



Research Article

Comparison of *Capsicum* Genotypes under *In Vitro* Saline Stress Induced by Different Concentrations of NaCl and CaCl₂

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Abstract | Five cultivated species of *Capsicum* are reported as moderately sensitive to salinity and their germination can be affected by high levels of salts in the soil. The objective of this study was to evaluate the *in vitro* response of different *Capsicum* spp. genotypes to salinity and to identify those with greater tolerance to salt stress. For this, eight *Capsicum* accessions were used, which were subjected to different levels (0.1, 0.3, 0.5, 0.7 and 0.9 g L⁻¹) of sodium chloride (NaCl) and calcium chloride (CaCl₂) application under *in vitro* conditions. The results show that the seeds of the different accessions respond in a varied way to saline treatments. Accessions ECU-2254b of *C. annuum* and ECU-9123 of *C. chinense* showed more tolerance to different levels of salinity, while accessions ECU-2237 and ECU-12970b of *C. frutescens* were the most susceptible. These results provide valuable information for genetic breeding programs seeking to develop *Capsicum* cultivars with greater tolerance to abiotic factors such as salinity, which is crucial to improve productivity in these areas.

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Introduction

The genus *Capsicum* includes sweet and spicy fruits that are grown and consumed as vegetables and/or spices throughout the world (Carrizo *et*

al., 2016). These provide a diverse range of beneficial compounds to the human diet such as vitamins, antioxidants, β -carotene and flavonoids (Liu *et al.*, 2013). Five cultivated species of this genus have been reported (*C. baccatum* Jacq., *C. pubescens* Ruiz and Pav., *C.*

frutescens L., *C. chinense* L., and *C. annuum* L.) and more than 30 wild relatives (Tripodi and Kumar, 2019). By 2023, the cultivation of peppers and chili peppers reached a global production of 39,972,494 t. In Ecuador, production totaled 8,665 t across an area of 2,240 hectares (FAOSTAT, 2024). According to the III Agricultural Census, approximately 950 hectares are dedicated to monoculture in Ecuador, managed by small and medium-sized producers. These producers are in the provinces of Santa Elena, Guayas, Manabí, El Oro, Imbabura, Chimborazo, and Loja. A total harvest of 5,900 tons was reported, with an average yield of 6.21 tons per hectare (Guevara, 2021; Amaiquema, 2020).

The genus *Capsicum*, which includes species such as chili peppers and bell peppers, holds significant importance for Ecuador both gastronomically and economically. These crops are an essential part of the local diet, and their demand is growing in domestic and international markets due to their nutritional and functional properties. *Capsicum* species not only enhance traditional Ecuadorian cuisine but also provide development opportunities for small-scale producers, particularly in rural regions of the country (Morales, 2011). However, *Capsicum* productivity in Ecuador faces significant challenges due to soil salinity, especially in coastal areas. The accumulation of salts in the soil impacts water and nutrient availability for plants, ultimately affecting crop productivity and quality (Lamz and González, 2013).

Salinity is the excessive accumulation of soluble salts (Ca^{2+} , Mg^{2+} , Na^{1+} , NaCl , CaCl_2 , Na_2CO_3 and others) in water, constituting one of the main abiotic factors that affect the cellular functioning of plants, inhibiting the growth and development of many crops (Hameed et al., 2021). This condition can cause the tips and edges of leaves to suffer cell death (burned appearance) and plants to appear dehydrated, even when the soil is moist. This is because salinity in the soil alters water absorption, causing crops to wither and die due to their inability to absorb enough water (Devi and Arumugam, 2019; Sheldon et al., 2017). As a result, agricultural productivity is negatively affected, leading producers to obtain insufficient and low-quality harvests (Daba and Qureshi, 2021; Egea et al., 2023).

Salinity tolerance is a complex characteristic that involves multiple physiological, biochemical and mo-

lecular mechanisms. In response to salt stress, plants can undergo physiological, anatomical, biochemical and genetic changes (Zhang and Shin, 2013; Adem et al., 2014). According to Chartzoulakis and Klapaki (2000), the presence of high concentrations of salts in the soil can result in a decrease in the germination rate of seeds, particularly in species of the genus *Capsicum*. Different species of *Capsicum* turn out to be sensitive to the effect of salinity (Penella et al., 2015) and their germination can be negatively compromised by high levels of salts in the soil (Munns and Tester, 2008). Despite the above, there are differences in the response of *Capsicum* genotypes to salinity, which depends on various factors, such as the species, the duration of exposure to salt stress and specific growth conditions (Acosta-Motos et al., 2017; Hao et al., 2021). Additionally, not all *Capsicum* genotypes act in the same way, either due to influence on genetic expression or due to different plant defense mechanisms (Álvarez et al., 2019).

Genetic improvement of *Capsicum* spp. to increase their tolerance to salinity is an important objective in agricultural research. *In vitro* culture techniques constitute alternative procedures to field experiments for the study and selection of promising genotypes with tolerance to salt stress in a controlled environment (Custódio et al., 2022). These techniques have proven effective in assessing salinity tolerance in various plant species, representing a promising approach in the process of genetic improvement of *Capsicum* spp. genotypes. Therefore, this study aims to evaluate the phenotypic response of different genotypes of *Capsicum* spp. to salt stress under *in vitro* conditions.

Materials and Methods

Location and description of the experimental site

The research was conducted in the Biotechnology Laboratory of the Faculty of Agricultural Engineering at the Technical University of Manabí. The laboratory is situated at an altitude of 60 m.a.s.l, with geographic coordinates of 1°12'25" S latitude and 80°22'15" W longitude. The area is characterized by the following climatic conditions: an average annual temperature of 26.5°C, annual precipitation of 669.3 mm, relative humidity ranging from 70% to 90%, and an annual solar radiation of 1,146 hours (INAMHI, 2024).

Plant material

For the experiment, eight accessions of *Capsicum* spp.

were used: two accessions of *C. frutescens* (ECU-2237 and ECU-12970b), three of *C. chinense* (ECU-9123, ECU-12984, and ECU-2239b), and three of *C. annuum* (ECU-2254b), all originating from the Gene Bank of the National Institute of Agricultural Research (INIAP). These accessions represent various regions of Ecuador. Additionally, two accessions of *C. annuum* (A2a and 2Cf), collected from home gardens in the Santo Domingo parish of Esmeraldas province, were also included in the study.

