Mitigation of salinity-induced growth inhibition of maize by seed priming and exogenous application of salicylic acid

ABSTRACT

Soil salinity has come to be recognized as a global challenge to the sustainability of farming systems due to its adverse effects on crop quality and production, particularly in coastal regions of the world. Although maize (*Zea mays* L.), a C4 plant, has the capacity to withstand a medium degree of salinity, maize is vulnerable to salinity stress during its early growth phases. Therefore, in order to develop strategies to improve maize adaptability in saline settings, it is essential to increase our understanding of maize response to salt stress and tolerance mechanisms.According to reports, plants are known to be able to withstand salt stress when phytohormones are applied. Salicylic acid (SA), a widely-used plant hormone, has been shown to increase a plant's ability to withstand salt stress.In order to reduce the growth inhibition of maize caused by salt, the aim of this study was to clarify and assess the potential impact of priming and exogenous administration of salicylic acid (SA). The results revealed that salt stress decreased seed germination, seedling fresh and dry weight, leaf relative water content (RWC), and photosynthetic pigments of maize. Salicylic Acid (SA) treatment increased germination percentage (43-69%), shoot (24-56%) and root length (13-37%), dry weight of shoot and root (35-171%), RWC (34-39%),and chlorophyll contents (12-36%) of maize under salt stress. The findings highlight the need for additional research to fully understand the underlying mechanisms and explore SA's potential mitigating effects in lowering salt stress as well as its potential practical applications in agricultural practices.

Keywords: salinity; maize; early growth stage; RWC; photosynthetic pigments.

1. INTRODUCTION

“Salt stress has been identified as one of the most serious threats to modern commercial oriented and profit-driven agricultural production on a global scale”(Javed et al., 2020; Seleiman et al., 2021a). “Aside from soil salinity, irrigation with salty water, particularly in low-lying coastal regions of several nations, has been identified as a major yield limiting factor for increasing agricultural productivity”(Daliakopoulos et al., 2016). The negative effects of salt stress appear as a decrease in plant relative water potential, which causes a decrease in plant growth (EL Sabagh et al., 2020a), as well as a negative influence on soil and water quality in the short and long term (Seleiman et al., 2021b).“Salt stress is linked to moisture stress, which affects plant growth and, ultimately, plant yield even when soil moisture content is not limiting for crop productivity”(EL Sabaghet al., 2019). “Because of its stress adaptive ability and relative tolerance to salinity, maize, like other C4 plants, can grow in both saline and non-saline situations"(Farooqet al., 2015; Khaliq et al., 2019)."Although salinity has a negative impact on maize development and yield qualities throughout the plant cycle, the final impact on plant productivity is determined by the length and severity of the stress, as well as the growth phase during which the stress occurs"(Billah et al., 2017; Feng et al., 2017)."In general, and like with other row crops, maize's early growth stage is very vulnerable to salt stress" (Qu et al., 2012).

The establishment of seedlings is a crucial stage in the plant's life cycle. Salt stress has a negative impact on seed germination (Borlu et al., 2018) owing to a decrease in the osmotic potential formed in the soil solution, which prevents water from entering the seed(Taiz and Zeiger, 2018). "Sodium and chloride ion intake during seedling establishment produces toxicity in plant cells, lowering seed germination rates and the growth of previously sprouted seedlings"(Carpycy et al.,2009). "Salinity stress, in addition to having a detrimental impact on germination rates, also delays the total germination process, lowering the survival prospects of those seeds that did germinate" (Ashraf et al., 2005; Bojović et al., 2010). "Because of the potential to significantly lower agricultural output, it is critical to notice the early negative effects of soil and water salinity on plant growth and development"(Goldsworthy 1994)"Salinity inhibits seedling establishment through increasing oxidative stress in the seeds due to the absorption of Na+ and Cl ions, which cause toxicity in embryogenesis and protein synthesis. The maximum oxidative stress generated by Na+ and Cl ions toxicity during germination reduces or slows plant germination"(Khajeh-Hosseiniet al., 2003). In the instance of maize production, only Na+ toxicity was revealed to be more harmful in lowering germination under salt-stressed conditions.

