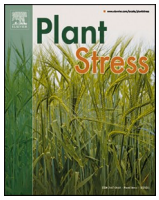
Plant Stress 15 (2025) 100767

Contents lists available at ScienceDirect

Plant Stress

journal homepage: www.sciencedirect.com/journal/plant-stress



Synergistic effects of glutathione and zinc seed priming in alleviating salt stress on maize seed germination, metabolite levels, seedling vigor, and nutrient acquisition

Rehan Ahmad Kasana [a], Muhammad Iqbal [a,*], Qasim Ali [b], Farah Saeed [c], Muhammad Rizwan [d,*], Rashida Perveen [e], Jean Wan Hong Yong [f,*]

[a] Department of Botany, University of Okara, Okara, Pakistan
[b] Department of Botany, Government College University Faisalabad, 38000, Pakistan
[c] Department of Botany, Government College Women University Faisalabad, 38000, Pakistan
[d] Department of Environmental Sciences, Government College University Faisalabad, 38000, Pakistan
[e] Department of Physics, University of Agriculture, Faisalabad, 38040, Pakistan
[f] Department of Biosystems and Technology, Swedish University of Agricultural Sciences, Alnarp, 23456, Sweden

ARTICLE INFO

Keywords:
Glutathione
Photosynthetic pigments
Seed germination
Salt stress
Oxidative stress

ABSTRACT

The comparative effects of reduced glutathione (GSH) and 0.5 % Zn (applied separately or in combination) were assessed on maize in relation to seed metabolite levels, seedling growth, antioxidative defense mechanism, levels of biochemicals and nutrient acquisition under NaCl stress. The level of applied salinity was 120 mM in Hoagland's nutrient solution. Salinity negatively affected seed germination and the emergence of seedlings; associated with altered seed metabolic activities. The high salinity also elevated the levels of malondialdehyde (MDA), increased reactive oxygen species (ROS) levels, altered metabolite levels, reduced uptake of mineral nutrients and increased the uptake of Na^+ in maize seedlings. Interestingly, the GSH seed priming protocol, when applied alone or with Zn, ameliorated the physiological negativities associated with high salinity upon maize germination, emergence and seedling development. The three millimolar GSH concentration in combination with Zn (0.5 %) improved the germination attributes and emergence of seedlings. The GSH level of 3 mM with Zn was also effective in mitigating the negative impacts of NaCl salinity on seedling growth, associated with better maintenance of physio-biochemical activities, reduced uptake or translocation of Na^{2+}, and better maintenance of the increased K^+/Na^+ and Ca^{2+}/Na^+. The improvement in maize salt stress tolerance, attributed to 3 mM GSH with 0.5 % Zn as seed treatment, was associated with reduced Na^+ uptake that decreased its toxicity. Based on this study, it is plausible to use a combination of GSH and Zn as seed priming agentsto enhance the physiological resilience of maize growing in areas with high salinity.

1. Introduction

Along with other abiotic factors, increasing levels of salinity in soil is also a major factor, along with freshwater deficiency, limiting the crop development and production worldwide (Munns and Gilliham, 2015; Shabala and Munns, 2017; Sheikhalipour et al., 2023). Salinity stress is also responsible for imbalance in soil and plant osmotic potential, disturbances in ionic balance that results in increased oxidative damages (Choudhary et al., 2023; dos Santos et al., 2022; He et al., 2023). Disturbances in plant and soil water relations is the foremost impact of salt stress at any plant growth stage. In order to counteract the adverse salinity effects on cellular water levels, a well-known mechanism of osmotic adjustment has been working in plants by accumulating/biosynthesis of organic and inorganic osmolytes/osmotica, including amino acids, peptide, protein and sugars etc. (Munns and Tester, 2008; Seleiman et al., 2023). Salinity, among the different stresses, is being considered more toxic one abiotic stress that negatively impacts every phase of plant growth, biochemical mechanisms, molecular changes as well as in plant tissues, due to accumulation of excessive concentrations of salt, more abundantly the sodium chloride (NaCl) (Suzuki et al., 2014). In this regard, sodium (Na^+) ions replace potassium (K^+) ions, causing a disturbance in the activity of major cytosolic

* Corresponding authors.
E-mail addresses: iqbal_shakir@uo.edu.pk (M. Iqbal), mrazi1532@yahoo.com (M. Rizwan), jean.yong@slu.se (J.W.H. Yong).

https://doi.org/10.1016/j.stress.2025.100767
Received 28 November 2024; Received in revised form 22 January 2025; Accepted 6 February 2025
Available online 7 February 2025

enzymes, because of osmotic stress, due to limiting water absorption from soil solution and also causes the ionic toxicity (Duan et al., 2024; He et al., 2023; Naz et al., 2023; Wu et al., 2023).

Rooting medium salinity causes the oxidative stress in shoot tissues through over accumulation of active oxygen species (AOS). These ROS produce as a byproduct through an aerobic metabolism and accumulates in various subcellular compartments (Rajput et al., 2021; Shabala and Munns, 2017; Wang et al., 2023). However, their excessive generation and accumulation beyond the normal physiological limits causes severe damages to cellular membrane and other macromolecules (Cakmak and Horst, 1991; Das et al., 2024; Hasanuzzaman et al., 2020).

To cope with high levels of salinity, sophisticated adaptations have been developed by plants; involving the osmoregulation to maintain cellular water levels for proper functioning of cellular metabolic activities (Kaur et al., 2024; Munns and Tester, 2008). Alhough salt stress affects every phase of plant growth and development, theseed germination and early emergence are considered the most vulnerable phases to high salt levels (Munns and Tester, 2008; Tebini et al., 2022). To overcome the issue of poor germination in highly saline soils, different ways are in practice/use such as selection of crop genotypes with better germination capacity in salinity or exogenous of different chemicals as treatment of seeds to reduce the salt stress adverse impacts on seed germination and seedlings emergence related attributes as well as its ameliorating impacts on later growth stages for better yield. It includes the seed treatment with organic and inorganic substances, plant-based extracts, micro-nutrients, chelates, antioxidant, biostimulants and phytohormones, etc. (Ali et al., 2020; Sani et al., 2025; Wong et al., 2020; Saeed et al., 2023; Shehzad et al., 2022)

Application of micro-nutrients through different modes are also found the effective one technique for reducing the negative stress impacts on plants at any growth stage (de Bang et al., 2021; Kumar et al., 2020; Sani and Yong, 2022; Wu et al., 2023). However, their use alone as in chemical form has some drawbacks in term of their chances of oxidation during their uptake and translocation (Alsafran et al., 2023). To overcome this problem for their better efficiency, these micro-nutrients are being applied in combination (Saeed et al., 2023) or in chelated form with different organic substances, that found very beneficial after their absorption (Shehzad et al., 2022; Saeed et al., 2023, 2024; Duan et al., 2024; Sani et al., 2025).

Among the essential micro-nutrients for normal plant development, Zn have important functions in regulating the proper cell division, cell expansion, role in carbohydrates biosynthesis, nucleic acids and protein biosynthesis as well as it also regulates the lipid metabolism (Hacisalihoglu, 2020; Jalil et al., 2023; Umair Hassan et al., 2020). Zinc also regulates the biosynthesis of growth regulators such as auxins, an important plant hormone that controls the plant growth (Castillo-González et al., 2018), have important role in seedling emergence and seed germination related attributes and also regulates the activities of other plant hormones (Taiz et al., 2015). Zinc deficiency in plants causes a sharp reduction in transcription process through abnormalities in ribosomes (Singh et al., 2023). Regarding its role in plant tolerance to different stresses, Zn biofortification increases the mobility of nutrients by binding to metal transcription factors (TFs), necessary for its activation (Jalil et al., 2023; Krishna et al., 2020; Xie et al., 2019). Zinc exogenous application also effects the uptake of water from soil and its transport from root to shoot (Iqbal et al., 2018; Zhang et al., 2021). Independently, Zn maintains the negative impacts of oxidative stress by acting as cofactor of different enzymes having roles against oxidative stress, including superoxide dismutase (SOD) and glutathione peroxidase (Cabot et al., 2019; Wu et al., 2015; Hassan et al., 2020).

Reduced glutathione, that have role as an antioxidant and reduces the cellular lipid peroxidation, naturally is a tripeptide (γ-Glu-Cys-Gly) (Hasanuzzaman et al., 2017). It also has numerous other functions, most importantly the stress relieving impacts by the regulation of redox balance of cell, modulation in the activity of genes, regulating the stress impacts, production of thiol group, enhances the activities of enzymes, regulates the biosynthesis of nucleotides and proteins as well as controls the process of senescence (Hossain et al., 2017; Gietler and Nykiel, 2017; Dawood et al., 2020; Hasanuzzaman et al., 2017; Saeed et al., 2023). Such diverse roles of GSH in different metabolic activities shows that its metabolism has great impact in tolerating abiotic stresses through diverse ways (Ramzan et al., 2023). Available reports depict well that exogenously-applied GSH through any mode (foliar or seed priming) enhances tolerance ability of plants against the stresses (Dawood et al., 2020; Zhou et al., 2019; Hossain et al., 2017; Nahar et al., 2015a, 2015b, 2015c; Saeed et al., 2023, 2024). It has also been found that GSH reduces the oxidation of divalent metal ions that is helpful for their better mobility especially in stressful environment (Eteshola et al., 2020).

Among the agronomic crops, maize is gaining prime importance in fulfilling the human food demand and feed requirements of animal in the present scenario and in future too. It is considered a raw source of food industry as well as in the production of energy. After wheat, it has gained the top position in cultivation at global level (Erenstein et al., 2022) and numbered at the third position in Pakistan in cultivation. In Pakistan, maize is cultivated on an area of 1.60 Mha during 2023/2024 (https://finance.gov.pk/survey/chapter-24/2-agriculture.pdf. In Pakistan, maize cultivation is facing the problem of soil salinity and water shortage (Priya et al., 2016; Shabaan et al., 2022). Under such circumstances maize crop is facing the problem of poor crop stand establishment due to bad seed germination, necessary for better emergence. It is being taken as main problem for gaining good yield of maize in Pakistan.

Based on existing knowledge, we hypothesize that the combined application of glutathione (GSH) and zinc (0.5 %) may prove more effective in mitigating the adverse effects of salt stress on maize. The study aims to evaluate the influence of this combination on kernel germination traits, seedling emergence, biomass accumulation, antioxidant activity, and nutrient ion uptake under saline conditions. Additionally, the research focuses on assessing alterations in kernel metabolite levels during the germination process, along with examining photosynthetic pigments in seedlings.

2. Materials and methods

Controlled growth room conditions were employed for conducted the experiment at room temperature (25±2 °C). Experiment was conducted in glass Petri-dishes, arranged in CRD. There were four petri-dishes for each treatment corresponding to number of replicates. There was total 72 glass Petri-dishes in two sets (36 corresponding to saline and other 36 to non-saline ones). Before the supply of solution, all the Petri-plates were equipped with filter paper in double layer. The set of non-saline Petri-dishes were given only 12 mL Hoagland's solution (Full strength) in Petri-dishes. The remaining 36 sets of Petri dishes corresponding to salt treatment was given 12 mL of Hoagland's nutrient solution having 120 mM NaCl. Different treatments applied as seed soaking were Zn (0.5 %) as $ZnSO_4$, 1.5 mM GSH, 3 mM GSH, 4.5 mM GSH, 1.5 mM GSH in combination with Zn (0.5 %), 3 mM GSH in combination with Zn (0.5 %), and 4.5 mM GSH in combination with Zn (0.5 %), including water soaking (WS) and no soaking (NS). The source of Zn was $ZnSO_4$. The solutions corresponding to each treatment were prepared in dH2O. Before seed sowing the soaking was done for 14 h in each respective treatment solution as mentioned above. After that the soaked seeds were blotted using filter paper, dried in shade by providing continuous air till there was no further change in seed weight. Surface sterilization of seeds was done with $HgCl_2$ (0.1 % solution) for 5 min before soaking, followed by thorough washing using dH_2O. In each Petri-dish ten seeds were sown and 4 Petri-dishes were allocated for each treatment. The whole experimental unit was set in twice. The repeated 2nd set of experimental units was allocated for the estimation of different seed metabolites. The detailed methodology is given in supplementary file.

2.1. Determination of different seed germination related parameters

For the quantification of attributes related to seed germination, counting of germinated seeds was done as per the instructions as described by Association of Official Seed Analysis (1990). Then for the determination of parameters regarding seed germination vigor and seedling health then obtained data was used. The estimation of seed germination percentage (G%) was done by the given formula as (G%) = (Number of seeds germinated/total seed grown) × 100. The estimation of time taken to complete 50 % emergence (E_{50}) was done using given formula as reported by Coolbear et al. (1984). The formula was employed to estimate mean emergence time (MET) as given by Ellis and Roberts (1981) and formula given by Bewley and Black (1985) was followed for the estimation of coefficient of uniformity of emergence (CUE). For the estimation of emergence index (EI), formula used by Association of Official Seed Analysis (1983) was followed. While to study changes in germination energy (GE) of germinating seeds, Ruan et al. (2002) method was used.

2.2. Estimation of morphological and growth parameters of maize seedlings

For the determination of biomasses of root and shoot four plants per treatment were used. Root and shoot lengths were also measured of the same plants. After that the dried masses of roots and shoots were measured of these samples after oven-drying at 65 °C for 48h. These dried roots and shoot samples after grinding well, used to measure the nutrients.

2.3. Quantification of leaf chlorophyll contents

For the quantification of chlorophyll *b* (Chl. *b*), total chlorophyll (T. Chl.), as well as chlorophyll *a* (Chl. *a*), the Arnon (1949) method was employed, while the levels of carotenoid (Car.) was determined by Kirk and Allen (1965) method. Extraction of photosynthetic pigments was conducted in 80 % acetone solution.

2.4. Quantification of different metabolites at different time interval of maize kernel during germination

For extracting T. Chl. and carotenoids during germination of seeds the method of Arnon (1949) was used. The extraction of sugars in kernels was done using technique as employed by Tongue et al. (2012) and the quantification was made spectrophotometrically of total soluble sugars (TSS) (Dubois et al.,1956), by phenol sulfuric acid method. While the method given by Somogyi (1952) was followed to quantify the reducing sugars (RS). The quantification of total soluble proteins (TSP) in germinating seeds was made following method given by Bradford (1976), using the extract obtained from seeds following the method as given by Larson and Beevers (1963). For the estimation of free amino acids (FAA) in seeds during germination was made from extract as obtained by using phosphate buffer, following Noctor et al. (2007) and the FAA content quantified following Lee and Takahashi (1966) using ninhydrin method. The quantitative analysis of oil in seeds during seed germination was made by Nuclear magnetic resonance (NMR), while total free fatty acids (TFFA) was quantified following Lowry and Tinsley (1976).

2.5. Estimation of activities of different antioxidant enzymes

The leaf material (0.5 g) was grinded using the liquid N_2 and then ten milliliter buffer (Potassium phosphate) was added to the material. After that the centrifugation was made using centrifugation force of 10,000 × *g*, for 20 min at 4 °C. Ascorbate peroxidase (APX) activity from buffer extracted leaf samples was made in a time scan manner using the method of Nakano and Asada (1981) and the absorbance was made at 290 nm. The method by Giannopolitis and Ries (1977) was followed to measure the superoxide dismutase (SOD) activity, using buffer extracted samples. The prepared mixture for SOD was used to read absorbance at 560 nm spectrophotometrically. Activities of catalase (CAT) and peroxidase (POD) were estimated following Chance and Maehly (1955) methods. The assessment of TSP was done as described by Bradford (1976).

2.6. Quantification of leaf total flavonoid (TFC), phenolics (TPC), anthocyanin TAC, ascorbic acid (AsA) contents and total free amino acids (TFAA) of maize seedlings

The TPC of seedling was quantified following the protocol as ascribed by Julkunen-Titto (1985). The seedling TFC were also determined by spectrophotometer according to Zhishen et al. (1999). The method given by Mirecki and Teramura (1984) was used for the quantification of TAC in maize seedlings using methanol extraction. The estimation of TFAA contents was done following the protocol of Hamilton et al. (1943). While AsA quantification in maize seedlings was done using method as employed by Mukherjee and Choudhuri (1983).

2.7. Estimation of leaf total reducing sugars (TRS), total soluble sugars (TSS) and total non-reducing sugars (NRS) in maize seedlings

The TSS was quantified as reported by Dubois et al. (1956). The content of RS was quantified following the method given by Henson and Stone (1988) and the quantification of NRS was done following the given formula

$$NRS = TSS \; - \; -RS$$

2.8. Quantification of leaf MDA and H_2O_2 contents in maize seedlings

Estimation of leaf MDA was done following Cakmak and Horst (1991), using trichloroacetic acid as the extractant. In case of estimation of H_2O_2 levels, was done following Velikova et al. (2000).

2.9. Quantification of mineral nutrients in shoot and root of maize seedlings

Dry powdered material of maize shoot and root separately was digested separately, using the digestion mixture as reported by Parkinson and Allen (1975). After digestion, the volume was made 50 mL with dH_2O. A flame photometer was used to measure K^+, Na^+ and Ca^{2+}, made of Jenway PFP 7, Cole-Parmer, Vernon Hills, IL, USA. While Mg^{2+} and Fe^{2+} were quantified using AAS (TRACE AI1200, Aurora Biomed, Vancouver, Canada). Phosphorus content in samples was quantified spectrophotometrically using the Barton's Reagent. The N quantification in digested samples was made following Bremner and Keeney (1965) method.

2.10. Statistical analysis of parameters studied

For measuring significant differences of the applied treatments on measured parameters, Computer Program CoStat (Window Version 6.303, PMB 320) applied. To find out the significant differences among means of parameter done, the Least Significant Difference (LSD) test was used following 5 % level of probability. For the principal component analysis (PCA), R-studio version 4.2.2 computer package was used to estimate the correlations among the studied parameters. To measure correlations among studied parameter and treatment, heatmap study was done. The significance among correlations value was find out using the values given by Pearson's correlation.

3. Results

3.1. Seed germination attributes

Different parameters related to seed germination and emergence of seedlings, including GE, EI, CUE and G% of maize decreased significantly due to imposition of salt stress. Seed soaking with different GSH concentrations, when applied separately or with Zn in combination, significantly ameliorating the salt stress negative impacts on seed GE, GI, CUE and G%. Soaking of seeds with GSH when applied separately or with Zn in combination also positively impacted the seed germinating and seedling emergence related attributes when grown without salt stress. Seed priming with GSH, when applied @ 3 mM with Zn in combination, was found the best one in improving the GE, GI, CUE and G %, followed by the 1.5 mM concentration of GSH with 0.5 % Zn as compared with other treatments (Fig. 2).

