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Comparative analysis of salinity tolerance mechanisms in two maize genotypes: growth performance, ion regulation, and antioxidant responses

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Abstract

This study investigates the differential responses of two maize genotypes, SC180 and SC168, to salt stress, aiming to elucidate the mechanisms underlying salinity tolerance and identify traits associated with improved stress resilience. Salinity stress, imposed by 150 mM NaCl, adversely affected various growth parameters in both genotypes. SC180 exhibited a more pronounced reduction in shoot length (13.6%) and root length (13.6%) compared to SC168, which showed minimal reductions (3.0% and 2.3%, respectively). Additionally, dry weight losses in SC180's leaves, stems, and roots were significantly greater than those in SC168. Under salinity stress, both genotypes accumulated Na^+ in all organs, with SC168 showing higher Na + concentrations. However, K^+ levels decreased more significantly in SC180's leaves than in SC168's. The study also assessed physiological responses, noting that SC180 experienced a substantial reduction in relative water content (RWC) in leaves (22.7%), while SC168's RWC remained relatively stable (5.15%). Proline accumulation, a marker for osmotic adjustment, increased 2.3-fold in SC168 compared one-fold in SC180. Oxidative stress indicators, such as electrolyte leakage and hydrogen peroxide levels, were elevated in both genotypes under salt stress, with SC180 showing higher increases (48.5% and 48.7%, respectively) than SC168 (35.25% and 22.0%). Moreover, antioxidant enzymes (APX, CAT, POD, SOD, GR) activities were significantly enhanced in SC168 under salinity stress, whereas SC180 showed no significant changes in these activities. Stress indices, used to quantify and compare salinity tolerance, consistently ranked SC168 as more tolerant (average rank = 1.08) compared to SC180 (average rank = 1.92). Correlation analyses further confirmed that SC168's superior tolerance was associated with better Na + regulation, maintenance of K^+ levels, and a robust antioxidant defense system. In conclusion, SC168 demonstrated greater resilience to salinity stress, attributed to its efficient ion regulation, stable water status, enhanced osmotic adjustment, and strong antioxidant response. These findings provide valuable insights for breeding and developing salinity-tolerant maize varieties.

Keywords Antioxidant enzymes, Maize, Oxidative stress, Proline, Na^+ exclusion

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Introduction

Saline soil is characterized by high concentrations of soluble salts, primarily Na^+ and Cl^-, with an electrical conductivity of the saturated paste extract typically exceeding 4 dS/m, equivalent to approximately 40 mM NaCl [1–3]. In arable land, increased salinization is the major global concern as more than 800 million hectares worldwide are affected negatively by salt stress, and this area represents about 6% of the world's total land area [2, 3].

Salinity stress has been demonstrated to be one of the most deleterious abiotic stresses that sharply decrease global agriculture's productivity [4, 5]. This decrease in production is caused by both osmotic and ionic stress, the two phases of salinity stress [6, 7]. The osmotic component of the stress is induced in the soil by the accumulation of high concentrations of salts, which decreases the ability of plants to absorb water through the roots, causing various physiological defects in the plant [8, 9] including decreased cell expansion [2, 3] and lower stomatal conductance [10, 11]. It also reduced photosynthesis, biomass accumulation and plant yield [8]. Moreover, osmotic stress reduces the uptake of many important minerals required for plant growth and development, such as N, K, Ca, Mg, and Fe [12]. The ionic effects (toxicity) of salinity stress occur when Na^+ concentration reaches a high level inside plant cells, especially in leaves or blades, where most metabolic processes in plants occur [2, 3, 10] and that affects its growth and development [13]. For maize (*Zea mays* L.) and most crops, Na is the most toxic ion [12]. Excess Na^+ causes ion imbalance and disrupts cellular metabolism [14, 15]. Also, elevated Na^+ accumulation induces Reactive Oxygen Species (ROS) production such as hydrogen peroxide (H_2O_2) [16, 17]. Induced ROS production leads to oxidative damage to proteins, membrane lipids and nucleic acids, thereby disrupting critical cellular functions of plants [16, 18]. At low concentration, H_2O_2 acts as a signal molecule in plant cells, involved in acclamatory signaling against abiotic stress [19, 20]. However, at high concentration, H_2O_2 induces programmed cell death [21].

Plants have evolved many strategies to cope with salinity through pathways involved in osmotic balance, ion homeostasis, ROS detoxification and hormone regulation [22, 23]. Plants tolerate osmotic stress by using different mechanisms to reduce water loss while increasing water uptake [23, 24]. One of these mechanisms is the synthesis of compatible solutes, such as proline, which is involved in osmotic adjustment [25, 26], ROS detoxification and enzyme and protein stabilization [27]. Another way of combating osmotic stress is the maintaining a balanced ratio of Na^+ or K^+ ions, as an osmoticum to generate osmotic potentials sufficient to drive water influx [5, 28]. Thus, maintaining this ratio is crucial for plant survival under salinity stress [6]. Moreover, the upregulation of osmoticum such as K, soluble sugar and proline contributes to ion compartmentalization and maintaining Na^+/K^+ ratio by restriction of Na^+ uptake [5, 28]. For plant cells, K^+ is more important than Na^+, and the contrast is true for animal cells [13, 14, 29]. K^+ is necessary to regulate osmotic pressure, stomatal movement, tropisms, enzymes activity, membrane potential and turgor pressure [30, 31]. Since Na^+ often disrupts cellular functions by interfering in K^+ regulated processes, the balance between Na and K is therefore crucial for the growth and development of plants, under salt stress. Furthermore, plants can adapt to ionic stress by either excluding the Na^+ ions, reducing its accumulation in the leaf blades (shoot Na^+ exclusion) [32], or by compartmentation of the excess Na^+ from the cytosol into vacuoles (vacuolar Na^+ sequestration) [10, 33, 34]. Na^+ sequestration in vacuoles reduces or prevents the toxicity of sodium in the cytosol, reason for which maintaining a stable cytosolic Na^+/K^+ ratio has become a salt stress tolerant trait [13]. The sequestration of excessive Na into vacuoles is mediated by tonoplast Na^+/H^+ antiporters driven by the proton gradient [12]. Another mechanism of salt stress tolerance involves the use of antioxidant enzyme defense system to detoxify ROS [16]. Antioxidant enzymes such as catalase (CAT), superoxide dismutase (SOD), peroxidase (POD), glutathione reductase (GR), and ascorbate peroxidase (APX) [18, 35]. In the ROS detoxification pathway, SOD initially dismutase the superoxide anion and converts it into H_2O_2 and O_2, through the Asada-Halliwell pathway [36]. In subsequent reactions, CAT, APX, POD, and GR participate in H_2O_2 detoxification, converting it to H_2O and O_2 [25, 37–39]. Furthermore, the enhancement of these antioxidant enzyme activities does not only mitigate ROS induced-oxidative damage, but also preserves the functionality of ion transport systems affecting Na^+/K^+ ratio [40].Maize (*Sea mays* L.) is considered to be one of the most important cereal crops in the world after rice and wheat and it grows in large scale under different soil and climatic conditions [12, 41–43], nonetheless it is sensitive to salinity stress, especially at the seedling stage [4, 44]. Consequently, maize production is being affected significantly by soil salinization [25]. Therefore, breeders must develop new hybrids, tolerant to salt stress in order to avoid or reduce the harmful effects of salinity. Being highly cross pollinated, maize has become a broad genetic base in which many various salinity tolerance mechanisms may exist, with reduced shoot Na^+ accumulation accounting for the tolerance of many of the hybrids, including Pioneer 32B33 and Pioneer 30Y87 [35]. SC168 is one of the most widespread hybrids in Egypt, which cultivated in large

areas in different regions because it is resistant to major diseases (late wilt, downy mildew and leaf blight), stable under different conditions and has high productivity from grain yield. On contrary, SC180 is a new hybrid with high yield and great plant height (3.55 cm), so it is used as bi-cropping fodder maize. The behavior of both hybrids of SC168 and SC180 under salinity stress has not been studied yet. The present study aimed to determine the effects of salt stress on growth, RWC, Na^+, K^+ levels, Na^+/K^+ ratio, oxidative stress indicators (electrolyte leakage and H_2O_2), osmoticum (proline) and antioxidant enzyme activities (APX, CAT, POD, SOD, GR) parameters of the two maize hybrids of SC168 and SC180, and to ascertain the mechanisms employed by the plants to adapt to salt stress.

