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Gibberellic Acid Enhances the Germination and Growth of Maize under Salinity Stress

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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

Soil salinity is the major limiting factor restricting plant growth and development. Little is known about the comparative and combined effects of gibberellic acid (GA_3) seed priming and foliar application on maize under salt stress. The current study determined the impact of different concentrations of GA_3 on morpho-physiological and photosynthetic attributes of maize seedlings under salinity stress treatments (no salinity and severe salinity-15 dSm^{-1}). The GA_3 treatments consisted of 1mM, 2mM, 3mM, 4mM and 5mM GA_3 seed priming and exogenous application in salt condition. Salt stress particularly at 15 dSm^{-1} reduced the length of shoots and roots, fresh and dry

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weights, chlorophyll, lycopene, beta-carotine and carotenoid contents in maize plants. Nevertheless, the application of GA_3 improved maize growth under salt stress. Compared with salt, the 2mM GA_3 treatment (T4) recorded the highest increase in roots and shoots length, roots fresh and dry weights, shoots fresh and dry weights, chlorophyll content under salt stress compared to other concentrations. These results indicated that 2mM GA_3 priming and exogenous application could be used as an effective tool for improving the maize growth and development in salt contaminated soils.

Keywords: Salinity; gibberellic acid; maize; seed priming; exogenous application; growth; photosybthetic attributes.

1. INTRODUCTION

Zea mays L., known as maize, is a common cereal crop that is grown all over the world for use as forage and as a source of food grains for both human and animal consumption. Additionally, it gives various industries access to raw materials [1]. Under field conditions, the maize crop is exposed to a number of abiotic challenges, including soil salinity, drought, light, and temperature, which can significantly reduce its production [2]. “Soil salinity is one of the main abiotic stress that restricts crop growth and productivity. According to estimates, soil salinity affects around 6% of all arable land worldwide” [3]. “The growth, development, and yield of field crops are all significantly hampered by soil salinity, in addition to lowering seed emergence and germination rates” [4,5]. Additionally, high salt levels in the soil cause stomata to close and harm the photosynthetic apparatus and chlorophyll content [6–8]. Reactive oxygen species (ROS) are continuously created by metabolic processes in plants, with production increasing in response to environmental stress [4]. When ROS generation rises, lipids, membranes, nucleic acids, and proteins can be destroyed, which causes cellular machinery to fail [9–11]. Ion equilibrium is disturbed as a result of the overproduction of ROS during salt stress [12,13]. “Plants have evolved a powerful antioxidant defence system, including the antioxidant enzymes superoxide dismutase (SOD), peroxidase (POD), ascorbate peroxidase (APX), and catalase (CAT), to limit the oxidative damage” [14].

“Chemical addition to plants, either as an exogenous foliar application or seed treatment, may stimulate their physiological systems, resulting in an improvement in plant growth” [15]. “The phenotypes of plants can change from seed germination through senescence, for instance, when seed priming with plant growth regulators is used” [16]. Gibberellic Acid (GA_3), a type of growth hormone that occurs naturally, controls how plants grow and develop [17]. “The GA_3 are linked to numerous aspects of plant growth and development, including seed germination, hypocotyl lengthening, leaf expansion, floral initiation, uniform flowering, floral organ development, shortened flowering times, increased flower number and size, and induction of a few hydrolytic enzymes in the aleurone of cereal grains” [18–20]. According to reports, growth regulators such as GA_3 can lessen the salinity-related germination inhibitory effect [21–24]. According to Kaur et al. [25], GA_3 at a concentration of 6 µM promotes enhanced seedling development under salt stress. According to Ashraf et al. [26], “treatment of GA_3 increased wheat's nutrient uptake, dry weight, plant height, leaf area, and yield in saline conditions”. “Additionally, there is proof that GA_3 can significantly reverse the growth inhibition caused by NaCl in rice” [27]. According to Starck and Kozinska [28], the GA_3 slightly changed the ion ratios in beans while increasing P and Ca2+ absorption and decreasing Na+ absorption. According to Bejaoui [29], “the benefits of exogenously given GA_3 in reducing salt stress may be brought on by the activation of particular enzymes involved in RNA and protein synthesis”. Aloni and Pressman [30] suggested “a potential relationship between salt and the GA_3 effect on petiole elongation, cellular disintegration, and bolting in celery as a defence response to stressors in plants”.

