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Physiological potential of *Phaseolus lunatus* L. seeds subjected to water stress at different temperatures

Potencial fisiológico de sementes de *Phaseolus lunatus* L. submetidas a estresse hídrico em diferentes temperaturas

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Abstract

Germination is negatively influenced by water scarcity and by temperatures above or below the optimal range of the species, so it is necessary to know the critical point of absorption for the species or cultivar. The objective of this work was to evaluate the effect of water stress, simulated by solutions of polyethylene glycol 6000 (PEG 6000) at different temperatures, on the germination and vigor of seeds of four cultivars (Branca, Orelha de Vó, Rosinha and Roxinha) of beans (*Phaseolus lunatus* L.). The water stress simulation was performed using PEG 6000 solutions at concentrations of 0.0, -0.2, -0.4, -0.6, -0.8, -1.0, and -1.2 MPa at temperatures of 25, 30, and 35 ° C. The analyzed variables were percentage of germination at first count, germination speed index, length of aerial part and primary root of seedlings, and dry mass of the respective parts of the seedlings. The seeds of the cultivar Orelha de Vó were more sensitive to water stress, with 88% germinating up to the potential of -0.6 MPa at the tested temperatures. The cultivars Branca, Rosinha, and Roxinha germinated (94, 100, and 100%, respectively) up to the potential of -0.8 MPa. Water stress simulated by PEG (6000) negatively affects seed twinning and seed vigor of bean cultivars.
Key words: Lima beans. Water stress. Germination. Vigor.

Resumo

A germinação é influenciada negativamente pela escassez de água e por temperaturas abaixo ou acima da faixa exigida pela espécie, por isso é necessário saber qual o ponto crítico de absorção das espécies ou cultivares. O objetivo nesse trabalho foi avaliar os efeitos do estresse hídrico, simulado com soluções de polietileno glicol 6000 (PEG 6000) em diferentes temperaturas, na germinação e vigor de sementes de quatro cultivares (Branca, Orelha de Vó, Rosinha e Roxinha) de feijão-fava (*Phaseolus lunatus* L.). Foram submetidas a simulação do estresse hídrico foi feita com soluções de PEG 6000, nas concentrações de 0,0; -0,2; -0,4; -0,6, -0,8, -1,0 e -1,2 MPa, nas temperaturas de 25, 30 e 35 °C. As variáveis analisadas foram percentuais de germinação e primeira contagem, índice velocidade de germinação, comprimentos de parte aérea e raiz primária de plântulas e massa seca das respectivas partes de plântulas. As sementes do cultivar Orelha de Vó são mais sensíveis ao estresse hídrico, germinando 88 % até o potencial de -0,6 MPa na temperatura, os cultivares Branca, Rosinha e Roxinha germinaram (94; 100 e 100

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%, respectivamente) até o potencial de -0,8 MPa. Estresse hídrico simulado por PEG (6000) afeta negativamente a geminação das sementes e o vigor das sementes de cultivares de feijão-fava.
Palavras-chave: Feijão-fava. Estresse hídrico. Germinação. Vigor.

Introduction

Seed germination may be influenced by several external factors. Seed hydration is considered the most important factor, since water intake triggers most of the biochemical and physiological processes that result in the protrusion of the primary root (BRAY, 1995). The use of tools to simulate natural stress conditions allows preliminary evaluation of the adaptation and survival limits of the species (GUEDES et al., 2013).

Studies that were carried out to evaluate the vigor of seeds of several species were conducted under conditions of simulated water deficiency (FORTI et al., 2009). For this, polyethylene glycol 6000 (PEG 6000), whose formula is $HOCH_2(CH_2OCH_2)_nCH_2OH$, is the mostly used osmotic conditioning solute because it is chemically inert and non-toxic to the seeds (QUEIROZ et al., 1998).

The sensitivity of the species to salinity or water stress is differentiated, and is determined by the speed and/or percentage of germination, as well as by seedling formation, because the osmotic potential of a solution makes it difficult for the water to be absorbed, being inferior to the osmotic potential of the embryo cells (CARVALHO; NAKAGAWA, 2012).

Another environmental element that influences germination is temperature, because it influences the speed of absorption and distribution of water by the seed and the rate of biochemical reactions. When varying temperatures are added to the effects of water stress, it interferes with the biochemical reaction rate, influencing the physiology of the seeds and consequently the formation of seedlings (CASTRO et al., 2004; MARCOS FILHO, 2015).

The effect of temperature on plants occurs on the vegetative stages, since growth and mainly the expansion of the cells are dependent on cell water content. In an environment with reduced temperature, there may be a decrease in the water potential gradient between the intracellular spaces and the ambient atmosphere (LIMA et al., 2016). An increase in temperature can cause several disorders that can lead to the rupture of the cell membrane, causing leakage of intracellular electrolytes (SILVA et al., 2015).

The increase in the intensity of climate change, which causes the reduction of water availability has necessitated the adoption of agricultural species that are water efficient (ARAÚJO et al., 2018). Thus, studies carried out to evaluate the behavior of seeds of *Phaseolus* during germination under osmotic conditions at different temperatures and how this relationship interferes with germination are of fundamental importance. Physiologically, the main interest in studying the dependence on isothermal germination is the search for limiting and partial factors in the processes of seed germination (LABOURIAU, 1983).