In vitro establishment of capsicum seeds

Capsicum seeds were sanitized with liquid soap and sterile distilled water. Subsequently, for disinfestation, under sterile conditions inside a laminar flow chamber, the seeds were immersed in 70% ethyl alcohol for 30 seconds, after which they were rinsed with sterile distilled water, and immediately placed in a solution of 1% Sodium hypochlorite (NaClO) with 0.1% Tween-20 for 20 minutes. Finally, they were rinsed three times with sterile distilled water. Next, the seeds were placed in 150 x 25 mm glass test tubes containing Murashige and Skoog (MS) culture medium (Murashige and Skoog, 1968), supplementing with NaCl and CaCl₂ salts at concentrations of 0.1, 0.3, 0.5, 0.7 and 0.9 g L⁻¹. The MS culture medium was solidified with the addition of 7 g L⁻¹ of agar (Plant TC) and was adjusted to a pH of 5.8 ± 0.2 before being sterilized in an autoclave at 121 °C for 20 minutes at 1.05 kg/cm². The culture conditions were adjusted to a photoperiod of 16 hours, with a temperature of 24 ± 2 °C and a light intensity of 3000 lumens.

Experimental design

The experiment was evaluated under a completely randomized design (CRD) with three factors: eight *Capsicum* genotypes, two types of salts, and six con-

centrations of salts, resulting in a total of 96 treatment combinations. Each treatment was replicated four times, leading to a total of 384 experimental units, which consisted of test tubes containing the previously described culture medium. The details of the factors are provided in Table 1.

Table 1: Evaluated factors in the study of salt stress tolerance in capsicum genotypes under in vitro conditions.

Capsicum genotype	Salt type	Salt concentration (g L ⁻¹)
ECU-2254b	NaCl	0
A2a	CaCl ₂	0.1
2Cf		0.3
ECU-9123		0.5
ECU-12984		0.7
ECU-2239b		0.9
ECU-2237		
ECU-12970b		

Variables evaluated

The variables associated with the different germination indices of *Capsicum* seeds were calculated following the procedures of Lozano-Isla et al. (2018), which are detailed in Table 2.

To determine the effect of salinity levels on the growth of *Capsicum* genotypes, the following variables were evaluated:

Seedlings height (cm)

The height measurement of each seedling was carried out at 60 days, from the base of the stem to the apical part. The following equation was used (Lozano-Isla et al., 2019):

$$SH = \frac{SHS}{TS}$$

Table 2: Germination indices used during the evaluation of salt stress tolerance in *Capsicum* genotypes.

Variables	Abbreviation	Formula	Detail	Units
Germination percentage	G	$G = \frac{GS}{TS} \times 100$	GS: Germinated seeds; TS: Total number of seeds sown	%
Emergency percentage	E	$E = \frac{EP}{TS} \times 100$	EP: Emerged plants; TS: Total number of seeds sown	%
Average germination time	MGT	$MGT = \sum \frac{n \times g}{N}$	n: Number of germinated seeds; g: Number of days from sowing to the respective count; N: Total number of germinated seeds.	Time
The mean germination rate (MGR) is expressed as the reciprocal of MGT				
Germination speed	GSP	$GSP = \sum \frac{n}{(n \times g)} \times 100$	n: Number of germinated seeds; g: Number of days from sowing to the respective count	%

Where; SHS: Sum of the height of the seedlings. TS: Total of seedlings.

Number of leaves

The number of leaves was evaluated 60 days after sowing. Only seedlings that had the first embryonic leaves were considered for the recording of this variable. The formula used was (Lozano-Isla *et al.*, 2019):

$$NL = SNL/TS$$

Where; SNL: Sum of the number of leaves. TS: Total of seedlings.

Statistical analysis

The experiment was set with an 8 x 2 x 6 factorial scheme (*Capsicum* genotypes x Salts x Salt Concentrations). Assumptions of normality were analyzed using the Shapiro-Wilk test and homogeneity of variances with the Bartlett test. In cases where these assumptions were not confirmed, the data were transformed using the formula $\sqrt{y \text{ obs} + 0.5}$. Subsequently, an analysis of variance (ANOVA) was performed. When the ANOVA was significant, the Scott-Knott multiple comparison test was applied ($p > 0.05$). Linear regression analysis was performed to establish the relationship between salt concentration and plant growth. All graphs and statistical analyzes were performed using the ggplot2, ExpDes.pt packages and Treatments.ad, available in R 4.3.2 software (R Core Team, 2023).

Results and Discussion

Seed germination indices

Germination indices showed significant statistical

differences according to the effect of the salt factor (NaCl and CaCl₂), salt concentration, genotype and the interactions of these three factors. The germination percentage presented significant differences ($p < 0.001$) for all factors except for the salt x genotype interaction ($p = 0.1241$). The mean germination time (MGT), mean germination rate (MGR) and germination speed (GSP) presented statistical differences ($p < 0.0001$) in the simple effects and interactions between the study factors (Table 3).

Sodium chloride (NaCl) concentrations significantly affected ($p < 0.001$) the germination percentage of seeds of *Capsicum* spp. (Figure 1A). The highest percentages of germination were observed in the control treatments (0 g L⁻¹ of NaCl) in relation to those treated with NaCl, where the highest percentage of germination was presented by the genotypes ECU-2254b, A2a, ECU-9123, ECU-12984 and ECU-2239b with an average of 74%, while the lowest value was observed for ECU-2237 with 45%. At the concentration level of 0.1 g L⁻¹ of NaCl, the genotypes ECU-2254b, ECU-2239b and ECU-9123 presented 95% germination, followed by the genotypes A2a, ECU-12984 and ECU-12970b with average of 72%. Similar results were observed at the concentration of 0.3 and 0.5 g L⁻¹ of NaCl. At the level of 0.7 g L⁻¹ of NaCl, the genotypes ECU-2254b, A2a, ECU-2239b, ECU-9123 and ECU-12984 presented an average of 89% germination, while the rest of the genotypes exhibited germination of less than 20%. Similar behavior was observed in the treatment with 0.9 g L⁻¹ (Figure 1A).

Table 3: Probability values (*p*-values) of the analysis of variance (ANOVA) for germination indices in the study of the effect of salinity on genotypes of *Capsicum* spp. under in vitro conditions.

