SOUZA, R. C.; ROMANOSKI, V. S.; SOUZA FILHO, P. R. M.; PIMENTEL, A. J. B. Effect of salt stress on the imbibition curve of cowpea seeds. **Revista de Agricultura Neotropical**, Cassilândia-MS, v. 10, n. 3, e7555, July/Sep., 2023. ISSN 2358-6303. DOI: https://doi.org/10.32404/rean.v10i3.7555.

Revista de Agricultura Neotropical

Effect of salt stress on the imbibition curve of cowpea seeds

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Received: 11/05/2023; Accepted: 20/07/2023.

ABSTRACT

The salinity of soil and water of irrigation can cause negative effects to agriculture by affecting the development and productivity of the cultivated species. These effects can be mitigated with the cultivation of salinity-tolerant varieties. The objective of this study was to evaluate the effect of salinity on the water imbibition curve during the germination process in cowpea seed and the response of different cultivars. An experiment was conducted in a factorial scheme, composed of three cultivars (BRS Marataoã, BRS Pajeu and BRS Tumucumaque) and four levels of salt stress, obtained by NaCl solutions at concentrations of 50, 100 and 150 mM and control, distilled water. The treatments were arranged in a completely randomized design, with four repetitions. The variables analyzed were the pattern of imbibition curve and final seed water content. It was found that the evaluated cultivars do not present the three-phase pattern for the seed soaking curve, in the absence or presence of salinity. There is variability for the pattern of the seed soaking curve among BRS Marataoã, BRS Pajeu and BRS Tumucumaque cowpea cultivars. Salinity acts by reducing the water content of cowpea seeds during the germination process and causes alteration in the pattern of the water soaking curve by the seeds. The cultivar BRS Tumucumaque shows higher tolerance to the reduction of seed water content under salt stress conditions when compared to the cultivars BRS Pajeu and BRS Marataoã.

Keywords: *Vigna unguiculata,* Abiotic stress, Salinity, Germination.

Efeito do estresse salino na curva de embebição de sementes de feijão-caupi

RESUMO

A salinidade do solo e da água de irrigação pode causar efeitos negativos à agricultura por afetar o desenvolvimento e a produtividade das espécies cultivadas. Esses efeitos podem ser mitigados com o cultivo de variedades tolerantes à salinidade. O objetivo desse trabalho foi avaliar o efeito da salinidade sobre a curva de embebição de água durante o processo de germinação em sementes de distintas cultivares de feijão-caupi. Para isso, foi conduzido um experimento em esquema fatorial, composto por três cultivares (BRS Marataoã, BRS Pajeu e BRS Tumucumaque) e quatro níveis de estresse salino, induzidos por soluções de NaCl nas concentrações 50, 100 e 150 mM e o controle com água destilada. Os tratamentos foram dispostos em delineamento inteiramente casualizado, com quatro repetições. As variáveis analisadas foram o padrão de curva de embebição; e o conteúdo de água final das sementes. Constatou-se que as cultivares avaliadas não apresentam o padrão trifásico para a curva de embebição de sementes, na ausência ou na presença de salinidade. Há variabilidade para o padrão de curva de embebição de sementes entre as cultivares BRS Marataoã, BRS Pajeu e BRS Tumucumaque de feijoeiro-caupi. A salinidade atua reduzindo o conteúdo de água das sementes de feijão-caupi durante o processo de germinação e causa alteração no padrão da curva de embebição de água pelas sementes. A cultivar BRS Tumucumaque demonstra maior tolerância à redução do conteúdo de água da semente em condições de estresse salino, quando comparada às cultivares BRS Pajeu e BRS Marataoã.

Palavras-chave: *Vigna unguiculata,* Estresse abiótico, Salinidade, Germinação.

1. Introduction

The culture of cowpea [*Vigna unguiculata* (L.) Walp] has great importance in the Northern and Northeastern regions of Brazil. Its cultivation is concentrated in small farms, where it plays a relevant socioeconomic role for the population (Silva et al., 2018a). However, grain yield is still low due to low investment in technology (Freire Filho et al., 1999) and to the occurrence of biotic and abiotic stresses in cultivation areas, such as saline stress (Tagliaferre et al., 2018).

