



Seed priming with Salicylic Acid (SA) and Hydrogen Peroxide (H₂O₂) Improve Germination and Seedling Growth of Wheat (*Triticum aestivum*) under Salt Stress

Shaila Shermin Tania^a, Md. Moklasur Rahaman^a, Farjana Rauf^a,
Mehera Afroj Suborna^a, Muhammad Humayun Kabir^a, Md. Anamul Hoque^b,
and Mohammad Saidur Rhaman^{a*}

^a Department of Seed Science and Technology, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh.

^b Department of Soil Science, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aim: Salinity is a major barrier to successful crop production. Seed priming and exogenous application of different signaling molecules can efficiently confer salinity tolerance. Wheat is a major cereal crop in the world and salinity drastically reduces the wheat seedling growth and yield. Therefore, the present study was conducted to explore the potentiality of different signaling molecules such as salicylic acid (SA) and H₂O₂ to alleviate the salinity-induced growth inhibition of wheat.

Place and Duration of the Study: The study was conducted in the Department of Seed Science and Technology, Bangladesh Agricultural University, from September-October, 2021.

Methodology: The wheat (cv. BARI-Gom 24) seeds were soaked in normal tap water (hydro-priming), 1 mM SA, 2 mM SA, 0.1 mM H₂O₂, and 0.15 mM H₂O₂ solutions for 30 minutes. The

untreated seeds were used as control. Eventually, primed seeds were exposed to 150 mM NaCl in Petri dishes during germination. Primed and non-primed seedlings were grown for 15 days under 150 mM NaCl stress condition.

Results: The result revealed that salt stress significantly reduced germination percentage (GP), germination index (GI), seed vigor index (SVI), shoot and root length. The results also exhibited that photosynthetic pigments, total chlorophyll, carotenoids, lycopene, and beta-carotene contents were significantly reduced by salt stress. Seed priming with SA and H₂O₂ and hydro-priming promoted the germination percentage, seedling growth (including shoot and root length), SVI, and photosynthetic pigments.

Conclusion: Pretreatment with 1 mM SA and 0.1 mM H₂O₂ was observed to be relatively more efficient in conferring salinity tolerance of wheat compared with other treating conditions. Overall, this study suggests that wheat seed priming with SA and H₂O₂ and hydro-priming can improve salinity tolerance.

Keywords: Abiotic stress; germination; priming; salinity; photosynthetic pigments.

1. INTRODUCTION

Wheat is a very common and demandable cereal crop in the world for its grain [1]. In Bangladesh, wheat is the second most important staple food crop after rice having an annual production of 3.3 metric tons per hectare [2]. The consumption of wheat grain for dietary purposes increasing daily and it is expected that it will increase at least 50% by 2050. However, the abiotic stresses continuously hamper agricultural production [3,4]. Among the stresses, soil salinity seriously reduces grain production [5]. Due to the ever-changing climates, salinity is increasing day by day in Bangladesh. In Bangladesh, 30% of cultivable lands are in the coastal area, which is seriously affected by soil salinization [6,7]. Soil salinity causes numerous physio-biochemical changes in plants which result in growth reduction, poor yield, and quality [8,9]. Though wheat is moderately salt tolerant, salt stress adversely affects germination percentage, growth, and yield of wheat [10]. Recently, several approaches such as seed priming, exogenous application of plant growth stimulants, and organic amendment have been implemented to mitigate the injurious effects of stresses like salinity [9,11,12]. Among the approaches, seed priming with exogenous chemicals is an easy and efficient method that is regarded as a practical approach to overcoming the detrimental effects of stresses [13,14]. Seed priming enhances seed performance by conferring positive physiological amendment leading to faster and more harmonized germination of seeds [15] apart from inducing early resistance. Adequate reports are showing that priming causes change of different cellular, subcellular and molecular processes in seeds, consequently boosting germination and growth of various

plants under diverse environmental stress conditions [16].