Materials and methods

Plant material and growth conditions

The experiment was performed at the greenhouse (Plant Nutritional Physiology Laboratory, Faculty of Integrated Science for Life, Hiroshima University, Japan). The growth conditions were 12/12 h (day/light) and 25° / 18° C day and night). Seven from Egyptian yellow single crosses were obtained from The Agricultural Research Center, Sakha Station, Egypt. From the maize kernels tested in a preliminary experiment conducted in 2022 to assess their sensitivity to salinity stress, SC168 and SC180 were selected. Maize seeds were sterilized for 30 min using a 2% Na-hypochlorite (NaClO) solution, and then they were thoroughly cleaned with distilled water. First, seeds were allowed to germinate for 48 h at 28° on petri plates. Following germination, the seedlings were placed in pots with soil for five days. Afterwards, they were moved once more to a hydroponic medium made of Hoagland solution at half strength (KH_2PO_4 (1.07 mM), $NH_4H_2PO_4$ (2.1 mM), $MgSO_4{\cdot}7H_2O$ (2.2 mM), M KNO_3 (5.9 mm), $Ca(NO_3)_2{\cdot}4H_2O$ (4.1 mM), $MnCl_2{\cdot}4H_2O$ (9.155 µM), $CuSO_4{\cdot}5H_2O$ (3.52 µM), H_3BO_3 (46.256 µM), $Na_2MoO_4{\cdot}2H_2O$ (0.489 µM), $ZnSO_4{\cdot}7H_2O$ (0.772 µM) and Fe-EDTA (136.05 µM)) in two separate 45-L containers. Pumps were used to continuously aerate the nutritional solution, which was replaced every four days. The pH was also adjusted every day to maintain a range of 5.5–6.0. To prevent osmotic shock, a salt treatment (150 mM) was gradually administered to 16-day-old seedlings (50, 100, and finally 150 mM NaCl). Control seedlings were grown under 0 mM NaCl. This stepwise approach minimizes osmotic shock, reflects natural environmental changes and help plant to activate and upregulate stress response mechanisms. 150 mM NaCl was selected to induce significant stress with the goal of maintaining plant viability for investigating their physiological and biochemical responses. The electrical conductivity (EC) of the hydroponic solution was regularly checked to ensure the expected salt concentration, and in case of high increased EC level the nutrient solution was replaced with a new nutrient solution of the same salinity concentration. The plants were grown in the Hoagland nutrient solution for twelve days starting from the point when the desired concentration of 150 mM NaCl was achieved [45]. Then seedlings were harvested for growth, mineral and biochemical analyses.

Growth measurements

At the end of treatments, plant leaves, stems, and roots were separated to measure dry weight (DW). The three parts were oven-dried at 70° C for three days and then weighed. Also, shoot and root lengths (cm) were recorded.

Measurement of Na^+ and K^+ concentrations

Fine-ground powder of maize samples of leaves, stem, and roots (100 mg) were digested in 0.1 N HCl for 24 h. The digested extracts were used to determine Na^+ and K^+ concentrations, using a flame photometer (a high-sensitivity instrument for measuring Na^+ and K^+ concentrations with featuring easy calibration and precise digital readout ANA-135; Tokyo Photoelectric, Tokyo, Japan). This instrument employs flame emission spectroscopy to measure Na^+ and K^+ concentrations in extracts. The concentrations of Na^+ and K^+ were calculated from their standard curves.

Relative water content (RWC) determination

Leaf relative water content (RWC) was determined [16], and it was estimated as follows: RWC = [(FW—DW)/(TW—DW)] × 100. Where, FW and DW are sample fresh and dry mass, respectively. TW (turgid weight) was obtained after soaking the fresh sample in fresh deionized water (24 h) followed by reweighing.

Electrolyte leakage ratio (ELR %) measurement

For ELR determination, about 0.5 g tissue from the leaf no. 4 was soaked in deionized water and shaken (12 h). Then the electrical conductivity of the solution (EC) was measured, using an EC meter (an equipment that provides precise measurements of solution salinity, CM-21P; DKK-TOA Corporation, Tokyo, Japan). The leaf samples were later autoclaved to measure the total EC (EC2) and the ELR was calculated (ELR = (EC1/EC2) × 100).

Hydrogen peroxide (H_2O_2) concentration

Xylenol Orange (FOX) was used to measure H_2O_2 concentration [46]. Briefly, 0.5 g of leaf tissue were homogenized using liquid nitrogen, and the homogenized samples were centrifuged (8000 g for 15 min at 4° C).

Then, 100 µL of the supernatant was mixed with FOX reagent for 1 h. H_2O_2 absorbance was read at 560 nm.

Determination of proline concentration

The proline was extracted in 0.5 g of ground leaf tissue and its concentration was measured by using ninhydrin method [47]. Then the proline concentration was calculated using standard curve.

Antioxidant enzyme activities

The activities of APX (EC 1.11.1.11), CAT (EC 1.11.1.6), POD (EC 1.11.1.7), SOD (EC:1.15.1.1) and G (EC 1.6.4.2), were measured according to the methods described in previous studies [48, 49]. By utilizing 0.5 g of leaf tissues that have been frozen. The protein concentration was measured (Protein Assay kit) with bovine serum albumin as the standard.

Experimental design and statistical analysis

The experimental design used for this study was a completely randomized design with four replications. The SPSS statistical package, version 22 (IBM Inc., USA), was used to analyze all the collected data by one-way ANOVA, using the Duncan's multiple range test at $p < 0.05$ to separate the means ($n = 4$). The results are presented as means ± SE. In addition, R Statistical Software [50] was used.

Stress indices, heatmap, and density plots

Stress indices serve as quantitative measures to evaluate a crop's response to abiotic stress, offering simpler interpretation compared to raw data. Numerous indices for salinity tolerance have been developed (Table 1), employing mathematical equations that relate trait performance under stress and control conditions. These indices fall into two categories: one where higher value signify greater salinity tolerance and another where lower values indicate the same.

A heatmap was created to depict the relationship between treatments and studied traits, using standardized data represented in a color scale. In this heatmap, red cells denote high trait values, while blue cells indicate low values. Prior to constructing the heatmap, data standardization was performed by subtracting the mean of each trait from each value and dividing the result by the trait's standard deviation, ensuring comparability across traits measured in different units.