Different *Phaseolus* genera can be grown in a wide range of environmental conditions. However, all cultivars of *Phaseolus vulgaris* may be susceptible to a certain degree of abiotic stress during the period of germination (COELHO et al., 2010).

The lima bean (*Phaseolus lunatus* L.) is a species of great economic and social importance in Brazil due to its rusticity, with prolonged harvests throughout the dry season, especially in the Northeast, and it has been established as an important alternative source of food and income for the population (AZEVEDO et al., 2003; GUIMARÃES et al., 2007). According to data from IBGE (2018), in 2013, *P. lunatus* planting, due to its potential as a protein source, reached an area of

25,542 hectares, with a production of 7,957 tons (SOARES et al., 2010).

In common beans, the percentage of normal seedlings in the first germination count was reduced by 60% when the seeds were subjected to PEG 6000 osmotic solutions of -0.05 MPa (MORAES et al., 2005). However, it is worth noting that reduction in seed germination and vigor depends on extrinsic and intrinsic factors such as genotype, plant age and intensity and duration of the stress (OLSOVSKA et al., 2016).

Therefore, this work was carried out with the objective of evaluating the effect of water stress, simulated with polyethylene glycol 6000 solutions, and different temperatures on seed germination and vigor of *Phaseolus lunatus.*

The work was carried out with seeds of four cultivars of lima bean (Branca, Orelha de Vó, Rosinha and Roxinha), after packing them inside plastic bags and storing in a refrigerator for a period of five months. In the water stress simulation, osmotic solutions of polyethylene glycol 6000 (PEG 6000) were used at concentrations of 0.0 (control), -0.2, -0.4, -0.6, -0.8, -1.0, and -1.2 MPa, according to the table proposed by Villela et al. (1991).

Germination test – The germination test was conducted with four replications of 50 seeds each that were previously treated with the active ingredient, captan, in the proportion of 240 g 100 kg^{-1} of seeds for each cultivar and treatment. The seeds were distributed over two sheets of paper towel, covered with a third sheet of paper towel and arranged in a roll form. The paper was pre-sterilized in an autoclave at 120 °C for 30 minutes (BRASIL, 2009) and then moistened with water (control treatment) or PEG 6000 solutions at the above concentrations by an amount equivalent to three times its dry mass, without subsequent moistening. The rolls were wrapped in transparent plastic bags to avoid the loss of water by evaporation (COIMBRA et al., 2007) and placed in germination chambers of the type Biological Oxygen Demand (BOD), which were regulated to the constant temperatures of 25, 30 or 35 °C, and a photoperiod of 8 hours using daylight fluorescent lamps (4 x 20 W). The percentage of germination was determined nine days after the start of the test (BRASIL, 2009), those seeds that had emitted primary roots and aerial parts (normal seedlings) were considered as germinated seeds, which also determined the percentage of abnormal seedlings.

First germination count – The first germination count was conducted in conjunction with the germination test by counting the normal seedlings (primary root and aerial part present) on the fifth day after the implementation of the test, with the results expressed as a percentage (BRASIL, 2009).

Germination speed index (GSI) - was performed in conjunction with the germination test by daily counts of the normal seedlings from the fifth to the ninth day of the test at the same time each day. The GSI was calculated using the formula proposed by Maguire (1962) ($GSI = \frac{G_1}{N_1} + \frac{G_2}{N_2} + \ldots + \frac{Gn}{Nn}$), where GSI = germination speed index; G1, G2, and G_n = number of seeds germinated in the first count, second count and last count; N_1, N_2, and N_n = number of days after sowing in the first, second and last counts.

Length and dry mass of seedlings – This was determined at the end of the experiment, the normal seedlings of each replication had their roots and aerial parts measured separately with a ruler graduated in centimeters, the results were expressed as cm $seedlings^{-1}$. After being measured, the roots and aerial parts of each replication were placed in kraft paper bags and conditioned in an oven at 65 °C for 72 hours. After this period, the samples were weighed on an analytical balance with an accuracy of 0.001 g, and the results were expressed in g $seedlings^{-1}$ (NAKAGAWA, 1999).

Statistical procedure - the experimental design was a completely randomized design, with the treatments distributed in a factorial scheme 4 × 7 ×

3 (cultivars, osmotic potentials and temperatures). The data were submitted for analysis of variance by the F-test at 1% probability and polynomial regression by the software SAS® 9.1.3. (2000-2004).

The analysis of variance of the data referring to the variables to evaluate the germination and vigor of seeds of different cultivars of *Phaseolus lunatus* submitted to different temperatures and osmotic potentials is summarized in Table 1. The interaction between the factors (cultivars x temperature x osmotic potentials) was significant at 1% or 5% by the F-test for all variables studied.