Seed priming with different signaling molecules has been shown positive results in different crop plants such as *Triticum aestivum* (wheat) [17], *Saccharum officinarum* (sugarcane) [15], *Helianthus annuus* (sunflower) [18], and *Abelmoschus esculentus* (okra) [19]. Seed priming and exogenous application of salicylic acid (SA) enhanced maize productivity under salt stress [20]. On the other hand, seed priming and exogenous H₂O₂ application enhanced salinity resistance in rice seedlings [21] and pretreatment with H₂O₂ also prevented the oxidative damage of wheat seedlings [22]. The effects of pretreatment with different signaling molecules and growth regulators on different plant species have been reported from time to time, however, few reports exist on the comparative effects of seed priming with SA, H₂O₂, and hydro-priming on seed germination and seedlings characteristics of wheat under salinity stress. Considering the above facts, the present experiment was undertaken to explore the potential roles of Seed priming with, SA, and H₂O₂ and hydro-priming to ameliorate the salinity-induced growth inhibition of wheat.

2. MATERIALS AND METHODS

2.1 Seed Priming with Experimental Treatments

The experiment was accomplished at the laboratory of the Department of Seed Science and Technology, Bangladesh Agricultural University, Mymensingh, using BARI-Gom 29, a high-yielding potential variety of wheat. Initially, uniform-sized seeds surface were sterilized with 1% sodium hypochlorite for 5 min followed by

washing several times with double-distilled water to sterilize the seeds. Firstly, for priming, the seeds were soaked in 1 mM, and 2 mM SA, and 0.1 mM and 0.15 mM H₂O₂ in separate screw-capped bottles, and the seeds were immersed in double-distilled water in case of hydro-priming treatment. Untreated seeds were used for control. The used concentration of SA and H₂O₂ were selected based on previous reports [20,21]. Each Petri dish contained 30 wheat seeds. Subsequently, after washing seeds several times with double-distilled water, the seeds were positioned on Petri dishes (150x20 diameter) having three layers of Whatman filter papers and kept in normal laboratory (the room temperature was 25±1°C and relative humidity was 95%) conditions. Then 15 ml of 150 mM NaCl solution was poured into each Petri dish for salt treatments. To get the non-saline condition, a Petri dish containing 20 ml distilled water acted as a control in the experiment. The following treatments were maintained: i. control ii. 150 mM NaCl iii. Hydro-primed seed iv. Hydro-primed+ salted seed v. 1 mM SA primed seed vi. 1 mM SA primed + 150 mM NaCl-treated seed vii. 2 mM SA primed seed viii. 150 mM NaCl + 2 mM SA primed seed ix. 0.1 mM H₂O₂ primed seed; x. 150 mM NaCl+ 0.1mM H₂O₂ primed seed; xi. 0.15 mM H₂O₂ primed seed; xii, 150 mM NaCl + 0.15 mM H₂O₂ primed seed. The chemicals, SA (Sigma-Aldrich), sodium hypochlorite (Sigma-Aldrich), and Hyponex (Osaka, Japan) nutrient solution were used in this study. At 12 h interval, the number of germinated seeds was recorded up to the 7th day after sowing (DAS). Seeds were counted as germinated when the radicle length was 1 mm or more. At 10 DAS, seedling characteristics like shoot and root length were and at 15 days the samples were collected for further photosynthetic pigment analysis. The experiment was performed in a completely randomized block design with three independent replicates.

2.2 Germination Indices

Germination percentage (GP), mean germination time (MGT), germination index (GI), and seedling vigor index (SVI) were determined. The number of germinated seeds was counted every morning. The appearance of plumule over the paper layer was considered as germination. GP, MGT, GI, and SVI were calculated as following the equations of Tania et al [13,19]:

$$\text{Germination percentage (GP)} = \frac{\text{Total number of seeds germinated}}{\text{Total number of seeds placed in germination}} \times 100$$

$$\text{Mean Germination Time (MGT)} = \sum \frac{Dn}{n}$$

where, n is the number of seeds germinated on day D and D is the number of days counted from beginning of germination.

