Acta Scientiarum

http://periodicos.uem.br/ojs
ISSN on-line: 1807-8621
https://doi.org/10.4025/actasciagron.v48i1.75998

CROP PRODUCTION

Optimal NaCl concentration for screening popcorn inbred lines for salt tolerance at the germination stage

Magaji Usman [1,2*], José Marcelo Soriano Viana[1], Laércio Junio da Silva[3], Matheus Pereira Ribeiro[1], Ibrahim Musa[2], Cleberson Ribeiro[1] and Vitor Batista Pinto[4]

[1]Departamento de Biologia Geral, Universidade Federal de Viçosa, Av. Peter Henry Rolfs, s/n, Campus Universitário, 36570-900, Viçosa, Minas Gerais, Brazil. [2]Departamento de Agronomia, Universidade Federal de Kashere, P.M.B. 0182, Estado de Gombe, Nigeria. [3]Departamento de Agronomia, Universidade Federal de Viçosa, Viçosa, Minas Gerais, Brazil. [4]Centro de Biociências e Biotecnologia, Universidade Estadual do Norte Fluminense, Campos dos Goytacazes, Rio de Janeiro, Brazil. *Author for correspondence. E-mail: usman.magaji@ufv.br

ABSTRACT. Approximately 85000 hectares of popcorn, which is a C4 glycophytic and salt-sensitive crop, is cultivated in Brazil. Agricultural land area is decreasing because of increasing population and salinity stress, leading scientists to devise a means to develop salt-tolerant crops using genetic approaches. This study was carried out to determine the most suitable NaCl concentration for screening popcorn inbred lines for salt tolerance. Four NaCl solutions (0, 60, 120, and 180 mmol L^{-1}) were used on four popcorn inbred lines in a completely randomized design. The experimental treatments were replicated four times in a seed-germinating chamber over seven days. Twenty-five seeds from each line were treated with Maxim® before being placed on germination paper and soaked in an appropriate NaCl solution. Significant ($p < 0.05$) responses of the inbred lines to different NaCl concentrations in terms of shoot length, root length, fresh and dry shoot and root weight, root diameter, root surface area and germination percentage were observed. The salt tolerance index based on dry seedling weight was significantly correlated with the other traits under 60 and 120 mmol L^{-1} NaCl. The most concentrated NaCl solution (180 mmol L^{-1}) resulted in the greatest reduction in the performance of the inbred lines, indicating that NaCl is toxic to the inbred lines evaluated. Regression analysis indicated that shoot length and root length are the most significant traits for assessing salt stress tolerance in popcorn inbred lines. The results suggest that NaCl solutions of 60 and 120 mmol L^{-1} could be used for screening salt tolerance in popcorn, as these provide a strong ability to distinguish among the inbred lines studied.

Keywords: abiotic stress; *Zea mays* L. var. Everta; sodium chloride; salinity stress; seedling growth.

Received on March 8, 2025.
Accepted on October 8, 2025.

Introduction

Popcorn (*Zea mays* L. var. Everta) is a special type of maize cultivated for human consumption (Batool et al., 2020). At the germination and seedling stages, salt stress has a significant effect on popcorn, whose resistance to salt is limited (Tian et al., 2024). Soil with elevated salt levels (ECe > 4dSm^{-1}) is generally defined as saline (Ismayilov et al., 2021). Soil with an ECe between 2 and 4 dSm^{-1} is considered slightly saline, that with an ECe between 4 and 16 dSm^{-1} is considered moderately saline, and if the ECe is greater than 16 dSm^{-1}, the soil is considered strongly saline (Kokebie et al., 2024). Up to 954 million hectares of saline land exist worldwide (Li et al., 2020). The physiology and biochemistry of plants growing in saline soils, from seed germination to nutritional and reproductive development, are affected by salt stress (Hasanuzzaman et al., 2013).

Globally, agricultural productivity and product quality have significantly declined because of soil salinization, which has had a major impact on the sustainable development of agriculture (Hussain et al., 2023). Soil NaCl concentration has a greater negative effect on shoot growth than on root growth, decreasing plant output (Lauchli & Epstein, 1990). Scientists are utilizing genetic methods to create salt-tolerant crops since agricultural field areas are shrinking because of population growth and salinity stress (Munns et al., 2006). Plant breeders have used a range of strategies to create salt-tolerant varieties; for example, they have examined genetic diversity among current genotypes to identify tolerant and sensitive genotypes under conditions impacted by salt (Ashraf et al., 2006; Masuda et al., 2021).

Salt damage dramatically decreases the stability of popcorn yield (Chen et al., 2019). Salt stress during germination primarily causes osmotic stress and ion toxicity, which significantly reduce the germination rate and plant vigor (Hussain et al., 2023). The most apparent morphological response is a decrease in the germination percentage and delayed germination time. Seeds may fail to imbibe enough water because of the lower water potential of saline solutions. This leads to shorter radicle (root) and coleoptile (shoot) lengths, smaller seedling size, and lower fresh weight (Tian et al., 2024). Physiologically, high concentrations of sodium (Na^+) and chloride (Cl^-) ions can be toxic to developing embryos, interfering with metabolic processes. To cope, some C4 plants, including popcorn, may activate specific osmoprotective mechanisms at this early stage, such as the synthesis of compatible solutes such as proline and glycine betaine, to maintain cellular turgor and protect cellular structures (Hussain et al., 2023). At two crucial growth stages—seed germination and seedling growth—plants are very susceptible to mortality and yield decreases from several stressors, including salt (Barnabas et al., 2008).

Soil salinization in Brazil has been exacerbated by poor management during irrigation with highly salinized water and by poor drainage. As a result, saline zones are mostly found in irrigated perimeters in semiarid regions in northeastern Brazil (Pessoa et al., 2016). Although this is a highly prevalent problem in these areas, Tian et al. (2024) reported that understanding how popcorn maize responds to salt stress in various growth phases is crucial. When popcorn varieties with high salt tolerance are screened, the groundwork is laid to produce salt-tolerant germplasms that can be grown in saline and alkaline soils; to improve saline and alkaline soils; to promote sustainable agricultural development; and to breed, produce, and use salt-tolerant germplasm resources (Masuda et al., 2021).

According to Freire et al. (2014) and Zahra et al. (2020), most current reports on salt tolerance in popcorn are at the local or, at most, irrigated perimeter scale. The majority of glycophytic plant species, such as popcorn, are salt sensitive, while a few are quite tolerant (Borsai et al., 2018). Only a small number of halophyte species are currently domesticated and widely used in agriculture; nearly all crops of agricultural significance are glycophytic and extremely sensitive to salt.

A paper roll germination experiment in which seeds are germinated on moist paper with varying levels of salt concentration can effectively reveal whether salt affects germination rate and early seedling growth (Zulkadir, 2025). The emergence of the radicle (root length) and hypocotyl (shoot length) can be compared, and any signs of stress, such as stunted growth or browning, can be observed in the presence of salt. This method is useful for quick and easy screening of germinability under salt stress (Masuda et al., 2021).

A helpful metric for identifying salt-tolerant genotypes at high NaCl concentrations (>8 dSm^{-1}) is the salt tolerance index (STI). More salt tolerance was found among genotypes with the highest STI values than among those with lower STI values (Fernandez, 1992; Sultana et al., 2016; Masuda et al., 2021). This approach is predicated on each genotype's total seedling dry weight under salt stress in comparison to a control. The effectiveness of this approach has been reported by different researchers in popcorn (Masuda et al., 2021), Italian ryegrass (Xie et al., 2021), and wheat (Irshad et al., 2022; Xu et al., 2024). To lessen the effects of salt stress, a thorough understanding of salt tolerance mechanisms and the selection of reliable screening indices are crucial for popcorn breeding programs. This study aimed to determine a suitable NaCl concentration for screening popcorn inbred lines for salt tolerance.