Table 1. Mean squares of the analysis of variance for germination (G), first germination count (FGC) and germination speed index (GSI) of seeds, aerial part length (APL) of primary root (PR), dry mass of the aerial part (DMAP) and of the roots (DMR) of normal seedlings, and percentage of abnormal seedlings (PAN) of cultivars of *Phaseolus lunatus* L. at different temperatures and osmotic potentials

Source of variation	Mean Squares								
	L	**G**	**GC**	**GSI**	**APL**	**PR**	**PDMAP**	**DMR**	**PAN**
Cultivar (C)	3	1.56**	1.102**	458.185**	4.3378**	74.024**	0.009*	0.011*	131.644**
Temperature (T)	2	0.081**	0.029**	3.350ns	5.239**	108.634**	0.018*	0.020*	26.434**
Potential (P)	6	11.070**	10.367**	18.976**	19.648**	17.881**	0.034**	0.031**	30.767**
C x T	6	0.067**	0.047**	15.961*	0.893**	24.994**	0.023**	0.028**	18.501**
C x P	18	0.260**	0.172**	140.715**	0.739**	4.057**	0.020**	0.026**	19.696**
T x P	12	0.107**	0.106**	35.002**	1.603**	17.246**	0.015**	0.019**	58.398**
C x T x P	36	0.046**	0.038**	21.678**	0.248**	5.914**	0.022**	0.027**	47.311**
Residues	252	0.005	0.004	6.817	0.093	0.0593	0.003	0.004	90.308
CV (%) =		12.793	12.896	37.609	45.716	30.492	32.340	41.629	29.776

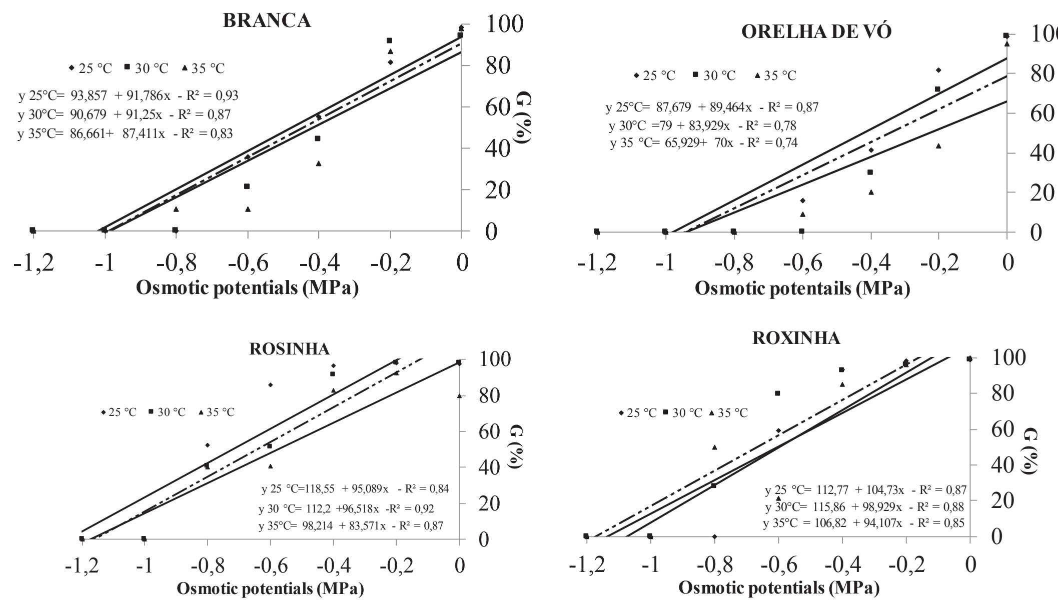
nsnot significant, *significant at 5% and, **significant at 1% of probability of F.

Figure 1 shows that the cultivars Rosinha and Roxinha obtained 100% of germination, regardless of the temperature tested; whereas, the cultivars Branca and Orelha de Vó obtained higher (94 and 88%, respectively) and lower (87 and 66%, respectively) germination percentages at the temperatures of 25 and 35 °C, respectively. In relation to water stress, there was a reduction in seed germination in all evaluated cultivars from the osmotic potential -0.2 MPa; however, the cultivar Orelha de Vó was shown to be more sensitive to water stress, with no germination occurring at the potential of -0.6 MPa at the temperatures of 25, 30, and 35 °C.

The seeds performed better at the less negative osmotic potentials, probably due to slower imbibition, allowing the reorganization of the embryo tissues over a longer period of time (LIMA; MARCOS FILHO, 2010). The decrease in germination under water stress conditions is attributed to the reduction in enzymatic activity, which promotes less meristematic development (PELEGRINI et al., 2013).

Seeds of the common bean (*Phaseolus vulgaris* L.) submitted to water stress simulated with PEG 6000 solution showed a reduction in germination at the osmotic potential -0.4 MPa (FORTI et al., 2009). Similarly, *P. vulgaris* seeds of the cultivar IAC-Carioca 80SH were also severely affected by the osmotic potential of -0.9 MPa of the PEG 6000 and NaCl solutes, with values of 0 and 15% for germination, respectively (MACHADO NETO et al., 2006).

Figure 1. Seed germination (G) of lima bean cultivars submitted to water stress at different temperatures.



Seed germination and initial development of seedlings of the cowpea (*Vigna unguiculata*), BRS Tumucumaque cultivar, were negatively affected by water stress simulated with PEG 6000 and mannitol at potentials -0.2 and -0.8 MPa, respectively, and with greater severity when induced by PEG-6000 (FERREIRA et al., 2017).