The saline and sodium soils are distributed throughout the world and cause stress in the most varied species cultivated. In Brazil, these soils are predominantly in the natural region of the semiarid region, where their development is due to imperfect drainage conditions resulting from low rainfall, associated with high evapotranspiration, and the presence of impermeable layers in the soil (Ribeiro et al., 2016). Also, the use of water with high level of salts for irrigation is the reality of millions of small producers (Porto et al., 2019). Thus, along with the intrinsic soil and water conditions of areas of cultivation of the Brazilian semiarid, it is found that part of the agricultural activity developed in the Northeast occurs under some level of salt stress.

Saline stress has a negative impact on plant growth, causing metabolic changes and impairment of biochemical, physiological and anatomical functions (Assis Júnior et al., 2007; Dutra et al., 2017). These impacts can be of osmotic and ionic origin (Dias et al., 2019). The osmotic effect directly influences the absorption capacity of water and mineral salts in the soil solution (Hanin et al., 2016). The ionic effect results from the high absorption of ions such as Na^+ and Cl^-, which modify cellular homeostasis, interfering with the assimilation and transport of nutrients, as well as with metabolic processes (Marques et al., 2011; Taiz et al., 2017; Wani et al., 2020).

Cultures are affected differently by salt stress. Some are more tolerant, can develop and produce satisfactorily at high salinity levels, while others are more sensitive (Brito et al., 2015). For cowpea, there is evidence that low levels of salinity can harm the crop throughout the cycle (Aquino et al., 2017; Fonseca et al., 2017; Tsague et al., 2017). Notably, for the seed germination phase, it is urgent to evaluate the behavior of genotypes under saline stress (Ravelombola et al., 2017), in order to understand its effect on crop establishment. Differential capacity of establishing cowpea cultivars, in function of the level of salinity in the culture substrate, was found by Romanoski et al. (2022).

Germination begins with water absorption by the seed, or imbibition, followed by the resumption of embryo growth, root protrusion and seedling establishment (Nonogaki et al., 2010). The imbibition process follows a characteristic three-phase pattern observed by seed mass gain (Bewley et al., 2013). The first phase is characterized by the entry of water into the seed tissue, usually being fast and physical, and it ends with the stabilization in water absorption. In phase II, processes of metabolic reactivation occur, and in phase III begins the growth of the embryonic axis indicated by mass gain (Castro and Hilhorst, 2004; Carvalho and Nakagawa, 2012).

The duration and occurrence of each phase are affected by endogenous and exogenous factors and determine the curve of water absorption by the seed (Marcos Filho, 2005). Thus, it is understood that the curve of imbibition can vary according to the species and cultivar, as well as the conditions of the medium to which the seeds were induced to germinate. Dantas et al. (2002) reported that the behavior and the degree of tolerance to salt stress are dependent on the genotype analyzed, with behavioral variability between different genotypes, especially among materials of different origins. Under salinity conditions, seeds tend to reduce water absorption resulting in disturbances that affect the germination process and seedling development (Rocha et al., 2019).

In view of the above, it is glimpsed that salt stress can alter the water absorption curve by cowpea seeds and that these changes are dependent on the cultivar considered. Dantas et al. (2005) and Lima and Torres (2009) reported that the evaluation of germination in saline solutions is an indicator of the potential performance of seeds in cultivation areas where salt stress is present. Thus, this study aimed to evaluate the effect of salinity on the imbibition curve, the first stage of the seed germination process, of different cowpea cultivars.

2. Material and Methods

To meet the objective proposed, an experiment was conducted in the Seed Laboratory of the Federal University of West Bahia - Campus de Barra, in factorial scheme 3 x 4, composed of three cultivars (BRS Marataoã, BRS Pajeu and BRS Tumucumaque) and four levels of saline stress, induced by NaCl solutions at concentrations 50, 100 and 150 mM, which correspond to 4.7, 8.5 and 12.3 dS/m, respectively, and for the treatment Control distilled water was used. The treatments were arranged in a completely randomized design, with 4 replications. The plot consisted of a transparent polypropylene container, 11 cm in diameter and 4.5 cm high, with a lid, where 20 seeds were placed.

Prior to the installation of the experiment, the seed lot of each cultivar was standardized (Figure 1), eliminating those seeds that had atypical size, color and presence of physiological or physical damage perceptible to the naked eye.

Figure 1. Characteristics of the seeds of cowpea cultivars evaluated in the experiment regarding shape, size and color. A: BRS Tumucumaque; B: BRS Pajeu; and C: BRS Marataoã.

