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Genetic and biochemical adaptive responses of some Egyptian maize (*Zea mays* L.) hybrids to salinity stress

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Maize (*Zea mays* L.) is one of the most important strategic crops in Egypt and globally, yet its cultivation in newly reclaimed land in Egypt faces challenges due to saline groundwater. Therefore, developing new maize genotypes with true resilience to salt stress is a sustainable strategy for addressing the gap in maize production. This work investigates the physiological and molecular responses of two Egyptian maize hybrids (SC 168 and SC 176) with contrasting reactions to long-term saline agriculture. SC 168 (salt tolerant) exhibited less severe wilting and discoloration in shoot tissues compared to SC 176, which completely perished. The accumulated Na+ in shoots significantly increased in SC 176 compared to SC 168 under salt stress, with a 3.5-fold increase. In the same context, SC 168 accumulated 1.8 times more K^+ than SC 176 under salt stress. Lipid peroxidation of the cell membrane was much more pronounced in the salt-sensitive genotype SC 176 than in SC 168, with a 1.7-fold increase. At the level of gene expression profile, several salt-stress marker genes were examined. The salt-tolerant maize hybrid SC 168 accumulates more mRNA of ZmNHX5 (sodium sequestration in vacuoles), the catalase gene (antioxidative enzyme), and ZmNR (nitrogen assimilation) under salt stress compared to SC 176. Collectively, it is concluded that minimizing sodium ions uptake, enhancing antioxidative power, and maintaining nutritional balance are the main maize salt-resilience strategies that should be considered in developing maize genotypes suitable for saline agriculture.

Keywords: Maize; salt stress; gene expression; sodium content

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INTRODUCTION

Salinity presents a persistent destructive challenge to crop productivity. With most of the Earth's water being saline (approximately 30,000 ppm), salinity continually impacts soils used for current and potential crop cultivation (Flower, 2004). The climate changes in the form of elevated atmospheric temperature, high levels of evapotranspiration, and expected sea level rise significantly led to noted unprecedented levels of topsoil salinity (Hazman et al., 2022).

Egypt, an arid and semi-arid agroecosystem, showed ~ 26% of salinized land areas in the Nile Delta and Nile Valley (~ 2000 ppm in topsoil) (Mohamed et al., 2007; Mohamed et al., 2011). This is probably due to improper agricultural practices as a massive application of inorganic fertilizers accompanied by inefficient agricultural drainage system (Abdel-Mawgoud et al., 2005; Kubota et al., 2017). The increasing demand for food due to the growing global and local population has led to the cultivation of marginal lands which previously left uncultivated due to high natural soil salinity and saline groundwater, which are now being brought into agricultural production yet with serious challenges (Shin et al., 2022). Therefore, it is demanded to develop a sustainable system that combines enhancing soil/water properties with a satisfied genetic knowledge of plants under edaphic stresses as salinity.

Salinity stress triggers a range of responses in plants, including morphological, physiological, biochemical, and molecular changes (Ambede et al., 2012; Abreu et al., 2013). It causes ionic toxicity, osmotic stress, nutrition deficiency, and the overproduction of reactive oxygen species (ROS) (Chawla et al., 2013; Hazman et al., 2016). The accumulation of Na^+ under salinity stress competes with K^+ for active binding sites in proteins, thus inhibiting protein synthesis and negatively affecting metabolic enzymic functions (Schachtman and Liu, 1999; Pardo and Quintero, 2002). Elevated NaCl concentrations outside the roots decrease potential water, making water uptake more challenging which causes osmotic stress; a case which is known as "physiological drought". In leaves, high salt levels lead to stomatal closure, disruption of electron transport, and impairment of the photosynthetic apparatus, and thus productivity (Abreu et al., 2013; Deinlein et al., 2014). Additionally, high salinity triggers an over-production of ROS within plant cells, leading to oxidative damage of membrane lipids, proteins, and nucleic acids which eventually causes plant death (Pérez-López et al., 2009; Gill and Tuteja, 2010).

