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ROLE OF ASCORBIC ACID AS A SEED PRIMING AGENT IN ALLEVIATING SALT INDUCED DAMAGES IN GERMINATION AND EARLY SEEDLING GROWTH OF RICE (*Oryza sativa* L.)

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Abstract

Salinity is one of the major brutal abiotic stresses that affect crop productivity worldwide. Seed priming with different bio-regulators has emerged as an effective and practical approach to inducing the plant tolerance against different abiotic stress including salinity. Therefore, a study was conducted at the Agroforestry Field Laboratory, Sher-e-Bangla Agricultural University, Dhaka during July 2022 to September 2022 to find out potential of seed priming with ascorbic acid inalleviation of germination and growth inhibition of rice (*Oryza sativa* L.) seedlings under salt stress. The study included four levels ascorbic acid *viz*. i) 0 mM (A_0), ii) 0.125 mM (A_1) iii) 0.250 mM (A_2) iv) 0.5 mM (A_3) for seed priming and four levels of salinity: i) 0 dS m^{-1} (S0), ii) 5 dS m^{-1}(S1), iii) 10 dS m^{-1}(S2), iv) 15 dS m^{-1} (S3) NaCl set up in petridishes and pots. Salinity levels significantly affected germination and growth attributes like germination percentage, germination speed, germination energy, radical length, plumule length, plant height, plant fresh weight, plant dry weight, SPAD value and leaf relative water content. However, ascorbic acid primed seeds provided higher germination percentage, germination speed, germination energy, radical length, plumule length, plant height, plant fresh weight, plant dry weight, SPAD value and leaf relative water content in contrast to respective salt stress. Among the doses of ascorbic acids 0.250 mM and 0.5 mM performed better than the 0.125 mM. Thus, this study suggests that use of ascorbic acid as a seed priming agent with appropriate dose can enhance the tolerance of rice seedlings against salt stress.

Keywords: Ascorbic acid, Germination and growth, Salinity stress, Seed priming.

Introduction

Crop production is vulnerable to climate variability, and climate change associated with increases in different abiotic stresses which may lead to a considerable decline in crop yield (Mall *et al*. 2017). Abiotic stresses such as salinity, drought, flooding, heat, cold, freezing, excess light, UV radiation, and heavy metal toxicity have a significant impact on seed which reduce germination rate, seedling growth and yield with

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significant variations from crop to crop worldwide (Zhao *et al*., 2007). Among them salinity is one of the major abiotic stresses that affect crop production in arid and semiarid areas (Ibrahim, 2016) including southern part of Bangladesh. Salt stress adversely affects physiology and biochemistry of plants primarily by creating osmotic stress, ionic imbalance, and toxicity (Hajlaoui *et al.*, 2009). As Reactive oxygen species (ROS) are responsible for damaging plant cells under salt stress, elimination of these toxic compounds is considered a strategy for developing salt stress tolerance. Seed priming has emerged as an effective and practical approach to induce the plant tolerance against different stress factors including salinity. Priming of seed enables the faster and better germination in plants under stressful conditions as they have potential to upregulate the antioxidant defense system.

Different cellular and metabolic events are involved in induction of salinity tolerance after seed priming. Primed seeds can activate the signal pathways during the early growth stage and triggered the faster stress response. Faster emergence and uniform stand establishment in primed seeds ultimately increases the crop productivity under salinity conditions (Hussain *et al*., 2022). Several priming strategies have been tested in rice under salinity stress. For example, bio-priming with *Trichoderma harzianum* alleviates salinity stress by improving physiological and biochemical traits (Rawat *et al*., 2012). Halo-priming or osmo-priming with salts such as potassium nitrate (KNO_3) or calcium chloride ($CaCl_2$) enhances germination and seedling establishment in saline environments (Ahmandvand *et al*., 2012; Farooq *et al*., 2019). Even low-cost hydro-priming (soaking seeds in water followed by drying) has proven effective in improving germination and vigor under stress (Casenave and Toselli, 2007; Afzal *et al*., 2005). More recently, nano-priming approaches using chitosan nanoparticles (CNPs) have shown superior enhancement of germination and seedling vigor in rice under salinity stress (Abdel-Aziz *et al*., 2019).

Despite the availability of these techniques, ascorbic acid (AsA) remains particularly attractive because of its dual role as a potent antioxidant and signaling molecule. AsA regulates enzymatic and non-enzymatic antioxidant activities, modulates gene expression, and improves stress adaptation (Roy *et al*., 2016). Furthermore, experimental studies on different plants have showed that exogenous application of AsA may reduce salt-induced adverse effects and significant increment of growth and yield (Athar *et al.*, 2009; Salama, 2009; Khan *et al*. 2010). Although there have been few studies regarding the impact of ascorbic acid as seed priming agent on alleviating salt stress in a variety of plants, but no investigation of the roles of ascorbic acid priming agent in seed germination, growth, physiology and yield of aman rice variety under salt stress has been reported. Therefore, this study was designed to investigate the roles of ascorbic acid as priming agents at different concentrations in seed germination, seedling growth and physiological performance of rice (*Oryza sativa* L.) of *T. aman* variety) under different salinity conditions.

Materials and Methods

Experimental site

The study was conducted from July to September 2022 at the Field Laboratory, Department of Agroforestry and Environmental Science, Sher-e-Bangla Agricultural University, Dhaka, Bangladesh. It included both laboratory (petri-dish) and pot experiments. The site, situated at 23°74'N and 90°35'E, 8 m above sea level, lies in the "Madhupur Tract" agro-ecological zone (AEZ-28).