In the first germination count (Figure 2), a drastic linear reduction was observed from the osmotic potential of -0.2 MPa for all evaluated cultivars. The highest percentages obtained in the control (0.0) occurred in the Rosinha cultivar with 100% at temperatures 25 and 35 °C and the Roxinha cultivar with 100% at 30 °C. The lowest percentage occurred in the cultivar Orelha de Vó with 61.75% at the temperature of 35 °C.

This result can be explained by the fact that water stress causes a decrease in the percentage of germination as well as the speed of germination of the seeds, showing that for each species, there is a value and a water potential in the substrate where germination does not take place (BEWLEY et al., 2013).

In the first count, the vigor of common bean seeds remained around 90% using PEG 6000 up to the potential of -0.2 MPa, but showed a drastic decrease after this potential (MORAES et al., 2005).

The seed germination speed index of the Branca, Orelha de Vó and Roxinha cultivars was linearly reduced from the osmotic potential starting at -0.2 MPa at all temperatures tested. However, the Rosinha cultivar showed a quadratic adjustment with a 9.32 increase at a temperature of 35 °C at the osmotic potential of -0.44 MPa (Figure 3).

This occurred because of the water stress tolerance characteristics peculiar to each cultivar. Similar to what occurred for lima bean seeds, Carneiro et al. (2011) observed that the germination speed index of sunflower seeds (*Helianthus annuus* L.) decreased as the osmotic potentials became more negative.

Cell dehydration reduces the metabolic activity and the synthesis of new tissues in the seeds, due to the reduction of water availability. Based on the time of exposure to water stress and different temperatures, and the genetics of the cultivars, a plant species can demonstrate its resilience to adverse conditions (TAIZ; ZEIGER, 2013; MARCOS FILHO, 2015).

Figure 2. First seed germination count (FGC) of lima bean cultivars submitted to water stress at different temperatures.

