

# GERMINATION AND BIOMASS PARTITIONING IN CREOLE VARIETIES OF COWPEA (*Vigna unguiculata* (L.) WALP.) UNDER SALINITY CONDITIONS

Francisco H. Alves de Andrade<sup>1</sup>, Ronimeire Torres da Silva<sup>2</sup>, Maria de F. de Queiroz Lopes<sup>2</sup>, Miguel A. Barbosa Neto<sup>2</sup>, Antonia D. C. de Lima Ferreira<sup>3</sup>, Maria I. Batista Clemente<sup>4</sup> and Erisvaldo Silva de Oliveira<sup>5</sup>

## ABSTRACT

Saline soils can limit seedling germination and growth of the crops. This research sought to identify whether the cowpea varieties Pingo-de-ouro and Coruja are tolerant to salinity in terms of germination and biomass partitioning. A completely randomized design in a 2 x 4 factorial arrangement, totaling eight treatments with four replications was used. The treatments consisted of two varieties of cowpea in addition to NaCl salinity with four levels of electric conductivity (0.0, 3.3, 6.6 and 9.9 dS·m<sup>-1</sup>). The variables analyzed were percentage of germination (PG), first germination count (FGC), germination speed index (GSI), average germination time (AGT), percentage of seedling (PSB), shoot (PSHB), root (PRB) and cotyledon (PCotB) biomass, PCotB/PSB ratio and salinity tolerance index (STI). The Pingo-de-ouro variety showed tolerance to 3.3 dS·m<sup>-1</sup> displaying an increase in PSB and PSHB, while the Coruja variety tolerated 6.6 dS·m<sup>-1</sup> with a more significant investment in PRB. Both displayed no significant statistical reduction in percentage of G, FGC, GSI, AGT, and STI up to 3.3 dS·m<sup>-1</sup> for the Pingo-de-ouro variety and 6.6 dS·m<sup>-1</sup> for the Coruja one. Thus, those genetic materials can be used to breed tolerant plants. The present research also provides results for further studies at physiological, molecular, and field conditions.

**Additional keywords:** Electric conductivity, local variety, seed physiological quality

## RESUMEN

### Germinación y partición de biomasa en *Vigna unguiculata* (L.) Walp. bajo condiciones de salinidad en variedades criollas

Los suelos salinos pueden limitar la germinación de las plántulas y el crecimiento de los cultivos. Esta investigación buscó identificar si las variedades de caupí, Pingo-de-ouro y Coruja, son tolerantes a la salinidad en términos de germinación y partición de biomasa. Se utilizó un diseño completamente al azar con arreglo factorial 2 x 4, totalizando ocho tratamientos con cuatro repeticiones. Los tratamientos consistieron en dos variedades de caupí además de salinidad de NaCl con cuatro niveles de conductividad eléctrica (0,0; 3,3; 6,6 y 9,9 dS·m<sup>-1</sup>). Las variables analizadas fueron porcentaje de germinación (PG), primer conteo de germinación (FGC), índice de velocidad de germinación (GSI), tiempo promedio de germinación (AGT), porcentaje de biomasa de plántula (PSB), brote (PSHB), raíz (PRB) y cotiledón (PCotB), relación PCotB/PSB e índice de tolerancia a la salinidad (STI). La variedad Pingo-de-ouro presentó tolerancia a 3,3 dS·m<sup>-1</sup> mostrando un aumento en PSB y PSHB, mientras que la variedad Coruja toleró 6,6 dS·m<sup>-1</sup> con una inversión más significativa en PRB. Ambos no mostraron reducción estadística significativa en PG, FGC, GSI, AGT y STI hasta 3,3 dS·m<sup>-1</sup> para la variedad Pingo-de-ouro y 6,6 dS·m<sup>-1</sup> para Coruja. Por lo tanto, esos materiales genéticos pueden usarse para producir plantas tolerantes. La presente investigación también proporciona resultados para futuros estudios en condiciones fisiológicas, moleculares y de campo.

**Palabras clave adicionales:** Calidad fisiológica de semillas, conductividad eléctrica, variedad local

## INTRODUCCION

Cowpea (*Vigna unguiculata* (L.) Walp.) is a versatile crop widely used for human consumption

of its fresh or dry grains (Freitas et al. 2016), especially in Northeast Brazil, as a subsistence culture. Due to the predominant characteristics of low rainfall and high rates of evapotranspiration in

Received: November 8, 2022

Accepted: May 22, 2023

<sup>1</sup>Programa de Pós-Graduação em Fisiologia Vegetal, Universidade Federal de Lavras. Lavras, MG, Brasil. e-mail: helioalvesuepb@gmail.com (corresponding author).

<sup>2</sup>Programa de Pós-Graduação em Agronomia, Universidade Federal da Paraíba. Areia, PB, Brasil. e-mail: ronimeireufc@gmail.com; atimaqueiroz0@gmail.com; miguelavelinoneto18@gmail.com

<sup>3</sup>Empresa Brasileira de Pesquisa Agropecuária. Brasília, DF, Brasil. e-mail: deboracamilla1@hotmail.com

<sup>4</sup>Programa de Pós-Graduação em Manejo de Solo e Água, Universidade Federal Rural do Semi-árido. Mossoró, RN, Brasil. e-mail: mariaisabelabclmente@gmail.com

<sup>5</sup>Instituto de Educação, Ciência e Tecnologia do Maranhão. Amarante do Maranhão, MA, Brasil. e-mail: erisvaldo.tobirama@gmail.com

that region (Medeiros et al., 2016), there is an increase and accumulation of salts on the soil surface, directly influencing the entire crop cycle, especially in the early stages.