Climate and soil

The Field Laboratory experiences a subtropical climate, marked by hot, humid, and rainy summers with gusty monsoon winds, and cooler, drier winters with scattered rainfall.

Planting materials

Rice var. BRRI dhan78 (slightly salinity tolerant) seed was collected from BRRI, Gazipur on June, 2022.

Treatments of the experiment

The following treatments were maintained in this study for each studies. Priming treatments: i) 0 mM (A0), ii) 0.125 mM ((A1)) iii) 0.250 mM (A2) iv) 0.5 mM (A3) ascorbic acid. Before sowing, seeds were sterilized with 70% ethanol, then primed with ascorbic acid for 12 hours. Control seeds were soaked in distilled water for the same duration. After treatment, all seeds were rinsed three times, dried on filter paper, and air-dried for 48 hours at room temperature.Salinity treatments: i) 0 dS m-1 (S0), ii) 5 dS m-1(S1), iii) 10 dS m-1(S2), iv) 15 dS m-1 (S3) NaCl were maintained in growing media of both experiments. So, the total no. of treatments was 16. The treatments were: i. A0S0, ii. A0S1, iii. A0S2, iv. A0S3, v. A1S0, vi. A1S1, vii. A1S2, viii. A1S3, ix. A2S0, x. A2S1, xi. A2S2, xii. A2S3, xiii. A3S0, xiv. A3S1, xv. A3S2, xvi. A3S3

Design and layout of the experiment

July to September 2022, two experiments were conducted to assess ascorbic acid seed priming effects on rice (*Oryza sativa* L.) under salinity. Both experiments were laid out in Completely Randomized Design (CRD) with 16 treatment combinations based on four ascorbic acid levels and four salinity levels, each with three replications (48 pots) Germination was tested in the lab (petri-dish), and morpho-physiology and early seedling growth were evaluated in the pot experiment.

Petri-dish and pot preparation

The experiment was conducted in a Completely Randomized Design (CRD) with three replications. In the petri-dish experiment, 9 cm dishes with six layers of blotting paper were used, and 20 seeds per dish were considered an experimental unit. Germination parameters were recorded for all seeds within each dish. In the pot experiment, pots (35 cm height × 30 cm top diameter × 20 cm bottom diameter) were filled with sun-dried soil mixed with recommended organic manures, fertilizers, and Furadan 5G. Ten seedlings were sown per pot, later thinned for uniform growth, and each pot was considered an experimental unit. Growth and physiological parameters, including plant height, biomass, SPAD, and leaf RWC, were recorded on the plants within each pot.

Manure and fertilizer application

In the petri-dish experiment, a nutrient solution of Hyponex (Japan) was used; its composition included N, P_2O_5, and K_2O at 6%, 10%, and 5%, respectively, along with essential micronutrients such as calcium and trace elements. For the pot experiment, soil was mixed with well-decomposed cow dung and supplemented with additional nutrients, with fertilizer doses calculated based on field rates per bigha according to the fertilizer recommendation by BRRI for BRRI dhan 78. Each pot received the recommended doses of urea (3.0 g), TSP (2.0 g), MoP (1.25 g), gypsum (1.2 g), and zinc (0.15 g) before seedling transplantation.

Application of salt treatment

Rice plant is evaluated in different level of salt treatment. S_0, S_1, S_2 and S_3 indicate 0, 5, 10 and 15 dSm^{-1} NaCl, respectively.

Intercultural operations

Irrigation

After transplanting, seedlings were given light watering immediately to prevent water stress, followed by regular watering every two days.

Weeding

Weeding was carried out to maintain clean plots and ensure proper soil aeration, promoting better growth. Newly emerged weeds were carefully removed, and mulching was applied as needed to break soil crust.

Data recording

Data were recorded from each pot based on growth and yield parameters. Data were recorded in respect of the following parameters:

Petri-dish experiment

Germination parameters

Germination percentage, speed of germination, germination energy, radical and plumule length were measured (for first experiment).Final germination percent (FGP), speed of germination (SG) and germination energy percentage (GE %) were calculated by the following formulae (Ellis and Robert, 1981; Ruan *et al*., 2002).

$$SG = \frac{\text{Number of germinated seeds}}{\text{Days of first count}} + \ldots\ldots\ldots + \frac{\text{Number of germinated seeds}}{\text{Days of final count}}$$

$$GE\ (\%) = \frac{\text{Number of germinated seeds at 4 DAS}}{\text{Total number of seed tested}} \times 100$$

$$FGP = \frac{\text{Number final germinated seeds}}{\text{Total number of seed tested}} \times 100$$

Plumule and radicle length:Plumule and radicle length were measured in cm basis.

Pot experiment

Plant height

Plant height (cm) was measured at 15 and 30 DAS from each replication were averaged to obtain mean plant height.

Plant fresh weight

Fresh weight (mg) was measured after uprooting plants at 15 and 30 DAS using an electronic balance.

Plant dry weight

Dry weight (mg) was recorded after drying plants in an oven at 70°C for 48 hours at 15 and 30 DAS.

SPAD value

SPAD value was estimated using a portable SPAD 502 Plus meter (Konica-Minolta, Tokyo, Japan) at 15 and 30 DAS. Each leaf was measured five times from tip to base, and the average SPAD value was used.

Leaf relative water content (RWC)

RWC (%) was determined following Barrs and Weatherly (1962) at 15 and 30 DAS. Leaf laminas were weighed for fresh weight (FW), floated on distilled water for 8 h to get turgid weight (TW), and then dried at 80°C for 48 h for dry weight (DW). RWC was calculated as: RWC (%) = [(FW-DW)/ (TW-DW)] ×100

Statistical analysis

Data were analyzed using Statistix 10. Data for all parameters were subjected to analysis of variance (ANOVA) to determine the effects of ascorbic acid priming (AsA) and salinity level (NaCl) alone and in combination, using the statistical software Statistix 10. Where the ANOVA indicated significant F-values ($p \leq 0.05$), treatment means were separated using Tukey's Honest Significant Difference (HSD) test.