Table 1 Abiotic stress screening indices

INDEX	FORMULA	REFERENCE
INDICES WITH MAXIMUM VALUES CORRESPONDING TO MORE TOLERANT		
MEAN PRODUCTIVITY (MP)	$(Y_S + Y_{NS}) / 2$	[51]
GEOMETRIC MEAN PRODUCTIVITY (GMP)	$(Y_{NS})^{(1/2)} * Y_S$	[52]
HARMONIC MEAN (HM)	$2 * (Y_S * Y_{NS}) / (Y_S + Y_{NS})$	[53]
STRESS TOLERANCE INDEX (STI)	$(Y_S * Y_{NS}) / (Y_{NS \cdot m})^2$	[52]
YIELD INDEX (YI)	$Y_S / Y_{S \cdot m}$	[54]
MODIFIED STRESS TOLERANCE INDEX-I (MSTI1)	$((Y_{NS})^2 / (Y_{NS.m})^2) * ((Y_S * Y_{NS}) / (Y_{NS \cdot m})^2)$	[55]
MODIFIED STRESS TOLERANCE INDEX- II (MSTI2)	$((Y_S)^2 / (Y_{S \cdot m})^2) * ((Y_S * Y_{NS}) / (Y_{NS \cdot m})^2)$	[55]
YIELD STABILITY INDEX (YSI)	Y_S / Y_{NS}	[56]
RELATIVE STRESS INDEX (RSI)	$(Y_S / Y_{NS}) / (Y_{S \cdot m} / Y_{NS.m})$	[57]
DROUGHT INDEX (DI)	$(Y_S * (Y_S / Y_{NS})) / Y_{S.m}$	[53]
STRESS/NON-STRESS PRODUCTIVITY INDEX (SNPI)	$((Y_{NS} + Y_S) / (Y_{NS} - Y_S))^{(1/3)} * (Y_{NS} * Y_S * Y_S)^{(1/3)}$	[58]
RELATIVE EFFICIENCY INDEX (REI)	$(Y_S * Y_{NS}) / (Y_{S \cdot m} * Y_{NS.m})$	[59]
MEAN RELATIVE PERFORMANCE (MRP)	$(Y_S / Y_{S \cdot m}) + (Y_{NS} / Y_{NS.m})$	[59]
GOLDEN MEAN (GM)	$(Y_{NS} + Y_S) / (Y_{NS} — Y_S)$	[60]
INDICES WITH MINIMUM VALUES CORRESPONDING TO MORE TOLERANT		
TOLERANCE INDEX (TOL)	$Y_{NS} - Y_S$	[51]
STRESS SUSCEPTIBILITY INDEX (SSI)	$(1 — (Y_S / Y_{NS})) / (1 — (Y_{S \cdot m} / Y_{NS.m}))$	[61]
STRESS SUSCEPTIBILITY PERCENTAGE INDEX (SSPI)	$(Y_{NS} — Ys) / (2 * Y_{NS.m})$	[58]
YIELD REDUCTION (YR)	$1 - (Ys / Y_{NS})$	[62]
ABIOTIC STRESS TOLERANCE INDEX (ATI)	$((Y_{NS} — Ys) / (Y_{NS.m} / Y_{S \cdot m})) * (Y_{NS} * Ys)^{(1/2)}$	[58]
MEAN PRODUCTIVITY INDEX (MPI)	$(Y_{NS} — Ys)/2$	[51]
SCHNIEDERS STRESS SUSCEPTIBILITY INDEX (SSSI)	$1-(Ys / Y_{NS}) — (1 - (Y_{S \cdot m} / Y_{NS.m}))$	[61]
SENSITIVITY DROUGHT INDEX (SDI)	$(Y_{NS} - Ys) / Y_{NS}$	[63]

Density plots for the studied traits were created using a smoothed kernel density function, showing both the density of values and their relative probability. The area under the curve represents the density of trait values, and the Y-axis shows the relative probability. The X-axis value at the curve's peak represents the average value of the trait. All the figures were generated by R Statistical Software [50].

Results

Growth performance under salt stress

At 150 mM NaCl, growth parameters of the two genotypes were influenced to varying degrees. The growth of SC180 was more significantly affected by salinity stress than the SC168 hybrid (Fig. 1). The reduction in shoot length was more in SC180 (13.6%) than in SC168 (3.0%), while a similar trend was observed for root length with a 13.6% reduction in SC180 and 2.3% in SC168 compared to plants under control conditions (Fig. 1A and B). Dry weight reduction of leaf, stem and root (14.4%, 10.3% and 8.9%, respectively) of SC168 was less than that of SC180 (39.2%, 40.0% and 26.4%, respectively) under salt stress (Fig. 1C).

Na^+ and K^+ accumulation under salinity treatment

The data showed that both hybrids accumulated high amounts of Na^+ in all organs of plant involving leaves, stems and roots under salinity stress. SC168 accumulated more Na^+ in all organs than those of SC180 (Fig. 2A–C).

On the contrary, salinity treatment decreased the K^+ concentration in leaves, stems and roots of SC168 and SC180 (Fig. 2D–F). Leaf K^+ concentration declined significantly in SC180, but was not significantly altered in SC168 under salinity stress (Fig. 2D). Stem K^+ concentration of SC168 was more significantly affected by salinity treatment than that of SC180 (Fig. 2E). Salt stress markedly reduced the K^+ concentration in roots of both hybrids (Fig. 2F). The Na^+/K^+ ratios in the leaves, stems, and roots of genotype SC168 were greater than 1 exceeding those in all tissues of genotype SC180, except for the roots (Fig. 2 G–I).

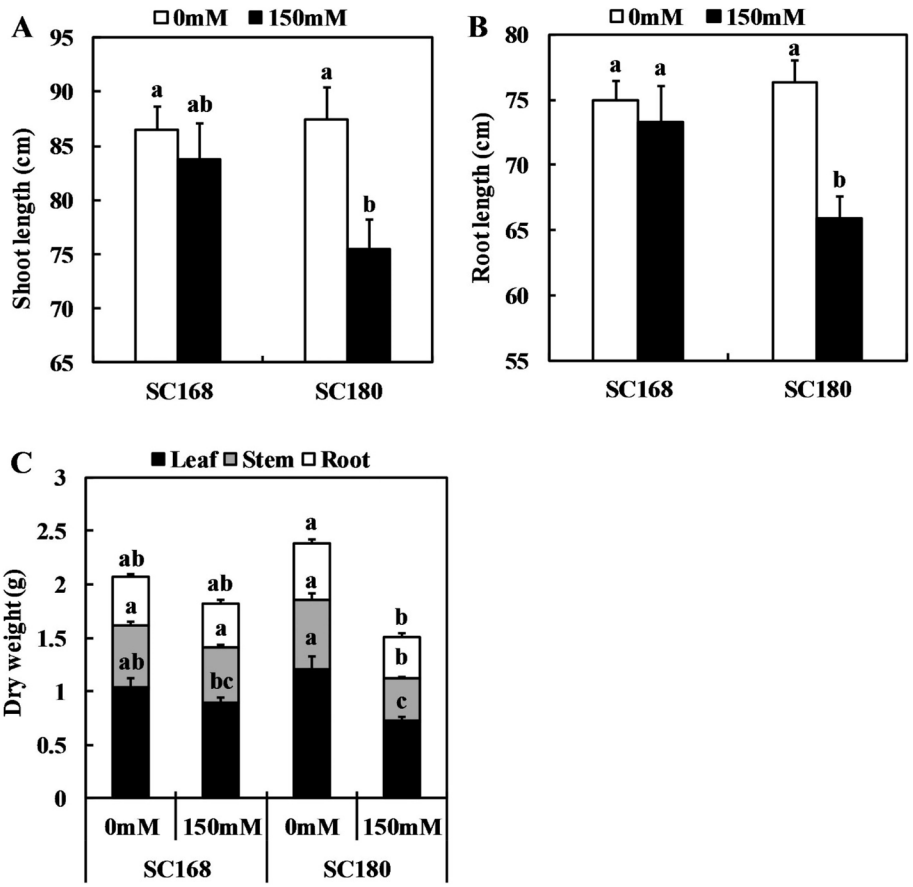

Fig. 1 **A** Shoot length, (**B**) Root length, and (**C**) Dry weight of the maize hybrids SC168 and SC180 grown under control and salinity (150 mM NaCl) conditions for 12 days. Data represent the means of four replicates ± SE. The different letters indicate significant differences ($P < 0.05$)