Source of variance	Degrees of freedom	Germination (%)		Mean germination time (MGT)		Mean germination rate (MGR)		Germination speed (GSP)	
		Mean square	p-value	Mean square	p-value	Mean square	p-value	Mean square	p-value
Salt	1	8719.8067	0.00001	509.6410	0.00001	0.0967	0.00001	876.6258	0.00001
Salt concentration	5	2794.6698	0.00001	25.2952	0.00001	0.0041	0.00001	37.7666	0.00001
Genotype	7	52085.0241	0.00001	256.8478	0.00001	0.1650	< 0.0001	1677.51	0.00001
Salt x Salt concentration	5	427.5201	0.00001	28.8236	0.00001	0.0049	0.00001	41.7804	0.00001
Salt x Genotype	7	2585.0241	0.1241	39.2756	0.00001	0.0553	< 0.0001	590.4339	0.00001
Salt concentration x Genotype	35	742.5322	0.00001	8.6583	0.00001	0.0039	0.00001	32.2199	0.00001
Salt x Salt concentration x Genotype	35	470.3099	0.0012	7.9193	0.00001	0.0044	0.00001	38.8056	0.00001

$p < 0.05$, statically significant values; $p \geq 0.05$ no statically significant values.

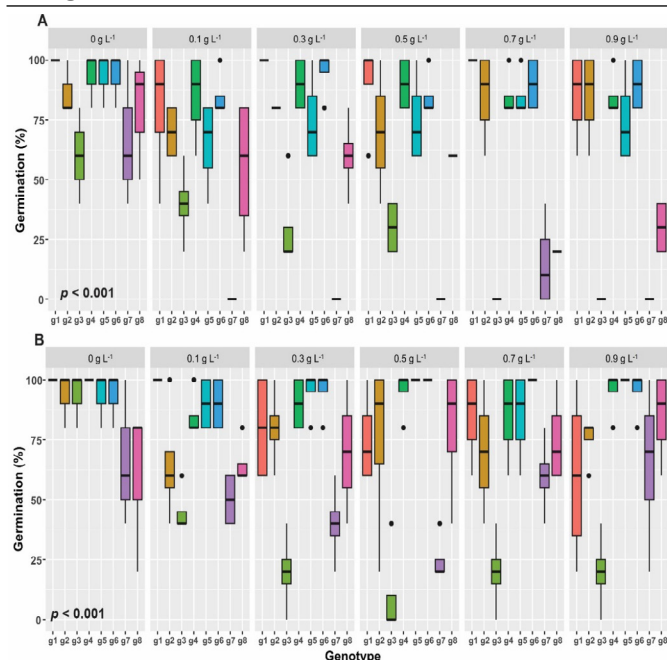


Figure 1: Effect of NaCl (A) and CaCl_2 (B) concentration on the germination percentage (%) of eight *Capsicum* genotypes under in vitro conditions. Genotypes: g1 = ECU-2254b; g2 = A2a; g3 = 2Cf; g4 = ECU-9123; g5 = ECU-12984; g6 = ECU-2239b; g7 = ECU-2237; and g8 = ECU-12970b. $p < 0.001$, indicates highly significant differences according to the Scott-knott test.

Calcium dichloride (CaCl_2) concentrations also significantly affected ($p < 0.001$) seed germination of *Capsicum* spp. (Figure 1B). At the level of 0.1 g L^{-1} of CaCl_2 , the genotypes ECU-2254b, ECU-12984, ECU-2239b and ECU-9123 showed 91% germination, followed by the genotypes A2a and ECU-12970b with an average of 65%; while the lowest germination percentage was observed in genotype 2Cf with 45%. At the concentration of 0.3 g L^{-1} the genotypes ECU-12984, ECU-2239b, ECU-9123, ECU-2254b, A2a and ECU-12970b showed an average of 85%, unlike the rest of the genotypes with values less than 40%. The 0.5 g L^{-1} concentration showed that ECU-12984, ECU-2239b and ECU-9123 exhibited average values of 97% germination, followed by ECU-12970b, ECU-2254b and A2a genotypes with 78%, opposite to the rest of the genotypes that presented germination less than 25%. The genotypes ECU-2239b, ECU-2254b, ECU-9123 and ECU-2239b treated with 0.7 g L^{-1} of CaCl_2 showed an average of 89% germination, followed by the genotypes ECU-12970b, A2a and ECU-2237 with an average of 68%, higher than the 2Cf genotype with 20%. Finally, ECU-12984, ECU-9123 and ECU-2239b and ECU-12970b presented germination averages of 94% at the 0.9 g L^{-1} concentration of CaCl_2 , different from the genotypes A2a, ECU-2237 and ECU-2254b with an average of 67%, these in turn being

higher than 2Cf with 20% (Figure 1B).

The mean germination time (MGT) of the genotypes of *Capsicum* differed significantly ($p < 0.001$) depending on the different concentrations of the salts studied. At the 0.1 g L^{-1} concentration of NaCl, the highest MGT was observed in genotype 2Cf with an average of 9 days, followed by genotypes A2a, ECU-9123, ECU-12984, ECU-2239b and ECU-12970b with average of 5.4 days; ECU-2237 did not present MGT values for this salt concentration. At the concentration of 0.3 g L^{-1} , the highest MGT was observed in the genotypes A2a, 2Cf, ECU-2239b and ECU-12970b with 7.10 days, higher than the genotypes ECU-9123 and ECU-12984 with 4.7 days, while the lowest values were shown by ECU-2254b with 3 days. The genotypes 2Cf and ECU-12970b with the concentration of 0.5 g L^{-1} showed MGT of 7.8 days, followed by the genotypes A2a, ECU-9123, ECU-12984 and ECU-2239b with an average of 5.9 days, both groups were superior to the ECU-2237 genotype. In the case of 0.7 g L^{-1} of CaCl_2 , the genotypes ECU-2254b, A2a, ECU-9123, ECU-12984, ECU-2239b, ECU-2237 and ECU-12970b presented averages of MGT 5.3 days. Finally, genotypes A2a, ECU-2239b and ECU-12970b exhibited an average of 6.5 days of MGT at doses of 0.9 g L^{-1} , while the lowest MGT values were shown by genotypes ECU-2254b, ECU-9123 and ECU-12984 with 4.01 days. In general, in relation to the salt treatments, the controls showed higher MGT values (Figure 2A).

When the genotypes were treated with CaCl_2 at a concentration of 0.1 g L^{-1} , all were statistically similar to each other, with an average MGT of 6.95 days. Similar results to the previous case were observed with the concentration of 0.3 g L^{-1} , where all genotypes showed statistically similar values. In treatments with 0.5 g L^{-1} of CaCl_2 , the MGT was higher in the genotypes ECU-2254b, A2a, ECU-9123, ECU-12984, ECU-2239b, ECU-2237 and ECU-12970b with 8.84 days, different from genotype 2Cf with 1.75 days. Meanwhile, genotypes treated with 0.7 g L^{-1} of CaCl_2 were statistically equal with MGT of 7.93 days. Finally, at the level of 0.9 g L^{-1} CaCl_2 the genotypes ECU-2254b, A2a, ECU-9123, ECU-12984, ECU-2239b, ECU-2237 and ECU-12970b resulted in the average production of 8.23 days, being higher than 2Cf (Figure 2B).