Results and Discussion

Final germination percentage (FGP)

Germination of rice seeds declined with increasing salt stress, with the lowest value in S_3. In non-primed seeds, germination was 96.3, 92.0, 81.3 and 51.3% under S_0,

S_1, S_2 and S_3, respectively representing 4.5, 12.5 and 46.7% reductions compared to control. Ascorbic acid priming significantly improved germination under all stress levels (Fig. 1), with the greatest gains from A_2 and A_3. Under S_3, germination increased by 43.7% with A_2 and 44.8% with A_3 compared to the respective control.

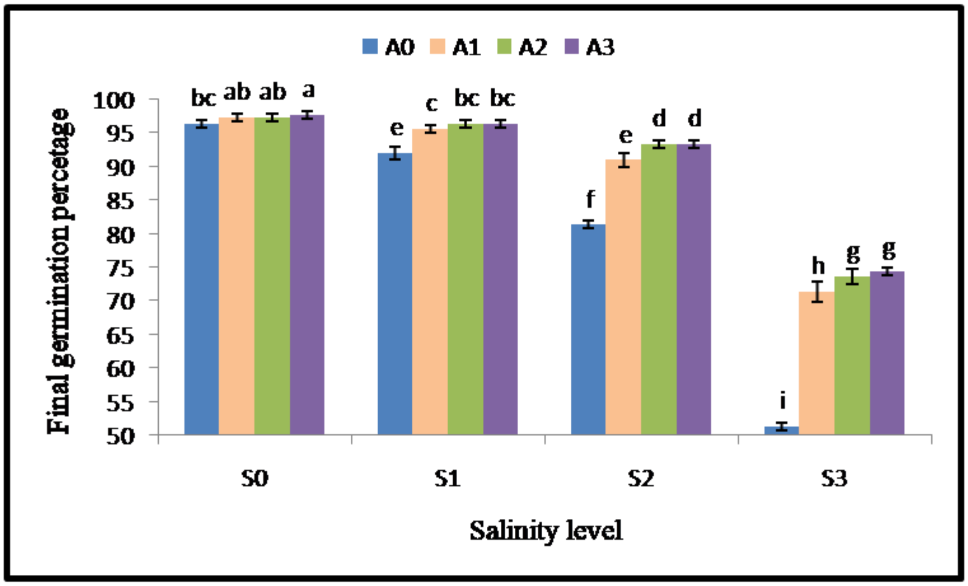

Fig. 1. Effect of ascorbic acid as seed priming agent on final germination percentage of rice under different levels of salt stress. Here, A_0, A_1, A_2, A_3 indicate 0 mM, 0.125 mM, 0.250 mM, 0.5 mM ascorbic acid, respectively, and S_0, S_1, S_2, S_3 indicate 0 dS m^{-1}, 5 dS m^{-1}, 10 dS m^{-1}, 15 dS m^{-1} NaCl stress, respectively. Bars sharing the same letter within a salinity level are not significantly different according to Tukey's HSD test ($p \leq 0.05$). Bars with different letters indicate significant differences at $p \leq 0.05$ according to Tukey HSD test.The interaction effect (AsA x NaCl) was significant ($p \leq 0.05$)

Speed of germination (SG)

Salinity delayed rice seed germination, reducing germination speed in a dose-dependent manner. In non-primed seeds, germination speed was 20.2, 18.2, 12.1 and 3.0 for S_0, S_1, S_2 and S_3, respectively, representing reductions of 9.9, 40.1 and 85.1% compared to control. Ascorbic acid priming accelerated germination under all stress levels (Fig. 2). Under S_1 and S_2, A_2 and A_3 performed similarly, while under S_3, A_3 showed the highest improvement, increasing germination speed by 336.7% compared to the respective control followed by A_2 with 303.3%.

