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RESEARCH ARTICLE

Effects of different seed priming agents on seed germination and physiological characteristics of wheat under saline-alkali stress

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ABSTRACT

Soil salinization restricts crop growth and yield, thereby adversely affecting agricultural development. The stage of seed germination is the most crucial and sensitive stage in the plants' life cycle and is particularly sensitive to saline-alkali stress. We investigated the effects of different hormonal priming agents, namely melatonin (MT), abscisic acid (ABA) and brassinosteroid (BR), as well as the osmopriming agent, calcium chloride ($CaCl_2$), on the germination of wheat (*Triticum aestivum* L.) seeds under saline-alkali stress. Saline-alkali stress was simulated with the solution of 100 mM NaCl and 50 mM $NaHCO_3$/Na_2CO_3 (9:1). The results indicated that hormonal priming agents (ABA, MT, or BR) significantly alleviated saline-alkali stress-induced inhibition of wheat seed germination. The germination rate of seeds primed with ABA, MT, or BR increased by 21.0%, 11.0%, and 10.5%, respectively. The seeds primed with ABA, MT or BR showed improved activities of α- and β-amylase under saline-alkali stress, with corresponding increases in starch hydrolysis and soluble sugar content, which contributed to seed germination and embryo growth. Hormonal priming (ABA, MT, or BR) also significantly improved antioxidase activities to alleviate oxidative damage in germinating seeds under saline-alkali stress. Seeds primed with ABA (38.7%), MT (37.0%), and BR (31.3%) displayed lower malondialdehyde (MDA) content than the H_2O-primed seeds. The ABA exerted the most significant promoting effect on wheat seed germination under saline-alkali stress. The promotional effect of $CaCl_2$ on seed germination was nonsignificant compared with that of hydropriming. The results offer a theoretical and practical basis for applying seed priming to enhance the saline-alkali tolerance of wheat in production.

Key words: Antioxidant system, hormonal priming, osmopriming, starch hydrolysis, *Triticum aestivum*.

INTRODUCTION

Soil salinization and alkalization is one of the important problems limiting agricultural production and has become increasingly severe because of inappropriate irrigation and agricultural management measures (Nachshon and Levy, 2023). According to the Global Map of Salt-Affected Soils launched by the Food and Agriculture Organization (FAO), more than 833 million hectares of soils are affected by salt worldwide. Moreover, 20% to 50% irrigated soils in all continents are too salty, which means over 1.5 billion people worldwide face substantial challenges in growing food owing to soil degradation (FAO, 2021). The stress effects of soil salinization and alkalization on plants include the effects of both salt stress and alkali stress. Salt stress can cause osmotic stress and ion toxicity to plants, thus restricting water absorption by plants; and disturbing ion homeostasis and the normal physiological function of cells (Liang et al., 2018; Shunkao et al., 2022). Besides osmotic stress and ionic toxicity, alkali stress is also harmful to plants in terms of the effects of high pH stress on plants. High pH can severely disturb cell pH stability, disrupt cell membrane integrity, and reduce root vitality

in plants (Fang et al., 2021). High pH also reduces soil nutrient availability, further affecting plant growth (Zhang et al., 2023).

Wheat (*Triticum aestivum* L.) is one of the most important food crops in the world, and it is also one of the main cultivated food crops in saline-alkali land. Studies on improving wheat saline-alkali tolerance are of important theoretical and practical significance for the full and reasonable development and utilization of saline-alkali land and ensuring food security. Most studies have currently focused on segmenting the salt tolerance of wheat, and few studies have investigated on salt-alkali mixed stress tolerance. However, in the realm of nature, excessive salt concentrations and elevated pH levels often occur simultaneously, synergistically having a detrimental effect on plant growth and development compared with the impact of either stress factor in isolation.

Seed priming is a pre-sowing treatment with natural or synthetic compounds and has garnered marked attention as a potential strategy for enhancing crop performance under stress. Seed priming involves controlled hydration and dehydration of seeds, which triggers various physiological and biochemical changes that improve crop resistance and yield. Different seed priming techniques, such as osmopriming, hormonal priming, and bio-priming, have been reported to effectively enhance seed germination and seedling growth under stresses (Paparella et al., 2015; Farooq et al., 2019). In plant defense responses, seed priming enables plants to respond faster and better to upcoming stresses, and the primed plants show more robust and rapid cellular defense responses to stresses (Farooq et al., 2019). It has been reported that seeds primed with 5-aminolevulinic acid mitigated temperature and drought stresses of wheat at germination and early seedling growth (Suliman et al., 2022). However, studies investigating on improving wheat saline-alkali tolerance through seed priming are few, and those investigating the effects of treatments with different seed priming agents are lacking.