Salts and their concentrations significantly affected (p

< 0.0001) the mean germination rate (MGR) in the eight genotypes of *Capsicum* spp. The dose of 0.1 g L⁻¹ of NaCl generated lower MGR in the 2Cf and ECU-2237 genotypes with values lower than 0.10, while the highest values were observed in ECU-2254b and ECU-12984 genotypes with 0.29. In the case of the concentration of 0.3 g L⁻¹, the highest MGR was observed in the ECU-2254b genotype with 0.3, followed by ECU-12984 with an index of 0.25, while values lower than 0.20 were found in the rest of the genotypes. In the same way, with doses of 0.5 g L⁻¹ the genotype ECU-2254b showed higher MGR with 0.28, while the rest of the genotypes presented values below 0.20. This index in conditions of 0.7 g L⁻¹ was higher in the genotypes ECU-2254b, A2a, ECU-9123, ECU-12984, ECU-2239b and ECU-12970b with an average of 0.20, while the genotypes ECU-2237 and 2Cf reported an MGR of 0.05 and 0.00, respectively. Finally, ECU-2254b and ECU-9123 genotypes showed the highest MGR with indices of 0.30 and 0.28, respectively, with a concentration of 0.9 g L⁻¹ of NaCl, followed by the response of the ECU-12984 with 0.23, while the other genotypes presented an MGR less than 0.18. On the other hand, the genotypes treated with CaCl₂ did not present statistical differences between themselves in any of the concentrations studied (Table 4).

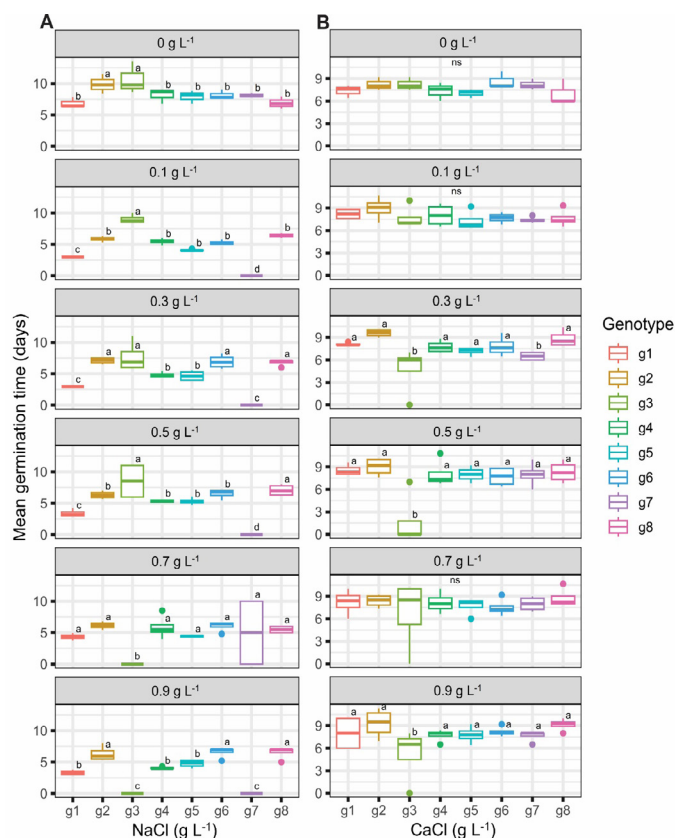


Figure 2: Effect of NaCl (A) and CaCl₂ (B) on the mean germination time (days) for eight *Capsicum* genotypes under in vitro conditions. Genotypes: g1 = ECU-2254b; g2 = A2a; g3 = 2Cf; g4 = ECU-9123; g5 = ECU-12984; g6 = ECU-2239b; g7 = ECU-2237; and g8 = ECU-12970b. Boxes with same letters are not statistically different according to the Scott-Knott test ($p \leq 0.05$); ns = Not significant differences.

Table 4: Effect of NaCl and CaCl₂ on the mean germination rate (days⁻¹) of eight *Capsicum* genotypes under in vitro conditions.

Genotype	Salt concentration											
	0 g L ⁻¹		0.1 g L ⁻¹		0.3 g L ⁻¹		0.5 g L ⁻¹		0.7 g L ⁻¹		0.9 g L ⁻¹	
	NaCl	CaCl ₂	NaCl	CaCl ₂	NaCl	CaCl ₂	NaCl	CaCl ₂	NaCl	CaCl ₂	NaCl	CaCl ₂
ECU-2254b	0.15 ± 0.01 NS	0.14 ± 0.01 NS	0.30 ± 0.001 a	0.12 ± 0.01 NS	0.30 ± 0.00 a	0.12 ± 0.00 NS	0.28 ± 0.02 a	0.12 ± 0.01 NS	0.23 ± 0.02 a	0.13 ± 0.01 NS	0.30 ± 0.00 a	0.13 ± 0.02 NS
A2a	0.10 ± 0.01	0.12 ± 0.01	0.20 ± 0.00 b	0.11 ± 0.01	0.13 ± 0.03 d	0.10 ± 0.00	0.18 ± 0.03 b	0.11 ± 0.01	0.18 ± 0.03 a	0.12 ± 0.01	0.18 ± 0.01 c	0.11 ± 0.01
2Cf	0.10 ± 0.02	0.12 ± 0.01	0.10 ± 0.00 c	0.13 ± 0.01	0.15 ± 0.03 d	0.12 ± 0.04	0.15 ± 0.00 c	0.04 ± 0.04	0.00 ± 0.00 c	0.09 ± 0.03	0.00 ± 0.00 e	0.11 ± 0.04
ECU-9123	0.13 ± 0.01	0.14 ± 0.01	0.20 ± 0.00 b	0.13 ± 0.01	0.20 ± 0.00 c	0.13 ± 0.01	0.20 ± 0.00 b	0.13 ± 0.01	0.20 ± 0.04 a	0.13 ± 0.01	0.28 ± 0.02 a	0.13 ± 0.01
ECU-12984	0.12 ± 0.01	0.14 ± 0.01	0.28 ± 0.02 a	0.14 ± 0.01	0.25 ± 0.03 b	0.14 ± 0.01	0.20 ± 0.00 b	0.13 ± 0.01	0.20 ± 0.00 a	0.13 ± 0.01	0.23 ± 0.02 b	0.13 ± 0.01
ECU-2239b	0.12 ± 0.01	0.12 ± 0.01	0.20 ± 0.001 b	0.13 ± 0.01	0.15 ± 0.03 d	0.13 ± 0.01	0.15 ± 0.03 c	0.13 ± 0.01	0.20 ± 0.00 a	0.14 ± 0.01	0.13 ± 0.03 d	0.12 ± 0.01
ECU-2237	0.13 ± 0.001	0.12 ± 0.01	0.00 ± 0.001 d	0.14 ± 0.001	0.00 ± 0.00 e	0.16 ± 0.01	0.00 ± 0.00 d	0.13 ± 0.01	0.05 ± 0.03 b	0.13 ± 0.01	0.00 ± 0.00 e	0.13 ± 0.01
ECU-12970b	0.15 ± 0.01	0.15 ± 0.02	0.18 ± 0.03 b	0.13 ± 0.01	0.13 ± 0.03 d	0.11 ± 0.01	0.15 ± 0.03 c	0.12 ± 0.01	0.20 ± 0.00 a	0.12 ± 0.01	0.13 ± 0.02 d	0.11 ± 0.01

