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The Efficiency of Seed Priming with Dead Sea Water for Improving Germination and Early Seedling Growth of Wheat (*Triticum aestivum* L.) under Salinity

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Abstract

Salinity is considered the most critical environmental factor which negatively affects the germination and growth of plants. In this study, the potential of using Dead Sea water (DS) as a seed priming agent for the mitigation of the adverse effects of salinity on seed germination and growth performance of wheat (*Triticum aestivum* L.) was investigated. Germination of wheat seeds primed with different doses of DS; 0%, 5%, 10%, 15%, and 20% were evaluated under different saline conditions (0, 100, 200, and 300 mM NaCl). High salinity (300 mM NaCl) remarkably inhibited germination attributes and reduced seedling length. However, seeds primed with DS exhibited improved germination parameters and seedling growth. Among the different DS concentrations used, the 10% DS priming achieved the highest increase in final germination percentage tolerance, germination index, relative germination salt tolerance, and seedling length. The increased tolerance to salinity was associated with improved water imbibition, α-amylase activity, antioxidant capacity and osmotic homeostasis correlated with high proline and soluble sugar levels. In addition, DS priming increased the membrane stability index, and reduced malondialdehyde content and K^+ leakage besides lowering Na^+/K^+ ratio. Overall, priming with DS could be a promising strategy for minimizing the damaging effects of salinity in wheat.

Keywords: abiotic stress; halopriming; plant physiology; seed germination; seedling growth

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INTRODUCTION

Salinity poses a major environmental threat to agriculture, limiting the growth and yield of many crop plants (Chele et al., 2021). Wheat (*Triticum aestivum* L.) is categorized as a species moderately sensitive to salt and salinity is known to inhibit its growth. Plants under salinity stress commonly face disturbances in plant water relations and accumulation of toxic ions thereby profoundly restricting their germination performance and seedling emergence. Several previous studies have shown that salt stress reduces seed vigor and inhibits germination and early seedling growth of many plant species including wheat (Uçarlı, 2021). Some studies suggested that inhibition of seed germination and seedling growth under saline conditions is correlated with decreased yield and provides an accurate estimation of growth and yield potential under salt conditions (Barichello et al., 2021; Mahboob et al., 2023).

Improving the tolerance of wheat to salinity is a promising strategy to overcome yield reductions caused by salt stress and could positively contribute to global food security. Many techniques have been applied in agricultural practice to improve plant growth under stress conditions (Costa et al., 2018). Nowadays, priming techniques are widely used for improving

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germination performance and seedling growth of many plant species (Biswas et al., 2023; Louis et al., 2023). Seed priming has much significance in sustainable agriculture due to its multiple benefits in agriculture and crop production, like rapid germination, good seed vigor, and abiotic and biotic stress tolerance (Majeed et al., 2019). This technique is a pre-germinating stress exposure that develops cellular defense mechanisms in seeds as 'priming memory', which could improve the physiological and biochemical responses to salinity in post-priming stages (Singh et al., 2015).

Plants can be primed by various organic and inorganic stimulants for inducing toughness under stressful circumstances (Turhan and Asgher, 2023; Khetsha et al., 2024). Depending on the priming agents, they are classified as hydropriming, osmopriming, halopriming, nutripriming, and hormone priming. Among the priming treatments, halopriming is a common practice that involves soaking seeds in a solution containing an inorganic salt followed by air drying before sowing. In previous studies, seed halopriming with salts such as NaCl, $CaCl_2$, KNO_3, $MgSO_4$, and $CuSO_4$ has been found efficient in alleviating the negative influences of salinity during seed germination of many plant species including wheat (Hmissi et al., 2024). However, in most cases, 1 single salt was used in the priming solution. Few studies, on the other hand, considered the complementary advantages of using a mixture of salts that could act in a positive synergistic way (Lutts et al., 2016). For instance, in a recent study, Khan et al. (2020) demonstrated that priming wheat seeds with solution containing a mixture of $ZnSO_4$ and $CuSO_4$ improved all growth parameters of wheat plants under salinity stress more than seeds primed with solutions of each salt separately. Accordingly, the potential value of solutions from mixtures of salts rather than a solution of 1 single salt as a seed halopriming agent is attracting more attention and is now being considered for further investigation.

Seawater is an excellent natural mixture of several salts that proved to be useful for plant growth (Javeed et al., 2021). The fact that seawater contains several salts in combination entitles it to evaluation as a natural halopriming agent. In line with this rationale, Maswada et al. (2016) reported that priming seeds with diluted Mediterranean Sea water significantly improved all growth traits of wheat under salt stress. Likewise, the Dead Sea water (DS) contains a unique mixture of salts but is significantly different from the composition of the salts of other seas. When compared with other oceans and seas, the DS is more abundant in many elements, including chloride (212.4 g l^{-1}), magnesium (40.65 g l^{-1}), sodium (39.15 g l^{-1}), calcium (16.86 g l^{-1}), potassium (7.26 g l^{-1}), and bromide (5.12 g l^{-1}). Conversely, it has a lower concentration of sulfate (0.47 g l^{-1}) and bicarbonate (0.22 g l^{-1}) (Nissenbaum, 1977).

Apparently, the unique salt composition of the DS makes it potentially ideal for use as a natural halopriming and nutripriming agent. However, according to the literature review, DS was never tested as a seed priming agent for the subsequent improvement of seed germination and seedling growth in salt-stress conditions. Therefore, the present work aimed to assess the potential of using diluted DS as a seed priming agent for the improvement of seed germination and seedling growth of wheat under induced salinity stress. Furthermore, the influence of salinity on some physiological and biochemical indices of wheat seedlings grown from unprimed and primed seeds was also investigated.

MATERIALS AND METHOD

Plant material and priming treatments

All experiments were carried out from October 2023 to May 2024 at the Plant Physiology Research Laboratory, the Department of Biological Sciences, The University of Jordan, Amman, Jordan. Seeds of a local wheat cultivar (*Triticum aestivum* L.) were surface sterilized with 5% sodium hypochlorite solution containing 1 drop of Tween-20 for 15 minutes and thoroughly washed with distilled water. The surface sterilized seeds were then subjected to priming treatments by direct immersion in 4 levels of DS (5%, 10%, 15%, and 20%) for 12 hours in darkness at 25 °C using a 1:5 ratio of seed weight (g) to solution volume (ml). The required DS concentration was obtained by diluting DS (345 g of mineral per liter) (collected from approximately 50 to 70 cm below the surface of the northern part of the DS shore, Jordan side), with distilled water until the treatment level was reached. Seeds soaked in distilled water for the same duration; hydroprimed and unprimed seeds were used as controls. After removal from the priming solution, seeds were washed 4 times with distilled water, re-dried under shade to their original weight and finally stored in the refrigerator at 5 °C until future use. The

experiments were arranged in a completely randomized block design with 3 to 4 replicates per treatment.