Seed germination involves a series of complex metabolic activities and is a necessary stage in the development of the plant. This stage has a decisive impact on plant growth, development, and yield. Seed germination is considered the most vulnerable stage of the plant life cycle because of its susceptibility to mechanical injury, disease, and environmental stress. Under saline-alkali stress, seed germination is usually inhibited and may even be terminated. Therefore, improving seed germination is crucial for improving crop yields in saline-alkali soils.

In this study, we determined the effects of different seed priming agents on wheat seed germination under saline-alkali stress. The effects of hormonal priming agents, namely melatonin (MT), abscisic acid (ABA), and brassinosteroid (BR), and the osmopriming agent calcium chloride ($CaCl_2$) on the germination rate (GR), germination index (GI), soluble sugar content, total starch content, amylase activity, and antioxidase activities of wheat seeds under saline-alkali stress were measured. Understanding the role of seed priming agents in improving wheat performance under saline-alkali stress can offer valuable insights for developing sustainable strategies to enhance crop productivity in saline-alkali affected areas.

MATERIALS AND METHODS

Experimental setup

The moderately saline-alkali tolerant wheat (*Triticum aestivum* L.) 'Jimai 22' was selected as the test material in this study. It is the most extensively planted cultivar in the North China Plain that produces a high yield (Meng et al., 2015).

Seed priming: One thousand uniform seeds were selected for each seed priming treatment. The seeds were surface sterilized by soaking in 1% sodium hypochlorite solution for 10 min and washed with distilled water for several times. The seeds were then soaked in 1 µM melatonin solution (MT), 1 µM brassinosteroid solution (BR), 1 µM abscisic acid solution (ABA), 100 µM calcium chloride solution ($CaCl_2$), or distilled water (H_2O) respectively at 20 °C under dark for 18 h. Subsequently, the seeds were removed from the solutions and washed with distilled water for several times. Absorbed the surface moisture. The seeds were then placed in a dark place to blow dry to the original weight.

Seed germination: The primed seeds were placed in 14-cm-diameter Petri dishes with filter paper. Saline-alkali (SA) stress was simulated using a solution of 100 mM NaCl and 50 mM $NaHCO_3$/Na_2CO_3 (9:1). The stress solution was added to the Petri dishes at an equal volume, and five treatments were established as follows: H_2O-SA, MT-SA, BR-SA, ABA-SA, and $CaCl_2$-SA. An equal volume of distilled water was added to the Petri dishes with distilled

water-primed seeds. These seeds were considered as control (CK). The Petri dishes were then transferred to a growth chamber at 22 °C/18 °C (day/night, and the length of day/night = 16:8 h). The experiment was a randomized complete block design with three biological replicates for each treatment. The samples were harvested at 3, 5, and 7 d, immediately placed in liquid nitrogen, and stored at -80 °C for further measurements.

Germination rate (GR) and germination index (GI)

After 7 d of treatment, GR and GI were determined as described by Liu et al. (2016). The GR is defined as the percentage of germinated seeds. GI = Σ(Gi/Ti), where Gi is the germination percentage at the ith d and Ti is the day of the germination test. The seed was defined as germinated when radicle and coleoptile lengths reach the full and half lengths of the seed, respectively.

Soluble sugar and total starch content

The soluble sugar content was measured according to the method of Moya et al. (1993). Dried seed powder (100 mg) was added to 8 mL 80% ethanol in a 10 mL centrifuge tube, heated at 80 °C for 30 min, cooled down, and centrifuged at 3000 r for 10 min. The supernatant was poured into a 25 mL tube. The extraction was repeated twice, and all the supernatant was collected. The supernatant was diluted with distilled water to 25 mL. Next, 0.1 mL extract solution was reacted with 5 mL anthranone-sulfuric acid solution at 90 °C for 15 min. The reaction solution was measured using a spectrophotometer (Cary 60 UV-Vis spectrophotometer, Agilent Technologies, Santa Clara, California, USA) at 620 nm.