Columns with same letters are not statistically different according to the Scott-Knott test ($p \leq 0.05$); NS = Not significant differences.

Table 5: Effect of NaCl and CaCl₂ on the germination speed (GSP) (%) of eight *Capsicum* genotypes under in vitro conditions.

Genotype	Salt concentration											
	0 g L ⁻¹		0.1 g L ⁻¹		0.3 g L ⁻¹		0.5 g L ⁻¹		0.7 g L ⁻¹		0.9 g L ⁻¹	
	NaCl	CaCl ₂	NaCl	CaCl ₂	NaCl	CaCl ₂	NaCl	CaCl ₂	NaCl	CaCl ₂	NaCl	CaCl ₂
ECU-2254b	14.69 ± 0.93 NS	13.76 ± 0.95 NS	33.30 ± 0.00 a	12.26 ± 0.52 NS	33.30 ± 0.00 a	12.35 ± 0.15 NS	29.95 ± 2.25 a	11.74 ± 0.50 NS	23.20 ± 1.31 a	12.63 ± 1.44 NS	30.58 ± 1.70 a	13.33 ± 1.92 NS
A2a	10.47 ± 0.98	12.23 ± 0.72	17.23 ± 0.68 c	11.43 ± 1.04	14.20 ± 0.53 c	10.46 ± 0.28	15.95 ± 0.74 c	11.27 ± 0.77	16.40 ± 0.80 b	12.03 ± 0.64	16.20 ± 1.39 d	11.10 ± 1.23
2Cf	10.42 ± 1.51	12.23 ± 0.72	11.18 ± 0.43 d	13.21 ± 1.07	13.88 ± 1.81 c	11.91 ± 4.01	12.90 ± 2.19 c	3.57 ± 3.57	0.00 ± 0.00 d	8.57 ± 3.03	0.00 ± 0.00 e	10.86 ± 3.72
ECU-9123	12.51 ± 1.21	13.91 ± 1.43	18.40 ± 0.86 c	12.80 ± 1.20	20.93 ± 0.87 b	13.01 ± 0.67	18.70 ± 0.29 b	12.80 ± 1.21	18.30 ± 2.70 b	12.52 ± 1.05	24.63 ± 0.38 b	13.07 ± 0.78
ECU-12984	12.82 ± 1.01	14.28 ± 0.69	24.53 ± 0.48 b	10.04 ± 1.10	21.85 ± 1.83 b	14.00 ± 0.56	19.03 ± 0.96 b	12.66 ± 0.83	22.93 ± 0.42 a	13.24 ± 1.15	21.30 ± 1.45 c	13.04 ± 0.99
ECU-2239b	12.41 ± 0.59	11.67 ± 0.83	19.15 ± 0.61 c	13.11 ± 0.61	14.80 ± 1.19 c	13.05 ± 1.06	15.55 ± 0.92 c	13.26 ± 1.11	16.85 ± 1.33 b	13.57 ± 0.99	15.53 ± 1.23 d	12.26 ± 0.49
ECU-2237	12.38 ± 0.30	12.26 ± 0.60	0.00 ± 0.00 e	13.51 ± 0.37	0.00 ± 0.00 d	15.48 ± 0.69	0.00 ± 0.00 d	12.92 ± 1.38	5.00 ± 2.89 c	12.64 ± 0.78	0.00 ± 0.00 e	13.22 ± 0.72
ECU-12970b	14.77 ± 1.09	14.82 ± 1.85	15.73 ± 0.41 c	13.34 ± 0.97	14.90 ± 0.60 c	11.43 ± 0.69	14.28 ± 0.89 c	12.29 ± 1.06	18.35 ± 0.95 b	11.54 ± 0.74	15.73 ± 1.43 d	11.02 ± 0.53

Columns with same letters are not statistically different according to the Scott-Knott test ($p \leq 0.05$); NS = Not significant differences.

The germination speed (GSP) presented significant differences ($p < 0.0001$) considering the type of salt and concentrations. In relation to the genotypes treated with 0.1 g L⁻¹ of NaCl, ECU-2254b and ECU-12984 presented the highest GSP with 33.30% and 24.53%, respectively, followed by the genotypes ECU-2239b, ECU-9123, A2a and ECU-12970b with 19.15, 18.40, 17.23 and 15.74%, respectively. The lowest GSP values were observed in 2Cf and ECU-2237. At the concentration of 0.3 g L⁻¹, like the previous case, ECU-2254b genotype showed the highest GSP with 33.30%, followed by ECU-12984 and ECU-9123 genotypes with 21.85 and 20.93%, respectively; the rest of the genotypes showed GSP lower than 14.90%. In treatments with 0.5 g L⁻¹ of NaCl, ECU-2254b genotype showed 29.95% GSP, followed by ECU-9123 and ECU-12984 with an average of 18.86%, different from the A2a, 2Cf, ECU-2239b and ECU-12970b with average of 14.67% GSP. Genotypes with 0.7 g L⁻¹, the highest GSP was achieved with ECU-2254b and ECU-12984 genotypes with an average of 23.20 and 22.93%, followed by the A2a, ECU-9123, ECU-2239b and ECU-12970b genotypes with an average of 17.60%, different from the rest of the genotypes that showed GSP less than 5%. Finally, at the highest dose studied (0.9 g L⁻¹), the most significant GSP response was observed in ECU-2254b with 30.58%, followed by ECU-9123, ECU-12984, A2a, ECU-12970b and

ECU-2239b genotypes with 24.63, 21.30, 16.20, 15.73 and 15.53%, respectively. On the other hand, the different levels of CaCl₂ did not affect the GSP in the *Capsicum* genotypes, with no statistical differences between them (Table 5).