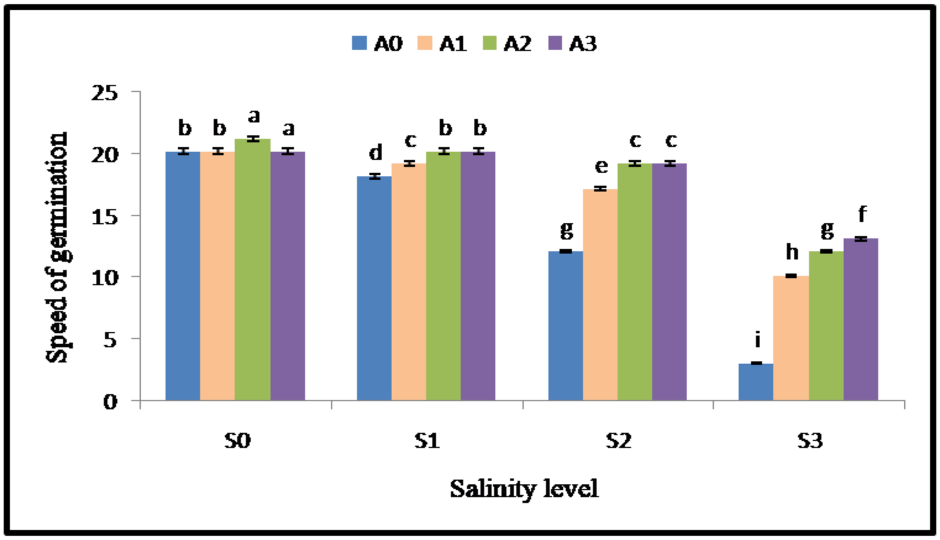

Fig. 2. Effect of ascorbic acid as seed priming agent on speed of germination of rice under different levels of salt stress. Here, A_0, A_1, A_2, A_3 indicate 0 mM, 0.125 mM, 0.250 mM, 0.5 mM ascorbic acid, respectively, and S_0, S_1, S_2, S_3 indicate 0 dS m^{-1}, 5 dS m^{-1}, 10 dS m^{-1}, 15 dS m^{-1} NaCl stress, respectively. Bars sharing the same letter within a salinity level are not significantly different according to Tukey's HSD test ($p \leq 0.05$). Bars with different letters indicate significant differences at $p \leq 0.05$ according to Tukey HSD test.The interaction effect (AsA x NaCl) was significant ($p \leq 0.05$)

Germination energy percentage (GE %)

Germination energy (GE%) measured on the 4th day after soaking declined significantly with increasing salinity, reaching 0% in S_3 under non-primed conditions. Ascorbic acid priming improved GE% under all stress levels (Fig.3), including S_3, where A_1, A_2, and A_3 achieved 24.0, 33.3, and 35.0%, respectively. The maximum GE (77.3%) was observed in A_3S_0, followed by A_2S_0.

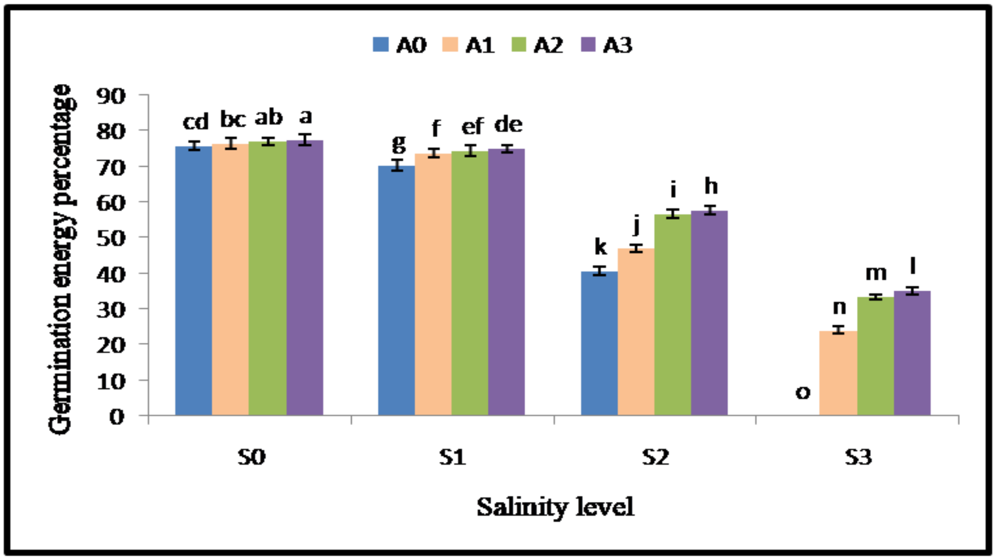

Fig. 3. Effect of ascorbic acid as seed priming agent on germination energy percentage of rice under different levels of salt stress. Here, A_0, A_1, A_2, A_3 indicate 0 mM, 0.125 mM, 0.250 mM, 0.5 mM ascorbic acid, respectively, and S_0, S_1, S_2, S_3 indicate 0 dS m^{-1}, 5 dS m^{-1}, 10 dS m^{-1}, 15 dS m^{-1} NaCl stress, respectively. Bars sharing the same letter within a salinity level are not significantly different according to Tukey's HSD test ($p \leq 0.05$). Bars with different letters indicate significant differences at $p \leq 0.05$ according to Tukey HSD test.The interaction effect (AsA x NaCl) was significant ($p \leq 0.05$)

Plumule length

Plumule length of rice declined under all salt treatments, with greater reductions at higher salinity. In non-primed seeds, plumule length was 5.6, 4.5, 3.1 and 2.0 cm for S_0, S_1, S_2 and S_3, representing reductions of 19.6, 29.0 and 64.3% compared to control. Ascorbic acid priming improved plumule length under all stress levels (Fig. 4). Under S_1, S_2 and S_3, A_1 increased plumule length by 6.7–35.0%, A_2 by 13.5–70.0%, and A_3 by 17.8–80.0% relative to respective stress treatments.

