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Original Article

The physiological quality of *Vigna unguiculata* L. seeds shows tolerance to salinity

A qualidade fisiológica das sementes de *Vigna unguiculata* L. apresenta tolerância à salinidade

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Abstract

Salinity reduces feijão-caupi production, and the search for tolerant varieties becomes important within the agricultural context, as, in addition to being used in the field, they can be used in genetic improvement. The objective was to for a identify variety that is tolerant to salinity considering the physiological quality of seeds and seedling growth. A 2 × 4 factorial scheme was used, referring to the varieties Pingo-de-ouro and Coruja, and four electrical conductivities of water (0; 3.3; 6.6 and 9.9 dS m^{-1}). The physiological quality of seeds and the growth of seedlings were analyzed, in addition to the cumulative germination. The Pingo-de-ouro variety showed no germination, length of the shoot and root, dry mass of the shoot and root compromised up to electrical conductivity of 6 dS m^{-1} in relation to 0.0 dS m^{-1}. On the other hand, the Coruja variety showed reduced germination, increased shoot and root length. The creole variety Pingo-de-ouro proved to be tolerant to salinity.

Keywords: *Vigna unguiculata* L., electrical conductivity, landrace varieties.

Resumo

A salinidade reduz a produção de feijão-caupi, e a busca por variedades tolerantes torna-se importante dentro do contexto agrícola, pois, além de serem utilizadas no campo, podem utilizar-se no melhoramento genético. Com isso, objetivou-se buscar alguma variedade crioula que seja tolerante à salinidade considerando a qualidade fisiológica de sementes e crescimento da plântula. Utilizou-se um esquema fatorial 2 × 4, correspondendo às variedades Pingo-de-ouro e Coruja, e quatro condutividades elétricas da água de irrigação (0; 3,3; 6,6 e 9,9 dS m^{-1}). Analisou-se a qualidade fisiológica de sementes e o crescimento das plântulas, além da germinação cumulativa. A variedade Pingo-de-ouro, não apresentou germinação, comprimento da parte aérea e raiz, massa seca da parte aérea e raiz comprometida até condutividade elétrica de 6 dS m^{-1} em relação a 0,0 dS m^{-1}. Por outro lado, a variedade Coruja apresentou redução da germinação, aumento no comprimento da parte aérea e raiz. A variedade crioula Pingo-de-ouro se mostrou tolerante à salinidade.

Palavras-chave: *Vigna unguiculata* L, condutividade elétrica, variedades crioulas.

1. Introduction

The feijão-caupi (*Vigna unguiculata* L.) is a bean of African origin and shows excellent growth and development in arid and semi-arid regions, being one of the main sources of protein and income for families in the northeastern countryside (FAO, 2019). Crops respond differently to salinity, ranging from being sensitive to highly tolerant of saline environments. This process may vary among species, with biochemical, physiological, and morphological changes (Santos et al., 2009), leading to stomatal closure as a plant response to stress, decreasing water loss through transpiration and reducing CO_2 absorption (Silva et al., 2024). Furthermore, changes occur in water relations, inhibiting leaf expansion (Lima et al., 2023). Seed germination is one of the most crucial stages of plant development since the progress of this phase determines the establishment of seedlings and, consequently,

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the formation of vigorous plants (Kubala et al., 2015). Seed germination capacity under salinity reflects the tolerance of plants to salts during subsequent developmental stages (Taiz et al., 2017).

Besides the ability of feijão-caupi seeds to germinate under salinity, the cotyledon biomass for seedlings, following the germination process, can also influence salinity tolerance as salinity-sensitive materials exhibit reduced seedling dry matter (Nunes et al., 2019). Feijão-caupi exhibits moderate tolerance to several abiotic stresses, such as saline. For instance, Souza et al. (2011) reported that for feijão-caupi, the primary salinity tolerance mechanisms are based on excluding the Na^+ ion from the leaves, retaining it in the root system during the initial days of exposure to salt stress, and maintaining available soluble carbohydrates, even when exposed to high concentrations of NaCl.

Therefore, identifying feijão-caupi varieties tolerant to saline stress aid in expanding the crop and improving crop yields. The use of traditional (creole) seeds is a promising strategy for cultivation in semi-arid regions, as they have been selected by producers for their adaptability and productivity under limiting conditions (Souza et al., 2019).