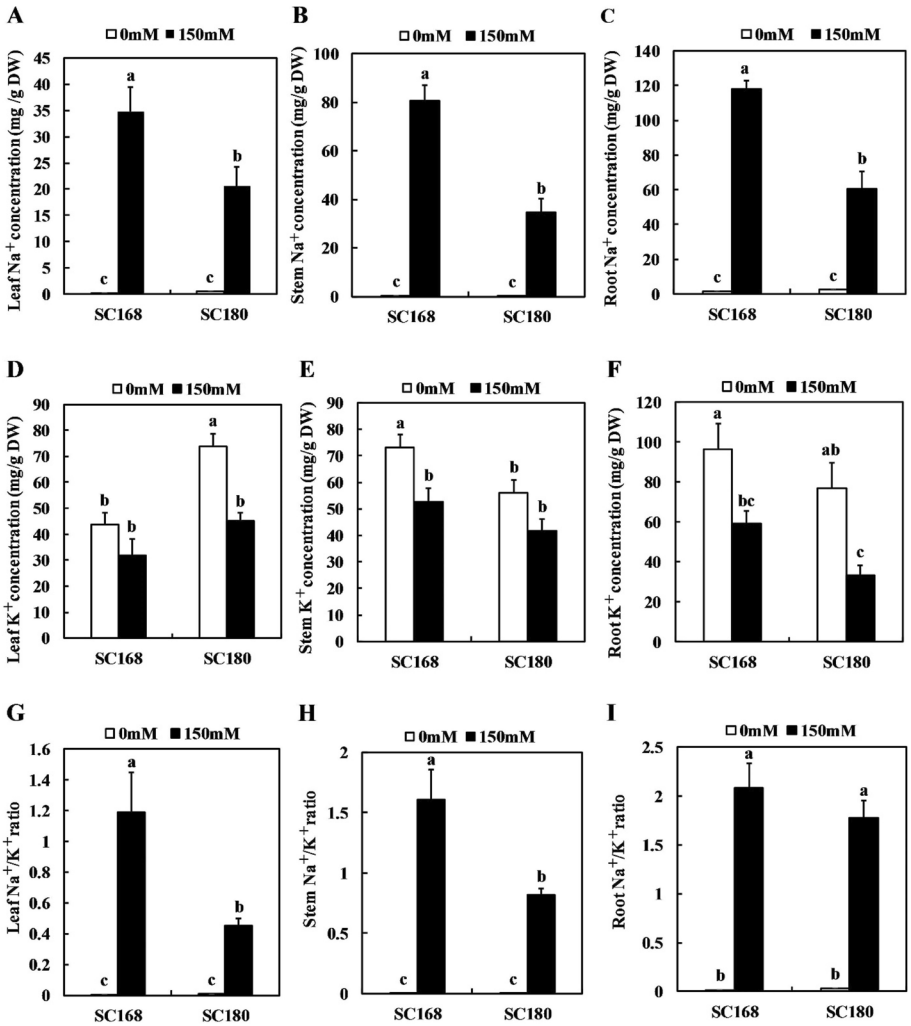

Fig. 2 **A** Leaf Na + concentration, (**B**) stem Na^+ concentration, (**C**) root Na^+ concentration, (**D**) leaf K^+ concentration, (**E**) stem K^+ concentration, (**F**) root K^+ concentration (**G**) leaf Na^+/ K^+, (**H**) stem Na^+/ K^+ and (**I**) root Na^+/ K^+ of the maize hybrids SC168 and SC180 grown under control and salinity (150 mM NaCl) conditions for 12 days. Data represent the means of four replicates ± SE. The different letters indicate significant differences ($P < 0.05$)

Water status and proline accumulation

The relative water content (RWC) decreased significantly in leaves of SC180 under salinity stress (22.7%) compared to control, while RWC in SC168 leaves was not significantly altered (5.15%) under salt stress, compared to control (Fig. 3A). Salinity stress led to enhanced proline accumulation in the leaves of both hybrids. Specifically, the increase in proline content was greater in SC168 leaves (2.3-fold) compared to SC180 leaves (onefold), as shown in Fig. 3B.

Oxidative stress markers

Electrolyte leakage and H_2O_2 accumulation (Fig. 4) are major signs of oxidative damage caused by salinity stress. In the current study, salt treatment significantly increased the electrolyte leakage in leaves of both hybrids comparing to control treatment. On the other side, the increase was more marked in SC180 (48.5%) than in SC168 (35.25%) (Fig. 4A). In addition, salt treatment resulted in a significant increase in H_2O_2 concentration in leaves of SC180 (48.7%), compared to that in SC168 leaves (22.0%), in comparison to the controls (Fig. 4B).

Antioxidant enzyme activities

The activities of the antioxidant enzymes APX, CAT, POD, SOD and GR are presented in Fig. 5 (A-E). For the direct ROS scavenging enzymes (APX, CAT, POD, and SOD), they were significantly increased in SC168 under salt stress, whereas these activities were not significantly altered in SC180. Specifically, in the leaves

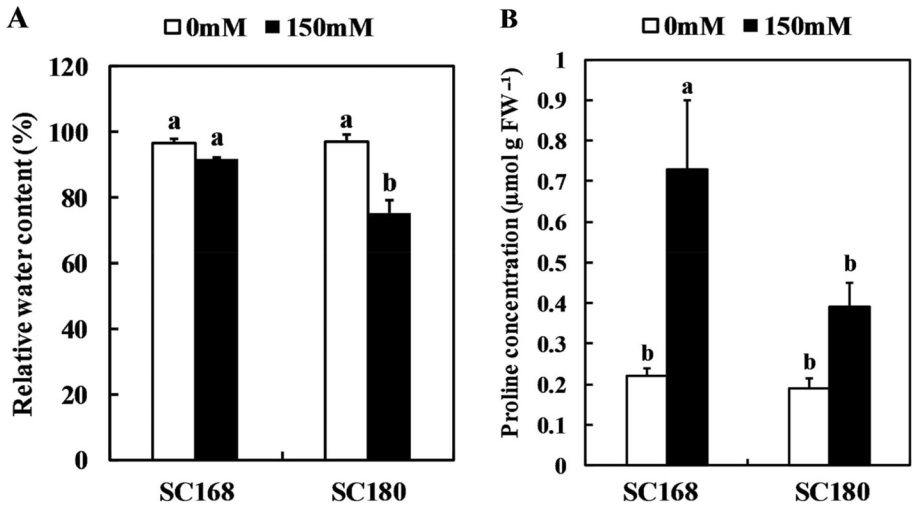

Fig. 3 **A** Relative water content and (**B**) proline concentration in leaves of the maize hybrids SC168 and SC180 grown under control and salinity (150 mM NaCl) conditions for 12 days. Data represent the means of four replicates ± SE. The different letters indicate significant differences ($P < 0.05$)

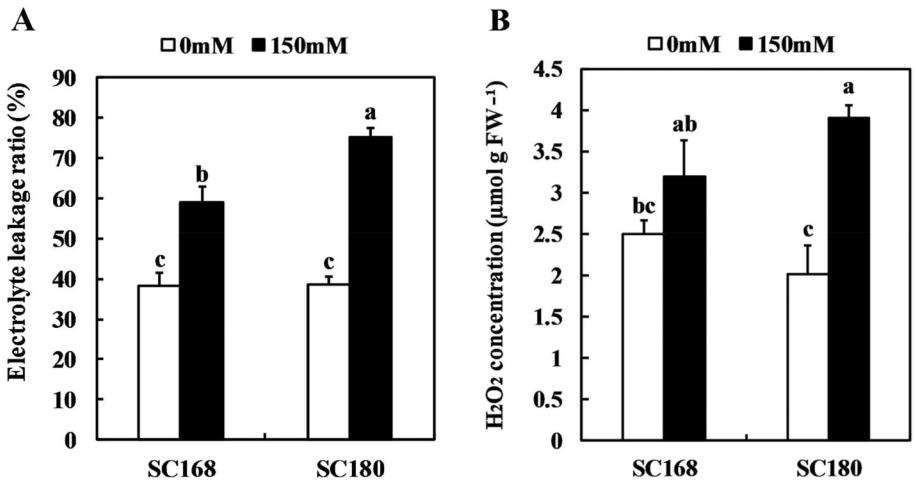

Fig. 4 **A** Electrolyte leakage ratio and (**B**) H2O2 concentrations in the leaves of the maize hybrids SC168 and SC180 grown under control and salinity (150 mM NaCl) conditions for 12 days. Data represent the means of four replicates ± SE. The different letters indicate significant differences ($P < 0.05$)

of SC168, salinity stress induced the activities of CAT, APX, POD, and SOD by 57.0%, 68.0%, 45.0%, and 56.0%, respectively, compared to the control (Fig. 5 (A-D)). However, the activities of these enzymes in the leaves of SC180 were not significantly altered by salt treatment (Fig. 5 (A-D)). For the glutathione reducing enzyme (GR), the activity was also significantly increased in SC168 under salt stress, while it remained unchanged in SC180. In the leaves of SC168, the activity of GR was induced by 62.0% compared to the control (Fig. 5 (E)). Conversely, the activity of GR in the leaves of SC180 did not show significant changes under salt stress (Fig. 5 (E)).