"The capacity of seeds to germinate in soil with high salt concentrations is critical for the survival of many plant species. Seed priming accelerates many metabolic processes involved in the early stages of germination, and seedlings from primed seeds have been shown to develop more vigorously and perform better under adverse environmental conditions than non-primed seeds" (Rhaman et al., 2020a; Rauf et al., 2022a)."Salicylic acid (SA) improves crop performance under abiotic and biotic conditions by acting as both a plant growth regulator and an antioxidant"(Hayat et al., 2010; Rhaman et al., 2020b)."Foliar SA treatment in maize has the capacity to raise relative water content and membrane stability index in maize cultivated in water-stressed situations" [90]."Furthermore, exogenous administration of SA increased plant growth, antioxidant enzyme levels, and stabilized the overall photosynthetic process in salt challenged maize plants"(Rhaman et al., 2021; Tania et al., 2022).Foliar application of SA in maize seedlings corrected the deleterious effects of soil salinity on plant gas exchange, rubisco activity, and photosynthetic efficiency (Khan et al., 2003; Khdarey et al., 2004), while significantly enhancing soluble sugar, proline, and nutrient uptake, particularly K+ (Fahad etal., 2012)."Increases in photosynthetic rates, gas exchange levels, internal CO2 exchange, and grain yield of maize were reported in saline soils when SA was given to roots" (Vazirimehr etal., 2014).

Seed germination and stand establishment in maize farms are usually poor due to high amounts of salt in irrigation water. As a result, the purpose of this study was to see how priming and exogenous administration of SA treatments on maize affected their ability to sustain normal germination and growth under salinity stress.

2. MATERIALSAND METHODS

2.1 Plant Materials and Chemicals

The investigation took place at Bangladesh Agricultural University's Department of Seed Science and Technology in Mymensingh, making use of maize as maize is widely cultivated in various parts of Bangladesh. The analytical grade chemicals Salicylic acid (SA), SA (Sigma-Aldrich), sodium hypochlorite (Sigma-Aldrich), and Hyponex (Osaka, Japan) nutritional solution were utilized in this work.

2.2 Experiment at pre-seedling stage

Maize seeds with uniform appearance were sorted and surface sterilized with 1% sodium hypochlorite for 5 minutes before being rinsed 3-4 times with dH2O. The seeds were immersed in 1mM, 2mM, 3mM, 4mM, and 5mM SA for 60 minutes for the seed priming experiment, and the control experiment seeds were washed many times in normal laboratory settings (room temperature was 251^{o}C and relative humidity was 95%).Following that, treated seeds were placed in a petri-dish (150*20 mm diameter) with three layers of wetted-Whatman filter papers and stored for seven days for germination testing. Each petri dish included fifteen treated seedlings. As a result, the therapies used in this investigation are listed below

List 1: Treatment details

Treatment	Concentration of priming agent
T1	Control
T2	Salt
T3	Salt+1mM SA
T4	Salt+2mM SA
T5	Salt+3mM SA
T6	Salt+4mM SA
T7	Salt+5mM SA

The experiment was conducted with a completely randomized block design having three replicates. Germination percentage (GP) and seed vigor index (Rauf et al. 2020) were computed with the following equations:

Germination percentage (GP) = Total number of seeds germinated/Total number of seeds placed in germination× 100 …….(1)

Seed vigor index SVI =GP×seedling length (cm)………..(2)

2.3 Experiment at seedling stage

Seedlings were planted in plastic pots (22 cm in height and 25 cm in diameter) filled with water and uniformly germinated seeds (4 seedlings per pot). The water was blended with Hyponex (Osaka, Japan) fertilizer solution including nitrogen, phosphorous, potassium, and other micronutrients. The fertilizer solution (2 mL mixed with water per plant) was applied to the pots twice a week. Seedlings were exogenously sprayed with varied concentrations of SA four times in four days after 12 days (8 ml per plant every spray). Three plants were studied morphologically and physiologically after four days of SA treatment.