The starch content was measured as described by Jiang et al. (2003). Dried seed powder (100 mg) was mixed with 10 mL 0.5 M KOH and stirred at 35 °C for 15 min. The mixture was transferred to a 50 mL volumetric bottle and diluted with distilled water to a 50 mL volume. Next, 2.5 mL supernatant was diluted with 20 mL distilled water and adjusted to pH 3.5 with 0.1 M HCl. Then, 0.5 mL I_2-KI reagent was added to the solution, diluted with distilled water to a 50 mL volume, and allowed to stand for 30 min. The mixture was measured using the spectrophotometer at 461.5, 555.0, 656.5 and 760.0 nm. The total starch content is the sum of amylose and amylopectin contents.

Amylase activities

Activities of α- and β-amylase were measured according to Kishorekumar's method (Kishorekumar et al., 2007). One gram of the fresh sample of germinated seeds was homogenized with 10 mL cold distilled water at 4 °C in a prechilled pestle and mortar. The homogenate was centrifuged at 15 000×*g* for 30 min at 4 °C. The supernatant was used to estimate the activities of α- and β-amylase.

α-Amylase: First, 3 mL 3 mM $CaCl_2$ solution was added to the 5 mL enzyme extract and heated at 70 °C for 5 min to passivate β-amylase. Then, 1 mL 0.1 M citrate buffer (pH 5.0) and 0.5 mL 2% soluble starch were added to the 0.5 mL enzyme treatment solution, and reacted at 30 °C for 5 min. The reaction was terminated by adding 2 mL color reagent. The mixture was reacted in a water bath at 50 °C for 5 min, cooled, and fixed to a 10 mL volume with distilled water. The absorbance measured at 540 nm was recorded.

β-Amylase: Low pH and 0.1 M EDTA passivation of α-amylase were used to determine β-amylase activity. One mL 0.1 M citrate buffer (pH 3.4) and 0.5 mL 2% soluble starch solution were added to 0.5 mL EDTA-treated enzyme extract, and the reaction was terminated by adding 2 mL color reagent. The mixture was reacted in a water bath at 50 °C for 5 min, cooled, and fixed to a 10 mL volume. The absorbance measured at 540 nm was recorded.

Antioxidase activities and malonaldehyde (MDA) content

First, 0.5 g fresh sample of germinated seeds was homogenized in the 5 mL enzyme extract (50 mM HEPES buffer, pH 7.8, containing 1 mM $EDTANa_2$, 1 mM ascorbic acid, 1 mM reduced glutathione, 5 mM $MgCl_2$, 1 mM dithiothreitol, and 20% v/v glycerol) on ice. The homogenate was centrifuged at 10 000×*g* for 30 min at 4 °C, and the supernatant was used for enzyme activity determination.

Superoxide dismutase (SOD, EC 1.15.1.1) activity was determined according to the method of Tan et al. (2008). First, 1.5 mL 50 mM HEPES buffer (pH 7.8), 0.3 mL 130 mM methionine solution, 0.3 mL 750 µM nitro-blue tetrazolium, 0.3 mL 100 µM EDTA-Na_2, 0.3 mL 20 µM riboflavin, and 100 µL enzyme extract were added to a 10 mL glass tube. The mixture was rapidly mixed and placed under 4000 lx light intensity for 15 min. Then, the absorption of the mixture was measured using a spectrophotometer at 560 nm.

Catalase (CAT, EC1.11.1.6) activity was measured as described by the method of Patra et al. (1978). First, 1.9 mL pH 7.0 buffer, 1 mL 0.075% H_2O_2 solution, and 0.1 mL enzyme extract were added sequentially in a cuvette, mixed rapidly. Then, the change in absorbance at 240 nm was monitored for calculation of CAT activity.

The MDA content was measured as described by Du and Bramlage (1992). First, 2 mL enzyme extract solution was reacted with 4 mL trichloroacetic acid-thiobarbituric acid (TCA-TBA) mixture (101.25 g TCA + 2.5 g TBA, heated and stirred, completely dissolved, and then cooled and fixed to a 500 mL volume) in a centrifuge tube for 20 min in boiling water bath, cooled, and centrifuged at 4000×*g* for 10 min. The absorption of the supernatant was measured using the spectrophotometer at 450, 532, and 600 nm.