In each plot, the seeds were soaked by immersion in the containers that constituted the experimental unit, containing 100 mL of distilled water or saline corresponding to the predetermined stress levels. After submission to imbibition, the containers were kept in laboratory benches at a controlled temperature of 25 ± 2 °C. Throughout the experiment, the mass of the seeds of each plot was determined, in predetermined time intervals, using an analytical scale with precision of 0.001 g. The first weighing occurred immediately before the immersion of the seeds to obtain the fresh weight. The other weighings were performed in 11 predefined periods: 1, 2, 3, 9, 15, 27, 39, 63, 87, 111 and 135 hours after immersion.

For weighing, the seeds were removed from the solution, submitted to surface drying with paper towel gently, weighed and placed again to soak in the middle of the renewed solution. With the data of mass of the seeds over the time of evaluation, the water content of the seeds was determined and we constituted the Water Imbibition Curves for each cultivar at different salinity levels. The water content of the seeds, expressed in % of water, was calculated for each evaluation period. At the end of 135 hours, the final water content was obtained. The following expression was used to calculate the seed water content:

$$Water\ content\ (\%) = \frac{Pf - Pi}{Pi}\ x\ 100$$

where Pf is the final weight of the seeds (g) in each evaluation period and Pi the initial fresh weight of the seeds (g) before the start of imbibition. The imbibition curves were constituted by lines constructed in an orthogonal cartesian system, where the imbibition time was represented in the x-axis and the water content of the seeds for each evaluation period, in the y-axis. The Final Water Content data, after 135 hours, were submitted to analysis of variance, using the F test at 5% probability. Complementary procedures were adopted according to the significance of the interaction and the nature of the factors under analysis. For comparison between means, Tukey test was applied, with significance level of 0.05. Data analysis was performed in the Genes Program - Computational application in genetics and statistics (Cruz, 2013).

3. Results and Discussion

Through the characterization and evaluation of the seed quality predicted, it was found that the weight of one hundred seeds of cultivars BRS Pajeu, BRS Marataoã and BRS Tumucumaque were 17.90, 21.16 and 21.12 g, respectively. The variable seed weight is a physical attribute that can be influenced by genetic variation between cultivars and interferes with the growth of cowpea (Silva et al., 2019). Germination was 86% for cultivar BRS Pajeu, 72% for BRS Marataoã and 85% for BRS Tumucumaque, noting that seed lots showed good germination percentage.

The imbibition curves of the cultivars BRS Pajeu, BRS Marataoã and BRS Tumucumaque, in the different levels of saline stress, are presented in Figures 2, 3 and 4, respectively. A similar water absorption pattern was observed between the cultivars BRS Pajeu and BRS Marataoã and a different behavior of the cultivar BRS Tumucumaque. However, for all of them, the three-phase model characteristic of the imbibition curves described by Bewley et al. (2013) was not observed.

Nonogaki et al. (2010) affirm that the use of the phases is practical to exemplify the events that happen in the seed germination process. However, in some species the differentiation between the phases is not well defined. In larger seeds, for example, water absorption in the cotyledons may continue to occur after the emergence of the radicle. Pereira et al. (2022) state that the three-phase standard of seed imbibition is not a general principle; therefore, it cannot be observed in all species.

When analyzing the imbibition curves of the cultivars BRS Pajeu (Figure 2) and BRS Marataoã (Figure 3), one can observe a similar behavior, varying only the water content of the seeds depending on the cultivar or the level of salt stress. The cultivars presented overlapping curves until the end of phase I. This phase lasted nine hours, when there is a sudden reduction in the rate of water absorption in the seeds.

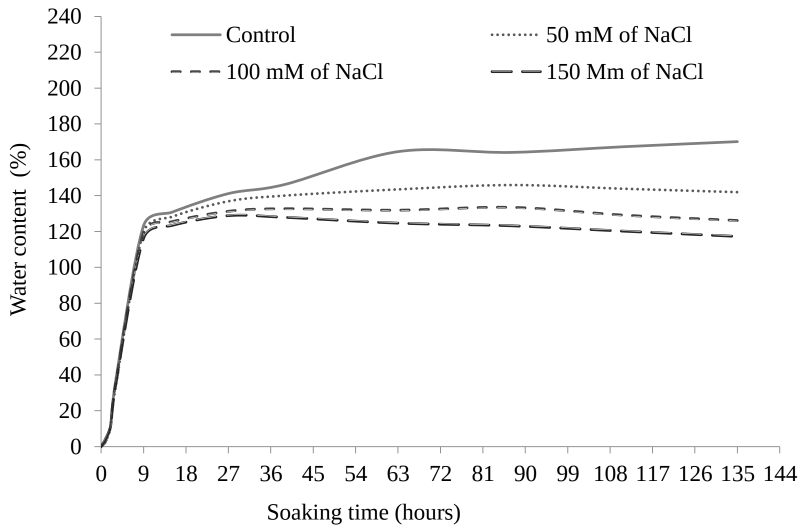

Figure 2. Imbibition curves of cowpea seeds of the cultivar BRS Pajeu, submitted to four levels of salt stress, over 135 hours.