Material and methods

The experiments were carried out at the Seed Research Laboratory in the Department of Agronomy, Federal University of Viçosa (UFV), Minas Gerais State, Brazil. Four popcorn inbred lines from the Popcorn Breeding Program of Federal University of Viçosa were chosen at random (21-2001-1, 21-2002-1, 21-2003-1, and 21-2005-1).

Four NaCl solutions (0, 60, 120, and 180 mmol L^{-1}) were used in this study in accordance with reports in previous literature (Rizk et al., 2024) and USDA soil salinity classification. The concentrations of the NaCl solutions were measured using a bench electrical conductivity meter (Digimed DM-32) to determine their ECe. The electrical conductivities of the salt samples were 0 dS m^{-1}, 6.987 dS m^{-1}, 12.453 dS m^{-1}, and 18.359 dS m^{-1}. The mass of the salt at each level was calculated using the molarity formula:

Mass = molarity × volume × molar mass.

The experiment was laid out in a completely randomized design with four replications. The seeds were first treated with Maxim® fungicide to prevent infection. Twenty-five seeds were used for each inbred line and salt level. The seeds were first spread on germinating paper (three papers per roll) soaked in the appropriate NaCl solution, and then the paper was rolled-up (Figure 1). The seeds were germinated in an automated seed germination chamber (25°C) at the same time to ensure homogeneous experimental conditions. The seeds were allowed to germinate for seven days after exposure to salt stress before evaluation. The germinated inbred lines under the different salt concentrations and the control are shown in Figure 2.

Figure 1. From left to right, germinating paper soaked in plastic bowl for each salt level, the 25 seeds for an inbred line spread on the paper, and four rolls rolled over before placing inside the germinating chamber.

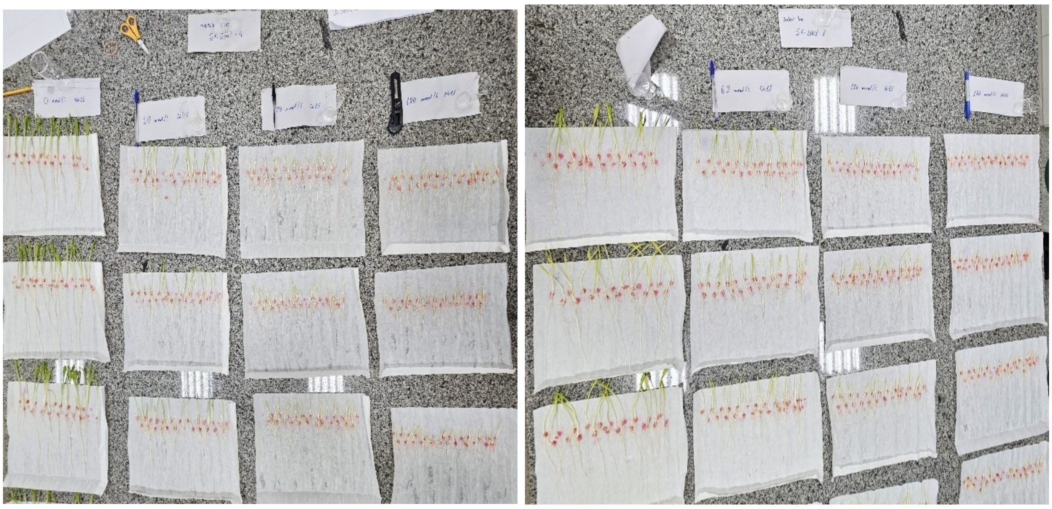

Figure 2. Showing the germinated inbred lines after 7 days of exposure to different NaCl concentrations; 1st frame is 21-2001-1 inbred line and 2nd frame is 21-2003-1 inbred line.

After they were harvested, the whole plants were divided into shoots and roots. The germination index, shoot length, fresh root weight, fresh shoot weight, dry root weight, dry shoot weight, and shoot water content were recorded. The distance from the crown to the leaf tip was measured as the shoot length. Shoot length was measured using a ruler. Fresh weight (FW) was determined using an electronic balance. Fresh samples were then washed with deionized water and dried at 80°C to a constant weight (DW), and the water content (WC) was calculated with the following formula: $WC = (FW - DW)/FW\ 100$. The abovementioned measurements and calculations were performed according to methods in Liu et al. (2015). An EPSON Expression 11000XL scanner (EPSON, São Paulo, São Paulo State, Brazil) was used to scan the roots, and WinRHIZO Pro 2009a software was used to analyze the images and determine the total root length, root surface area, and root diameter following the protocol of Ribeiro et al. (2024). The germination index provides the germination percentage in relation to the speed of germination as affected by the different salt concentrations:

Germination index (GI) = no. of germinated seeds day^{-1} of first count (4 days) + no. of germinated seeds day^{-1} of last count (7 days).

One-way analysis of variance was performed on the data to determine traits that were significant and affected by the salt stress treatments, and the Tukey test ($p < 0.05$) was used to compare significant mean differences among the inbred lines and salt concentrations. The salt tolerance index (STI) was calculated according to Tao et al. (2021):

$STI = (Yp \times Ys)/\hat{Y}p^2$

where *Yp* is the average seedling dry weight of the inbred line under nonstress conditions, *Ys* is the average seedling dry weight of the inbred line under stress conditions, and $\hat{Y}p$ is the grand mean dry weight of the seedlings under nonstress conditions. The dry weight of the inbred line was determined by summing the shoot dry weight and root dry weight. Using STI estimates, it is possible to determine the relative tolerance or stress intensity of the inbred lines under salt stress.

The responses of the inbred lines to varying salt concentrations were investigated using linear regression. The Pearson correlation coefficient was used to analyze correlations among the 14 characteristics studied and their association with the STI. The SAS 9.4v package was used to conduct the statistical analysis.

Results and discussion

The interactions between NaCl concentration and popcorn inbred lines significantly differed ($p < 0.05$) among the studied traits, especially at 60 and 120 mmol L^{-1} NaCl. Ten out of the eleven traits were significantly different between the inbred lines at 60 mmol L^{-1} NaCl and 120 mmol L^{-1} NaCl, except for root length and dry root weight (Table 1). At a higher dose of 180 mmol L^{-1}, 50% of the traits showed no significant ($p < 0.05$) difference among the studied inbred lines, indicating a negative effect on the germination and early growth performance of the popcorn lines. Compared with the control (0 mmol L^{-1}), 60 and 120 mmol L^{-1} NaCl resulted in more significant differences, indicating that the differences may be due to the response of the inbred lines to salt stress. Furthermore, the analysis of variance indicated a significant difference ($p < 0.05$) among the different salt concentrations for all the traits (Table 1). These findings indicate that salt concentration affected all the studied traits in the inbred lines. Furthermore, at 0 mmol L^{-1}, the studied inbred lines differed significantly ($p < 0.05$) in terms of the germination index, average root diameter, fresh root weight, dry shoot weight and root water content but not in terms of the remaining 5 traits (Table 1). The performance of the inbred line 21-2001-1 was good (Table 2). With respect to the results at 60 mmol L^{-1} NaCl, all the studied traits differed significantly ($p < 0.05$) among the inbred lines except for root length. These findings revealed that a low-concentration NaCl solution could be used to screen for salt tolerance among popcorn inbred lines.