The hypothesis of this research was that one of the feijão-caupi varieties tolerates the salinity of irrigation water in relation to the physiological quality of the seeds. In this research, the objective was to evaluate the physiological quality of seeds and seedling growth of creole feijão-caupi varieties cultivated in the semi-arid region of the Brazilian Northeast.

2. Materials and Methods

The experiment was conducted at the Seed Analysis Laboratory, at the Federal University of Paraíba (SAL-UFPB), Campus II, Areia, state of Paraíba, Brazil. Seeds of two creole feijão-caupi varieties (Pingo-de-ouro and Coruja) were used, sourced from traditional farmers of Sítio Córrego (5° 39'39.4"S 37° 52'42.3"W), located in the rural area of the Municipality of Apodi, RN.

The design of the experiment was completely randomized, utilizing a 2 × 4 factorial scheme. This scheme consisted of two varieties of Creole feijão-caupi (Pingo-de-ouro and Coruja) and four values of electrical conductivity (EC) (0.0; 3.3; 6.6 and 9.9 dS m^{-1}). Four replicates were used, with experimental units comprised of 50 seeds. The treatments involved various ECs, which were determined using a portable conductivimeter (Water quality detector - Yieryi®).

Following the harvest, seeds were stored in PET bottles with capacity of the two liters for approximately one year, a period consistent with the practices of farmers until the next planting. Post storage, the seed's water content was ascertained via the greenhouse method, involving exposure to 105 °C ± 3 °C for 24 hours (Brasil, 2009), after which tests were conducted to evaluate the physiological quality.

Sowing occurred within rolls of Germitest® paper, which were saturated with a NaCl solution amounting to twice the paper's initial weight. Subsequently, the seeds were placed in a B.O.D (Biological Organism Development) chamber for germination, with a constant temperature of 25 °C and a 12-hour photoperiod. To analyze the impact of salinity on both varieties, we evaluated the following variables: i) germination percentage, ii) first germination count (FGC), iii) germination speed index (GSI), and iv) mean germination time (MGT).

The percentage of germination was assessed on the fifth at eighth days post sowing (Brasil, 2009). Cumulative germination was considered from the second day post sowing until the tenth day. Alongside twinning, the first germination count took place, factoring in the percentage of normal seedlings on the fifth day after sowing (Brasil, 2009). Seedlings with all essential structures were classified as normal.

The germination speed index was concurrently analyzed with the germination test, evaluating the seedlings daily, from the first day post sowing until the eighth day. This followed the formula proposed by Maguire (1962). The mean germination time was calculated using Labouriau's (1983) equation, which necessitated a daily count of the germinated seeds until the eighth day after sowing.

Measurements of the shoot length (SL) and the root length (RL) were taken using a ruler graduated in centimeters. The shoot dry matter (SDM) and root dry matter (RDM) were measured with a precision balance after the seedling matter had remained for 72 hours. The salinity tolerance index (STI) was determined in accordance with Sá et al. (2016) (Equation 1),

$$STI = \frac{ST}{CT} x100 \tag{1}$$

CT = control treatment (0.0 dS m^{-1}); ST = saline treatment.

Data were analyzed on the R platform, utilizing the ExpDes.pt packages (Ferreira et al., 2018) to perform the Shapiro-Wilk normality tests and Bartlett homoscedasticity tests. Subsequently, ANOVA, regression tests, and the Tukey test were conducted using the same package. The cumulative germination was analyzed using modeling with the sigmoid function.

3. Results

Table 1 presents the results of the variance analysis for all variables studied. The variables that demonstrated interaction between the factors were MGT, RL, SL and RDW, the others demonstrated isolated significance.

Cumulative germination with increasing saline concentrations was not affected throughout the experiment. However, it was observed that the Coruja variety exhibited a reduction of 18.72% on the 10th day when the seeds were subjected to an EC of 9.9 dS m^{-1}, compared to an EC of 0 dS m^{-1} (Figure 1A).

The varieties, when evaluated separately can be observed which for the Pingo-de-ouro variety led to an increase of 40%, 43% and 39% in germination, IGS and FGC in relation to the Coruja variety, respectively (Figure 1B, C and E). On the other hand, increasing electrical conductivity resulted in a unit reduction of 0.244 and 1.32 with increasing EC in both varieties for GSI and FGC, respectively (Figure 1D and F).