$$\text{Germination index (GI)} = \frac{\text{number of germinated seeds}}{\text{day of first count}} + \dots + \frac{\text{number of germinated seeds}}{\text{day of final count}}$$

At 10 DAS, 20 seedlings from each replicate were selected randomly selected to measure shoot and root length. Seedling vigor index (SVI) was calculated with the following equations:

$$\text{Seedling vigor index (SVI)} = \text{GP} \times \frac{\text{seedling length (cm)}}{100}$$

where, seedling length= shoot length+ root length

2.3 Determination of Photosynthetic Pigment Contents

The contents of photosynthetic leaf pigments chlorophyll, lycopene, beta carotene, and carotenoids were determined spectrophotometrically based on the method described by Lichtenthaler [23]. 0.5g fresh leaves were collected into a small vial containing 10 mL of 80% ethanol. The containers were covered by aluminum foil and preserved in the dark for 7 days for extraction of pigments. The absorbance was measured from leaf extraction at 663, 645, 505, and 453 nm wavelength for chlorophyll, lycopene, beta carotene, and carotenoids contents by using a spectrophotometer (Shimadzu UV-2550, Kyoto, Japan). The photosynthetic pigments were calculated using the following equations:

$$\text{Total Chlorophyll} = \text{Chlorophyll a} + \text{Chlorophyll b}$$

$$\begin{aligned} \text{Chlorophyll a} &= 0.999 \times A_{663} - 0.0989 \times A_{645} \\ \text{Chlorophyll b} &= -0.328 \times A_{663} + 1.77 \times A_{645} \end{aligned}$$

$$\text{Lycopene} = 0.0458 \times A_{663} + 0.204 \times A_{645} + 0.372 \times A_{505} - 0.0806 \times A_{453}$$

$$\text{Beta-carotene} = 0.216 \times A_{663} - 1.22 \times A_{645} - 0.304 \times A_{505} + 0.452 \times A_{453}$$

$$\text{Carotenoids} = A_{480} + (0.114 \times A_{663} - 0.638 \times A_{645})$$

2.4 Statistical Analysis

Data collected for each parameter were subjected to a one-way ANOVA using Minitab 17.0 statistical software (Minitab Inc., State College, PA, USA). The statistical differences among the mean values of different treatments and salt stress were compared using Tukey's pair-wise comparisons ($P < 0.05$).

3. RESULTS

3.1 Seed Priming Improves Germination under Salt Stress

The effects of hydro-priming, SA-priming, and H_2O_2 -priming on germination of wheat seedlings under 150 mM NaCl stress are presented in Fig. 1. The GP and GI varied considerably among the treatments. Salinity reduced GP by 76% compared with that of the control condition (Fig. 1A). In both saline and non-saline environments, priming with water, SA and H_2O_2 showed a significant effect on GP compared to stress conditions. Whereas, priming with 2 mM SA and 0.15 mM H_2O_2 performed non-significantly with the control but significantly with salted seedlings. However, the highest GP was recorded for 0.1 mM H_2O_2 among the priming treatments and it was 56.73%. Again, under normal conditions, pretreatment with water, SA, and H_2O_2 significantly increased GI, where, GI increased by 81% and 78% for 0.1 mM H_2O_2 and control, respectively, in comparison with salted conditions (Fig. 1C). In the case of MGT priming treatments did not show any significant results compared to salt stress. Also, no significant difference among the treatments was observed under normal and saline conditions. Only hydro-priming reduced MGT by 16% compared to stress conditions (Fig. 1B).

3.2 Priming Enhances the Growth of Wheat Seedlings under Salt Stress

To evaluate the consequences of salt stress and stress alleviating functions of SA and H_2O_2 priming on the growth of wheat seedlings and to find out the effect of these agents on seedling vigor, we monitored the shoot and root length (Fig. 2A, B).