Adaptation strategies are essential for survival in saline conditions; while they exhibit broad similarities

across plant species, they are most pronounced in halophytes (salt-tolerant plants) and present to varying degrees in glycophytes, i.e., salt-sensitive plants (Flowers & Flowers, 2005). A crucial adaptive mechanism for plants under salt stress is ions homeostasis which could be achieved through fine-tuned ion uptake and compartmentalization (Zhu, 2003). Excess Na^+ is either transported to the vacuole via tonoplast Na^+/H^+ antiporters driven by the proton gradient or sequestered in older tissues; thus, mitigating the impact of sodium toxicity (Zhu, 2003). In maize, salt tolerance is associated with higher K^+ levels, lower Na^+ and Cl^- fluxes, and a higher K^+/Na^+ ratio (Hajibagheri et al., 1989; Cerda et al., 1995). Salt-resistant maize plants more effectively exclude Na^+ from the cytoplasm of leaf cells compared to salt-sensitive plants (Wakeel et al., 2011). Enhanced enzyme activities are significantly greater in salt-tolerant maize plants compared to their salt-sensitive counterparts (de Azevedo Neto et al., 2006). For example, the combined activity of CAT, GPX, and APX enzymes, along with SOD, shows superior H_2O_2 scavenging capability in the leaves and roots of salt-tolerant maize plants under salt stress. Maize employs a range of defense mechanisms, including osmolyte production, activation of antioxidant enzymes, and regulation of ion transport, to counteract the effects of salt stress (Li et al., 2023).

Under salt stress, maize plants also increase the synthesis of endogenous growth hormones, such as abscisic acid (ABA), which may promote stomatal closure and reduce water loss due to osmotic stress induced by salinity (Younis et al., 2003). Molecular research has provided valuable insights, including the identification of key genes involved in the mechanisms of salt tolerance (Luo et al., 2019; Ali et al., 2023).

In this study, we investigate the adaptive genetic and physiological responses of two Egyptian hybrids of maize (SC 168 and SC 176) to salinity stress. We examined the content of malondialdehyde (MDA) as an indicator of lipid peroxidation, measured the Na^+ and K^+ content to assess ionic balance, and analyzed the expression of several stress key genes involved in salinity response.

MATERIAL AND METHODS

Plant Materials, Growth, and Stress Conditions

The seeds of two Egyptian maize hybrids (*Zea mays* L), SC 168 and SC 176, were sowed and grown in pots (≈ 5000 cm^3) under controlled environmental conditions in the greenhouse. In each pot, seven seeds were pre-germinated and then reduced to the most four healthy ones after 10 days. The experiment's location latitude was 300127 N, and the longitude was 311211.3 E. First, all plants were irrigated with tap water until the age of two months. Thereafter, the plants were exposed to salinity stress by applying nearly 500 ml of solution containing 75 mM NaCl, while the control group was continuously given tap water. The salinity treatment was given for one month with one time per weak intervals. After the salinity stress course was finished, plants were photographed using a high-quality professional camera (Nikon, USA). Subsequently, leaves were collected from the treated and untreated plants to measure ion content. For molecular and biochemical analysis, collected leaves were immediately transferred to liquid nitrogen and then kept at −80°C.

Sodium and Potassium Ions Content Determination

The amounts of sodium (Na^+) and potassium (K^+) ions in the leaf sheath tissues were analyzed using the Teledyne Leeman Labs Prodigy7 Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES). The measurements were conducted at the Micro Analytical Center, Faculty of Science, Cairo University according to (Kumar et al., 2021). The samples were first dried in an oven at 80℃ for three days, ground to fine powder, and then digested with a mixture of acids to dissolve the organic matter and get a clear solution. The digested samples were diluted with deionized water to an appropriate concentration for analysis expressed in mg/gDW. These prepared solutions were then injected into the ICP-OES spectrometer where the samples were atomized and ionized in high-temperature argon plasma. The intensity values of light at the defined wavelengths for Na^+ and K^+ were recorded, and due to this, their concentration in the samples was determined. Calibration was done with standard solutions of known concentration to minimize the error of the measurements.

Determination of Lipid peroxidation

Lipid peroxidation in control and stressed leaves was assessed by measuring the malondialdehyde (MDA) content using the thiobarbituric acid (TBA) method, as outlined by Heath and Packer (1968). In summary, 500 mg of maize leaves were homogenized in 1 ml of 0.1% trichloroacetic acid (TCA, w/v) using a mortar and pestle. The resulting homogenate was centrifuged at 10,000 g for 20 minutes at room temperature, and then 0.5 ml of the supernatant was mixed with 1 ml of 0.5% TBA in 20% TCA (w/v). This mixture was heated in a boiling water bath for 1 hour. The reaction

was halted by placing the tubes in a water bath at room temperature for 10 minutes, followed by a second centrifugation at 10,000 g for 10 minutes. The absorbance of the supernatant was measured at 532 nm, with the non-specific absorbance at 600 nm subtracted. The concentration of the MDA–TBA complex (red pigment) was determined using an extinction coefficient of 155 mM–1 cm^{-1}.