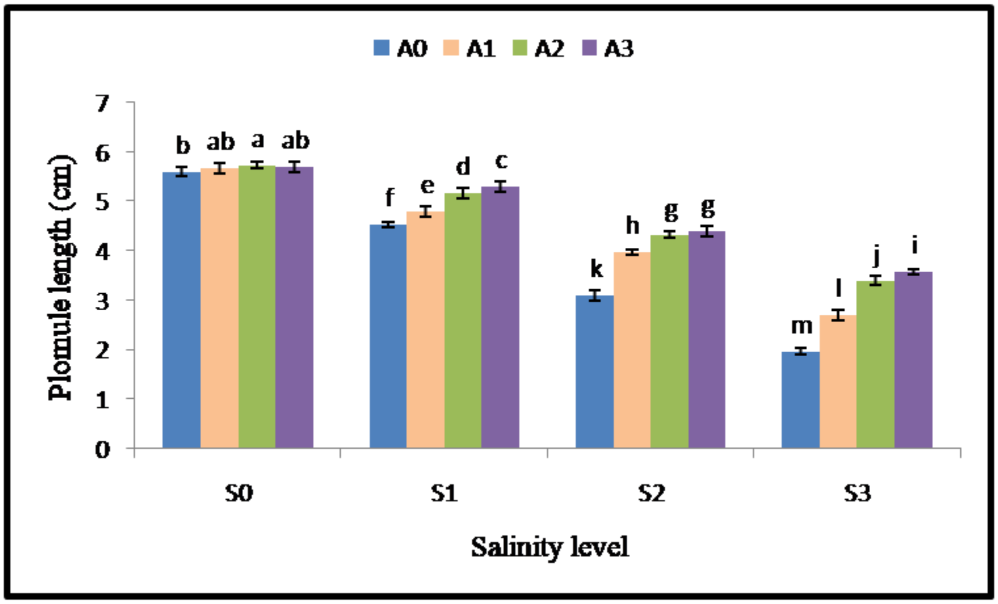

Fig. 4. Effect of ascorbic acid as seed priming agent on plumule length of rice under different levels of salt stress. Here, A_0, A_1, A_2, A_3 indicate 0 mM, 0.125 mM, 0.250 mM, 0.5 mM ascorbic acid, respectively, and S_0, S_1, S_2, S_3 indicate 0 dS m^{-1}, 5 dS m^{-1}, 10 dS m^{-1}, 15 dS m^{-1} NaCl stress, respectively. Bars sharing the same letter within a salinity level are not significantly different according to Tukey's HSD test ($p \leq 0.05$). Bars with different letters indicate significant differences at $p \leq 0.05$ according to Tukey HSD test.The interaction effect (AsA x NaCl) was significant ($p \leq 0.05$)

Radicle length

Similar to plumule length, radicle length decreased with increasing salinity, showing greater suppression than plumule at each salt level. In non-primed seeds, radicle length was 5.7, 3.2, 1.8, and 0.9 cm for S_0, S_1, S_2, and S_3, representing reductions of 19.6, 46.6 and 64.3% compared to control. Ascorbic acid priming improved radicle length under all stress levels (Fig. 5). Under S_1, S_2, and S_3, A_1 increased radicle length by 25.0, 33.3 and 33.3%, A_2 by 40.6, 77.8 and 111.1%, and A_3 by 43.8, 77.8 and 111.1% relative to the respective stress treatments.

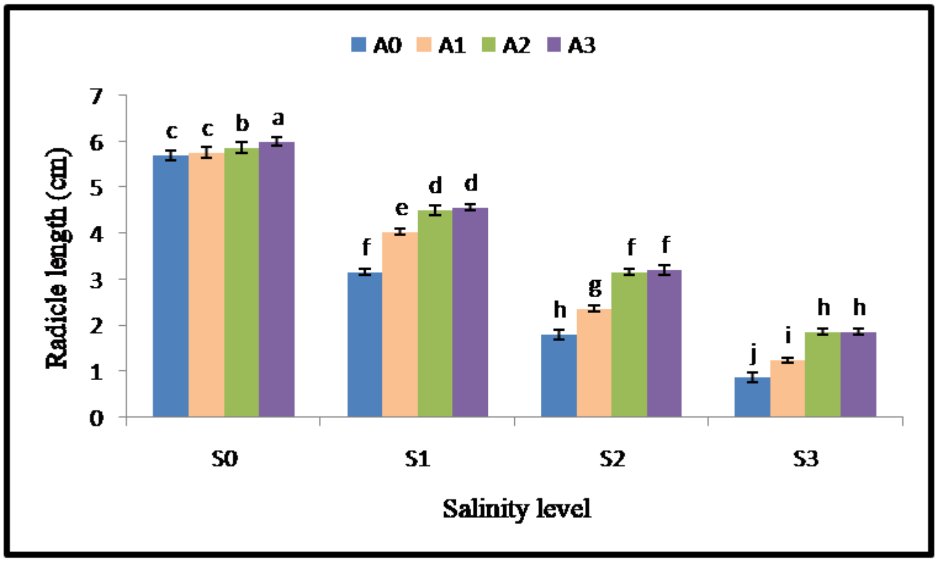

Fig. 5. Effect of ascorbic acid as seed priming agent on radicle length of rice under different levels of salt stress. Here, A_0, A_1, A_2, A_3 indicate 0 mM, 0.125 mM, 0.250 mM, 0.5 mM ascorbic acid, respectively, and S_0, S_1, S_2, S_3 indicate 0 dS m^{-1}, 5 dS m^{-1}, 10 dS m^{-1}, 15 dS m^{-1} NaCl stress, respectively. Bars sharing the same letter within a salinity level are not significantly different according to Tukey's HSD test ($p \leq 0.05$). Bars with different letters indicate significant differences at $p \leq 0.05$ according to Tukey HSD test.The interaction effect (AsA x NaCl) was significant ($p \leq 0.05$)

Plant height

Plant height declined under all salt stress levels in a dose-dependent manner at both 15 and 30 DAS (Fig. 6), with the greatest reduction in S_3. In non-primed plants, height was 22.2, 21.3, 19.1 and 17.4 cm at 15 DAS, and 41.1, 35.1, 32.1 and 28.1 cm at 30 DAS for S_0, S_1, S_2 and S_3, respectively, representing 4.1,13.9 and 21.6% and 14.6, 21.9 and 31.6% reductions compared to control. Ascorbic acid priming improved plant height under stress, with A_2 and A_3 performing better than A_1 at 15 DAS, and A_3 showing the greatest improvement at 30 DAS, increasing height by 14.2, 21.8 and 21.4% under S_1, S_2 and S_3 stress.