Table 1. Summary of analysis of variance for germination (G), germination speed index (GSI), mean germination time (MGT), first germination count (FGC), shoot length (SL), root length (RL), shoot dry weight (SDW), root dry weight (RDW), germination salt tolerance index (GSTI) and seedling dry weight salt tolerance index (SDWSTI) of Pingo-de Ouro and Coruja subjected to salinity.

SV	DF	MS				
		G	GSI	MGT	FGC	SL
Variety (V)	1	10878.1**	510.24**	0.089	6216.1**	0.132
Salinity (S)	3	22.1	9.87*	0.329*	315.8*	4.444**
V × S	3	133.5	3.36	0.287*	117.5	0.651**
Residue	24	57.8	2.30	0.071	72.3	0.051
CV		10.28	10.29	9.30	15.13	8.05
SV	**DF**	**MS**				
		RL	**SDW**	**RDW**	**GSTI**	**SDWSTI**
Variety (V)	1	83.689**	94.463*	103.284**	675.83	238.8
Salinity (S)	3	31.698**	126.074**	22.100	91.33	3458.5**
V × S	3	3.252**	12.375	48.155**	297.58	956.4
Residue	24	0.352	18.623	7.553	181.42	577.3
CV		10.83	24.80	28.9	13.8	24.18

** and * significant difference at 1 and 5% by the F test. MS = mean square; SV = source of variation; DF = degree of freedom; CV = Coefficient of variation.

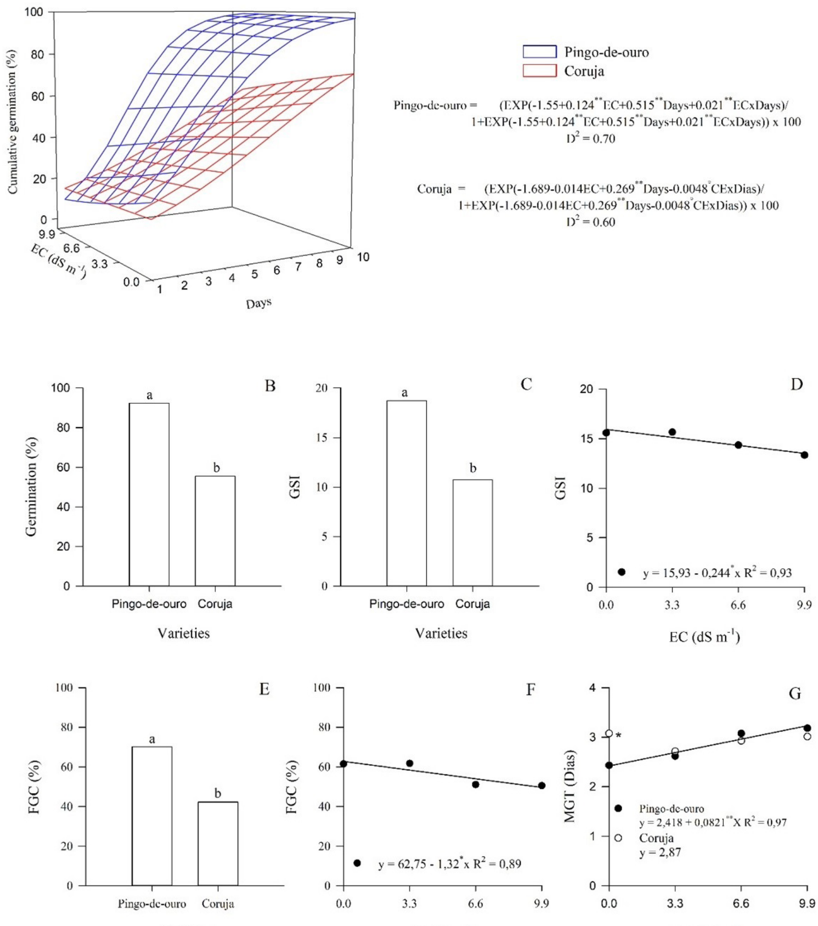

Figure 1. Cumulative germination (A), germination (B), germination speed index (C and D), first germination count (E and F), mean germination time (G) of Pingo-de Ouro and Coruja in function of electrical conductivity (EC) of irrigation water. Deviance = D^2.