2.4 Relative water content measurement

The conventional approach of Mostofa and Fujita,2013 was used to determine the relative water content (RWC). In the instance of RWC measurement, leaf samples were obtained 14 days after transplanting, and fresh weight (FW) of leaves were extracted and soaked in dH_2O for 4 hours. The surplus water was then wiped away from the turgid leaves with a paper towel, and the turgid weight (TW) was immediately recorded. After that, the leaves were oven dried for 48 hours at 70 °C and their dry weight (DW) was recorded. The RWC was computed using the following formula:

RWC (%) = (FW – DW)/(TW – DW)× 100…………(3)

2.6 Measurement of leaf chlorophyll contents

The amounts of the photosynthetic leaf pigments chlorophyll, lycopene, beta carotene, and carotenoids were quantified spectro-photometrically using the procedure defined by Lichtenthaler, 1987. Picked fresh leaves weighing 0.5g were inserted in a small vial with 10 mL of 80% ethanol. The containers were covered with aluminum foil and kept in the dark for 7 days to extract the pigments. A spectrophotometer (Shimadzu UV-2550, Kyoto, Japan) was used to measure the absorbance from leaf extraction at wavelengths of 663, 645, 505, and 453 nm for the amounts of chlorophyll, lycopene, beta carotene, and carotenoids. The photosynthetic pigments were calculated using the following formulas:

Total Chlorophyll = Chlorophyll a +Chlorophyll b……….(4)

Chlorophyll a = $0.999\times A_{663} - 0.0989\times A_{645}$………..(5)

Chlorophyll a = $-0.328\times A_{663} + 1.77\times A_{645}$……………(6)

Lycopene=$0.0458\times A_{663}+0.204\times A_{645}+0.372\times A_{505}-0.0806\times A_{453}$……….(7)

Beta-carotene=$0.216\times A_{663}$- $1.22\times A_{645}$-$0.304\times A_{505}$+$0.452\times A_{453}$…………….(8)

Carotenoids= A_{480}+($0.114\times A_{663}$ –$0.638\times A_{645}$…………(9)

2.8 Statistical Analysis

Data collected for each parameter were subjected to one way ANOVA using Minitab 17 statistical software (Minitab Inc., State College, PA, USA). The statistical differences among the mean values of different treatments were compared using Tukey's pair-wise comparisons ($P < 0.05$).

3. RESULT

3.1. Priming boosts germination indices and traits of seedlings under salt stress

The impacts of SA priming and exogenous application on the germination indices of maize under salt stress are displayed in Figure 1. The findings show that salt stress significantly reduced GP by 33.33% compared to the control condition. Priming and exogenous application of SA showed a significant effect on GP (Figure 1a). While the highest GP (90%) was recorded for T4 (2mM SA) and T5 (3mM SA)treatment, the lowest GP (53.33%)was recorded for salt stressed seeds (Figure 1a). In the case of shoot and root length, significant variations were found for different priming treatments compared to salt stress. The highest shoot and root length under stress condition with priming was observed in treatment T4(Salt+2mM SA) 42.2cm and 24.8cm, respectively. Shoot length decreased by 28.94% in stress condition while SA treatments increased shoot length by 30, 56.29, 44.44, 31.48 and 24.81%, respectively for 1mM, 2mM, 3mM, 4mM and 5mM SA treated seeds under stress condition (Figure 1b). Salt stress significantly reduced root length while priming with SA increased root length under salt settings. Root length decreased by 31.69% in stress condition while SA treatments increased shoot length by 13.25, 37.01, and 25.41% respectively, for 1mM, 2mM, and 3mM SA treated seeds under stress condition (Figure 1c). Similarly, salt stresssignificantly reduced SVI (53.38%) compared with the control. But priming with SA significantly increased the SVI compared with salt condition(Figure 1d).