Germination tests

Surface sterilized primed and control seeds (unprimed and hydroprimed) (25 each) were separately sown on 2 sheets of Whatman No.1 filter paper placed in 9-cm diameter sterile petri dishes. The filter papers in each plate were moistened with 6 ml of different saline concentrations consisting of 0, 100, 200, and 300 mM NaCl. These treatments are referred to as non-saline; low salinity (100 mM); moderate salinity (200 mM) and high salinity (300 mM) stress. Control groups were wetted with distilled water. All germination tests were carried out in an incubator (national variable temperature incubator) at 25 °C in the dark for 5 days. During the germination period, distilled water, equal to the mean loss from dishes, was added to filter paper to maintain moisture and avoid salt accumulation. All petri dishes were arranged in a completely randomized design with 4 replications per treatment. Germination of seeds was recorded daily for 5 days and all seeds with a radicle length of 2 mm were considered germinated. Final germination percentage (FG%) and germination index (GI) were calculated according to Espanany et al. (2016), based on the Equation 1 and 2.

$$FG = \frac{n}{N} \times 100\% \quad (1)$$

Where, n is the number of seeds that germinated and N is the total number of seeds.

$$GI = \sum \frac{Gt}{Dt} \quad (2)$$

Where, Gt refers to the number of seeds germinated on day t and Dt is day 1, 2, 3, etc.

Relative germination salt tolerance (RST) was also calculated according to Bolton et al. (2019) using the Equation 3.

$$RST = \frac{PG_{NaCl}}{PG_{Control}} \quad (3)$$

Where, $PG_{Control}$ is the final percent of germination under non-stress conditions and PG_{NaCl} is the final percent of germination under salt stress.

The seedling length was measured as the total length of shoot and root from 4 randomly selected seedlings per treatment at day 5 after sowing.

Water imbibition measurement

Measurement of water imbibition by primed and control seeds at different salinity levels was carried out as described by Patanè et al. (2009). The identified initial weight of seeds (2 to 2.5 g) was placed in small petri dishes and treated as described for the germination experiments. After 24 hours of incubation at 25 °C in darkness, each seed group was blotted with absorbent paper and reweighed for final weight determination. Measurement was performed 3 times per treatment and the percentage of water imbibition was calculated using Equation 4.

$$\text{Percentage of water imbibition} = \frac{\text{final weight-initial weight}}{\text{initial weight}} \times 100\% \quad (4)$$

Root vitality

Root vitality was estimated by measuring the activity of the dehydrogenase enzyme by using the 2,3,5-triphenyl tetrazolium chloride (TTC) reduction technique (Clemensson-Lindell, 1994). A sample of 500 mg of fresh roots from 4-day-old seedlings was cut into small pieces, put into test tubes with 2.5 ml of TTC (0.6% w/v) and 2.5 ml of phosphate buffer (10 mM, pH 7.0), and incubated for 3 hours at 30 °C. The samples were then extracted in 5 ml ethyl acetate for 15 minutes. The absorbance of samples was measured at 485 nm. Results were expressed as absorbance concerning root fresh weight (A485 nm g^{-1} FWt).

α-amylase activity

Alpha amylase activity was estimated using the method of Nie et al. (2022) with some modifications. For enzyme extraction, 3 days after sowing, 100 mg fresh germinating seeds from each treatment were individually homogenized in 10 ml of 0.1 M phosphate buffer (pH 6.9). The homogenate was centrifuged (1,000 g at 4 °C) for 10 minutes. The supernatant was collected and the volume was maintained up to 10 ml by adding phosphate buffer. Enzyme activity was determined in triplicate using a reaction medium containing 1 ml of 1% (w/v) starch dissolved in 0.2 M acetate buffer (pH 4.7) and 1 ml of extracted enzyme. The mixture was incubated at 30 °C for 15 minutes. The reaction was stopped by the addition of 2 ml of DNSA reagent (12 g of sodium potassium tartrate tetrahydrate in 8.0 ml of 2 M NaOH and 20 ml of 96 mM of 3,5-dinitrosalicylic acid solution) and the mixture was boiled for 10 minutes. After cooling, absorbance

was read at 560 nm and amylase activity was calculated and expressed as µM glucose g^{-1} FWt.

Total soluble sugar content

Total soluble sugar content was assessed by the method of Irigoyen et al. (1992). Fresh 4-day-old seedling tissue (200 mg) was homogenized with 1.5 ml of 96% methanol and the homogenate was centrifuged at 10,000 g for 20 minutes at 4 °C. From the supernatant, 100 µl was transferred to the test tube and 400 µl distilled water was added. The content of the tube was mixed with 3 ml of 150 mg dissolved anthrone in 100 ml of 72% H_2SO_4. The solutions were boiled in a water bath for 15 minutes and cooled at room temperature. Finally, the sample absorbance was measured at 625 nm, and then total soluble sugar was expressed as µg g^{-1} FWt using the calibration curve of glucose.

Free proline content

The free proline content was determined according to Bates et al. (1973). The germinating seedling tissue (0.5 g) was homogenized with 10 ml of 3% sulfosalicylic acid at 4 °C for 4 days. The extract was centrifuged at 10,000 g for 20 minutes at 4 °C and 2 ml of the supernatant, 2 ml of acid-ninhydrin, and 2 ml of glacial acetic acid were mixed in a test tube and incubated at 100 °C for 1 hour. The reaction was terminated on ice, and the reaction mixture was then extracted with 4 ml of toluene. The chromophore-containing toluene was separated from the hydrated phase. The absorbance at 520 nm was spectrophotometrically determined with toluene as the blank. The proline concentration was calculated using a standard curve and expressed as µg proline g^{-1} FWt.

