

## CHANGES IN PROLINE AND MINERAL CONCENTRATION IN CHILI (*Capsicum spp.*) GENOTYPES FROM CHIAPAS, MEXICO, IN RESPONSE TO NaCl

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### ABSTRACT

Salt stress tolerance is an important quality in breeding cultivated plants to reduce crop loss. In this work, the tolerance to sodium chloride (NaCl) salinity of two *Capsicum* genotypes was evaluated: *C. annuum* (Blanco and Simojovel chili) and *C. frutescens* (Siete Caldos chili) from the state of Chiapas, Mexico. Seedlings were hydroponically maintained with Hoagland nutrient solution supplemented with 0, 60, and 120 mM NaCl for 8 days. Physiological response variables (SPAD units, dry and fresh weight of root and aerial part), percentage survival, proline concentration, and relative Na<sup>+</sup>, K<sup>+</sup>, and Cl<sup>-</sup> concentration in the aerial part and roots were measured. The results showed that the Siete Caldos genotype was the most sensitive to NaCl stress, with a survival rate of 66.66 % at 120 mM. Stress affected chlorophyll by decreasing SPAD units and increasing proline concentrations at 60 mM and above. Furthermore, there was a high concentration of Na<sup>+</sup> in leaves and a low concentration in roots, indicating its translocation. On the other hand, the Simojovel genotype was the most tolerant, with 100 % survival and 86.66 % survival at 60 and 120 mM, low Na<sup>+</sup> accumulation in the aerial part, and a significant increase in proline concentration. Moderately salinity-tolerant genotypes could be an alternative for plant breeding. The results obtained here lay the basis for the selection of salt stress-tolerant chili genotypes in order to transfer this tolerance to other commercially important cultivars and to minimize the damage caused by excess salts in the soil through genetic improvement and the use of tolerant rootstocks.

**Keywords:** salinity, sodium, wild genotypes, tolerance.

## INTRODUCTION

Plants, being sessile organisms, are exposed to constant environmental changes that are sometimes unfavorable to their growth and development. Adverse environmental conditions cause different types of stress in them, including biotic stress, caused by plant pathogens and herbivore attacks, and abiotic stress, caused by drought, heat, cold, nutrient deficiency and excess, metal toxicity, and excess salts or salinity in the soil (Yoshida *et al.*, 2014). Drought, salinity, and temperature extremes are some of the major types of abiotic stresses that cause adverse effects on crop growth and productivity (Dutta *et al.*, 2018). Salinity stress is one of the most limiting factors in agricultural production (Wang *et al.*, 2022).

A saline soil has an electrical conductivity of more than 0.4 dS m<sup>-1</sup> at 25 °C, an exchangeable sodium percentage of less than 15, and a pH of less than 8.5. The main cause of natural soil salinity is attributed to saltwater intrusion and salt deposition through wind; however, irrigation with high levels of salts in the water, inadequate irrigation, excessive fertilizer use, and poor soil drainage increase it (Chakdar *et al.*, 2019). The excess of soluble salts in the water reduces the leaching fraction in the soil, generating a saline over-accumulation of sodium sulfate (Na<sub>2</sub>SO<sub>4</sub>), sodium nitrate (NaNO<sub>3</sub>), sodium chloride (NaCl), sodium bicarbonate (NaHCO<sub>3</sub>), sodium carbonate (Na<sub>2</sub>CO<sub>3</sub>), potassium sulfate (K<sub>2</sub>SO<sub>4</sub>), calcium sulfate (CaSO<sub>4</sub>), magnesium sulfate (MgSO<sub>4</sub>), and magnesium chloride (MgCl<sub>2</sub>). In general, these salts are necessary for plant development; however, their surplus is harmful when they accumulate in high concentrations in the cytosol (Mosa *et al.*, 2017; Pandey *et al.*, 2019).

Excess soluble salts in the soil, mainly NaCl, can cause osmotic, ionic, and oxidative stress in plants. These three factors reduce water and nutrient uptake and induce massive efflux of water and K<sup>+</sup> ions in plant cells, leading to water and nutrient imbalances. Sodium entry and accumulation in glycophytic species and the production of reactive oxygen species (ROS) reduce growth, yield, and production in economically important crops (Munns and Tester, 2008; Bojórquez-Quintal *et al.*, 2012).

To counteract these negative effects of salt stress, plants possess different tolerance mechanisms, among which are the accumulation of compatible solutes such as proline, which participates in osmotic regulation; Na<sup>+</sup> homeostasis through the regulation of absorption; long-distance transport; compartmentalization in vacuoles and intracellular compartments; and flow or extrusion to the external environment. Another mechanism is the maintenance of K<sup>+</sup> homeostasis in plant cells through retention of this cation in the cytosol, remobilization, and uptake of K<sup>+</sup> (Deinlein *et al.*, 2014; Shabala and Pottosin, 2014).

In cultivated plants, wild relatives constitute an important reservoir of genes that can contribute to the solution of agricultural problems, such as resistance to pests and diseases and tolerance to abiotic factors. It has been reported that the development of *Capsicum chinense* grafts with wild *Capsicum annuum* Amashito and Muela chilis (*C. annuum* var. *glabriusculum*) as rootstocks shows better agronomic performance and crop productivity, as well as greater resistance to virosis (Navarrete-Mapen *et al.*,

2020). In Mexico, there are wild or poorly domesticated populations of species that are closely related to cultivated plants of great economic and food importance, which have been little studied and are at risk of being lost. Hence the importance of working with backyard or semi-domesticated species of *Capsicum* from the state of Chiapas, Mexico, since these could have more efficient mechanisms of tolerance to salinity and be considered for the genetic improvement of plants of commercial interest.