Statistical analysis

The presented data represents the mean ± standard error of three independent measurements. The collected data was analyzed using one-way ANOVA with the SPSS package Ver. 24.0 (SPSS Inc., Chicago, Illinois, USA). Duncan's multiple range test was used to determine significance differences among treatments, with a significance level set at $P < 0.05$.

RESULTS

Effects of different seed priming agents on GR and GI of wheat seeds under saline-alkali stress

Compared with the control, GR and GI of wheat seeds germinated under saline-alkali stress were significantly reduced, and the change amplitude varied in different treatments (Figure 1). Under saline-alkali stress, GR and GI of seeds primed with ABA, MT, and BR were significantly higher than those of seeds primed with $CaCl_2$ and H_2O. The GR under the ABA-SA, MT-SA, and BR-SA treatments increased by 21.0%, 11.0%, and 10.5%, respectively, compared with that under the H_2O-SA. There was nonsignificant difference in GR and GI between the MT-SA and BR-SA treatments.

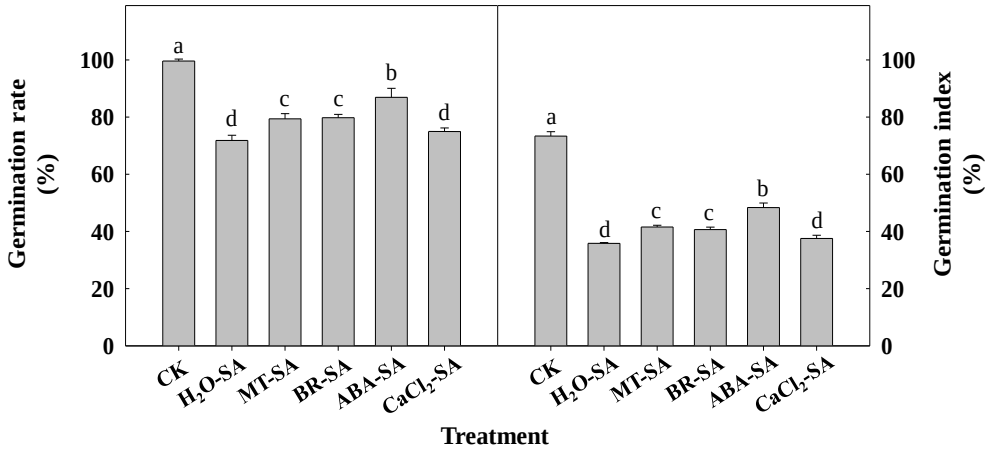

Figure 1. Effects of different seed priming agents on germination rate and germination index of wheat seeds under saline-alkali stress. CK: Seeds primed with distilled water germinated in distilled water; H_2O-SA: seeds primed with distilled water germinated under saline-alkali stress; MT-SA: seeds primed with MT germinated under saline-alkali stress; BR-SA: seeds primed with BR germinated under saline-alkali stress; ABA-SA: seeds primed with ABA germinated under saline-alkali stress; $CaCl_2$-SA: seeds primed with $CaCl_2$ germinated under saline-alkali stress. Data are means ± SE (n = 3). Different letters indicate significant difference at $p < 0.05$ level.

Effects of different seed priming agents on total soluble sugar content of germinating wheat seeds under saline-alkali stress

As can be seen from Figure 2, the soluble sugar content of the seeds at 5 d increased significantly compared with that at 3 d, and under saline-alkali stress, the increase in the soluble sugar content of the seeds was significantly restricted. The overall trend of the soluble sugar content under the treatments at 3 and 5 d was basically the same, with CK > ABA-SA > MT-SA > BR-SA > $CaCl_2$-SA > H_2O-SA. Under saline-alkali stress, the soluble sugar content of the germinating seeds primed with ABA, MT, and BR was significantly higher than that of seeds primed with H_2O. There was nonsignificant difference in soluble sugar content between the $CaCl_2$-SA and H_2O-SA treatments.