Due to high levels of salt in irrigation water, seed germination and stand establishment in maize farms are frequently poor. Therefore, the goal of this experiment was to investigate how priming and exogenous application of GA_3 treatments on maize affected their ability to maintain normal germination and growth under salinity stress.

2. MATERIALS AND METHODS

2.1 Plant Materials and Chemicals

The experiment was carried out at the Department of Seed Science and Technology of

Bangladesh Agricultural University, Mymensingh, using BARI Hybrid Maize 11, widely cultivated in many regions of Bangladesh. The chemicals, Gibberellic Acid (GA_3), SA (Sigma-Aldrich), sodium hypochlorite (Sigma-Aldrich), and Hyponex (Osaka, Japan) nutrient solution were used as an analytical grade in this study.

2.2 Experiment at Seedling Stage

Uniform in appearance maize seeds were sorted out and surface sterilized with 1% sodium hypochlorite for 5 min and then washed 3-4 times with dH2O. For the seed priming experiment, the seeds were soaked in 1mM, 2mM, 3mM, 4mM and 5mM GA_3 for 60 minutes, and the control experiment seeds were washed in distilled water for several times in normal laboratory (the room temperature was 25±1ºC and relative humidity was 95%) conditions. After that, fifteen treated seeds were placed in a petri-dish (150×20 mm diameter) having three layers of wetted-Whatman filter papers and kept for 7 days for the germination study. For salt treatments 150 mM NaCl solution was sprayed daily upto 7th day.

"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" [31]:

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)

Then, uniformly germinated seeds were placed in plastic pots (22 cm in height and 25 cm in diameter) filled with water (4 seedlings per pot) and the water was mixed with nutrient solution Hyponex (Osaka, Japan) containing nitrogen, phosphorous, potassium, and other micronutrients. The nutrient solution (2 ml/2.5L) was applied twice in a week in the pots. After 3 days of transplanting, seedlings 150mM NaCl was added in water of pot and were also exogenously treated with different concentrations of GA_3 for four times in four days (8 ml per plant per spray). After 7 days of treatment, morphological and physiological data were collected from three plants.

2.3 Relative Water Content Measurement

"Relative water content (RWC) was determined followed by the standard procedure of Mostofa & Fujita" [32]. "In the case of RWC measurement, leaf samples were collected after 14 days of transplanting and then fresh weight (FW) of leaves were taken and immersed in dH2O and kept for 4 hr. After that, excess water was removed from the turgid leaves with a paper towel and turgid weight (TW) was recorded instantly. After that leaves were oven dried at 70 °C for 48hrs and dry weight (DW) was recorded" [32]. The RWC was calculated according to the following formula:

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

2.4 Measurement of Leaf Chlorophyll Contents

Based on the procedure outlined by Lichtenthaler [33], the concentrations of the photosynthetic leaf pigments chlorophyll, lycopene, beta carotene, and carotenoids were measured spectro-photometrically. Fresh leaves weighing 0.5g were picked at 21th day of sowing and placed in a tiny vial with 10 mL of 80% ethanol. For the purpose of extracting the pigments, the containers were covered with aluminum foil and kept in the dark for 7 days. A spectrophotometer was used to measure the absorbance from leaf extraction at wavelengths of 663, 645, 505, and 453 nm for the concentrations of chlorophyll, lycopene, beta carotene, and carotenoids (Shimadzu UV-2550, Kyoto, Japan). The following formulae were used to compute the photosynthesis pigments:

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

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

$$\text{Chlorophyll b} = -0.328\times A_{663} + 1.77\times A_{645} \quad (6)$$

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

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

$$\text{Carotenoids}= A_{480}+(0.114\times A_{663} -0.638\times A_{645} \quad (9)$$

Table 1. List of treatments and concentration of priming agent

Treatment	Concentration of priming agent
T1	Control
T2	Salt (150mM NaCl)
T3	Salt (150mM NaCl)+1mM GA_3
T4	Salt (150mM NaCl)+2mM GA_3
T5	Salt (150mM NaCl)+3mM GA_3
T6	Salt (150mM NaCl)+4mM GA_3
T7	Salt (150mM NaCl)+5mM GA_3

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. RESULTS

3.1 Priming Boosts Germination Indices and Traits of Seedlings under Salt Stress

The impacts of GA_3 priming and exogenous application on the germination indices of maize under salt stress are displayed in Fig. 1. The findings show that salt stress significantly reduced GP by 17.39% compared to the control condition. Priming and exogenous application of GA_3 showed a significant effect on GP (Fig. 1a). While the highest GP (90%) was recorded for T2 (2mM GA_3) treatment, the lowest GP (63.33%) was recorded for salt stressed seeds (Fig. 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 was observed in treatment T4 (Salt+2mM GA_3) 43.1cm and 18.1cm, respectively. Shoot length decreased by 24.61% in stress condition while GA_3 treatments increased shoot length by 46.59, 66.67, 40.54, 35.03 and 23.37%, respectively for 1mM, 2mM, 3mM, 4mM and 5mM GA_3 treated seeds under stress condition (Fig. 1b). Salt stress significantly reduced root length while priming with GA_3 increased root length under salt settings. Root length decreased by 56.98% in stress condition while GA_3 treatments increased shoot length by 58.77, 94.73, 53.51, 34.21 and 6.14% respectively, for 1mM, 2mM, 3mM, 4mM and 5mM GA_3 treated seeds under stress condition (Fig. 1c). Similarly, different priming conditions increased SVI but salt stress significantly reduced SVI (48.54%) compared with the control. The results indicated that SVI increased by 89.47, 147.98, 97.28, 77.37 and 49.75%, respectively, for 1mM, 2mM, 3mM, 4mM and 5mM GA3 priming compared to salt stress (Fig. 1d).

3.2 Priming Increases Fresh and Dry Weight of Seedlings under Salt Stress

To assess the effects of salt stress and stress-decreasing acts of GA_3 on the weight of maize seedlings, we recorded the fresh and dry weight of seedlings. All the priming conditions significantly increased fresh and dry weight of shoot and root under salt stress. Shoot fresh weight (SFW), shoot dry weight (SDW), and root fresh and dry weight (RFW, RDW) were decreased by salt stress (Fig. 2). In case of shoot fresh and dry weight was highest at T4 treatment 2.78g and 0.17g, respectively under stress condition (Fig. 1a, c). Similarly, root fresh and dry weight was highest at T4 treatment 0.75g and 0.11g, respectively under salt stress (Fig. 1b, d). All other treatments increased shoot and root fresh and dry weight under stress condition when seeds were treated with GA_3 (Fig. 2).

3.3 Exogenous GA_3 Enhance RWC of Plants under Salt Stress

The water status of maize plants was studied in this work by measuring RWC with and without salt stress using priming agents. The results showed that salt stress significantly reduced RWC by 10%.29 (Fig. 3). The application of priming agents responded strongly to RWC at both times in comparison to salt conditions. The highest RWC increased by T4 treatment by 11%.