Table 1. Analysis of variance of the 11 traits of the four studied popcorn inbred lines under control and different salt level conditions.

Traits	Salt levels (3df)	Inbred Lines × Salt level (9df)	Inbred lines within 0 mmol L^{-1} (3df)	Inbred lines within 60 mmol L^{-1} (3df)	Inbred lines within 120 mmol L^{-1} (3df)	Inbred lines within 180 mmol L^{-1} (3df)	Error (48df)	CV (%)
Germination Index	8.01**	2.30**	10.85**	7.22**	1.61**	0.36^{NS}	0.39	14.58
Shoot Length	251.72**	1.35**	0.57^{NS}	7.48**	2.40**	0.71*	0.32	7.28
Root length	2068.89**	25.611*	89.11^{NS}	0.67^{NS}	6.63**	1.33**	10.75	14.59
Average Root Surface area	1136.40**	23.06**	1.99^{NS}	115.51**	35.34**	11.05**	3.63	12.52
Average Root Diameter	0.12**	0.002*	0.002*	0.001**	0.004**	0.009**	0.00055	3.33
Fresh Root Weight	0.55**	0.09*	0.34**	0.22*	0.12**	0.15*	0.0431	13.96
Fresh Shoot Weight	52.36**	0.453**	0.41^{NS}	1.52**	0.01**	0.10*	0.1112	9.97
Dry Root Weight	0.022**	0.0006^{NS}	0.001^{NS}	0.002**	0.001^{NS}	0.0002^{NS}	0.00034	14.24
Dry Shoot Weight	0.1220**	0.0018*	0.004*	0.01**	0.0013**	0.003^{NS}	0.00068	9.84
Root Water Content	39.016**	5.4429**	34.82**	3.82**	4.71**	2.23^{NS}	0.9695	1.079
Shoot Water Content	49.158**	1.458^{NS}	0.53*	0.62*	1.54**	6.25^{NS}	0.9149	1.039

NS = Not significant, ** = highly significant at $p < 0.01$, * = significant at $p < 0.05$, df = degree of freedom, CV = coefficient of variation.

Table 2. Mean performance of the four inbred lines of the studied traits under no salt stress condition: 0 mmol L^{-1}.

Traits	21-2001-1	21-2002-1	21-2003-1	21-2005-1
Germination Index	6.62^{a}	5.80^{a}	3.31^{b}	3.53^{b}
Shoot Length	12.73^{a}	12.66^{a}	12.16^{a}	11.95^{a}
Root length	18.20^{a}	20.3^{a}	20.01^{a}	17.86^{a}
Average Root Surface area	7.23^{a}	8.00^{a}	7.65^{a}	6.36^{a}
Average Root Diameter	0.66^{a}	0.61^{b}	0.65^{ab}	0.66^{ab}
Fresh Root Weight	1.30^{b}	1.79^{a}	2.00^{a}	1.64^{ab}
Fresh Shoot Weight	5.87^{a}	5.29^{a}	5.78^{a}	5.26^{a}
Dry Root Weight	0.20^{a}	0.19^{a}	0.17^{a}	0.16^{a}
Dry Shoot Length	0.37^{a}	$0.34a^{b}$	0.34^{ab}	0.29^{b}
Root Water Content	84.87^{b}	89.71^{a}	91.48^{a}	90.57^{a}
Shoot Water Content	93.71^{b}	93.66^{b}	94.07^{ab}	94.45^{a}

Means followed by same letters on the same row are not significantly different following Tukey test ($p < 0.05$).

Although the root is a sensitive part of the plant affected by salt stress, the inbred lines may have been able to tolerate low levels of salt stress and hence showed no differences (Table 3). Similarly, significant variation was observed among the different four inbred lines at 120 mmol L^{-1} for all the studied traits except dry shoot weight. Moderate salt stress affected the performance of the studied inbred lines (Table 4), indicating that screening for salt tolerance was possible. After 7 days of exposure to salt stress at 180 mmol L^{-1}, only 5 out of the 11 studied traits significantly ($p < 0.05$) affected the performance of the popcorn inbred lines. This could be attributed to the fact that strong salt stress may have negatively affected (slow growth) all the inbred lines (Table 5). This may not be a suitable salt level for screening salt tolerance among different inbred lines. Compared with the other inbred lines, the inbred lines 21-2001-1 and 21-2002-2 consistently exhibited good germination rates. The germination index reflects the percentage of germination on each day of the germination period. It provides information about the germination percentage and speed of germination of each inbred line at different salt levels.

Table 3. Mean performance of the four inbred lines of the studied traits under 60 mmol L^{-1} salt stress condition.

Traits	21-2001-1	21-2002-1	21-2003-1	21-2005-1
Germination Index	5.41^{a}	6.54^{a}	3.87^{b}	3.71^{b}
Shoot Length	8.32^{b}	10.80^{a}	8.10^{b}	10.29^{a}
Root length	11.88	11.59	11.64	10.925
Average Root Surface area	27.26^{ab}	32.34^{a}	25.43^{bc}	19.32^{c}
Average Root Diameter	0.62^{ab}	0.65^{b}	0.65^{a}	0.66^{a}
Fresh Root Weight	1.43^{b}	1.86^{a}	1.69^{ab}	1.36^{b}
Fresh Shoot Weight	4.71^{a}	5.17^{a}	4.70^{a}	3.71^{b}
Dry Root Weight	0.15^{ab}	0.17^{a}	0.14^{bc}	0.11^{c}
Dry Shoot Length	0.36^{ab}	0.38^{a}	0.31^{b}	0.26^{c}
Root Water Content	89.84^{b}	90.80^{ab}	92.02^{a}	91.71^{a}
Shoot Water Content	92.45^{b}	92.66^{ab}	93.36^{a}	92.98^{ab}

Means followed by same letters on the same row are not significantly different following Tukey test ($p < 0.05$).

Table 4. Mean performance of the four inbred lines of the studied traits under 120 mmol L^{-1} salt stress condition.

Traits	21-2001-1	21-2002-1	21-2003-1	21-2005-1
Germination Index	4.82^{a}	4.41^{ab}	3.73^{bc}	3.43^{c}
Shoot Length	5.52^{b}	7.00^{a}	5.64^{b}	5.29^{b}
Root length	7.71^{ab}	7.91^{a}	8.21^{a}	5.40^{b}
Average Root Surface area	20.14^{a}	17.53^{a}	18.88^{a}	13.29^{b}
Average Root Diameter	0.69^{b}	0.69^{b}	0.73^{ab}	0.75^{a}
Fresh Root Weight	1.33^{b}	1.97^{a}	1.66^{ab}	1.36^{b}
Fresh Shoot Weight	2.97^{b}	3.84^{a}	2.89^{b}	2.23^{c}
Dry Root Weight	0.12^{a}	0.11^{a}	0.12^{a}	0.09^{a}
Dry Shoot Length	0.27^{b}	0.39^{a}	0.33^{a}	0.25^{b}
Root Water Content	91.14^{b}	91.1^{b}	92.93^{a}	93.11^{a}
Shoot Water Content	90.92^{b}	90.82^{b}	92.15^{a}	91.58^{ab}

Means followed by same letters on the same row are not significantly different following Tukey test ($p < 0.05$).