Seed MET and E_{50} increased significantly of maize seeds when grown in NaCl stress. Use of different GSH levels as seed soaking, applied alone or with Zn found significant for reducing negativities of salt stress on MET as well as E_{50}. GSH-applied this amelioration in E_{50} and MET also recorded in maize seeds grown without saline condition. But the ameliorating effect was treatment and parameter specific. Regarding MET and E_{50} the maximum improvement was found by seed soaking with GSH when applied @ 3 mM, either used separately or with Zn under saline condition but under non-saline conditions this improvement in MET and E_{50} was maximum due to seed soaking with GSH when applied @ 3 mM either used with Zn (0.5 %) in combination followed by the 3 mM GSH level alone (Fig. 2; Table S1).

3.2. Growth and morphological attributes of maize seedlings

Ameliorating impacts of seed soaking with GSH, either used separately or with Zn (0.5 %), on the growth of radicle and plumule as well as on morphology of seedlings are given in Fig. 1A, B, C and D. It clearly shows that soaking of seeds in GSH when used @ 3 mM either alone or with Zn (0.5 %) in combination, resulted in the speedy growth of radicles and plumules than other treatments. Different morphological and growth-related attributes as presented in Fig. 3 and Table S1 for maize seedling showing that these parameters significantly negatively impacted due to NaCl salinity. Seed soaking with reduced glutathione with different levels, either applied alone and with Zn, ameliorated the negative effects of NaCl salinity on these morphological as well as

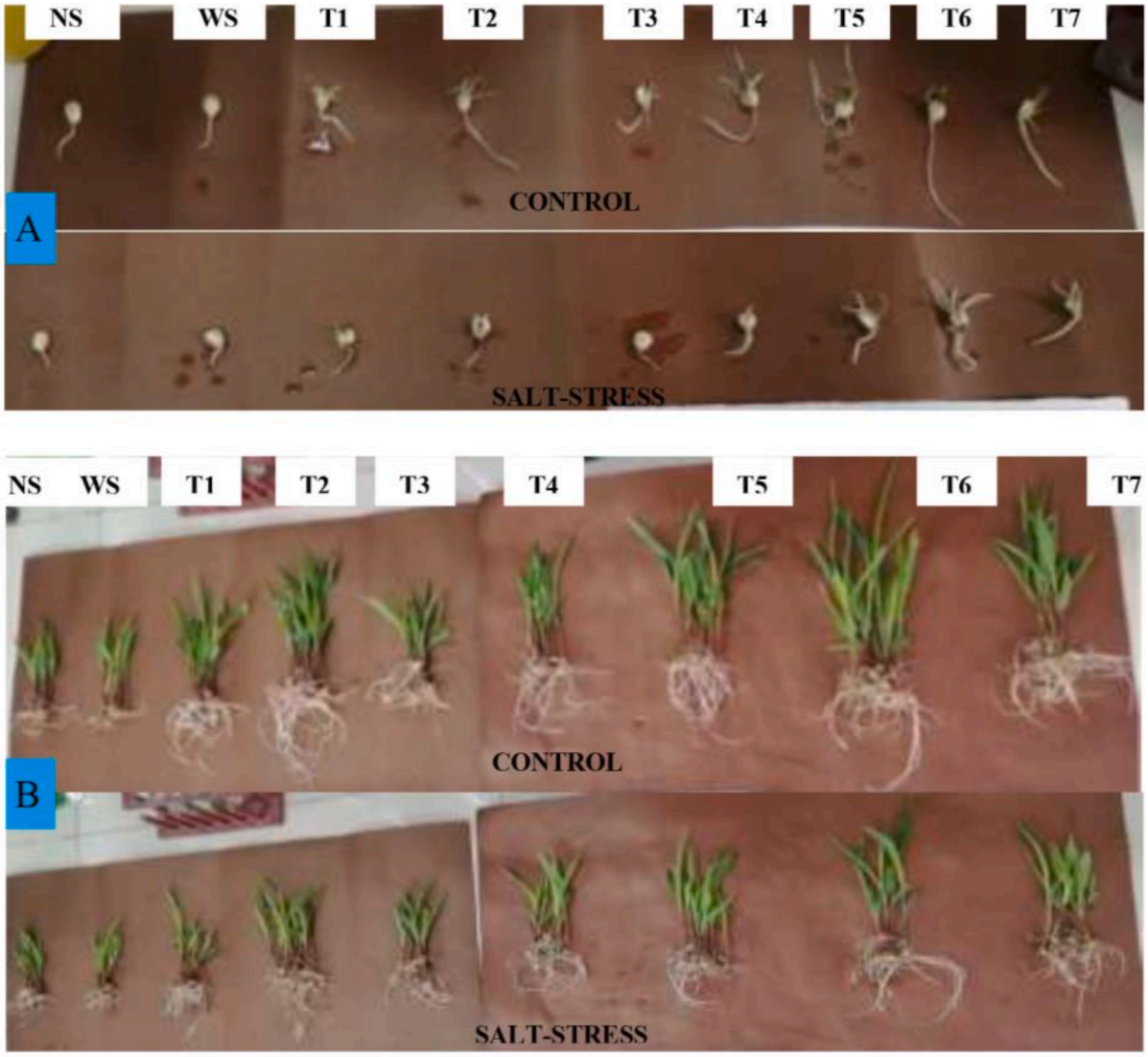


Fig. 1. Plumule and radical protrusion patterns, growth patterns (A) shoot and root lengths (B) of maize seedlings under salt stress and non-stress conditions when grown from seeds priming with different levels of GSH alone and in combination with Zn. **NS**= No soaking; **WS**=Water soaking; **T1**=1.5 mM GSH; **T2**=3 mM GSH; **T3**=4.5 mM GSH; **T4**=0.5 % Zn; **T5**=1.5 mM GSH+0.5 % Zn; **T6**=3 mM GSH+0.5 % Zn; **T7**=4.5 mM GSH+0 .5 %Zn.

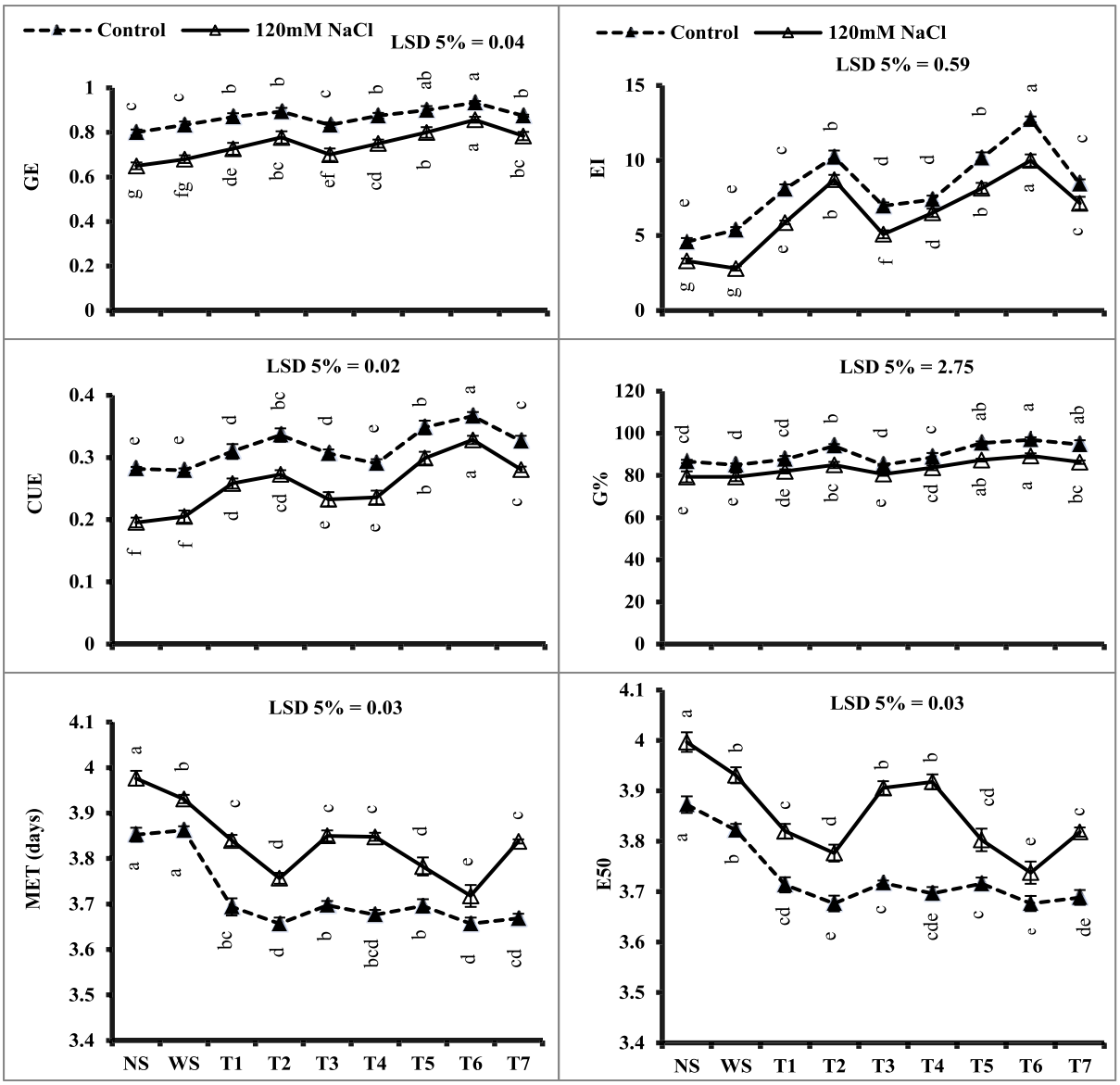


Fig. 2. Effects of different levels of GSH when applied separately or in combination with 0.5 % Zn as seed priming on different seed germination and seedling emergence related attributes of maize when grown under non-saline and NaCl salinity (Mean±SE; $n = 4$).
NS= No soaking; **WS**=Water soaking; **T1**=1.5 mM GSH; **T2**=3 mM GSH; **T3**=4.5 mM GSH; **T4**=0.5 % Zn; **T5**=1.5 mM GSH+0.5 % Zn; **T6**=3 mM GSH+0.5 % Zn; **T7**=4.5 mM GSH+0.5 % Zn; *(Values against treatments on lines with same alphabets do not differ significantly)*

growth attributes. However, amelioration impact was treatment specific. Zinc and GSH-applied increments in growth parameters also recorded in maize seedlings grown without salt stress. Comparatively more increment in these growth attributes recorded, when GSH was applied @ 3 mM with 0.5 % Zn followed by 4.5 mM and 1.5 mM concentration of GSH with Zn in combination. Similar treatments were also found better in improving the growth attributes under non-saline condition but their extent of improvement was less than the plants grown under salt stress condition.

3.3. Leaf AsA, H_2O_2 and MDA contents of maize seedlings

Data for MDA, H_2O_2 and ASA contents showing that their levels significantly increased in maize seedlings grown in NaCl salinity stress than maize plants without any treatment (Fig. 3; Table S1). Less rise in MDA and H_2O_2 levels was recorded in maize seedlings grown from seeds soaked in GSH solutions with different levels, either alone or with Zn (0.5 %) in combination that confers the stress ameliorating impacts of these seed soaking treatments. Among different studied treatments, the maximum amelioration regarding the reduction in MDA and H_2O_2 levels found by soaking of seeds with GSH when applied @ 1.5 mM level, alone or with Zn in combination followed by 3 and 4.5 mM concentrations of GSH with Zn in combination. Regarding leaf AsA content, a further increment in the levels of AsA recorded in plants grown from seeds soaked in GSH, either applied separately or with Zn in combination but the combined application was more effective than the alone applications of GSH and Zn. More increment in seedling AsA level recorded due to seed soaking with 3 mM solution of GSH prepared in 0.5 % Zn solution.

3.4. Leaf chlorophyll content, TAC, TFC and TPC of maize seedlings

Data shows that T. Chl., Chl. *a/b* ratio, Chl. *b*, and Chl. *a* reduced significantly due to imposition of NaCl salinity. Significant improvements in leaf chlorophyll contents were recorded due to seed soaking with solutions of reduced glutathione, used either alone or with Zn (0.5 %) under salt stress. Seed soaking using different GSH concentrations

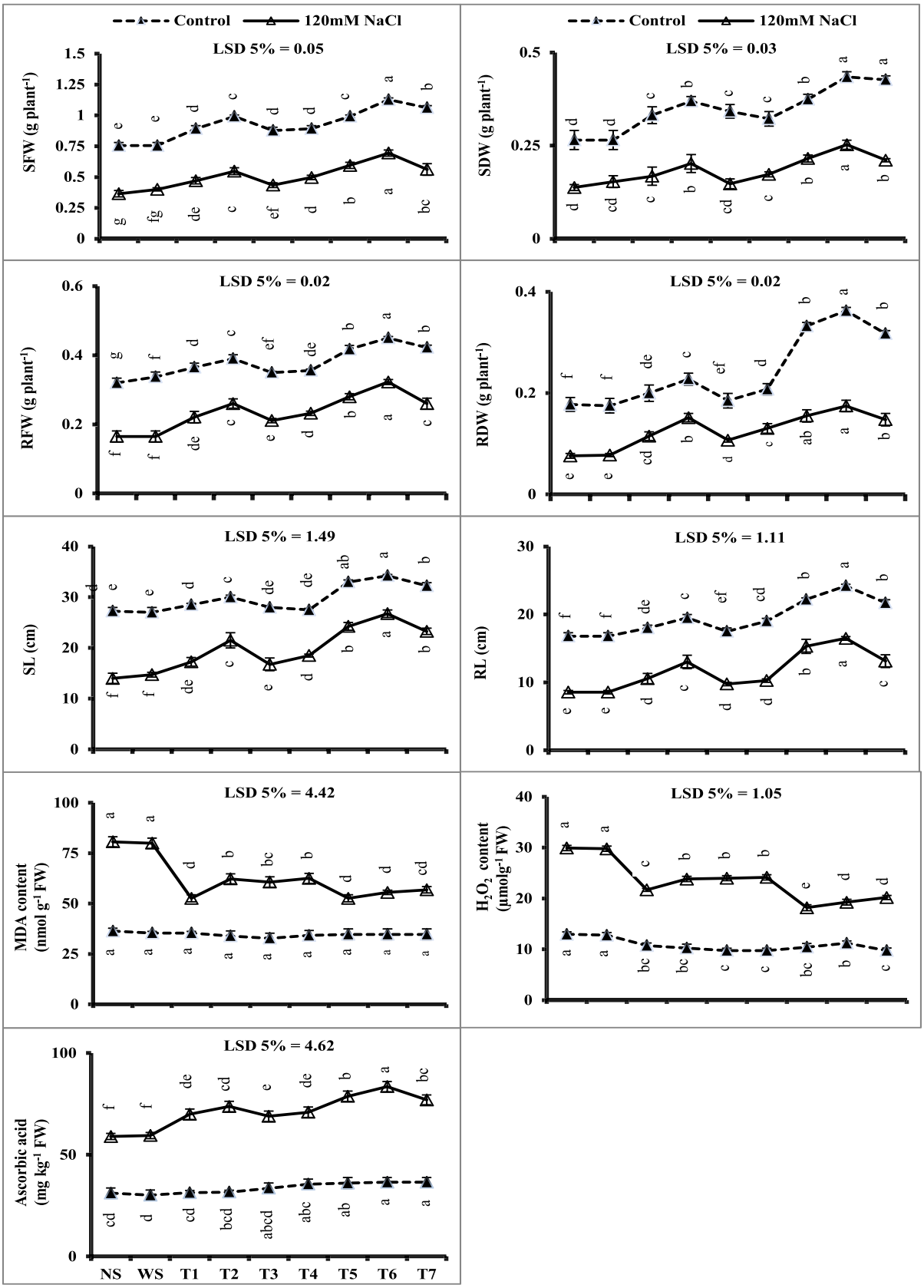


Fig. 3. Effects of different levels of GSH when applied separately or in combination with 0.5 % Zn as seed priming on growth attributes MDA, H_2O_2 and AsA contents of maize seedlings when grown under non-saline and NaCl salinity (Mean±SE; $n = 4$).
NS= No soaking; **WS**=Water soaking; **T1**=1.5 mM GSH; **T2**=3 mM GSH; **T3**=4.5 mM GSH; **T4**=0.5 % Zn; **T5**=1.5 mM GSH+0.5 % Zn; **T6**=3 mM GSH+0.5 % Zn; **T7**=4.5 mM GSH+0.5 % Zn; *(Values against treatments on lines with same alphabets do not differ significantly)*

either alone or with Zn (0.5 %), found significant in improving leaf chlorophyll levels as well as the Chl. *a/b* ratio of maize seedlings when grown without NaCl salinity but this increment was less in comparison with plants grown under NaCl salinity. However, the increasing extent was specific to GSH level, either applied separately or with Zn in combination. In comparison, more increment in leaf photosynthetic pigments due to seed soaking with GSH was found when used with 0.5 % Zn in combination as compared with the alone application of GSH. More increments in leaf chlorophyll as well as *a/b* ratio recorded due to GSH treatment when applied @ 3 mM, either used separately or with Zn in combination under both salt regimes, followed by the 1.5 and 4.5 mM GSH concentrations when applied with Zn in combination (Fig. 4).

Rooting medium salinity caused significant increment in leaf Car. levels of maize plants. Seed soaking using various concentrations of GSH either applied with Zn (0.5 %) or alone, further increased the leaf Car. content of maize seedlings. Treatment-applied this increment in leaf Car. was also recorded in plants grown in non-saline environment. However, treatment-applied, this increment in Car. content was GSH level specific, used either alone or with Zn in combination, under both salt regimes. Among different treatments, 3 mM GSH level, following 1.5 and 4.5 mM treatments in combination with Zn showed more increasing impact on Car. level, under both salt regimes (Fig. 4; Table S1).

Significant increment was also found in ratio of T. Chl./Car. under NaCl salinity. Soaking of maize kernels using varying levels of reduced glutathione either used alone or with 0.5 % Zn in combination significantly decreased the T. Chl./Car. under salt stress. This decrease in T. Chl./Car. ratio as a result of seed soaking with different GSH concentrations also found significant in plants when grown without salinity. However, the increment in T. Chl./Car. found comparatively less than that found under salt stress. Among different levels of GSH, 1.5 mM level was found most effective one in reducing the T. Chl./Car. ratio (Fig. 4; Table S1).

Leaf TAC and TFC contents significantly increased due to rooting medium salinity. Seed soaking with GSH, either applied separately or with Zn in combination found effective in further increasing the TFC and TAC levels of maize seedling under NaCl salinity. However, seed soaking with 3 mM reduced glutathione + 0.5 % Zn was found more effective one than other treatment under both salt levels (Fig. 4; Table S1).