RNA Extraction and Quantitative Gene Expression Profile

Total RNA was extracted from the harvested leaf samples using a Direct-zol™ RNA Miniprep kit (USA). 200 ng of isolated total RNA was then used for cDNA synthesis and PCR with the help of DlaStar ™ one step RT-PCR Kit (South Korea) according to the following thermal program: 55 °C for 30 min, 95 °C for 15 min, 30 cycles of 95 °C 30 s, 60 °C for 30 s and 72 °C for 1 min, and the final extension was at 72 °C for 5 min. PCR reaction was stopped by holding the tubes at 4 °C for at least 10 min. The PCR product samples were separated and visualized in a 1% agarose gel stained with ViSafe Red Gel Stain (Vivantis, Malaysia) against a 100 bp ready-to-use DNA ladder (GeneDirex, Tiwan). The quantification of the relative gene expression profile was calculated for both control and salt stress-treated samples according to the Gel Express method (Hazman, 2022) using the following equation: $[E^{IntDen(ref)}/E^{IntDen(target)}]_{sample}$. The target genes whose expressions were quantified in this work are NHX5, SOS1, Catalase, APX, GR, NCED9, Dehydrin, and NR, with 18S used as a reference gene (Figure S1 and Table S1 in the supplementary materials).

Experimental Design and Statistical Analysis

The experiment plots were arranged in a complete randomized design (CRD). SPSS (IBM Statistics, USA) software was used for statistical tests including mean separations by Duncan's multiple range test (DMRT) (Duncan, 1955), with a significance level of $P \leq 0.05$. All analyzed data represented three independent biological replications, each in technically the pool of triplicates.

RESULTS AND DISCUSSION

In this work, we challenged two Egyptian maize hybrids, SC168 and SC176, with salinity stress by irrigating the plants with 500 ml of a 75 mM NaCl solution once per week for one month. SC168 and SC176 showed distinct responses to the saline irrigation regime (Figure 1). SC168 exhibited fewer damage symptoms compared to SC176, which displayed more severe salt stress symptoms such as complete leaf discoloration and full shoot stunting (from base to top), eventually leading to plant death. Although maize is considered a salt-sensitive crop (Shahzad et al., 2015), we screened several Egyptian maize hybrids and found that SC168 could be described as salt-tolerant, while SC176 could be salt-sensitive, based on germination ratios in 150 mM NaCl in the dark at room temperature for one week. In the greenhouse pot experiment, the results supported the previous screening test and showed that the salt-sensitive variety (SC176) exhibited more severe wilting and chlorosis symptoms compared to the salt-tolerant variety (SC168). (Jiang et al., 2018) observed similar results, where a salt-sensitive maize variety exhibited more severe wilting and chlorosis symptoms compared to a salt-tolerant variety. These chlorosis and stunting symptoms are primarily attributed to the massive accumulation of Na^+ ions in the leaves, particularly in the appendicular areas (Hazman et al., 2016).

Ion content determination in leaves showed that Na+ accumulation in SC168 (salt-tolerant) was significantly lower than in the salt-sensitive maize hybrid SC176 (Figure 2A). SC176 accumulated more Na+ in leaves under saline irrigation, with a nearly 13-fold increase, while the salt-tolerant hybrid SC168 showed approximately a 2.2-fold increase compared to control conditions. Collectively, the salt-sensitive maize hybrid SC176 accumulated 3.3 times more Na+ than the salt-tolerant hybrid SC168. The degree of salinity stress in glycophytes (salt-sensitive plant species) is influenced by the uptake and translocation of NaCl (Hu et al., 2022). (Wang et al., 2020) indicated that salt tolerance is inversely related to the amount of Na^+ accumulated in tissues, particularly in leaves. Thus, we conclude that the superior salt tolerance phenotype of SC168 over SC176 is due to the lower accumulation of sodium in the leaves (Figures 1 and 2). In the same context, SC168 was able to influx more K^+ into leaves than SC176 (Figure 2B). SC176 significantly accumulated less K^+, with a reduction ratio of ~44% compared to SC168 under salt stress. As a result, the Na^+/K^+ ratio in SC176 was much higher than in SC168 under salt stress (Figure 2C). Salt-tolerant maize genotypes exhibit lower K^+/Na^+ ratios compared to sensitive ones (Akram et al., 2007; Wang et al., 2020). It is suggested that the greater depletion of potassium, an essential macronutrient, in SC176 is a central contributing factor to the retarded growth symptoms observed in shoot tissues (Hazman et al., 2016).