Salt stress significantly reduced shoot and root length by 28% and 14%, respectively, in wheat

seedlings compared with the non-primed normal plants. The priming with 1 mM SA showed the highest shoot length and the length was recorded as 21.27 cm. Again hydro-priming, 2 mM SA and 0.1 mM H_2O_2 increased the shoot length significantly by 45%, 46% and 47% respectively, in comparison with salt-treated plants. Moreover, under NaCl stress conditions, seed priming with 1 mM SA and 0.15 mM H_2O_2 enhanced shoot length non-significantly. Likewise, water, 1 mM SA and 2 mM SA priming increased root length significantly by 65%, 44% and 62% respectively, compared to saline condition but H_2O_2 priming did not show any significant effect on root length elongation. Moreover, salt stress significantly decreased seed vigor index (82%) compared to non-saline control plants (Fig. 2C). Priming with every agent except 0.15 mM H_2O_2 , significantly increased SVI both in saline and non-saline conditions. Anyway, among the significant treatments, the highest SVI was recorded for hydro-priming and the lowest result was found for 0.1 mM H_2O_2 . On the other hand, there was no significant difference between hydro-primed and unprimed control in the case of SVI.

3.3 Priming Regulates Photosynthetic Pigments in Wheat Seedlings under Salt Stress

A considerable variation of chlorophyll pigment content was observed as a result of salt stress (Fig. 3A). A sharp decrease in total chlorophyll content (7.62%) in wheat leaves was noticed in salt-stressed seedlings compared to the non-stressed control seedlings. Prior to salt-stress treatment, increased chlorophyll pigment content was recorded due to priming with water, SA and H_2O_2 . The maximum total chlorophyll content was observed in 2 mM SA among the priming treatments. Every agent increased total chlorophyll contents to more than that in the control condition. Under non-saline conditions, water, SA and H_2O_2 pretreatment improved total chlorophyll content significantly compared with stress-free control plants. Pigment analysis also revealed that the findings of other pigment contents named lycopene, beta-carotene, and carotenoids also responded significantly with the priming agent's application. From the obtained results we observed that lycopene, beta carotene, and carotenoids increased by 59%, 47% and 16% respectively in comparison to salt stress (Fig. 3B, C, and D).