Concerning cowpea development, the excess of salts in the soil may affect seed germination by restrictions to cell division and elongation, the mobilization of essential reserves for the germination process (Larré et al., 2011), and negatively interfering with physiological and metabolic processes related to embryonic tissue and root elongation (Esteves and Suzuki, 2008). Salinity also reduces seed vigor by diminishing the osmotic potential of the soil solution, which limits seed hydration due to the toxic effect of salts in the cells of endosperm and embryo (Duarte et al., 2006).

It is estimated that cowpea tolerates saline irrigation water with electrical conductivity up to  $3.3 \text{ dS}\cdot\text{m}^{-1}$  being, therefore, considered a crop moderately tolerant to salinity (Ayers and Westcot, 1999). However, various authors have reported that the degree and effect of the salinity stress vary between cowpea genotypes during the different stages of their development, as Nunes et al. (2019) report limitations to seed germination; Oyetunji and Imade (2015), to plant growth and development; Carvalho et al. (2016), to grain yield; and Predeepa and Ravindran (2010), to nodules formation for biological nitrogen fixation.

There must be a constant search for new materials tolerant to salinity (Almeida et al., 2011). The selection of varieties of *V. unguiculata* moderately tolerant to salinity will minimize problems arising from salinization (Lima et al., 2007) since they can be incorporated into the breeding programs of this culture. Seed germination is considered one of the most fundamental stages of plant development since the proper course of this phase determines the establishment of the seedlings and, consequently, the formation of vigorous plants (Kubala et al., 2015).

The seed ability to germinate under salinity reflects the plant tolerance to salts during subsequent stages of their development (Taiz et al., 2017). In addition to cowpea seed ability to germinate under salinity, the partition of cotyledon biomass to the seedling after germination can also influence salinity tolerance since salinity-sensitive materials have reduced

seedling dry mass (Nunes et al., 2019; Sá et al., 2016). Thus, this study aimed to evaluate whether the germination and biomass partitioning from cotyledons to seedlings increases tolerance of the Creole varieties of cowpea Pingo-de-ouro and Coruja in crescent levels of salinity.

## MATERIALS AND METHODS

The experiment was conducted at the Seed Analysis Laboratory at the Federal University of Paraíba (SAL-UFPB), Campus II Areia, PB, and used seeds of two Creole varieties of cowpea (Pingo-de-ouro and Coruja) from traditional farmers of Sitio Córrego ( $5^{\circ}39'39'' \text{ S}$ ,  $37^{\circ}52'42'' \text{ W}$ ) located in the rural area of the municipality of Apodi, RN, Brasil.

The experimental design was a randomized  $2 \times 4$  in factorial arrangement with eight treatments and four replications of 50 seeds. The treatments consisted of two creole varieties of cowpea (Pingo-de-ouro and Coruja) and four levels of electric conductivity (EC) (0.0, 3.3, 6.6 and  $9.9 \text{ dS}\cdot\text{m}^{-1}$ ) obtained by adding increasing amounts of NaCl to the irrigation water. The EC was defined employing a portable conductivity meter (Water quality detector-Yieryi).

After harvest, the seeds were stored in PET bottles, where they remained for approximately one year, the same storage time used by farmers until the next sowing. After this period, the seed moisture content was determined by drying at  $105 \pm 3^{\circ} \text{ C}$  for 24 hours, and assessing their physiological quality afterward.

Sowing was carried out in Germitest paper rolls soaked with the NaCl solution. Subsequently, the seeds were germinated in a chamber at a constant temperature of  $25^{\circ} \text{ C}$  and a photoperiod of 12 hours. To assess the effect of salinity on both varieties, the following evaluations were carried out: percentage of germination (PG), first germination count (FGC), germination speed index (GSI), and average germination time (AGT). The PG was evaluated on the fifth and eighth days after sowing, and cumulative germination was measured from the second until the tenth day. The FGC was carried out considering the percentage of normal seedlings (all essential structures) on the fifth day after sowing (Brasil, 2009). The GSI was analyzed along with the germination percentage test,

evaluating the seedlings daily from the first until the eighth day, employing the formula proposed by Maguire (1962). The AGT was calculated using the formula proposed by Labouriau (1983) through the daily count of the germinated seeds until the eighth day after sowing.

The percentage of biomass was evaluated by the dry shoot weight (DSHW), dry root weight (DRW), dry cotyledons weight (DCotW) and dry seedling weight (DSHW+DRW) in percentage, according to the following equations:

Percentage of seedling biomass (PSB):

$$PSB = \frac{DRW + DSHW}{DRW + DSHW + DCotW} \times 100$$

Percentage of shoot biomass (PSHB):

$$PSHB = \frac{DSHW}{DRW + DSHW + DCotW} \times 100$$

Percentage of root biomass (PRB):

$$PRB = \frac{DRW}{DRW + DSHW + DCotW} \times 100$$

Percentage of cotyledon biomass (PCotB):

$$PCotB = \frac{DCotW}{DRW + DSHW + DCotW} \times 100$$

Salinity tolerance index (STI) (Sá et al., 2016):

$$STI = \frac{DSHW_{st} + DRW_{st}}{DSHW_c + DRW_c} \times 100$$

The relationship between the percentage of cotyledon and seedling biomass (PCot / PSB) was given by:

$$PCotB/PSB = \frac{MSCot}{PSB}$$

Where:

st: saline treatment;

c: treatment with 0.0 dS·m<sup>-1</sup>.

MSCot: Dry mass of cotyledon

The PCotB/PSB ratio indicates the amount of cotyledonary biomass transferred to the seedling.

The data were analyzed on the R platform using the ExpDes.pt package (Ferreira et al., 2018) for the Scott-Knott average cluster test, the Hmisc package for the correlation (t-test) (Harrell, 2020), and the ggfortify package with the prcomp function: for the graphical analysis of the principal component analysis (Tang et al., 2016).