Salinity stress tolerance

Stress indices are quantitative measure used to quantify crop stress response. Its advantage came from its easily useable than raw data because they can be directly interpreted. Many stress indices of abiotic tolerance such as salinity and drought have been proposed (Table 1). They are used for estimating abiotic stress tolerant through assessing the association between growth under control and stress conditions. Abiotic stress indices are divided

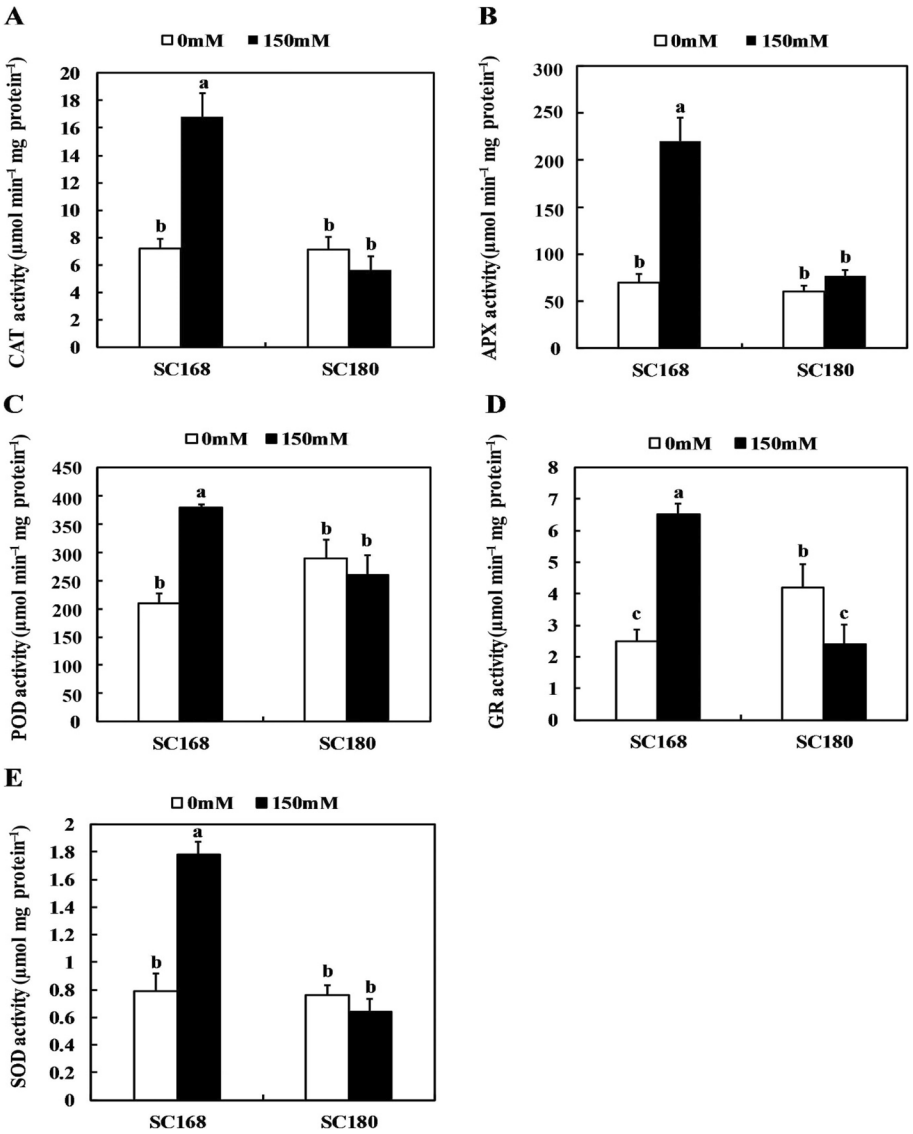

Fig. 5 Activity of the antioxidant enzymes, (**A**) catalase (CAT), (**B**) ascorbate peroxidase (APX), (**C**) peroxidase (POD), (**D**) glutathione reductase (GR), and (**E**) superoxide dismutase (SOD), in the leaves of the maize hybrids SC168 and SC180 grown under control and salinity (150 mM NaCl) conditions for 12 days. Data represent the means of four replicates ± SE. The different letters indicate significant differences ($P < 0.05$)

into two categories. The first category includes indices where higher values indicate greater stress tolerance, while the second category comprises indices where lower values suggest higher stress tolerance. By relying on these stress indices, we can recognize tolerant and sensitive genotypes in addition to their stability [64].

The results in Tables (2 & 3) reveal that SC168 genotype was more tolerant than SC180 with an average rank (AR = 1.08) (Fig. 6) where almost all the stress indices ranked it the first except the index MSTI1. However, the genotype SC180 was the second in tolerance (AR = 1.92). It was useful to account for the average of all ranks of all abiotic stress indices due to the different results of them. The heatmap cluster analysis (Fig. 7) displays the correlation between SC168 and SC180 and the analyzed characteristics, using scaled (standardized) data represented by a color scale for both control and salinity conditions. The heatmap uses red cells to indicate high trait values and blue dots to indicate low trait values. Prior to building the heatmap, the data underwent standardization by subtracting the mean of each trait from each corresponding value and thereafter dividing by the standard deviation. The standardization process is employed to enable comparability, as the researched features may be measured

Table 2 Values of 22 abiotic stress indices based on shoot dry weight under stress (Ys) and control (Yns)

	YNS	YS	MP	GMP	HM	STI	YI	MSTI1	MSTI2	YSI	RSI	DI	SNPI	REI	MRP	GM	TOL	SSI	SSPI	YR	ATI	MPI	SSSI	SDI
SC168	0.58	0.53	0.56	0.40	0.55	0.81	1.14	0.72	1.06	0.91	1.21	1.04	1.54	1.07	2.08	22.20	0.05	0.35	0.04	0.09	0.02	0.03	-0.16	0.09
SC180	0.65	0.40	0.53	0.32	0.50	0.69	0.86	0.77	0.51	0.62	0.81	0.53	0.76	0.91	1.92	4.20	0.25	1.58	0.20	0.38	0.10	0.13	0.14	0.38

Table 3 Rank of SC168 and SC180 by 22 abiotic stress indices and shoot dry weight under stress (Ys) and control (Yns) as well as their average rank (AR)

	YNS	YS	MP	GMP	HM	STI	YI	MSTI1	MSTI2	YSI	RSI	DI	SNPI	REI	MRP	GM	TOL	SSI	SSPI	YR	ATI	MPI	SSSI	SDI	AR
SC168	2	1	1	1	1	1	1	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1.08
SC180	1	2	2	2	2	2	2	1	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	1.92

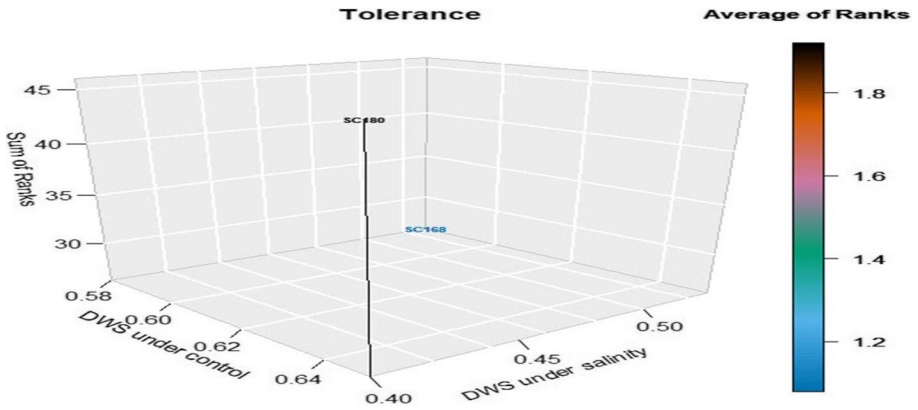

Fig. 6 Tolerance of SC168 and SC180 according to the average rank of 22 abiotic stress indices (small number of average ranks means tolerant)

using multiple units of measurement. Under control conditions, the two genotypes exhibit similar values for the studied variables. However, when exposed to salinity conditions, the genotype SC168 demonstrates greater values for all the studied traits, except for ELR and leaf H_2O_2. The low scores of these two features indicate salinity tolerance.