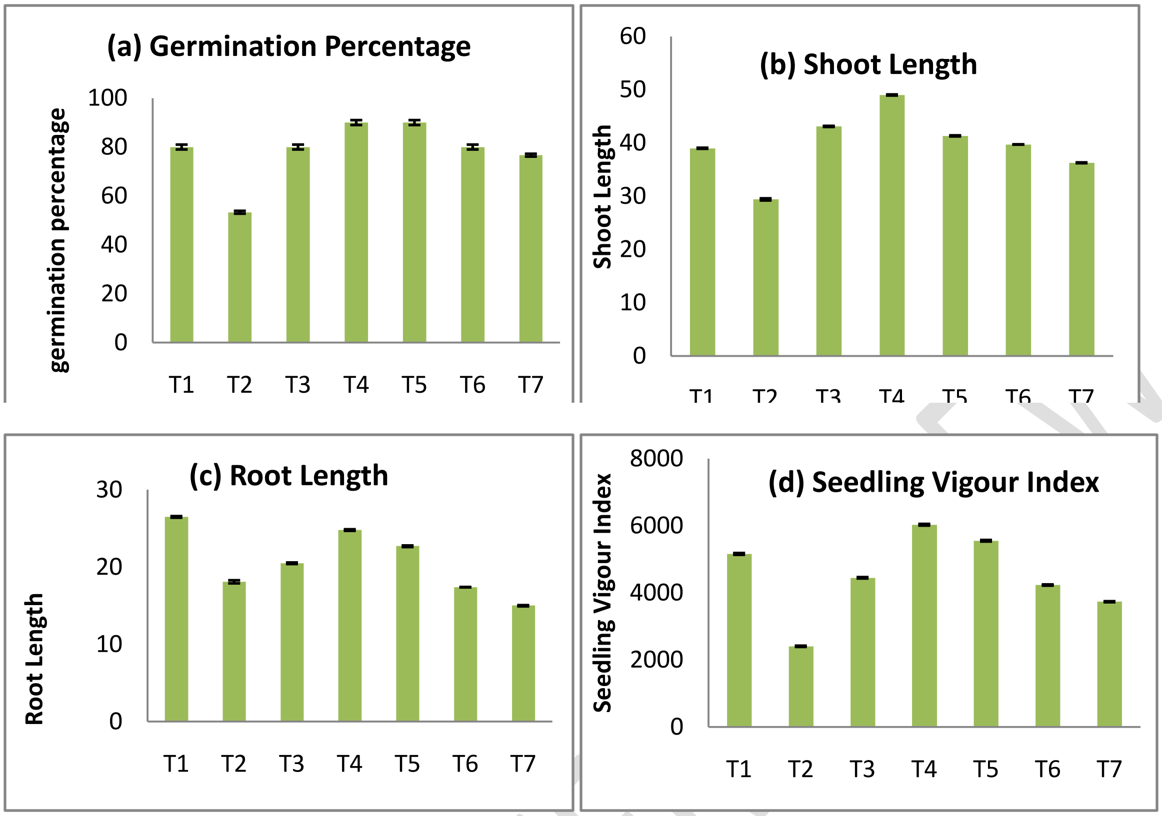


Figure 1. Effects of SApriming and exogenous application on the germination and growth indices of maize under salt stress. (a) Germination percentage; (b) Shoot Length; (c) Root Length; (d) Seed vigor index. The error bar represents standard error.

3.2 Priming increases fresh and dry weight of seedlingsunder salt stress

To assess the effects of salt stress and stress-decreasing acts of SA on the weight of maize seedlings, we recorded the fresh and dry weight of seedlings. Shoot fresh weight (SFW), shoot dry weight(SDW), and root fresh and dry weight (RFW, RDW) were decreased by salt stress (Figure 2).All the priming conditions significantly increased fresh and dry weight of shoot and root under salt stress.Priming of seeds with 2mM SA showed highest fresh and dry weight of both shoot and root. All other treatments increased shoot and root fresh and dry weight under stress condition when seeds were treated with SA (Figure 2).