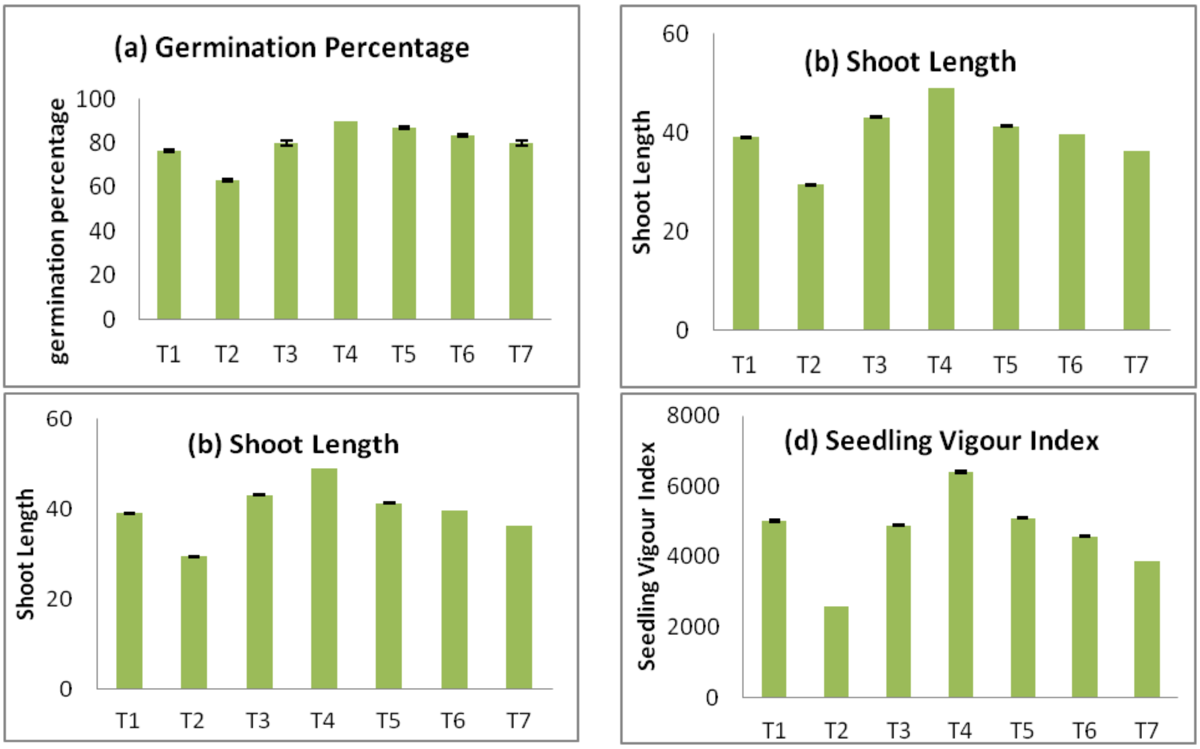

Fig. 1. Effects of GA_3 priming and exogenous application on the germination and growth indices of maize under salt stress. (a) Germination percentage; (b) Shoot Length; (c) Root Length; (d) Seed vigor index. The error bar represents standard error