Malondialdehyde content (MDA)

MDA content was determined as an indication of lipid peroxidation according to Hichem et al. (2009). Fresh 5-day-old seedling tissue (500 mg) from each treatment was homogenized in 5 ml of 0.1% trichloroacetic acid (TCA). The homogenate was centrifuged at 10,000 g for 20 minutes at 4 °C. One milliliter aliquot of the supernatant was mixed with 3 ml of 0.5% thiobarbituric acid (TBA) prepared in 20% TCA and incubated at 90 °C for 20 minutes. After stopping the reaction in an ice bath, samples were centrifuged at 10,000 g for 5 minutes. The supernatant absorbance at 532 nm was measured using LKB Novaspec Model 4049 Digital Spectrophotometer and corrected for non-specific turbidity by subtracting the absorbance at 600 nm. MDA concentration was determined and expressed as µmole g^{-1} FWt.

Membrane stability index (MSI)

MSI was determined by recording the electrical conductivity of seedling tissue leakages in double distilled water at 40 and 100 °C (Sairam and Srivastava, 2002). Four days after sowing, seedlings were rinsed several times with distilled water and 200 mg of the washed seedling tissue, representing each treatment, were taken in test tubes containing 20 ml of double-distilled water in 2 sets. One set was kept at 40 °C for 30 minutes. Initial electrical conductivity (EC1) was then recorded using a conductivity meter after bringing the sample to 25 °C. Another set was placed in a boiling water bath (100 °C) for 15 minutes, cooled to 25 °C, and final electrical conductivity (EC2) was recorded. The MSI was then calculated using Equation 5.

$$MSI = \left[1-\left(\frac{EC_1}{EC_2}\right)\right]\times 100 \quad (5)$$

Determination of total antioxidant capacity

Three days after sowing, 0.5 g of fresh seedling tissue from each treatment was homogenized in 2 ml chilled distilled water and centrifuged at 10,000 g for 30 minutes. All steps were performed at 4 °C and the supernatants were used to determine the total antioxidant capacity by the spectrophotometric method of Prieto et al. (1999). A mixture of 0.5 ml of the supernatant (tissue extract) and 1 ml of reagent solution (0.6 M H_2SO_4, 28 mM sodium phosphate, 4 mM ammonium molybdate mixture) was boiled for 1 hour. After cooling to room temperature, absorbance was read at 695 nm against a blank (mixture without tissue extract). The assay was conducted in triplicate and the total antioxidant activity expressed as the absorbance of the sample at 695 nm. The higher the absorbance value, the higher the antioxidant activity.

K^+ leakage from roots

K^+ leakage from roots was estimated according to Chen et al. (2005). Four days after sowing, 5 seedlings of each treatment were randomly chosen, their roots were washed 3 times in deionized water and immersed in a small beaker with 5 ml of 80 mM NaCl solution for 2 hours. Roots were then surface dried with paper towels and their fresh weight was measured. The amount of K^+ released into the solution was determined using a flame photometer and expressed as PPM K^+ g^{-1} FWt.

Sodium and potassium ratio

Determination of sodium and potassium ion concentrations was carried out following the extraction method described by Asch et al. (2022). At 4 days after sowing, roots and shoots from 10 seedlings of each treatment were collected and dried in an oven at 70 °C for 48 hours. Finely ground roots and shoots (50 mg) were homogenized with 5 ml of deionized water. Samples were heat extracted with an autoclave at 120 °C for 60 minutes and centrifuged at 10,000 g for 5 minutes. From the supernatant, 4.5 ml was collected and made up to 50 ml with deionized water. Na^+ and K^+ concentrations were estimated separately using a flame photometer and the ratio of Na^+:K^+ was calculated from the content of these ions in shoot and root, based on the concentration and dry matter weight.

Statistical analysis

Data are presented as the mean of all replicates ±SE (standard error). Significant differences between means were analyzed using Duncan's multiple range test (DMRT) at the $p < 0.05$ level.

RESULTS AND DISCUSSION

FG%, GI, RST, and seedling length of wheat were recorded under low (100 mM NaCl), medium (200 mM NaCl), and high (300 mM NaCl) salinity stresses. In addition, the effect of priming wheat seeds with different concentrations of DS on these parameters was examined by comparing them with hydroprimed and unprimed seeds. Figure 1 showed that in the absence of DS priming, increasing salinity to 300 mM NaCl led to a sharp reduction in FG%. The FG% decreased to 20.1% and 27.3% in unprimed and hydroprimed seeds, respectively. However, DS-primed seeds recorded significantly higher FG% with the highest value of 79.62% observed in the 10% DS-primed seeds followed by the 5% DS-primed groups (65%), 15% DS-primed group (60%) and 20% DS-primed group (58%). The markedly higher FG% in DS-primed seeds compared to that of hydroprimed and unprimed seeds and the small differences in FG% between hydroprimed and unprimed seeds clearly indicated that the alleviation of the negative influence of salinity on wheat seed germination was due to DS priming and not due to seed soaking treatment.

All salinity levels decreased the GI of unprimed and primed wheat seeds in a concentration-dependent manner (Figure 2). However, at each salinity level, the GI was higher in seeds primed with DS. The highest GI value was achieved with 10% DS. In line with its effect on GI, seeds primed with 10% DS showed the highest relative RST at 300 mM NaCl salinity level followed by seeds primed with 5%, 15%, and 20% DS (Figure 3). On the other hand, unprimed and hydroprimed seeds exhibited the lowest relative RST.

According to the results presented in Figure 4, increasing NaCl stress led to a progressive reduction in the length of wheat seedlings in all of

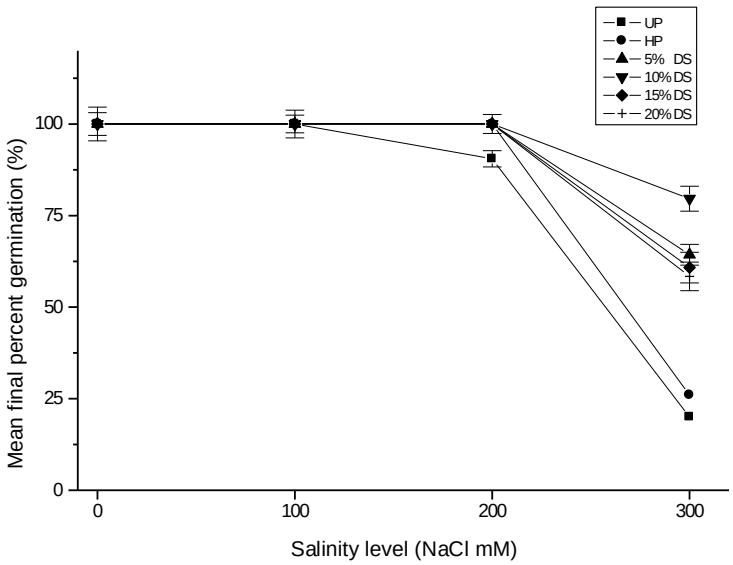

Figure 1. Effects of different salinity levels and priming treatments on the FG% of wheat seeds. Unprimed (UP), hydroprimed (HP), and seeds primed with different concentrations of DS. Values presented are the means from 4 replicates ±SE