The two genotypes under control and salinity conditions using kernel density estimation for estimating the probability density function (PDF) (Figs. 8 & 9, show the density plots of the studied traits). Each density plot shows the density of the values as relative probability. The area under the curve illustrates the distribution shape of each trait's values, indicating whether the distribution is unimodal (one peak), bimodal (two peaks), or multimodal (more than two peaks), as well as its skewness. The Y-axis values represent the relative probability of the corresponding X-axis values, with the dashed line on the X-axis indicating the trait's average. The figures demonstrate that peaks in the density plots correspond to higher concentrations of trait values, signifying higher relative probability. Conversely, the tails of the plots indicate lower concentrations of values, representing lower relative probability. Under salinity, the means of the traits of the two genotypes were profoundly different from each other than under control where SC168 tended to have higher values than SC180 under salinity. While RDW mean values were too close for the two genotypes. Both magnitude and distribution of the studied traits values were affected by salinity. The most distinguished traits that identify the tolerance between the two genotypes were ELR and LH_2O_2 where higher values of these traits indicate low tolerance as in SC180. Also, the distribution of these traits had changed from unimodal (one peak) under control to multimodal (more than two peaks) for ELR and bimodal shape for leaf H_2O_2 under salinity for SC180 which imply genetic variation or heterogeneity (polymorphic) [65]. Additionally, Leaf Na^+, Stem Na^+, Root Na^+ were also distinguished between the two genotypes where higher values of these traits indicate high tolerance as in SC168.

Correlation analysis

Figures 10 & 11 show the Spearman correlation coefficients among the studied traits under control and salinity conditions. In general, the correlation coefficients among the studied traits were more profoundly under salinity than under control. For example, under control DWS was significantly and positively correlated with Root Na^+ (0.67) and Root K^+ (0.57), however, DWS was significantly and negatively correlated with ELR (-0.7) and Stem K^+ (-0.52). On the other hand, DWS correlation with Root Na^+ was (0.74) and (0.67) with Root K^+.

Discussion

To understand the bases underlie the salinity stress tolerance, interspecific and intraspecific differences between sensitive and tolerant cultivars or hybrids of same species [28, 66]. Therefore, here we screened the salinity tolerance screening maize hybrids (SC180 and SC168) of contrasting stress selectivity. The results presented here show clearly that on the basis of biomass accumulation, SC168 is more tolerant than SC180 when both are subjected to a high salinity of 150 mM NaCl in a hydroponic medium. Following, we will explore the physiological and biochemical bases by which this differential tolerance may occur.

SC168 exhibits greater Na^+ exclusion from the leaf blade and possibly vacuolar Na^+ sequestration to withstand high salinity

Maize is a glycophyte and one of the main adaptive characteristics of glycophytes under salt stress is shoot Na^+ exclusion [34]. Furthermore, glycophytes restrict

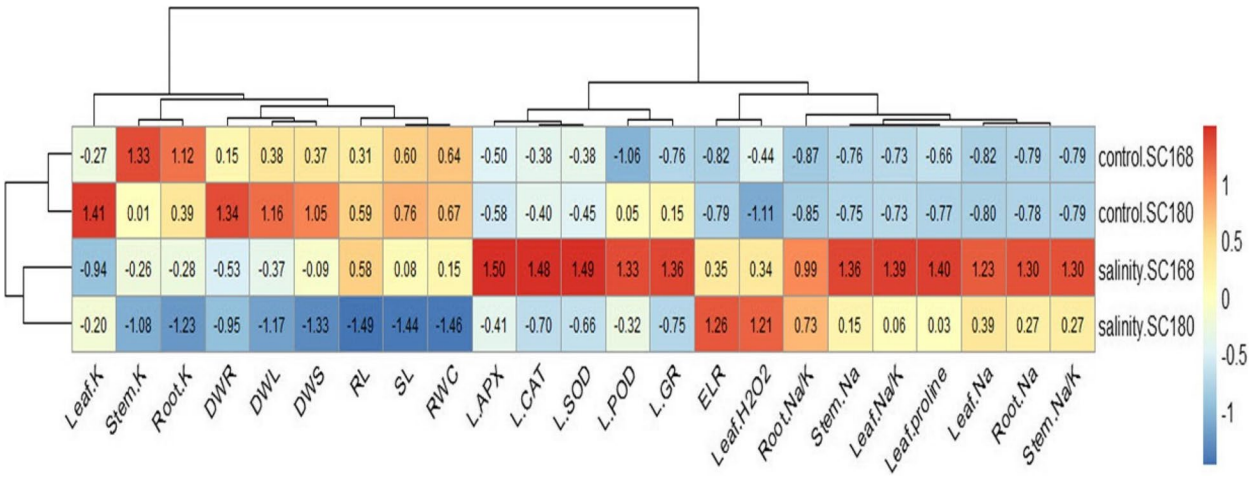

Fig. 7 Heatmap of the relationship between genotypes and the studied traits under control

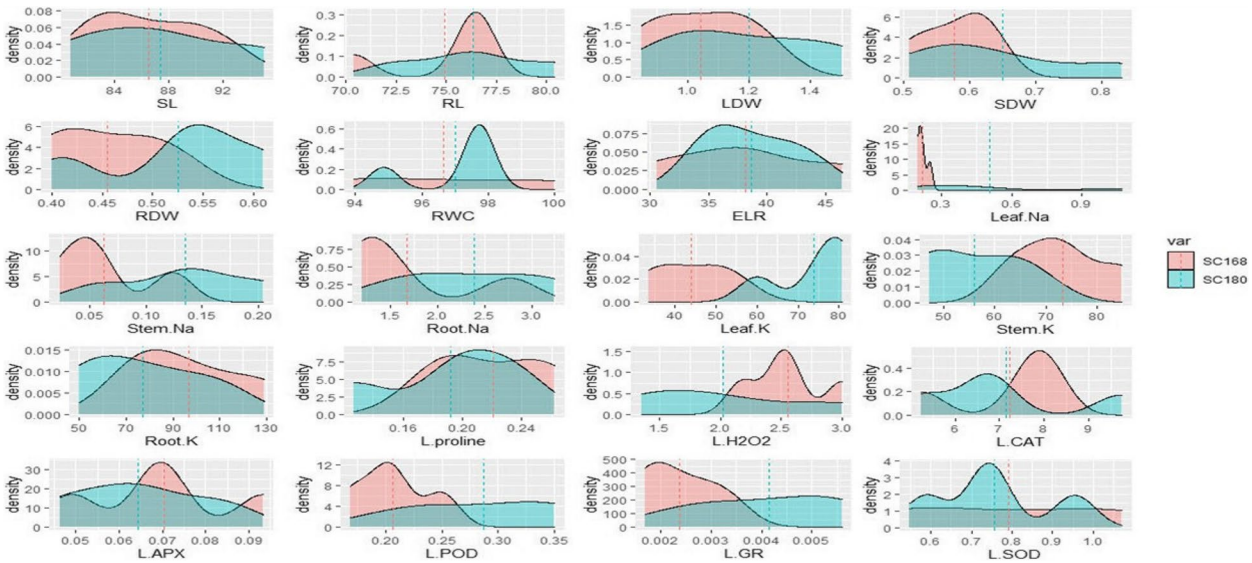

Fig. 8 Density plots of the studied traits under control

Na^+ movement from root to shoot by mediating various HKT1 transporters and SOS1 antiporters [66, 67]. Na^+ from xylem sap retrieval is an effective approach to preventing excessive root-to-shoot Na + translocation, where *HKT1* transporters play a key role in this process [68]. Interestingly, the salt-tolerant hybrid SR03 displayed higher of *ZmNHX* genes expression under salinity stress [34].