Leaf TPC also significantly increased due to rooting medium salinity. A further increment in TPC was recorded due to seed soaking with GSH, either applied separately or with Zn (0.5 %) in combination and treatment-applied this increase in TPC levels recorded in non-stressed plants too. However, treatment-applied this increment in TPC was less than in plants grown under saline conditions. Maximum increment in TPC, under both salt regimes was recorded due to seed soaking with GSH, when applied @ 3 mM with Zn in combination (Fig. 4; Table S1).

3.5. Antioxidant enzyme activities, and some leaf metabolites of maize seedlings

Date presented in Fig. 5 for the activities of antioxidants enzymes and TSP shows that their activities significantly increased when grown under saline condition. Pre-sowing treatment of seeds, using reduced glutathione separately or with 0.5 % Zn found effective in further increasing the activities of antioxidant enzymes as well as levels of TSP under both salt regimes. The more increment in antioxidative enzyme activities was recorded when seeds were primed using 1.5 mM solution of reduced glutathione concentration and applied with 0.5 % Zn, under both salt regimes.

Rooting medium salinity significantly increased the levels of metabolites, such as NRS, RS and NRS in maize seedlings. Seed soaking using varying GSH concentrations, either used with Zn (0.5 %) in combination or as separate application, significantly increased the NRS, RS and TSS of maize plants grown under NaCl salinity as well as of plants grown in non-saline condition. More increment in NRS, RS and TSS found due to seed soaking with 3 mM concentration of reduced glutathione with Zn (0.5 %) (Fig. 5; Table S1).

Rooting medium salinity increased significantly the leaf TFAA in maize seedlings. However, a significant reduction in TFAA was recorded in plants due to priming of seeds with reduced glutathione, either used with Zn (0.5 %) as well as separately. However, comparatively more decrease in TFAA was recorded due to priming of seeds with reduced glutathione in combination with Zn (0.5 %) and all combined treatment of GSH with 0.5 % Zn found equally effective in decreasing the TFAA contents under salt stress (Fig. 5).

3.6. Uptake of nutrient in root and shoot

Rooting medium salinity significantly decreased the root and shoot nutrient contents such as K^+, Ca^{2+}, N, P, Mg^{2+} and Zn^{2+} in maize plants. Seed soaking with varying concentrations of GSH, either applied separately or with Zn in combination, reduced the negativities of imposition of NaCl salt stress in uptake of examined root and shoot nutrient. However, the extent of this amelioration in root and shoot nutrient uptake was treatment specific. Seed soaking with various concentration of GSH, either used alone or with Zn in combination, also increased the nutrient uptakes in root and shoot under non-saline condition too. However, this increase in root and shoot nutrient contents was not same at all GSH concentration, either applied alone or with Zn in combination. Among different levels of GSH, 3 mM level of GSH was found most effective one in combination with Zn for maintaining root and shoot nutrient contents under both salt regimes (Table 3; Table S1). Regarding the Zn content in root and shoot relatively more increment found in plants raised from GSH treated seed, when used with Zn in combination.

Imposition of NaCl salinity resulted in a significant increase in Na^+uptake as well as and Na^+/k^+ ratio. However, this increment in uptake of Na^+decreased significantly in root and shoot as well as the Na^+/k^+ ratios due to seed treatment with GSH of different concentrations, either applied alone or with Zn in combination. Among different treatments, maximum decrease in uptake of Na^+ and Na^+/k^+ ratio both in shoot and root was resulted, when seeds were primed with 3 mM GSH level, either applied separately or with Zn in combination, followed by 1.5 mM and 4.5 mM concentrations of GSH with Zn in combination (Fig. 6; Table S1).

Data presented in Fig. 6 and Table S1 showing that root and shoot Ca^{2+}/Na^+ ratio significantly decreased when grown under saline conditions. Seed soaking with GSH either applied separately or with Zn in combination, ameliorated the negative impacts of NaCl salinity on root and shoot Ca^{2+}/Na^+ratio. The maximum amelioration in maintaining Ca^{2+}/Na^+ratio was found when seeds were soaked in solution of 3 mM level of reduced glutathione with 0.5 % Zn in combination.

Shoot and root Cl^- contents significantly increased due to rooting medium salinity. Seed soaking with reduced glutathione, used separately or with Zn (0.5 %), decreased significantly the shoot and root Cl^- content, when grown in NaCl salinity. Maximum decrease in the uptake of Cl^- was found in plants, when seeds were primed using GSH of 3 mM strength, either applied separately as well as with 0.5 % Zn, under salt stress conditions. However, no significant impact of GSH seed priming was found on Cl^-, either applied alone or with Zn in combination on their uptake in root and shoot when grown under non-saline condition (Fig. 6; Table S1).

3.7. Metabolite levels of germinating seeds of maize

Data for Chl. and Car. contents, for germinating seed/seedlings showing a gradual increment in these parameters, as seed germination started under both salt regimes and a gradual increase was recorded when data recorded at different timing (24 h, 48 h and 72 h) after seed sowing (Table 1). Moreover, the increments in Chl. as well as the Car. of seeds during germinating or seedlings after start of seed germination, with passage of time, was more in seeds grown under non-saline condition than saline ones. Seed soaking with GSH either used alone or with

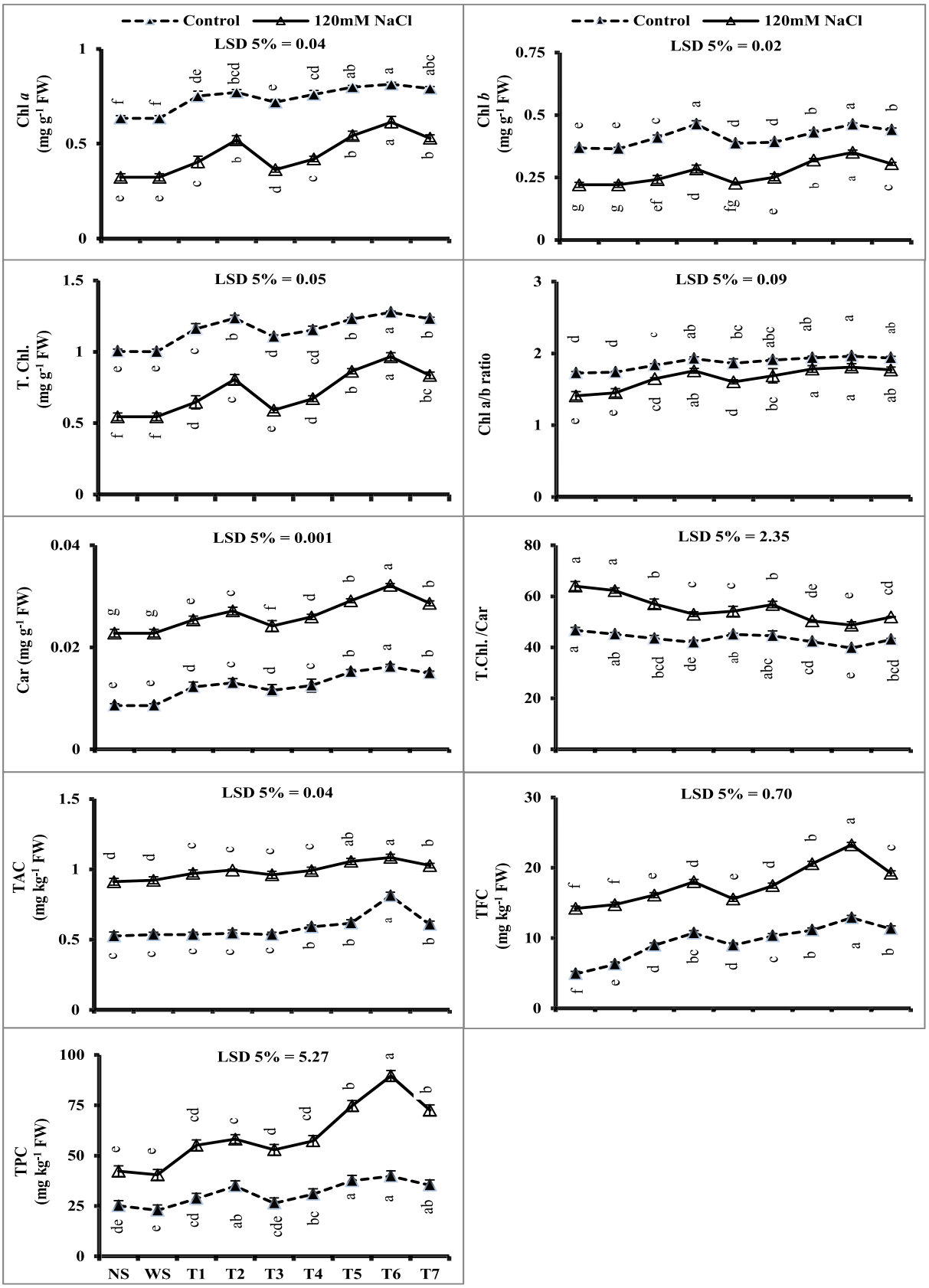


Fig. 4. Effects of different levels of GSH when applied separately or in combination with 0.5 % Zn as seed priming on photosynthetic pigments, TAC, TFC and TPC contents of maize seedlings when grown under non-saline and NaCl salinity (Mean±SE; $n = 4$).
NS= No soaking; **WS**=Water soaking; **T1**=1.5 mM GSH; **T2**=3 mM GSH; **T3**=4.5 mM GSH; **T4**=0.5 % Zn; **T5**=1.5 mM GSH+0.5 % Zn; **T6**=3 mM GSH+0.5 % Zn; **T7**=4.5 mM GSH+0.5 % Zn; *(Values against treatments on lines with same alphabets do not differ significantly)*

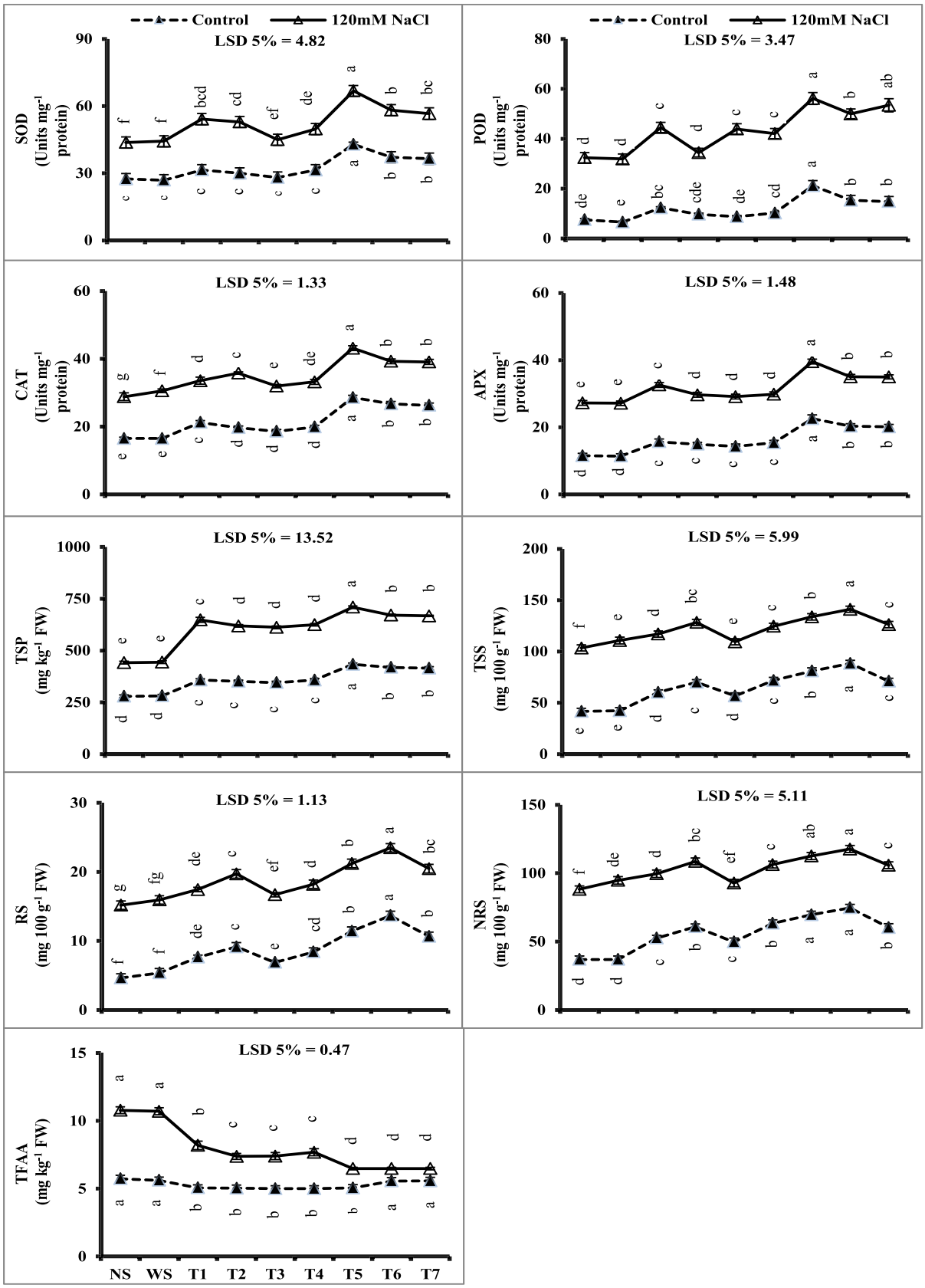


Fig. 5. Effects of different levels of GSH when applied separately or in combination with 0.5 % Zn as seed priming on the activities of SOD, POD, CAT, APX and the contents of TSP, TSS, RS, NRS and TFAA of maize seedlings when grown under non-saline and NaCl salinity (Mean±SE; $n = 4$).
NS= No soaking; **WS**=Water soaking; **T1**=1.5 mM GSH; **T2**=3 mM GSH; **T3**=4.5 mM GSH; **T4**=0.5 % Zn; **T5**=1.5 mM GSH+0.5 % Zn; **T6**=3 mM GSH+0.5 % Zn; **T7**=4.5 mM GSH+0.5 % Zn; *(Values against treatments on lines with same alphabets do not differ significantly)*

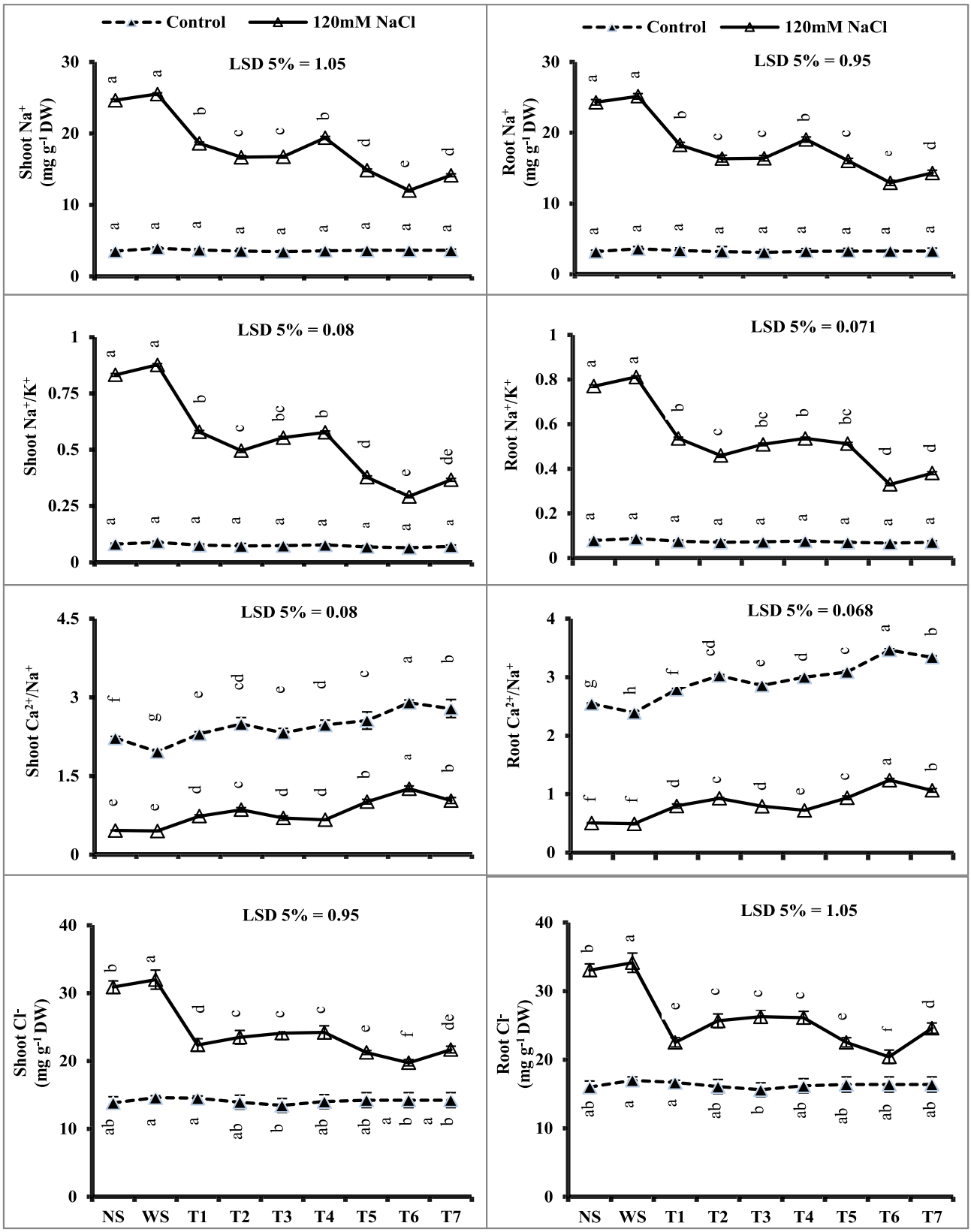


Fig. 6. Effects of different levels of GSH when applied separately or in combination with 0.5 % Zn as seed priming on content of Na^+, ratios of Na^+/K^+, and Ca^+/Na^+ and Cl^- contents in root and shoot of maize seedlings when grown under non-saline and NaCl salinity (Mean±SE; $n = 4$).
NS= No soaking; **WS**=Water soaking; **T1**=1.5 mM GSH; **T2**=3 mM GSH; **T3**=4.5 mM GSH; **T4**=0.5 % Zn; **T5**=1.5 mM GSH+0.5 % Zn; **T6**=3 mM GSH+0.5 % Zn; **T7**=4.5 mM GSH+0.5 % Zn; *(Values against treatments on lines with same alphabets do not differ significantly)*

Table 1
Effects of different levels of GSH when applied separately or in combination with 0.5 % Zn as seed priming on carotenoid (Car.), chlorophyll (Chl.), total soluble sugars (TSS), and total reducing sugar (TRS) contents of germinating maize seeds under non-saline and NaCl salinity (Mean±SE; $n = 4$).