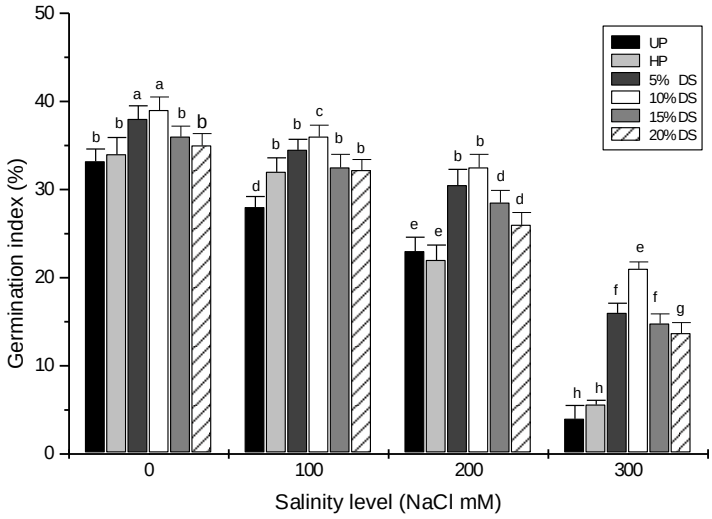

Figure 2. The effects of various salinity levels and priming treatments on the GI of wheat seeds were examined. Unprimed (UP), hydroprimed (HP), and seeds primed with different concentrations of DS were assessed. Error bars represent the ±SE. Means indicated by different letters in each column are significantly different at $p < 0.05$ according to DMRT (n = 4)

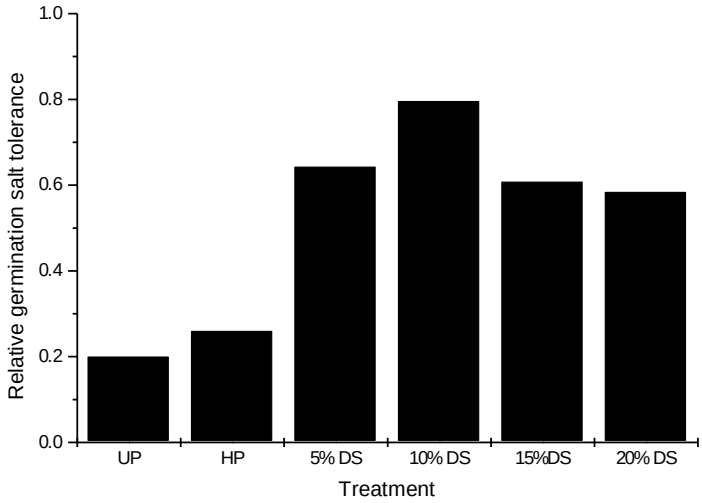

Figure 3. The effects of different priming treatments on the relative RST of wheat seeds germinated at 300 mM NaCl were assessed. Unprimed (UP), hydroprimed (HP), and seeds primed with various concentrations of DS were assessed. Values presented are the means from 4 replicates

the studied populations. However, priming seeds with DS could decrease the extent of reduction in shoot length as compared to seedlings raised from unprimed seeds. In fact, all concentrations of DS priming, except the 20% treatment, enhanced seedling length with or without salt treatment with the highest seedling length recorded in the 10% DS-primed group. Compared to its unprimed and hydroprimed equivalents, the 10% DS priming increased seedling length by 69%, 100%, 132%, and 415% at 0, 100, 200, and 300 mM NaCl, respectively. Priming with 5% and 15% DS resulted in a similar trend of increased seedling length but to a lower extent compared to the 10% DS priming treatment.

Most plant species under high salinity incurred a significant reduction in seed germination and seedling growth (Uçarlı, 2021). Seed priming is often reported to alleviate the effect of salinity stress on different plants during emergence and subsequent growth (Forni and Borromeo, 2023). The positive influence of priming wheat seeds with DS on FG%, GI, relative RST, and seedling length under salt stress are in line with

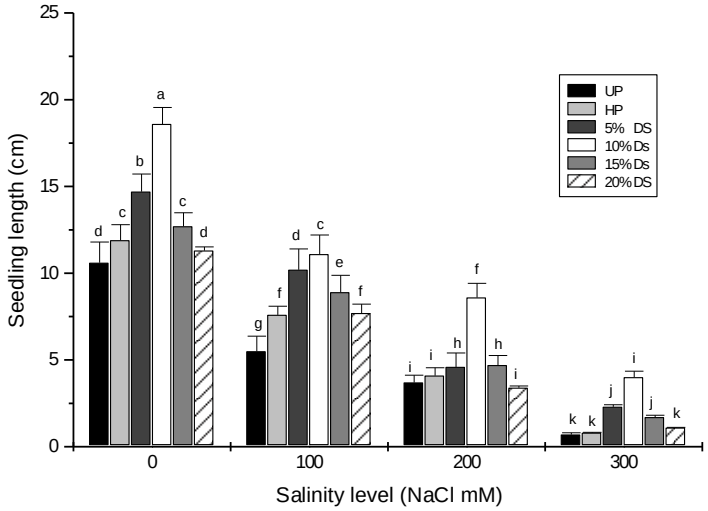

Figure 4. Effects of different salinity levels on the length of wheat seedlings raised from unprimed (UP), hydroprimed (HP), and seeds primed with various concentrations of DS were examined. Error bars represent the ±SE. Means indicated by different letters in each column are significantly different at $p < 0.05$ according to DMRT (n = 4)

previously published positive results of different priming treatments on seed germination and growth of various plants (Saddiq et al., 2019; Zulfiqar et al., 2022). Nevertheless, this is the 1st study to report evidence suggesting that priming with DS mitigated the negative effects of salinity on germination and seedling growth in wheat.