Table 5. Mean performance of the four inbred lines of the studied traits under 180 mmol L^{-1}salt stress condition.

Traits	21-2001-1	21-2002-1	21-2003-1	21-2005-1
Germination Index	3.25^{a}	3.74^{a}	3.47^{a}	3.04^{a}
Shoot Length	2.92^{b}	3.85^{a}	3.07^{ab}	3.46^{ab}
Root length	4.83^{ab}	4.99^{a}	5.24^{a}	3.92^{b}
Average Root Surface area	11.56^{a}	10.12^{ab}	10.59^{a}	7.66^{b}
Average Root Diameter	0.79^{b}	0.78^{b}	0.85^{ab}	0.88^{a}
Fresh Root Weight	1.05^{b}	1.19^{ab}	1.51^{a}	1.32^{ab}
Fresh Shoot Weight	1.67^{a}	1.45^{ab}	1.29^{b}	1.40^{ab}
Dry Root Weight	0.08^{a}	0.09^{a}	0.10^{a}	0.09^{a}
Dry Shoot Length	0.18^{a}	0.16^{a}	0.14^{a}	0.12^{a}
Root Water Content	92.17^{a}	92.02^{a}	93.38^{a}	93.39^{a}
Shoot Water Content	89.47^{a}	89.15^{a}	89.38^{a}	91.82^{a}

Means followed by same letters on the same row are not significantly different following Tukey test ($p < 0.05$).

In countries where popcorn is grown, salinity stress is a major abiotic factor that significantly affects popcorn yields (Epstein et al., 1980). Plants under salt stress are affected by both water shortages and excess ions (Giambalvo et al., 2022). Under salt stress, plants suffer from disruption of cell structure, suppressed growth and development, and interference with essential physiological functions (Frukh et al., 2020). Under NaCl stress, plants exhibit reduced osmotic potential, which causes the cell expansion pressure to decrease quickly, the plasma membrane to shrink, and eventually, growth to be suppressed (Munns & Tester, 2008). In previous studies on the ability of popcorn to withstand salt, researchers have mostly focused on a single growing season and have not considered different growth periods. Additionally, the use of different germplasms may lead to different results. Fu and Zhang (2015) reported that treatments with NaCl at concentrations of 200 mmol L^{-1} and greater hindered popcorn seed germination, but 100 mmol L^{-1} NaCl promoted it. Furthermore, our findings are in agreement with those of Xie et al. (2021), who reported that a less than 50% decrease in the performance of different studied cultivars at 255 mmol L^{-1} NaCl could be used as an indicator for screening salt tolerance.

Linear regression analysis revealed that all the popcorn inbred lines responded significantly negatively to the different concentrations of salt (Figures 3, 4 and 5) except for average root diameter (Figure 6) which responded significantly positively. Similarly, Figures 7, 8, 9 and 10 showed that the popcorn inbred lines responded significantly negatively to the different concentrations of salt except for root water content (Figure 11) which responded significantly positively to the different concentrations of salts. Again, Figure 12 showed that the popcorn inbred lines responded significantly negatively to the different concentrations of salt. As the salt concentration increases, the growth of the inbred lines decreases significantly, indicating the detrimental effect of NaCl. In our own investigation, the value of a number of seed germination indicators, including shoot length and root and shoot weight, decreased as the quantity of salt increased. The reduction in the coefficients of determination for the regression equations of some traits indicated that those traits may not be suitable for screening salt tolerance and may not serve as selection indices. Shoot length, root length, fresh shoot weight, dry root weight, and root and shoot water contents are highly reliable and can be considered for preliminary salt tolerance selection. Salinity causes delayed germination by upsetting the hormone and nutritional balance during seed germination, especially gibberellin/abscisic acid (Sulaiman et al., 2023). In line with our study, Xu et al. (2023) investigation revealed that the relative water content increased significantly at a concentration of 120 mmol L^{-1} NaCl rather than decreasing as salt stress increased. This increase could be explained by the ongoing salt stress, which decreased the water absorption efficiency in popcorn inbred lines and caused swelling disorders (Pooja et al., 2020).

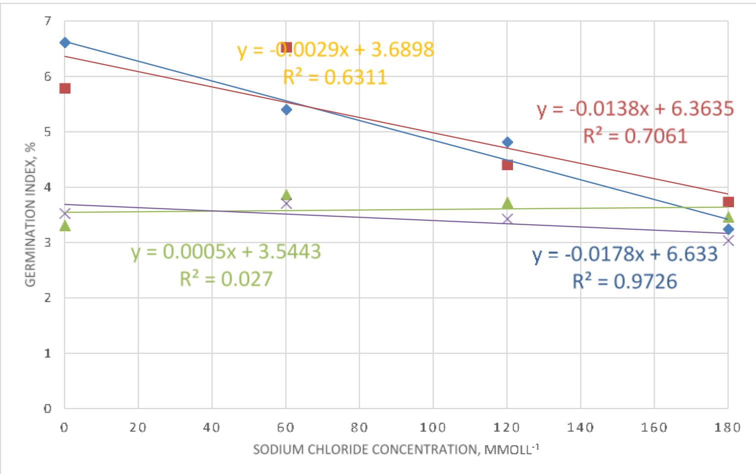

Figure 3. Response of the four (4) different inbred lines exposed to four different salt levels using linear regression model on the germination index. 21-2005-1 (Yellow), 21-2002-1 (Brown), 21-2003-1 (Grey), and 21-2001-1 (Blue).

Table 6 presents the salt tolerance index (STI) of each inbred line based on dry seedling weight. A higher STI value indicates greater salt tolerance and yield potential. Inbred lines 21-2001-1 and 21-2002-2 demonstrated better germination rates compared to the other lines. The STI classified the inbred lines into two groups under different NaCl concentrations: tolerant (STI > 0.65) and moderately tolerant (STI between 0.50 and 0.65). At 120 mM L^{-1} NaCl, inbred lines 21-2001-1, 21-2002-2, and 21-2003-1 were categorized as tolerant, while 21-2005-1 was moderately tolerant.

A significant relationship ($p < 0.05$) was observed between the salt tolerance index (STI) and several morphological traits influencing the salt tolerance of the inbred lines (Table 7) at 60, 120, and 180 mmol L^{-1} NaCl. This suggests that the STI based on dry seedling weight could serve as an effective tool to identify contrasting popcorn inbred lines. Salt stress negatively affects almost every stage of popcorn growth, with early seedling establishment being particularly vulnerable (Munns & Tester, 2008). Although popcorn exhibits high genotypic diversity for salt tolerance, it remains susceptible to salt stress (Magar et al., 2021). The crop adapts by making crucial morphological changes in its roots and shoots (Chen et al., 2020). Therefore, quantifying shoot and root morphological characteristics at the seedling stage is essential for using these traits as selection criteria for salt tolerance in popcorn.