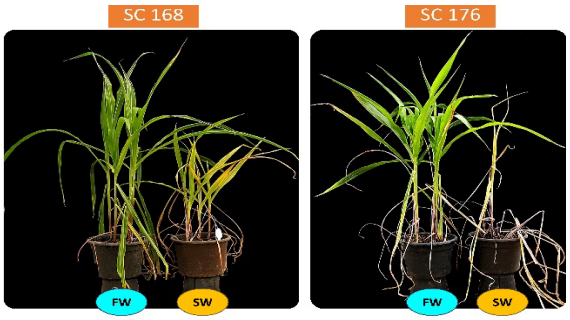

Figure 1. Phenotyping of maize plants in response to salt stress. Two Egyptian maize hybrids (SC 168 and SC 176) were grown under freshwater and saltwater irrigation regimes for three months under greenhouse conditions.

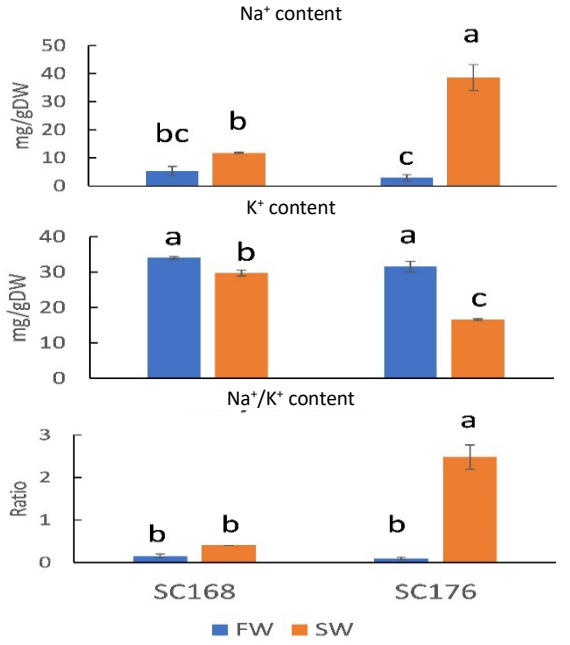

Figure 2. Contents of sodium (A), potassium ions (B), and sodium/potassium content ratio (**C**) in shoots of maize plants (SC 168 and SC 176) under freshwater (FW) and saltwater (SW) irrigation regimes. Values represent the mean of at least three independent experiments ±SE. Significant differences amongst different treatments are indicated by other letters, according to Duncan's multiple range test (DMRT) ($p \leq 0.05$).

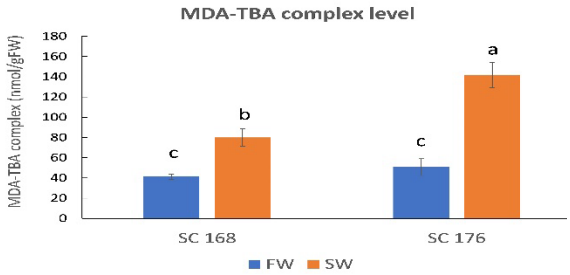

Figure 3. Lipid peroxidation levels in maize hybrids SC 168 and SC 176 under freshwater (FW) and saltwater (SW) irrigation regimes. Values represent the mean of at least three independent experiments ±SE. Significant differences amongst different treatments are indicated by other letters, according to Duncan's multiple range test (DMRT) ($p \leq 0.05$).

Salinity typically triggers the overproduction of reactive oxygen species (ROS), which can damage various biological molecules, including lipids, proteins, and cell membranes. To assess membrane damage in the two maize hybrids, we quantified malondialdehyde (MDA), a stable marker of lipid peroxidation under abiotic stress (including osmotic and alkalinity stress) (Apel and Hirt, 2004). The sensitive maize hybrid SC176 accumulated more MDA than SC168, with a 2.8- and 1.95-fold increase, respectively, under saltwater (SW) irrigation compared to freshwater (FW) conditions (Figure 3). It is suggested that SC168 accumulated less MDA due to lower Na^+ accumulation in leaves, thus retaining more antioxidative power (Hu et al., 2022).

Investigating the molecular response to salt stress is essential for understanding plant adaptive mechanisms and developing salt-resistant crops (Ma et al., 2022). Therefore, we quantified the relative gene expression of several salt-stress-related maize genes covering various aspects of plants' adaptive response to salt stress. Sodium sequestration into vacuoles is a well-known salt tolerance mechanism (Pabuayon et al., 2021) demonstrated that the gain-of-function mutation of AtNHX1 enhances salt tolerance in Arabidopsis. As shown in Figure 4A, ZmNHX5 (encoding the Na^+/H^+ tonoplast antiporter) was significantly upregulated in SC168 under salt stress, with a 485-fold increase compared to SC176 (Rizk et al., 2024).

