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INFLUENCE OF SEED PRIMING WITH NaCl ON GROWTH AND BIOCHEMICAL RESPONSES OF WHEAT (*TRITICUM AESTIVUM* L.) UNDER SALINITY STRESS

ABDULBAKI SHEHU ABDULBAKI[1,2*], TAHANI MOHAMMAD ALQHTANI[1], YAHYA ALZAHRANI[1] AND HAMEED ALSAMADANY[1*]

[1]Department of Biological Sciences, Faculty of Science, King Abdulaziz University, Jeddah, Saudi Arabia.
[2]Department of Plant Science, Faculty of Natural and Applied Sciences, Federal University of Agriculture and Development Studies Iragbiji, Osun State, Nigeria.

*Corresponding author's email: abdulbakias@gmail.com; halsamadani@kau.edu.sa

Abstract

Salinity constrains wheat growth through osmotic imbalance, ion toxicity, oxidative damage, and disruption of photosynthetic metabolism. This study evaluated whether 30 mM NaCl seed priming could improve the response of a wheat genotype (*Triticum aestivum* L., var. Halba) to 0, 100, 150, and 200 mM NaCl. A completely randomized 2 × 4 factorial experiment was conducted with three replications. Relative to their respective controls, saline treatments reduced plant height, leaf area, shoot fresh and dry mass, and total chlorophyll, although some traits showed non-monotonic differences between intermediate and severe salinity levels. Priming was associated with higher SOD activity and generally higher APX activity, although APX declined from 8.0 to 7.1 U mg^{-1} protein between 150 and 200 mM NaCl in primed plants. CAT activity peaked at 100 mM NaCl after priming and declined at higher concentrations. Primed plants, under salinity, accumulated more proline and had lower MDA concentrations than corresponding unprimed plants, consistent with stronger osmotic adjustment and reduced lipid peroxidation. The most distinctive response was total soluble protein (TSP): primed plants maintained high TSP under moderate salinity (100–150 mM) but showed a marked decline at 200 mM, whereas unprimed plants showed the opposite trend. This pattern suggests a shift in protein allocation or turnover under severe stress, although protein identity and turnover were not measured. Overall, the data support NaCl priming as a physiological preconditioning treatment, while molecular validation and field experiments are required to establish the underlying mechanism and agronomic value.

Key words: NaCl seed priming; Halo-priming; Salinity stress; Antioxidant enzymes; Proline; Total soluble protein; Lipid peroxidation

Introduction

Wheat (*Triticum aestivum* L.) is a major cereal crop and an important source of dietary calories and protein. Its productivity is increasingly constrained by abiotic stresses, particularly salinity in irrigated and arid production systems. Salinity imposes both an early osmotic constraint on water uptake and a later ionic/oxidative burden that can disrupt photosynthesis, membrane stability, nutrient relations, and growth (Gupta & Huang, 2014; Zia *et al.,* 2021).

Plants respond to salinity through coordinated osmotic adjustment, ion regulation, antioxidant protection, and metabolic reprogramming. Compatible solutes such as proline can contribute to cellular osmotic balance and protection of macromolecules, while antioxidant enzymes including SOD, CAT, and APX limit the accumulation of reactive oxygen species (ROS). However, the magnitude and coordination of these responses depend on genotype, stress intensity, developmental stage, and the type and timing of the priming treatment.

Seed priming is a pre-sowing treatment that initiates metabolic processes before radicle emergence and can improve subsequent establishment under adverse conditions. Halo-priming with inorganic salts such as NaCl is of particular interest because a controlled, sub-inhibitory salt exposure can act as a form of physiological preconditioning. Evidence from cereals indicates that priming can enhance antioxidant capacity, osmolyte accumulation, and growth under subsequent salinity; for example, NaCl priming improved antioxidant activity, proline accumulation, and biomass in salt-stressed sorghum (Guo *et al.,* 2022). Recent wheat studies have increasingly examined alternative priming agents, including salicylic acid, calcium/nitric-oxide combinations, and biological extracts, demonstrating that priming-induced salt tolerance can involve coordinated changes in antioxidant defense, osmoprotection, water relations, and stress-responsive pathways (Maqsood *et al.,* 2023; El-Shazoly *et al.,* 2024; Al-Huqail *et al.,* 2024).

NaCl seed priming in wheat is not a new concept: earlier studies have examined seed-vigour enhancement and halo-priming under saline conditions (Jamal *et al.,* 2011), while Islam *et al*., (2015) reported cultivar-dependent antioxidative responses in two wheat cultivars. The present experiment therefore evaluates the response of the Halba genotype to a 30 mM NaCl pre-sowing treatment followed by a graded salinity series (0–200 mM), using growth traits and selected biochemical indicators. Particular attention is given to whether the priming-associated response is consistent across traits and salinity levels. The study is thus framed as a physiological assessment.

Materials and Methods

Plant material: The wheat genotype (*Triticum aestivum* L., var. Halba) used in this study was obtained from the Department of Biological Sciences, Faculty of Science, King Abdulaziz University, Jeddah, Saudi Arabia.

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Seed priming treatment: Surface sterilization of the grains was performed using 0.01% mercuric chloride followed by thorough washing with distilled water. For halo-priming, grains were immersed in 30 mM NaCl for 12 h at room temperature (Jamal *et al.,* 2011). Control grains were immersed in distilled water for the same duration. Seeds were subsequently allowed to dry before sowing.