High levels of relationship were observed between the different germination indices of *Capsicum* genotypes with the addition of NaCl (Figure 3A-C) and CaCl₂ (Figure 3D-F). As the germination speed increased, the percentage of germination also increased in treatments with NaCl ($R^2 = 0.80$), while for treatments with CaCl₂ the correlation was lower (0.53), with a significant correlation ($p < 0.001$) in both cases (Figure 3A, D). Likewise, a significant positive correlation ($p < 0.001$) was presented between germination percentage and average germination rate for both NaCl and NaCl₂ (Figure 2B, E), while the increase in germination speed showed high correlation with the average index germination (NaCl: $R^2 = 0.98$; CaCl₂: $R^2 = 1.00$) (Figure 3C, F).

Seedling growth

The growth of the different genotypes of *Capsicum* spp. was affected by increasing concentrations of NaCl (Figure 4A) and CaCl₂ (Figure 4B). To the extent that when NaCl concentration increased, the genotypes 2Cf, ECU-9123, and ECU-12970b were negatively affected, that is, the increase in NaCl

doses reduced plant growth. On the contrary, genotype A2a showed an increase in growth, resulting in high tolerance to NaCl. The genotypes ECU-2254b, ECU-12984, ECU-2239b and ECU-2237 were not significantly affected by the increase in the NaCl concentrations studied.

erage of 4.62 leaves, on the contrary, the lowest number of leaves was observed in 2Cf, ECU-12984 and ECU-2237 genotypes, with a mean of 1.80. Similar to the previous case, ECU-2254b presented the highest number of leaves (6.75) with 0.3 g L⁻¹ of NaCl, differing significantly from the rest of the genotypes, where the most affected genotypes were 2Cf and ECU-12970b with 1.75 and 1.50 sheets, respectively.

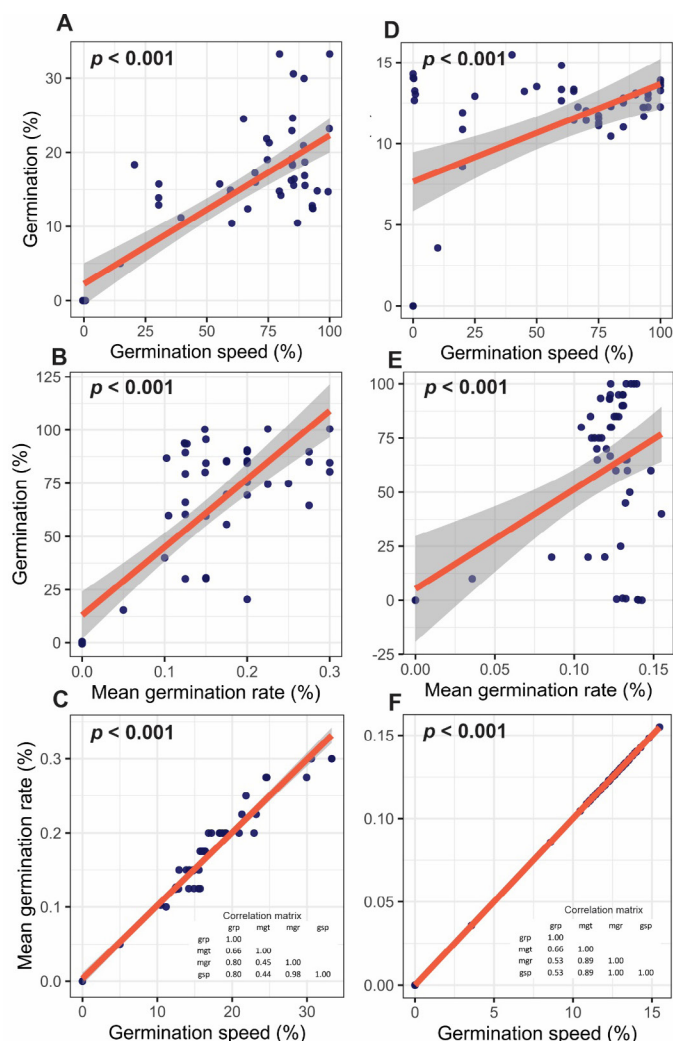


Figure 3: Correlations between germination indices associated with the effect of NaCl (A-C) and CaCl₂ (D-F) on *Capsicum* plants under in vitro conditions. (GRP = Germination percentage; MGT = Mean germination time; MGR = Mean germination rate; GSP = Germination speed). Matrixes correlations indicate the coefficient of Pearson (R^2).

When the plants were treated with CaCl₂, only the genotype ECU-2254b was negatively affected by the increase in the concentrations of this salt. Increasing CaCl₂ concentrations did not significantly affect the growth of the rest of the *Capsicum* genotypes.

The number of leaves was significantly affected by the effect of NaCl and its concentrations ($p < 0.001$) (Figure 5A). At the 0.1 g L⁻¹ concentration of NaCl, the highest number of leaves was observed in the ECU-2254b and ECU-9123 genotypes with an av-

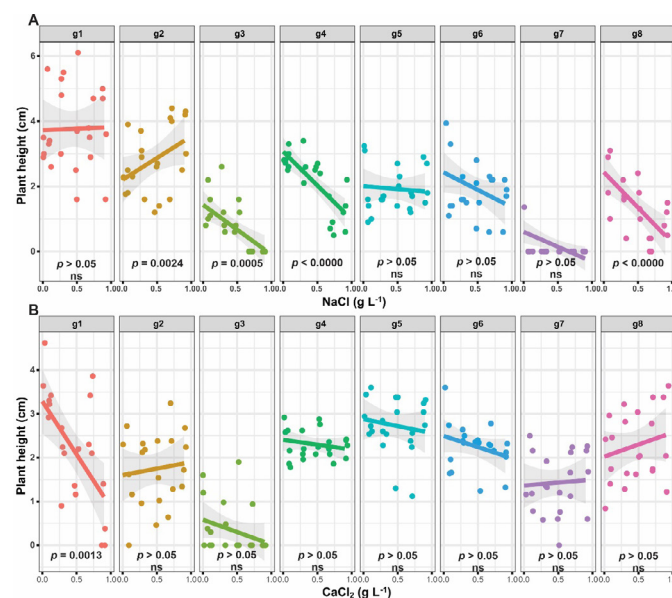


Figure 4: Relationship between concentrations of NaCl (A) and CaCl₂ (B) on the seedling height of eight *Capsicum* genotypes under in vitro conditions. Genotype: g1 = ECU-2254b; g2 = A2a; g3 = 2Cf; g4 = 9123; g5 = ECU-12984; g6 = ECU-2239b; g7 = 2237; g8 = ECU-12970b. p -values indicate statistical significance or not for the linear regression analysis; ns, indicates non-significant differences.