## RESULTS

The percentage of germination of the Pingo-de-ouro was constant from the seventh day of

evaluation at EC of 0.0 and 3.3 dS·m<sup>-1</sup>, and on the eighth day at 6.6 and 9.9 dS·m<sup>-1</sup> with germinations of 90, 90, 93 and 94 %, respectively. The Coruja, in turn, reached peak germination from the seventh day of evaluation, with values of 60, 59, 55 and 47 % at 0.0 through 9.9 dS·m<sup>-1</sup>, respectively (Figure 1).

It is observed that the germination of the two varieties was not affected by an increase in salinity since the EC treatments did not differ statistically according by the Scott-Knott test ( $P > 0.05$ ). However, Pingo-de-ouro's germination percentage was higher than in Coruja within each EC, with increments of 30, 29, 37 and 41 % at 0.0 through 9.9 dS·m<sup>-1</sup>, respectively (Figure 2a).

Both varieties, Pingo-de-ouro and Coruja, kept a constant FGC, which was not found to be statistically different with the increase in EC. However, a higher percentage of normal seedlings was observed in the first count for Pingo-de-ouro, with values of 90, 88, 91, and 86 % on the fifth day after sowing, while Coruja provided seedling percentages of 57, 57, 51 and 49 % at 0.0, 3.3, 6.6 and 9.9 dS·m<sup>-1</sup>, respectively (Figure 2b).

The salinity increase neither influenced the GSI nor the variables germination and FGC in Pingo-de-ouro. Whereas in Coruja, it was found that the EC of 9.9 dS·m<sup>-1</sup> reduced the germination rate. In addition, an increase in germination speed was observed in Pingo-de-ouro compared to Coruja within each level of EC (Figure 2c).

As the salinity increased, there was an increase in the germination time of Pingo-de-ouro, from two days at EC of 0.0 and 3.3 dS·m<sup>-1</sup> to 3 days at 6.6 and 9.9 dS·m<sup>-1</sup>. However, the average germination time of Coruja was not affected by the increase in salinity (Figure 2d).

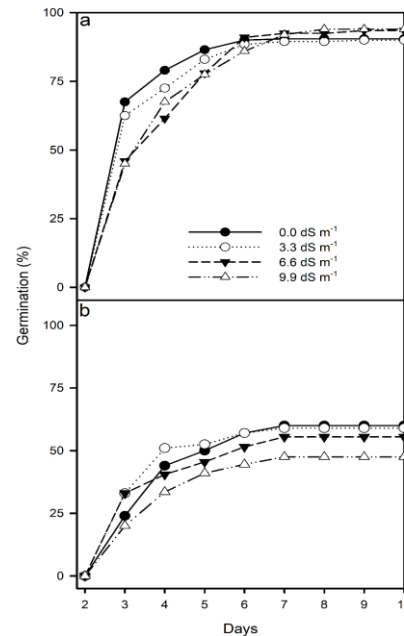
Pingo-de-ouro reached its maximum potential of seedling biomass (PotSB) at EC of 3.3 dS·m<sup>-1</sup> with an increase of 25.4 %, nonetheless, with a reduction of 19.6 and 32.0 % at EC 6.6 and 9.9 dS·m<sup>-1</sup>, respectively, compared to 0.0 dS·m<sup>-1</sup>. Coruja, on the other hand, was not sensitive to EC of 0.0, 3.3 and 6.6 dS·m<sup>-1</sup>, but the EC 9.9 dS·m<sup>-1</sup> promoted a 43 % reduction compared to the control (Figure 3a).

The percentage of shoot biomass of Pingo-de-ouro was not negatively affected by salinity increase, besides being stimulated at EC 3.3 dS·m<sup>-1</sup> with a maximum peak of 20.2 %, and the EC of

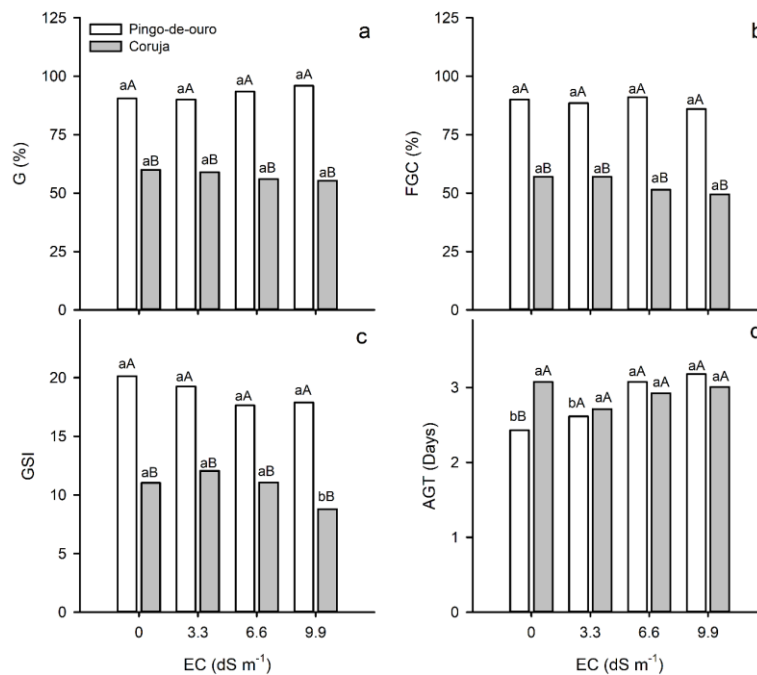
0.0, 6.6 and 9.9  $\text{dS}\cdot\text{m}^{-1}$  showed values of 14.9, 13.4 and 8.4 %, respectively. Coruja's PSHB was not influenced by the elevation of the saline levels at 0.0 and 3.3  $\text{dS}\cdot\text{m}^{-1}$  since no statistical difference was observed by the Skott-Knott test. Nonetheless, a 22 and 48 % reduction was observed at EC 6.6 and 9.9  $\text{dS}\cdot\text{m}^{-1}$  compared to the control, respectively (Figure 3b).