Experimental design and growth conditions: The study adopted a completely randomized design with three biological replications. A 2 × 4 factorial arrangement was used, with seed priming (primed and unprimed) and salinity (0, 100, 150, and 200 mM NaCl) as the experimental factors; replication was not treated as a third factor. A total of 24 pots (3 kg each) were used, with sand and peat moss mixed at 1:1 (v/v).

Three to five primed or non-primed grains were planted per pot. Salinity treatments were initiated after the three-leaf stage. Temperature and humidity were maintained at 20 ± 2°C and 60% ± 5% relative humidity, respectively, with a 14 h photoperiod and light intensity of 600 µmol m^{-2} s^{-1} (photosynthetically active radiation). The treatment combinations were:

T1: 0 mM NaCl + unprimed grains (Control)
T2: 100 mM NaCl + unprimed grains
T3: 150 mM NaCl + unprimed grains
T4: 200 mM NaCl + unprimed grains
T5: 0 mM NaCl + primed grains (Control)
T6: 100 mM NaCl + primed grains
T7: 150 mM NaCl + primed grains
T8: 200 mM NaCl + primed grains

Morphological measurements: Plant height: Measured from the base of soil to the tip of the highest shoot using a calibrated ruler.

Number of leaves: Number of leaves was counted manually for each plant.

Leaf area: Using the formula LA (cm^2) = 0.75 × L × W, where L is leaf length, and W is leaf width (Chanda & Singh, 2002).

Fresh and dry weight of shoots: Fresh weight was immediately determined after harvest using highly sensitive scales. Dry shoot weight was measured after 72-hour incubation at 80°C in an oven.

Biochemical analyses: Fresh leaf samples of all replicates were instantly frozen in liquid nitrogen and kept in –80°C for biochemical analyses.

Enzymatic antioxidants: Superoxide dismutase (SOD): SOD activity was assayed by monitoring epinephrine autoxidation to adrenochrome in alkaline carbonate buffer, following the principle of Misra & Fridovich (1972). Leaf tissue (2 g) was homogenized in 50 mM phosphate buffer (pH 7.0) and centrifuged at 5000 ×g for 10 min at 4°C. The reaction mixture contained 100 µL extract, 125 µL carbonate buffer (0.05 M, pH 10.2) and 15 µL epinephrine (0.30 mM). Absorbance was recorded at 480 nm at 30 s intervals over 150s. One unit was defined as the amount of extract causing 50% inhibition of the epinephrine-autoxidation rate relative to the no-extract control; specific activity was expressed as U mg^{-1} protein.

CAT: CAT activity was assayed using the ammonium-molybdate method of Hadwan & Abed (2016). The reaction contained 5 µL leaf extract and 50 µL H_2GO_2 (20 mM), was incubated for 3 min at 37°C, and was then treated with 200 µL ammonium molybdate (32.40 mmol L^{-1}); absorbance was read at 374 nm. CAT activity should be expressed as µmol H_2O_2 decomposed min^{-1}, with specific activity reported as U mg^{-1} protein (1 U = 1 µmol H_2O_2 decomposed min^{-1}).

APX: APX activity was assayed following Nakano & Asada (1981) by monitoring the decrease in absorbance at 290 nm. The reported reaction contained sodium phosphate buffer (pH 7.0), 0.25 mM ascorbate, 0.10 mM EDTA, 1.00 mM H_2O_2 and 0.20 mL leaf homogenate. The ascorbate extinction coefficient (ε_{290} = 2.8 mM^{-1} cm^{-1}) should be used to calculate the rate of ascorbate oxidation. Specific activity is expressed as U mg^{-1} protein, where 1 U corresponds to 1 µmol ascorbate oxidized min^{-1}.

Non-enzymatic antioxidants: Free proline was determined using the acid-ninhydrin method of Bates *et al*., (1973). Leaf tissue (0.50 g) was homogenized in 5 mL of 3% sulfosalicylic acid and centrifuged at 4000 ×g for 10 min. The supernatant (2 mL) was mixed with 2 mL glacial acetic acid and 2 mL acid ninhydrin, incubated at 98°C for 30 min, and extracted with 5 mL toluene; absorbance was read at 520 nm.

Oxidative stress parameters: MDA concentration: The level of MDA was determined according to the protocol suggested by Reilly & Aust (2001). In the course of the experiment, 50µL of leaf homogenate was mixed with 100µL of the TBA/TCA/HCl solution (0.37%/15%/0.2 N). After 10 minutes of centrifugation at 1000 g, absorbance was measured at 535 nm.

Total chlorophyll: Fresh leaf tissue (0.50 g) was extracted in 10 mL of 80% (v/v) acetone and centrifuged at 10,000 ×g for 15 min at 4°C. Absorbance was measured at 646 and 663 nm. For 80% acetone, chlorophyll a and b concentrations in the extract (mg L^{-1}) are calculated as Chl a = $12.21A_{663} - 2.81A_{646}$ and Chl b = $20.13A_{646} - 5.03A_{663}$; total chlorophyll is therefore Chl(a+b) = $7.18A_{663} + 17.32A_{646}$ (Lichtenthaler and Wellburn, 1983). Tissue content is then calculated as [Cextract (mg L^{-1}) × extraction volume (L) × dilution factor] / fresh mass (g), and reported as mg g^{-1} FW.