Seed germination is a complex physiological process that begins with water uptake by the dry seeds, mobilization of food reserves, and protein synthesis and ends with radical protrusion. While the exact mechanism of DS priming on germination and growth under salinity is difficult to envisage, yet, its positive effects on germination and growth under salt stress might be attributed to its unique mineral content. DS is rich in sulfur, calcium, and magnesium which were demonstrated to have a prominent role in salinity stress. Sulfur has been demonstrated to play a critical role in the response of plants to abiotic stress factors including salinity (Cao et al., 2014). In addition, calcium is considered as 1 of the most versatile and important elements in the response of plants to salinity (Seifikalhor et al., 2019). Furthermore, the role of magnesium in fortifying plants against environmental stress has been recently reported by Kumari et al. (2022). The combined synergistic effects of these elements may have contributed to the increased salinity tolerance of wheat through DS priming.

Nevertheless, seed priming has been frequently suggested to stimulate the germination process by the induction of a certain set of key physiological and biochemical processes in the seed. These processes include imbibition, enzyme activation, dormancy breaking, and metabolism of germination inhibitors.

In the present study, similar mechanisms seem to operate in the DS-primed seeds. Under non-saline and low salinity conditions, water uptake (imbibition) by seeds was similar between the primed and unprimed seed groups. However, as the level of salinity increased, the level of water uptake was significantly reduced in the unprimed and hydroprimed groups but significantly improved in the DS groups (Figure 5). In the 10% DS-primed group, water uptake of seeds increased by 40% and 28% under moderate and high salinity, respectively. Other DS treatments caused a lower increment of water uptake (20%) compared to unprimed and hydroprimed controls. These results align with reports suggesting that priming seeds with a mineral solution enables them to quickly absorb water, reinitiate metabolism, and enhance germination (Johnson and Puthur, 2021).

A significant increase in root vitality with increasing salt concentration was observed in primed and unprimed wheat seedlings although the increase was more prominent in the primed groups (Figure 6). All priming treatments (hydroprimed and DS) strongly promoted root vitality with increasing severity of salt stress compared to the unprimed control.

At the highest salinity level (300 mM NaCl), the 10% DS priming treatment caused an increase in root vitality by 95% and 54% over the unprimed and hydroprimed plants, respectively.

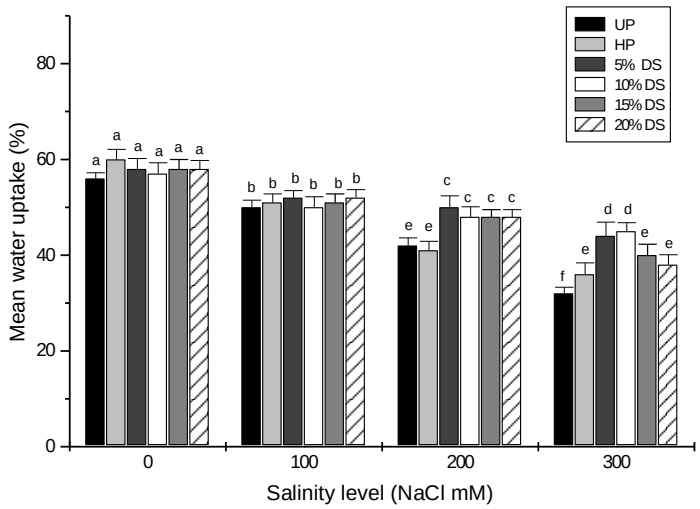

Figure 5. The effects of different salinity levels and priming treatments on % water uptake (imbibition) by wheat seeds were examined. Unprimed (UP), hydroprimed (HP), and seeds primed with different concentrations of DS were assessed. Error bars represent the ±SE. Means indicated by different letters in each column are significantly different at $p < 0.05$ according to DMRT (n = 3)

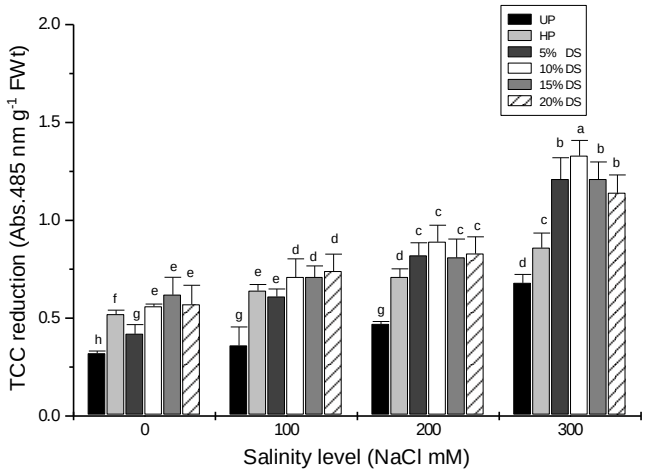

Figure 6. Effects of different salinity levels on root vitality of wheat seedlings raised from unprimed (UP), hydroprimed (HP), and seeds primed with different concentrations of DS. The error bars indicate ±SE. Means indicated by different letters in each column are significantly different at $p < 0.05$ according to DMRT (n = 3)

A comparable trend but a lower increase in root vitality was observed in seedlings primed with other concentrations of DS. Roots are considered as the most sensitive organ for plants to sense and initiate the stress response (Li et al., 2017; Li et al., 2023). Plants that show higher root activity have a greater ability to absorb water and nutrients, with a more vigorous metabolism and stronger stress resistance. According to Karlova et al. (2021), root vitality normally changes under stress conditions but, in fact, the capacity of roots for recovery and maintaining their vigor and vitality under salt stress has been considered a reliable and sensitive indicator for the assessment of salinity tolerance of plants. The dehydrogenase activity of roots is regarded as an indicator of vigor and used as a comprehensive assessment index reflecting the metabolic activity level and the root's ability to absorb nutrients and water (Johnson and Puthur, 2021). It appears that the higher root vigor in the DS-primed seedlings is likely to be involved in the alleviation of wheat seedling growth under salt stress.