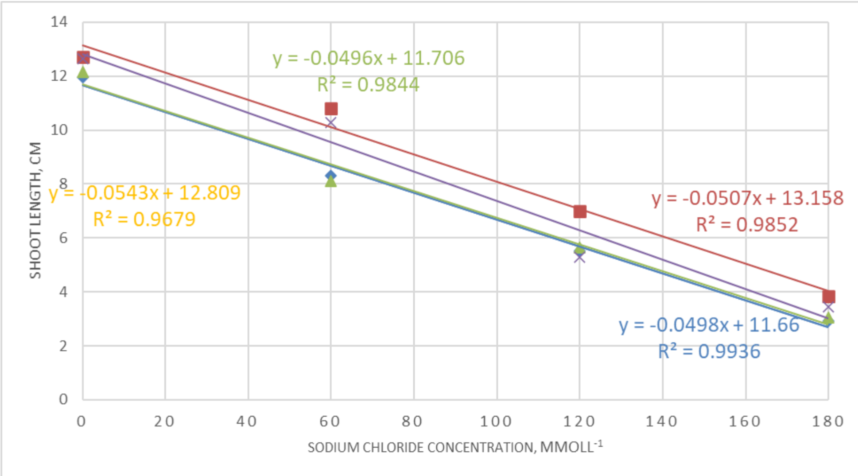

Figure 4. Response of the four (4) different inbred lines exposed to four different salt levels using linear regression model on the shoot length. 21-2005-1 (Yellow), 21-2002-1 (Brown), 21-2003-1 (Grey), and 21-2001-1 (Blue).

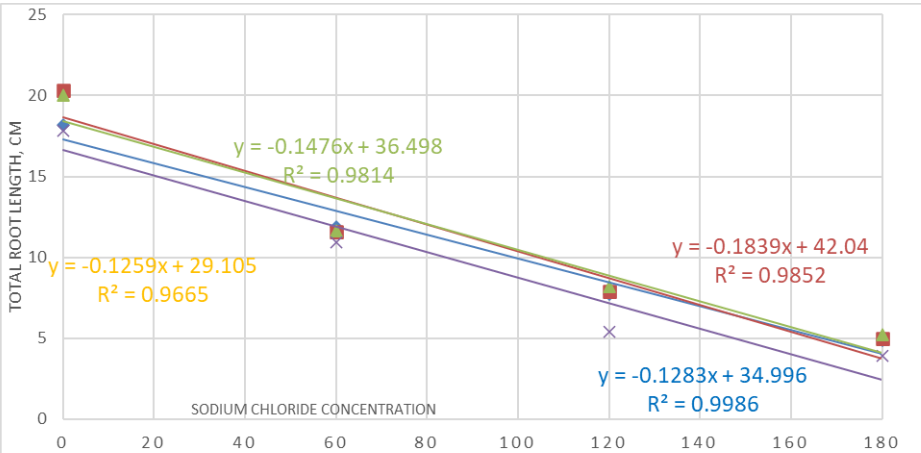

Figure 5. Response of the four different inbred lines exposed to four different salt levels using linear regression model on the total root length. 21-2005-1 (Yellow), 21-2002-1 (Brown), 21-2003-1 (Grey), and 21-2001-1 (Blue).

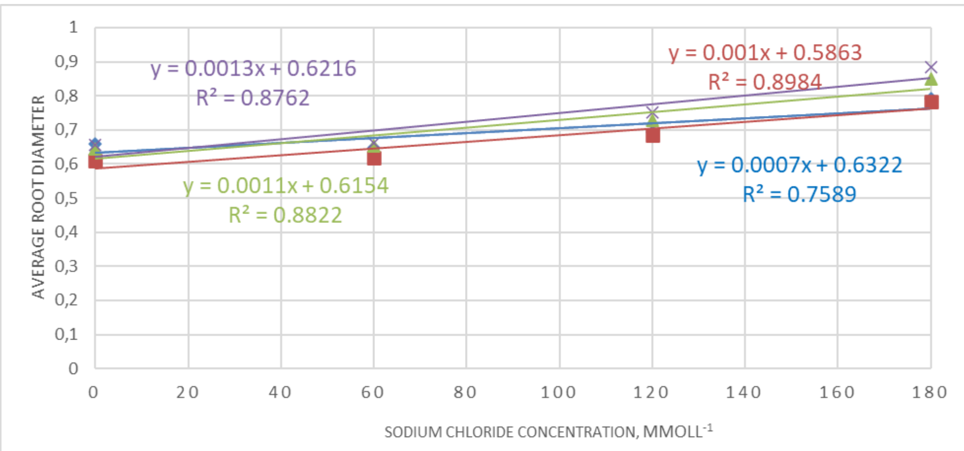

Figure 6. Response of the four different inbred lines exposed to four different salt levels using linear regression model on the average root diameter. 21-2005-1 (Yellow), 21-2002-1 (Brown), 21-2003-1 (Grey), and 21-2001-1 (Blue).

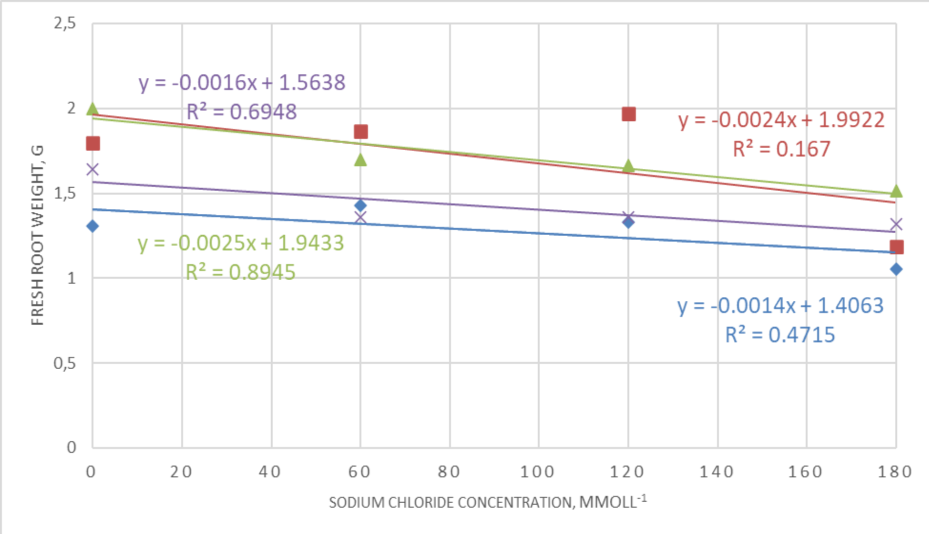

Figure 7. Response of the four different inbred lines exposed to four different salt levels using linear regression model on the fresh root weight. 21-2005-1 (Yellow), 21-2002-1 (Brown), 21-2003-1 (Grey), and 21-2001-1 (Blue).

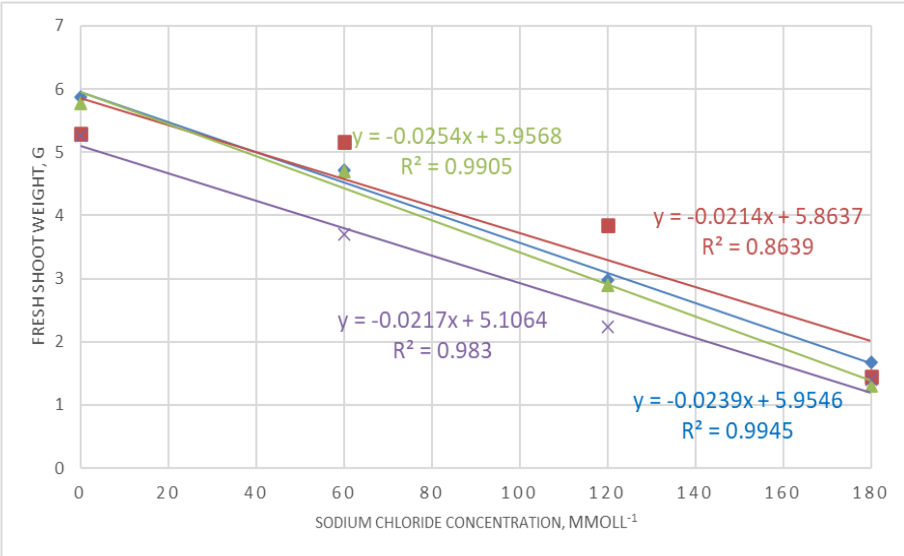

Figure 8. Response of the four different inbred lines exposed to four different salt levels using linear regression model on the fresh shoot weight. 21-2005-1 (Yellow), 21-2002-1 (Brown), 21-2003-1 (Grey), and 21-2001-1 (Blue).