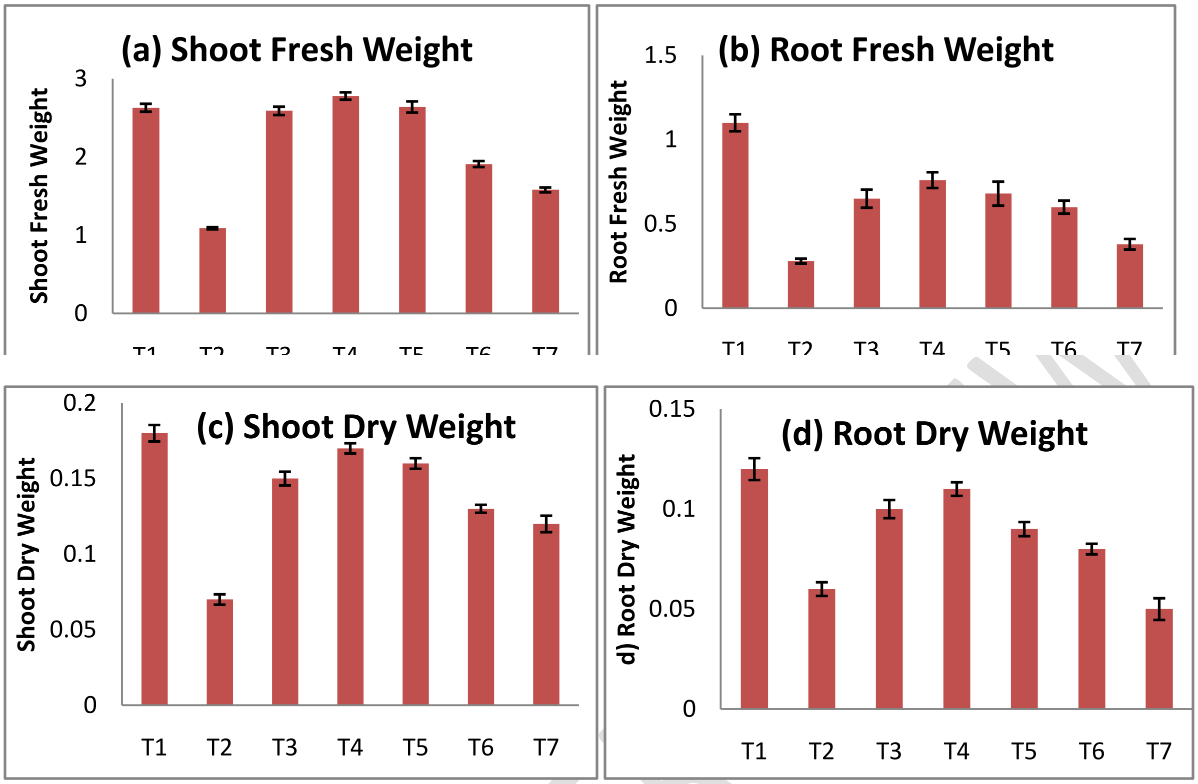


Figure 2. Effects of SApriming and exogenous application on the germination and growth indices of maize under salt stress. (a) Shoot fresh weight; (b) Root fresh weight; (c) Shoot dry weight; (d) Root dry weight. The error bar represents standard error.

3.3 Exogenous SAenhance RWC of plants under salt stress

The water status of maize plants was studied in this work by measuring RWCwith and without salt stress using priming agents. The results showed that salt stresssignificantly reduced RWC by 28.87%(Figure 3). The application ofpriming agents responded strongly to RWC at both times in comparison to salt conditions.The highest RWC increased by T4 treatment by 39.2% compared with salt condition.