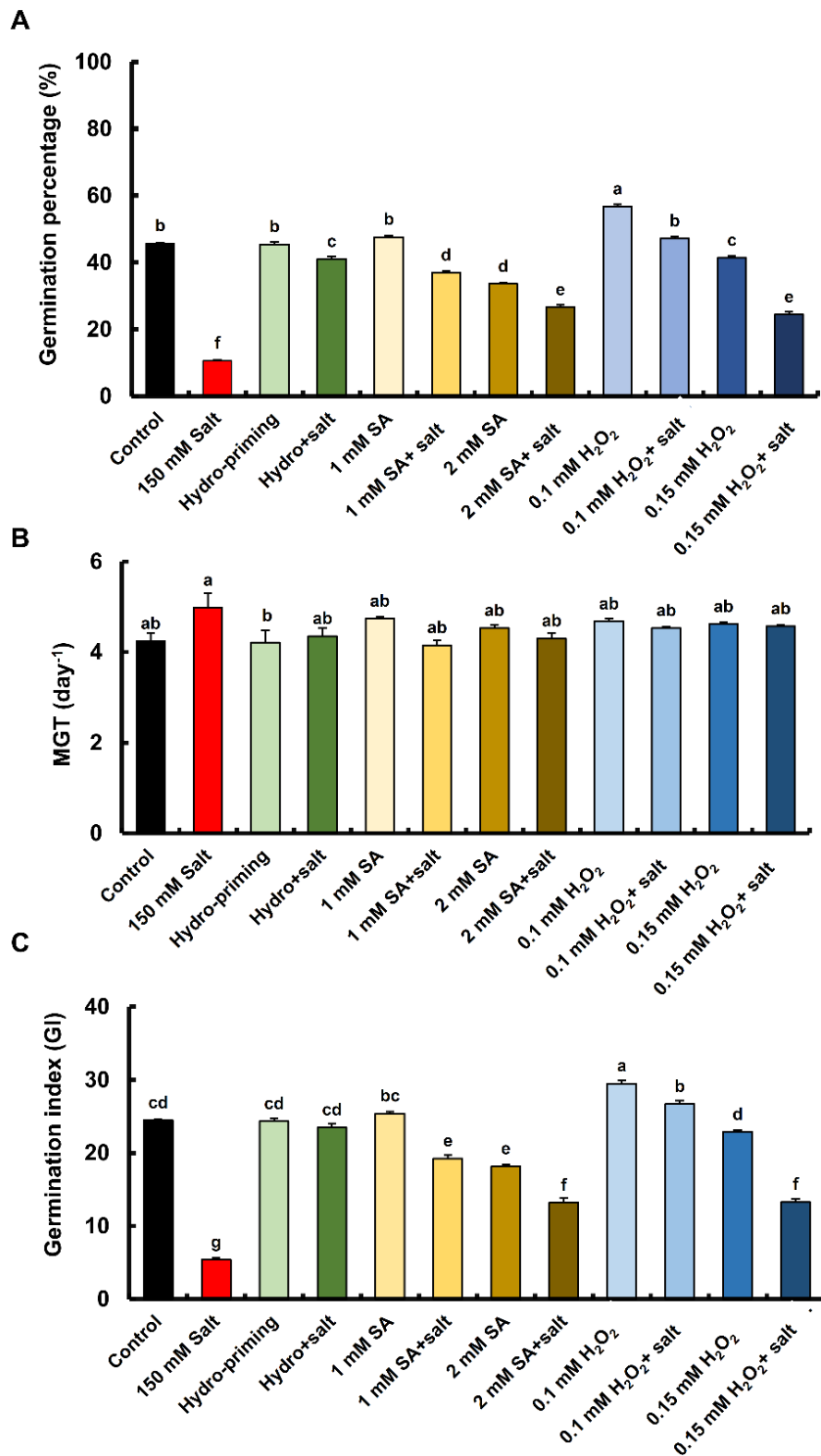


Fig. 1. Effects of seed priming with SA, H₂O₂, and hydro-priming on the germination indices of wheat under salt stress. (A) Germination percentage (B) Mean germination time (C) Germination index. The error bar represents standard error. Differences among treatments were analyzed by Tukey's test: P<0.05