Total soluble protein (TSP): Protein content was measured following Bradford (1976).

Fresh leaf samples (0.50 g) were groundin liquid nitrogen and homogenized in 10 mL extraction buffer (50 mM sodium phosphate buffer pH 7, 1 mM EDTA-Na_2, 2% PVP-40). After centrifugation at 11,000 g for 15 minutes at 4°C, the crude extract was mixed with Bradford solution, and absorbance was measured at 595 nm.

Statistical analysis

The experiment was a 2 × 4 factorial design, with seed priming and salinity as fixed factors and three biological replicates per treatment combination. Data were analysed using two-way ANOVA (factors: priming treatment and salinity level) using SPSS 25.0 software to test both main effects and the priming × salinity interaction. Means were compared using Tukey's HSD (Honestly Significant Difference) post-hoc test at $p \leq 0.05$ level of significance. Data are presented as means ± standard error (SE) of three replicates.

Results

Morphological parameters

Plant height: Salinity progressively reduced plant height in both primed and unprimed plants (Fig. 1). Under control conditions, plant height was 34.6 cm in unprimed plants (T1) and 37.0 cm in primed plants (T5). At 200 mM NaCl, height was decreased declined to 26 cm in unprimed plants (T4) but was maintained at 30 cm in primed plants (T8), a 4 cm difference. Thus, priming attenuated the salinity-associated decrease in height, although the magnitude of protection varied with salinity.

Number of leaves: The number of leaves was less sensitive to salinity stress compared to other morphological parameters (Fig. 2). Both control treatments (T1 and T5) produced 5 leaves per plant. Under salinity stress, leaf number was decreased slightly to 4 leaves per plant across all salinity treatments (100-200 mM NaCl) for both primed and unprimed plants. No significant differences were observed between primed and unprimed treatments at any salinity level.

Leaf area: Salinity significantly reduced leaf area (Fig. 3). Leaf area was 10.3 cm^2 in unprimed controls (T1) and 14.0 cm^2 in primed controls (T5), declining to 4.3 and 5.0 cm^2, respectively, at 200 mM NaCl. Primed plants therefore retained a larger photosynthetic surface than corresponding unprimed plants across the salinity gradient.

Fresh shoot weight: Salinity stress caused a marked reduction in fresh shoot weight (Fig. 4). Control plants had 0.96 g fresh weight in both primed and unprimed treatments. At 150 mM NaCl, fresh weight was 0.29 g in unprimed plants but 0.46 g in primed plants; at 200 mM NaCl, the corresponding values were 0.29 and 0.40 g. These data indicate a substantial preservation of shoot hydration/ biomass after priming under moderate-to-severe salinity.

Dry shoot weight: Dry shoot weight followed the same general pattern as fresh weight (Fig. 5). At 150 mM NaCl, dry shoot weight was 0.02 g in unprimed plants and 0.04 g in primed plants, while at 200 mM NaCl it was 0.03 and 0.04 g, respectively. The priming-associated advantage was therefore retained after drying and was not restricted to tissue water content.

Biochemical parameters

Enzymatic antioxidants: SOD activity was increased with increasing salinity in both primed and unprimed plants (Fig. 6). In unprimed plants, activity was increased from 113 to 135 U mg^{-1} protein between 0 and 200 mM NaCl. Primed plants showed higher activity throughout, increasing from 130 to 160 U mg^{-1} protein. The consistently elevated SOD response indicates that NaCl priming enhances the capacity for superoxide dismutation under subsequent salinity.

CAT activity showed a priming-specific, non-monotonic response (Fig. 7). In primed plants, CAT activity peaked at 77.12 U mg^{-1} protein at 100 mM NaCl and declined to 66.90 and 65.70 U mg^{-1} protein at 150 and 200 mM NaCl, respectively. Nevertheless, CAT remained higher at 200 mM NaCl in primed plants than the maximum value recorded in unprimed plants (58.73 U mg^{-1} protein at 150 mM). This pattern differs from the monotonic increase observed for SOD and is therefore an important feature of the priming response.

APX activity increased with salinity in both groups, and primed plants consistently showed higher activity than corresponding unprimed plants (Fig. 8). Activity reached 8.00 U mg^{-1} protein at 100 and 150 mM NaCl in primed plants compared with 6.00 and 6.50 U mg^{-1} protein, respectively, in unprimed plants. At 200 mM NaCl, APX activity remained elevated in primed plants (7.10 U mg^{-1} protein) relative to the unprimed treatment (6.44 U mg^{-1} protein).

Non-enzymatic antioxidants: Proline accumulation increased under salinity and was consistently higher in primed plants than in corresponding unprimed plants (Fig. 9). Primed plants reached 23.73 μg g^{-1} FW at 100 mM NaCl, 21.69 μg g^{-1} FW at 150 mM, and 22.81 μg g^{-1} FW at 200 mM. In unprimed plants, proline peaked at 18.65 μg g^{-1} FW at 150 mM and declined at 200 mM. The sustained proline accumulation after priming, particularly at the highest salinity, contrasts with the decline observed in unprimed plants.