For Pingo-de-ouro, there was a 33 and 28 % reduction in the percentage of root biomass at the EC of 6.6 and 9.9  $\text{dS}\cdot\text{m}^{-1}$  and no significant difference between 3.3  $\text{dS}\cdot\text{m}^{-1}$  and the control by the Skott-Knott test. On the other hand, there was an increase in the percentage of root biomass in Coruja at the EC of 0.0, 3.3 and 6.6  $\text{dS}\cdot\text{m}^{-1}$ , with values of 4.2, 6.9 and 8.7 %, respectively (Figure 3c).

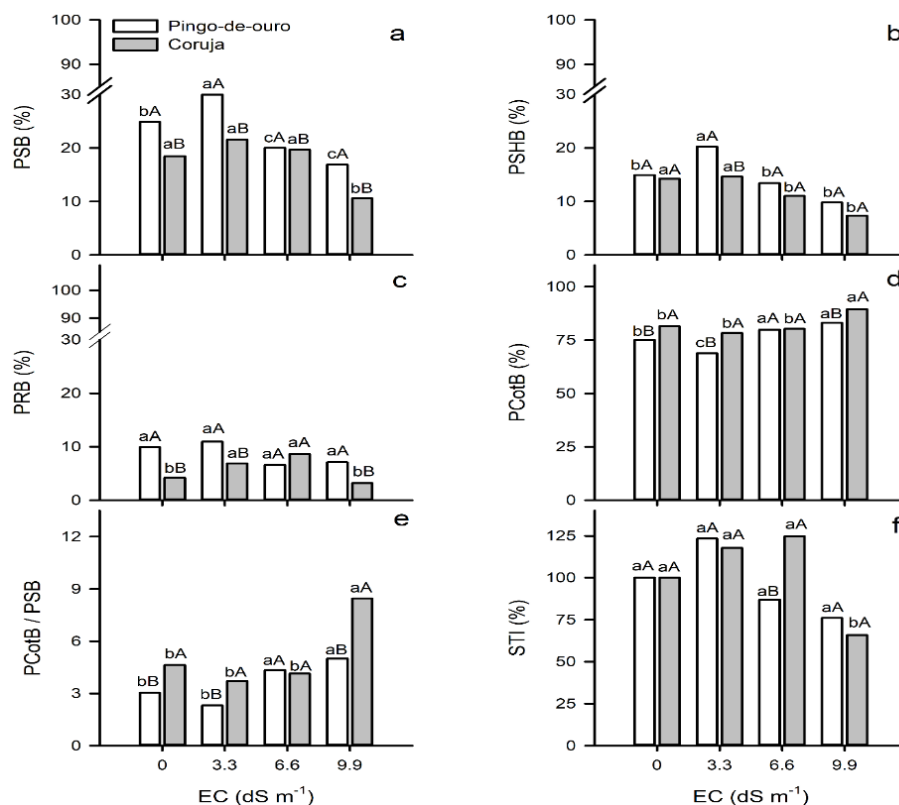
The highest percentage of cotyledon biomass (PCotB) in Pingo-de-ouro was observed at EC 6.6 and 9.9  $\text{dS}\cdot\text{m}^{-1}$  with 80 and 83 %. Coruja, in turn, displayed 89 % of PCotB at 9.9  $\text{dS}\cdot\text{m}^{-1}$  EC (Figure 3d).



**Figure 1.** Germination of creole varieties of cowpea, Pingo-de-Ouro (a) and Coruja (b), with time under salinity



**Figure 2.** Germination percentage (a), first germination count at 5<sup>th</sup> day (b), germination speed index (c) and average germination time (d) of cowpea varieties under salinity. Columns with different literals means differences according Scott-Knott test ( $P \leq 0.05$ ); lower case letters for comparisons among EC treatments; uppercase letters for comparisons between cowpea varieties (Pingo-de-ouro and Coruja)



**Figure 3.** Biomass percentage of seedling (a), shoot (b), root (c) and cotyledons (d), ratio between the percentage of cotyledon and seedling biomass (e), and salinity tolerance index (f) of two cowpea creole varieties under salinity. Columns with different literals means differences according Scott-Knott test ( $P \leq 0.05$ ); lower case letters for comparisons among EC treatments; uppercase letters for comparisons between cowpea varieties (Pingo-de-ouro and Coruja)

In the relationship between the percentage of cotyledon and seedling biomass the Pingo-de-ouro variety was high in the largest EC of 6.6 and 9.9 dS·m<sup>-1</sup> with values of 4 and 5, while in Coruja, the highest value was found at 9.9 dS·m<sup>-1</sup> with a value of 8 (Figure 3e). The PcotB/PSB ratio may indicate biomass partitioning from cotyledonary reserves (source) to the seedling (sink). Thus, the treatments displaying lower PcotB/PSB ratios point to variety tolerance under saline conditions since the saline treatment did not interfere negatively with the partitioning.

The salinity tolerance index of the Pingo-de-ouro variety presented no significant effect in any of the EC studied. However, a reduction in the STI of 13 and 24 % was observed in EC of 6.6 and 9.9 dS·m<sup>-1</sup> compared to the 0.0 dS·m<sup>-1</sup>, respectively. For the same variable, Coruja displayed a significant reduction of 34 % at the EC of 9.9 dS·m<sup>-1</sup> and other non-significant STI

increases of 17.8 and 24.6 % at 3.3 and 6.6 dS·m<sup>-1</sup>, respectively (Figure 3f). Pearson's linear correlation coefficient ( $r$ ) fluctuated between -0.1 and 0.98. The germination speed index obtained a positive correlation with the first count, germination and percentage of root biomass and a negative correlation with PCotB and PCotB/PSB. In turn, PCotB correlated negatively with STI, PSB, PSHB, PRB, GSI and FGC (Figure 4).