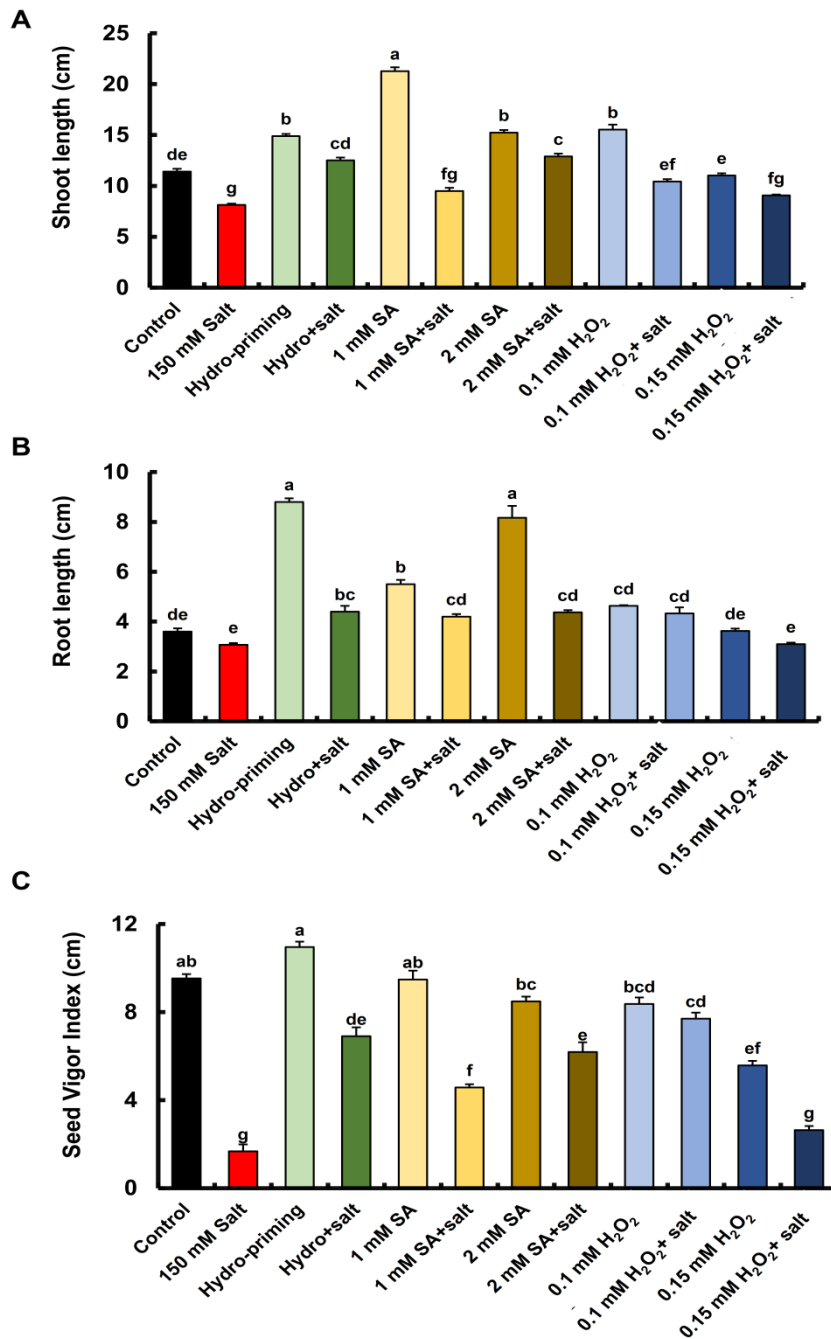


Fig. 2. Effects of seed priming with SA, H₂O₂, and hydro-priming on the seedling growth of wheat under salt stress. (A) Shoot length (B) Root Length (C) Seed Vigor Index. The error bar represents standard error. Differences among treatments were analyzed by Tukey's test: P<0.05

4. DISCUSSION

Seed germination is the fundamental and crucial step in the plant development cycle, as it plays an important role in the acclimatization of seedlings to the ever-changing environment and its subsequent productivity [14,19]. In this fact, different pre-treatment application techniques are

widely being used on the germination and seedlings establishment. Seed priming is one of the most widely used techniques that promote seed germination, enhance morphological parameters, and improve plant growth and development either under stress or normal conditions [14,24]. Seed germination, as well as plant growth, are severely hindered by salt-

induced stress and seed priming could be used to improve the salt-stress tolerance of cereals [25-27]. A similar phenomenon was also noticed in our experiment, where salinity showed a deleterious effect on germination and growth performances of wheat seedlings. Seed priming with normal water, SA and H₂O₂, has previously been testified in different crops to recover crop growth under various stressed conditions [13,28,29]. In our current experiment, seed priming with such materials in different concentrations efficiently mitigated the harmful consequences of salt-induced stress on the germination of wheat seeds (Figs. 1 and 2). Findings from our experiment showed that 0.1 mM H₂O₂ increased GP, GI, and SVI than the treatment 0.15 mM H₂O₂ which indicates that the lower concentration of H₂O₂ is more effective on wheat priming than higher concentration. These results are consistent with several studies that have found that low concentrations of signaling molecules are effective in stress alleviation [30-32].

Photosynthetic attributes such as pigments play important role in plant growth and development by regulating different mechanisms such as

transpiration by regulating stomata [20,33-35]. It has been reported that photosynthetic pigments like chlorophyll, lycopene, beta carotene, and carotenoids gave better performance due to seed priming. Our results showed that salt stress significantly reduced the photosynthetic pigments and seed priming with SA, H₂O₂ and hydro-priming enhanced the pigments content (Fig. 3). We found that 2 mM SA exhibited more pigment contents in most cases than the other priming materials even that of 1 mM SA. Similar results have been reported by several studies, they showed that reduced photosynthetic pigments might be associated with stress factor and concentration [36,37]. Overall, all priming agents improved salinity tolerance and enhanced germination and seedling growth of wheat. However, lower concentration was found more effective than higher concentration, because higher concentrations may have an inhibitory effect. After all, although, there was a difference among different priming agents due to the variation of concentration, every treatment reduced salt stress in any concentrations. Taken together the results indicate that seed priming with SA and H₂O₂ improves seed germination and seedlings' growth under salt stress.