In the present study, both plants (specifically SC168) showed ability to exclude Na^+ from the leaf blade, while retaining higher concentrations in the stem and roots (Figs. 2, 3, 4, 5). In line with our results, most of Na^+ of shoot maize plants is compartmentalized in the stem, reducing Na^+ accumulation in leaf blades [69]. Na^+ exclusion from the leaf blade is important for stress adaptation so as to preserve important metabolic processes such as photosynthesis. Although Na^+ concentration in the leaf blade of SC168 was significantly higher than that of SC180, the proportion of the leaf Na^+ concentration to that of stem and root was smaller in SC168 than in SC180. In SC168, Leaf Na^+: Stem Na^+ = 1:2.5, and Leaf Na^+:Root Na^+ = 1:4, whereas in SC180, Leaf Na^+ stem Na^+ = 1:1.5, and Leaf Na^+:Root Na^+ = 1:3. This result indicates that the capacity to exclude Na^+ from the leaf blade is more effective in

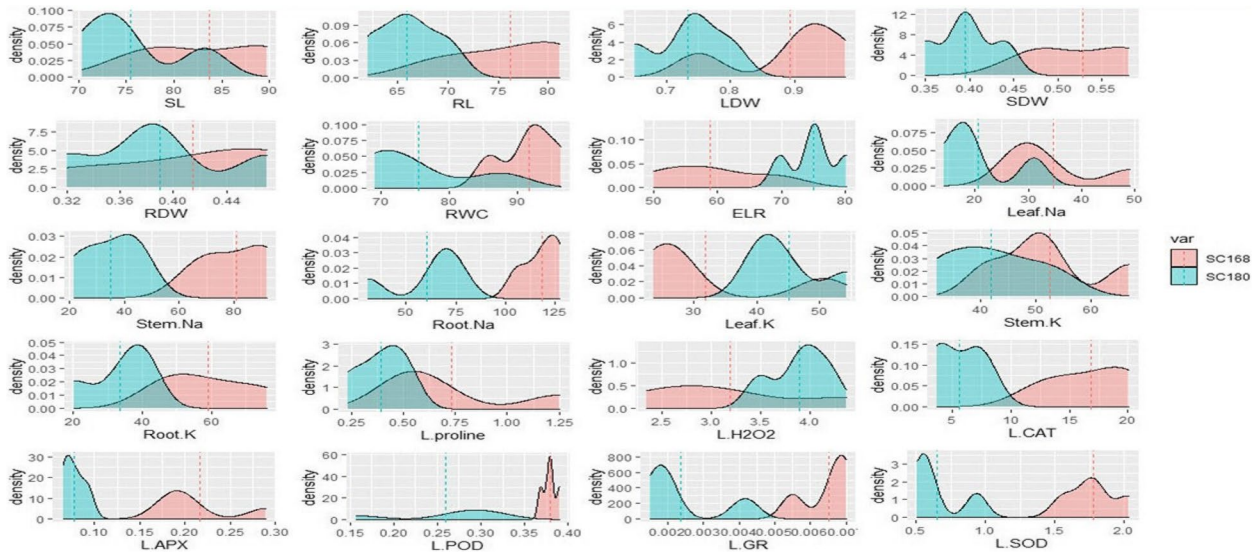

Fig. 9 Density plots of the studied traits under salinity

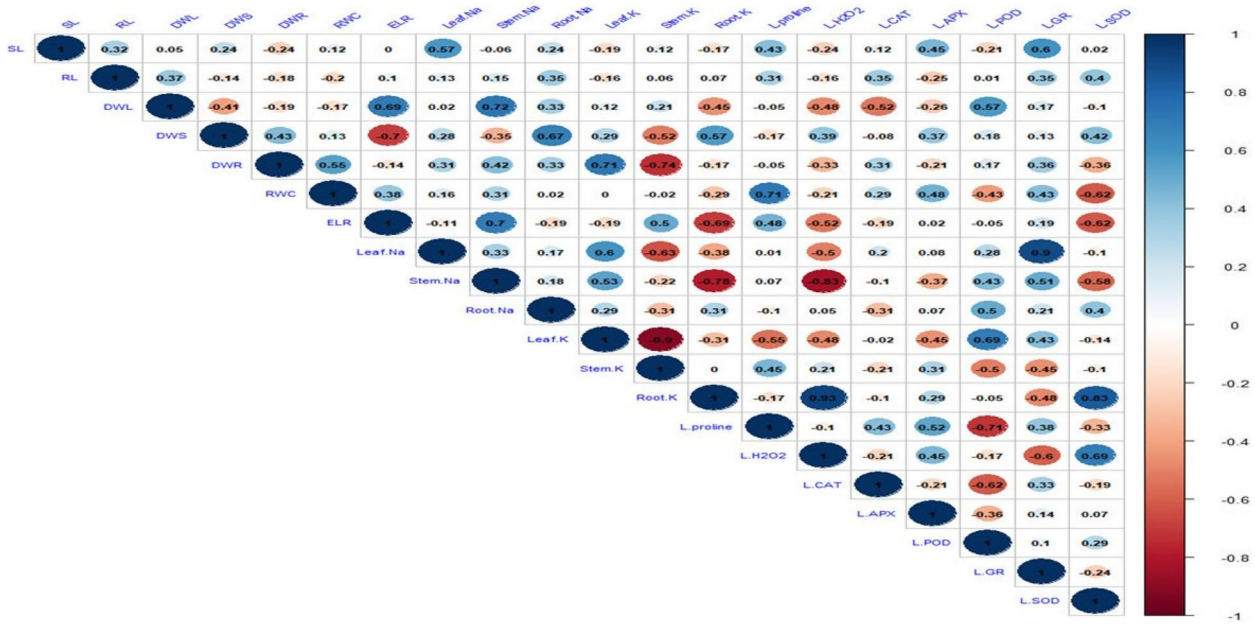

Fig. 10 Spearman correlation matrix among the studied traits under control

SC168 than in SC180 and could contribute in the superior tolerance of SC168 over SC180.

Variation in leaf Na^+ exclusion has been shown to account for differential tolerance in maize cultivars [70], as well as in other plant species such as, *Solanum scabrum* [71], rice [14], flax [72], *Talinum paniculatum* [13]. Furthermore, the higher accumulation of Na^+ in leaf blade of SC168, corresponding to better growth indicates the existence of another stress tolerance mechanism, possibly vacuolar Na^+ sequestration. Vacuolar Na^+ sequestration is very important for salt stress tolerance as it helps maintain a constant cytosolic Na^+/K^+ ratio for optimal cellular functions. This sequestration is mediated by tonoplast Na^+/H^+ antiporters, which have been shown to be crucial for salt stress tolerance in some maize hybrids [34]. It is possible that this mechanism is operational in SC168 but absent in SC180. Thus, the higher leaf Na^+/K^+ ratio (greater than 1.0, Figs. 2, 3, 4, 5)