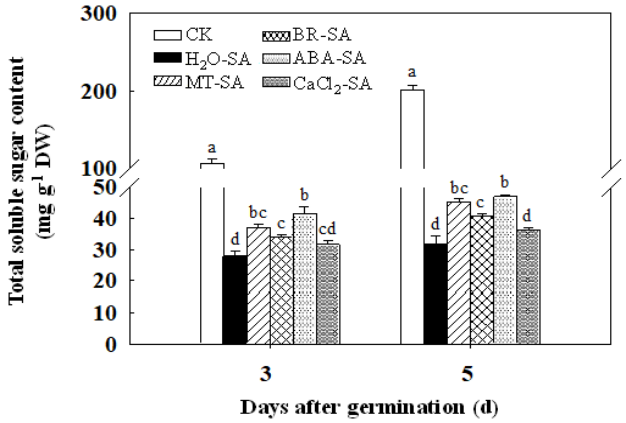

Figure 2. Effects of different seed priming agents on the total soluble sugar content of germinating wheat seeds under saline-alkali stress. CK: Seeds primed with distilled water germinated in distilled water; H_2O-SA: seeds primed with distilled water germinated under saline-alkali stress; MT-SA: seeds primed with MT germinated under saline-alkali stress; BR-SA: seeds primed with BR germinated under saline-alkali stress; ABA-SA: seeds primed with ABA germinated under saline-alkali stress; $CaCl_2$-SA: seeds primed with $CaCl_2$ germinated under saline-alkali stress. Data are means ± SE (n = 3). Different letters indicate significant difference at $p < 0.05$ level.

Effects of different seed priming agents on starch content of germinating wheat seeds under saline-alkali stress

The starch content of seeds in CK at 5 d was significantly decreased compared with that at 3 d (Figure 3). Saline-alkali stress significantly increased starch content of the germinating seeds. Under saline-alkali stress, starch content under each treatment was H_2O-SA > $CaCl_2$-SA > BR-SA > MT-SA > ABA-SA. The starch content under the ABA-SA, MT-SA, and BR-SA treatments were significantly lower than that under H_2O-SA and $CaCl_2$-SA treatments. The starch content under ABA-SA treatment was significantly lower than that under BR-SA treatment. There was nonsignificant difference in starch content between $CaCl_2$-SA and H_2O-SA treatments.

Effects of different seed priming agents on amylase activity in germinating wheat seeds under saline-alkali stress

The α- and β-amylase activities in germinating seeds increased without saline-alkali stress (Figure 4). Saline-alkali stress slightly affected α-amylase activities in the germinating seeds at 3 d after germination, whereas significantly decreased β-amylase activities. At 5 d after germination, saline-alkali stress significantly decreased α- and β-amylase activities in the germinating seeds. Under saline-alkali stress, α- and β-amylase activities under the ABA-SA, MT-SA and BR-SA treatments were significantly higher than those under the H_2O-SA treatment. There was nonsignificant difference in β-amylase activities between the $CaCl_2$-SA and H_2O-SA treatments.

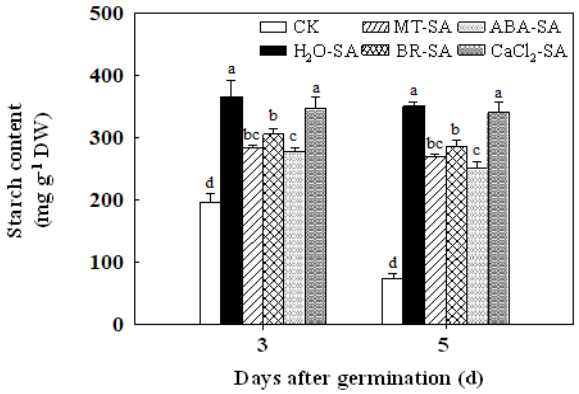

Figure 3. Effects of different seed priming agents on the starch content of germinating wheat seeds under saline-alkali stress. CK: Seeds primed with distilled water germinated in distilled water; H_2O-SA: seeds primed with distilled water germinated under saline-alkali stress; MT-SA: seeds primed with MT germinated under saline-alkali stress; BR-SA: seeds primed with BR germinated under saline-alkali stress; ABA-SA: seeds primed with ABA germinated under saline-alkali stress; $CaCl_2$-SA: seeds primed with $CaCl_2$ germinated under saline-alkali stress. Data are means ± SE (n = 3). Different letters indicate significant difference at $p < 0.05$ level.