In this work, *C. annuum* and *C. frutescens* were selected for study since they have limited information on salt stress tolerance and could possess characteristics that allow them to grow and develop in abiotic stress situations, in this case NaCl salinity. Therefore, the objective of our research was to characterize in a hydroponic system the response to salinity at the seedling level of *C. annuum* (Blanco and Simojovel chili) and *C. frutescens* (Siete Caldos chili) genotypes and to determine the most tolerant genotype to salt stress.

## MATERIALS AND METHODS

### Plant material and growing conditions

Chili seeds were obtained from the ripe fruits of Simojovel and Blanco chili peppers (*C. annuum*) and Siete Caldos (*C. frutescens*), which were collected in local markets in the city of Tuxtla Gutiérrez, Chiapas, Mexico. Seeds were hydrated for 72 h at 4 °C (Ruiz-Lau *et al.*, 2016) and germinated in polystyrene seedbeds using peat (Peat Moss® peat) as substrate. The seedlings were irrigated at field capacity and kept in darkness at 25 °C. Emergence occurred approximately 10 days after sowing. Once the seedlings presented the first pair of true leaves, they were transplanted into polystyrene seedbeds using vermiculite as substrate. Irrigation was carried out with a 20 % Hoagland nutrient solution at pH 6.8 as the only source of nutrients. The photoperiod was 16 h light and 8 h dark, at a temperature of  $28 \pm 2$  °C during 40 d. The Hoagland solution used (Hoagland and Arnon, 1950) contained 1.2 mM  $\text{KNO}_3$ , 0.8 mM  $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ , 0.2 mM  $\text{KH}_2\text{PO}_4$ , 0.2 mM  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , 50  $\mu\text{M}$   $\text{CaCl}_2$ , 12.5  $\mu\text{M}$   $\text{H}_3\text{BO}_3$ , 1  $\mu\text{M}$   $\text{MnSO}_4 \cdot \text{H}_2\text{O}$ , 1  $\mu\text{M}$   $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ , 0.5  $\mu\text{M}$   $\text{CuSO}_4$ , 0.1  $\mu\text{M}$   $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 2\text{H}_2\text{O}$ , 0.1  $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$ , and 10  $\mu\text{M}$  Fe-EDTA (Sigma Aldrich® A.C.S.) in distilled water.

### Salt stress with NaCl

To determine the effects of NaCl on *Capsicum* spp., Siete Caldos (CSC) seedlings of the species *C. frutescens* and Blanco (CB) and Simojovel (CS) seedlings of the species *C. annuum* were utilized at 40 d after emergence (DAE). Each seedling represented an experimental unit. Seedlings were grown hydroponically in plastic containers (30 seedlings each) containing 500 mL of 20 % Hoagland nutrient solution for acclimatization for 10 d (Ruiz-Lau *et al.*, 2016). Permanent oxygenation was applied by means of air pumps with a maximum capacity of 108 L h<sup>-1</sup> (HiDOM® HD-8800). For each NaCl treatment, 30 seedlings were placed in a 500 mL capacity plastic container

of nutrient supplemented with 0, 60, and 120 mM NaCl (Sigma Aldrich). To avoid osmotic shock, the NaCl concentration was gradually increased every 24 h by adding 30 mM until the desired concentration was reached, and from then on, the 8 days were counted for each treatment; the seedlings were maintained under saline conditions with a photoperiod of 16 h light and 8 h dark at an ambient temperature of  $28 \pm 2$  °C. At the end of the stress treatment, the percentage survival and dry weight of the aerial part (leaf and stem) and roots were determined.

#### **Electrical conductivity and measurement of SPAD units**

To confirm that the seedlings were under salt stress, electrical conductivity (CE) was measured with a portable conductivity meter (CE/TDS/temperature HANNA DIST® 6; Italy) at each increase in NaCl concentration and at the end of the experiment. Chlorophyll estimation was determined by measuring leaf greenness with a SPAD-502 PLUS chlorophyllometer (Guzmán-Albores *et al.*, 2020) and choosing three leaves at random from 10 seedlings per treatment for a total of 30 readings.

#### **Relative concentration of Na<sup>+</sup>, K<sup>+</sup>, Cl<sup>-</sup> by energy dispersive X-ray spectroscopy (EDS)**

The relative elemental concentration was determined in the aerial part and roots previously dried in an oven at 60 °C for 5 days. These dried samples were ground, homogenized, and stored in polyethylene bags until further analysis by SEM-EDS. Determination of the relative concentration of Na<sup>+</sup>, K<sup>+</sup>, and Cl<sup>-</sup> was performed with a scanning electron microscope (SEM) (JEOL model JSM6390LV, Japan; Software INCA Suite 4.08) equipped with an energy dispersive electron probe X-ray (EDS) system (LK-IE250 Oxford INCA Energy 250) for qualitative and quantitative elemental microanalysis. For the analysis, 10 and 2.5 mg of homogenized powder of the aerial part and roots, respectively, were weighed. Before SEM-EDS analysis, the sample was carefully placed on a metal support using double-sided carbon tape. The EDS scan time was set at 70 s in an area of 3.01 × 2.32 mm at 40X magnification and a scan energy of 20 kV (Procacci *et al.*, 2021). The relative element concentration for each plant sample is shown as a percentage. Each sample (aerial part and roots) from each treatment was analyzed in triplicate.