Oxidative stress parameters: MDA content was increased with increasing salinity in both groups, but remained lower in primed than in corresponding unprimed plants (Fig. 10). In unprimed plants MDA was increased from 12.11 to 20.00 nmol g^{-1} FW, whereas in primed plants was increased from 11.80 to 17.00 nmol g^{-1} FW. The lower MDA accumulation after priming was consistent with reduced lipid peroxidation under salinity.

Total chlorophyll was decreased with increasing salinity in both groups (Fig. 11). Primed plants retained 2.11 mg g^{-1} FW at 200 mM NaCl, compared with 0.84 mg g^{-1} FW in unprimed plants. The greater pigment retention after priming paralleled the stronger antioxidant and osmolyte responses.

TSP showed the most distinctive divergence between primed and unprimed plants (Fig. 12). In unprimed plants, TSP increased progressively from 0.37 mg g^{-1} FW in the control to 1.53 mg g^{-1} FW at 200 mM NaCl. In contrast, primed plants started at a substantially higher control value (1.88 mg g^{-1} FW) and declined from 1.75 mg g^{-1} FW at 100 mM to 1.73 mg g^{-1} FW at 150 mM and 0.64 mg g^{-1} FW at 200 mM. Thus, priming maintained higher TSP than unprimed plants under moderate salinity but reversed to a lower TSP value under severe salinity. This crossover response is a potentially informative physiological signature of priming, although the present assay cannot determine whether it reflects altered protein synthesis, degradation, or changes in the composition of the soluble protein pool.

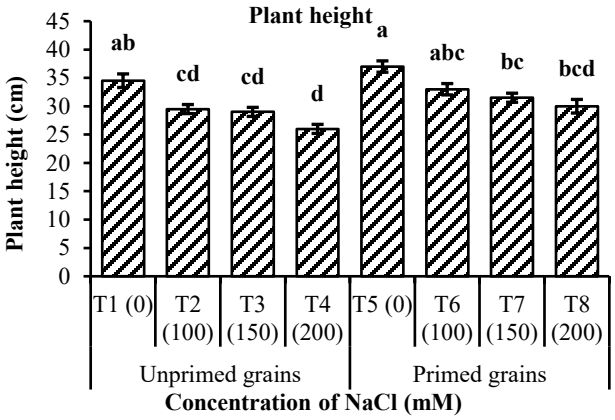


Fig. 1. Plant height (cm) at 0, 100, 150 and 200 mM NaCl in plants from unprimed (T1–T4) and primed (T5–T8) grains. Bars are mean ± SE (n = 3). Different letters indicate significant differences among all eight treatment combinations (T1–T8).
Impact of salinity stress and NaCl seed priming on plant height of wheat. Each bar represents the mean value and mean with different letters among the salinity treatments indicate significant difference at p≤0.05.

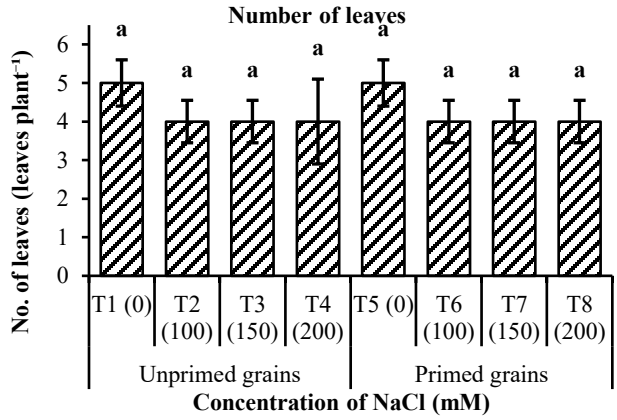


Fig. 2. Number of leaves (leaves plant^{-1}) at 0, 100, 150 and 200 mM NaCl in plants from unprimed (T1–T4) and primed (T5–T8) grains. Bars are mean ± SE (n = 3). Different letters indicate significant differences among all eight treatment combinations (T1–T8).
Impact of salinity stress and NaCl seed priming on leaves number of wheat. Each bar represents the mean value and mean with different letters among the salinity treatments indicate significant difference at p≤0.05.

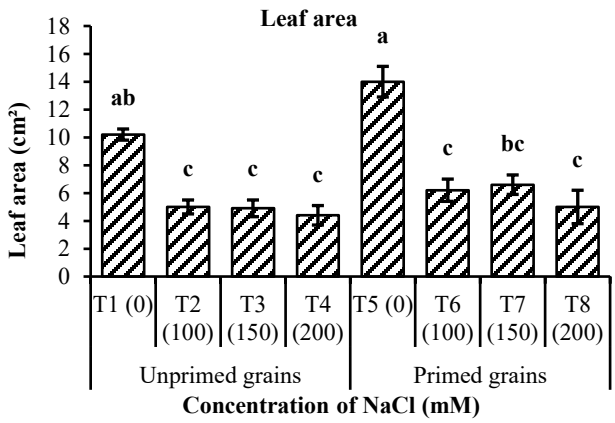


Fig. 3. Leaf area (cm²) at 0, 100, 150 and 200 mM NaCl in plants from unprimed (T1–T4) and primed (T5–T8) grains. Bars are mean ± SE (n = 3). Different letters indicate significant differences among all eight treatment combinations (T1–T8).
Impact of salinity stress and NaCl seed priming on leaves area of wheat. Each bar represents the mean value and mean with different letters among the salinity treatments indicate significant difference at p≤0.05.