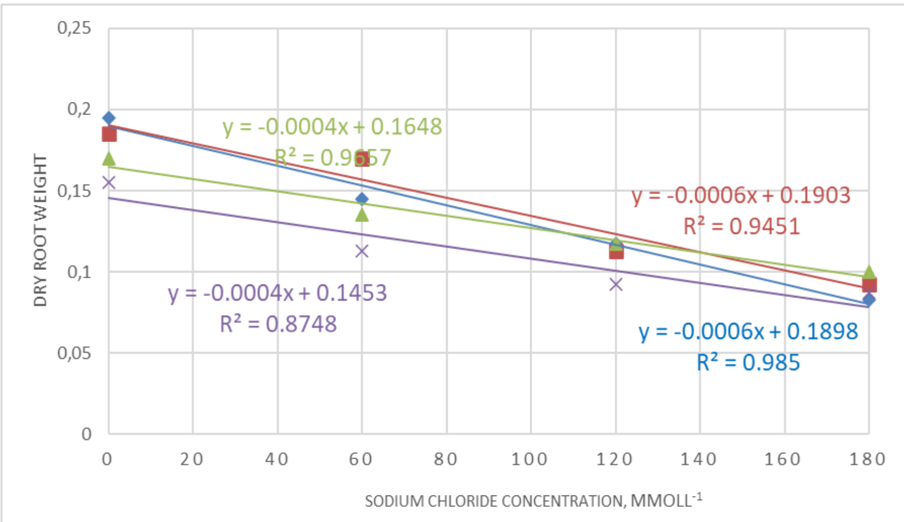

Figure 9. Response of the four different inbred lines exposed to four different salt levels using linear regression model on the dry root weight. 21-2005-1 (Yellow), 21-2002-1 (Brown), 21-2003-1 (Grey), and 21-2001-1 (Blue).

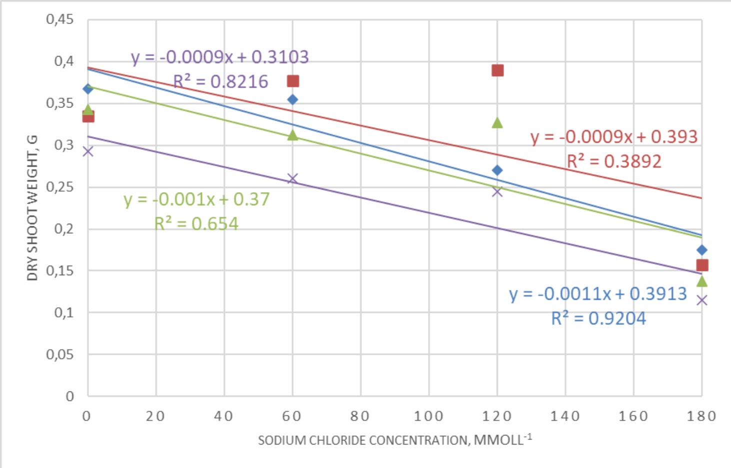

Figure 10. Response of the four different inbred lines exposed to four different salt levels using linear regression model on the dry shoot weight. 21-2005-1 (Yellow), 21-2002-1 (Brown), 21-2003-1 (Grey), and 21-2001-1 (Blue).

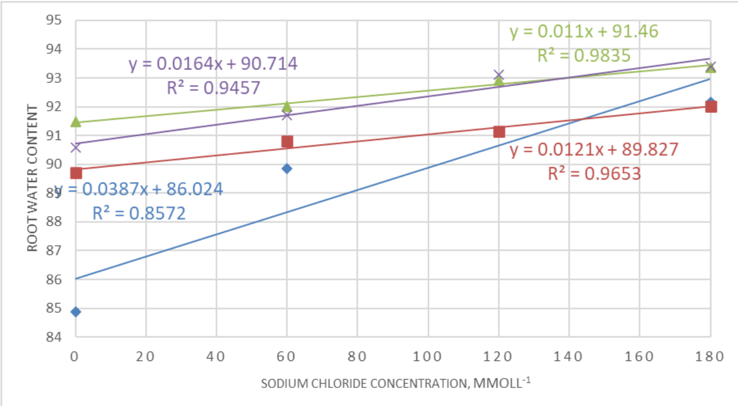

Figure 11. Response of the four different inbred lines exposed to four different salt levels using linear regression model on the root water content. 21-2005-1 (Yellow), 21-2002-1 (Brown), 21-2003-1 (Grey), and 21-2001-1 (Blue).

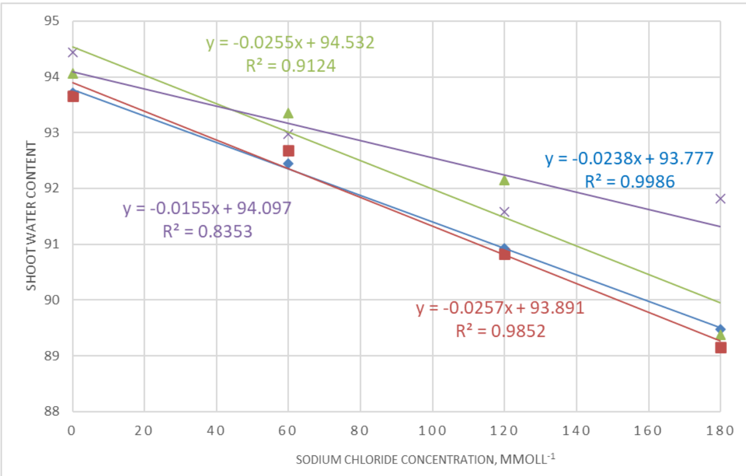

Figure 12. Response of the four different inbred lines exposed to four different salt levels using linear regression model on the shoot water content. 21-2005-1 (Yellow), 21-2002-1 (Brown), 21-2003-1 (Grey), and 21-2001-1 (Blue).

Table 6. Inbred Lines ranking according to the salt tolerance index (STI) following Tao et al. (2021).

Inbred Lines	60 mmol L^{-1}	Ranking	120 mmol L^{-1}	Ranking	180 mmol L^{-1}	Ranking
21-2001-1	1.08	T	0.84	T	0.56	MT
21-2002-1	1.09	T	0.94	T	0.72	T
21-2003-1	0.88	T	0.71	T	0.63	MT
21-2005-1	0.64	MT	0.58	MT	0.53	MT

MT = Moderately Tolerant (STI ranging from 0.50 – 0.65), T = Tolerant (STI > 0.65)

Table 7. The correlation between the salt tolerance index (STI) at different salt concentrations and different characters of the inbred lines.