#### **Quantification of proline**

Proline concentration was determined in leaves and roots following the method of Bates *et al.* (1973), with modifications reported by Bojórquez-Quintal *et al.* (2014). Acid ninhydrin was used for the extraction (Sigma Aldrich®, Germany; A. C. S.) and toluene (Meyer®, México; A. C. S.). Readings were taken with a spectrophotometer at 520 nm using toluene as a blank. Proline concentration was determined from an L-proline curve (Sigma Aldrich®, Germany; HPLC grade) and calculated based on fresh weight (usually expressed as μmol per gram of fresh weight, respectively). Calculations were performed with the following formula:

$$\mu\text{moles proline} * \text{g}^{-1} = \left( \frac{\left( \frac{\mu\text{g proline} * \text{mL}^{-1}}{115.5 \mu\text{g} + \mu\text{mol}^{-1}} \right) (\text{mL toluene})}{\frac{\text{g sample}}{5}} \right)$$

### Statistical analysis

A 3<sup>2</sup> factorial design was used, and the data were statistically analyzed with a two-way analysis of variance using chili genotypes (CSC, CB, and CS) and NaCl concentrations (0, 60, and 120 mM) as factors. Treatment means were compared with Tukey's multiple range test (STATGRAPHICS Centurion XVI, Inc., Madrid, Spain).

## RESULTS AND DISCUSSION

### Tolerance and physiological effects of NaCl salinity

To determine NaCl tolerance, *C. annuum* and *C. frutescens* seedlings of approximately 50 DAE were exposed to different concentrations of NaCl for 8 days. The CE in the solution increased at each concentration, reaching values of 10 and 12.13 dS m<sup>-1</sup> at 60 and 120 mM NaCl, respectively, indicating that the plants were under stress. In general, the results showed that chili seedling survival and SPAD values for chlorophyll at different NaCl concentrations depended on genotype, according to which there were significant statistical differences. The lowest survival percentages were found in CSC (66.6 %) and CB (73.3 %) with the 120 mM NaCl dose. On the other hand, CS seedlings presented 100 and 86.6 % survival with 60 and 120 mM NaCl, being the least sensitive chili genotype under the evaluated salt stress conditions (Table 1).

**Table 1.** Effect of NaCl on survival percentage, chlorophyll content, and dry weight of *Capsicum* spp. genotypes.

Treatment	Survival (%)	Chlorophyll (SPAD)	Dry weight reduction (%)	
			Aerial part	Root
CSC0	100.00 ± 0.00 a	31.36 ± 1.68 b	0 ab	0 cd
CB0	100.00 ± 0.00 a	32.26 ± 2.41 ab	0 ab	0 cd
CS0	100.00 ± 0.00 a	36.5 ± 2.79 a	0 ab	0 cd
CSC60	80.00 ± 5.77 c	24.64 ± 0.62 cd	-12.38 ± 11.21 c	21.05 ± 9.51 a
CB60	86.66 ± 1.28 b	22.13 ± 0.56 d	22.97 ± 6.81 a	30.35 ± 6.72 a
CS60	100.00 ± 0.00 a	31.23 ± 1.25 b	1.46 ± 6.02 bc	-8.97 ± 5.91 d
CSC120	66.66 ± 1.28 e	26.50 ± 0.57 cd	17.90 ± 3.87 ab	1.70 ± 7.53 bcd
CB120	73.33 ± 1.28 d	22.63 ± 0.41 d	28.47 ± 1.91 a	19.60 ± 8.36 ab
CS120	86.66 ± 1.92 b	28.86 ± 0.92 bc	19.98 ± 13.77 ab	12.25 ± 8.04 abc
LSD	6.41	4.44	20.21	18.84

Means ± standard error with different letters in each column indicate significant statistical difference between treatments (Tukey,  $p < 0.05$ ). LSD: least significant difference. Genotypes: CSC (Siete Caldos chili), CB (Blanco chili), CS (Simojovel chili); NaCl concentration at 0, 60, and 120 mM.

It was observed that seedlings exposed to salt stress showed symptoms of toxicity such as chlorosis, defoliation of old leaves, and loss of turgor, which were reflected in the SPAD readings (Table 1). SPAD values decreased with increasing NaCl concentration in all genotypes; however, statistical analysis showed no difference between 60 and 120 mM NaCl in each genotype, but at 120 mM NaCl, there is a difference between the two *C. annuum* genotypes (CB = 22.63 and CS = 28.86) (Table 1). These results show that there is a decrease in survival percentage and SPAD values as NaCl concentrations increased.

The chlorophyll content in the leaf is an indicator of the photosynthetic capacity of the plant. Chloroplasts are the first organelles to be damaged by ionic stress, and this manifests as chlorosis. Also, Na<sup>+</sup> mainly affects osmoregulation and is involved in turgor loss in plants (Isayenkov, 2012). During the experiment, leaf chlorosis was observed at 60 mM NaCl and a loss of turgor accompanied by defoliation at 120 mM NaCl. Because of this, plants suffered damage and even death, with the lowest survival rates for CSC and CB, as mentioned above (Table 1).