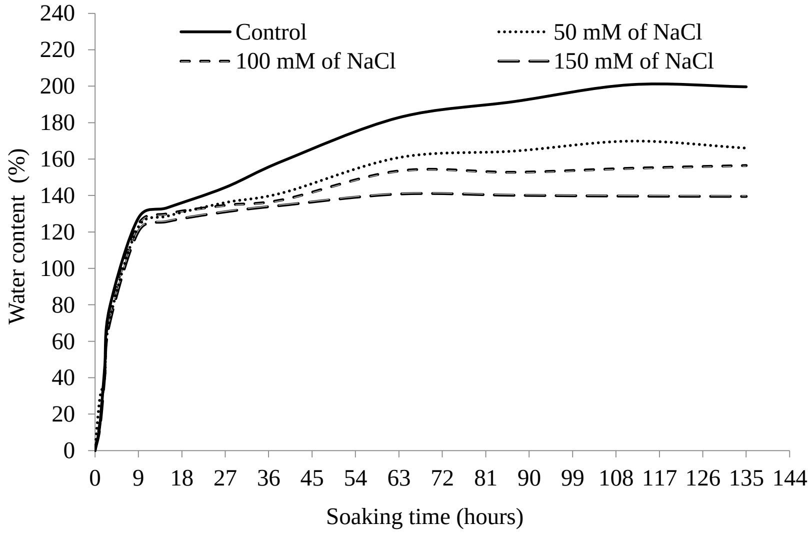

Figure 3. Imbibition curves of cowpea seeds of the cultivar BRS Marataoã, submitted to four levels of salt stress, over 135 hours

Phase I imbibition results in the hydration of cell walls and reserve polymers within cells (Hanin et al., 2016). The increase in water absorption over time is a function of the number of cells being hydrated and this process does not occur uniformly between cells (Hanin et al., 2016). Observing Figures 2 and 3, it is noticed that phases II and III are not characterized as expected. One can observe the transition between phases I and II; however, the change from phase II to III is not noticeable, since stabilization in the water content does not occur, followed by the resumption of embryo growth.

The absence of an evident three-phase pattern in seed imbibition was also observed by Silva et al. (2018b) for the culture of peas (*Pisum sativum* L.) and lentils (*Lens culinaris* Medik). Dias et al. (2019) did not detect a clear difference between the phases for seeds of chickpea cultivars. In a work developed by Portes et al. (2018), with desert rose (*Adenium obesum* (Forssk.) Roem. & Schult.), it was found that the period of phase I was nine to twelve hours.

From the end of phase I, the separation between the lines of the curves for the different stress levels shows the effect of salinity on the water content of the seeds of the cultivars BRS Pajeu and BRS Marataoã. For both cultivars, as the saline concentration of the soaking solution increases, the water content of the seeds decreases (Figure 2 and 3), so that the water content was higher in the control treatment, followed by the solutions with concentrations of 50, 100 and 150 mM NaCl. According to Neto et al. (2020), salinity levels above 5.0 dS/m (about 54 mM NaCl) affect the germination performance of cowpea. Thus, it is evident that saline stress acted by reducing the final water content of the seeds of these two cultivars.

According to Coelho et al. (2017), the reduction of seed water content under saline stress conditions is related to the reduction of the osmotic potential of the saline solution, which consequently reduces the availability of water to the seeds. Osmotic effect due to the accumulation of salts in the solution, reducing the soaking process of the seeds, has already been reported in the following cultures: cowpea (Coelho et al., 2017), cabbage (Lopes and Macedo, 2008), jatropha (Andreo-Souza et al., 2010) and watermelon (Torres, 2007). The imbibition curve of the cultivar BRS Tumucumaque (Figure 4) presented a different behavior in relation to the cultivars BRS Pajeu and BRS Marataoã, for some aspects. BRS Tumucumaque finished phase I in three hours after induction of imbibition, while for BRS Pajeu and BRS Marataoã, the duration of phase I was nine hours.