Na^+ efflux is a major sodium detoxification mechanism in plants in response to salt stress, with the salt overly sensitive (SOS) pathway being the most well-studied mechanism (Zhang & Yang, 2023). While the SOS pathway has been extensively studied in the model dicot plant Arabidopsis, its role in crop species remains largely unknown (Vega & Melino, 2022). In this work, ZmSOS1 expression was significantly reduced in both SC168 and SC176 (Figure 4B) under long-term salinity stress. However, the reduction was much higher in SC168 (approximately 64%) compared to SC176 (42%). Despite this level of reduction, the expression level in SC168 (salt tolerant) remains higher in SC176 (salt sensitive). ZmSOS1 is known to confer salinity tolerance in maize (Zhou et al., 2022); nevertheless, several reports indicate that transcriptional changes in SOS1 do not always correlate with functional outcomes. Additionally, post-transcriptional modifications, rather than gene transcription, are often key determinants of SOS1 activity in plants (Ishikawa et al., 2022; Li et al., 2023).

Salt stress can disrupt plant cellular balance in the homeostasis of ROS, i.e., the balance between production and scavenging capacity (Li et al., 2023). One of the main defense mechanisms for detoxifying ROS under salt stress is the increased activity of antioxidant enzymes such as catalase, ascorbate peroxidase, and glutathione reductase (Kesawat et al., 2023). The quantified gene expression profile of catalase (CAT), ascorbate peroxidase (APX), and glutathione reductase (GR) displayed varied expression patterns between SC 168 and SC 176 under salinity stress (Figure 5), directly impacting their ability to manage oxidative damage (Hazman et al., 2016). Figure 5A shows that salt stress significantly reduced the expression of the APX gene in both SC 168 and SC 176. However, the level of APX transcripts in SC 168 was still much higher, with a 66-fold increase compared to SC 176, indicating that SC 168 might retain higher APX activity, which could contribute to its superior salt stress tolerance. In SC 168, catalase gene expression increased 3.5-fold in the treated plants (saline water) compared to the control (freshwater), indicating robust upregulation in response to salinity. In contrast, SC 176 showed no significant difference in the treated plants relative to the control.

The expression level of the catalase gene in treated SC 168 was approximately five times higher than in treated SC 176 plants, highlighting a pronounced difference in the antioxidative response between the two hybrids under salinity stress (Figure 5B). For Glutathione Reductase (GR) gene expression, Figure 5C shows that SC 168 maintained a comparable level under both control (freshwater) and salt stress (saltwater), whereas SC 176 exhibited a decrease in GR gene expression with a reduction ratio of 46%. The increased GR activity in SC 168 under salt stress suggests a more effective glutathione recycling system to counteract oxidative damage. The higher upregulation of antioxidant genes (catalase, ascorbate peroxidase, and glutathione reductase) in SC 168, compared to the limited or decreased expression in SC 176, indicates that SC 168 is better equipped to neutralize ROS and mitigate oxidative damage, contributing to its greater salinity tolerance (Hu et al., 2022; Ali et al., 2024).

The expression of the ZmNCED9 gene (a key gene in abscisic acid synthesis) and dehydrin genes revealed critical differences between SC 168 and SC 176 under salinity stress, reflecting their varied responses to salinity tolerance mechanisms (Figure 7A). For NCED9 gene expression, long-term salinity stress significantly diminished the mRNA level in both SC 168 and SC 176 (Figure 7A), with the reduction being more severe in SC 176 than in SC 168 compared to control freshwater conditions, at 53% and 97%, respectively. It is worth noting that the level of NCED9 transcripts is still higher in SC 168 than in SC 176 under salinity stress, with a 61-fold increase. Abscisic acid plays an essential role in plant adaptation to challenging environmental conditions, including salt stress. The induction of ABA synthesis is one of the fastest phytohormonal responses of plants to abiotic stress, thereby triggering ABA-inducible gene expression, such as stomatal closure, thus reducing Na^+ influx (Riemann et al., 2015). Nevertheless, the role of ABA in plants under abiotic stress is still far from being fully understood, with many questions remaining unanswered. (Sreenivasulu et al., 2012) stated that elevated ABA in plants under stress could impair growth and development parameters. It is speculated that under prolonged salt stress, plants might reduce ABA synthesis to achieve ABA homeostasis and avoid a severe reduction in yield (Lozano-Juste and Cutler, 2016; Kishor et al., 2022). On the other hand, Dehydrin (DHN) gene expression showed no significant difference between treatments in both SC 168 and SC 176, indicating that dehydrin protein is not a major factor in the response to salinity in these hybrids (Figure 7B).