Chl. (mg/g FW) (LSD at 5 % level = 0.03)									
Time (h)	NS	WS	T1	T2	T3	T4	T5	T6	T7
Control									
0	0	0	0	0	0	0	0	0	0
24	0.05±0.003^{de}	0.04±0.003^{e}	0.09±0.007^{c}	0.14±0.060^{b}	0.07±0.006^{cd}	0.09±0.006^{c}	0.16±0.002^{b}	0.19±0.002^{a}	0.15±0.002^{b}
48	0.08±0.002^{e}	0.07±0.002^{e}	0.15±0.029^{d}	0.39±0.038^{bc}	0.13±0.028^{e}	0.15±0.028^{d}	0.41±0.005^{b}	0.44±0.003^{a}	0.37±0.003^{c}
72	0.31±0.31^{d}	0.30±0.31^{d}	0.81±0.070^{c}	1.03±0.074^{b}	0.79±0.68^{c}	0.81±0.06^{c}	1.05±0.016^{b}	1.13±0.004^{a}	1.04±0.004^{b}
120 mM NaCl									
0	0	0	0	0	0	0	0	0	0
24	0.04±0.003^{c}	0.04±0.002^{c}	0.06±0.03^{c}	0.12±0.057^{b}	0.05±0.005^{c}	0.06±0.03^{c}	0.13±0.002^{ab}	0.15±0.003^{a}	0.11±0.003^{b}
48	0.06±0.002^{e}	0.05±0.002^{e}	0.12±0.04^{d}	0.18±0.0s36^{c}	0.11±0.028^{d}	0.12±0.04^{d}	0.27±0.004^{a}	0.29±0.002^{a}	0.24±0.002^{b}
72	0.26±0.23^{f}	0.25±0.22^{f}	0.79±0.05^{e}	0.96±0.072^{d}	0.78±0.067^{e}	0.79±0.05^{e}	1.02±0.012^{b}	1.11±0.004^{d}	0.99±0.005^{c}
Car. (µg/g FW) (LSD at 5 % level = 0.009)									
Control									
0	0	0	0	0	0	0	0	0	0
24	0.011±0.005^{c}	0.012±0.004^{c}	0.032±0.003^{a}	0.021±0.003^{b}	0.019±0.002^{bc}	0.021±0.002^{b}	0.037±0013^{a}	0.035±0.005^{a}	0.033±0.003^{a}
48	0.026±0.001^{g}	0.027±0.006^{g}	0.153±0.008^{d}	0.109±0.004^{e}	0.082±0.003^{f}	0.102±0.004^{e}	0.214±0.002^{a}	0.183±0.008^{b}	0.162±0.004^{c}
72	0.082±0.006^{h}	0.092±0.005^{g}	0.302±0.016^{d}	0.204±0.013^{e}	0.183±0.004^{f}	0.202±0.012^{e}	0.353±0.004^{a}	0.333±0.012^{b}	0.313±0.005^{c}
120 mM NaCl									
0	0	0	0	0	0	0	0	0	0
24	0.006±0.003^{b}	0.007±0.003b	0.012±0.002^{b}	0.021±0.003^{a}	0.008±0.007^{b}	0.012±0.002^{b}	0.024±0.004^{a}	0.027±0.008^{a}	0.023±0.015^{a}
48	0.013±0.003^{g}	0.014±0.004^{g}	0.052±0.004^{e}	0.102±0.006^{d}	0.023±0.003^{f}	0.052±0.004^{e}	0.143±0.005^{b}	0.174±0.007^{a}	0.123±0.014^{c}
72	0.043±0.004^{h}	0.052±0.005^{g}	0.114±0.011^{e}	0.202±0.014^{d}	0.095±0.005^{f}	0.112±0.011^{e}	0.233±0.002^{b}	0.262±0.002^{a}	0.222±0.014^{c}
TSS (mg/g FW) (LSD at 5 % level = 1.95)									
Control									
0	14±0.86^{d}	15±0.88^{d}	15.50±0.89^{d}	19.00±0.96^{c}	15.23±0.91^{d}	15.52±0.89^{d}	21.04±0.87^{b}	24.57±0.98^{a}	18.92±0.98^{c}
24	15±0.88^{e}	16±0.90^{e}	20.00±1.99^{d}	22.00±2.04^{c}	19.07±1.95^{d}	20.00±1.99^{d}	30.06±1.44^{b}	33.00±1.46^{a}	22.83±0.96^{c}
48	29±1.64^{f}	31±1.66^{e}	39.00±2.05^{bc}	44.00±2.35^{a}	37.04±2.03^{d}	39.02±2.04^{bc}	37.52±1.30^{cd}	40.08±1.34^{b}	44.79±2.44^{a}
72	43±1.98^{f}	45±2.01^{e}	51.00±2.36^{c}	57.00±2.55^{b}	49.02±2.31^{d}	51.02±2.35^{c}	58.02±2.02^{ab}	59.42±2.06^{a}	52.01±2.55^{c}
120 mM NaCl									
0	13.09±0.82^{e}	14.02±0.86^{de}	15.70±0.77^{cd}	17.25±0.87^{c}	15.00±0.73^{de}	14.70±0.77^{de}	20.50±0.68^{b}	22.45±0.77^{a}	15.90±0.65^{cd}
24	12.56±0.86^{e}	14.05±0.87^{e}	16.00±1.92^{d}	19.21±0.46^{bc}	16.54±1.85^{d}	16.00±1.92^{d}	21.00±1.22^{b}	29.54±0.44^{a}	19.05±1.20^{c}
48	26.00±1.53^{d}	27.90±1.63^{d}	26.00±1.99^{d}	36.90±0.88^{ab}	34.01±1.35^{c}	36.00±1.98^{b}	37.45±2.03^{ab}	38.87±1.24^{a}	36.96±1.95^{ab}
72	32.09±1.96^{e}	33.67±1.89^{e}	38.45±2.13^{cd}	39.02±2.08^{c}	37.06±2.02^{d}	38.45±2.13^{cd}	41.10±1.88^{b}	49.60±2.02^{a}	39.94±1.76^{bc}
TRS (mg/g) (LSD at 5 % level = 1.71)									
Control									
0	9.01±0.32^{ab}	9.32±0.30^{ab}	9.51±0.34^{ab}	8.86±0.32^{ab}	8.46±0.27^{b}	9.50±0.34^{ab}	9.90±0.36^{ab}	10.23±0.41^{a}	9.76±0.33^{ab}
24	9.36±0.33^{b}	9.67±0.33^{ab}	9.76±0.32^{ab}	8.97±0.37^{b}	8.72±0.23^{b}	9.75±0.34^{ab}	10.01±0.39^{ab}	11.33±0.45^{a}	9.97±0.35^{ab}
48	13.02±0.48^{c}	13.92±0.43^{c}	17.01±0.53^{b}	19.01±0.62^{a}	16.86±0.44^{b}	17.00±0.51^{b}	19.76±0.65^{a}	20.57±0.73^{a}	19.67±0.57^{a}
72	18.02±0.79^{d}	18.88±0.73^{d}	28.01±1.71^{c}	31.02±1.82^{b}	27.01±1.64^{c}	28.00±1.71^{c}	31.92±1.84^{b}	34.74±2.04^{a}	31.45±1.79^{b}
120 mM NaCl									
0	8.00±0.12^{b}	8.26±0.13^{ab}	9.15±0.16^{ab}	8.98±0.12^{ab}	9.00±0.15^{ab}	9.15±0.16^{ab}	9.23±0.27^{ab}	9.90±0.33^{a}	8.99±0.20^{ab}
24	8.32±0.11^{b}	8.56±0.14^{b}	9.78±0.17^{ab}	9.32±0.13^{ab}	9.65±0.12^{ab}	9.78±0.17^{ab}	10.02±0.22^{ab}	10.51±0.26^{a}	9.45±0.15^{ab}
48	9.00±0.06^{e}	9.76±0.09^{de}	10.54±0.11^{cde}	11.00±0.09^{bcd}	9.40±0.12^{de}	10.54±0.11^{cde}	12.68±0.44^{b}	17.75±0.64^{a}	11.87±0.33^{bc}
72	10.43±0.38^{d}	10.90±0.42^{cd}	12.43±0.63^{c}	16.54±0.82^{b}	11.75±0.45^{cd}	12.43±0.63^{c}	17.87±1.82^{b}	20.38±1.84^{a}	17.12±1.24^{b}

NS=No soaking; WS=water soaking; T1=1.5 mM GSH; T2=3 mM GSH; T3=4.5 mM GSH; T4=Zn 0.5 %; T5=Zn+1.5 mM GSH; T6=Zn+3.0 mM GSH; T7=Zn+4.5 mM GSH; *(Values with same alphabets in superscript in a row do not differ significantly)*.
Means in a row with same letter (a, b, c,……) do not differ significantly.

Zn in combination, significantly speed up this increase in seedling Chl. and Car contents under both salt regimes and comparatively GSH-applied this increase was found more under salt stress conditions than non-saline ones, in comparison to their non-treated ones. Moreover, GSH-applied this increment was not found same due to all GSH levels, either used alone as well as with 0.5 % Zn under both salt regimes. The speedier rise in Chl. and Car contents of germinated seed/seedlings recorded, when seed soaking was done with 3 mM strength of GSH, either applied separately or with Zn in combination. In comparison with other GSH concentrations under both salt regimes. Furthermore, the fast increment in Chl. and Car. levels also recorded due to seed soaking with Zn that was similar as with GSH applied @ 1.5 mM.

The levels of TRS and TSS started to rise significantly of germinating seeds when grown in both salt regimes and that increment was found when studied with different timings after seed sowing. Moreover, this increment in TRS and TSS was recorded more under non-saline conditions. Seed soaking with GSH either applied alone or with Zn further speed up these increments in TRS and TSS contents under both salt regimes. However, the extent of increment was found treatment specific in both salt regimes. Comparatively this increment in TSS and TRS was more, when soaking of seeds was done using the GSH @ 3 mM, either used with Zn (0.5 %) in combination or separately under both salt regimes (Table 1).

Contents of TSP and seed oil as presented in Table 2 during seed germination decreased significantly under both salt regimes, when the data was noted at different time intervals, after seed sowing. Moreover, reduction in seed oil and TSP during germination process was less in seeds grown under NaCl salinity. Soaking of seeds in reduced glutathione solution either used alone or in combination with Zn (0.5 %) further increased the decrease in seed oil and TSP contents under both salt regimes, but the speedy decrease was treatment specific under both salt regimes. The speedier reduction in seed oil and TSP found in germinated seed soaked in 3 mM level of GSH either used with Zn (0.5 %) or separately, in comparison with other treatments, under both salt regimes. Moreover, the speedy decrease in seed oil and TSP also recorded in germinating seeds or seedlings treated using Zn (0.5 %) as $ZnSO_4$ that found comparatively similar with impacts of GSH applied @ 1.5 mM.

Data for TFAA contents of germinating seeds during germination of seeds (Table 2), shows that a gradual increment was recorded as seed

Table 2
Effects of different levels of GSH when applied separately or in combination with 0.5 % Zn as seed priming on total soluble proteins (TSP), total free amino acids (TFAA), free fatty acids (FFA), and oil contents of germinating maize seeds under non-saline and NaCl salinity (Mean±SE; $n = 4$).

TSP (mg/kg FW) (LSD at 5 % level = 16.57)									
Time (h)	NS	WS	T1	T2	T3	T4	T5	T6	T7
Control									
0	900±8.9^{a}	860±8.6^{c}	790±5.5^{d}	700±5.7^{e}	880±6.4^{b}	790±5.4^{d}	680±2.4^{f}	600±3.6^{g}	690±2.3^{ef}
24	800±4.8^{a}	800±5.7^{a}	700±4.8^{b}	690±6.6^{b}	660±3.6^{c}	700±4.8^{b}	650±4.2^{c}	530±3.3^{d}	660±3.5^{c}
48	650±2.6^{b}	750±3.4^{a}	615±3.8^{c}	570±4.3^{d}	600±3.7^{c}	615±3.8^{c}	550±4.8^{e}	480±2.3^{f}	600±6.5^{c}
72	500±3.5^{b}	600±4.4^{a}	480±6.5^{c}	450±4.4^{d}	470±4.5^{c}	480±6.5^{c}	400±2.4^{e}	350±8.1^{f}	480±3.2^{c}
120 mM NaCl									
0	910±6.5^{a}	850±9.2^{b}	800±2.4^{c}	720±6.3^{d}	900±6.7^{a}	800±3.6^{c}	700±5.5^{e}	620±3.7^{f}	720±2.8^{d}
24	850±3.9^{a}	820±5.1^{b}	750±5.3^{d}	740±6.5^{d}	790±3.8^{c}	750±5.6^{d}	700±6.3^{f}	570±3.1^{h}	680±3.9^{g}
48	700±3.6^{b}	800±4.3^{a}	665±4.2^{cd}	715±4.8^{b}	680±4.3^{c}	665±5.4^{cd}	600±4.7^{e}	530±2.5^{f}	650±6.2^{d}
72	590±4.9^{b}	650±4.4^{a}	580±3.3^{b}	530±3.1^{c}	590±4.6^{b}	580±3.1^{b}	450±2.6^{d}	400±6.3^{e}	530±3.1^{c}
TFAA (mg/kg FW) (LSD at 5 % level = 66.25)									
Control									
0	50±3.35^{b}	55±2.45^{b}	65±2.50^{b}	85±2.33^{b}	60±2.55^{b}	65±2.60^{b}	100±3.54^{b}	225±2.33^{a}	95±3.43^{b}
24	70±3.45^{b}	75±2.52^{b}	95±2.44^{b}	98±2.48^{b}	90±2.40^{b}	95±2.43^{b}	215±2.57^{a}	240±2.46^{a}	210±2.53^{a}
48	250±2.37^{c}	253±2.41^{c}	275±4.47^{bc}	295±3.54^{abc}	270±2.41^{bc}	275±3.47^{bc}	320±2.67^{ab}	350±2.75^{a}	315±2.67^{abc}
72	415±4.28^{b}	420±3.33^{b}	435±3.65^{ab}	455±3.69^{ab}	430±3.60^{ab}	435±3.65^{ab}	470±3.73^{ab}	490±3.80^{a}	465±4.65^{ab}
120 mM NaCl									
0	48±2.34^{b}	49±2.45^{b}	60±2.42^{b}	75±2.30^{b}	58±2.54^{b}	61±3.40^{b}	92±3.47^{b}	215±2.15^{a}	86±3.23^{b}
24	60±2.54^{b}	65±2.55^{b}	75±2.23^{b}	85±2.53^{b}	70±2.36^{b}	75±4.20^{b}	200±2.34^{a}	225±2.46^{a}	100±2.32^{b}
48	240±2.44^{b}	245±2.30^{b}	265±3.35^{ab}	275±3.62^{ab}	260±2.43^{ab}	265±4.32^{ab}	290±2.67^{ab}	320±2.76^{a}	285±2.63^{ab}
72	405±3.36^{a}	410±3.45^{a}	425±3.50^{a}	445±3.68^{a}	420±3.54^{a}	425±4.60^{a}	450±2.45^{a}	470±2.76^{a}	445±3.54^{a}
Oil (%) (LSD at 5 % level = 0.28)									
Control									
0	4.09±0.12^{a}	4.15±0.16^{a}	3.50±0.16^{b}	3.57±0.04^{b}	3.40±0.11^{b}	3.65±0.18^{b}	3.45±0.09^{b}	3.65±0.18^{b}	3.60±0.16^{b}
24	3.80±0.11^{ab}	3.85±0.21^{a}	3.80±0.26^{ab}	3.37±0.10^{de}	3.10±0.13^{e}	3.55±0.19^{bcd}	3.75±0.10^{abc}	3.55±0.19^{bcd}	3.50±0.18^{cd}
48	3.50±0.13^{a}	2.55±0.16^{b}	3.40±0.06^{a}	2.38±0.03^{b}	2.45±0.12^{b}	2.45±0.15^{b}	2.05±0.08^{c}	2.45±0.15^{b}	2.40±0.22^{b}
72	2.15±0.15^{ab}	2.20±0.19^{a}	1.90±0.09^{bc}	1.75±0.07^{c}	1.95±0.04^{abc}	1.75±0.14^{c}	1.90±0.04^{bc}	1.75±0.14^{c}	1.70±0.13^{c}
120 mM NaCl									
0	4.19±0.17^{a}	4.25±0.20^{a}	3.75±0.21^{b}	3.75±0.16^{b}	3.55±0.07^{bc}	3.60±0.21^{bc}	3.75±0.23^{b}	3.45±0.13^{c}	3.77±0.08^{b}
24	4.00±0.14^{a}	4.05±0.17^{a}	3.05±0.16^{c}	3.45±0.18^{b}	3.25±0.04^{bc}	3.30±0.28^{bc}	3.05±0.14^{c}	3.35±0.17^{b}	3.27±0.06^{bc}
48	3.25±0.16^{ab}	3.30±0.19^{a}	2.95±0.15^{c}	2.90±0.25^{cd}	2.60±0.02^{e}	2.65±0.06^{de}	2.55±0.17^{e}	2.10±0.26^{f}	3.02±0.02^{bc}
72	2.65±0.18^{ab}	2.70±0.22^{a}	2.25±0.11^{c}	2.20±0.16^{cd}	2.40±0.06^{bc}	2.40±0.07^{bc}	2.25±0.11^{c}	1.95±0.07^{d}	2.20±0.03^{cd}
FFA (mg/g FW) (LSD at 5 % level = 2.38)									
Control									
0	36±2.27^{d}	37±2.72^{cd}	39±2.44^{bc}	41±2.42^{ab}	39±2.46^{bc}	39±2.44^{bc}	42±2.44^{a}	43±2.43^{a}	41±2.44^{ab}
24	55±1.53^{e}	61±1.51^{d}	67±2.33^{c}	70±2.35^{b}	65±2.25^{c}	67±2.33^{c}	72±2.35^{ab}	73±2.32^{a}	71±2.31^{ab}
48	95±2.30^{f}	101±1.61^{e}	116±2.54^{d}	120±2.59^{c}	115±2.54^{d}	116±2.50^{d}	125±2.58^{ab}	126±2.17^{a}	123±2.56^{b}
72	103±1.64^{f}	119±1.22^{e}	129±1.25^{d}	133±1.31^{c}	127±1.23^{d}	129±1.22^{d}	137±1.28^{ab}	139±1.27^{a}	135±1.24^{bc}
120 mM NaCl									
0	30±2.10^{d}	34±2.72^{c}	39±2.43^{b}	42±2.52^{a}	39±2.44^{b}	39±2.40^{b}	42±2.37^{a}	40±2.41^{ab}	39±2.13^{b}
24	32±1.41^{e}	37±1.48^{d}	57±2.28^{bc}	58±2.43^{b}	55±2.33^{c}	57±2.27^{bc}	62±2.17^{a}	63±2.22^{a}	61±2.23^{a}
48	72±2.20^{e}	78±1.60^{d}	104±2.62^{c}	112±2.54^{b}	102±2.46^{c}	104±2.60^{c}	114±2.43^{ab}	116±2.12^{a}	113±2.53^{b}
72	80±1.40^{f}	96±1.10^{e}	114±1.14^{d}	119±1.25^{c}	112±2.32^{d}	114±1.13^{d}	124±1.17^{b}	139±1.22^{a}	123±1.24^{b}

NS=No soaking; WS=Water soaking; T1=1.5 mM GSH; T2=3 mM GSH; T3=4.5 mM GSH; T4=Zn 0.5 %; T5=Zn+1.5 mM GSH; T6=Zn+3.0 mM GSH; T7=Zn+4.5 mM GSH; *(Values with same alphabets in superscript in a row do not differ significantly)*.
Means in a row with same letter (a, b, c,……) do not differ significantly.

germination started, with the passage of time, under both salt regimes. However, this increment in TFAA during germination was less in germinating seeds grown under NaCl salinity. Soaking the seeds with GSH, either applied alone or with Zn in combination, caused a speedy increase in TFAA under both salt regimes. However, comparatively more increment in TFAA was treatment specific under both salt regimes. The maximum speedy increment in TFAA found due to priming of seeds using GSH @ 3 mM, either used separately or in combination with 0.5 % Zn as compared with other treatments under both salt regimes. Moreover, the speedy increment in TFAA due to priming of seeds with GSH as well as with 0.5 % Zn as seed soaking, was less under non-saline conditions than saline condition in comparison with their non-treated ones.