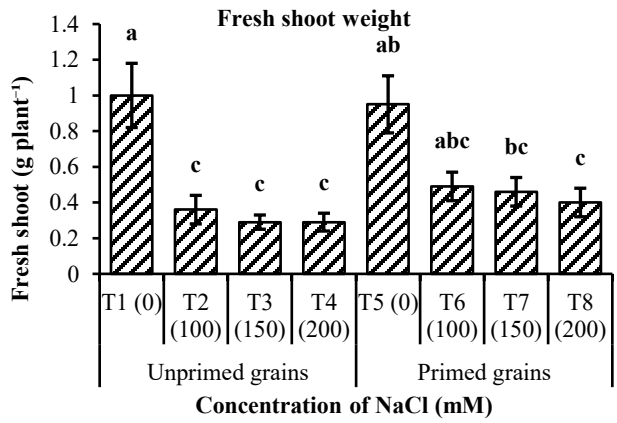


Fig. 4. Fresh shoot weight (g plant^{-1}) at 0, 100, 150 and 200 mM NaCl in plants from unprimed (T1–T4) and primed (T5–T8) grains. Bars are mean ± SE (n = 3). Different letters indicate significant differences among all eight treatment combinations (T1–T8).
Impact of salinity stress and NaCl seed priming on Fresh Shoot weight of wheat. Each bar represents the mean value and mean with different letters among the salinity treatments indicate significant difference at p≤0.05.

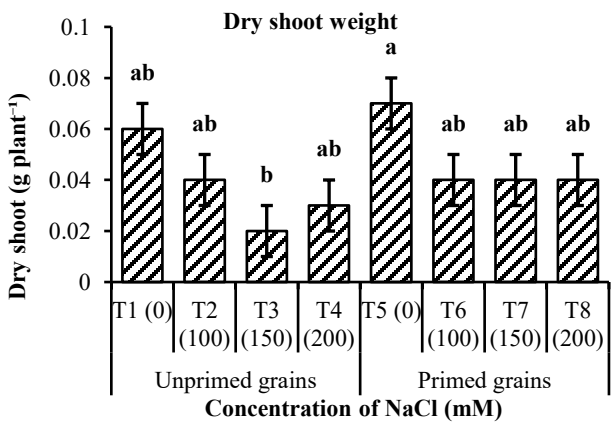


Fig. 5. Dry shoot weight (g plant^{-1}) at 0, 100, 150 and 200 mM NaCl in plants from unprimed (T1–T4) and primed (T5–T8) grains. Bars are mean ± SE (n = 3). Different letters indicate significant differences among all eight treatment combinations (T1–T8).
Impact of salinity stress and NaCl seed priming on Dry Shoot weight of wheat. Each bar represents the mean value and mean with different letters among the salinity treatments indicate significant difference at p≤0.05.

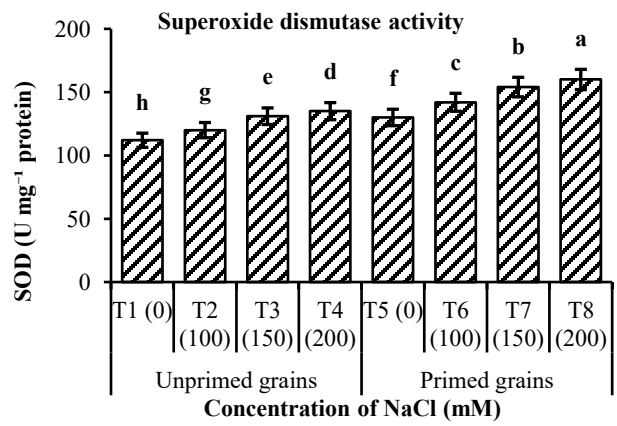


Fig. 6. Superoxide dismutase activity (U mg^{-1} protein) at 0, 100, 150 and 200 mM NaCl in plants from unprimed (T1–T4) and primed (T5–T8) grains. Bars are mean ± SE (n = 3). Different letters indicate significant differences among all eight treatment combinations (T1–T8).
Impact of salinity stress and NaCl seed priming on Superoxide dismutase (SOD). Each bar represents the mean value and mean with different letters among the salinity treatments indicate significant difference at p≤0.05.

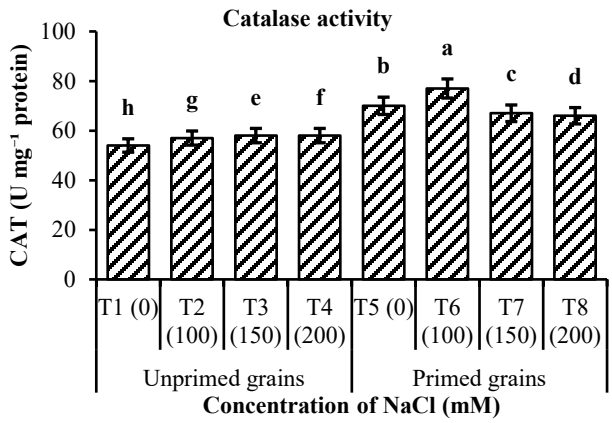


Fig. 7. Catalase activity (U mg⁻¹ protein) at 0, 100, 150 and 200 mM NaCl in plants from unprimed (T1–T4) and primed (T5–T8) grains. Bars are mean ± SE (n = 3). Different letters indicate significant differences among all eight treatment combinations (T1–T8).
Impact of salinity stress and NaCl seed priming on Catalase (CAT). Each bar represents the mean value and mean with different letters among the salinity treatments indicate significant difference at p≤0.05.