A similar behavior was found by Beckert et al. (2000) with soybean seeds, where phase I ended in the period of three hours. Another difference presented by the cultivar BRS Tumucumaque in relation to the other two cultivars is the reduction in the water content of the seeds in the transition from phase I to II. This reduction, over the time of imbibition, is subtle for control treatment and prominent for treatments with salinity. That may be associated with loss of solutes and loss of water. The structures of the cell membrane pass from the crystalline phase to the liquid phase when they are rehydrated. With this, some membranes are damaged, resulting in the leakage of solutes from cells (Wojtyla et al., 2006).

In addition to the shorter time to reach phase II and the reduction in the water content of seeds in the transition from phase I to II, the cultivar BRS Tumucumaque demonstrated a differentiated response to increased saline concentration of the imbibition solution, compared to cultivars BRS Pajeu and BRS Marataoã. Observing Figure 4, there are two patterns of imbibition: one for the control treatment and another for the treatments with saline stress. That is, the response of the cultivar to stress levels of 50, 100 and 150 mM NaCl was similar, with a slight superiority to the level of 50 mM NaCl. For the cultivars BRS Pajeu and BRS Marataoã, the reduction in water content of the seeds was proportional to the concentration of NaCl in the soaking solution, presenting greater variability in the response to salt stress (Figure 5).

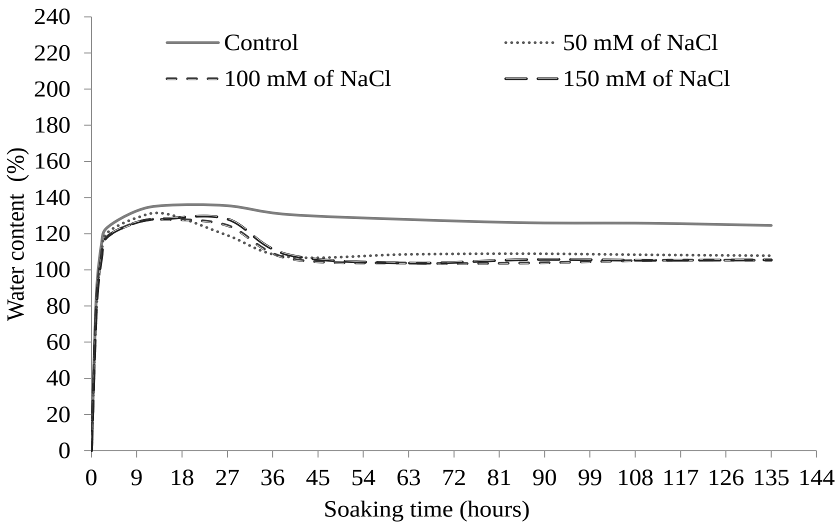

Figure 4. Imbibition curves of cowpea seeds of the cultivar BRS Tumucumaque, submitted to four levels of salt stress, over 135 hours.

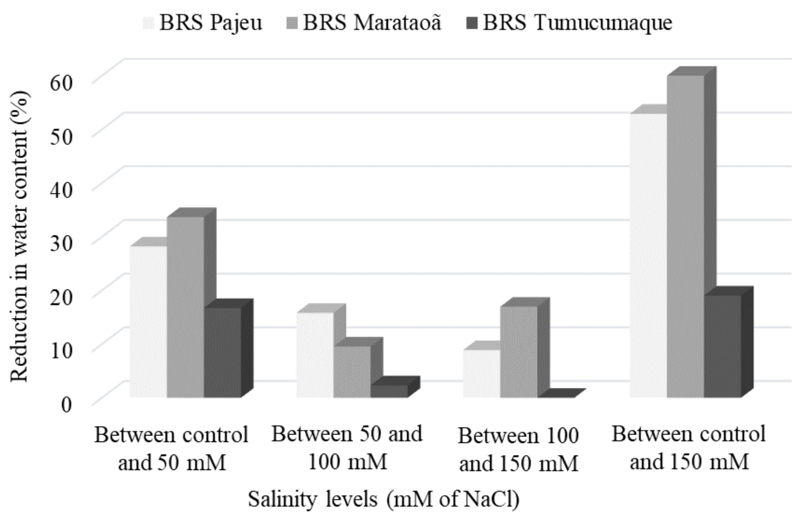

Figure 5. Reduction in the Final Water Content of cowpea seeds of the cultivars BRS Pajeu, BRS Marataoã and BRS Tumucumaque between different levels of salinity of the seed soaking medium.