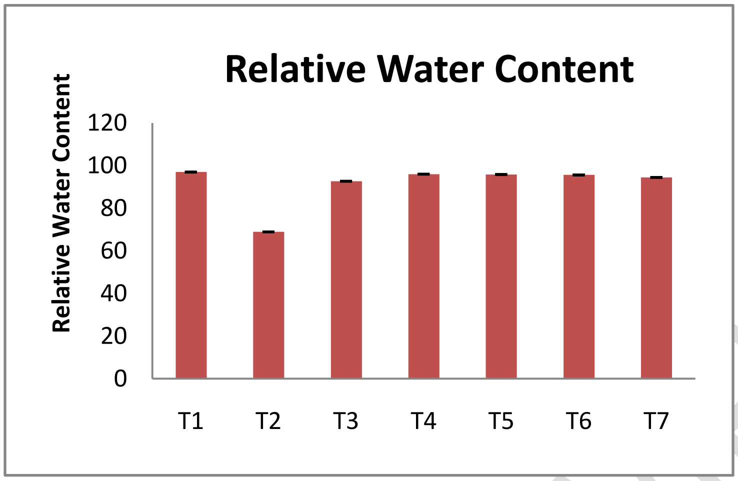
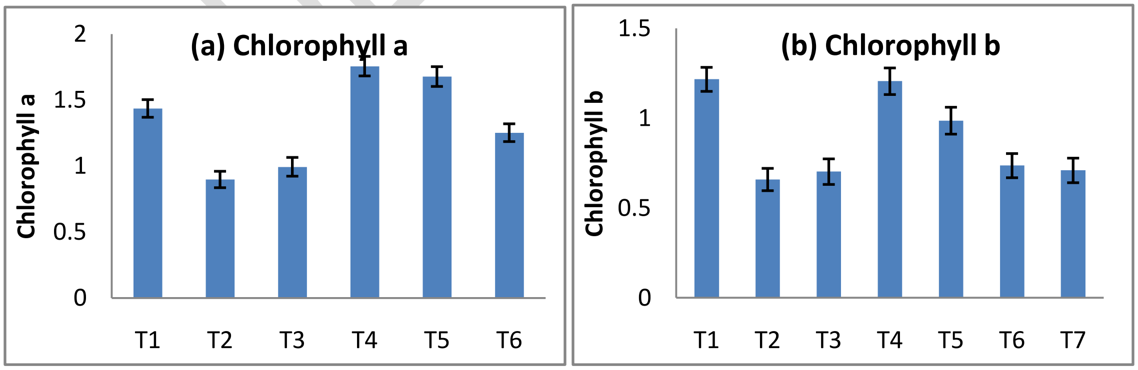


Figure 3. Effects of SApriming and exogenous application on the relative water content of maize under salt stress. The error bar represents standard error.

3.4. Pretreatment of GA$_3$ regulate photosynthetic pigment of maize under salt stress

A significant fluctuation of Chl pigment contents was detected due to applied salt stress (Figure 4). A considerable decline in total Chl content (41.31%) including Chl a (37.49%)and Chl b (45.82%) in maize leaves due to salt stress compared to the control (Figure 4a–c). Thesupplementation of different concentrations of SAremarkably augmented Chl a,Chl b, and total Chl contents. Pigment analysis also revealed that lycopene (51.22%), beta-carotene (74.83%) and carotenoids (50.35%) were also reduced due to salt stress, and thesupplementation of SA significantly increased the pigments (Figure 4c–f). Thesupplementation of 2mM SA under salt stress increased the maximum lycopene (72.58%), beta-carotene (178.17%) and carotenoids (5.35%) contents.