Sugar metabolism during germination and early seedling growth plays a crucial role in determining seedling vigor, particularly under

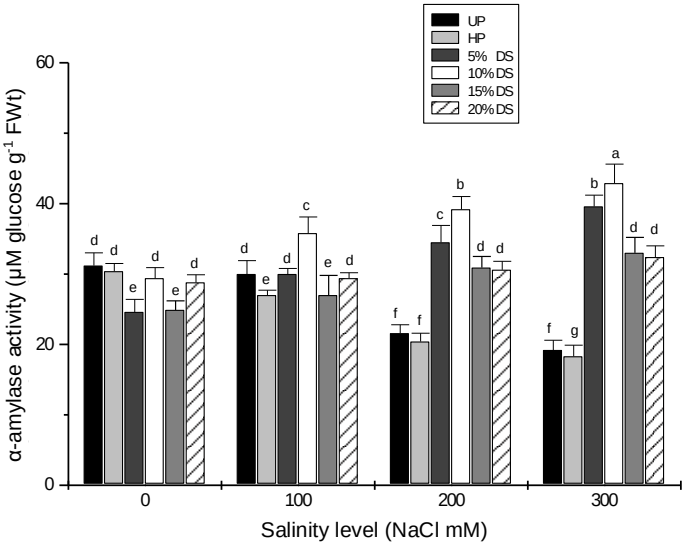

Figure 7. The effects of various salinity levels and priming treatments on α-amylase activity in 3-day-old germinated wheat seeds were analyzed. Unprimed (UP), hydroprimed (HP), and seeds primed with different concentrations of DS were assessed. Error bars represent the ±SE. Means indicated by different letters in each column are significantly different at $p < 0.05$ according to DMRT (n = 3)

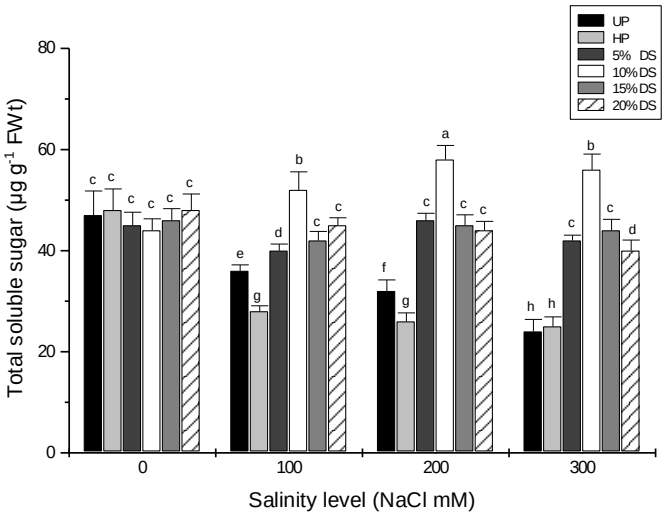

Figure 8. The effects of various salinity levels on total soluble sugar content in 4-day-old wheat seedlings from unprimed (UP), hydroprimed (HP), and seeds primed with different concentrations of DS were assessed. Error bars represent the ±SE. Means indicated by different letters in each column are significantly different at $p < 0.05$ according to DMRT (n = 3)

stress conditions (Hussain et al., 2016). Generally, sugar metabolism during wheat seed germination involves the degradation of storage starch and the generation of small molecular substances such as glucose and sucrose. In this study, sugar metabolism in wheat seedlings was evaluated by measuring α-amylase activity (a key enzyme involved in the starch mobilization process) and total soluble sugar content. Compared with unprimed control, salinity stress significantly reduced α-amylase activity and decreased the total soluble sugar contents in wheat seedlings (Figure 7 and 8). In contrast, priming seeds with DS brought about an obvious increase in α-amylase activity accompanied by a significant increase in the accumulation of soluble sugars particularly at the medium (200 mM NaCl) and high (300 mM NaCl) salinity levels. Compared with unprimed control, the 10% DS priming was the most effective treatment, increasing α-amylase activity

by 19%, 81%, and 123% at 100, 200, and 300 mM NaCl respectively (Figure 7).

Furthermore, the contents of total soluble sugars followed the increasing trend of α-amylase activity (Figure 8). Compared to the unprimed and hydroprimed groups, the 10% DS priming group achieved a 45 to 50%, 80%, and 130% increase in total soluble sugars under 100, 200, and 300 mM NaCl stress, respectively. These findings are similar to those obtained by Bajwa et al. (2018) and indicated that increasing α-amylase activity in DS-primed seeds improved metabolic and growth processes making seeds ready for radicle protrusion thus, improving seed germination and subsequent seedling growth under NaCl stress.

Proline content of the wheat seedlings raised from unprimed and primed seeds increased with increasing salinity stress (Figure 9). However, there were significant differences in the level of proline accumulation between DS-primed and hydroprimed seedlings with the maximum

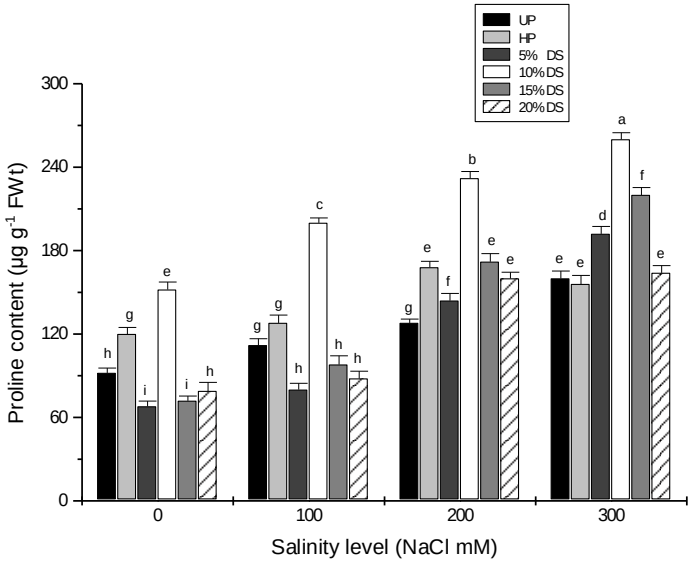

Figure 9. The effects of various salinity levels on proline content in 4-day-old wheat seedlings raised from unprimed (UP), hydroprimed (HP), and seeds primed with different concentrations of DS were evaluated. Error bars represent the ±SE. Means indicated by different letters in each column are significantly different at $p < 0.05$ according to DMRT (n = 3)