Salinity, drought, and temperature extremes are the main abiotic factors responsible for causing stress and severe damage to many crops worldwide. Stress can cause morphological, physiological, metabolic, and molecular changes that are detrimental to plant growth and survival. Abiotic stress, mainly salinity, leads to inhibition of plant growth (Dutta *et al.*, 2018). It has been reported that when NaCl concentration is above 40 mM, osmotic pressure above 0.2 MPa is generated. This increase in soil osmotic pressure negatively affects plant development, mainly the root, and can lead to a yield drop of 30 % (Chakdar *et al.*, 2019). Of all the energy acquired by photosynthesis and fixed in carbon compounds, only a small portion (10–40 %) is used directly for biomass accumulation, even under optimal conditions (Munns and Gilliam, 2015).

On the other hand, the reduction of aerial part dry weight at different NaCl concentrations depends on the genotype, according to which there are significant statistical differences. Taking as 100 % the treatment without NaCl for each chili genotype, it could be observed that there is a decrease in the dry weight (DW) of the aerial part and in the root in the presence of NaCl in the genotypes evaluated. However, this reduction was not significant among all treatments. At 60 mM NaCl, the aerial part of CSC is not affected; however, root DW is significantly decreased by 21.05 % (Table 1).

One of the major consequences of NaCl stress is the loss of intracellular water, so many plant species accumulate compatible solutes in response to drought and salinity (Escalante-Magaña *et al.*, 2020). The accumulation of proline is considered a mechanism of tolerance to salt stress and can even prevent the K<sup>+</sup> efflux from the roots (Bojórquez-Quintal *et al.*, 2016). Hence the importance of determining the proline concentration in chili seedlings under salinity conditions and whether there are differences between genotypes and concentrations. In the aerial part, CB and CS showed a significant increase in proline concentration only with the 120 mM NaCl dose (Table 2). In the CSC genotype, only with the 60 mM NaCl dose was the proline concentration (20.24 mg proline g<sup>-1</sup> dry weight) statistically higher (Table 2).

**Table 2.** Proline concentration in *Capsicum* spp. genotypes under NaCl stress conditions.

Treatment	Proline in aerial part (mg g <sup>-1</sup> dry weight)	Proline in roots (mg g <sup>-1</sup> dry weight)
CSC0	1.88 ± 0.35 c	0.73 ± 0.16 e
CB0	1.83 ± 0.43 c	0.57 ± 0.09 e
CS0	2.56 ± 0.34 c	0.52 ± 0.09 e
CSC60	20.24 ± 5.63 b	1.50 ± 0.37 de
CB60	8.00 ± 0.42 c	3.50 ± 0.90 cd
CS60	5.30 ± 0.52 c	1.90 ± 0.13 de
CSC120	2.38 ± 1.08 c	12.26 ± 2.29 a
CB120	28.02 ± 1.86 a	8.54 ± 0.41 b
CS120	28.76 ± 0.65 a	5.13 ± 0.99 c
LSD	6.21	2.40

Means ± standard error with different letters in each column indicate significant statistical difference between treatments. (Tukey,  $p < 0.05$ ). LSD: least significant difference. Genotypes: CSC (Siete Caldos chili), CB (Blanco chili), CS (Simojovel chili); NaCl concentration at 0, 60, and 120 mM.

Salt stress limits plant development due to several adverse effects on biochemical and physiological processes such as photosynthesis, antioxidant metabolism, mineral homeostasis, and osmolyte accumulation, among others (Khan *et al.*, 2012). The primary effects of salt stress are caused by the osmotic effect (increased solutes) and the presence of ions in the rhizosphere, limiting water uptake by the root system and causing an ionic imbalance, which reduces plant growth, while secondary effects are caused by enzyme inactivation, nutrient deficiency, ionic toxicity in tissues, and oxidative stress (ROS) (Nazar *et al.*, 2011; Khan *et al.*, 2012).

To reverse these adverse effects caused by salinity, plants have developed different tolerance strategies. One of them is the accumulation of compatible compounds, also called osmolytes (Nazar *et al.*, 2011; Khan *et al.*, 2012; Bojórquez-Quintal *et al.*, 2014). These compounds are low molecular weight, water soluble, and non-toxic at millimolar concentrations, including proline, glycine betaine, trehalose, and others (Hayat *et al.*, 2012). Osmolytes accumulate mainly in the cytoplasm without causing inhibition of enzyme activity and prevent dehydration of the cytosol by reducing water potential due to the accumulation of ions in vacuoles (Dutta *et al.*, 2018). Furthermore, they are related to salinity tolerance due to their ability to counteract water and osmotic stress and maintain ion homeostasis as well as ion compartmentalization (Nazar *et al.*, 2011; Khan *et al.*, 2012).

Proline is one of the most studied osmolytes and accumulates in response to salt stress. This amino acid maintains osmotic balance by facilitating water uptake or reducing

water loss, protecting cellular structures, and reducing oxidative damage (Alhasnawi, 2019; Escalante-Magaña *et al.*, 2020; Palchetti *et al.*, 2021). This explains the increased proline in plant tissue of chili genotypes exposed to NaCl. The concentration of proline present in the aerial part without salt treatment is low in all genotypes (Table 2) and is due to the naturally occurring proline synthesis of the plants.