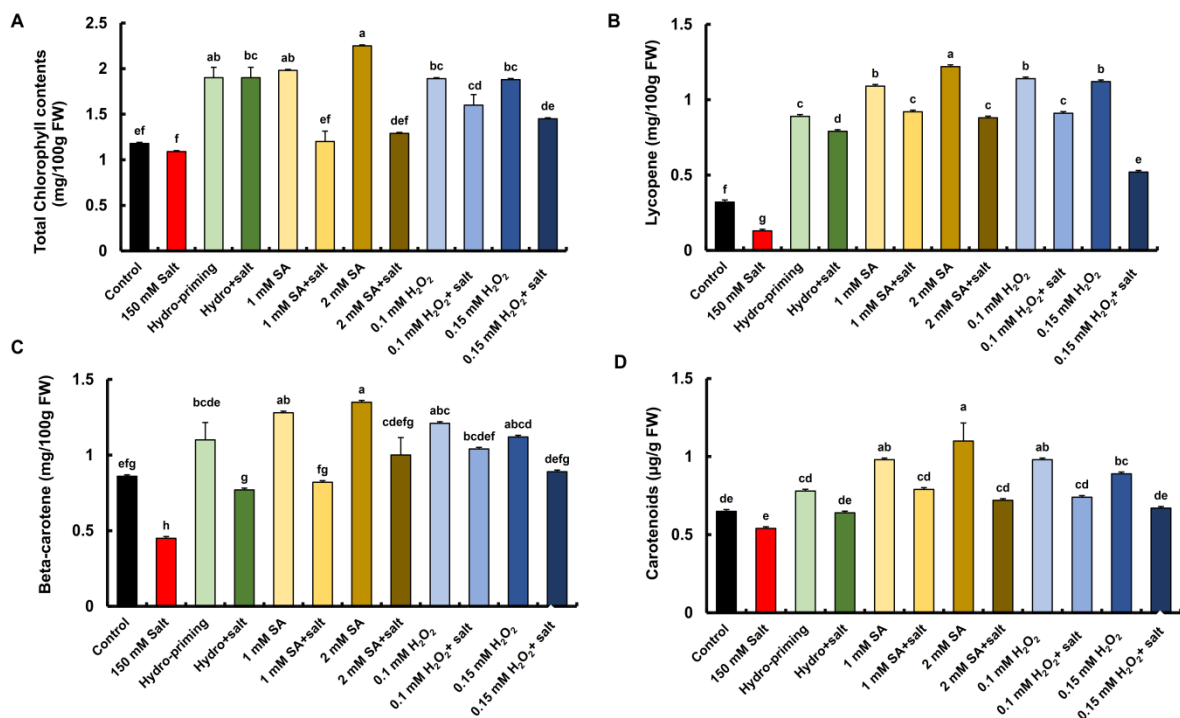


Fig. 3. Effects of seed priming with SA, H₂O₂, and hydro-priming on the photosynthetic pigments of wheat under salt stress. (A) Total chlorophyll (B) Lycopene (C) Beta-carotene (D) Carotenoids. The error bar represents standard error. Differences among treatments were analyzed by Tukey's test: P<0.05

5. CONCLUSION

It is concluded that seed priming with SA and H₂O₂ and hydro-priming enhances wheat seed germination, seedling growth, and photosynthetic pigments. Our results also suggest that 1 mM SA or 0.1 mM H₂O₂ or hydro-priming can be used for successful wheat production. However, it is highly recommended to conduct the same experiment at the field level to validate our results.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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