Nitrogen (N) is a crucial macronutrient for optimal plant growth and development; it is a primary component of amino acids, chlorophyll, adenosine triphosphate, and nucleic acids such as DNA and RNA molecules (Wang et al., 2017). Additionally, maize has a significant demand for nitrogen fertilization for proper growth and development under both standard and stress conditions (Sheoran et al., 2021). The gene expression of the nitrogen assimilation key gene nitrate reductase (ZmNR) was investigated in treated maize genotypes under freshwater and saline water conditions (Figure 7C). The enzyme nitrate reductase (NR) is a substrate-inducible enzyme controlling nitrogen assimilation levels in plants (Harper and Paulsen, 1968). In this study, the nitrate reductase gene was significantly upregulated in SC 168, showing approximately a 3-fold increase compared to freshwater treatment. However, salinity stress significantly reduced the expression of the nitrate reductase gene in SC 176 by 33% compared to the control. The expression level of ZmNR remained significantly higher in SC 168 than in SC 176, with a 4.7-fold increase. Since salt stress dramatically disturbs the nutritional balance in crops and causes

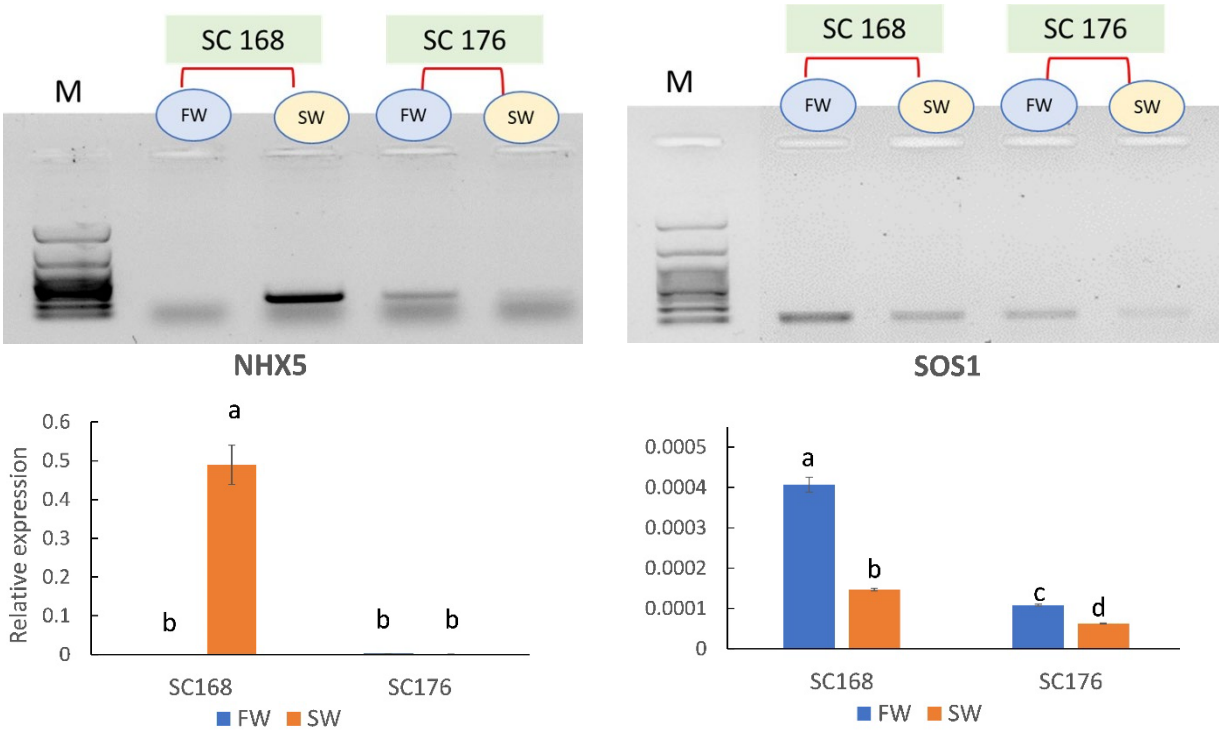

Figure 4. The relative expression profile of selected stress marker genes in shoots of Egyptian maize hybrids SC 168 and SC 176 under freshwater (FW) and saltwater (SW) irrigation regimes. A: NHX5 (encoding tonoplast Na^+/H^+ antiporter), and B: SOS1 (encoding salt overlay sensitive 1 protein). An agarose gel image representing the migration of PCR amplicons for each gene was added. Values represent the means of three replications ± SE. Means with the same letters are not significantly different according to Duncan's multiple range test (DMRT) ($p \leq 0.05$).