Observing Figure 5, it is noteworthy that the cultivar BRS Tumucumaque showed the lowest reduction in water content when compared to the control treatment and the 50 mM solution, as compared to the control treatment and the highest level of saline stress (150 mM NaCl). Among the treatments of 50 mM and 100 mM NaCl, the reduction in water content was 2.31% and among the treatments of 100 mM and 150 mM NaCl there was no reduction. The cultivars BRS Pajeu and BRS Marataoã showed more similar reduction percentages among them, compared to BRS Tumucumaque, along the salinity levels. Furthermore, the reduction of water content of these two cultivars was considerably higher than the cultivar BRS Tumucumaque, when compared to the control treatment at the level of stress induced by the solution with concentration of 150 mM NaCl.

It is known that the water content of the three cultivars in the transition from phase I to phase II varied between 120 and 140%. In phase II, the water content of the cultivar BRS Tumucumaque did not exceed 140% and for the cultivars BRS Pajeu and BRS Marataoã, the range was 180 to 200%, after 135 hours, in the control treatment curve. For the Final Water Content, the analysis of variance showed a statistically non-significant effect for the interaction between Cultivars and Saline Stress (Table 1). This fact indicates that there is no differential expression between cultivars, compared to different levels of saline stress. Thus, each factor was analyzed separately. Romanoski et al. (2022) also found no significant interaction between salinity level and cultivar for the percentage and germination speed of 11 cowpea cultivars, including the three present in this experiment. For the Final Water Content, the cultivar BRS Marataoã surpasses BRS Tumucumaque, while BRS Pajeu does not differ statistically from these two cultivars (Figure 6).

The highest Final Water Content of BRS Marataoã in relation to BRS Tumucumaque may be related to the chemical composition of the seeds. Bezerra et al. (2019), Cordeiro et al. (2021) and Pessoa et al. (2023) evaluated the chemical composition of the seeds of the cultivars BRS Marataoã, BRS Pajeu and BRS Tumucumaque and observed that the carbohydrate and protein contents are similar. However, the cultivars differ in lipid content, and BRS Marataoã, BRS Pajeu and BRS Tumucumaque have in their composition 0.68, 1.21 and 1.70% of lipids, respectively. That is, BRS Tumucumaque has 1% more lipids when compared to BRS Marataoã.

One of the common characteristics of lipids is insolubility in water and these molecules exert cellular structural function and energy reserve, being important constituents of cell membranes and organelles (Jorge, 2009). In the germination process, the degradation of carbohydrates and lipids occurs, due to the energy supply in the seedling development (Zhan et al., 2015). Considering the high proportion of lipids in the seeds of BRS Tumucumaque, it can be suggested that the degradation of lipids in the cultivar is late, which is the factor that hinders the process of water absorption in the seeds.

The effect of saline stress on the Final Water Content, considering the average of the three cultivars, is presented in Figure 7. It is observed that the water content of seeds presents a negative linear relationship with the increase in salinity. It can be inferred that the salt stress reduces the final water content of the seeds, with high coefficient of determination (0.84). The water content in the seeds is reduced with the increase in the saline level due to the excess of soluble salts in the water, which promote the reduction of the water potential and induce the lower capacity of water absorption by the seeds. This process interferes negatively in the germination and growth of seedlings (Nóbrega et al., 2021).

The concentration of ions, specifically sodium and chlorine, promote physiological disorders, due to the reduction of water availability (Motos et al., 2017), and causes a reduction in germination percentage, germination speed index and seed deterioration, due toxicity to the embryonic axis, in cowpea seeds (Tsague et al., 2017). Studies like this one, which describe the water imbibition curve of cowpea seeds under saline stress, are relevant to determine cultivars with higher stress tolerance.

Table 1. Summary of the analysis of variance for the final water content in seeds of cowpea cultivars, after 135 hours of imbibition, under different salinity levels.

Source of Variation	GL	QM	F-valor	Pr>F
Cultivar	2	91.57	3.6744	0.0353*
Salt stress	3	152.53	6.1202	0.0017**
Cultivar x Salt stress	6	34.88	1.3996	0.2415^{ns}
Residue	36	24.92		
Total	47			
C.V.(%)	38.39			

*,** significant at 5 and 1% probability by the F test, respectively.