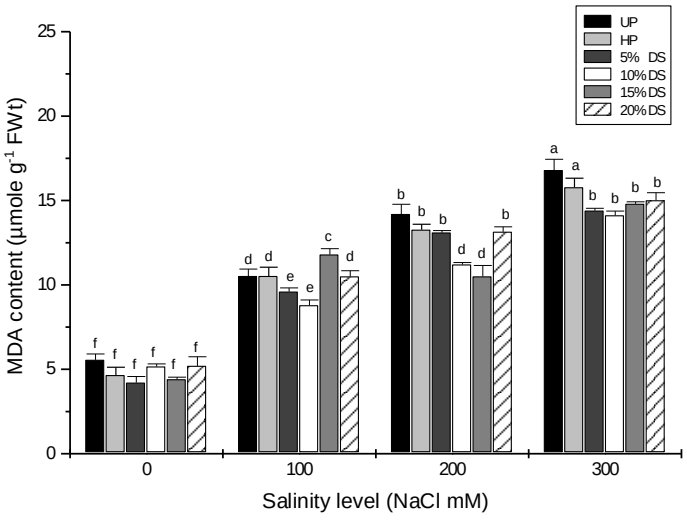

Figure 10. The effects of various salinity levels on MDA content in wheat seedlings grown from unprimed (UP), hydroprimed (HP), and seeds primed with different concentrations of DS were examined. Error bars represent the ±SE. Means indicated by different letters in each column are significantly different at $p < 0.05$ according to DMRT (n = 3)

increase in proline content recorded in seedlings primed with 10% DS. Proline accumulation in the 10% DS-primed group increased by 78%, 81%, and 62% at 100, 200, and 300 mM NaCl, respectively, over their unprimed control. These findings are similar to those of Zhu et al. (2020) who suggested that the increase in the synthesis of proline by environmental stresses decreases protein breakdown, detoxifies the augmented level of reactive oxygen species (ROS), and maintains membrane integrity, thus suggesting an important role in the increased proline synthesis in the improvement of salt tolerance by DS priming.

Environmental stresses normally lead to the generation of a large amount of ROS including O_2^-, HO_2^-, and H_2O_2 in plant cells as well as elevated levels of MDA by increasing lipid peroxidation. ROS are known to be very harmful for seed germination and seeding establishment by impairing cellular functions through oxidative reactions with different biomolecules such as proteins, nucleic acid, and lipids and eventually lead to cell death. Despite this, seed priming treatments have been shown to ameliorate the salt-induced toxicity by scavenging ROS to non-injurious level and by controlling the level of MDA synthesis, which accelerates cell membrane damage. In the present study, MDA accumulation in primed and unprimed seedlings was significantly progressive with increased salinity levels and significantly decreased when seeds were primed with DS (Figure 10). On the contrary, the total antioxidant capacity of seedlings was found to increase with increased salinity and was further enhanced by DS priming treatments. The highest increment in total antioxidant capacity resulted from the 10% DS priming treatment.

This treatment increased the antioxidant capacity of seedlings by 35%, 28%, and 43% under low, moderate, and high salinity, respectively, relative to unprimed control. The MSI of all primed groups under non-saline and low salinity (100 mM NaCl) treatments remained almost comparable with those of unprimed plants. However, a significant increase in MSI was noted in the primed groups at higher salinity levels, 8% (hydroprimed) and 23 to 25% (DS-primed) over the unprimed group, respectively (Figure 11).

The increase in antioxidant capacity of DS-primed seedlings over unprimed control (Figure 12) and the negative correlation of priming treatments with MDA production agree with reports indicating that priming treatments stimulated the synthesis or activity of non-enzymatic and enzymatic antioxidants, hence reducing the oxidative damage of ROS, and improving the ability of plants to adapt to saline environment (Mansour et al., 2019). Furthermore, the lower MDA content in the DS-primed seedlings as were in unprimed control might be due to the membranes having been repaired in the DS-primed seedlings. The data obtained

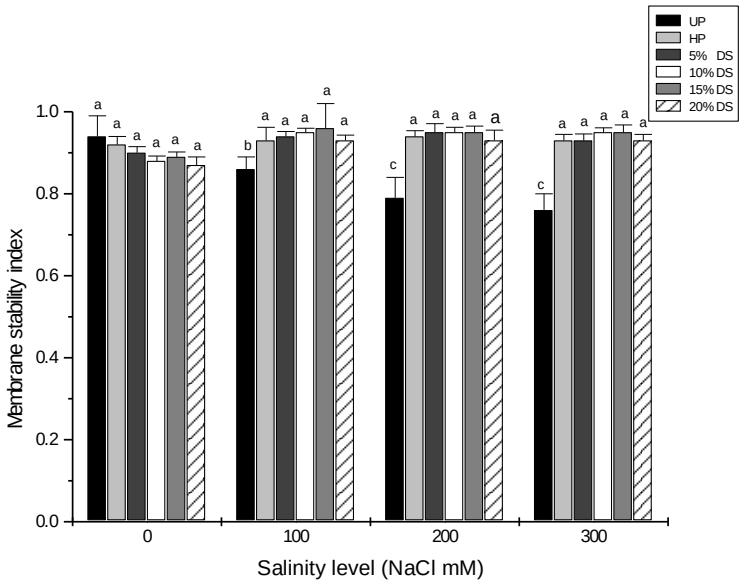

Figure 11. The effects of various salinity levels on the MSI of wheat seedlings raised from unprimed (UP), hydroprimed (HP), and seeds primed with different concentrations of DS were examined. Error bars represent the ±SE. Means indicated by different letters in each column are significantly different at $p < 0.05$ according to DMRT (n = 3)

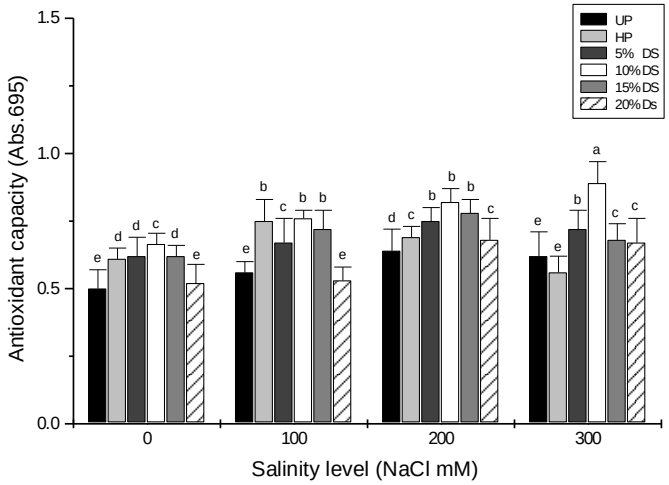

Figure 12. The effects of salinity levels on the total antioxidant capacity of wheat seedlings raised from unprimed (UP), hydroprimed (HP), and seeds primed with different concentrations of DS were examined. Error bars represent the ±SE. Means indicated by different letters in each column are significantly different at $p < 0.05$ according to DMRT (n = 3)