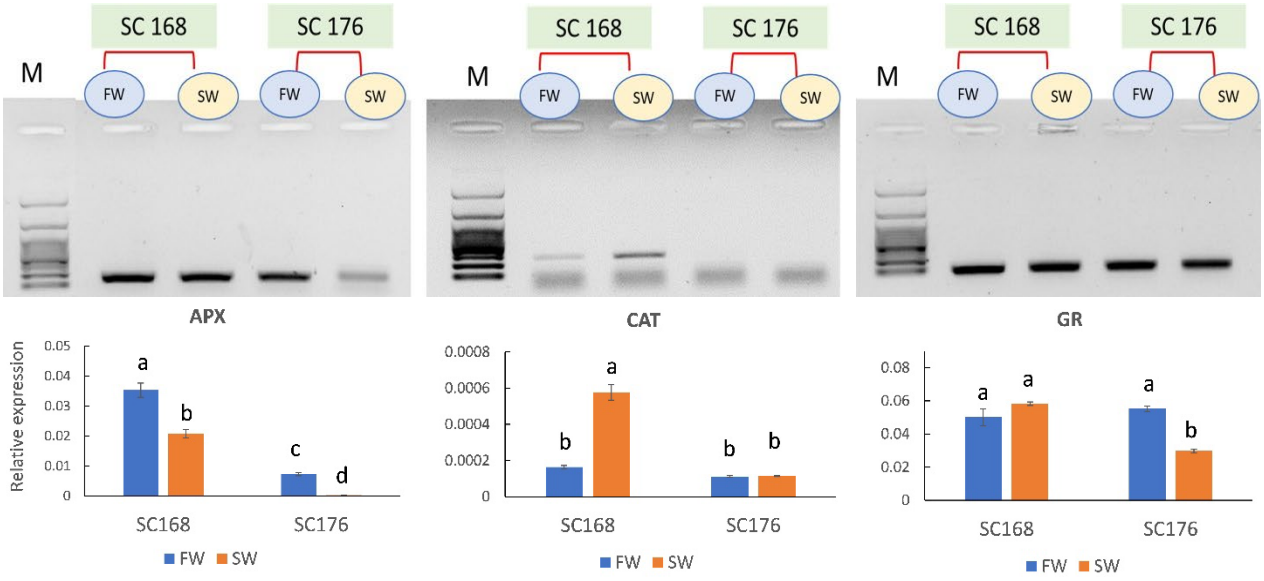

Figure 5. The relative expression profile of selected stress marker genes in shoots of Egyptian maize hybrids SC 168 and SC 176 under freshwater (FW) and saltwater (SW) irrigation regimes. A: APX (encoding ascorbate peroxidase enzyme), B: CAT (encoding catalase enzyme), and C: GR (encoding glutathione reductase). antiporter). An agarose gel image representing the migration of PCR amplicons for each gene was added. Values represent the means of three replications ± SE. Means with the same letters are not significantly different according to Duncan's multiple range test (DMRT) ($p \leq 0.05$).