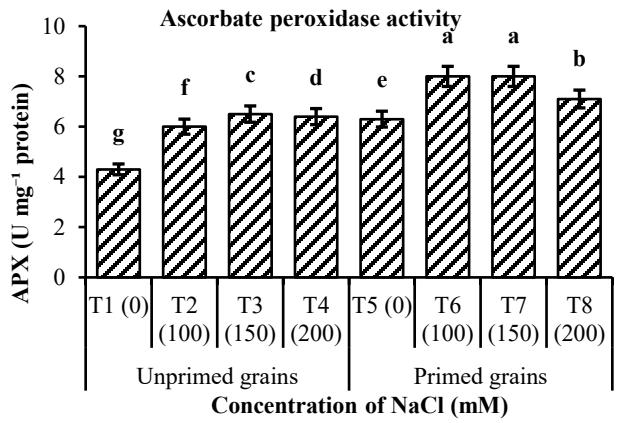


Fig. 8. Ascorbate peroxidase activity (U mg⁻¹ protein) at 0, 100, 150 and 200 mM NaCl in plants from unprimed (T1–T4) and primed (T5–T8) grains. Bars are mean ± SE (n = 3). Different letters indicate significant differences among all eight treatment combinations (T1–T8).
Impact of salinity stress and NaCl seed priming on Ascorbate Peroxidase (APX). Each bar represents the mean value and mean with different letters among the salinity treatments indicate significant difference at p≤0.05.

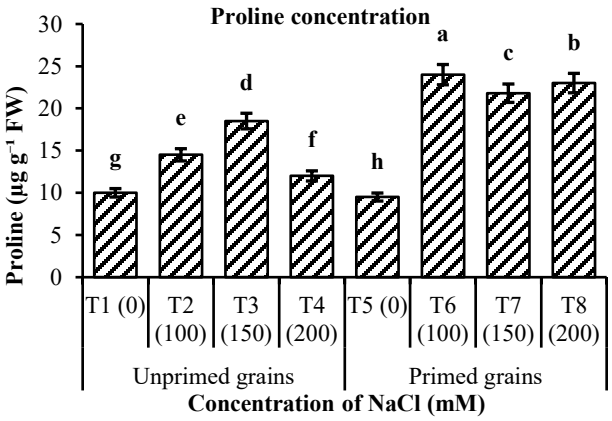


Fig. 9. Proline concentration (µg g⁻¹ FW) at 0, 100, 150 and 200 mM NaCl in plants from unprimed (T1–T4) and primed (T5–T8) grains. Bars are mean ± SE (n = 3). Different letters indicate significant differences among all eight treatment combinations (T1–T8).
Impact of salinity stress and NaCl seed priming on Proline concentration. Each bar represents the mean value and mean with different letters among the salinity treatments indicate significant difference at p≤0.05.

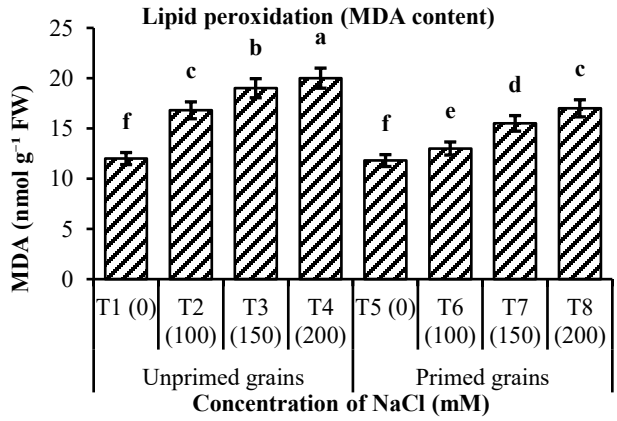


Fig. 10. Lipid peroxidation (MDA content) (nmol g⁻¹ FW) at 0, 100, 150 and 200 mM NaCl in plants from unprimed (T1–T4) and primed (T5–T8) grains. Bars are mean ± SE (n = 3). Different letters indicate significant differences among all eight treatment combinations (T1–T8).
Impact of salinity stress and NaCl seed priming on Lipid Peroxidation (MDA content). Each bar represents the mean value and mean with different letters among the salinity treatments indicate significant difference at p≤0.05.

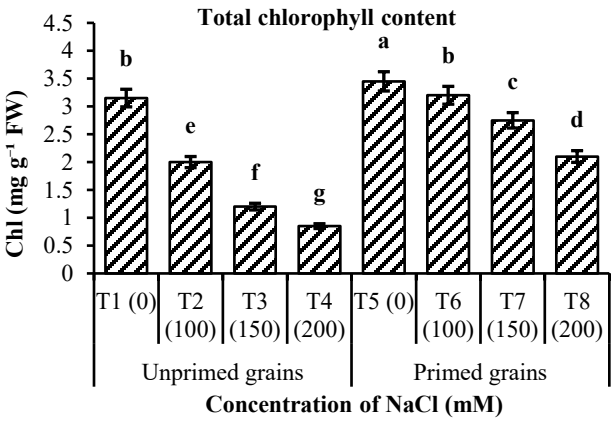


Fig. 11. Total chlorophyll content (mg g⁻¹ FW) at 0, 100, 150 and 200 mM NaCl in plants from unprimed (T1–T4) and primed (T5–T8) grains. Bars are mean ± SE (n = 3). Different letters indicate significant differences among all eight treatment combinations (T1–T8).
Impact of salinity stress and NaCl seed priming on Total Chlorophyll. Each bar represents the mean value and mean with different letters among the salinity treatments indicate significant difference at p≤0.05.