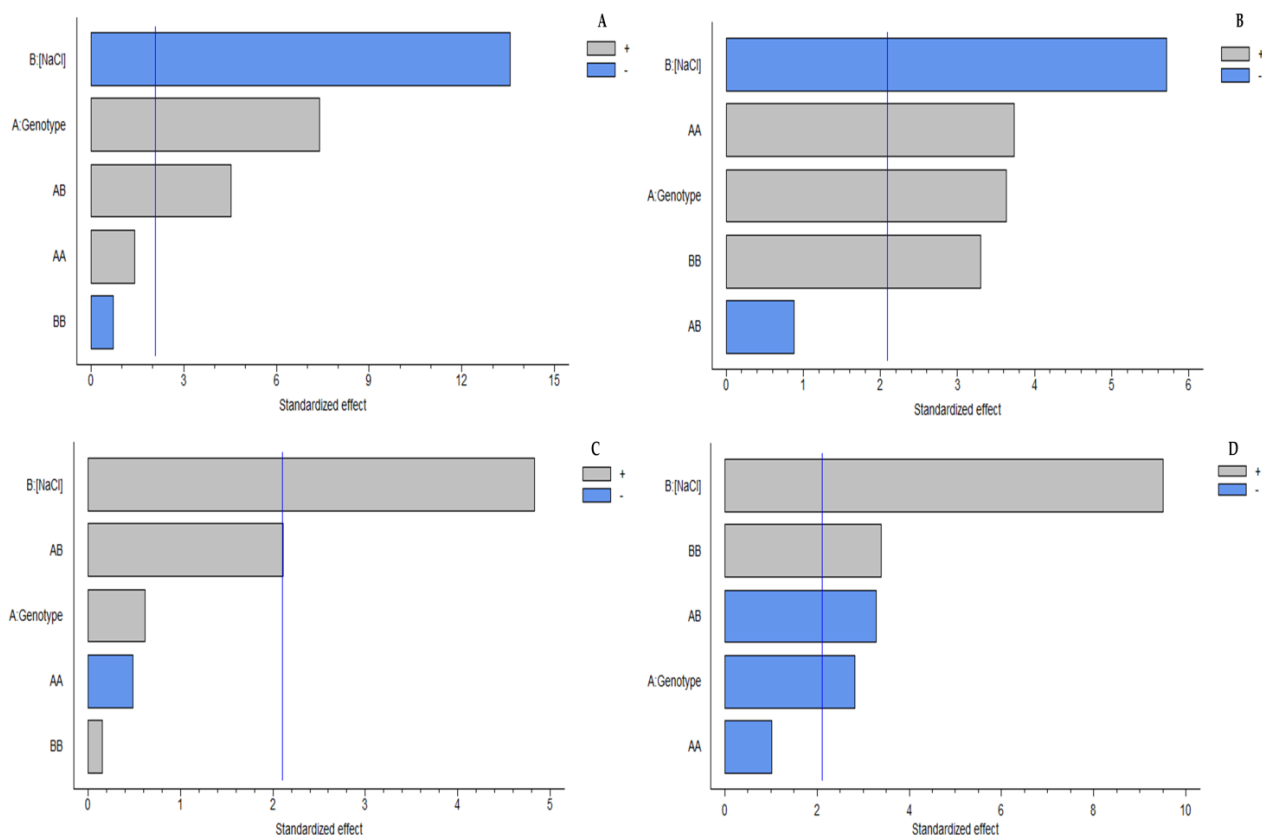
The proline values obtained in the root were lower than those recorded in the aerial part; also, increasing values are observed in all genotypes with increasing NaCl levels (Table 2). The CSC genotype had the highest proline concentration in roots with 120 mM NaCl, indicating that when exposed to severe stress that diverts the main mechanisms such as biomass production and chlorophyll synthesis, this genotype allocates nutrients to increase proline synthesis and accumulation. This forces the plant to use the glutamate present in the cell as a precursor for proline production (Escalante-Magaña *et al.*, 2020).

The survival of the CSC genotype is affected at 60 mM NaCl and above (80 % survival) (Table 1) because the main metabolic pathways that help it maintain normal growth and development are affected. This is mainly due to the high concentration of Na<sup>+</sup> in the plant tissue. Under these conditions, CSC activates mechanisms such as osmoregulation and protection of some enzymes so as not to suffer greater damage. This suggests that each genotype has different tolerance mechanisms according to the concentrations tested. Salinity tolerance is a trait controlled by many genes and involves several physiological, biochemical, and molecular mechanisms. Therefore, a combination of several of them is necessary to achieve a considerable increase in this (Adem *et al.*, 2014).

Pareto analysis showed a main effect between NaCl concentration and genotype and an interaction between these two variables. NaCl concentration has a negative effect, and genotype has a positive effect on survival percentage and SPAD chlorophyll units. As for proline concentration, NaCl has a positive effect on proline concentration; however, genotype has a negative effect on root proline concentration (Figure 1).

#### **Analysis of relative Na<sup>+</sup>, K<sup>+</sup> and Cl<sup>-</sup> concentrations**

Salinity affects mineral nutrient uptake due to over-accumulation of Na<sup>+</sup> and Cl<sup>-</sup>, which can lead to nutritional imbalance (Chakdar *et al.*, 2019), affecting plant growth and development. To determine whether the effect of NaCl on dry weight and SPAD readings was related to the accumulation of Na<sup>+</sup> and Cl<sup>-</sup> in the aerial part and roots, a relative concentration analysis of these elements was carried out by SEM-EDS. This semi-quantitative elemental analysis made it possible to determine the organ with the highest accumulation of Na<sup>+</sup>, K<sup>+</sup>, and Cl<sup>-</sup>. The results show that the relative concentration of Na<sup>+</sup> and Cl<sup>-</sup> in the aerial part and root increases significantly with increasing NaCl concentration (Table 3). However, in the CSC genotype, the Na<sup>+</sup> and Cl<sup>-</sup> concentrations are lower in the aerial part than in the roots at 120 mM NaCl (Table 3) compared to CB and CS. This suggests that CSC seedlings exposed to high NaCl concentrations accumulate more Na<sup>+</sup> (3.15 %) and Cl<sup>-</sup> (4.3 %) in the roots, preventing their translocation to the aerial part, which represents a tolerance mechanism.