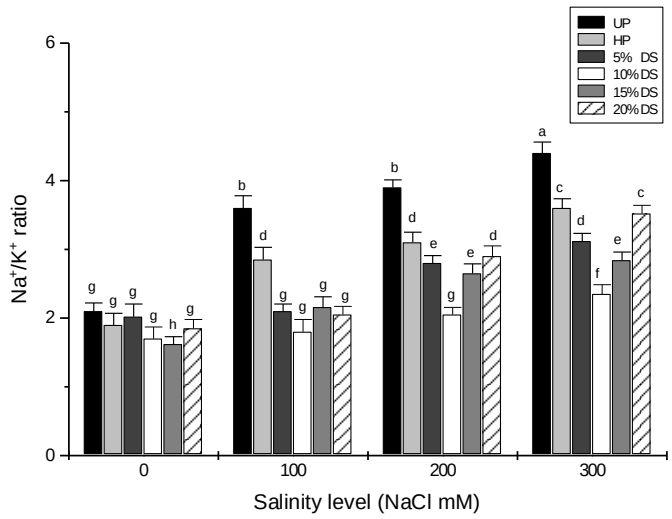

Figure 13. The effects of salinity levels on the Na^+/K^+ ratio in wheat seedlings raised from unprimed (UP), hydroprimed (HP), and seeds primed with different concentrations of DS were examined. Error bars represent the ±SE. Means indicated by different letters in each column are significantly different at $p < 0.05$ according to DMRT (n = 3)

in this work showed that the intactness of membrane i.e., MSI was more stabilized in plants from seeds primed with DS compared to unprimed control, thus representing an additional factor by which DS priming may have alleviated the adverse effects of salinity.

A low Na^+/K^+ ratio in plants has been considered a physiological trait indicator of salt tolerance (Morsy et al., 2007). The results presented in Figure 13 showed insignificant differences in the Na^+/K^+ ratio between primed and unprimed seedlings under non-saline conditions. However, the Na^+/K^+ ratio gradually increased with increasing salinity levels and unprimed seedlings exhibited the highest ratio at all salinity levels. On the contrary, the Na^+/K^+ ratio declined in primed seedlings with increasing concentration of NaCl and the DS-primed seedlings showed the highest reduction followed by hydroprimed seedlings. Among the DS-primed groups, the maximum reduction in Na^+/K^+ ratio (45 to 50% relative to unprimed control) under the different salinity levels was brought about by the 10% DS priming treatment.

The magnitude of salinity-induced K^+ leakage in roots of unprimed and primed seedlings is presented in Figure 14. Similar levels of K^+ leakage from roots of unprimed and primed

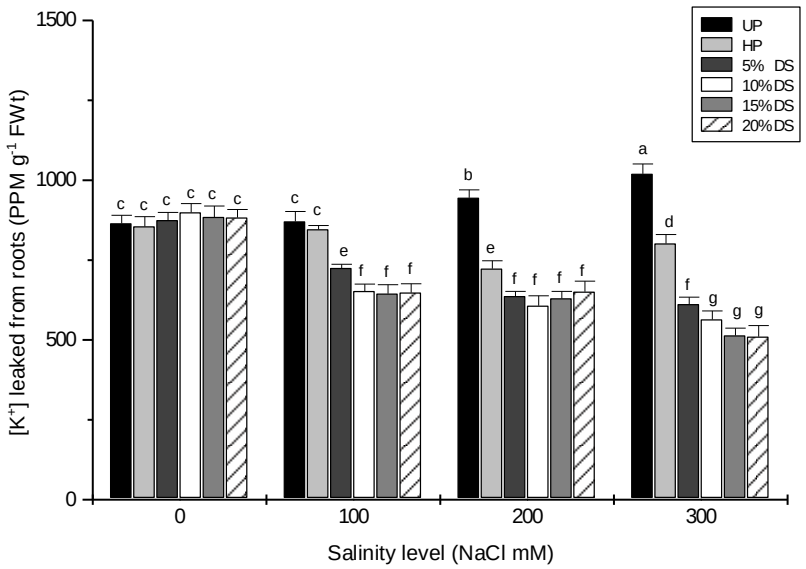

Figure 14. The effects of salinity levels on K^+ leakage were examined as the concentration of K^+ leaked from roots of wheat seedlings raised from unprimed (UP), hydroprimed (HP), and seeds primed with different concentrations of DS. Error bars represent the ±SE. Means indicated by different letters in each column are significantly different at $p < 0.05$ according to DMRT (n = 3)

seedlings were observed under non-saline conditions. However, increasing salinity increased the level of K^+ leakage from roots of unprimed seedlings by 9.2% at 200 mM NaCl and 17.9% at 300 mM NaCl as compared with that under non-saline conditions. Meanwhile, all priming treatments reduced K^+ leakage under salt stress. Compared to unprimed control, 10% DS priming treatments reduced K^+ leakage by 32% at 200 mM NaCl and (40 to 50%) at 300 mM NaCl, meanwhile, the lowest reduction in K^+ leakage (approximately 20%) was observed in roots of seedlings originated from hydroprimed seeds. These results confirm those obtained by Zhang et al. (2018) that NaCl salinity produces high ratios of Na^+/K^+ in plants, causing them to be susceptible to osmotic and specific-ion injury, as well as to nutritional disorders. Nevertheless, priming with DS significantly reduced the Na^+/K^+ ratio.

It has been suggested that the beneficial effects of reduced Na^+/K^+ ratio by priming treatments are likely to be due to increased accumulations of K^+ with simultaneous decreases in Na^+ uptake (Cuin et al., 2003; Yang et al., 2018). The findings that DS priming reduced K^+ leakage from the roots of salinity-stressed seedlings may have contributed to the enhanced K^+ content of DS-primed seedlings under salinity. These findings suggested that seed priming with DS improved ionic equilibrium under salinity, potentially reducing Na^+ toxicity and its diverse effects on cellular metabolism.

CONCLUSIONS

Priming seeds with DS at a concentration of 10% effectively minimized the adverse effects of salinity stress on wheat. This improvement was linked to enhanced seed germination and seedling establishment, as well as better salt tolerance indices. It is suggested that priming seeds with DS is a feasible approach for improving wheat seed germination and subsequent growth in the presence of salinity stress.

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