Seed/seedlings TFFA content also increased significantly when grown in different regimes. This increment in TFFA recorded less under salinity. Soaking of seeds with GSH either used alone or with Zn in combination caused a speedy increase in TFFA as compared with non-primed seeds, but this speedy increase in TFAA following priming of seed using GSH, either used with Zn (0.5 %) or without Zn was comparatively less in seeds grown under non-saline conditions with respect to their non-treated seeds. Comparatively, seed treatment with 3 mM and 1.5 mM GSH levels either applied with 0.5 % Zn or separately were found best under both, non-saline and salt stressed conditions (Table 2).

3.8. Correlation studies and PCA

The correlation studies of studied parameters are shown in Table S2 and Fig. 7a. Strong significance correlations were recorded of SFW, SDW with G%. Significant negative correlation was found of SFW, SDW with E50 and MET. SFW and SDW found significantly positively correlated with CUE and GI, respectively. Photosynthetic pigments including Chl. *a/b*, T. Chl., Chl. *b* and Chl. *a*, found significantly positively correlated with SFW and SDW, respectively but T. Chl./Car. and Car. found negatively correlated with SDW and SFW, respectively, Moreover, T. Chl., Chl. *b*, Chl. *a/b* and Chl. *a* found positively correlated with G% but negatively correlated with E_{50}. Leaf MDA, H_2O_2, AsA, SOD, POD, CAT and APX found negatively correlated with SDW and SFW, respectively. Nutrient ions, including Sh K, R K, R P, Sh P, Sh Mg, R Mg, Sh Ca, R Ca, Sh N, R N, Sh P, R P, Sh Mg, R Mg, Sh Zn, R Zn, have positive and R Na, Sh Na, Sh Na/K, R Na/K, Sh Cl^- and R Cl^- showed negative correlations

Table 3
Effects of different levels of GSH when applied separately or in combination with 0.5 % Zn as seed priming on K, Ca, N, P, Mg and Zn contents in shoot and root of maize seedlings when grown under non-saline and NaCl salinity (Mean±SE; $n = 4$).

Treatments	S K^+ (mg g^{-1} DW)		R K^+ (mg g^{-1} DW)		S Ca^{2+} (mg g^{-1} DW)		R Ca^{2+} (mg g^{-1} DW)		S N (mg g^{-1} DW)		R N (mg g^{-1} DW)	
	Control	120 mM NaCl	Control	120 mM NaCl	Control	120 mM NaCl	Control	120 mM NaCl	Control	120 mM NaCl	Control	120 mM NaCl
NS	43.64 ±1.09^{f}	29.61±1.09^{f}	40.57 ±1.64^{f}	31.54 ±1.64^{ef}	11.39 ±0.30^{e}	7.88 ±0.30^{e}	12.25 ±0.41^{e}	8.15 ±0.24^{f}	45.32 ±1.13^{c}	30.27 ±0.67^{f}	35.54 ±0.56^{fg}	23.48 ±0.33^{e}
WS	44.18 ±1.09^{f}	29.09±1.09^{ef}	41.11 ±1.64^{f}	31.01 ±1.64^{f}	11.53 ±0.30^{e}	7.75 ±0.30^{e}	12.38 ±0.41^{e}	8.63 ±0.39^{e}	45.82 ±1.13^{bc}	31.70 ±1.06^{e}	36.04 ±0.56^{ef}	22.16 ±0.46^{f}
T1	48.82 ±1.07^{d}	32.14±1.09^{d}	44.74 ±1.61^{d}	34.07 ±1.64^{d}	13.68 ±0.30^{c}	8.52 ±0.30^{cd}	14.54 ±0.40^{c}	9.37 ±0.41^{cd}	46.80 ±1.12^{b}	32.68 ±1.14^{e}	35.02 ±0.56^{g}	24.90 ±0.57^{d}
T2	49.07 ±1.09^{d}	33.64±1.03^{c}	45.99 ±1.64^{c}	35.57 ±1.53^{c}	14.30 ±0.38^{b}	8.89 ±0.28^{bc}	15.15 ±0.51^{b}	9.75 ±0.38^{bc}	49.07 ±1.42^{a}	36.07 ±1.06^{b}	39.29 ±0.71^{c}	26.54 ±0.56^{c}
T3	46.87 ±1.09^{e}	30.25±1.09^{e}	42.79 ±1.64^{e}	32.17 ±1.63^{e}	11.70 ±0.30^{e}	8.04 ±0.30^{de}	13.00 ±0.41^{de}	8.90 ±0.40^{de}	46.45 ±1.14^{b}	33.93 ±1.13^{d}	36.67 ±0.57^{e}	23.14 ±0.56^{e}
T4	46.62 ±1.09^{e}	33.59±1.09^{c}	42.55 ±1.64^{e}	35.52 ±1.63^{c}	12.89 ±0.30^{d}	8.88 ±0.30^{bc}	13.74 ±0.41^{d}	9.74 ±0.40^{bc}	46.85 ±1.14^{b}	34.77 ±1.10^{cd}	38.07 ±0.57^{d}	25.99 ±0.55^{c}
T5	53.09 ±1.08^{b}	39.32±1.09^{b}	47.02 ±1.62^{b}	31.25 ±1.64^{f}	15.00 ±0.30^{a}	9.31 ±0.30^{b}	15.00 ±0.40^{bc}	10.17 ±0.41^{b}	48.68 ±1.12^{a}	35.32 ±1.14^{bc}	40.90 ±0.56^{b}	31.54 ±0.57^{b}
T6	56.45 ±1.22^{a}	41.07±0.40^{a}	49.37 ±1.84^{a}	39.20 ±0.59^{a}	15.12 ±0.25^{a}	10.55 ±0.11^{a}	16.00 ±0.33^{a}	11.41 ±0.14^{a}	49.00 ±1.06^{a}	40.90 ±0.41^{a}	42.70 ±0.53^{a}	36.12 ±0.20^{a}
T7	51.51 ±1.15^{c}	38.64±1.14^{b}	46.43 ±1.73^{bc}	37.57 ±1.70^{b}	14.61 ±0.32^{ab}	10.14 ±0.31^{a}	15.21 ±0.41^{b}	11.00 ±0.42^{a}	48.29 ±1.14^{a}	40.69 ±1.18^{a}	41.51 ±0.57^{b}	30.91 ±0.59^{b}
LSD 5 %	0.86		0.85	0.54		0.48			1.04		0.90	
	S P (mg g^{-1} DW)		**R P (mg g^{-1} DW)**		**S Mg (mg g^{-1} DW)**		**R Mg (mg g^{-1} DW)**		**S Zn^{2+} (mg kg^{-1} DW)**		**R Zn^{2+} (mg kg^{-1} DW)**	
	Control	120 mM NaCl	Control	120 mM NaCl	Control	120 mM NaCl	Control	120 mM NaCl	Control	120 mM NaCl	Control	120 mM NaCl
NS	10.30±0.44^{f}	6.56 ±0.26^{f}	9.80 ±0.29^{d}	6.06 ±0.17^{e}	14.21 ±0.47^{e}	8.78 ±0.28^{e}	13.79 ±0.23^{e}	8.36 ±0.14^{e}	43.29 ±1.50^{e}	26.25 ±0.89^{f}	44.23 ±1.50^{g}	27.19 ±0.89e
WS	10.45±0.44^{f}	6.45 ±0.34^{f}	9.95 ±0.29^{d}	5.95 ±0.23^{e}	14.42 ±0.47^{e}	8.63 ±0.37^{e}	14.00 ±0.23^{e}	8.20 ±0.18^{e}	43.95 ±1.50^{e}	25.76 ±1.17^{f}	44.89 ±1.50^{g}	26.70 ±1.17^{e}
T1	12.76±0.43^{d}	7.22 ±0.44^{e}	11.26 ±0.28^{c}	6.72 ±0.29^{d}	15.78 ±0.47^{cd}	9.74 ±0.47^{d}	15.35 ±0.23^{cd}	9.32 ±0.23^{d}	46.49 ±1.47^{d}	29.25 ±1.50^{e}	48.43 ±1.48^{e}	20.94 ±1.46^{f}
T2	13.42±0.54^{bc}	7.69 ±0.43^{d}	12.92 ±0.36^{b}	7.24 ±0.29^{c}	16.25 ±0.34^{c}	11.25 ±0.67^{b}	15.83 ±0.17^{bc}	11.33 ±0.33^{c}	47.63 ±1.92^{d}	31.32 ±2.78^{d}	48.82 ±1.91^{e}	32.27 ±1.54^{d}
T3	11.63±0.44^{e}	6.71 ±0.43^{f}	11.13 ±0.29^{c}	6.71 ±0.29^{d}	15.69 ±0.47^{d}	10.00 ±0.47^{cd}	14.26 ±0.23^{e}	8.58 ±0.23^{e}	46.78 ±1.50^{d}	30.94 ±1.49^{d}	46.72 ±1.50^{f}	30.88 ±1.49^{d}
T4	11.90±0.44^{e}	7.53 ±0.42^{d}	11.40 ±0.29^{c}	7.03 ±0.28^{cd}	15.53 ±0.47^{d}	10.50 ±0.46^{c}	15.11 ±0.23^{d}	9.78 ±0.23^{d}	52.59 ±1.50^{c}	35.69 ±1.45^{c}	55.53 ±1.50^{d}	36.64 ±1.45^{c}
T5	13.17±0.43^{c}	8.50 ±0.44^{c}	12.67 ±0.29^{b}	8.14 ±0.29^{b}	16.83 ±0.47^{b}	10.53 ±0.47^{c}	16.40 ±0.23^{b}	12.11 ±0.23^{b}	60.93 ±1.48^{b}	38.01 ±1.50^{b}	61.87 ±1.48^{b}	39.96 ±1.50^{b}
T6	15.25±0.36^{a}	9.47 ±0.15^{a}	14.32 ±0.24^{a}	9.20 ±0.10^{a}	17.92 ±0.27^{a}	12.46 ±0.17^{a}	17.50 ±0.13^{a}	14.78 ±0.08^{a}	64.39 ±1.45^{a}	40.17 ±0.61^{a}	63.33 ±1.23^{a}	42.11 ±0.61^{a}
T7	13.48±0.44^{b}	8.96 ±0.45^{b}	13.98 ±0.29^{a}	8.46 ±0.30^{b}	16.82 ±0.48^{b}	11.27 ±0.49^{b}	16.14 ±0.24^{b}	11.84 ±0.24^{bc}	60.72 ±1.63^{b}	39.19 ±1.56^{ab}	57.41 ±1.62^{c}	41.14 ±1.56^{ab}
LSD 5 %	0.30		0.38	0.55		0.63			1.49		1.42	

NS=No soaking; WS=Water soaking; T1=1.5 mM GSH; T2=3 mM GSH; T3=4.5 mM GSH; T4=Zn 0.5 %; T5=Zn+1.5 mM GSH; T6=Zn+3.0 mM GSH; T7=Zn+4.5 mM GSH; *(Values with same alphabets in superscript in a row do not differ significantly)*.
S *K* = shoot potassium; **R *K*** = root potassium; **S Ca** = shoot calcium; **R Ca** = root calcium; **S *N*** =shoot nitrogen; **R *N*** = root nitrogen; **S *P*** = shoot phosphorous; **R *P*** = root phosphorous; **S Mg** = shoot magnesium; **R Mg** = root magnesium; **S Zn** = shoot zinc; **R Zn** = root zinc; **S Na** = shoot sodium; **R Na** = root sodium; **S Na^+/K^+** = shoot sodium potassium ratio; **R Na^+/K^+** = root sodium potassium ratio; **S Ca^{2+}/Na^+** = shoot calcium sodium ratio; **R Ca^{2+}/Na^+** = root calcium sodium ratio; **S Cl^-** = shoot chloride ion; **R Cl^-** = root chloride ion.

with growth attributes. Leaf Car., TRS and TSS are positively correlated with seed/seedlings Chl. of germinating seeds, and a positive correlation of TFAA, TFAA, recorded with Chl. of seedlings. While TSP negatively correlated with TSS, Car., Chl. and TRS. Oil content of germinating seeds negatively correlations with TFAA, TRS, TSS, Car., Chl. but positively with TSP (Table S3 and Fig. 7b).

3.9. PCA of different parameters of seedlings

This study revealed that the studied attributes were clustered into three groups. In one group, the activities of oxidative stress related enzymes, levels of non-enzymatic antioxidants, and TSS, RS and NRS were closely positively related to each other. In the 2nd group, the shoot and root Na^+ uptake, Na^+/K^+ in root and shoot, MDA, FAA, H_2O_2, that are apart from the attributes of the 1st group. Rest of the attributes such as growth, nutrient uptake Ca^{2+}/Na^+ in root and shoot, CUE, G.E, G% and G.E were grouped together and closely positively correlated to each other. However, the attributes of 3rd group were negatively correlated with the attributes of 2nd group, but less apart from the 1st group. In findings the variance among attributes the major contribution of Dim 1 (Factor 1) i-e 79.2 % followed by of factor 2 i-e 15.5 % with a cumulative variance of 94.7 %. PCA analysis of seed/seedling metabolites are presented in Fig. 7b; Table S3. It was clear from that the TRS, Car. and Chl. are strongly positively corelated, TSS, TFAA and TFFA are totally closely related; but these strongly negatively correlated to TSP and oil content (Fig. 7a; Table S2)

3.10. Heat map analysis

Heat map analysis of among studied attributes and given treatment

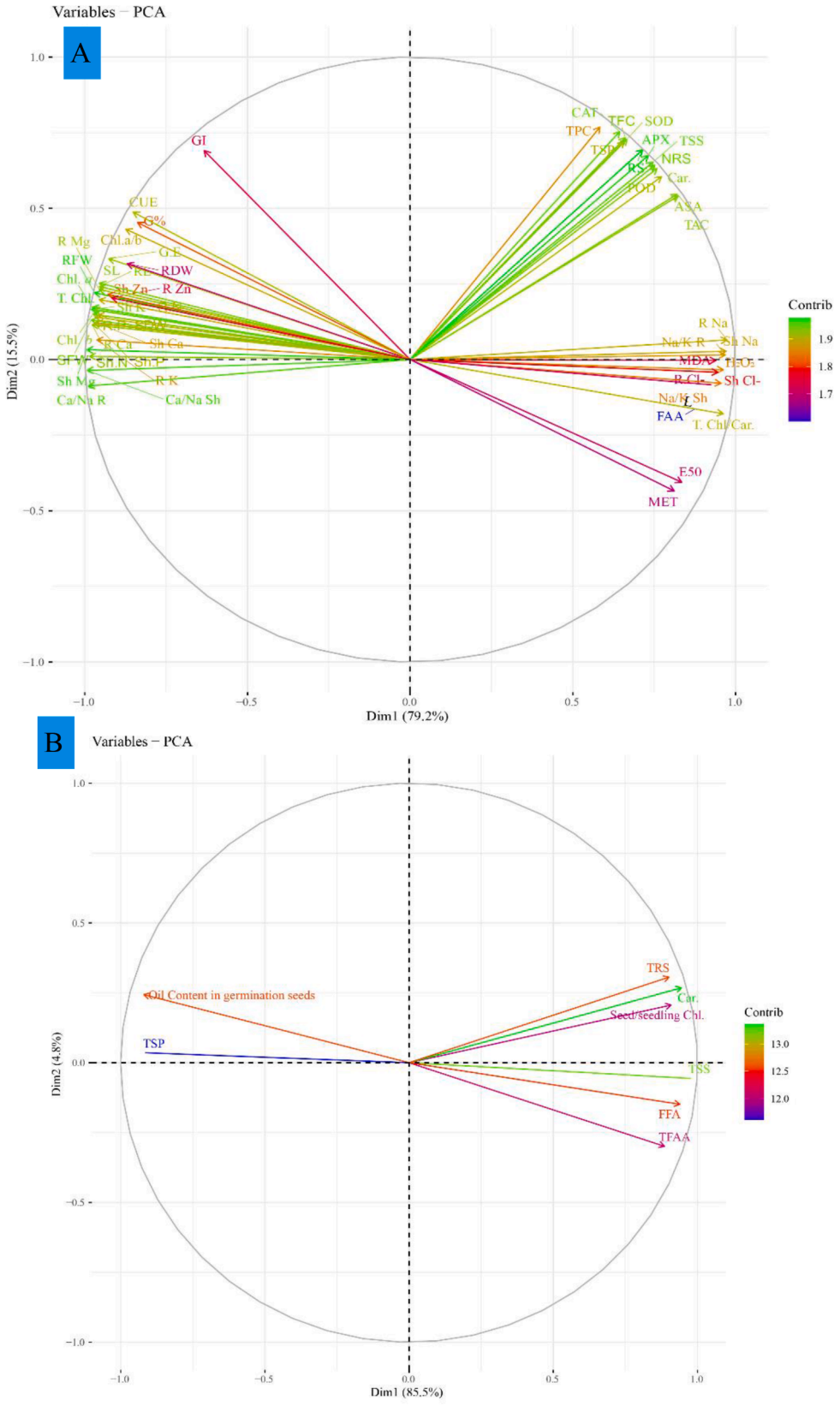


Fig. 7. The principal component analysis (PCA) **(A)** among the studied germination parameters, growth attributes, photosynthetic pigments, activities of antioxidant enzymes, contents of non-enzymatic antioxidants, nutrient ions and some metabolites of maize seedlings grown from seeds primed with glutathione (GSH) alone and in combination with Zn, under non-saline and NaCl salinity PCA analysis among studied seed metabolites (**B**).

are presented in Fig. 8. The intensity of color and color type in squares clearly shows its extent of correlation of effectiveness of specific treatment in relation with specific parameters. It shows that different combined treatment of glutathione with Zn under salt stress showed better result among other treatments under salt stress. Among these GSH with 3 mM with Zn are most effective followed by 1.5 mM GSH with Zn and there 4.5 mM with Zn. It clearly shows that better effectiveness of 0.5 % Zn^{2+} 3 mM GSH is associated with it strong correlation with NRS, TSS, TFC, CAT, SOD, APX, RS, TSP, POD, SOD, AsA, TAC, TPC and Car. followed by CUE, GI as clear from the color intensity along a line (Treatment) in a column (parameter) and Zn least effective one was Zn alone treatment under salt stress.