The salinity tolerance index correlated positively with PSB and PSHB, besides a negative correlation between PCotB and PCotB/PSB. Moreover, PRB correlated positively with PSB, and the variables PCotB and PCotB/PSB had negative correlations with STI, PSHB, PRB and PSB (Figure 4).

The GSI correlated positively with the physiological quality variables (FGC and PG), while PSHB correlated positively with PSB.

Furthermore, PRB also correlated positively with GSI (Figure 4).

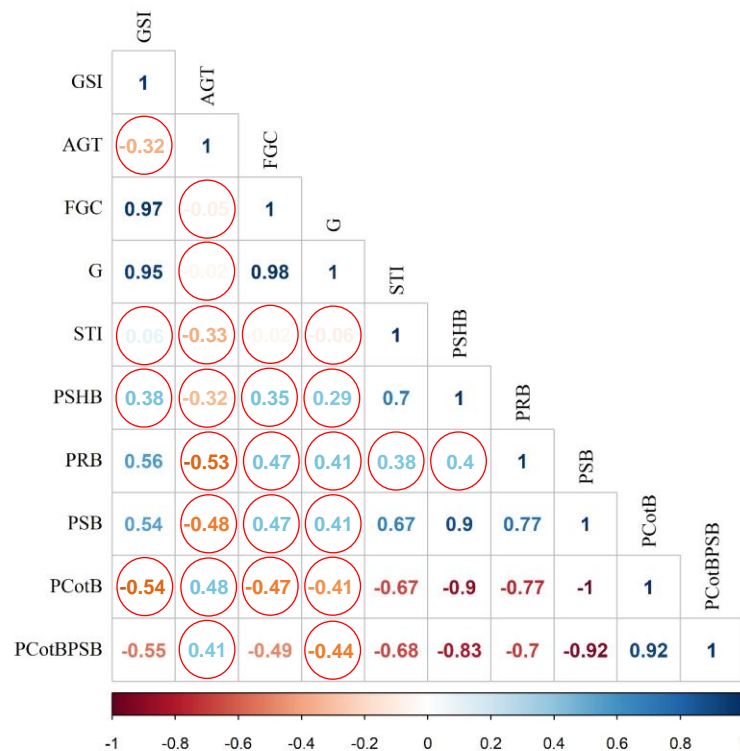
The principal component analysis (PCA) for Pingo-de-ouro revealed that the first two components hold 92.3 % of the accumulated variance, of which 82 % corresponds to PC1 and 10.4 % to PC2. It is observed that the germination variables are correlated with PC1, except the first germination count. Pingo-de-ouro also has higher STI, GSI, PRB, PSHB and PSB, besides observing that PCotB was lower in the EC of 3.3  $\text{dS}\cdot\text{m}^{-1}$  (Figure 5). This same figure also shows that Coruja variety held 81.4 % for PC1 and 12.9 % for PC2 of the accumulated variance.

The germination speed index and the PSB were higher in the EC 3.3  $\text{dS}\cdot\text{m}^{-1}$ , whereas the STI was higher in the EC treatments of 3.3 and

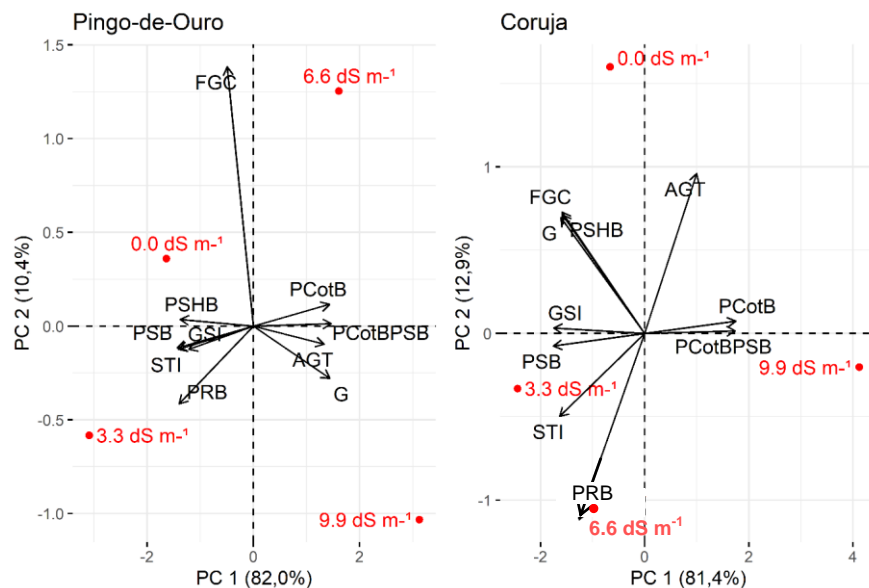
6.6  $\text{dS}\cdot\text{m}^{-1}$ , and the percentage of root biomass at 6.6  $\text{dS}\cdot\text{m}^{-1}$  (Figure 3).

The two varieties presented similar biomass partitioning indicating salinity tolerance, which may positively influence the seedlings' establishment in the field. Both varieties can significantly transfer nutrients to the seedlings under tolerable salinity conditions. It was observed that both Pingo-de-ouro and Coruja had the highest biomass partition to the shoot and root in 3.3 and 6.6  $\text{dS}\cdot\text{m}^{-1}$  (tolerant treatment), respectively. On the other hand, at the salinity of 9.9  $\text{dS}\cdot\text{m}^{-1}$ , the varieties studied have a lower PSB.