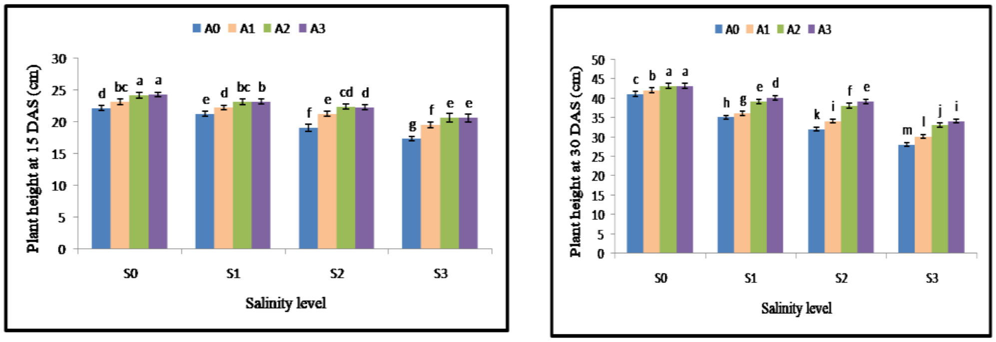

Fig. 6. Effect of ascorbic acid as seed priming agent on plant height of rice at 15 and 30 DAS (days after sowing) under different levels of salt stress. Here, A_0, A_1, A_2, A_3 indicate 0 mM, 0.125 mM, 0.250 mM, 0.5 mM ascorbic acid, respectively, and S_0, S_1, S_2, S_3 indicate 0 dS m^{-1}, 5 dS m^{-1}, 10 dS m^{-1}, 15 dS m^{-1} NaCl stress, respectively. Bars sharing the same letter within a salinity level are not significantly different according to Tukey's HSD test ($p \leq 0.05$). Bars with different letters indicate significant differences at $p \leq 0.05$ according to Tukey HSD test.The interaction effect (AsA x NaCl) was significant ($p \leq 0.05$)

Fresh weight of plant

Plant fresh weight declined under all salt treatments in a dose-dependent manner at both 15 and 30 DAS, with the highest reduction in S_3 (Fig. 7). In non-primed plants, fresh weight was 152.3, 142.3, 123.2 and 103.0 mg at 15 DAS, and 2067.3, 1851.3, 1553.3 and 1302.0 mg at 30 DAS for S_0, S_1, S_2 and S_3, respectively, showing reductions of 6.6, 19.1 and 32.4% and 10.4,24,9 and 37.0% compared to control. Ascorbic acid priming increased fresh weight under all stress levels. At 15 DAS, A_1, A_2 and A_3 increased fresh weight by 2.8, 6.5 and 6.9%, 5.6, 10.6 and 19.6% and 5.9, 11.4 and 22.5%, respectively, under S_1, S_2 and S_3 stress, with a similar trend at 30 DAS.

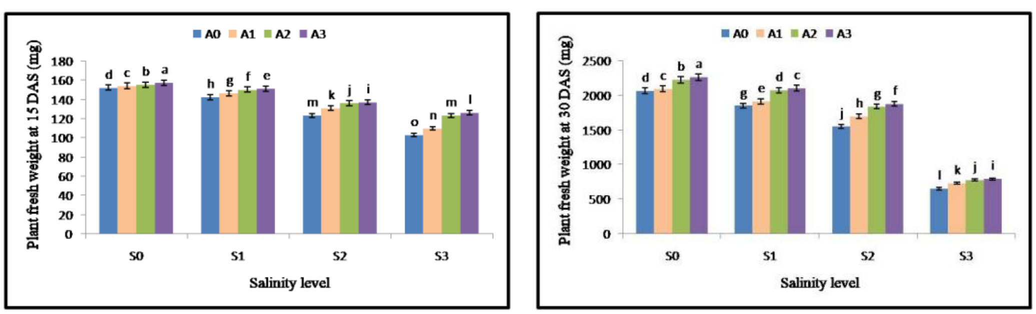

Fig. 7. Effect of ascorbic acid as seed priming agent on plant fresh weight of rice at 15 and 30 DAS (days after sowing) under different levels of salt stress. Here, A_0, A_1, A_2, A_3 indicate 0 mM, 0.125 mM, 0.250 mM, 0.5 mM ascorbic acid, respectively, and S_0, S_1, S_2, S_3 indicate 0 dS m^{-1}, 5 dS m^{-1}, 10 dS m^{-1}, 15 dS m^{-1} NaCl stress, respectively. Bars sharing the same letter within a salinity level are not significantly different according to Tukey's HSD test ($p \leq 0.05$). Bars with different letters indicate significant differences at $p \leq 0.05$ according to Tukey HSD test.The interaction effect (AsA x NaCl) was significant ($p \leq 0.05$)

Dry weight of plant

Like fresh weight, plant dry weight also declined under salinity in a dose-dependent manner at both 15 and 30 DAS (Fig. 8), with the greatest reduction in S_3. In non-primed plants, dry weight was 19.3, 18.4, 16.0 and 13.4 mg at 15 DAS, and 288.2, 267.7, 224.4 and 189.2 mg at 30 DAS for S_0, S_1, S_2 and S_3, respectively, representing 4.7,17.1 and 30.6% and 7.1, 22.1 and 34.4% reductions compared to control. Ascorbic acid priming improved dry weight under all stress levels. At 15 DAS, A_1, A_2, and A_3 increased dry weight by 3.3,6.3 and 6.7%, 6.0,10.6 and 19.4%, and 7.1,11.3 and 21.6%, respectively, under S_1, S_2 and S_3 stress, with a similar trend at 30 DAS.