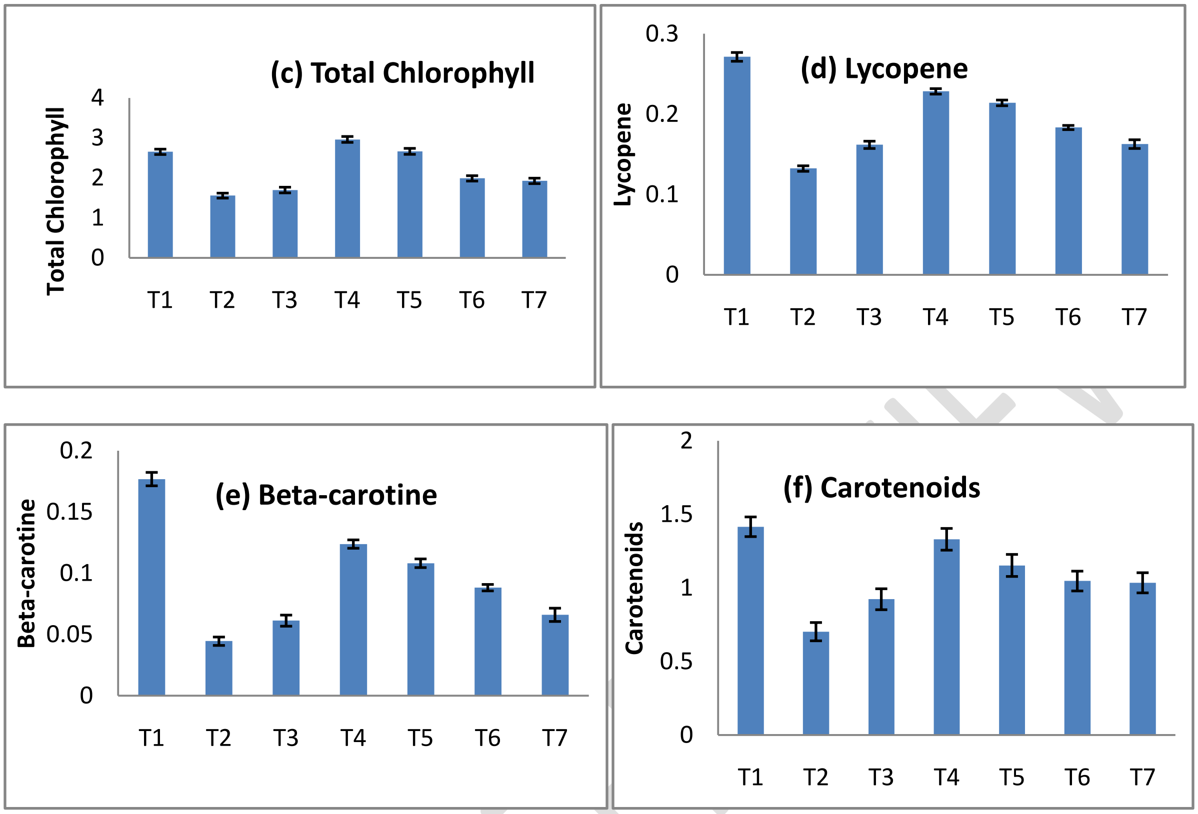


Figure 4. Effects of SApriming and exogenous application on the photosynthetic pigments of maize under salt stress. (a) Chlorophyll a; (b) chlorophyll a; (c) total chlorophyll; (d) lycopene; (e) beta-carotene; (f) carotenoids. The error bar represents the standard error.