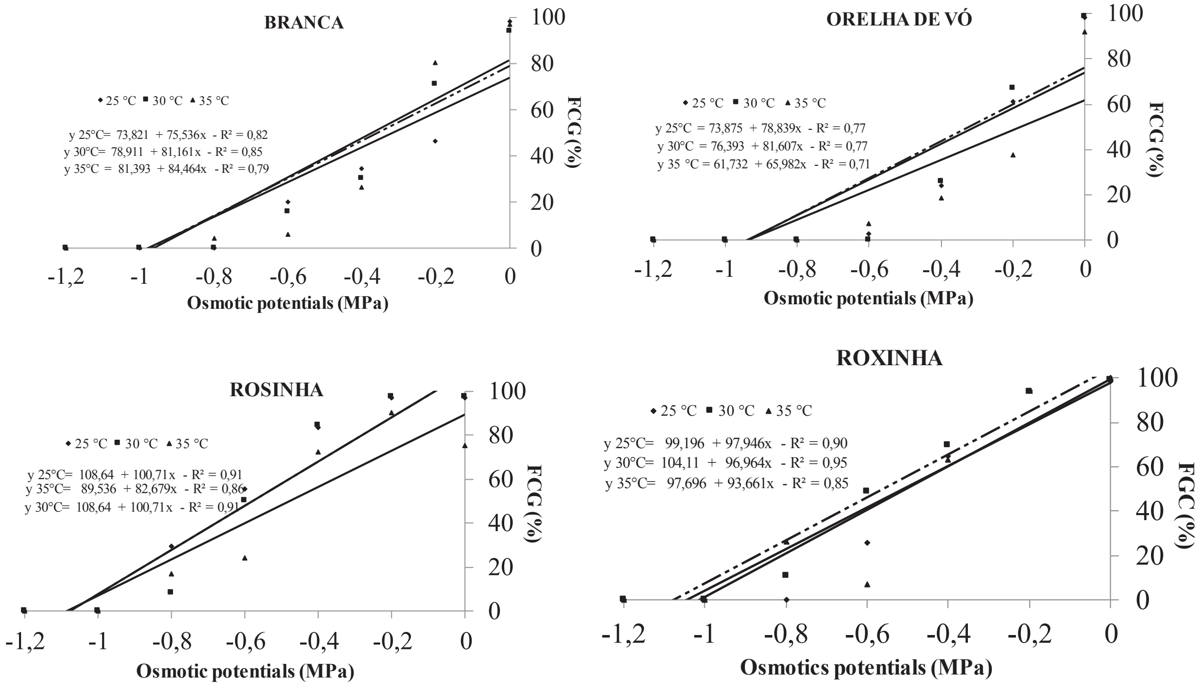
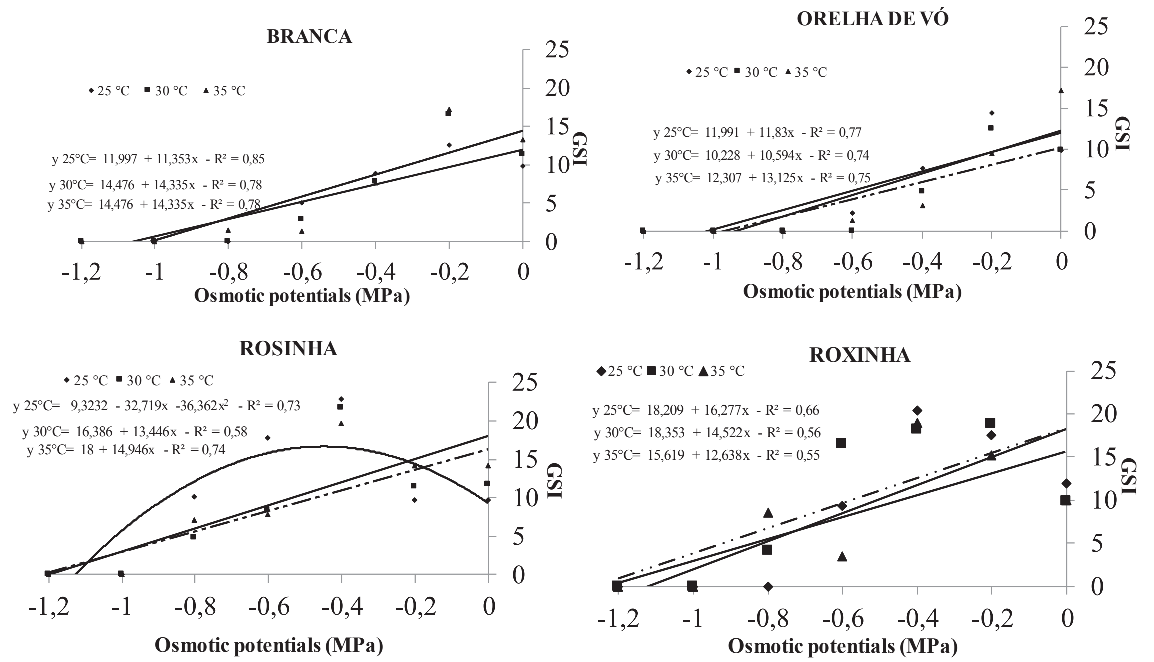
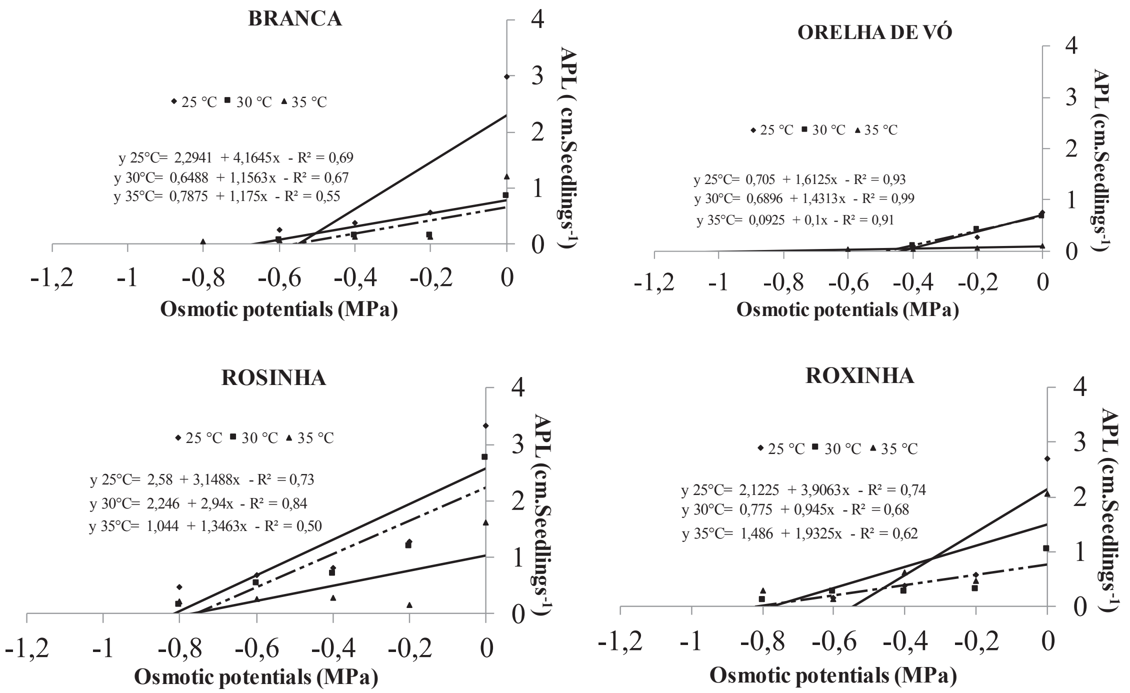


Figure 3. Seed germination speed index (GSI) of lima bean cultivars submitted to water stress at different temperatures.



The length of the aerial part of lima bean seedlings from seeds of all evaluated cultivars was linearly reduced at all temperatures and osmotic potentials (Figure 4). The length of the root of the seedlings of Rosinha seeds was longer at the temperatures of 25 and 30 °C (8.7 and 8.8 cm, respectively), while at 35 °C, Roxinha was the cultivar that stood out, with the primary root measuring 4.4 cm; these values were obtained from the equations at zero potential, but were drastically reduced when the potential became more negative (Figure 5).