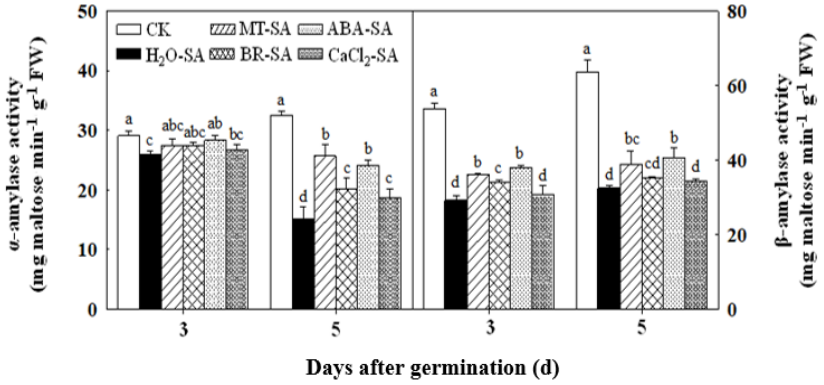

Figure 4. Effects of different seed priming agents on amylase activity in germinating wheat seeds under saline-alkali stress. CK: Seeds primed with distilled water germinated in distilled water; H_2O-SA: seeds primed with distilled water germinated under saline-alkali stress; MT-SA: seeds primed with MT germinated under saline-alkali stress; BR-SA: seeds primed with BR germinated under saline-alkali stress; ABA-SA: seeds primed with ABA germinated under saline-alkali stress; $CaCl_2$-SA: seeds primed with $CaCl_2$ germinated under saline-alkali stress. Data are means ± SE (n = 3). Different letters indicate significant difference at $p < 0.05$ level.

Effects of different seed priming agents on antioxidant system in germinating wheat seeds under saline-alkali stress

Under saline-alkali stress, the MDA content of seeds, which indicates the degree of lipid peroxidation, significantly increased (Figure 5). The MT-, BR-, ABA-, and $CaCl_2$-primed seeds (MT-SA, BR-SA, ABA-SA, and $CaCl_2$-SA) exhibited lower MDA contents than the H_2O-primed seeds (H_2O-SA), shown as ABA-SA< MT-SA < BR-SA < $CaCl_2$-SA < H_2O-SA.

Activities of SOD and CAT increased significantly under saline-alkali stress (Figure 6). The SOD activities in the germinating seeds under MT-SA, BR-SA, and ABA-SA treatments were significantly higher than those under $CaCl_2$-SA and H_2O-SA. There was nonsignificant difference in SOD activities between $CaCl_2$-SA and H_2O-SA treatments. The CAT activities in germinating seeds under saline-alkali stress were in the following order: ABA-SA = MT-SA = BR-SA > $CaCl_2$-SA > H_2O-SA.

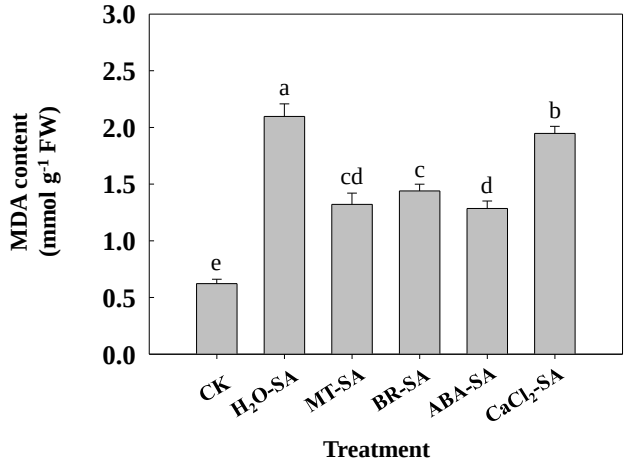

Figure 5. Effects of different seed priming agents on the malondialdehyde (MDA) content in germinating wheat seeds under saline-alkali stress. CK: Seeds primed with distilled water germinated in distilled water; H_2O-SA: seeds primed with distilled water germinated under saline-alkali stress; MT-SA: seeds primed with MT germinated under saline-alkali stress; BR-SA: seeds primed with BR germinated under saline-alkali stress; ABA-SA: seeds primed with ABA germinated under saline-alkali stress; $CaCl_2$-SA: seeds primed with $CaCl_2$ germinated under saline-alkali stress. Data are means ± SE (n = 3). Different letters indicate significant difference at $p < 0.05$ level.