4. DISCUSSION

Successful seed germination is the most crucial and essential phase of the plant life cycle for seedling development and subsequent productivity (Shiade et al., 2020). Numerous research have shown that seed priming is a common technique for promoting germination, enhancing morphological features, and accelerating plant development in both stress-free and stressful environments (Muhei et al., 2018; Rhaman et al., 2021). Salt stress is one of the main types of stress that drastically lowers seed germination and crop establishment. Numerous crops, including wheat, faba beans, bitter gourd, and rice, can develop salinity resistance through priming and the addition of different signalling molecules, according to the literature [Tania et al., 2021; Abdel latif et al., 2021; Rauf et al., 2022b; Tania et al., 20]. As we recently discovered (Rauf et al., 2022a), SA priming of maize seeds increased germination and seedling traits under salt stress.Understanding SA's optimum concentration during the priming of maize seeds under salt stress was the main objective of the current investigation. GP, SL, RL, and SVI were all significantly lower as a result of salt stress, according to the research (Figure 1). Additionally, SFW, SDW, RFW, and RDW were all reduced by salt stress (Figure 2). The results showed that under salt stress, SA priming increased the GP, SL, RL, and SVI of maize. These results are consistent with recent studies that found that salt stress had a negative impact on parameters related to seed germination in wheat (Farooq et al., 2008), maize (Ashraf et al., 2001), and rice (Tahjib-Ul Arif et al., 2019), but that a number of priming agents significantly reduced those effects. The exogenous administration of SA may promote plant development by increasing its endogenous synthesis (Aldesuquy et al., 2001).

Maintaining a sufficient water level is an essential physiological step for keeping normal development progression in plants under salt stress (Rauf et al., 2022a). RWC is a well-known indicator of water status in plants since it is a water-related feature (Rhaman et al., 2021). Salinity reduces the soil's water potential, which has been linked to a drop in photosynthesis and a decline in the RWC of leaves (Rhaman et al., 2021;Rauf et al., 2022a. Exogenous chemical supplementation has been found to improve the water status of many plant families (Tahjib-Ul-Arif et al., 2018). The results of the study showed that RWC dropped in response to salt stress (Figure 3), and this was due to the salt's destruction to the leaves' cell walls, which changed their structure and decreased their capacity to absorb water [50]. The results also demonstrated that when RWC is inhibited by salt, the addition of various signaling molecules, such as SA priming, inhibits RWC (Figure 3). This research suggests that a variety of priming and foreign chemicals may be involved in the uptake of more water from the soil to alter the water level within plant organs.

The primary pigment used in plant photosynthesis, chlorophyll, is crucial for many biological processes. Numerous physiological processes in plants depend on chlorophyll, the principal pigment employed in photosynthesis (Gu et al., 2016). When compared to plants that weren't treated, salinity stress significantly reduced the amount of leaf chlorophyll (measured as Chla, Chlb, and total chlorophyll) in this study (Figure 4). Additionally, salt stress has been shown to reduce the activity of photosynthetic pigments in a number of earlier investigations (El-Esawi et al., 2018; Qu et al., 2012). The decrease in chlorophyll content is caused by the emergence of proteolytic enzymes at high salt concentrations (Tuna et al., 2008). Additionally, these enzymes contribute to the breakdown of chlorophyll (Yaseen et al., 2021) and the cessation of photosynthesis in settings high in salt (Xia et al., 2015). Under salt stress, the levels of chlorophyll (Figure 4), along with the rate of photosynthesis(Xia et al., 2015), may have contributed to the decline in maize biomass (Figures 3). When SA was applied to maize plants under salt stress, the chlorophyll content increased, peaking with the T4 treatment (Figure 4). Higher chlorophyll content accumulation may be associated with lower Na+ buildup, reduced oxidative damage, and improved antioxidant defense in maize seedlings under salinity stress. These outcomes are consistent with past studies (Zang et al., 2016), which shown that applying GA3 increased the chlorophyll content of leaves. When maize was exposed to salt stress, foliar SA treatment significantly improved the amount of chlorophyll in the plant (Rauf et al., 2022a).

5. CONCLUSIONS

According to the study, maize's germination indicators, growth characteristics, leaf hydration state, and photosynthetic pigments all decrease under salt stress. By priming and applying exogenous SA to maize under salt stress, the germination percentage, seed vigour index, shoot and root length, shoot and root fresh and dry weight, leaf hydration status, and photosynthetic pigments are all increased. Additionally, our results suggest that under salt stress conditions, maize production may be highly successful when supplemented with 2mM SA. Despite this, it is advised that further study conduct a field trial to confirm our findings.

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