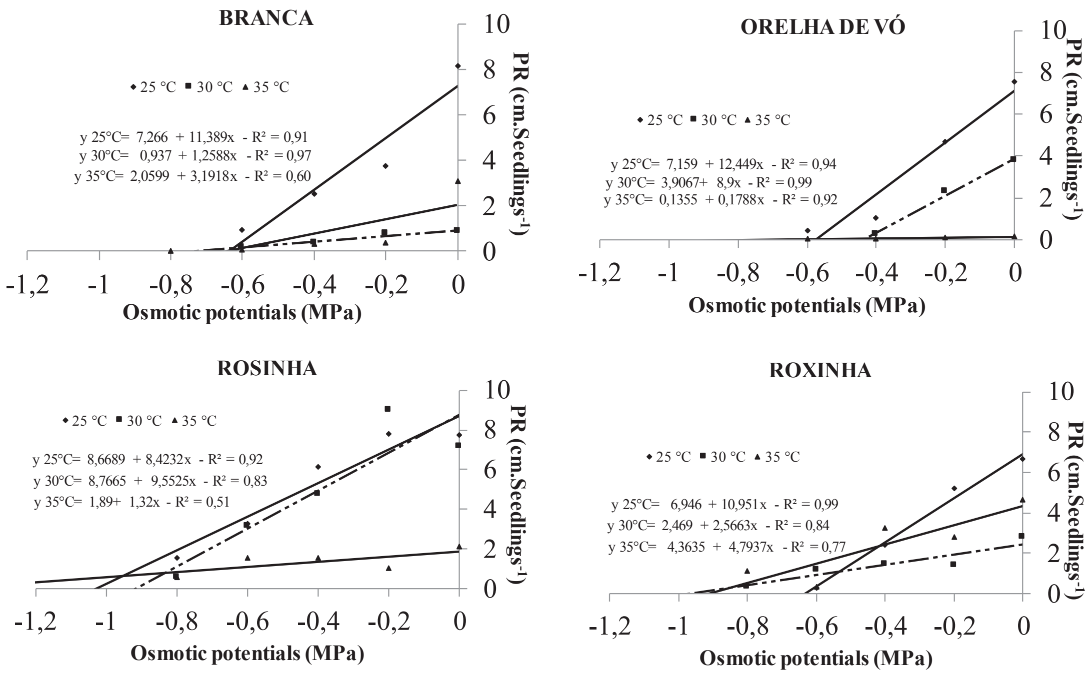
Figure 4. Aerial part length (APL) of the seedlings of lima bean cultivars from seeds submitted to water stress at different temperatures.



This reduction is because water deficits combined with temperature influence the processes related to growth, cell elongation and wall synthesis, causing a decrease in cell expansion, which affects the growth process of the seedlings (DANTAS et al., 2017).

Changes in plant behavior due to water deficiency depend on the cultivar, duration, severity and stage of development of the plant (PELEGRINI et al., 2013). The water deficiency influences the seed vigor, which can be observed in the growth of the seedlings, that is, more vigorous seeds produce well-developed seedlings, due to a greater capacity for transformation and supply of reserves from the storage tissues and the embryonic axis (DAN et al., 1987).

Figure 5. Length of the primary root (PR) of seedlings of lima bean cultivars from seeds submitted to water stress at different temperatures.



This is an important way to note that the seedling length is more sensitive to the reduction of water availability in comparison to seed germination (ABATI et al., 2014). The aerial part length of the common bean (*P. vulgaris*) decreased to zero when subjected to a potential of -1.2 MPa mannitol solution (AGOSTINI et al., 2013).

The germination of seeds of cowpea (*Vigna unguiculata* L. Walp.) was also affected when they were submitted to osmotic potentials equal to or lower than -0.6 MPa, preventing the development of normal seedlings (ARAUJO, et al., 2015).

Seedlings of improved genotypes of beans (*P. vulgaris*) showed a reduction in their length when submitted to mannitol concentrations of 0.0, -0.6, -1.2, and -1.8 MPa. Among the genotypes studied, BAF9 exhibited better performance than the others, demonstrating a greater potential to adapt to the water deficit, a characteristic relevant to breeding programs (GARCIA et al., 2012).

For the dry mass of the aerial part, the maximum values for the Branca cultivar at 25, 30, and 35 °C were 0.015, 0.04, and 0.469 g seedlings^{-1}, respectively. For the Orelha de Vó cultivar, the values of 0.014, 0.079, and 0.001 g seedlings^{-1} were obtained at temperatures of 25, 30, and 35 °C, respectively, while the Rosinha and Roxinha cultivars showed values lower than 0.03 g seedlings^{-1} at the temperatures tested (Figure 6). The dry mass content of the roots of the different lima bean seedlings varied between 0.0003 and 0.5 g seedlings^{-1} (Figure 7).

This fact can be explained by the lower production of G6PDH and consequently of NADPH, culminating in a decline in the activities of some antioxidant enzymes due to the unfavorable environments to which the plants are submitted such as water stresses, producing lower Calvin cycle activity, which results in severe damage to the cell membrane structure, affecting the quality of the seedlings (SZABADOS; SAVOURE, 2009; MARINI et al., 2012).

Figure 6. Dry mass of the aerial part (DMAP) of seedlings of lima bean cultivars from seeds submitted to osmotic potentials at different temperatures.