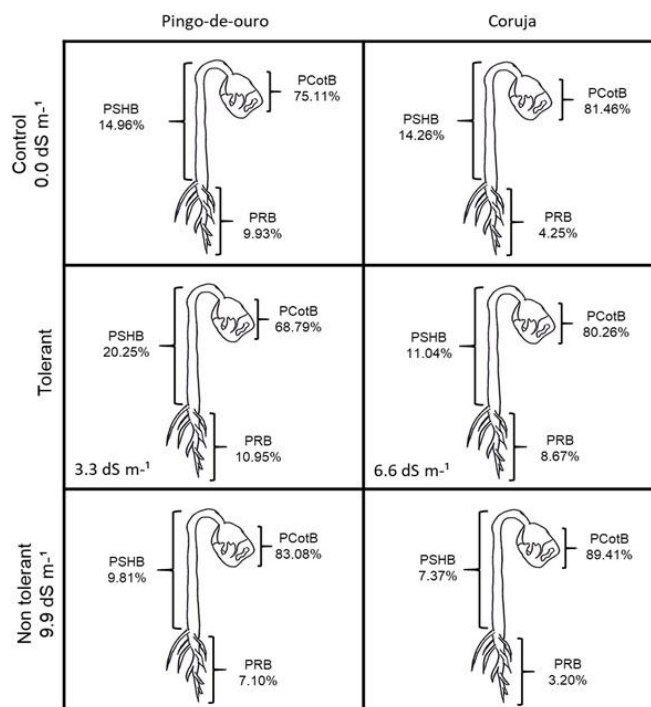
The Figure 6 shows a simplified model of the biomass partitioning of the two cowpea seedling varieties as a function of salinity.



**Figure 4.** Pearson's correlation between the germination speed index (GSI), average germination time (AGT), first germination count (FGC), germination (G), salinity tolerance index (STI), biomass percentage of the shoot (PSHB), root (PRB), seedling (PSB), and cotyledon (PCotB), and percentage ratio of cotyledon and seedling biomass (PCotB/PSB) related to two cowpea varieties (Pingo-de-ouro and Coruja) under salinity. The X represents the correlations that did not suffer significant effects (t-test,  $P \leq 0.05$ )



**Figure 5.** Principal component analysis on germination speed index (GSI), average germination time (AGT), first germination count (FGC), germination percentage (G), salinity tolerance index (STI), percentage of shoot biomass (PSHB), percentage of root biomass (PRB), percentage of seedling biomass (PSB), percentage of cotyledon biomass (PCotB) and percentage ratio between cotyledon biomass and percentage of seedling biomass (PCotB/PSB) as a function of salinity in Pingo-de-ouro and Coruja varieties



**Figure 6.** Biomass distribution of two varieties of cowpea seedlings as a function of salinity. Simplified model of biomass partitioning of the two varieties under control (0.0 dS·m<sup>-1</sup>), tolerant (Pingo-de-ouro = 3.3 dS·m<sup>-1</sup> and Coruja = 6.6 dS·m<sup>-1</sup>), and non-tolerant (9.9 dS·m<sup>-1</sup>) treatments, based on the previous results in Figures 3, 4 and 5



## DISCUSSION

The salinity of irrigation water is one of the factors that cause reduction of germination, growth and production in cowpea (Almeida et al., 2012; Ferreira et al., 2017; Oliveira et al., 2017). Saline stress limits water absorption, inhibits the growth of the embryonic axis, and induces secondary dormancy in the seeds (Farooq et al., 2017).

The genetic variability of salinity-tolerant material is decisive for agricultural production, given the difficulties encountered in the field (Nunes et al., 2019). Thus, searching for cowpea materials tolerant to salinity can be a strategy to deal its negative impact.

The germination capacity of cowpea seeds under saline conditions may indicate the tolerance of these materials. However, the capacity varies under different EC's, as observed in the cv. Marataoã, which presented a reduction at 3.0 dS·m<sup>-1</sup>; the genotypes Pujante and Epacé 10, which displayed germination reduction at 6 dS·m<sup>-1</sup> (Nunes et al., 2019), and the cv. Costela-de-vaca that behaved well at 8.0 dS·m<sup>-1</sup> (Sá et al., 2016), corroborating the results of this research, where both varieties germinated under salinity stress.

In a recent study with *V. unguiculata* cv. BRS Imponente, Tavares et al. (2021) found that NaCl stress lead to accumulation of hydrogen peroxide and increase in the respiratory activity of seeds, which acted as the main mechanisms of tolerance. NaCl concentrations above 75 mM (roughly 7.4 dS·m<sup>-1</sup>) did not affect the germination but were phytotoxic and reduced seedling growth.

In addition to germination, the FGC is a good parameter for understanding salinity tolerance. Since it is a seed vigor test, the higher the percentage of normal seedlings in the first count, the greater the seed batch vigor. Studies reveal that cowpea cultivars, such as Marataoã and Epacé 10, show a reduction in the FGC at EC of 1.5 and 4.5 dS·m<sup>-1</sup>, respectively (Nunes et al., 2019), whereas the cultivars BRS Guariba, BRS Potengi, BRS Itaim, BRS 17 Gurgueia and BRS Aracê present FGC reductions of 33, 31, 38, 34 and 34 % at EC of 8 dS·m<sup>-1</sup>, respectively (Sá et al., 2016).

The germination speed index, on the other hand, is considered one of the most rigorous tests to identify seed vigor, especially under salinity conditions, meaning that the higher the

germination speed, the greater the seed vigor (Larré et al., 2011). Research by Nunes et al. (2019a) pointed out that the BRS Marataoã and Setentão genotypes have a decrease in GSI from 1.5 dS·m<sup>-1</sup>, reaching a 46 % reduction in the EC of 7.5 dS·m<sup>-1</sup>. In turn, fava beans (*Phaseolus lunatus*), such as Roxinha and Orelha de vó, did not have an altered GSI at an EC of 9.0 dS·m<sup>-1</sup> (Nascimento et al., 2017).