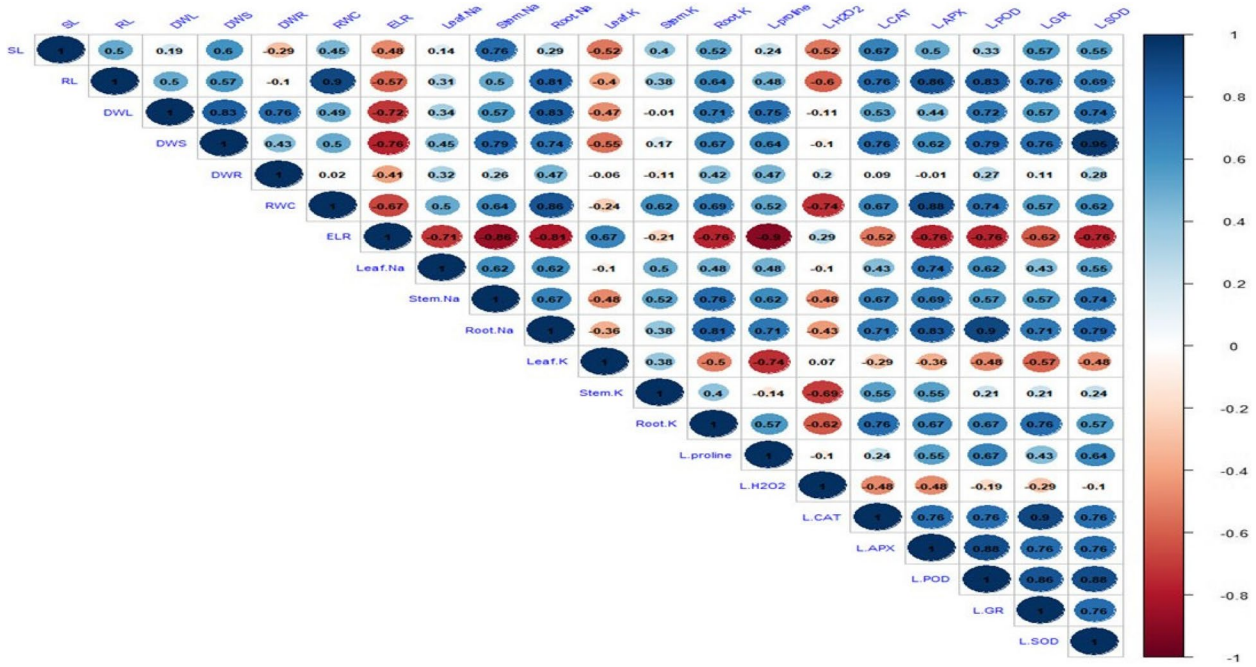

Fig. 11 Spearman correlation matrix among the studied traits under salinity

correlating with enhanced tolerance observed for SC168 lends more credence to the existence of a vacuolar Na^+ sequestration mechanism. On the contrary low Na^+/K^+ ratios, especially cytosolic ratios are often associated with stress tolerance [71, 73]. In agreement, the maize hybrids i.e., Pioneer 32B33 and Pioneer 30Y87 with high leaf K^+/Na^+ ratio showed high salinity stress tolerance, indicating the role of K^+ maintenance and K^+/Na^+ homeostasis in improving stress tolerance. However, although the ratio is low in SC180 (< 1), it does not correspond to stress tolerance, implying that the Na^+ present in the tissue could be mostly cytosolic and hence deleterious for the plant in general.

A defective ROS detoxification system may account for the sensitivity of SC180 to salt stress

Over accumulation of ROS generation is often one of the major responses of plant to salinity stress, leading to oxidative stress damage [74]. ROS is the product of compromised electron transport systems, whereby electrons leaked from the systems, oxidize O_2 molecules to very reactive species that induces damage to cellular components [24]. Plants with robust antioxidant defense systems can withstand environmental challenges including salinity stress, ensuring their survival. Thus, a poor antioxidant defense system would lead to induced susceptibility to salinity stress [75–77]. In this context, enhance antioxidant enzyme activity corresponding to enhanced salt stress tolerance has been observed in *Solanum scabrum* [71].

The maize hybrid (SC132) also showed high oxidative stress damage under salinity stress, possibly due to a less effective ROS scavenging system. This hybrid could not upregulate the activities of peroxidase, APX, and SOD enzymes activity to counteract the oxidative stress effectively [45]. On the other hand, the maize hybrid SC131, showed enhanced CAT and APX enzymes activity which provided more protection as they directly detoxify H_2O_2 [45]. In agreement, SC180 showed signs of oxidative stress damage as indicated by induced H_2O_2 and ELR level (Fig. 4). Induced oxidative stress damage in SC180 was consistent with the unchanged antioxidant enzyme activities under salt stress, indicating a potentially lower tolerance to salinity-induced oxidative stress (Figs. 6, 7, 8, 9). On the other hand, SC168 has a more robust antioxidant defense mechanism under salinity stress, which enhanced its ability to reduce oxidative damage, suggesting that antioxidant enzyme activity plays a major role in salinity tolerance of SC168, but offers no protection in SC180, hence contributing to its susceptibility. Similarly, a stress tolerant SC131 genotype showed increased CAT and APX activities, providing more protection against H_2O_2 induced oxidative damage [67].

In addition, the higher proline accumulation in SC168 could possibly play significant role in ROS scavenging under the stress, since proline has been shown to scavenge $OH^{\bullet}$ under salt stress conditions [78, 79]. It also

stabilizes proteins and boosts the cellular redox potential [80]. Exogenous proline increases antioxidant capacity in plants growing under stress condition, improving their stress resilience [80]. This stress tolerance can be explained, at least partially by proline induced high activity of SOD, CAT, and APX enzymes in stress tolerant plants [80]. It was also reported that elevated accumulation of proline participated clearly in osmotic adjustment and increased enzymes activities (SOD, POD and PPO), leading to maize salinity tolerance [81].

Osmotic adjustment in the leaf could contribute to maintain water uptake and translocation to the shoot in SC168

The ability of plants to maintain water absorption and translocation to the leaf is an important requirement to withstand osmotic stress. Osmotic stress is often induced by the presence of high concentrations of salts especially Na^+ in the soil, which inhibits water uptake by plant roots [1, 28]. Failure to acquire water leads to compromised metabolic functions such as photosynthesis, with a consequential production of excess ROS (as mentioned above). To overcome this problem, plants synthesize osmolytes such as proline [25, 82] as well as accumulate inorganic solutes such as Na^+ and K^+ in order to balance the external osmotic potential required to drive water influx [1, 9]. Thus, enhanced accumulation of proline and, K^+ (mostly in halophytes) has been associated with salt stress tolerance. Beside its role in ROS detoxification, proline is known as osmoregulatory. Interestingly, several studies showed a significant correlation between proline accumulation and salt stress tolerance [83]. K^+ is necessary to regulate osmotic pressure, stomatal movement, tropisms, enzymes activity, membrane potential and turgor pressure [30, 31].

In the present study, the relative water content (RWC) of SC168 remained unchanged, whereas that of SC180, significantly decreased (Fig. 5A). This unaltered RWC in SC168, coincided with more than threefold increase in proline concentration, which was about twofold that in SC180 (Fig. 5B). In line with our results the tolerant hybrid (SC131) showed higher proline content, indicating its role in salt stress adaptation [45].Thus, it is possible that proline accumulation in the SC168 hybrid for osmotic adjustment to sustain hydraulic conductivity is another key tolerance trait in the plant. Enhanced Na^+ accumulation may also contribute to enhanced water uptake in this hybrid.

Conclusions

In conclusion, the superior tolerance of SC168 over SC180, under 150 mM NaCl stress owes partially to: 1. Na^+ exclusion from the leaf blade and enhanced Na^+ accumulation which is possibly sequestered into vacuoles and used for osmotic adjustment, 2. Enhanced ROS scavenging as seen in the enhanced antioxidant enzyme activities for all measured enzymes. Proline accumulation could possibly play a role in this detoxification. Osmotic adjustment via the accumulation of proline for a stable water content. Stress indices consistently ranked SC168 as more tolerant, with an average rank of 1.08, compared to SC180's average rank of 1.92. Correlation analyses confirmed that SC168's superior tolerance was associated with better Na + regulation, maintenance of K^+ levels, and a robust antioxidant defense system. Future research should focus on identifying the specific genes and molecular pathways involved in the enhanced salinity tolerance observed in SC168. This could provide deeper insights into the genetic basis of stress tolerance. In addition, conducting field trials to evaluate the performance of SC168 and SC180 under natural saline conditions will help validate the findings and determine their practical applicability in agricultural settings. Moreover, Utilizing the tolerant hybrid SC168 in breeding programs could help develop new maize varieties with improved salinity tolerance, thereby enhancing crop productivity in saline-prone areas.

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Authors' contributions

MR drafted the work and made experiments, DA made experiments and revised the manuscipt , AM interpretated the data and revised the manuscript , NS interpretated the data and revised the manuscript, ER interpretated the data and revised the manuscript, AE analysed the data, OI analysed the data, HM revised the manuscript, MO interpretated the data and revised the manuscript, MM revised the manuscritp, HA analysed the data and revised the manuscritp, AU designed the work and revised the writing of the manuscript.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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