As for MGT, an interaction between the factors studied was observed. The Pingo-de-ouro variety did not respond to the increase in EC for MGT, while the Coruja variety exhibited a unit increase of 0.0821 (Figure 1G). In this context, the MGT is an adequate variable for indicating the salinity tolerance of seeds. It has been observed that the higher the percentage of seeds germinated in the FGC, the better the seed quality. As per the GSTI, seeds of the Pingo-de-ouro variety demonstrated tolerance to electrical conductivity up to 9.9 dS m^{-1}.

The SL of the Pingo-de-ouro variety was fitted to a second-degree polynomial function, and it reached its highest value at an electrical conductivity of 3.4 dS m^{-1}. Interestingly, the electrical conductivity of 6.6 dS m^{-1} did not negatively impact the SL, similar to that observed with an electrical conductivity of 0.0 dS m^{-1}; both conditions resulted in a SL of 3.1 cm (Figure 2A).

The RL was also fitted to a polynomial function, yielding a value of 8.65 cm at an electrical conductivity of 2.33 dS m^{-1} (Figure 2B). However, salinity negatively influenced the SL and RL of the Coruja variety, resulting in a unit reduction of 0.0163 and 0.384, respectively (Figure 2A and B). The Pingo-de-ouro variety demonstrated salinity tolerance, as both SL and RL were not negatively affected by electrical conductivity up to 6.6 dS m^{-1}. Conversely, the Coruja variety showed sensitivity to salinity in terms of SL and RL. In addition, the Pingo-de-ouro variety produced higher SL and RL results at ECs of 0.0; 3.3 and 6.6 dS m^{-1} compared to the Coruja variety, as per the 5% F test.

Regarding biomass production, the Pingo-de-ouro variety did not fit the salinity-dependent models studied for RDW. Furthermore, this variety promoted an increase of 58%, 37%, and 55% in RDW at electrical conductivity levels of 0.0; 3.3, and 9.9 dS m^{-1}, respectively, when compared to the Coruja variety, as per the 5% F test (Figure 2C).

SDW significantly responded to isolated salinity factors and was fitted to the second-degree polynomial function. Notably, both varieties showed a decrease in SDW starting from an electrical conductivity of 6 dS m^{-1} compared to an EC of 0.0 dS m^{-1}, with values equivalent to 19 mg $seedling^{-1}$ (Figure 2D). Additionally, the SDW showed a significant effect from the variety factor; it was observed that the Pingo-de-ouro variety promoted an increase of 18% compared to the Coruja variety (Figure 2F).

The SDWSTI is fitted to a second-degree polynomial model. This modeling suggests that the varieties exhibit tolerance up to 7.5 dS m^{-1}, given that the values between electrical conductivities of 0.0 and 8.5 dS m^{-1} are approximately 100% (Figure 2E).