Regarding the AGT, it was noticed that cowpea genotypes (Kegornatki HYV and Kegornatki green) have a delayed germination time starting from EC of 6 dS·m<sup>-1</sup> (Islam et al., 2019). The seeds of Pingo de Ouro and Coruja obtained favorable results on the physiological quality, where the variables PG, FGC, GSI, and AGT for Pingo-de-ouro were not negatively altered with up to 3.3 dS·m<sup>-1</sup>. And Coruja was tolerant up to 6.6 dS·m<sup>-1</sup> under the same variables.

The increase in salinity tolerance is decisive to seed germination and biomass partitioning from the cotyledons to the seedling. The starch hydrolysis by the enzyme  $\alpha$ -amylase in the endosperm is of fundamental importance in supplying energy for seedling growth (Kaneko et al. 2002). Thus, this enzyme plays a crucial role in tolerating salt stress, as verified by Adda et al. (2014), who confirmed an increase in  $\alpha$ -amylase activity at 50mM (equivalent to 5.0 dS·m<sup>-1</sup>) in two *Phaseolus vulgaris* cultivars (Djadida and Cocorese).

The present research's results suggest that the increase of PBP, PBCot, and STI in cowpea (Figure 4) is more pronounced at 3.3 dS·m<sup>-1</sup> in Pingo-de-ouro and 6.6 dS·m<sup>-1</sup> in Coruja (Figure 5). However, each variety has a different behavior concerning PSHB and PRB to increase salinity tolerance (Figure 3). The Pingo-de-ouro variety invested more in PSHB to increase STI at 3.3 dS·m<sup>-1</sup> (Figures 4 and 5), while Coruja invested more in PRB (Figure 5).

The starch hydrolysis into glucose in Pingo-de-ouro and Coruja varieties, respectively, at EC of 3.3 and 6.6 dS·m<sup>-1</sup>, was probably not affected by salinity and provided the mobilization of cotyledon reserves to the seedling. The relationship between cotyledon and seedling may give an understanding of reserve biomass partitioning for the seedling, so for growth to occur in the seedling stage, the cotyledon reserve



has to be directed to the seedling. However, when the seedling failed to mobilize those reserves satisfactorily, the salinity tolerance was compromised (Figure 4). This was evidenced in Pingo-de-ouro at the EC of 6.6 and 9.9 dS·m<sup>-1</sup> and the Coruja at 9.9 dS·m<sup>-1</sup> (Figure 3e).

Likewise, the cowpea genotype Pujante was tolerant at an EC of 3.0 dS·m<sup>-1</sup> since it did not have reduced DSW (Nunes et al. 2019). The Green Sper and Hai Jiang san genotypes are tolerant to 6 dS m<sup>-1</sup> with a shoot salinity tolerance index greater than 90 % in both genotypes (Islam et al., 2019).

The STI of the varieties Pingo-de-ouro and Coruja were similar to the STI of the commercial cultivars Ribs of cow, BRS Aracê, BRS Itaim and Canapu Branco which presented values below 70 % (Sá et al., 2016), giving indications of the potential of the Creole varieties Pingo-de-ouro and Coruja as parental donors of resistance genes to saline stress in a future genetic breeding program focusing *V. unguiculata*. However, more experiments are needed to fully understand the biomass partitioning and field establishment of the Creole varieties Pingo-de-ouro and Coruja.

## CONCLUSIONS

The variety Pingo-de-Ouro and Coruja displayed salinity tolerance to 3.3 and 6.6 dS·m<sup>-1</sup>, respectively, as they had better biomass partitioning to the shoot and root without reducing their physiological quality and salt tolerance index.

## LITERATURE CITED

1. Adda, A., Z. Regagba, A. Latigui, and O. Merah. 2014. Effect of Salt Stress on  $\alpha$ -amylase Activity, Sugars Mobilization and Osmotic Potential of *Phaseolus vulgaris* L. Seeds Var. 'Cocorose' and 'Djadida' During Germination. J. Biol. Sci. 4(5): 370-375.
2. Ayers, R.S. and D. Westcot. 1999. Qualidade de água na agricultura. Campina Grande: UFPB, FAO. 153 p.
3. Almeida, W.S., F.R.F. Belém, C.H.C. Bertini, M.S. Pinheiro, and E.M. Teófilo. 2011. Identificação de genótipos de feijão-caupi tolerantes a salinidade avaliada por meio de método multivariado. Ciência Rural 41(11): 1884-1889.
4. Almeida, W.S., F.R.B. Fernandes, C.H.C. Bertini, M.S. Pinheiro, and E.M. Teófilo. 2012. Emergência e vigor de plântulas de genótipos de feijão-caupi sob estresse salino. Rev. Bras. Eng. Agríc. Ambient. 16(10): 1047-1054.
5. Brasil. 2009. Ministério da Agricultura, Pecuária e Abastecimento. Regras para análise de sementes. MAPA/ACS, Brasília. 399 p. <https://n9.cl/5aow> (retrived November 2022).
6. Carvalho, J.F., E.F.F. Silva, G.F. Silva, M.M. Rolim, and E.M.R. Pedrosa. 2016. Produção dos componentes *Vigna unguiculata* L. (Walp) irrigados com água salobra sob diferentes frações de lixiviação. Revista Caatinga 29(4): 966-975.
7. Duarte, G.L., N.F. Lopes, D.M. Moraes, and R.N. Silva. 2006. Physiological quality of wheat seeds submitted to saline stress. Rev. bras. Sementes 28(1): 122-126.
8. Esteves, B.S and M.S. Suzuki. 2008. Efeito da salinidade sobre as plantas. Oecol. Bras. 12: 662-679.
9. Farooq, M., N. Gogoi, M. Hussain, S. Barthakur, S. Paul, N. Bharadwaj et al. 2017. Effects tolerance mechanisms and management of salt stress in grain legumes. Plant Physiol. Biochem. 118: 199-217.
10. Ferreira, A.C.T., R.A. Felito, A.M. Rocha, , M.A.C. Carvalho and O.M. Yamashita. 2017. Water and salt stresses on germination of cowpea (*Vigna unguiculata* cv. BRS Tumucumaque) seeds. Rev. Caatinga 30(4): 1009-1016.
11. Ferreira, E. B., P. P. Cavalcanti and D.A. Nogueira. 2018. ExpDes.pt: Pacote Experimental Designs (Portuguese). R package version 1.2.0.
12. Freitas, T.G.G., P.S.L. Silva, J.C. Vale and E.M. Silva. 2016. Green bean yield and path analysis in cowpea landraces. Rev. Caatinga 29(4): 866-877.
13. Harrell, F.E. J. with contributions from Charles Dupont and many others. 2020. Hmisc: Harrell Miscellaneous. R package version 4.4-0.
14. Islam, M.M., Haque, M.S. and A.G. Sarwar. 2019. Salt tolerance of cowpea genotypes during seed germination and seedling growth. J Bangladesh Agril Univ. 17(1): 39-44.
15. Kaneko, M., H. Itoh, M. Ueguchi-Tanaka, M.