**Figure 1.** Analysis of the effect of NaCl concentration and genotype. A: survival percentage; B: SPAD units; C: leaf proline concentration; D: root proline concentration.

As for  $K^+$ , this is significantly reduced in the genotypes due to the presence of salt in both tissues, with the root being the organ that accumulates the least. Among the genotypes, CSC retains more  $K^+$  in the aerial part at high salinity (120 mM NaCl) and in the root at 60 mM NaCl (Table 3). This ability to retain  $K^+$  in root and leaf cells has been suggested as a mechanism of salt tolerance in chili plants (Bojórquez-Quintal *et al.*, 2016). Potassium is a macronutrient for plants, constituting up to 10 % of their dry weight. Regulation of  $K^+$  homeostasis is essential for plant adaptation to harsh environments (Anschütz *et al.*, 2014; Shabala and Pottosin, 2014; Bojórquez-Quintal *et al.*, 2016).

Statistical analysis showed that NaCl concentration has a positive effect on the relative  $Na^+$  and  $Cl^-$  concentrations and a negative effect on  $K^+$  in the aerial part and root (Figures 2 and 3). In the case of the genotype, this was not significant in the aerial part (Figure 2). Genotype had a positive effect on the relative  $Na^+$  and  $Cl^-$  concentrations (Figure

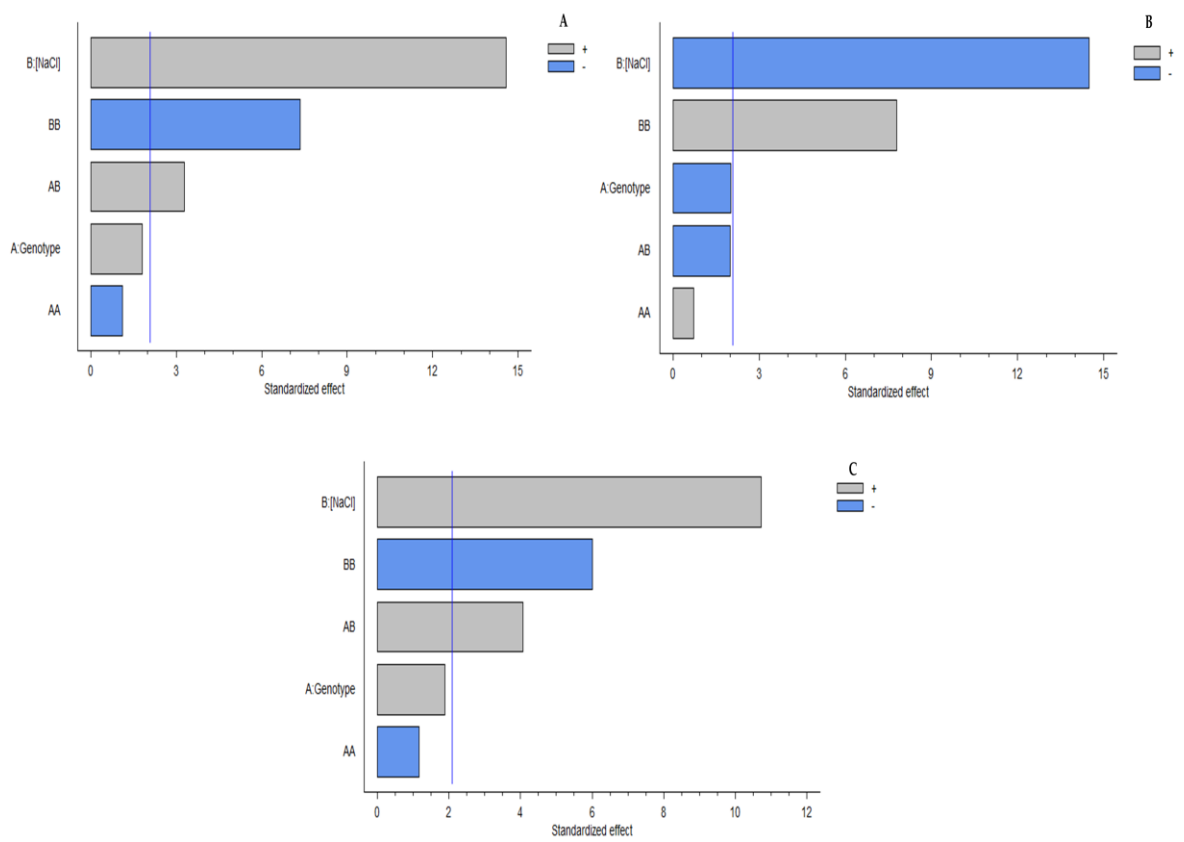
**Table 3.** Relative concentration of sodium, chlorine, and potassium in *Capsicum* spp. genotypes under NaCl salinity conditions.