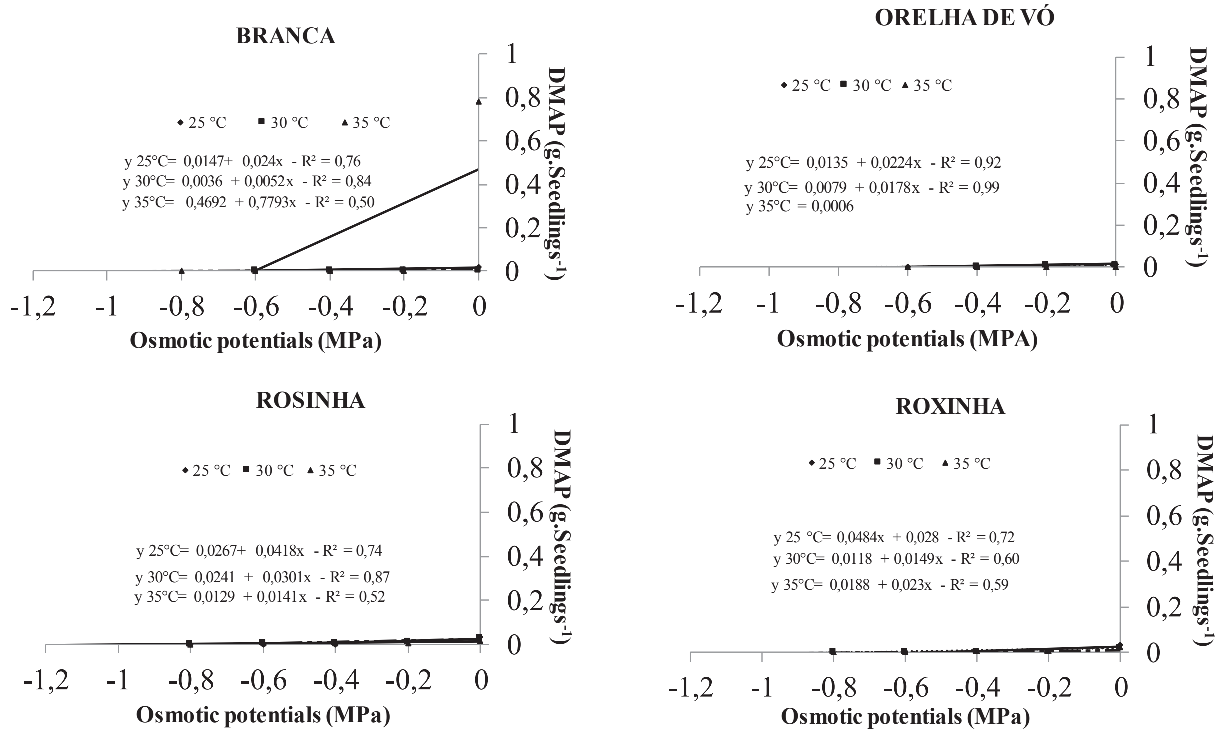
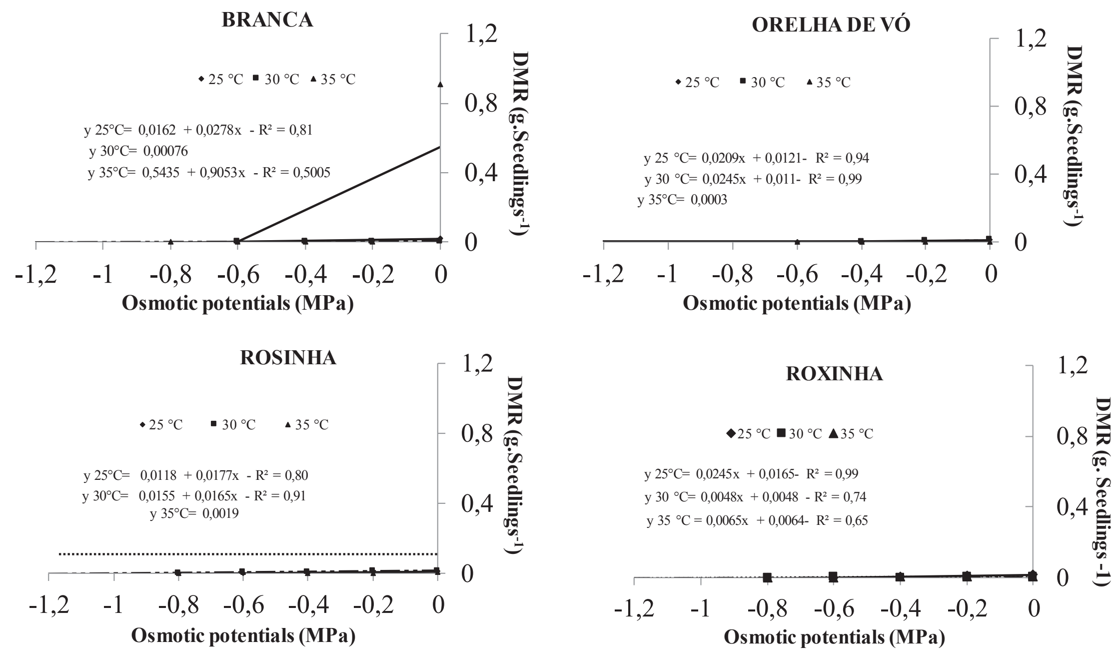


Figure 7. Dry mass of the roots (DMR) of seedlings of lima bean cultivars from seeds submitted to water stress at different temperatures.