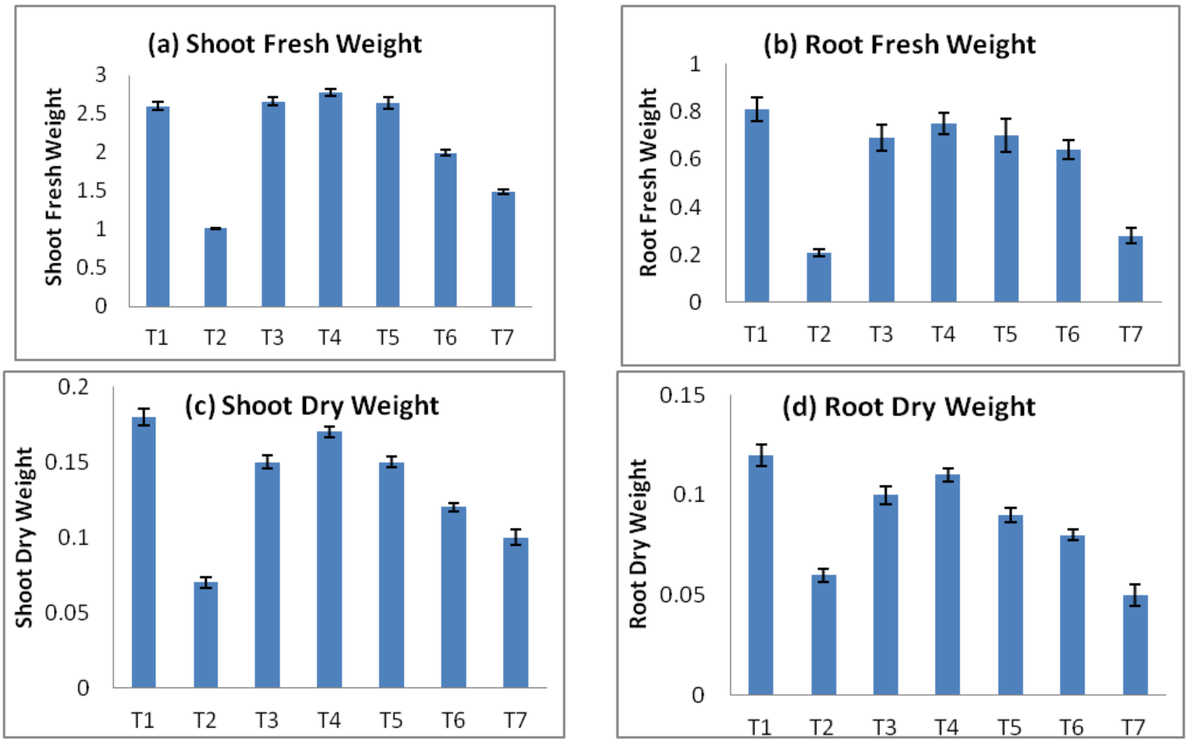

Fig. 2. Effects of GA_3 priming and exogenous application on the germination and growth indices of maize under salt stress. (a) Shoot fresh weight; (b) Root fresh weight; (c) Shoot dry weight; (d) Root dry weight. The error bar represents standard error

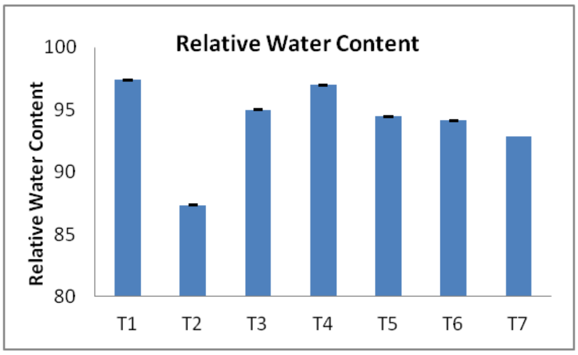

Fig. 3. Effects of GA_3 priming and exogenous application on the relative water content of maize under salt stress. The error bar represents standard error

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 (Fig. 4). A considerable decline in total Chl content (34.54%) including Chl a (29.42%) and Chl b (41.51%) in maize leaves due to salt stress compared to the control (Fig. 4a–c). The supplementation of different concentrations of GA_3 remarkably augmented Chl a, Chl b, and total Chl contents. Pigment analysis also revealed that lycopene (52.26%), beta-carotene (43.93%) and carotenoids (31.10%) were also reduced due to salt stress, and the supplementation of GA_3significantly increased the pigments (Fig. 4c–f). The supplementation of 2mM GA_3 under salt stress increased the maximum lycopene (96.68%), beta-carotene (57.71%) and carotenoids (34.92%) contents.