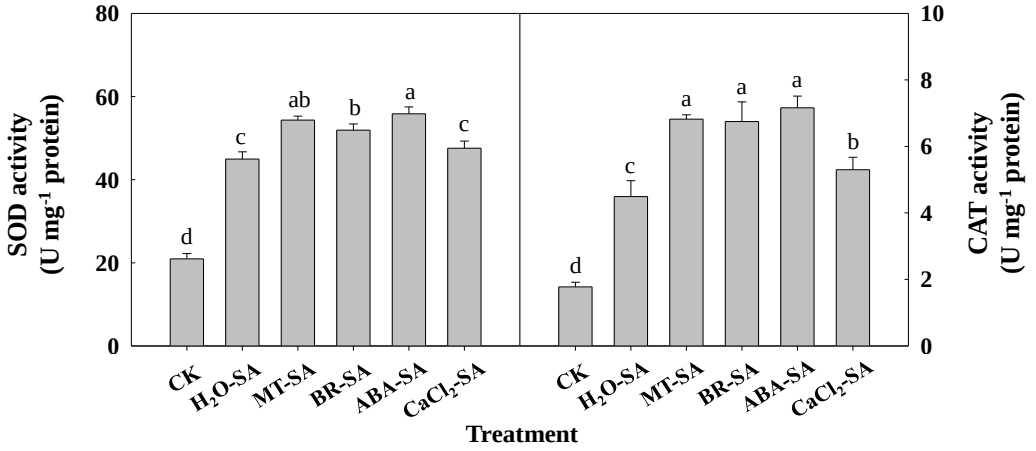

Figure 6. Effects of different seed priming agents on superoxide dismutase (SOD) and catalase (CAT) activities in germinating wheat seeds under saline-alkali stress. CK: Seeds primed with distilled water germinated in distilled water; H_2O-SA: seeds primed with distilled water germinated under saline-alkali stress; MT-SA: seeds primed with MT germinated under saline-alkali stress; BR-SA: seeds primed with BR germinated under saline-alkali stress; ABA-SA: seeds primed with ABA germinated under saline-alkali stress; $CaCl_2$-SA: seeds primed with $CaCl_2$ germinated under saline-alkali stress. Data are means ± SE (n = 3). Different letters indicate significant difference at $p < 0.05$ level.

DISCUSSION

The seed priming technology is simple, efficient, and affordable, which makes it highly promising for enhancing drought and salt tolerances of crops. Several priming agents such as $CaCl_2$ or KCl (Islam et al., 2015), ABA (Gao et al., 2002), BR (da Silva et al., 2015) and MT (Guo et al., 2022; Spolaor et al., 2022) have been reported to improve seed performance or seedling growth under abiotic stresses such as drought and saline stress. However, studies investigating on improving wheat tolerance to saline-alkali stress through seed priming are few, and those investigating the effects of treatments with different seed priming agents are lacking. Therefore, this study investigated the effects of the osmopriming agent ($CaCl_2$) and hormonal priming agents (ABA, MT, and BR) on wheat seed germination under saline-alkali stress.

Seed germination is the most crucial and sensitive stage in the plants' life cycle. In crop production, rapid and consistent seed germination is essential for successfully establishing the crop and achieving high yields. Saline-alkali stress is one of the widespread environmental problems limiting agricultural production and can significantly inhibit wheat seed germination (Wang et al., 2022; Gong et al., 2023). In this study, saline-alkali stress significantly decreased the seed GR and GI, whereas ABA-, MT-, and BR-primed seeds displayed higher GR and GI than the $CaCl_2$- and H_2O-primed seeds. This suggested that priming seeds with the hormonal priming agents ABA, MT, and BR significantly improved seed germination under saline-alkali stress. The ABA exhibited the most significant promoting effect on seed germination under saline-alkali stress.