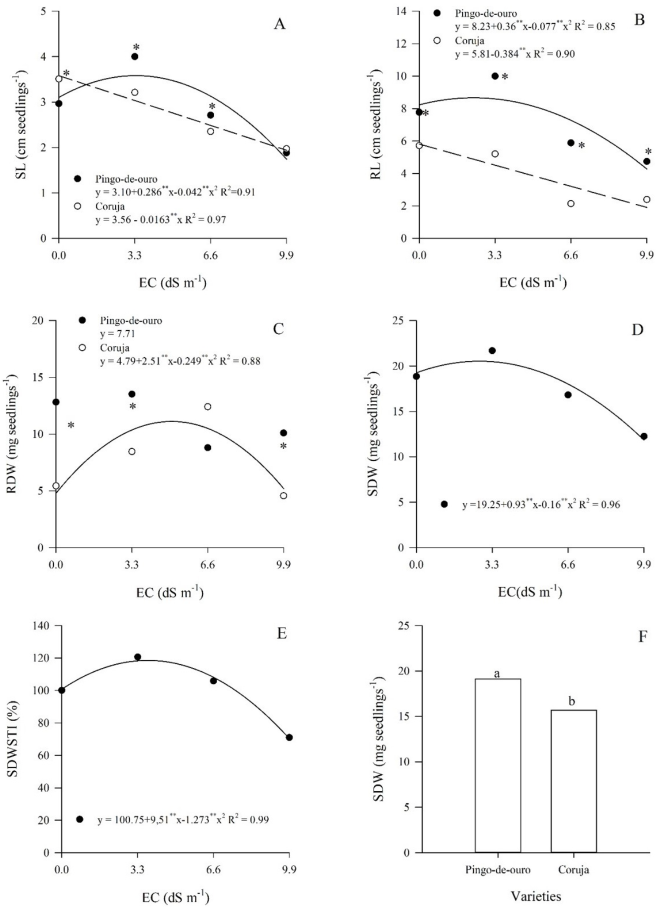

Figure 2. Illustrates the shoot length (A), root length (B), root dry weight (C), shoot dry weight (D and F), and seedling dry weight salt tolerance index (E) of Pingo-de-Ouro and Coruja when subjected to different electrical conductivity levels. *Statistical difference by Tukey test at 5%.

4. Discussion

Soil salinity is an abiotic factor that represents a serious threat to global food security, particularly in arid and semi-arid regions. It results in socioeconomic impacts associated with decreased crop productivity and depreciation of agricultural land (Dias et al., 2020; Mohammadi et al., 2023). Variables such as germination rate, radicle and seedling length, number of lateral roots, shoot and root dry weight, STI, and seed vitality are commonly used as indicators reflecting seed germination and vigor (Sá et al., 2016; Li et al., 2019).

Studies aim to understand the physiological regulation in the germination phase of seeds and seedlings under salinity stress. These include the exogenous application of growth regulators such as gibberellins, abscisic acid, strigolactone, and hydrogen sulfide (Vishal and Kumar, 2018; Liu et al., 2022; Ma et al., 2022; Turan et al., 2022; Zhang et al., 2022). In addition, researchers such as Sá et al. (2016) and others involved in the present research are examining feijão-caupi varieties and genotypes for potential use under salinity conditions.

The Pingo-de-ouro variety proved to be tolerant to salinity, as no reduction in germination and GSTI was seen. Additionally, there was no increase in MGT with salinity. Furthermore, it provided a higher germination rate, GSI, and FGC compared to the Coruja variety. Islam et al. (2019) reported that from CE dS, there was an increase in MGT in feijão-caupi genotypes (Kegornatki HYV and Kegornatki green).

A similar result that was observed by Tavares et al. (2021), who established that a salinity of 100 mM (10 dS m^{-1}) did not impede the germination of *Vigna unguiculata* seeds (cultivar BRS Imponente). The same researchers attributed salinity tolerance to the stimulation of mitochondrial electron transport chain enzymes and the regulation of ROS content, both of which act as signals in the germination process. Nonetheless, salinity tolerance assessments should not only include seed germination (Nabi et al., 2017; Ravelombola et al., 2017) as variations in salinity tolerance across different phenological stages of a crop have been reported.

According to Taiz et al. (2017), saline stress induces osmotic effects that restrict water transport, thereby reducing stomatal opening. Depending on a species' genotype capacity to manage saline ions within the vacuole and synthesize organic solutes, this may influence the gas exchange of crops (Pereira Filho et al., 2019).

Additionally, the Pujante genotype of feijão-caupi has shown tolerance to an electrical conductivity of 3.0 dS m^{-1}, as it did not exhibit a reduced seedling dry matter (Nunes et al., 2019). The Green Sper and Hai Jiang San feijão-caupi genotypes are tolerant to 6 dS m^{-1}, wherein the aerial part salinity tolerance index surpassed 90% for both genotypes (Islam et al., 2019). Conversely, the STI for cultivars such as Costela de Vaca, BRS Aracê, BRS Itaim, and Canapu Branco falls below 70% (Sá et al., 2016).

The local variety Pingo-de-ouro displays similarities to the aforementioned commercial cultivars, indicating potential as parental donors for genes resistant to saline stress in a prospective genetic improvement program for *V. unguiculata*. Nonetheless, additional experiments are required for a more comprehensive understanding such as establishment of the Pingo-de-ouro variety under saline conditions. These experiments should aim to assess the productivity and salinity tolerance mechanism.

5. Conclusions

The Pingo-de-ouro variety exhibits superior performance in terms of physiological quality parameters such as germination, germination speed index, first germination count and shoot dry weight, when compared to the Coruja variety. Considering that the physiological quality of the seeds was not negatively impacted up to an electrical conductivity of 6.6 dS m^{-1}, the Pingo-de-ouro variety can be considered salinity tolerant.

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