4. DISCUSSION

“For seedling development and subsequent productivity, successful seed germination is the most important and fundamental stage in the plant growth cycle” [34]. According to a number of studies, “seed priming is a widely used method for encouraging germination, boosting morphological traits, and speeding up plant development in both stress-free and stressful conditions” [35,36]. One of the main types of stress that significantly reduces seed germination and crop establishment is salt stress. According to the literature, many crops like wheat, faba beans, and rice can acquire salinity resistance through priming and the addition of various signaling molecules [37-39]. The priming of maize seeds with GA_3 improved germination and seedling characteristics under salt stress, as we recently observed [40]. “The goal of the current study was to understand GA_3's function in the priming of maize seeds under salt stress. The findings showed that salt stress significantly reduced GP, SL, RL, and SVI (Fig. 1). Additionally, salt stress decreased SFW, SDW, RFW, and RDW (Fig. 2). The results showed that under salt stress, GA_3 priming increased the GP, GI, SL, RL, and SVI of maize. Though lower concentration of 2mM GA_3 performed better than other treatments of high concentrations of GA_3 in mitigating salinity stress compared with salt condition. These findings are in line with other studies that discovered that a number of priming agents considerably lessened the detrimental effects of salt stress on parameters linked to seed germination in wheat” [41], maize [42], and rice [43]. Plant growth may be facilitated by the exogenous input of GA_3, which may increase its endogenous accumulation [44]. The higher seedling length and better growth of the GA_3-treated maize in the current study (Figs. 1 and 2) may be attributable to the increased stem and cell elongation because GA_3 is thought to be a key hormone for cell elongation [45,46].

A crucial physiological step for preserving normal growth progression in plants under salt stress is maintaining an adequate water level [41]. Since RWC is a water-related property, it is well recognized as a water status indicator in plants [42]. Salinity lowers the soil's water potential, which has been associated with a decline in the RWC of leaves and a drop in photosynthesis [43]. Numerous plant groups' hydration status has been shown to be improved by exogenous chemical supplementation [47–49]. The study's findings revealed that RWC decreased as a result of the salt stress (Fig. 3), and this was because the salt's damage to the leaves' cell walls altered their structure and reduced their ability to absorb water [50]. The outcomes also showed that the addition of these signaling molecules like GA_3 priming increase RWC when it is hindered by salt (Fig. 3). This finding suggests that the uptake of extra water from the soil to modify the water level within plant organs may include a number of priming and exogenous substances.

Chlorophyll is the main pigment of plant photosynthesis and plays an important role in plant growth. The primary pigment used in photosynthesis in plants, chlorophyll, is crucial to numerous physiological processes in plants [51]. “Salinity stress dramatically decreased the amount of leaf chlorophyll in this study (measured as Chla, Chlb, and total chlorophyll) compared to untreated plants (Fig. 4). Additionally, it has been demonstrated in numerous earlier studies that salt stress can decrease the activity of photosynthetic pigments” [52,53]. The development of proteolytic enzymes at high salt concentrations is the cause of the decrease in chlorophyll content [45]. These enzymes also cause the degradation of chlorophyll [54] and the loss of photosynthesis in salty environments [55]. The decrease in chlorophyll levels (Fig. 4) and the rate of photosynthesis [56] may possibly be contributing factors to the decrease in maize biomass under salt stress (Fig. 3). The chlorophyll content of maize leaves exposed to salt stress rose with the application of GA_3, reaching its highest rise with the T4 (2mM GA3) treatment (Fig. 4) compared to other treatments. In maize seedlings exposed to salinity stress, higher chlorophyll content accumulation may be related to decreased Na+ buildup, reduced oxidative damage, and enhanced antioxidant defense. “These results are in line with those of earlier research [57–59], which showed that the application of GA_3 raised the chlorophyll content of leaves. Under salt stress, foliar application of GA_3 considerably increased the chlorophyll content in maize” [45].