Traits	60 mmol L^{-1}	120 mmol L^{-1}	180 mmol L^{-1}
Germination Percentage	0.702**	0.471^{NS}	0.250^{NS}
Germination Index	0.718**	0.702**	0.233^{NS}
Germ Length	-0.197^{NS}	0.236^{NS}	-0.278^{NS}
Total Root Length	0.830**	0.659**	0.595*
Average Surface Area	0.830**	0.659**	0.595*
Average Root Diameter	-0.602*	-0.689*	-0.445^{NS}
Fresh Root Weight	0.403^{NS}	-0.191^{NS}	-0.189^{NS}
Fresh Germ Weight	0.783**	0.270^{NS}	0.433^{NS}
Seedling Fresh Weight	0.723**	0.089^{NS}	0.168^{NS}
Dry Root Weight	0.831**	0.349^{NS}	-0.019^{NS}
Dry Germ Weight	0.913**	0.651**	0.850**
Seedling Dry Weight	0.917**	0.726**	0.873**
Root Water Content	-0.700*	-0.609*	-0.208^{NS}
Germ Water Content	-0.498^{NS}	-0.567*	-0.749*
Fresh Root Shoot ratio	-0.308^{NS}	-0.282^{NS}	-0.279^{NS}
Dry Root Shoot ratio	0.057^{NS}	-0.089^{NS}	-0.635**

** = highly significant at $p < 0.01$, * = significant at $p < 0.05$, NS = not significant.

Conclusion

Determining a suitable NaCl concentration for screening salt tolerance is the first step in distinguishing among popcorn inbred lines at the germination stage. The shoot and roots of popcorn are severely affected by salt stress at the germination and seedling stages. On the basis of the results of this study, 60 and 120 mmol L^{-1} NaCl could be used as the optimum solutions for screening salt tolerance because they provide high discrimination among the studied inbred lines. Furthermore, a less than 50% decrease in the performance of the inbred lines was observed compared with NaCl solutions of higher concentrations (180 mmol L^{-1}).

Data availability

Not applicable.

Acknowledgements

We thank the Tertiary Education Trust Fund (TETFund), Nigeria, the National Council for Scientific and Technological Development (CNPq), the Coordination for the Improvement of Higher Education Personnel (CAPES; Finance Code 001), and the Foundation for Research Support of Minas Gerais State, Brazil (FAPEMIG) for financial support.

References

Ashraf, M. Y., Akhtar, K., Hussain, F., & Iqbal, J. (2006). Screening of different accessions of three potential grass species from Cholistan desert for salt tolerance. *Pakistan Journal of Botany*, *38*(5), 1589-1597.

Barnabás, B., Jäger, K., & Fehér, A. (2008). The effect of drought and heat stress on reproductive processes in cereals. *Plant, Cell & Environment*, *31*(1), 11-38. https//doi.org/10.1111/j.1365-3040.2007.01727.x

Batool, M., Saqib, M., Qayyum, M. A., Jan, M., Hameed, S. A., Khan, A. K., Khaliq, A., Naz, T., Hussain, A., Nawaz, A. (2020). Screening of different popcorn (*Zea mays* L.) genotypes against salinity under hydroponics. *Pure and Applied Biology*, *9*(1), 1118-1129. http://dx.doi.org/10.19045/bspab.2020.90117

Borsai, O. (2018). The genus Portulaca as a suitable model to study the mechanisms of plant tolerance to drought and salinity. *The EuroBiotech Journal, 2*(2), 104-113. https//doi.org/10.2478/ebtj-2018-0014

Chen, F., Fang, P., Peng, Y., Zeng, W., Zhao, X., Ding, Y., Zhuang, Z., Gao, Q., & Ren, B. (2019). Comparative proteomics of salt-tolerant and salt-sensitive popcorn inbred lines to reveal the molecular mechanism of salt tolerance. *International Journal of Molecular Sciences, 20*(19), 1-22. https//doi.org/10.3390/ijms20194725

Chen, X., R. Zhang, Y. Xing, B. Jiang, B. Li, X. Xu, & Y. Zhou (2020). The efficacy of different seed priming agents for promoting sorghum germination under salt stress, *PloS ONE, 16*(1), e0245505, https//doi.org/10. 1371/journal.pone.0245505

Epstein, E., Norlyn, J. D., Rush, D. W., Kingsbury, R. W., Kelley, D. B., Cunningham, G. A., & Wrona, A. F. (1980). Saline culture of crops: a genetic approach. *Science*, *210*(4468), 399-404.

Fernandez, G. C. J. (1992). Effective selection criteria for assessing stress tolerance. In C. G. Kuo (Ed.), *Proceedings of the international symposium on adaptation of vegetables and other food crops in temperature and water stress* (pp. 257-270). AVRDC.

Freire, M. B. G. S., Miranda, M. F. A., Oliveira, E. E. M., Silva, L. E., Pessoa, L. G. M., & Almeida, B. G. (2014). Agrupamento de solos quanto á salinidade no perímetro irrigado de Custódia em função do tempo. *Revista Brasileira de Engenharia Agrícola e Ambiental*, *18*(Suplemento), 86-91. https://doi.org/10.1590/1807-1929/agriambi.v18nsuppS86-S91

Frukh, A., Siddiqi, T. O., Khan, M. I. R., & Ahmad, A. (2020). Modulation in growth, biochemical attributes and proteome profile of rice cultivars under salt stress. *Plant Physiology and Biochemistry*, *146*, 55-70 https://doi.org/10.1016/j.plaphy.2019.11.011

Fu, C. F., & Zhang, H. Y. (2015). Effects of salt stress on seed germination and seedling Cglorophyll content and osmotic potential of popcorn. *Shandong Agricultural Sciences*, *47*(5), 27-30. https://link.oversea.cnki.net/doi/10.14083/j.issn.1001-4942.2015.05.007

Giambalvo, D., Amato, G., Borgia, D., Ingraffia, R., Librici, C., Lo Porto, A., Puccio, G., Ruisi, P., & Frenda, A. S. (2022). Nitrogen availability drives mycorrhizal effects on wheat growth, nitrogen uptake and recovery under salt stress. *Agronomy*, *12*(11), 1-14. https://doi.org/10.3390/agronomy12112823

Hasanuzzaman, M., Nahar, K., & Fujita, M. (2013). Plant response to salt stress and role of exogenous protectants to mitigate salt-induced damages. In P. Ahmad, M. Azooz, & M. Prasad (Eds.), *Ecophysiology and responses of plants under salt stress* (pp. 25-87). Springer. https://doi.org/10.1007/978-1-4614-4747-4_2

Hussain, S. S., Rasheed, M., Ahmed, Z. I., & Jilani, G. (2023). Characterizing Maize genotypes for salt tolerance using morphological and ionic traits at seedling stage. *International Letters of Natural Sciences*, *86*, 1-24. https://doi.org/10.56431/p-03pzu8

Irshad, A., Ahmed, R. I., Ur Rehman, S., Sun, G., Ahmad, F., Sher, M. A., Aslam, M. Z., Hassan, M. M., Qari, S. H., Aziz, M. K., & Khan, Z. (2022). Characterization of salt tolerant wheat genotypes by using morpho-physiological, biochemical, and molecular analysis. *Frontiers in Plant Science*, *13*, 1-13. https://doi.org/10.3389/fpls.2022.956298

Ismayilov, A. I., Mamedov, A. I., Fujimaki, H., Tsunekawa, A., & Levy, G. J. (2021). Soil salinity type effects on the relationship between the electrical conductivity and salt content for 1:5 soil-to-water extract. *Sustainability*, *13*(6), 1-11. https://doi.org/10.3390/su13063395

Kokebie, D., Enyew, A., Masresha, G., Fentie, T., & Mulat, E. (2024). Morphological, physiological, and biochemical responses of three different soybean (*Glycine max* L.) varieties under salinity stress conditions. *Frontiers in Plant Science*, *15*, 1-14. https://doi.org/10.3389/fpls.2024.1440445

Läuchli, A., & Epstein, E. (1990). Plant responses to saline and sodic conditions. In K. K. Tanji (Ed.), *Agricultural salinity assessment and management* (pp. 113-137). ASCE.