- Ashikari and M.Matsuoka, 2002. The alpha-amylase induction in endosperm during rice seed germination is caused by gibberellin synthesized in epithelium. *Plant Physiol.* 128(4): 1264-1270.
16. Kubala, S., Ł. Wojtyła, M. Quinet, K. Lechowska, S. Lutts and M. Garnczarska, 2015. Enhanced expression of the proline synthesis gene P5CSA in relation to seed osmopriming improvement of *Brassica napus* germination under salinity stress. *J Plant Physiol.* 183: 1-12.
  17. Labouriau, L.G.A. 1983. Germinação das sementes. Washington: Secretaria Geral da Organização dos Estados Americanos, 174 p.
  18. Larré, C.F., Moraes, D.M. and N.F. Lopes. 2011. Qualidade fisiológica de sementes de arroz tratadas com solução salina e 24-epibrassinolídeo. *Rev. bras. Sementes* 33(1): 86-94.
  19. Lima, C.J.G.S., F.A. Oliveira, J. F. Medeiros M.K.T. Oliveira and A.B Almeida Júnior. 2007. Resposta do Feijão Caupí à salinidade da água de irrigação. *Rev. Verde Agroecologia Desenvol. Sustentável* 2(2): 79-86.
  20. Maguire, J.D. 1962. Speeds of germination-aid selection and evaluation for seedling emergence and vigor. *Crop Science* 2(2): 176-177.
  21. Medeiros, J. F., R. Hans, H. R. Gheyi, A. R. F. C. Costa, V. Q. Halan and V. Q. Tomaz, 2016. Manejo do solo-água-planta em área afetadas por sais. In: Manejo da Salinidade na Agricultura. In: Gheyi, Dias and Lacerda (eds). INCT Sal, Fortaleza, CE. pp. 319-333,
  22. Nascimento, M. G. R., E. U. Alves, , M. L. M Silva and C. M. Rodrigues. 2017. Lima bean (*Phaseolus lunatus* L.) Seeds exposed to different salt concentrations and temperatures. *Rev. Caatinga* 30(3): 738-747.
  23. Nunes, L. R. L., P. R. Pinheiro, C. L. Pinheiro, K. A. P. Lima and A. S. Dutra. 2019. Germination and vigor in seeds of the cowpea in response to salt and heat stress. *Rev. Caatinga* 32(1): 143-151.
  24. Nunes, L. R. L., P. R. Pinheiro, F. A. Z., Cabral, J. B. Silva and A. S. Dutra. 2019a. Ascorbic acid of cowpea seeds under saline stress. *J. Seed Sci.* 41(4): 441-451.
  25. Oliveira, F. A., M. K. T. Oliveira, L. A. Lima, R. C. Alves, L. R. L. Régis, and S. T. Santos. 2017. Estresse salino e biorregulador vegetal em feijão caupí. *Irriga* 22(2): 314-329.
  26. Oyetunji, O.J. and F.N. Imade. 2015. Effect of different levels of NaCl and Na<sub>2</sub>SO<sub>4</sub> salinity on dry matter and ionic contents of cowpea (*Vigna unguiculata* L. Walp.). *Afr. J. Agric. Res.* 10 (11): 1239-1243.
  27. Predeepa, R. J and D.A. Ravindran,. 2010. Nodule formation, distribution and symbiotic efficacy of *Vicia faba* L. under different soil salinity regimes. *Emir. J. Food Agric.* 22(4): 275-284.
  28. Sá, F. V. S., E. P. Paiva, S.B. Torres, M.E.B. Brito, N.W.N. Nogueira, L.J.G. Frade and R.M.O. Freitas. 2016. Seed germination and vigor of different cowpea cultivars under salt stress. *Com. Sci.* 7(4): 450-455.
  29. Taiz, L., E. Zeiger, I.M Møller and A. Murphy 2017. *Fisiologia e Desenvolvimento Vegetal*. Artmed, Porto Alegre.
  30. Tang, B, M. Horikoshi and W. Li. 2016. ggfortify: Unified interface to visualize statistical results of popular R packages, *The R Journal* 8: 474-485.
  31. Tavares, D.S., T.E. Kanarski Fernandes, Y. Larissa Rita, D.C. Rocha, B.F. Sant'Anna-Santos, and M.P. Gomes. 2021. Germinative metabolism and seedling growth of cowpea (*Vigna unguiculata*) under salt and osmotic stress. *South African Journal of Botany* 139: 399-408.