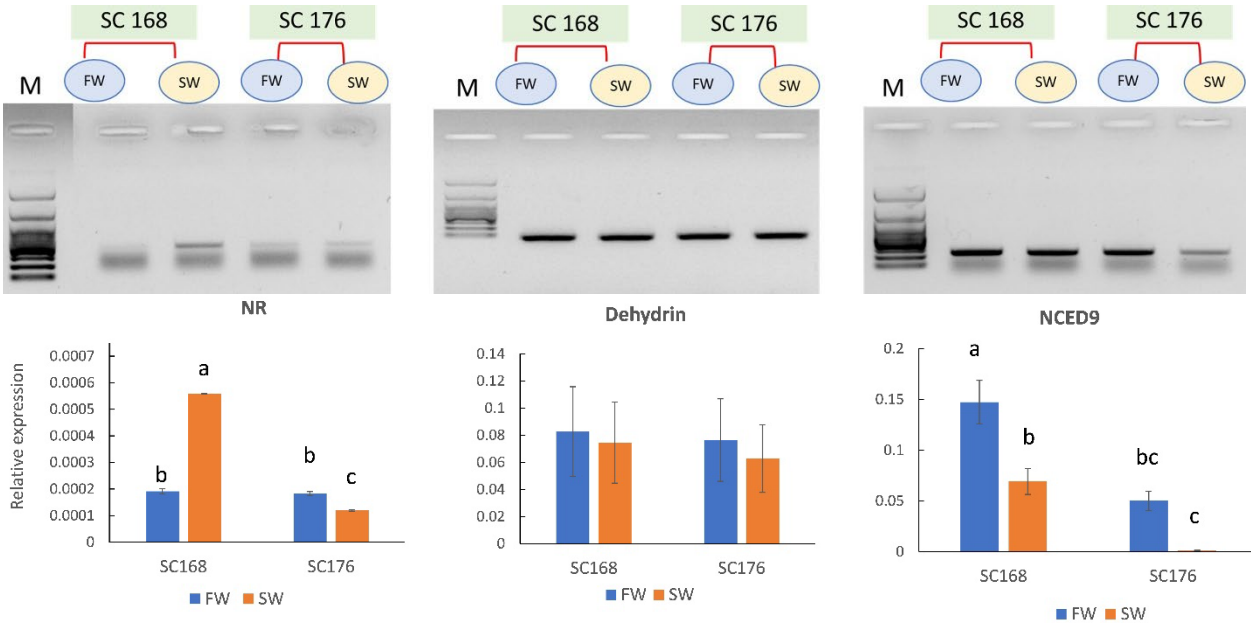

Figure 6. The relative expression profile of selected stress marker genes in shoots of Egyptian maize hybrids SC 168 and SC 176 under freshwater (FW) and saltwater (SW). A: NR (encoding nitrate reductase enzyme), B: dehydrin (encoding dehydrin protein), and **C**: NCED9 (encoding abscisic acid synthesis key enzyme). antiporter) irrigation regimes. An agarose gel image representing the migration of PCR amplicons for each gene was added. Values represent the means of three replications ± SE. Means with the same letters are not significantly different according to Duncan's multiple range test (DMRT) ($p \leq 0.05$).

severe ion toxicity, Na^+ competes with ammonia ($NH4^+$), while Cl- competes with nitrate ions ($NO3^-$). Additionally, better N assimilation could mitigate the effects of salinity stress by encouraging the synthesis of antioxidant enzymes for more efficient ROS scavenging (for review, see Zayed et al., 2023). This assumption seems to be confirmed, as some reports indicate that N application could exacerbate the negative impacts of salinity stress on plant growth due to increased sodium ions uptake, as Na+ may be co-transported with $NO3^-$ (Ye et al., 2022).

CONCLUSION

Maize (*Zea mays* L.) is an essential strategic cereal crop, yet it is very sensitive to saline irrigation. Therefore, developing maize genotypes with better adaptive responses to salt stress would be beneficial for cultivating newly reclaimed sandy soil using saline agriculture. It has been shown that salt-tolerant maize hybrids accumulate fewer sodium ions in leaves and higher amounts of potassium ions, in addition to better nitrogen assimilation compared to salt-sensitive hybrids. Furthermore, the high antioxidative power in salt-tolerant genotypes can scavenge ROS overproduced under salinity, thus lowering lipid peroxidation levels.

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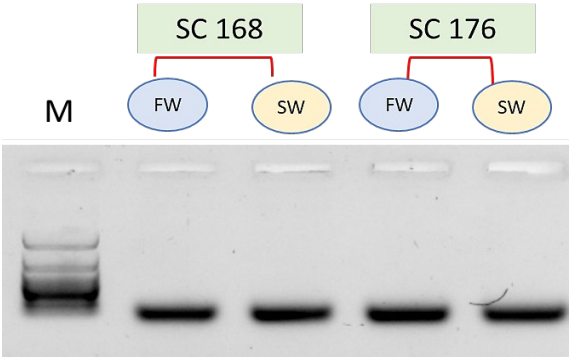

Figure S1. Agarose gel image represents the migration of PCR amplicons for reference gene (Zm18S ribosomal RNA). M: 100 bp DNA ladder

Table S1. The sequences of forward and reverse primers for investigated genes

Gene Name	Forward sequence (5' to 3')	Reverse sequence (5' to 3')	Amplicon size (bp)
18S ribosomal RNA (reference)	CCGGCGACGCATCATT	GGCCCCTATATCCTACCATCGA	58
Abscisic acid synthesis (NCED**9**)	TTCCACGGCACCTTCATC	AGCTGCCACTATCCCAATTT	250
Ascorbate peroxidase (APX)	CCTTCTTCAGCTCCCAAGTG	GGTGGGCCTCTTTGTAGTCA	177
Catalase (CAT)	TCTGGACGTGGTCCTCTAAT	CACCTTCTAATGTTGCTTGTATGC	355
Dehydrin	AGGAAGAAGGGAATCAAGGAGAAGA	CGTGCTGGTCGTCCTTGT	67
Glutathione Reductase (GR)	CACCAAAGCAGACTTCGACA	AAGTTCGTCTTTGGCTTGGA	115
Nitrate reductase (NR)	GTACGTCATCGACCAGGTAAAG	GAACACGACGAAAGAATTGGC	450
Sodium overlay sensitive1 (SOS1)	ACTTGCAGGAGGAATACAAC	CGAGAAGAGAAGACCACATC	156
Sodium/hydrogen antiporter (NHX5)	AGCTTCTGATGGTGCGTTAG	AGAGTGGATGTGCTGCTATTT	298