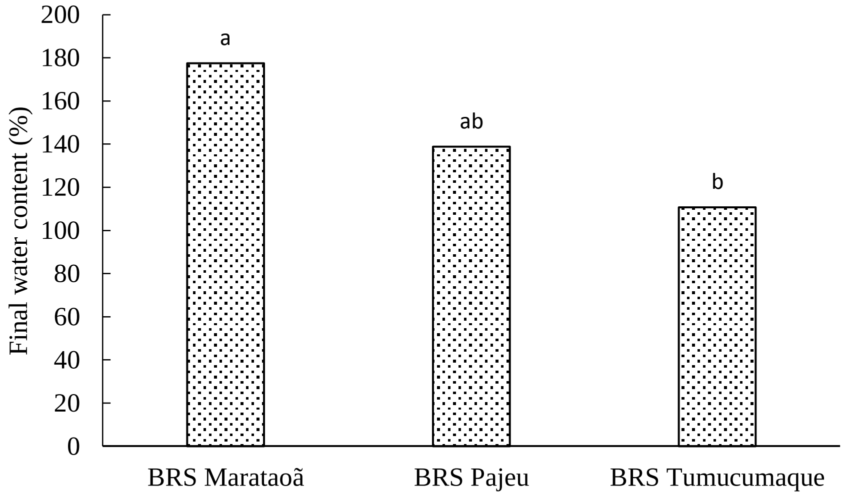

Figure 6. Final Water Content of seeds of cowpea cultivars, after 135 hours of imbibition, considering the average of five levels of salt stress. Columns accompanied by the same letter do not differ from each other by Tukey's test, at 5% probability.

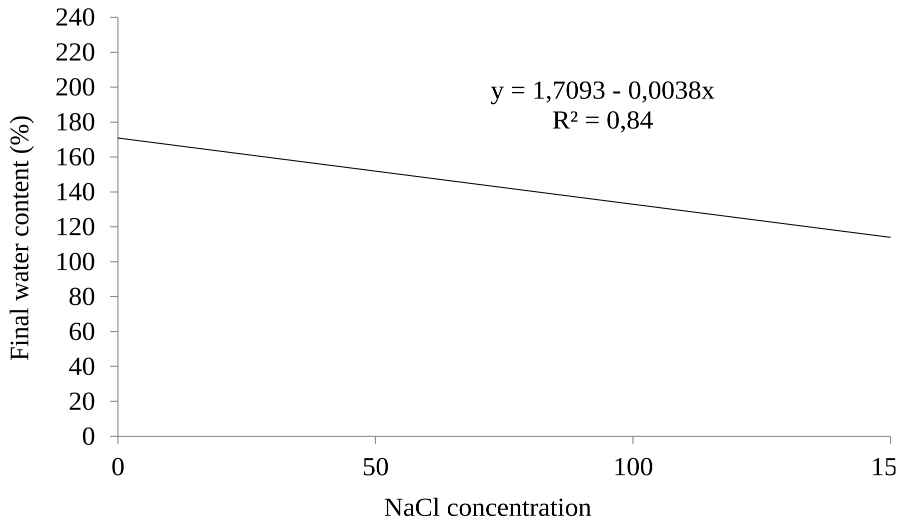

Figure 7. Effect of different levels of salt stress on the Final Water Content of cowpea cultivar seeds after 135 hours of imbibition, considering the average of three cultivars.

4. Conclusions

There is variability for the seed imbibition curve pattern among the cultivars BRS Marataoã, BRS Pajeu and BRS Tumucumaque of cowpea bean and these cultivars do not present the three-phase pattern described in the literature for the seed imbibition curve. Salinity reduces the water content of cowpea seeds during the germination process and changes the pattern of the water soaking curve by the seeds. The cultivar BRS Tumucumaque shows greater tolerance to the reduction of seed water content under saline stress conditions, when compared to cultivars BRS Pajeu and BRS Marataoã.

Authors' Contribution

Raéder Correia de Souza: data collection, data interpretation and writing article; Vanessa Silva Romanoski: data collection, data interpretation, writing article, and translation of the article; Paulo Roberto de Moura Souza Filho: orientation and data review; Adérico Júnior Badaró Pimentel: orientation, experiment setup, statistical analysis, data interpretation and review.

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