4. Discussion

Seed germination is a complex process, starts with imbibition after absorbing water, necessary for activating the hydrolyzing enzymes that play significant roles in emergence process by providing necessary metabolites (Saeed et al., 2023; Perveen et al., 2021; Taiz et al., 2015). Availability of these necessary metabolites is of prime importance for the biosynthesis of large structural molecules, where these metabolites play roles as building blocks. The activities of these hydrolyzing enzymes during this process also generates metabolic energy by the breakdown of stored reserves to simpler molecules that are being utilized in the development and emergence of new seedlings, necessary for uniform and good crop stand establishment (Perveen et al., 2021; Saeed et al., 2023).

It is well known that abiotic stresses including salt stress adversely affect the process of germination that also delays seedling emergence process (Suzuki et al., 2014; Tebini et al., 2022; Nikolić et al., 2023). It is a well-known fact that under salt stress, aside to the negative effects of toxic ions, it also results in lowering of osmotic potential of growth medium that reduces water uptake/absorption by seeds causes disturbances in imbibition that results in reduced seed germination and delayed emergence (Munns and Tester, 2008; Uçarlı, 2020; Kumar et al., 2021; Saeed et al., 2023; Naseer et al., 2022; Nikolić et al., 2023). This study demonstrated that 120 mM of NaCl affected the maize seed germination processes; activities of hydrolyzing enzymes related to germination and emergence were negatively affected (Uçarlı, 2020; Naseer et al., 2022; Perveen et al., 2021; Xiong et al., 2024). To overcome the negative effects of high salinity during germination and seedling emergence, different ways can be employed; including seed priming using different methods (Saeed et al., 2023; Sghayar et al., 2023). Seed soaking with GSH either used alone or with 0.5 % Zn in combination, significantly reduced the NaCl salinity negativities on attributes related to seedling emergence and seed germination parameters. The GSH applied @ 3 mM either applied alone or with Zn in combination was found superior one than other treatments. GSH-applied amelioration on seed germination attributes, seems the effect of its better uptake by seeds, when used in combination with Zn than its individual application that might have resulted in better water absorption due to lowering of seed osmotic potential, necessary for maintaining the better activities of hydrolyzing enzymes (Naseer et al., 2022; Raja et al., 2022; Saeed et al., 2023). Moreover, in relation to present findings, earlier it was found that exogenous application of GSH have significant role in seed germination related processes, because it is found a clear increase in levels of GSH of germinating seeds during radical formation (Ciacka et al., 2019; Moothoo-Padayachie et al., 2016). Furthermore, it is reported that high concentration of reduced glutathione during seed germination process plays significant role in maintaining cell division related metabolic process (Hossain et al., 2017). Similar findings are well reported by Saeed et al. (2023) on maize seed germination related attributes were significantly improved under stress when grown after soaking in GSH solution and more efficiency

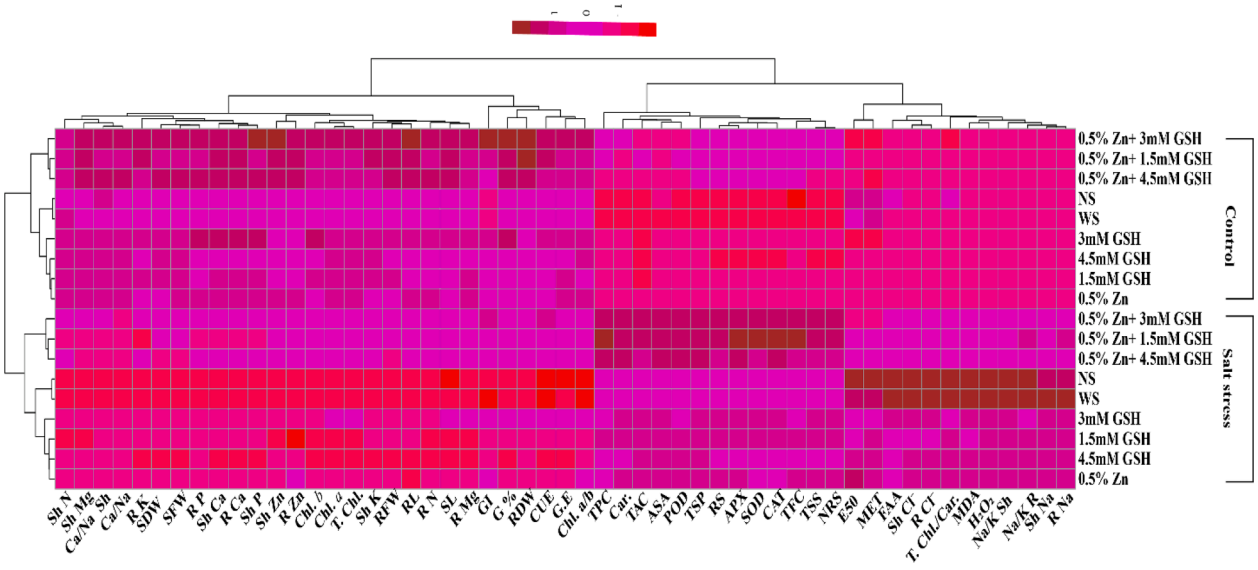


Fig. 8. Heatmap analysis of studied germination parameters, growth attributes, photosynthetic pigments, activities of antioxidant enzymes, contents of non-enzymatic antioxidants, nutrient ions and some metabolites.

E_{50}= time to 50 % seed emergence; **MET**= mean emergence time; **CUE**= coefficient of uniformity of emergence; **EI**= Emergence index; **G %**= germination %; **GE**= germination energy; **RFW**= root fresh weight; **RDW**= root dry weight; **SFW**= shoot fresh weight; **SDW**= shoot dry weight; **SL**= shoot length; **RL**= root length; **Car**= carotenoids; **Chl. *a***= chlorophyll *a*; **Chl. *b***= chlorophyll *b*; **T.Chl.**= total chlorophyll; **Chl *a/b***= ratio of chlorophyll *a*/chlorophyll *b*; **T. Chl./Car**= total chlorophyll/Carotenoids; **Seed/seedling Chl.**= seed/seedling chlorophyll; **TSS**= total soluble sugar; **TRS**= total reducing sugar; **TSP**= total soluble protein; **TFAA**= total free amino acid; **FFA**= free fatty acid; **MDA**= malondialdehyde; **H_2O_2**= hydrogen peroxide; **AsA**= ascorbic acid; **TAC**= total anthocyanin content; **TFC**= total flavonoid content; **SOD**= superoxide dismutase; **POD**= peroxide dismutase; **CAT**= catalase; **RS**= reducing sugar; **NRS**= non-reducing sugar; **TPC**= total phenolic content; **S *K***= shoot potassium; **R *K***= root potassium; **S Ca**= shoot calcium; **R Ca**= root calcium; **S *N***=shoot nitrogen; **R *N***= root nitrogen; **S *P***= shoot phosphorous; **R *P***= root phosphorous; **S Mg**= shoot magnesium; **R Mg**= root magnesium; **S Zn**= shoot zinc; **R Zn**= root zinc; **S Na**= shoot sodium; **R Na**= root sodium; **S Na^+/K^+**= shoot sodium potassium ratio; **R Na^+/K^+**= root sodium potassium ratio; **S Ca^{2+}/Na^+**= shoot calcium sodium ratio; **R Ca^{2+}/Na^+**= root calcium sodium ratio; **S Cl^-**= shoot chloride ion; **R Cl^-**= root chloride ion

was recorded due to seed priming with GSH in combination with Zn, where 1.5 mM concentration was found more effective. It is plausible that exogenously-applied GSH, during seed soaking, might have elevated the GSH content of the seed before seed germination; improving the effects on seedling emergence and seed germination through faster metabolic activities (Saeed et al., 2023; Ali et al., 2024). The improvement observed following seed soaking with GSH + 0.5 % Zn on germination and emergence related attributes, could be attributed to the presence of Zn (Seyedi et al., 2012; Imran et al., 2018; Choukri et al., 2022); that might also have played role in lowering the seed osmotic potential for better water absorption lead to better imbibition. Earlier it was reported that Zn have role in maintaining the better levels of GSH during process of germination helpful to play antistress role to counteract oxidative stress (Saeed et al., 2023). Moreover, it is found that the GSH application regulates the translocation of Zn in plants (Nakamura et al., 2019) that correlates well to present findings, where GSH+0.5 % Zn application showed better performance in improving seedlings emergence and germination especially when grown in salt stress.

Levels of simple metabolites such as FAA, FFA, TSS, Car., Chl. and TRS of germinating seeds/seedlings increased significantly after seed sowing under both salt regimes, with an alternate decrease in levels of macromolecules such as seed oil and TSP, conferring the increased activities of seed hydrolyzing enzymes (Perveen et al., 2022). Reduced increment in levels of these simple metabolites recorded in germinating seeds grown in NaCl salinity. Priming of seeds with GSH either applied in combination with 0.5 % Zn or separately as well as further increased the levels of these simple metabolites, in comparison to non-treated ones, with an alternate rapid reduction in seed oil and TSP. Among the different treatments, more rapid increase with the passage of time in the levels of these simple metabolites was recorded when seeds were primed using GSH (3 mM) with either Zn or separately than the other levels/treatments. Specifically, the changes in the levels of these simpler metabolites corresponded well to the activities of metabolic enzymes responsible for hydrolyzing activities (Perveen et al., 2021, 2022; Saeed et al., 2023) that confers the better significant impact of Zn and GSH in controlling seed hydrolyzing enzymes activities. The increase in metabolite levels with 3 mM level of GSH correlated well with the GSH concentration specific improvement in seed germination related enzymes (Kalemba and Ratajczak, 2018; Cheng et al., 2015; Nahar et al., 2015a; Pei et al., 2019; Zaki et al., 2019; Hoang et al., 2021; Jaiswal et al., 2021; Saeed et al., 2023). Rapid catabolism of large molecular weight, stored as seed metabolites, to simpler ones by priming of seeds with reduced glutathione when used with Zn (0.5 %), than with their sole applications; demonstrating that their combined application with 0.5 % Zn delivered better outcomes (Ciacka et al., 2019; Nakamura et al., 2019; Saeed et al., 2023). Furthermore, rapid conversion of seed reserves to smaller metabolites considered as most important one for rapid emergence of seedlings, due to their requirement as basic necessary material for the synthesis of new structural secondary molecules (Samreen et al., 2017; Ahmad and Tahir, 2017; Shao et al., 2022), considered as playing significant role in the speedy seedling emergence, through better seed germination (Uçarlı, 2020; Perveen et al., 2021; Naseer et al., 2022). Furthermore, it was earlier reported that increased accumulation of simple metabolites in seeds due to the breakdown of larger molecules, significantly lowers the seed osmotic potential necessary for further better uptake of water, necessary for speedy seedling emergence and growth (Saeed et al., 2023; Perveen et al., 2021) and might be the same phenomena occurred in case of present experiment, where better emergence and growth of seedlings were associated with high levels of simple metabolites, when seeds were grown after soaking in different concentration of GSH, either used alone or with Zn as combined application.

Significant amelioration on plant growth related attributes was found of seed soaking with different concentrations of GSH, either used alone or with Zn, but the impact was treatment specific. This amelioration in maintaining better growth of maize plants found better associated with speedy germination process, better protrusion of radicles and fast emergence of seedlings and rapid conversion of large-sized metabolites stored in seeds to smaller ones; that take part is the biosynthesis of structural molecules that resulted in healthy plants, is correlated well with earlier findings of Saeed et al. (2023) and Perveen et al. (2021) on maize.

Moreover, the rapid emergence of seedlings was due to the different concentration of glutathione with Zn; these correlated well to the speedy growth and higher levels of chlorophylls. The speedy growth of roots following germination better absorbs the mineral nutrients and water (Zaki et al., 2019; Imran et al., 2018; Taiz et al., 2015). In addition, the higher levels of chlorophylls provided greater gas exchange capacity and light utilization efficiency to drive the entire photosynthetic processes (Ma et al., 2021; Yong et al., 2010), and delivering better growth of seedlings, along with catabolism of seed metabolites as found in present study. Earlier, Afrin et al. (2021) reported that application of zinc treatment as seed priming resulted in speedy increment in plant height and root length of soyabean. They found that exogenous supplementation of zinc significantly reduced the negative impacts of NaCl salinity upon the growth of plants. Moreover, it was reported that in soybean Zn fertigation ameliorated the negativities of NaCl salinity on growth found associated with reduced uptake of Cl^- and Na^+. While in comparison with present results, regarding seed priming effects on growth attributes of maize seedlings, in earlier studies, it was reported that seed treatment with Zn significantly positively influenced the lengths of shoot and root of maize (Ambika and Balakrishnan, 2015). Furthermore, in comparison more biomass production in maize plants when priming was done using with 3 mM concentration of GSH with Zn (0.5 %), correlates well with more accumulation of NRS, RS, TSP and TFAA that might play significant roles in better growth of plants at any stage, including the cellular osmotic adjustment, water relation maintenance and many more cellular metabolic process (Aragão et al., 2015; Shao et al., 2022).

In our study, the reduction in growth of maize seedlings under salinity correlated negatively with high levels of MDA and H_2O_2; and also associated with lesser chlorophyll. Due to the overproduction of ROS, increased production of lipid peroxidation products is a common activity under stressful environment. It causes severe damages to cellular membranes, including chloroplastic membranes and photosynthetic units (Das et al., 2024). Lipid peroxidation under stressful environment also disturbs the cellular turgidity and water relations through leaky membranes and causes reduction in net photosynthesis leading to reduced growth (Nahar et al., 2015c; Shafiq et al., 2019; Das et al., 2024). Plants protect themselves from negative impacts of lipid peroxidation by scavenging the overly produced ROS through well-developed antioxidative defence mechanism, comprised of different antioxidative component (Rajput et al., 2021), that is plant species, life stage, severeness of stress and type of stress specific (Rajput et al., 2021; Suzuki et al., 2014; Hasanuzzaman et al., 2020; Shehzad et al., 2022). Imposition of NaCl salinity resulted in reduced biomass production in present study and negatively linked with increased levels of MDA and H_2O_2. Moreover, the activities of antioxidative enzymes also increased that depicts, the antioxidative mechanisms was not much effective to required level to reduce the lipid peroxidation through ROS scavenging. Seed soaking with different GSH levels used alone or with Zn found effective in decreasing the lipid peroxidation with an alternate growth improvement of maize plants when grown in NaCl salinity. Glutathione-applied this reduction in MDA and H_2O_2 levels has found linked with increased levels of antioxidative compounds and antioxidant enzymes activities. Because in the findings of present study, comparatively superior increase in growth found due to priming of seeds using 3 mM concentration of GSH, when used with 0.5 % Zn than other treatments under salinity in comparison to other treatments, found correlated well with better performance of POD, SOD, APX, CAT as well as increased levels of Car., TPC, AsA and TFC. The study demonstrated that the combination of GSH and Zn delivered better antioxidative defence mechanisms for maize and in line with earlier reports for other species

(Sadak et al., 2017; Ebtihal et al., 2018; Ali et al., 2022; Pei et al., 2019). Moreover, better effective role of reduced glutathione with Zn, depicts well their accumulative better impact for maintaining the better mechanism of antioxidation, resulted in decreased accumulation of MDA, that maintained better cellular water levels, lead to better maintenance in biomass production of maize seedlings when exposed to NaCl salinity.

Mineral nutrient status, especially with reference to nitrogen and phosphorus, is of key importance to maintain growth of plants not only under non-stressed conditions but also improves plant ability to tolerate the stressful environment (de Bang et al., 2021; Shi et al., 2020; Wang et al., 2020). During high salinity, the Na^+ over accumulation causes the osmotic imbalance and disturbs the uptake of other nutrients that causes growth and yield reductions (Hussain et al., 2022; Munns and Tester, 2008; Wu et al., 2023). However, the Na^+ accumulation or uptake is plants species, the genotype, or cultivar specific. To overcome the problem of toxicity of toxic ions plants have developed mechanisms of Na^+ exclusion or sequestration at shoot or root levels (Munns and Tester, 2008; Naz et al., 2023; Shabala and Munns, 2017). In present study, maize plants comparatively accumulated less Na^+ in roots than shoot when grown in NaCl salinity. It also reduced the mobility/uptake of essential minerals including Ca, K, Mg, P, N and Zn that is associated with reduced growth of maize seedlings. However, this reduction in nutrient uptake was found less in maize plants raised after soaking using different concentration of reduced glutathione, either used separately as well as with Zn or applied alone. The better results were found when primed the seeds using 3 mM concentration of glutathione applied either separately and with 0.5 % Zn in combination. The reduced adverse effects of NaCl stress in nutrient uptake was found associated with reduced uptake of Na^+ and Cl^- that resulted in increased ratios of K^+/Na^+ and Ca^{2+}/Na^+in root as well as in shoots. Comparatively more increment in Ca^{2+}/Na^+ and K^+/Na^+ was noted at root level showing restricted uptake of Na^+ at shoot level or increased translocation from root to shoot. It confers the better role of GSH used as seed soaking, either alone or with Zn, in reducing the Na^+ and Cl^- uptakes, alternatively increased nutrient uptake that is found positively linked with better growth maintenance, better levels of chlorophyll and good performance of antioxidative defence mechanism, metabolite levels and reduced lipid peroxidation. It also demonstrated that exogenous usage of GSH through seed soaking with Zn in combination, better reduced the toxicity of Cl^- as well as Na^+ ions to decrease stress inducible damages in maize plants under NaCl salinity. In the better interest of present study, Sadak et al. (2017) reported that exogenous use of glutathione increased the uptake, translocation and mobility of K, Ca, N, P, and Mg as well as K^+/Na^+ ratio in *Cicer arietinum* associated with increased levels of amino acids, TSS, antioxidative defence mechanisms with a reduced Na^+ uptake. Moreover, Safi-naz and Zaki (2018) reported that in *Vicia faba* plants, seed priming with GSH decreased the physiological negativities associated with salinity on growth, photosynthesis, and nutrient uptake; with better osmoprotection and antioxidative defence mechanism. In addition, Zaki et al. (2019) reported that GSH delivered better salt resilience in *Glycine* max; associated with higher level of chlorophylls, accumulation of osmolytes, better antioxidative defence mechanisms and nutrient uptake.