Li, W.-Y., Wang, C., Shi, H.-H., Wang, B., Wang, J.-X., Liu, Y.-S., Ma, J.-Y., Tian, S.-Y., & Zhang, W.-Y. (2020). Genome-wide analysis of ethylene-response factor family in adzuki bean and functional determination of VaERF3 under saline-alkaline stress. *Plant Physiology and Biochemistry*, *147*, 215-222. https://doi.org/10.1016/j.plaphy.2019.12.019

Liu, X., Zhou, X., Zeng, X., Zhang, L., Wang, Z.-H., & Di, H. (2015). Comparison of identification method of maize salt tolerence in germination and seedling stage. *Journal of Maize Sciences*, *23*(1), 115–121. https://doi.org/10.13597/j.cnki.maize.science.2015.01.019

Magar, B.T., S. Acharya, B. Gyawali, K. Timilsena, J. Upadhayaya, & J. Shrestha, (2021). Genetic variability and trait association in Popcorn (*Zea mays* L.) varieties for growth and yield traits. *Heliyon, 7*(10), e08144. https://doi.org/10.1016/j.heliyon.2021.e07939

Masuda, M. S., Azad, M. A. K., Hasanuzzaman, M., & Arifuzzaman, M. (2021). Evaluation of salt tolerance in popcorn (*Zea mays* L.) at seedling stage through morphological characters and salt tolerance index. *Plant Physiology Reports*, *26*(3), 419-427. https://doi.org/10.1007/s40502-021-00611-2

Munns, R., James, R. A., & Läuchli, A. (2006). Approaches to increasing the salt tolerance of wheat and other cereals. *Journal of Experimental Botany*, *57*(5), 1025-1043. https://doi.org/10.1093/jxb/erj100

Munns, R., & Tester, M. (2008). Mechanisms of salinity tolerance. *Annual Review of Plant Biology. 59*, 651-681. https://doi.org/10.1146/annurev.arplant.59.032607.092911

Pessoa, L. G. M., Freire, M. B. G. S., Wilcox, B. P., Green, C. H. M., Araújo, R. J. T., & Araújo Filho, J. C., (2016). Spectral reflectance characteristics of soils in northeastern Brazil as influenced by salinity levels. *Environmental Monitoring and Assessment, 188*(11), 616. https://doi. org/10.1007/s10661-016-5631-6

Pooja, A. S. N., Chand, M., Pal, A., Kumari, A., Rani, B., Goel, V., & Kulshreshtha, N. (2020). Soil moisture deficit induced changes in antioxidative defense mechanism of sugarcane (*Saccharum officinarum*) varieties differing in maturity. *The Indian Journal of Agricultural Sciences*, *90*(3), 507-512. https://doi.org/10.56093/ijas.v90i3.101458

Ribeiro, M. P., Viana, J. M. S., Gama, G. F. V., Silva, L. J., Oliveira, J. A., & Ribeiro, C. (2024). Uni- and Multivariate analyses for the characterization of popcorn inbred lines for drought tolerance at seedling and vegetative stages. *Agronomy*, *14*(11), 1-19. https://doi.org/10.3390/agronomy14112513

Rizk, M. S., Assaha, D. V. M., Mekawy, A. M. M., Shalaby, N. E., Ramadan, E. A., El-Tahan, A. M., Ibrahim, O. M., Metwelly, H. I. F., Okla, M. K., Maridueña-Zavala, M. G., AbdElgawad, H., & Ueda, A. (2024). Comparative analysis of salinity tolerance mechanisms in two popcorn genotypes: growth performance, ion regulation, and antioxidant responses. *BMC Plant Biology*, *24*(1),1-17. https://doi.org/10.1186/s12870-024-05533-3

Sultana, R., Uddin, Md. N., Rahim, Md. A., & Fakir, Md. S. A. (2016). Effect of salt stress on germination and seedling growth in four popcorn genotypes. *International Journal of Natural and Social Science*, *3*(4), 20-27.

Sulaiman, Ullah, S., Saud, S., Liu, K., Harrison, M. T., Hassan, S., Nawaz, T., Zeeshan, M., Nasar, J., Khan, I., Liu, H., Adnan, M., Kumar, S., Ali, M. I., Jamal, A., Zhu, M., Ali, N., Ali, S., El-Kahtany, K., & Fahad, S. (2023). Germination response of oat (*Avena sativa* L.) to temperature and salinity using halothermal time model. *Plant Stress, 100*, 1-11. https://doi.org/10.1016/j.stress.2023.100263

Tao, R., Ding, J., Li, C., Zhu, X., Guo, W., & Zhu, M. (2021). Evaluating and screening of agro-physiological indices for salinity stress tolerance in wheat at the seedling stage. *Frontiers in Plant Science*, *12*, 1-12. https://doi.org/10.3389/fpls.2021.646175

Tian, H., Liu, H., Zhang, D., Hu, M., Zhang, F., Ding, S., & Yang, K. (2024). Screening of salt tolerance of popcorn (*Zea mays* L.) lines using membership function value and GGE biplot analysis. *PeerJ*, *12*, 1-28. https://doi.org/10.7717/peerj.16838

Xie, Y., Liu, X., Amee, M., Yu, H., Huang, Y., Li, X., Chen, L., Fu, J., & Sun, X. (2021). Evaluation of salt tolerance in Italian ryegrass at different developmental stages. *Agronomy*, *11*(8), 1-18. https://doi.org/10.3390/agronomy11081487

Xu, B., Cao, L., Zhang, Z., Li, X., Zhao, X., Wang, X., Wang, Y., Wu, B., Zhou, W., Lin, C., Gao, Y., & Rong, L. (2023). Physiological effects of combined NaCl and $NaHCO_3$ stress on the seedlings of two maple species. *Frontiers in Plant Science*, *14*, 1-9. https://doi.org/10.3389/fpls.2023.1209999

Xu, Y., Weng, X., Jiang, L., Huang, Y., Wu, H., Wang, K., Li, K., Guo, X., Zhu, G., & Zhou, G. (2024). Screening and evaluation of salt-tolerant wheat germplasm based on the main morphological indices at the germination and seedling stages. *Plants*, *13*(22), 1-27. https://doi.org/10.3390/plants13223201

Zahra, N., Raza, Z. A., & Mehmood, S. (2020). Effect of salinity stress on various growth and physiological attributes on two contrasting popcorn genotypes. *Brazilian Archives of Biology and Technology, 63*, 1-10. http://doi.org/10.1590/1678-4324-2020200072

Zulkadir, G. (2025). Salt tolerance of different maize genotypes during germination and seedling stages. *Phyton*, *94*(6), 1879-1896. https://doi.org/10.32604/phyton.2025.064144

Associate Editor in charge:
Alessandro Lucca Braccini
ORCID: https://orcid.org/0000-0002-6915-4804
Carlos Alberto Scapim
ORCID: https://orcid.org/0000-0002-7047-9606