed at 16.7 and 22.7 dS m<sup>-1</sup> osmotic potentials, with NaCl as the solute (TORRES, 2007). The -1.2 MPa osmotic potential dramatically reduced the percentage of normal corn plants owing to decreased water absorption by seeds (KAPPES et al., 2010).



**Figure 8.** Abnormal seedlings of lima beans (*Phaseolus lunatus* L.) obtained from seeds exposed to different salt concentrations at different temperatures.

## CONCLUSION

Lima bean cultivar seed germination is not affected by salt concentrations up to 9 dS m<sup>-1</sup>; Roxinha cultivar seeds are the most tolerant to the simulated sodium chloride salinity; and seedling development of all evaluated cultivars was not enhanced under saline conditions at 35°C.

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