5. Conclusions

The combination of GSH and Zn delivered better outcomes for mitigating the negative effects of NaCl salinity on seed germination and seedlings emergence than the individually-applied Zn or GSH. The induction of salt tolerance in maize plants raised from GSH primed seeds in combination with Zn in comparison with other treatments for maintaining better growth; associated later with lesser MDA due to better antioxidative defence mechanism as well as the foliar photosynthetic pigments. Interestingly, the better salt tolerance in maize plants was attributed to the combination of GSH and Zn; these primed seeds had reduced uptake of Cl^- and Na^+, increased K^+/Na^+ at root or shoot level, especially at the root level with a concomitant increase in Mg, K, Ca, N, P as well as Zn uptake. Based on this study, the combined usage of 3 mM GSH and 0.5 % Zn for seed priming should be recommended for strengthening maize eco-physiological tolerance in areas with high salt levels. Moving forward, further studies are needed for all the growth stages of maize (with and without GSH + Zn application) and to conduct these trials under field conditions.

Declaration of funding

The authors gratefully acknowledge the funding from the Higher Education Commission (HEC) Pakistan (5603/Punjab/NRPU/R&D/HEC/2016).

CRediT authorship contribution statement

Rehan Ahmad Kasana: Writing – original draft, Methodology, Formal analysis, Data curation, Conceptualization, Funding acquisition. **Muhammad Iqbal:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization. **Qasim Ali:** Writing – review & editing, Visualization, Validation, Software, Resources, Writing – original draft, Methodology. **Farah Saeed:** Writing – review & editing, Writing – original draft, Supervision, Validation, Software, Resources, Conceptualization. **Muhammad Rizwan:** Writing – review & editing, Methodology, Validation, Project administration, Funding acquisition. **Rashida Perveen:** Writing – review & editing, Visualization, Validation, Methodology, Resources. **Jean Wan Hong Yong:** Writing – review & editing, Visualization, Methodology, Funding acquisition, Validation, Software, Resources.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgment

The authors gratefully acknowledge the facilities provided by the Experimental Botany Lab, Department of Botany, GC University Faisalabad

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.stress.2025.100767.

Data availability

Data will be made available on request.

References

Ahmad, A., Tahir, M., 2017. Role of zinc sulphate for maize (*Zea mays* L.) and mungbean (*Vigna radiata* L. Wilczek) yield improvement: a review Bull. Biol. All. Sci. Res. 2 (1), 1–17.

Ali, Q., Perveen, R., Saeed, F., Manzoor, H., Ali, S., Hussain, M.I., Ahmad, A., 2024. Enhancing water stress tolerance of bread wheat during seed germination and seedling emergence: caffeine-induced modulation of antioxidative defense mechanisms. Front. Plant Sci. 15, 1336639. https://doi.org/10.3389/fpls.2024.1336639.

Ali, Q., Perveen, R., El-Esawi, M.A., Ali, S., Hussain, S.M., Amber, M., Ahmad, P., 2020. Low doses of *Cuscuta reflexa* extract act as natural biostimulants to improve the germination vigor, growth, and grain yield of wheat grown under water stress: photosynthetic pigments, antioxidative defense mechanisms, and nutrient acquisition. Biomolecules 10, 1212. https://doi.org/10.3390/biom10091212.

Ali, B., Wang, X., Saleem, M.H., Sumaira, Hafeez, A., Afridi, M.S., Ali, S., 2022. PGPR-mediated salt tolerance in maize by modulating plant physiology, antioxidant

defense, compatible solutes accumulation and bio-surfactant producing genes. Plants 11 (3), 345.
Alsafran, M., Saleem, M.H., Rizwan, M., Al Jabri, H., Usman, K., Fahad, S., 2023. An overview of heavy metals toxicity in plants, tolerance mechanism, and alleviation through lysine-chelation with micro-nutrients—a novel approach. Plant Growth Regul. 100 (2), 337–354.
Ambika, S., Balakrishnan, K., 2015. Enhancing germination and seedling vigour in cluster bean by organic priming. Sci. Res. Essays 10 (8), 298–301. https://doi.org/10.5897/SRE2015.6197.
Aragão, V.P.M., Navarro, B.V., Passamani, L.Z., Macedo, A.F., Floh, E.I.S., Silveira, V., Santa-Catarina, C., 2015. Free amino acids, polyamines, soluble sugars and proteins during seed germination and early seedling growth of *Cedrela fissilis* Vellozo (Meliaceae), an endangered hardwood species from the Atlantic Forest in Brazil. Theor. Exp. Plant Physiol. 27, 157–169. https://doi.org/10.1007/s40626-015-0041-7.
Arfrin, S., Akhtar, N., Hossain, F., 2021. Application of zinc mitigates the salt-induced effects on growth of soybean (*Glycine* max L.). Int. J. Ecotoxicol. Ecobiol. 6 (3), 59–64.
Arnon, D.I., 1949. Copper enzymes in isolated chloroplasts. polyphenoloxidase in *Beta vulgaris*. Plant Physiol. 24 (1), 1. https://doi.org/10.1104/pp.24.1.1.
Association of Official seed Analysis (AOSA), 1990. Rules for testing seeds. J. Seed Technol. 12, 1–112.
Association of Official Seed Analysts Seed Vigor Testing Handbook: Contribution No. 32 to the Handbook on Seed Testing, (1983).
Bewley, J.B., Black, M., 1985. Seed Physiology of Development and Germination. Plenum Press, New York, NY, USA. https://doi.org/10.1007/978-1-4899-1002-8.
Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72 (1–2), 248–254. https://doi.org/10.1016/0003-2697(76)90527-3.
Bremner, J.M., Keeney, D.R., 1965. Steam-distillation methods for determination of ammonium, nitrate and nitrite. Anal. Chim. Acta 32, 485–495. https://doi.org/10.1016/S0003-2670(00)88973-4.
Cabot, C., Martos, S., Llugany, M., Gallego, B., Tolrà, R., Poschenrieder, C., 2019. A role for zinc in plant defense against pathogens and herbivores. Front. Plant Sci 10, 1171. https://doi.org/10.3389/fpls.2019.01171.
Cakmak, I., Horst, W.J., 1991. Effect of aluminium on lipid peroxidation, superoxide dismutase, catalase, and peroxidase activities in root tips of soybean (*Glycine* max). Physiol. Plant 83 (3), 463–468. https://doi.org/10.1111/j.1399-3054.1991.tb00121.x.
Castillo-González, J., Ojeda-Barrios, D., Hernández-Rodríguez, A., González-Franco, A. C., Robles-Hernández, L., López-Ochoa, G.R., 2018. Zinc metalloenzymes in plants. Interciencia 43 (4), 242–248.
Chance, B., Maehly, A.C., 1955. Assay of catalases and peroxidase. Methods Enzymol. 2 (1), 764–775. https://doi.org/10.1016/S0076-6879(55)02300-8.
Cheng, M.C., Ko, K., Chang, W.L., Kuo, W.C., Chen, G.H., Lin, T.P., 2015. Increased glutathione contributes to stress tolerance and global translational changes in Arabidopsis. Plant J. 83 (5), 926–939.
Choudhary, S., Wani, K.I., Naeem, M., Khan, M.M.A., Aftab, T., 2023. Cellular responses, osmotic adjustments, and role of osmolytes in providing salt stress resilience in higher plants: polyamines and nitric oxide crosstalk. J. Plant Growth Regul. 42 (2), 539–553.
Choukri, M., Abouabdillah, A., Bouabid, R., Abd-Elkader, O.H., Pacioglu, O., Boufahja, F., Bourioug, M., 2022. Zn application through seed priming improves productivity and grain nutritional quality of silage corn. Saudi J. Biol. Sci. 29 (12), 103456.
Ciacka, K., Krasuska, U., Otulak-Kozieł, K., Gniazdowska, A., 2019. Dormancy removal by cold stratification increases glutathione and S-nitrosoglutathione content in apple seeds. Plant Physiol. Biochem. 138, 112–120. https://doi.org/10.1016/j.plaphy.2019.02.026.
Coolbear, P., Francis, A., Grierson, D., 1984. The effect of low temperature pre-sowing treatment on the germination performance and membrane integrity of artificially aged tomato seeds. J. Exp. Bot. 35 (11), 1609–1617. https://doi.org/10.1093/jxb/35.11.1609.
Das, A., Pal, S., Chakraborty, N., Hasanuzzaman, M., Adak, M.K., 2024. Regulation of reactive oxygen species metabolism and oxidative stress signaling by abscisic acid pretreatment in rice (*Oryza sativa* L.) seedlings through sub1A QTL under salinity. Plant Stress 11, 100422.
Dawood, M.G., Sadak, M.S., Bakry, B.A., Kheder, H.H., 2020. Effect of glutathione and/or selenium levels on growth, yield, and some biochemical constituents of some wheat cultivars grown under sandy soil conditions. Bull. Natl. Res. Cent. 44, 158. https://doi.org/10.1186/s42269-020-00410-z.
de Bang, Husted, S., Laursen, K.H., Persson, D.P., Schjoerring, J.K., 2021. Themolecular–physiological functions of mineral macronutrients and theirconsequences for deficiency symptoms in plants. New Phytol. 229, 2446–2469.
dos Santos, T.B., Ribas, A.F., de Souza, S.G.H., Budzinski, I.G.F., Domingues, D.S., 2022. Physiological responses to drought, salinity, and heat stress in plants: a review. Stresses 2 (1), 113–135.
Duan, S., Al-Huqail, A.A., Alsudays, I.M., Younas, M., Aslam, A., Shahzad, A.N., Qayyum, M.F., Rizwan, M., Hamoud, Y.A., Shaghaleh, H., Yong, J.W.H., 2024. Effects of biochar types on seed germination, growth, chlorophyll contents, grain yield, sodium, and potassium uptake by wheat (*Triticum aestivum* L.) under salt stress. BMC Plant Biol. 24, 487. https://doi.org/10.1186/s12870-024-05188-0.
Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.T., Smith, F., 1956. Colorimetric method for determination of sugars and related substances. Anal. Chem. 28 (3), 350–356. https://doi.org/10.1021/ac60111a017.
Ebtihal, Mervat, S.S., Tawfik, M.M., 2018. Glutathione treatment alleviate salinity adverse effects on growth, some biochemical aspects, yield quantity and nutritional value of chickpea. Plant Sci. Fed. J. Global Warm. 2 (2), 1–11.
Ellis, M.E., Roberts, E.H., 1981. The quantification of ageing and survival in orthodox seeds. Seed Sci. Technol. 9, 373–409.
Erenstein, O., Jaleta, M., Sonder, K., Mottaleb, K., Prasanna, B.M., 2022. Global maize production, consumption and trade: trends and R&D implications. Food Secur. 14, 1295–1319. https://doi.org/10.1007/s12571-022-01288-7.
Eteshola, E.O., Haupt, D.A., Koos, S.I., Siemer, L.A., Morris Jr, D.L., 2020. The role of metal ion binding in the antioxidant mechanisms of reduced and oxidized glutathione in metal-mediated oxidative DNA damage. Metallomics 12 (1), 79–91. https://doi.org/10.1039/c9mt00231f.
Giannopolitis, C.N., Ries, S.K., 1977. Superoxide dismutases I. Occurrence in higher plants. Plant Physiol. 59 (2), 309–314. https://doi.org/10.1104/pp.59.2.309.
Gietler, M., Nykiel, M., 2017. Involvement of thiol-based mechanisms in plant growth, development, and stress tolerance. Glutathione in Plant Growth, Development, and Stress Tolerance. Springer, Cham, pp. 59–98. https://doi.org/10.1007/978-3-319-66682-2_3.
Hacisalihoglu, G., 2020. Zinc (Zn): the last nutrient in the alphabet and shedding light on Zn efficiency for the future of crop production under suboptimal Zn. Plants 9 (11), 1471. https://doi.org/10.3390/plants9111471.
Hamilton, P.B., Van Slyke, D.D., Lemish, S., 1943. The gasometric determination of free amino acids in blood filtrates by the ninhydrin-carbon dioxide method. J. Biol. Chem. 150, 231–250. https://doi.org/10.1016/S0021-9258(18)51268-0.
Hasanuzzaman, M., Nahar, K., Anee, T.I., Fujita, M., 2017. Glutathione in plants: biosynthesis and physiological role in environmental stress tolerance. Physiol. Mol. Biol. Plant 23 (2), 249–268. https://doi.org/10.1007/s12298-017-0422-2.
Hasanuzzaman, M., Bhuyan, M.H.M.B., Zulfiqar, F., Raza, A., Mohsin, S.M., Mahmud, J. A., Fujita, M., Fotopoulos, V., 2020. Reactive oxygen species and antioxidant defense in plants under abiotic stress: revisiting the crucial role of a universal defense regulator. Antioxidants, (Basel) 9 (8), 681. https://doi.org/10.3390/antiox9080681.
He, A., Ma, Z., Li, Y., Huang, C., Yong, J.W.H., Huang, J., 2023. Spatiotemporal, physiological and transcriptomic dynamics of wild jujube seedlings under saline conditions. Tree Physiol. 43, 832–850. https://doi.org/10.1093/treephys/tpad001.
Henson, C., Stone, J., 1988. Variation in a-amylase and a-amylase inhibitor activities in barley malts. J. Cereal Sci. 8 (1), 39–46. https://doi.org/10.1016/S0733-5210(88)80047-X.
Hoang, M.T.T., Doan, M.T.A., Nguyen, T., Tra, D.P., Chu, T.N., Dang, T.P.T., Quach, P.N. D., 2021. Phenotypic characterization of *Arabidopsis* ascorbate and glutathione deficient mutants under abiotic stresses. Agronomy 11 (4), 764. https://doi.org/10.3390/agronomy11040764.
Hossain, M.A., Mostofa, M.G., Diaz-Vivancos, P., Burritt, D.J., Fujita, M., Tran, L.S.P., 2017. Glutathione in Plant Growth, Development, and Stress Tolerance. Springer Int. Publish. https://doi.org/10.1007/978-3-319-66682-2
Hussain, N., Sohail, Y., Shakeel, N., Javed, M., Bano, H., Gul, H.S., Ajaj, R., 2022. Role of mineral nutrients, antioxidants, osmotic adjustment and PSII stability in salt tolerance of contrasting wheat genotypes. Sci. Rep. 12 (1), 12677.
Imran, M., Boelt, B., Mühling, K.H., 2018. Zinc seed priming improves salt resistance in maize. J. Agron. Crop Sci. 204 (4), 390–399.
Iqbal, M.N., Rasheed, R., Ashraf, M.Y., Ashraf, M.A., Hussain, I., 2018. Exogenously applied zinc and copper mitigate salinity effect in maize (*Zea mays* L.) by improving key physiological and biochemical attributes. Environ. Sci. Pollut. Res., 25 (24), 23883–23896. https://doi.org/10.1007/s11356-018-2383-6.
Jaiswal, D., Pandey, A., Agrawal, S.B., 2021. Role of glutathione in enhancing plant tolerance to organic pollutants. In: Hasanuzzaman, M., Prasad, M.N.V. (Eds.), Handbook of Bioremediation; Physiological, Molecular and Biotechnological Interventions. Academic Press, pp. 523–533. https://doi.org/10.1016/B978-0-12-819382-2.00033-8.
Jalil, S., Nazir, M.M., Ali, Q., Zulfiqar, F., Moosa, A., Altaf, M.A., Zaid, A., Nafees, M.A., Yong, J.W.H., Jin, X., 2023. Zinc and nano zinc mediated alleviation of heavy metals and metalloids in plants: an overview. Funct. Plant Biol. 50, 23021. https://doi.org/10.1071/FP23021.
Julkunen-Titto, R., 1985. Phenolic constituents in the levels of northern willows: methods for precursors of clarified apple juice sediment. J. Food Sci. 33 (4), 254–257. https://doi.org/10.1021/jf00062a013.
Kalemba, E.M., Ratajczak, E., 2018. The effect of a doubled glutathione level on parameters affecting the germinability of recalcitrant *Acer saccharinum* seeds during drying. J. Plant Physiol., 223, 72–83. https://doi.org/10.1016/j.jplph.2018.02.010.
Kaur, G., Sanwal, S.K., Kumar, A., Pundir, R.K., Yadav, M., Sehrawat, N., 2024. Role of osmolytes dynamics in plant metabolism to cope with salinity induced osmotic stress. Discov. Agric. 2 (59). https://doi.org/10.1007/s44279-024-00070-x.
Kirk, J.T.O., Allen, R.L., 1965. Dependence of chloroplast pigment synthesis on protein synthesis: effect of actidione Biochem. Biochem. Biophys. Res. Commun. 21 (6), 523–530. https://doi.org/10.1016/0006-291X(65)90516-4.
Krishna, T.P.A., Maharajan, T., Victor Roch, G., Ignacimuthu, S., Antony Ceasar, S., 2020. Structure, function, regulation and phylogenetic relationship of ZIP family transporters of plants. Front. Plant Sci 11, 662. https://doi.org/10.3389/fpls.2020.00662.
Kumar, S., Li, G., Yang, J., Huang, X., Ji, Q., Liu, Z., Hou, H., 2021. Effect of salt stress on growth, physiological parameters, and ionic concentration of water dropwort (*Oenanthe javanica*) cultivars. Front. Plant Sci 12, 660409.
Kumar, V., Singhal, R.K., Kumar, N., Bose, B., 2020. Micro-nutrient seed priming: a pragmatic approach towards abiotic stress management. In: Rakshit, A, Singh, H, Singh, A, Singh, U, Fraceto, L. (Eds.), New Frontiers in Stress Management For Durable Agriculture. Springer, Singapore. https://doi.org/10.1007/978-981-15-1322-0_14.