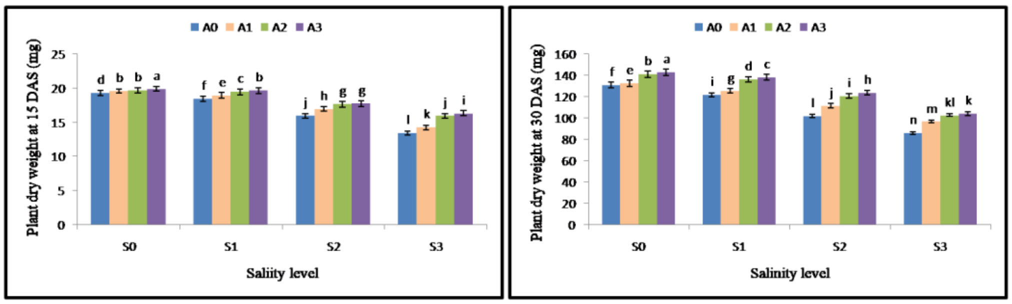

Fig. 8. Effect of ascorbic acid as seed priming agent on plant dry weight of rice at 15 and 30 DAS (days after sowing) under different levels of salt stress. Here, A_0, A_1, A_2, A_3 indicate 0 mM, 0.125 mM, 0.250 mM, 0.5 mM ascorbic acid, respectively, and S_0, S_1, S_2, S_3 indicate 0 dS m^{-1}, 5 dS m^{-1}, 10 dS m^{-1}, 15 dS m^{-1} NaCl stress, respectively. Bars sharing the same letter within a salinity level are not significantly different according to Tukey's HSD test ($p \leq 0.05$). Bars with different letters indicate significant differences at $p \leq 0.05$ according to Tukey HSD test.The interaction effect (AsA x NaCl) was significant ($p \leq 0.05$)

Leaf SPAD value

SPAD value declined under salt stress in a dose-dependent manner, with the greatest reduction in S_3 at both 15 and 30 DAS (Fig. 9).In non-primed plants, SPAD values were 41.0, 39.7, 35.0 and 31.0 at 15 DAS, and 45.3, 44.0, 38.7 and 34.0 at 30 DAS for S_0, S_1, S_2 and S_3, respectively, showing reductions of 3.2, 14.6 and 24.4% compared to control. Ascorbic acid priming improved SPAD under stress. At 15 DAS, A_1, A_2, and A_3 increased SPAD by 3.3,4.9 and 7.4%, 5.8,14.3 and 17.1%, and 6.5,16.3 and 19.4%, respectively; at 30 DAS, the increases were 3.0,4.1 and 7.9%, 5.2,14.5 and 17.6%, and 6.1, 16.3 and 20.6%, respectively, under S_1, S_2 and S_3 stress.

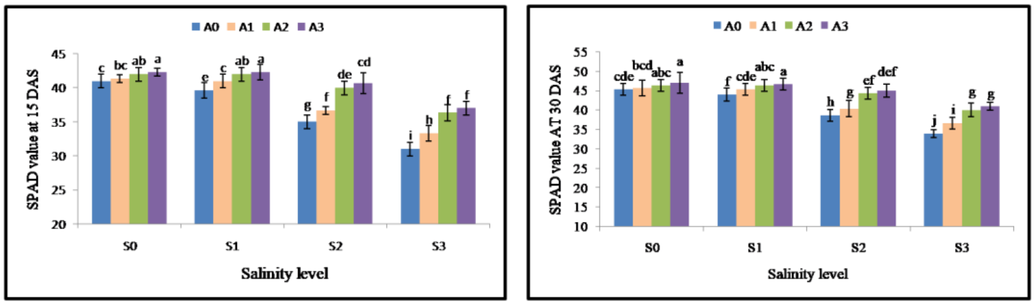

Fig. 9. Effect of ascorbic acid as seed priming agent on SPAD value of rice at 15 and 30 DAS (days after sowing) under different levels of salt stress. Here, A_0, A_1, A_2, A_3 indicate 0 mM, 0.125 mM, 0.250 mM, 0.5 mM ascorbic acid, respectively, and S_0, S_1, S_2, S_3 indicate 0 dS m^{-1}, 5 dS m^{-1}, 10 dS m^{-1}, 15 dS m^{-1} NaCl stress, respectively. Bars sharing the same letter within a salinity level are not significantly different according to Tukey's HSD test ($p \leq 0.05$). Bars with different letters indicate significant differences at $p \leq 0.05$ according to Tukey HSD test.The interaction effect (AsA x NaCl) was significant ($p \leq 0.05$)

Leaf relative water content (RWC) (%)

Leaf relative water content (RWC), an indicator of plant water status, declined significantly under salt stress in a dose-dependent manner at both 15 and 30 DAS, with the greatest reduction in S_3 (Figure 10). In non-primed plants, RWC was 97.5, 93.5, 87.5 and 82.4% at 15 DAS, and 94.9, 91.0, 85.1 and 80.3% at 30 DAS for S_0, S_1, S_2 and S_3, respectively, representing reductions of 4.1, 10.3 and 15.5% compared to the control. Ascorbic acid priming improved RWC under all stress levels. At 15 DAS, A_1 increased RWC by 1.9, 3.4 and 2.4%, A_2 by 4.0, 6.9 and 7.4%, and A_3 by 4.4, 7.1 and 8.6% under S_1, S_2 and S_3 stress, with similar trends at 30 DAS.