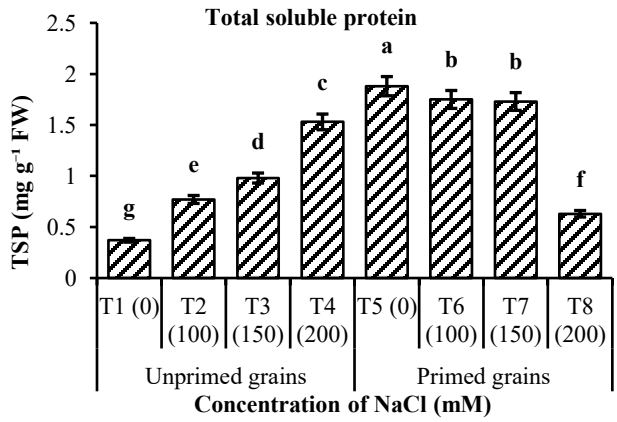


Fig. 12. Total soluble protein (mg g⁻¹ FW) at 0, 100, 150 and 200 mM NaCl in plants from unprimed (T1–T4) and primed (T5–T8) grains. Bars are mean ± SE (n = 3). Different letters indicate significant differences among all eight treatment combinations (T1–T8).
Impact of salinity stress and NaCl seed priming on Total Soluble Protein (TSP). Each bar represents the mean value and mean with different letters among the salinity treatments indicate significant difference at p≤0.05.

Discussion

Morphological responses to salinity and priming: Salinity reduced plant height, leaf area, fresh shoot mass, and dry shoot mass, confirming the strong growth penalty imposed by increasing NaCl concentration. The protective effect of priming was most apparent in biomass-related traits: at 150 mM NaCl, priming increased fresh shoot mass from 0.29 to 0.46 g and dry shoot mass from 0.02 to 0.04 g. Such responses are consistent with the broader cereal literature showing that salt priming can preserve growth by improving early metabolic readiness and subsequent stress responses. NaCl priming has similarly reduced oxidative injury and increased antioxidant activity and proline in salt-stressed sorghum (Guo *et al.,* 2022), while recent wheat studies using other priming agents have reported improved growth, chlorophyll, antioxidant capacity, and water relations under salinity (Maqsood *et al.,* 2023; El-Shazoly *et al.,* 2024).

The present results extend those observations in two ways. First, the priming treatment was applied before sowing at a relatively low NaCl concentration (30 mM), whereas the stress treatments reached 200 mM, creating a clear preconditioning-to-stress contrast. Second, the protection was not uniform across all physiological variables: CAT and TSP displayed priming-specific response trajectories rather than simply increasing with stress severity. Earlier wheat work also showed that the response to halopriming depends on genotype: Islam *et al*., (2015) compared the salt-tolerant Lu26s and salt-sensitive Lasani-06 cultivars and found cultivar-dependent antioxidant responses under 100 mM NaCl. A plausible interpretation is that NaCl priming establishes a biochemical "preparedness" state before exposure to severe salinity. Priming may accelerate the activation of antioxidant and osmotic-adjustment systems when stress begins, thereby reducing the metabolic cost of subsequent injury. However, the present experiment did not measure seed metabolic markers, ROS directly, ion concentrations, or gene expression; therefore, these processes should be regarded as mechanistic hypotheses rather than demonstrated molecular events. Recent wheat work has shown that priming-associated changes can extend to stress-responsive genes and osmolyte metabolism, illustrating the value of combining physiological assays with molecular measurements in future experiments (Al-Huqail *et al.,* 2024).

Leaf number was comparatively stable, declining only from five 5 leaves in the controls to approximately 4 leaves under salinity, with no reported significant difference between primed and unprimed plants. This contrasts with the stronger responses of leaf area and biomass and suggested that, over the experimental period, salinity affected leaf expansion and tissue accumulation more strongly than leaf initiation.

Antioxidant defense mechanisms: The antioxidant data provide a coherent but not completely uniform picture. Priming increased SOD and APX activities across the salinity gradient, while CAT showed a distinct peak at 100 mM NaCl followed by a decline at higher salinity. SOD converts superoxide radicals to H_2O_2, whereas CAT and APX contribute to subsequent H_2O_2 removal. Thus, the higher SOD and APX activities are consistent with greater antioxidant capacity after priming. The CAT pattern is particularly informative: its early maximum may indicate that CAT is was strongly recruited under moderate stress, whereas the decline at higher salinity may reflect changing substrate availability, enzyme regulation, or stress intensity.