Larson, L.A., Beevers, H., 1963. Amino acid metabolism in young pea seedlings. Plant Physiol. 40 (3), 424. https://doi.org/10.1104/pp.40.3.424.
Lee, Y.P., Takahashi, T., 1966. An improved colorimetric determination of amino acids with the use of ninhydrin. Anal. Biochem. 14 (1), 71–77. https://doi.org/10.1016/0003-2697(66)90057-1.
Lowry, R.R., Tinsley, I.J., 1976. Rapid colorimetric determination of free fatty acids. J. Am. Oil Chem. Soc. 53 (7), 470–472. https://doi.org/10.1007/BF02636814.
Ma, M., Liu, Y., Bai, C., Yang, Y., Sun, Z., Liu, X., Zhang, S., Han, X., Yong, J.W.H., 2021. The physiological functionality of PGR5/PGRL1-dependent cyclic electron transport in sustaining photosynthesis. Front. Plant Sci. 12, 702196. https://doi.org/10.3389/fpls.2021.702196.
Mirecki, R.M., Teramura, A.H., 1984. Effects of ultraviolet-B irradiance on soybean V. The dependence of plant sensitivity on the photosynthetic photon flux density during and after leaf expansion. Plant Physiol 74 (3), 475–480. https://doi.org/10.1104/pp.74.3.475.
Moothoo-Padayachie, A., Varghese, B., Pammenter, N.W., Govender, P., Sershen, 2016. Germination associated ROS production and glutathione redox capacity in two recalcitrant-seeded species differing in seed longevity. Botany 94 (12), 1103–1114. https://doi.org/10.1139/cjb-2016-0130.
Mukherjee, S.P., Choudhuri, M.A., 1983. Implications of water stress-induced changes in the levels of endogenous ascorbic acid and hydrogen peroxide in Vigna seedlings. Physiol. Plant 58 (2), 166–170. https://doi.org/10.1111/j.1399-3054.1983.tb04162.x.
Nahar, K., Hasanuzzaman, M., Alam, M., Fujita, M., 2015a. Glutathione-induced drought stress tolerance in mung bean: coordinated roles of the antioxidant defence and methylglyoxal detoxification systems. AoB Plants 7. https://doi.org/10.1093/aobpla/plv069.
Munns, R., Gilliham, M., 2015. Salinity tolerance of crops – what is the cost? New Phytol. 208, 668–673. https://doi.org/10.1111/nph.13519.
Munns, R., Tester, M., 2008. Mechanisms of salinity tolerance. Annu. Rev. Plant Biol. 59, 651–681. https://doi.org/10.1146/annurev.arplant.59.032607.092911.
Nahar, K., Hasanuzzaman, M., Alam, M.M., Fujit, M., 2015c. Exogenous glutathione confers high temperature stress tolerance in mung bean (*Vigna radiata* L.) by modulating antioxidant defense and methylglyoxal detoxification system. Environ. Exp. Bot., 112, 44–54. https://doi.org/10.1016/j.envexpbot.2014.12.001.
Nahar, K., Hasanuzzaman, M., Alam, M.M., Fujita, M., 2015b. Roles of exogenous glutathione in antioxidant defense system and methylglyoxal detoxification during salt stress in mung bean. Biol. Plant 59 (4), 745–756. https://doi.org/10.1007/s10535-015-0542-x.
Nakamura, S.I., Wongkaew, A., Nakai, Y., Rai, H., Ohkama-Ohtsu, N., 2019. Foliar-applied glutathione activates zinc transport from roots to shoots in oilseed rape. Plant Sci. 283, 424–434. https://doi.org/10.1016/j.plantsci.2018.10.018.
Nakano, Y., Asada, K., 1981. Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. Plant Cell Physiol. 22 (5), 867–880. https://doi.org/10.1093/oxfordjournals.pcp.a076232.
Naseer, M.N., Rahman, F.U., Hussain, Z., Khan, I.A., Aslam, M.M., Aslam, A., Iqbal, S., 2022. Effect of salinity stress on germination, seedling growth, mineral uptake and chlorophyll contents of three cucurbitaceae species. Braz. Arch. Biol. Technol. 65, e22210213.
Naz, M., Ghani, M.I., Atif, M.J., Raza, M.A., Bouzroud, S., Afzal, M.R., Fan, X., 2023. Sodium and abiotic stress tolerance in plants beneficial chemical elements of plants: recent. Develop. Future Prospects 13, 307–330.
Nikolić, N., Ghirardelli, A., Schiavon, M., Masin, R., 2023. Effects of the salinity-temperature interaction on seed germination and early seedling development: a comparative study of crop and weed species. BMC Plant Biol. 23 (446). https://doi.org/10.1186/s12870-023-04465-8.
Noctor, G., Bergot, G., Mauve, C., Thominet, D., Lelarge-Trouverie, C., Prioul, J.L., 2007. A comparative study of amino acid measurement in leaf extracts by gas chromatography-time of flight-mass spectrometry and high-performance liquid chromatography with fluorescence detection. Metabolomics 3 (2), 161–174. https://doi.org/10.1007/s11306-007-0057-3.
Parkinson, J.A., Allen, S.E., 1975. A wet oxidation procedure suitable for the determination of nitrogen and mineral nutrients in biological material. Commun. Soil Sci. Plant Anal. 6 (1), 1–11.
Pei, L., Che, R., He, L., Gao, X., Li, W., Li, H., 2019. Role of exogenous glutathione in alleviating abiotic stress in maize (*Zea mays* L.). J. Plant Growth Regul. 38 (1), 199–215.
Perveen, R., Wang, X., Jamil, Y., Ali, Q., Ali, S., Zakaria, M.Q., Fiaz, S., 2021. Quantitative determination of the effects of He-Ne laser irradiation on seed thermodynamics, germination attributes and metabolites of safflower (*Carthamus tinctorius* L.) in relation with the activities of germination enzymes. Agronomy 11 (7), 1411. https://doi.org/10.3390/agronomy11071411.
Perveen, R., Jamil, Y., Javed, Y., Shahid, M.A., 2022. A Comprehensive Study on pulsed Nd:YAG laser irradiation of maize kernels: measurement of biophoton emissions, metabolite levels and thermodynamical attributes during germination. Arab. J. Sci. Eng. 47, 7593–7607. https://doi.org/10.1007/s13369-021-06328-6.
Priya, P., Patil, V.C., Kumar, B.A., 2016. Cultivation practices and production constraints of maize under rainfed conditions. Res. Environ. Life Sci. 9 (2), 166169.
Raja, V., Wani, U.M., Wani, Z.A., Jan, N., Kottakota, C., Reddy, M.K., John, R., 2022. Pyramiding ascorbate–glutathione pathway in *Lycopersicum esculentum* confers tolerance to drought and salinity stress. Plant Cell Rep. 41 (3), 619–637.
Rajput, V.D., Singh, R.K.H., Verma, K.K., Sharma, L., Quiroz-Figueroa, F.R., Meena, M., Gour, V.S., Minkina, T., Sushkova, S., Mandzhieva, S., 2021. Recent developments in enzymatic antioxidant defence mechanism in plants with special reference to abiotic stress. Biology (Basel) 10, 267. https://doi.org/10.3390/biology10040267.
Ramzan, M., Shah, A.A., Ahmed, M.Z., Bukhari, M.A., Ali, L., Casini, R., Elansary, H.O., 2023. Exogenous application of glutathione and gamma amino-butyric acid alleviates salt stress through improvement in antioxidative defense system and modulation of CaXTHs stress-related genes. South Afr. J. Bot. 157, 266–273.
Ruan, S., Xue, Q., Tylkowska, K., 2002. The influence of priming on germination of rice (*Oryza sativa* L.) seeds and seedling emergence and performance in flooded soil. Seed Sci. Technol., 30 (1), 61–67.
Sadak, M., Abd Elhamid, E., Mahmoud, M., 2017. Glutathione induced antioxidant protection against salinity stress in chickpea (*Cicer arietinum* L.) plant. Egyp. J. Bot. 57 (2), 293–302.
Saeed, F., Kausar, A., Ali, Q., Mahmood, A., Javed, T., Shabbir, R., Ali, M.F., 2024. Glutathione and zinc application upregulate the antioxidant system increasing photosynthetic pigments and growth under water stress in maize (*Zea mays* L.). Braz. J. Bot. 47 (3), 795–808.
Saeed, F., Kausar, A., Ali, Q., Akhter, N., Tehseen, S., 2023. Impact of combined glutathione and Zn application for seed priming in ameliorating the adverse effects of water stress on maize seed germination attributes, metabolite levels, and seedling vigor. Gesunde Pflanzen 75 (5), 2147–2168.
Safi-naz, Z., Zaki, 2018. Alleviating effects of ascorbic acid and glutathione for faba bean plants irrigated with saline water. J. Plant Sci. 2 (2), 1–15.
Samreen, T., Shah, H.U., Ullah, S., Javid, M., 2017. Zinc effect on growth rate, chlorophyll, protein and mineral contents of hydroponically grown mungbeans plant (*Vigna radiate* L.) Arab. J. Chem. 10, S1802–S1807. https://doi.org/10.1016/j.arabjc.2013.07.005.
Sani, M.N.H., Amin, M., Bergstrand, K.J., Caspersen, S., Prade, T., Yong, J.W.H., 2025. Harnessing biostimulants from biogas digestates for high-value resource recovery: a review. Environ. Chem. Lett. 23 (1), 139–164. https://doi.org/10.1007/s10311-024-01801-8.
Sani, M.N.H., Yong, J.W.H., 2022. Harnessing synergistic biostimulatory processes: aplausible approach for enhanced crop growth and resilience in organic farming. Biology 11, 41. https://doi.org/10.3390/biology11010041.
Seleiman, M.F., Ahmad, A., Alhammad, B.A., Tola, E., 2023. Exogenous application of zinc oxide nanoparticles improved antioxidants, photosynthetic, and yield traits in salt-stressed maize. Agronomy 13 (10), 2645.
Seyedi, M., Hamzei, J., Fathi, H.A.D.I., Bourbour, A.M.I.N., Dadrasi, V., 2012. Effect of seed priming with zinc sulfate on germination characteristics and seedling growth of chickpea (*Cicer arietinum* L.) under salinity stress. Int. J. Agric. 2 (3), 108.
Sghayar, S., Debez, A., Lucchini, G., Abruzzese, A., Zorrig, W., Negrini, N., Morgutti, S., Abdelly, C., Sacchi, G.A., Pecchioni, N., Vaccino, P., 2023. Seed priming mitigates high salinity impact on germination of bread wheat (*Triticum aestivum* L.) by improving carbohydrate and protein mobilization. Plant Direct 7 (6), e497.
Shabaan, M., Asghar, H.N., Zahir, Z.A., Zhang, X., Sardar, M.F., Li, H., 2022. Salt-tolerant PGPR confer salt tolerance to maize through enhanced soil biological health, enzymatic activities, nutrient uptake and antioxidant defense. Front. Microbiol. 13, 901865. https://doi.org/10.3389/fmicb.2022.901865. PMID: 35633670; PMCID: PMC9136238.
Shabala, S., Munns, R., 2017. Salinity stress: physiological constraints and adaptive mechanisms. Plant Stress Physiol. 24–63. https://doi.org/10.1079/9781845939953.0059.
Shafiq, S., Akram, N.A., Ashraf, M., 2019. Assessment of physio-biochemical indicators for drought tolerance in different cultivars of maize (*Zea mays* L.). Pak. J. Bot. 51 (4), 1241–1247.
Shao, J., Wu, W., Rasul, F., Munir, H., Huang, K., Awan, M.I., Albishi, T.S., Arshad, M., Hu, Q., Huang, G., Hassan, M.U., Aamer, M., Qari, S.H., 2022. Trehalose induced drought tolerance in plants: physiological and molecular responses. Not. Bot. Horti. Agrobo. 50 (1), 12584. https://doi.org/10.15835/nbha50112584.
Shehzad, F., Ali, Q., Ali, S., Al-Misned, F.A., Maqbool, S., 2022. Fertigation with Zn-Lysine confers better photosynthetic efficiency and yield in water stressed maize: water relations, antioxidative defense mechanism and nutrient acquisition. Plants 11, 404. https://doi.org/10.3390/plants11030404.
Sheikhalipour, M., Gohari, G., Esmaielpour, B., Behnamian, M., Giglou, M.T., Milani, M.H., Vita, F., 2023. Effect of melatonin foliar sprays on morphophysiological attributes, fruit yield and quality of *Momordica charantia* L. under salinity stress. Plant Physiol. Biochem. 205, 108194.
Shi, Q., Pang, J., Yong, J.W.H., Bai, C., Pereira, C.G., Song, Q., Wu, D., Dong, Q., Cheng, X., Wang, F., Zheng, J., Liu, Y., Lambers, H., 2020. Phosphorus-fertilisation has differential effects on leaf growth and photosynthetic capacity of *Arachis hypogaea* L. Plant Soil. 447, 99–116. https://doi.org/10.1007/s11104-019-04041-w.
Singh, S., Kaur, J., Ram, H., Singh, J., Kaur, S., 2023. Agronomic bio-fortification of wheat (*Triticum aestivum* L.) to alleviate zinc deficiency in human being. Rev. Environ. Sci. Bio/Technol. 22, 505–526.
Somogyi, M., 1952. Notes on sugar determination. J. Biol. Chem. 195 (1), 19–23.
Suzuki, N., Rivero, R.M., Shulaev, V., Blumwald, E., Mittler, R., 2014. Abiotic and bioticstress combinations. New Phytol. 203, 32–43. https://doi.org/10.1111/nph.17074.
Taiz, L., Zeiger, E., Møller, I.M., Murphy, A., 2015. *Plant Physiology and Development* (No. Ed. 6). Sinauer Associates Incorporation.
Tebini, M., Rabaoui, G., M'Rah, S., Luu, D.T., Ben Ahmed, H., Chalh, A., 2022. Effects of salinity on germination dynamics and seedling development in two amaranth genotypes. Physiol. Mol. Biol. Plants 28 (7), 1489–1500.
Tonguc, M., Elkoyunu, R., Erbaş, S., Karakurt, Y., 2012. Changes in seed reserve composition during germination and initial seedling development of safflower (*Carthamus tinctorius* L.). Turk. J. Biol. 36 (1), 107–112. https://doi.org/10.3906/biy-1012-164.
Uçarlı, C., 2020. Effects of salinity on seed germination and early seedling stage. Abiotic Stress Plants 211, 1–12.

Umair Hassan, M., Aamer, M., Umer Chattha, M., Haiying, T., Shahzad, B., Barbanti, L., Nawaz, M., Rasheed, A., Afzal, A., Liu, Y., 2020. The critical role of zinc in plants facing the drought stress. Agriculture 10 (9), 396.

Velikova, V., Yordanov, I., Edreva, A., 2000. Oxidative stress and some antioxidant systems in acid rain-treated bean plants: protective role of exogenous polyamines. Plant Sci. 151 (1), 59–66. https://doi.org/10.1016/S0168-9452(99)00197-1.

Wang, F., Gao, J., Yong, J.W.H., Wang, Q, Ma, J., He, X., 2020. Higher atmospheric CO_2 levels favor C_3 plants over C_4 plants in utilizing ammonium as a nitrogen source. Front. Plant Sci. 11, e537443. https://doi.org/10.3389/fpls.2020.537443.

Wang, P., Liu, W.C., Han, C., Wang, S., Bai, M.Y., Song, C.P., 2023. Reactive oxygen species: multidimensional regulators of plant adaptation to abiotic stress and development. J. Integr. Plant Biol. 66, 330–367.

Wong, W.S., Zhong, H.T., Cross, A.T., Yong, J.W.H., 2020. Plant biostimulants invermicomposts: characteristics and plausible mechanisms. In: Geelen, D., Xu, L. (Eds.) (Eds.), The Chemical Biology of Plant Biostimulants. Wiley, pp. 155–180. https://doi.org/10.1002/9781119357254.ch6.

Wu, D., Chen, C., Liu, Y., Yang, L., Yong, J.W.H., 2023. Iso-osmotic calcium nitrate and sodium chloride stresses have differential effects on growth and photosynthetic capacity in tomato. Sci. Hortic. 312, 111883. https://doi.org/10.1016/j.scienta.2023.111883.

Wu, S., Hu, C., Tan, Q., Li, L., Shi, K., Zheng, Y., Sun, X., 2015. Drought stress tolerance mediated by zinc-induced antioxidative defense and osmotic adjustment in cotton (*Gossypium hirsutum*). Acta Physiol. Plant. 37 (8), 1–9. https://doi.org/10.1007/s11738-015-1919-3.

Xie, X., Hu, W., Fan, X., Chen, H., Tang, M., 2019. Interactions between phosphorus, zinc, and iron homeostasis in nonmycorrhizal and mycorrhizal plants. Front. Plant Sci. 10, 172. https://doi.org/10.3389/fpls.2019.01172.

Xiong, M., Xu, J., Zhou, Z., Peng, B., Shen, Y., Shen, H., Xu, X., Li, C., Deng, L., Feng, G., 2024. Salinity inhibits seed germination and embryo growth by reducing starch mobilization efficiency in barley. Plant Direct 8 (2), e564. https://doi.org/10.1002/pld3.564. PMID: 38312996; PMCID: PMC10835642.

Yong, J.W.H., Ng, Y.F., Tan, S.N., Chew, A.Y.L., 2010. Growth observations and the effects of fertilizer applications on physiology of *Jatropha curcas* L. Photosynthetica 48, 208–218. https://doi.org/10.1007/s11099-010-0026-3.

Zaki, S.S., Belal, E.E., Rady, M.M., 2019. Cyanobacteria and glutathione applications improve productivity, nutrient contents, and antioxidant systems of salt-stressed soybean plant. Int. Lett. Nat. Sci. 76, 72–85.

Zhang, L., Yan, M., Ren, Y., Chen, Y., Zhang, S., 2021. Zinc regulates the hydraulic response of maize root under water stress conditions. Plant Physiol. Biochem. 159, 123–134. https://doi.org/10.1016/j.plaphy.2020.12.014.

Zhishen, J., Mengcheng, T., Jianming, W., 1999. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chem. 64 (4), 555–559. https://doi.org/10.1016/S0308-8146(98)00102-2.

Zhou, Y., Diao, M., Chen, X., Cui, J., Pang, S., Li, Y., Hou, C., Liu, H., 2019. Application of exogenous glutathione confers salinity stress tolerance in tomato seedlings by modulating ions homeostasis and polyamine metabolism. Sci. Hort 250, 45–58. https://doi.org/10.1016/j.scienta.2019.02.026.