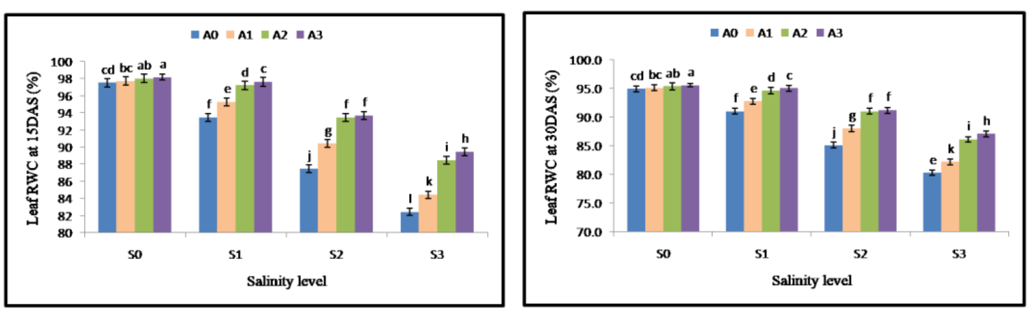

Fig. 10. Effect of ascorbic acid as seed priming agent on leaf relative water content of rice at 15 and 30 DAS (days after sowing)under different levels of salt stress. Here, A_0, A_1, A_2, A_3 indicate 0 mM, 0.125 mM, 0.250 mM, 0.5 mM ascorbic acid, respectively, and S_0, S_1, S_2, S_3 indicate 0 dS m^{-1}, 5 dS m^{-1}, 10 dS m^{-1}, 15 dS m^{-1} NaCl stress, respectively. Bars sharing the same letter within a salinity level are not significantly different according to Tukey's HSD test ($p \leq 0.05$). Bars with different letters indicate significant differences at $p \leq 0.05$ according to Tukey HSD test.The interaction effect (AsA x NaCl) was significant ($p \leq 0.05$)

Discussion

Seed germination, speed of germination, and germination energy are fundamental processes for successful crop establishment, ultimately determining yield potential (Almansouri *et al*., 2001; Murungu *et al*., 2003). These processes are often severely hampered under abiotic stress conditions in most crops (Omid *et al*., 2012). Among all developmental stages, seed germination is considered the most sensitive to environmental stresses, including salinity (Omid *et al*., 2012; Redmann, 1974).

In the present study, rice seeds were subjected to different levels of salt stress, and the mitigating role of ascorbic acid (AsA) priming was evaluated. Our results (Figures 1–3) show that the tested rice variety was sensitive to salinity, exhibiting marked reductions in final germination percentage, germination speed, and germination energy with increasing NaCl concentration. However, seed priming with AsA (0.125, 0.250, and 0.5 mM) significantly improved germination traits under all stress levels. The observed improvement is consistent with the known role of AsA as a non-enzymatic antioxidant that scavenges reactive oxygen species (ROS) and protects cellular components during germination (Baig *et al*., 2021). Specifically, the significant enhancement of radicle length under AsA priming (Figure 5) indicates that AsA protects the root-tip meristem from oxidative damage, which is essential for early root elongation (Afzal *et al*., 2005). Similarly, plumule and radicle lengths were substantially reduced under higher salinity, resulting in shorter seedlings. AsA priming partially restored these growth parameters (Figures4 and 5), likely due to its role in maintaining osmotic balance and promoting antioxidant defense, which supports cell division and elongation in young tissues. This aligns with previous findings that AsA can stabilize cell membranes and maintain turgor under stress, leading to improved shoot and root growth (Saboora and Kiarostami, 2006; Gupta and Srivastava, 1989).

Plant height, fresh weight, and dry weight were also adversely affected by salinity (Fig.6-8). The partial recovery of these growth parameters in AsA-primed plants can be explained by enhanced metabolic activity and improved biomass accumulation, as primed seeds exhibit faster germination and uniform seedling establishment. These findings are consistent with reports that priming accelerates seedling vigor and increases shoot and root biomass under stress (Ghoulam and Fares, 2001; Salim, 1991). In addition to growth, physiological traits such as SPAD value and leaf relative water content (RWC) were significantly reduced by salinity (Figures 9 and 10). The maintenance of chlorophyll in AsA-primed plants can be attributed to the antioxidant role of AsA, which prevents pigment degradation and protects pigment–protein complexes. Likewise, the improved RWC in primed plants suggests better osmotic regulation and water retention, helping plants maintain turgor and cellular hydration under salt stress. These physiological improvements clearly correlate with the enhanced growth and biomass observed in AsA-primed seedlings. These results demonstrate that salinity negatively impacts seed germination, seedling growth, and key physiological processes in rice. Exogenous application of AsA through seed priming mitigates these effects by enhancing antioxidant defense, protecting meristematic tissues, maintaining osmotic balance, and supporting chlorophyll and water status. The significant improvements observed in germination traits, radicle and plumule lengths, plant height, biomass, SPAD values, and RWC directly reflect the underlying physiological mechanisms, confirming that AsA priming is an effective strategy to improve salinity tolerance in rice at germination and early seedling stages.

Conclusion

Soil salinity is one of the most pressing challenges for agriculture worldwide, and in Bangladesh, it is a major constraint, especially in coastal areas where over half of the land is affected. This study revealed that priming rice seeds with ascorbic acid (AsA) improved germination, growth, and physiological traits under different salinity levels, with 0.25 mM and 0.5 mM proving more effective than 0.125 mM. These findings indicate that AsA seed priming can enhance rice tolerance to salinity, but further field-level research is needed to confirm its effects on overall growth, development, and yield.

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Conflicts of interest

The authors declare no conflicts of interest regarding publication of this paper.

Authors' contribution

Conceptualization, Supervision, Writing-original draft: JAM, MA and HA; Data curation: MA, HA and FA; Formal analysis: JAM; Funding acquisition: JAM; Investigation: HA, MA, FA and MGJH Methodology: HA and JA; Project administration: JAM and MGJH; Validation: JAM and HA; Review and editing: JAM, MA and MGJH.

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