The overall antioxidant pattern is consistent with recent cereal studies but also illustrates the importance of comparing response trajectories rather than individual enzyme values. Guo *et al*., (2022) reported that NaCl and other priming treatments improved SOD, CAT, POD, proline, and biomass in salt-stressed sorghum. In barley, likewise seed priming has been associated with altered antioxidant defense and reduced oxidative injury, although the magnitude of benefit depends on the priming compound (Ellouzi *et al.,* 2023). Recent wheat studies using biological or chemical priming similarly reported coordinated changes in CAT/APX, oxidative markers, and growth (Al-Huqail *et al.,* 2024; El-Shazoly *et al.,* 2024). The present study therefore supports the general priming paradigm but adds a useful CAT response pattern within a single wheat genotype exposed to a graded salinity series.

Proline accumulation: Proline accumulation was consistently greater in primed plants under salinity, and unlike unprimed plants, primed plants maintained high proline at 200 mM NaCl. This pattern is compatible with a role for proline in osmotic adjustment and cellular protection. Its accumulation can reflect both enhanced synthesis and altered utilization or degradation. The sustained proline response after priming is therefore best interpreted as evidence of altered osmotic/metabolic regulation, not proof of a single biochemical mechanism.

Lower MDA concentrations in primed plants indicate less accumulation of a lipid-peroxidation product under equivalent salinity. At 200 mM NaCl, MDA was 17.00 nmol g^{-1} FW after priming compared with 20.00 nmol g^{-1} FW without priming. This pattern was compatible with better control of oxidative damage and agreed with reports in other cereals in which priming lowered MDA while increasing antioxidant defenses (Guo *et al.,* 2022; Ellouzi *et al.,* 2023). Nevertheless, MDA is an indicator rather than a direct measurement of membrane integrity.

The most parsimonious explanation is that the stronger SOD/CAT/APX system limited the propagation of ROS-mediated lipid oxidation. The mechanism through which NaCl priming provides protection against lipid peroxidation is associated with the increased ability of the antioxidant defense system in scavenging ROS, thus preventing oxidation of lipids in the cell membrane (Yan *et al.,* 2015). Similar findings were reported by Guo *et al*., (2022) where it was shown that NaCl priming resulted in lower levels of MDA in stressed sorghum.

Total chlorophyll was declined with increase of salinity, but primed plants retained substantially more pigment at 200 mM NaCl than unprimed plants. Chlorophyll loss under salinity can arise from impaired biosynthesis, pigment degradation, ionic imbalance, or oxidative damage. In this study, the improved pigment retention occurred alongside stronger antioxidant and

proline responses, supporting a coordinated physiological protection model. Recent wheat studies also reported preservation of chlorophyll and improved antioxidant status after priming under salt stress (Maqsood *et al.,* 2023; Al-Huqail *et al.,* 2024). Gas exchange, fluorescence, and photosynthetic rate measurements would be valuable in future experiments to test whether the biochemical protection observed here translates into sustained photosynthetic function and ultimately yield.

Such results were justified in the work of Sheikh Abol-Hasani & Roshandel (2020) since these authors found an increase in chlorophyll content in NaCl-primed *Dracocephalum moldavica* under salinity stress. In addition, Aymen *et al*., (2014) recorded a 15-30% increase in chlorophyll content in NaCl-pretreated safflower under salt stress conditions. Such protection allows for ongoing photosynthetic activity, hence improves growth performance.

Total soluble protein: The TSP response is the most distinctive feature of the dataset. Unprimed plants showed a progressive increase in TSP from 0.37 mg g^{-1} FW in the control to 1.53 mg g^{-1} FW at 200 mM NaCl, whereas primed plants began with a much higher control TSP (1.88 mg g^{-1} FW), remained high at 100–150 mM NaCl, and then fell sharply to 0.64 mg g^{-1} FW at 200 mM. This crossover cannot be adequately described simply as "priming increases protein content." Instead, it suggests that priming altered the relationship between stress severity and the soluble protein pool.

One hypothesis is that unprimed plants accumulated soluble proteins progressively as part of a stress-associated response, whereas primed plants entered salinity with a metabolically prepared protein pool and maintained it during moderate stress. At severe salinity, the sharp fall in primed TSP could indicate accelerated protein turnover, reduced synthesis, or redistribution of nitrogen and carbon toward other stress-protective compounds. This interpretation is consistent with the concept of metabolic reprogramming during salinity.

Conclusion

NaCl seed priming at 30 mM improved the performance of the tested wheat material under 100–200 mM NaCl, as shown by greater plant height, leaf area, shoot biomass, chlorophyll retention, antioxidant enzyme activity, and proline accumulation, together with lower MDA accumulation. The response was not uniform across biochemical traits: CAT exhibited a priming-specific peak at 100 mM NaCl, whereas TSP displayed a marked crossover between primed and unprimed plants, with higher TSP after priming under moderate salinity but lower TSP at 200 mM NaCl. This TSP pattern should be regarded as a stage-specific, hypothesis-generating observation rather than evidence of a confirmed protein-turnover mechanism. Accordingly, the present study supports NaCl halo-priming as a physiological preconditioning approach for the tested wheat material, while its genotype specificity, molecular basis, response beyond the three-week vegetative stage, and field-level agronomic value require further validation.

Study limitations and implications: The experiment was conducted under controlled pot/glasshouse conditions using one wheat genotype. These limitations do not negate the physiological signal in the present dataset, but they constrain mechanistic and agronomic claims. Future work should test multiple genotypes, include molecular markers of antioxidant and osmolyte pathways, quantify Na^+/K^+ homeostasis and ROS, and validate the 30 mM priming treatment under field or semi-field salinity conditions.

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