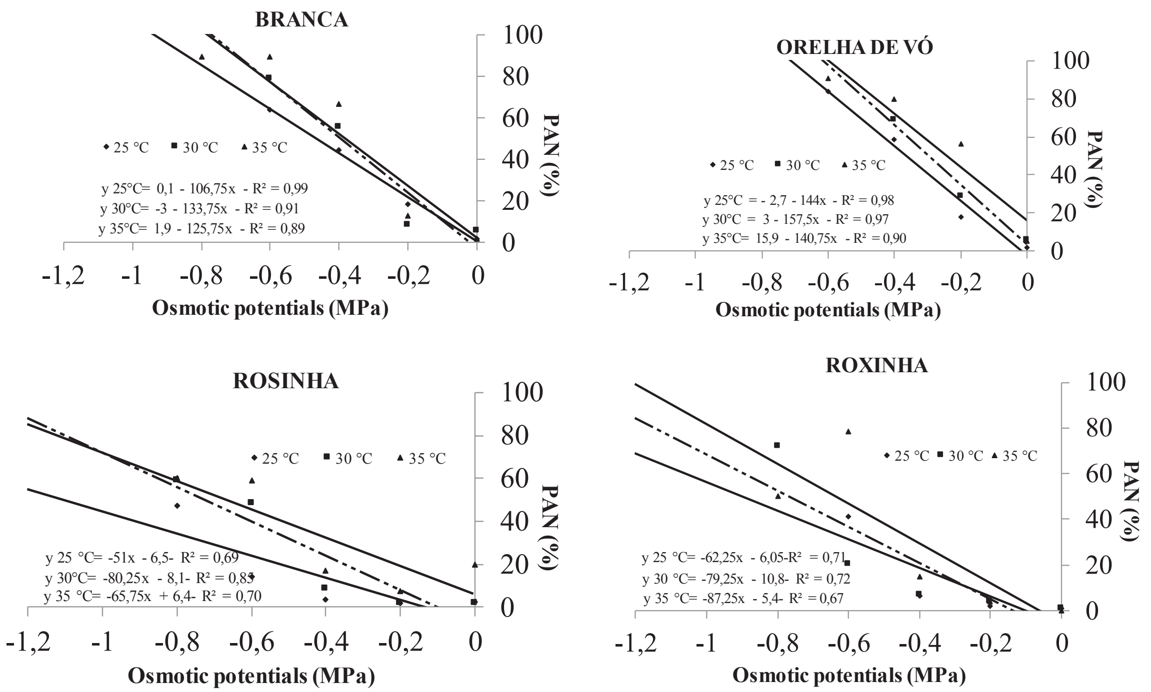


Seedlings of different soybean cultivars (*Glycine max* L. Merrill) (MG/BR 46 Conquista, Carajás, UFS Impacta) from seeds submitted to different osmotic potentials (0, -0.1, -0.2, -0.3, and -0.4 MPa) of PEG 6000 solutions had drastically reduced dry matter content (VIEIRA et al., 2013).

The reduction of cell expansion occurs because of inadequate turgor potential due to decreased cell growth, which is the first measurable effect when the plants are subjected to water stress conditions. This is a progressive reduction of the dry mass of the seedlings because of water restriction, due to the lower speed of physiological and biochemical processes or the difficulty of hydrolysis and mobilization of seed reserves (TAIZ and ZEIGER, 2013; BEWLEY; BLACK, 1994).

The highest percentage of abnormal seedlings occurred when the potentials became more negative by the following values in the respective cultivars: Branca, Orelha de Vó, Rosinha, and Roxinha at temperatures: 25 (0.1, -2.7, -6.5, and -6.1%), 30 (-3, 3, -8.1, and -10.8%), and 35 °C (1.9, 15.9, 6.4, and -5.4%) (Figure 8).

Figure 8. Percentage of abnormal seedlings (% PAN) of lima bean cultivars from seeds submitted to water stress at different temperatures.



The osmotic potentials of -0.25 and -0.30 MPa of PEG 6000 favored the appearance of abnormal seedlings of fennel (*Foeniculum vulgare* Miller) in the first germination count test (STEFANELLO et al., 2006), while for seeds of *Citrullus lanatus* L., when submitted to the osmotic potentials of -0.6 and -0.8 MPa of NaCl, caused a rise in abnormal seedlings (TORRES, 2007).

The germination and seed vigor of the lima bean cultivars Branca, Orelha de Vó, Rosinha, and Roxinha were negatively affected by the water stress simulations with PEG 6000 solutions. Seeds of the Orelha de Vó cultivar were the most sensitive to the simulated water stress, while seeds of the Rosinha cultivar were the most tolerant. The temperature of 35 °C affected the germination and vigor of lima bean cultivars Branca, Orelha de Vó, Rosinha, and Roxinha.

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