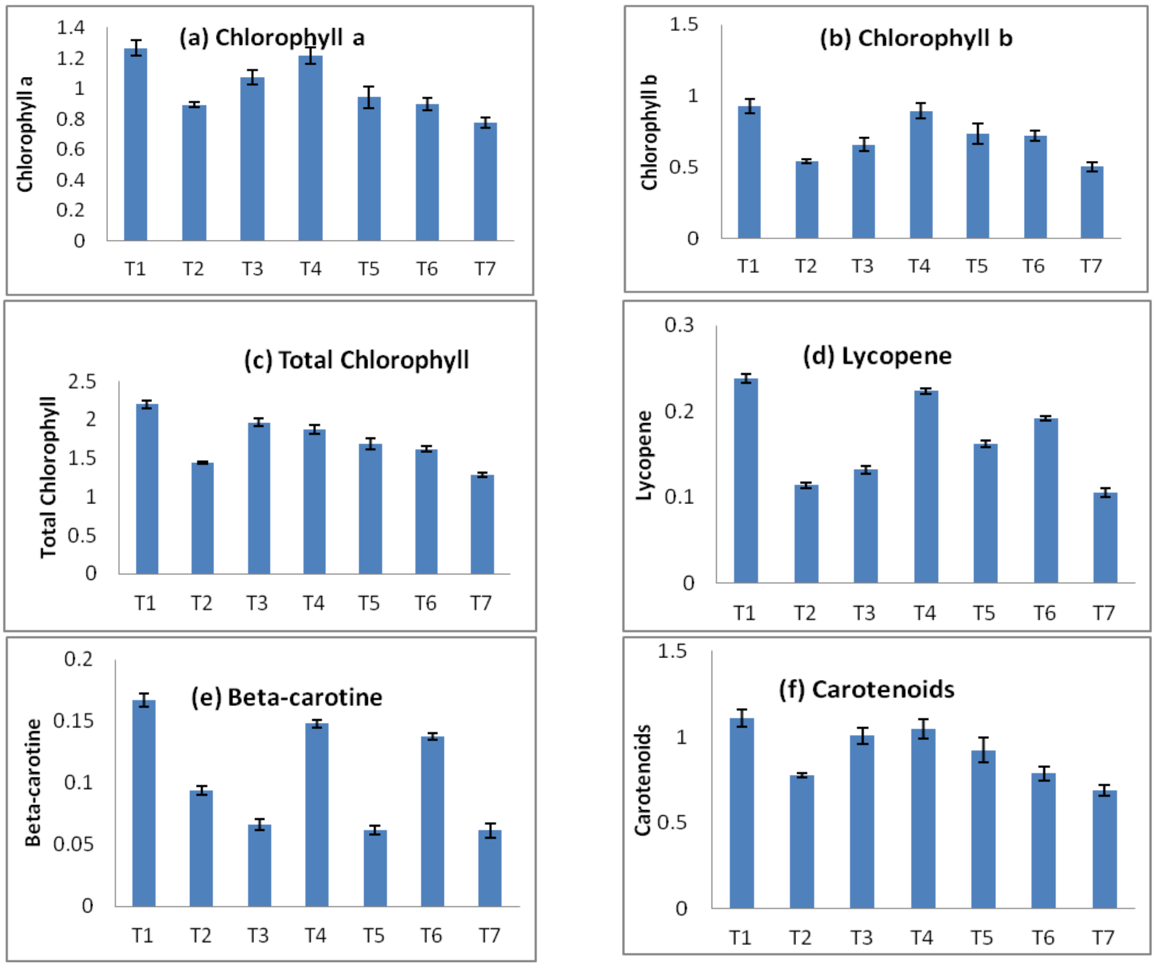

Fig. 4. Effects of GA3 priming and exogenous application on the photosynthetic pigments of maize under salt stress. (a) Chlorophyll a; (b) chlorophyll b; (c) total chlorophyll; (d) lycopene; (e) beta-carotene; (f) carotenoids. The error bar represents the standard error

5. CONCLUSIONS

According to the research, salt stress lowers the germination indices, growth features, leaf hydration status and photosynthetic pigments of maize. The germination %, seed vigor index, shoot and root length, shoot and root fresh and dry weight, leaf hydration status, and photosynthetic pigments of maize under salt stress are all improved by priming and exogenous application of GA_3. The 2mM concentration of GA_3 showed highest positive result in mitigating salinity stress compared to other concentrations. Therefore, from the findings it can be suggested that maize production might be successful with lower GA_3 concentrations under salt stress condition. In spite of this, it is recommended that future research conducts a trial at the field level to validate our findings.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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