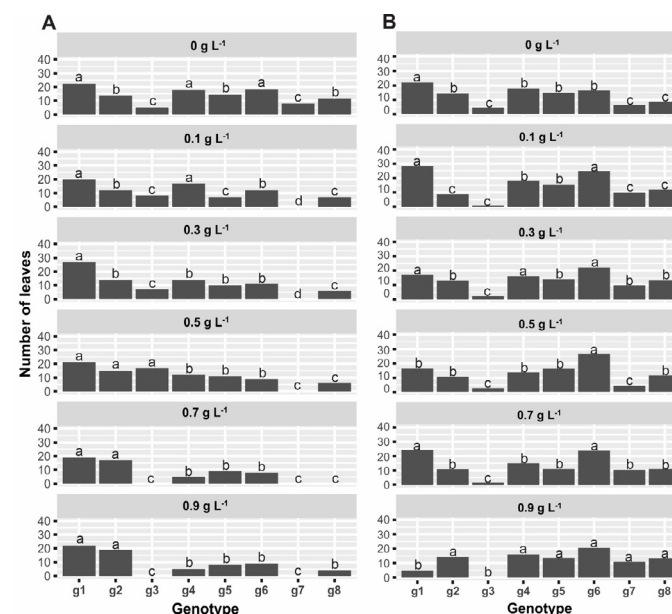


Figure 5: Effect of NaCl (A) and CaCl₂ (B) on the number of leaves for nine *Capsicum* genotypes under in vitro conditions. Genotypes: g1 = ECU-2254b; g2 = A2a; g3 = 2Cf; g4 = ECU-9123; g5 = ECU-12984; g6 = ECU-2239b; g7 = ECU-2237; g8 = ECU-12970b. Bars followed by same letters are not statistically different according to the Scott-Knott test ($p \leq 0.05$).

Meanwhile, at the 0.5 g L^{-1} concentration of NaCl, ECU-2254b and A2a genotypes showed a greater number of leaves with 5.25 and 3.75, respectively, contrary to the ECU-12970b genotype that was greatly affected by the effect of NaCl with 1.50 leaves. At the concentration 0.7 and 0.9 g L^{-1} of NaCl, the responses of the genotypes were similar, with genotypes 2Cf (0 leaves) and ECU-2237 (0 leaves) being the most affected by NaCl, unlike the genotypes ECU-2254b and A2a, which were not significantly affected by high doses of NaCl. Finally, the only genotype that did not show leaves at all NaCl concentrations was ECU-2237, except for the control where 2.66 leaves were counted. The control treatments, in general, showed a greater number of leaves in relation to the NaCl treatments.

On the other hand, the number of leaves of the *Cap-sicum* genotypes was also significantly affected ($p < 0.001$) by the different concentrations of CaCl_2 (Figure 5B). At the concentration of 0.1 g L^{-1} of CaCl_2 , the A2a genotype was mostly affected, producing on average just 0.2 leaves, contrary to the ECU-2254b and ECU-2239b genotypes that produced an average of 6.63 leaves. In turn, the 0.3 g L^{-1} concentration of CaCl_2 also affected the A2a genotype, while the genotypes ECU-2254b, ECU-9123 and ECU-2239b were not negatively influenced by CaCl_2 , with an average of 4.60 leaves. The number of leaves was also greatly reduced in genotypes 2cf and ECU-2237 at the doses of 0.5 g L^{-1} with 0.70 and 1.10 leaves, respectively. At concentrations of 0.7 g L^{-1} of CaCl_2 , the genotypes behaved very similar to the previous concentration, with genotype 2cf being the most affected by the CaCl_2 concentration. Finally, with 0.9 g L^{-1} of CaCl_2 , all genotypes showed an average of 3.68 leaves, except for the ECU-2254b and 2cf genotypes that showed 1.15 and 0.00 leaves, respectively.

Seed germination rates

Salt stress is an important environmental stress that affects plant growth and development (Zhao *et al.*, 2021). Peppers are sensitive to high salt levels, with approximately 14% of their yield reduction attributed to salinity (Abdelaal *et al.*, 2020). The response of *Capsicum* spp. seeds to different concentrations of NaCl and CaCl_2 salts reveals a complex interaction between genotype and salinity conditions. Our results highlight the importance of these factors in germination and early seedling development. The germination difference may be greater in the presence than in the

absence of salinity, attributing it to the fact that the genotypic variation in the germination response is expressed to a greater degree under salt stress conditions than under normal conditions (Yildirim *et al.*, 2011).

Sodium chloride (NaCl) treatment significantly affected seed germination, with concentrations of 0.1 g L^{-1} and 0.3 g L^{-1} showing higher germination percentages in genotypes, such as ECU-2254b and ECU-9123. These results are consistent with previous studies that indicate that low concentrations of NaCl can induce an adaptive response in some plant species, improving their germination capacity under saline conditions (Munns and Tester, 2008). However, at higher concentrations (0.7 g L^{-1} and 0.9 g L^{-1}), most genotypes showed a significant reduction in germination, suggesting a lower tolerance threshold for these conditions. The differential response between genotypes may be attributed to inherent genetic variations that confer different levels of salinity tolerance. For example, the A2a genotype of *C. annuum* showed high tolerance to NaCl, maintaining a good germination percentage even at high concentrations. This finding is consistent with research suggesting that genetic variability is crucial for adaptation to salt stress conditions (Ashraf and Foolad, 2013).