During germination, energy and nutrients required by wheat seeds are primarily acquired through the decomposition of storage substances. Starch is the key storage substance in wheat seeds, and amylase hydrolyzes 90% of this starch (Hu, 2015). The hydrolytic enzyme α-amylase is known to play a crucial role in degradation of starch to soluble sugars during germination (Perata et al., 1997). And β-amylase is considered to play an important role in early wheat seed germination (Safari et al., 2020). Yu et al. (2019) reported that saline-alkali stress significantly reduced α-amylase activities in germinating wheat seeds, and the α-amylase activity gradually decreased with an increase in the concentration of saline-alkali treatment. It was suggested that the reduction in α-amylase activity could serve as an indicator of germination vulnerability under salt stress, particularly in salt-sensitive genotypes compared to salt-tolerant genotypes (Hussain et al., 2022). Sghayar et al. (2023) reported that total amylolytic activity and α- and β-amylases activities were decreased under salt stress, which could be significantly enhanced by seed priming (hormonal priming, GA; halopriming, calcium chloride; osmopriming, mannitol), particularly hormonal priming. The present study revealed that under saline-alkali stress, soluble sugar content and α- and β-amylases activities of wheat seeds were inhibited, whereas starch content was significantly higher than that of the non-stress control. This indicated that saline-alkali stress inhibited α- and β-amylase activities, leading to a limited starch hydrolysis rate and a decrease in the energy available for seed germination. This corresponded with the significantly reduced seed GR and GI under saline-alkali stress. Compared with hydropriming and osmopriming ($CaCl_2$), hormonal priming (ABA, MT, or BR) significantly enhanced α- and β-amylases activities in wheat seeds under saline-alkali stress, which resulted in a lower starch content and a higher soluble sugar content, in line with the results of Sghayar et al. (2023). Furthermore, the enhancement of α- and β-amylases activities and soluble sugar content and the reduction in the starch content were more pronounced in the ABA-primed seeds, corresponding to its highest GR and GI under saline-alkali stress.

Excessive accumulation of reactive oxygen species (ROS) under salt stress leads to membrane lipid peroxidation and MDA production. This destroys membrane integrity and leads to several physiological and biochemical process disorders (Liang et al., 2018). The SOD can dismutate superoxide to H_2O_2, thereby acting as the first line of defense against ROS. Subsequently, CAT, the ascorbate-glutathione cycle, and glutathione peroxidase cycle detoxify H_2O_2 (Apel and Hirt, 2004). It has been reported that seed priming with Si could alleviate salt stress-induced damages by enhancing antioxidant responses in lettuce seedlings (Alves et al., 2020). Our results showed that, saline-alkali stress significantly increased the MDA content. However, this increase was considerably lower in the ABA-, MT-, BR-, or $CaCl_2$-primed seeds than in the H_2O-primed seeds. Saline-alkali stress significantly increased SOD and CAT activities to cope with the increased superoxide $O_2^{\bullet-}$ release rate and H_2O_2 content. The activities were considerably higher in the ABA-, MT-, and BR-primed seeds than in the $CaCl_2$- and H_2O-primed seeds. This suggested that compared with hydropriming and osmopriming ($CaCl_2$), hormonal priming (ABA, MT, and BR) significantly improved the antioxidant enzyme activities to

alleviate oxidative damage in germinating seeds under saline-alkali stress, which corresponded to the significantly higher GR and GI in the ABA-, MT-, and BR-primed seed.

Overall, the ABA-primed seeds possessed higher α- and β-amylases activities, which resulted in lower starch content and higher soluble sugar content, corresponding to its highest GR and RI under saline-alkali stress. Moreover, the ABA-primed seeds exhibited higher SOD and CAT activities and lower MDA content. Thus, in this study, seed priming with ABA exerted the most significant promotional effect on wheat seed germination under saline-alkali stress. The ABA plays a key role in the initiation and maintenance of seed dormancy. Furthermore, it is a crucial player in plant response to abiotic stresses, including salt stress (Rehman et al., 2021). It has been reported that ABA priming could improve tolerance to saline-alkali stress (Wei et al., 2017) or alkali stress (Wei et al., 2021) in crops. The mechanisms underlying ABA-priming-induced promotion of seed germination under saline-alkali stress need to be further investigated.

CONCLUSIONS

Under saline-alkali stress, compared with hydropriming and osmopriming ($CaCl_2$), hormonal priming (abscisic acid [ABA], melatonin, or brassinosteroid) significantly improved α- and β-amylase activities during seed germination, promoted starch hydrolysis, increased soluble sugar content, increased superoxide dismutase, catalase activities and reduced malonaldehyde content, and ultimately improved the seed germination index and germination rate. Among them, the promotional effect of ABA on wheat seed germination under saline-alkali stress was the most significant. However, the promotional effect of $CaCl_2$ on seed germination was nonsignificant compared with that of hydropriming in this study.

Author contribution

Conceptualization: Y.S., X.W. Methodology: X.W. Validation: Y.S. Formal analysis: X.W. Investigation: X.W. Data curation: X.W. Writing-original draft: X.W. Writing-review & editing: Y.S. Supervision: Y.S. Project administration: Y.S. Funding acquisition: Y.S., X.W. All co-authors reviewed the final version and approved the manuscript before submission.

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