Treatment	Relative [Na <sup>+</sup> ] (%)	Relative [K <sup>+</sup> ] (%) Aerial part	Relative [Cl <sup>-</sup> ] (%)
CSC0	0.18 ± 0.04 g	6.16 ± 0.22 a	0.76 ± 0.06 f
CB0	0.23 ± 0.03 g	5.83 ± 0.10 ab	0.85 ± 0.06 f
CS0	0.29 ± 0.04 g	5.81 ± 0.12 b	0.64 ± 0.07 f
CSC60	3.63 ± 0.12 c	3.15 ± 0.05 e	5.61 ± 0.08 b
CB60	3.17 ± 0.02 d	3.60 ± 0.08 d	4.74 ± 0.06 c
CS60	2.85 ± 0.06 e	3.65 ± 0.04 d	4.17 ± 0.05 d
CSC120	2.25 ± 0.09 f	4.12 ± 0.11 c	2.63 ± 0.07 e
CB120	3.89 ± 0.10 b	3.11 ± 0.03 e	5.69 ± 0.01 b
CS120	4.11 ± 0.03 a	2.90 ± 0.12 e	6.39 ± 0.13 a
LSD	0.20	0.33	0.21
		Root	
CSC0	0.21 ± 0.02 e	5.23 ± 0.11 a	0.65 ± 0.03 f
CB0	0.38 ± 0.03 e	4.29 ± 0.12 b	0.60 ± 0.03 f
CS0	0.35 ± 0.02 e	4.20 ± 0.09 b	0.60 ± 0.03 f
CSC60	1.35 ± 0.04 d	3.66 ± 0.07 c	2.75 ± 0.04 e
CB60	2.29 ± 0.01 c	2.90 ± 0.02 d	3.91 ± 0.04 c
CS60	2.54 ± 0.08 b	2.63 ± 0.01 e	3.69 ± 0.09 d
CSC120	3.15 ± 0.13 a	2.85 ± 0.01 d	4.37 ± 0.08 b
CB120	3.17 ± 0.02 a	2.76 ± 0.03 de	5.35 ± 0.05 a
CS120	3.02 ± 0.03 a	2.35 ± 0.04 f	4.05 ± 0.05 c
LSD	0.17	0.20	0.16

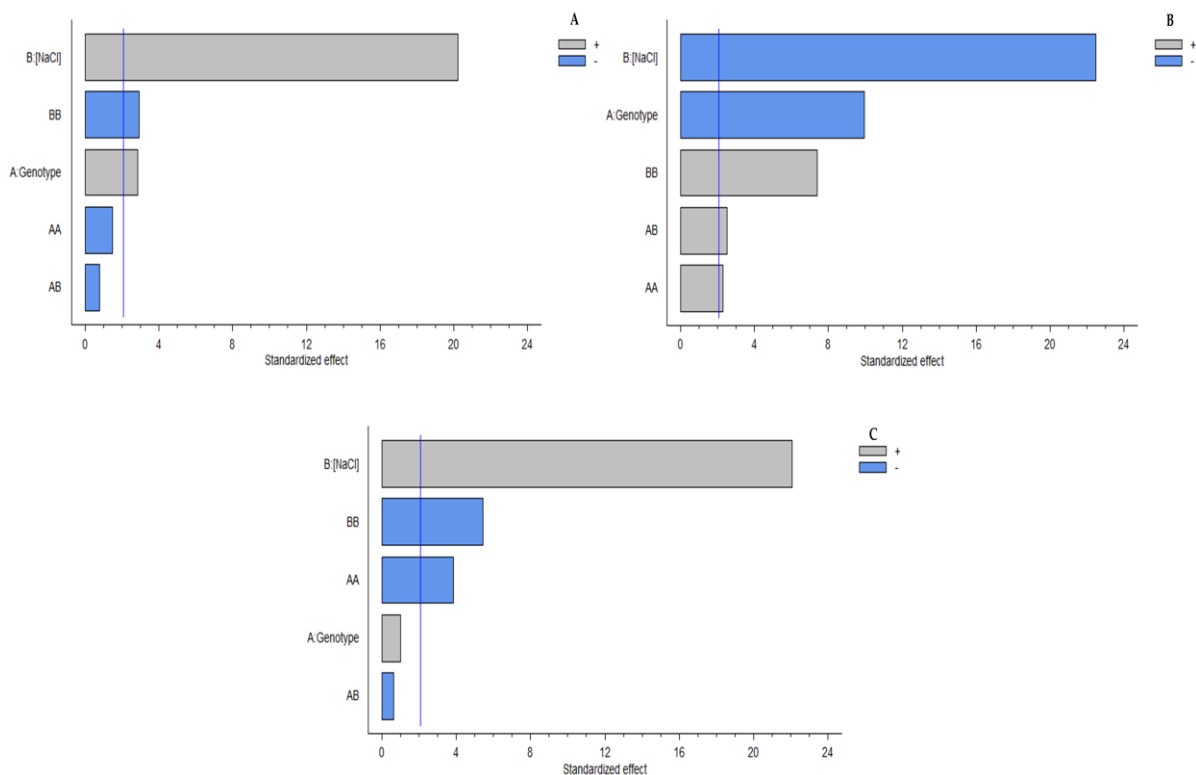
Means ± standard error with different letters in each column indicate significant statistical difference between treatments (Tukey,  $p < 0.05$ ,  $n = 3$ ). LSD: least significant difference. Genotypes: CSC (Siete Caldos chili), CB (Blanco chili), CS (Simojovel chili); NaCl concentration at 0, 60, and 120 mM.