Regarding CaCl_2 concentrations, the genotypes also showed significant variability in their germination capacity. The results indicate that CaCl_2 may have a mitigating effect on the growth inhibition caused by NaCl, as has been observed in other plant species (Tavakkoli *et al.*, 2010). At concentrations of 0.1 g L^{-1} and 0.3 g L^{-1} , genotypes such as ECU-2254b and ECU-2239b showed high germination percentages, which suggests that CaCl_2 helps to stabilize cell membranes since it does not generate greater damage to the cells of different species of *Capsicum*. Mean germination time (MGT) was significantly affected by salt concentrations, with genotypes 2Cf and ECU-12970b showing the highest MGT at higher NaCl concentrations. This increase in MGT could be related to the delay in imbibition and the start of germination due to osmotic stress, as reported in other studies such as Shabala and Munns (2017). The mean germination rate (MGR) and germination speed (GSP) also presented significant differences depending on the concentrations of NaCl and CaCl_2 . Genotypes ECU-2254b and ECU-12984 showed the highest MGR and GSP values, indicating greater germination efficiency under these conditions. These results are con-

sistent with studies that show that certain genotypes can develop adaptive mechanisms to improve germination under salt stress (Flowers and Colmer, 2015).

The significant positive correlation between the different germination indices suggests that the genotypes ECU-2254b and A2a of *C. annuum* and ECU-9123, ECU-12984 and ECU-2239b of *C. chinense* with higher germination percentage also tend to have a higher germination rate and speed. This pattern may be indicative of the inherent ability of certain genotypes to maintain superior performance under salt stress conditions (Acosta-Motos *et al.*, 2017).

Seedling growth

The growth of the different genotypes of *Capsicum* spp. was significantly affected by the increase in NaCl and CaCl₂ concentrations. The sensitivity of genotypes 2Cf, ECU-9123 and ECU-12970b to NaCl, evidenced by the reduction in their growth, coincides with studies that have shown that salinity can cause osmotic and toxic stress, affecting the absorption of water and nutrients (Munns and Tester, 2008). On the contrary, A2a genotype showed a notable tolerance to NaCl, suggesting the presence of genetic mechanisms that confer resistance to salinity, possibly through the regulation of genes that control ionic homeostasis and the synthesis of osmoprotectants (Parida and Das, 2005; Flowers and Colmer, 2015).

The analysis of the number of leaves also revealed significant differences between the genotypes. The number of leaves was affected by NaCl concentrations, being higher in genotypes treated with low concentrations of NaCl and CaCl₂. Genotype ECU-2254b showed a higher number of leaves at various NaCl concentrations, while 2Cf and ECU-12970b were the most affected. This suggests that the ECU-2254b genotype has a better capacity to handle salt stress, which could be related to better osmotic and physiological adaptation under moderate stress conditions (Zhu, 2001).

Regarding the effect of CaCl₂, it was observed that most of the genotypes were not significantly affected by the increase in the concentrations of this salt, except for genotype ECU-2254b. This result is consistent with research that has shown that Ca plays a crucial role in stabilizing cell membranes and reducing sodium toxicity (Hasegawa *et al.*, 2000; White and Broadley, 2003). The ability of Calcium to mitigate

the negative effects of NaCl on the germination and growth of *Capsicum* spp. could be related to its role in cell signaling and the activation of antioxidant defense mechanisms (Ranty *et al.*, 2016).

Flowers and Colmer (2015) and Ibrahimova *et al.* (2021) suggest that plants with a greater capacity to maintain leaf growth under salt stress also show greater accumulation of compatible solutes and efficient regulation of stomatal opening. While other works also highlight the role of Ca in cell signaling under stress conditions, improving membrane integrity and promoting root growth (White and Broadley, 2003; Zörb *et al.*, 2019). Ca supplementation has also been shown to improve membrane stability and nutrient uptake in tomato plants under salt stress, supporting the idea that calcium may play an important protective role (Tuna *et al.*, 2007).

The addition of salts to the culture medium made the use of NaCl more harmful compared to CaCl₂, because NaCl inhibits plant growth, and it is presumed that the inhibition of plant growth under salt stress is associated with alterations in water relations, osmotic effects, specific effects of ions and excess or deficiency in soil nutrient availability (Roy and Chowdhury, 2021; Ludwiczak *et al.*, 2021; Munns, 1993). On the contrary, CaCl₂ can counteract the negative effects of NaCl, improving the plant's tolerance to salinity and even water stress, since being a source of Ca it is one of the essential nutrients for the growth and development of plants (Voutsinos-Frantzis *et al.*, 2023). Since the different species of *Capsicum* spp. are sensitive to the effect of salinity, the plants are more tolerant to CaCl₂ compared to NaCl.

Conclusions and Recommendations

The response of *Capsicum* spp. seeds. to NaCl and CaCl₂ concentrations depends largely on the genotype and the inherent ability of plants to adapt to salinity conditions. The genotypes ECU-2254b of *C. annuum* and ECU-9123 of *C. chinense* proved to be more tolerant, while others such as 2Cf of *C. annuum* and ECU-2237 of *C. frutescens* were more susceptible. These results provide valuable information for genetic improvement programs, contributing to the search and development of *Capsicum* cultivars with greater tolerance to abiotic factors, which is crucial to improve production in areas affected by soil salinity.

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Novelty Statement

This study provides a novel and comprehensive analysis of the in vitro response of various *Capsicum* genotypes to saline stress induced by different concentrations of NaCl and CaCl₂. While previous research has addressed the salinity tolerance of *Capsicum* species, this work distinguishes itself by systematically evaluating eight distinct *Capsicum* accessions under a range of salinity levels, thereby expanding the understanding of genotype-specific responses to salt stress. Notably, this study identifies ECU-2254b (*C. annuum*) and ECU-9123 (*C. chinense*) as highly tolerant to salinity, contrasting with the high susceptibility observed in ECU-2237 and ECU-12970b (*C. frutescens*). This detailed evaluation of genotype-specific responses to saline conditions under controlled in vitro conditions provides critical insights for developing targeted genetic breeding strategies aimed at enhancing salt tolerance in *Capsicum* cultivars. The findings offer valuable guidance for improving productivity in saline-affected agricultural areas, contributing significantly to the field of plant stress physiology and agronomy.

Author's Contribution

Katherine D Leones, Álvaro Monteros-Altamirano, Francisco Arteaga-Alcívar, Luis Alberto Duicela Guambi: Conceptualization, investigation, methodology, writing original draft.

Dany J Coveña: Conceptualization, visualization, writing review and editing.

Liliana Corozo-Quinónez: Conceptualization, resources, supervision, validation, writing review and editing.

Fátima Macías-Ponce: Investigation, methodology, validation, visualization, writing review and editing.

Luis Alberto Saltos-Rezabala: Formal analysis, methodology, writing original draft.

Data availability

Data will be made available on request.

Statement of conflict of interest

The authors have declared no conflict of interest.

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