3). Regulation of homeostasis is essential for plant adaptation to abiotic stresses, and genetic variability in plants allows them to respond to stress in different ways within the same species.

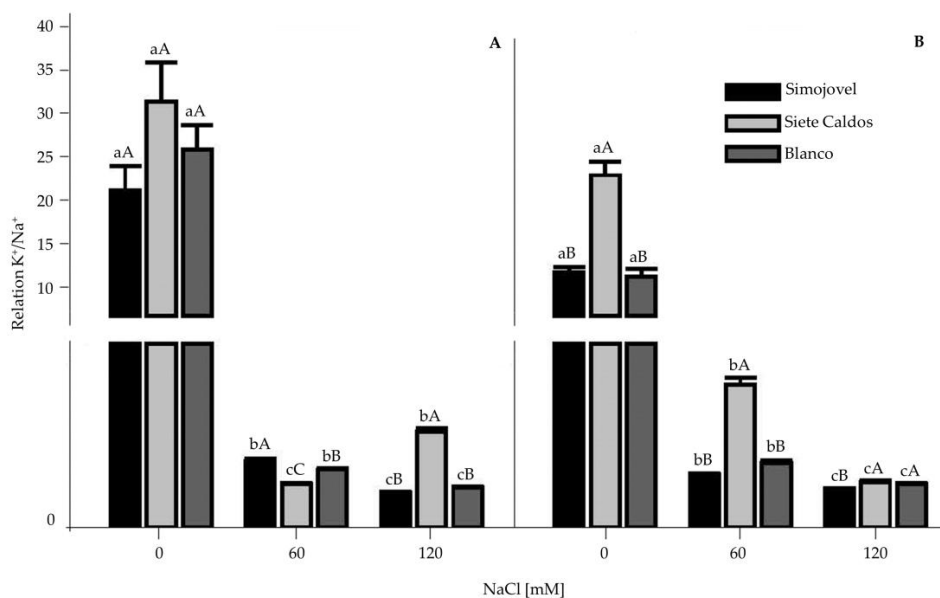
When analyzing the K<sup>+</sup>/Na<sup>+</sup> ratio, it decreased drastically, both in the aerial part and in the roots, as NaCl levels increased. This ratio was higher in the roots of the CSC genotype (*C. frutescens*) at all NaCl concentrations compared to *C. annuum*. A smaller decrease in the K<sup>+</sup>/Na<sup>+</sup> ratio could be observed with 60 mM NaCl in CS (aerial part) and in CSC at 120 mM NaCl (aerial part and roots) (Figure 4). The K<sup>+</sup>/Na<sup>+</sup> ratio is considered an indicator of tolerance to salt stress; low values of this ion ratio are indicators of sensitivity (Bojórquez-Quintal *et al.*, 2014; 2016).



**Figure 2.** Analysis of the effect of NaCl concentration and genotype on the relative element concentration in leaves. A: Na<sup>+</sup> concentration; B: K<sup>+</sup> concentration; C: Cl<sup>-</sup> concentration.



**Figure 3.** Analysis of the effect of NaCl concentration and genotype on the relative element concentration in the root. A: Na<sup>+</sup> concentration; B: K<sup>+</sup> concentration; C: Cl<sup>-</sup> concentration.



**Figure 4.** Potassium/sodium ratio in *Capsicum* spp. genotypes exposed to NaCl salinity. A: aerial part; B: roots. Capital letters indicate comparison between genotypes, and small letters between concentrations per genotype; different letters indicate significant statistical difference (Tukey,  $p < 0.05$ ,  $n = 5$ ).

The accumulation of salts in roots induces ionic stress by altering cellular ion homeostasis, as it causes an inhibition of ion uptake of essential elements such as  $K^+$ ,  $Ca^{2+}$ , and  $NO_3^-$  and an accumulation of  $Na^+$  and  $Cl^-$  (Machado and Serralheiro, 2017). Franco-Navarro *et al.* (2016) suggest that when  $Cl^-$  is supplied to glycophytic plants such as tobacco (*Nicotiana tabacum* L. var. Habana) at concentrations of 1 to 5 mol  $m^{-3}$ , it plays a role in regulating the osmotic and turgor potential of leaves, allowing plants to improve leaf water balance parameters. These concentrations are higher than necessary to meet requirements as a micronutrient but insufficient to cause toxicity.

### CONCLUSIONS

With the results obtained, it is possible to suggest that Siete Caldos is a genotype sensitive to salinity starting at 60 mM NaCl, so that by 120 mM NaCl it has already entered a stage of deterioration, presenting a 66.66 % survival rate. Furthermore, it shows a decrease in chlorophyll, an increase in proline, and a high  $Na^+$  concentration in leaves but low in roots, indicating a translocation of the ion. In response to stress, the Siete Caldos genotype retains more  $K^+$  in its aerial part, with the retention or prevention of this ion leakage being an important point in the survival and tolerance of the plants.

On the other hand, the Simojovel genotype proved to be the most tolerant, with a 100 % survival rate at 60 mM NaCl. This may be caused by two situations: the  $Na^+$  concentration in the aerial part and the root is lower, and proline accumulation increases considerably at 120 mM NaCl in the aerial part, suggesting that from this concentration onwards, this tolerance mechanism is activated. These results provide the basis for the selection of chili genotypes that are tolerant or moderately tolerant to salt stress, with the aim of transmitting this tolerance to other commercially important cultivars by means of different breeding techniques.

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