



# Glutathione and zinc application upregulate the antioxidant system increasing photosynthetic pigments and growth under water stress in maize (*Zea mays* L.)

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## Abstract

The current study was planned to compare the effects of foliar spray of glutathione and co-applied zinc (Zn) on growth, lipid peroxidation and photosynthetic pigments in relation to alterations in antioxidative potential and biochemicals under drought induced water stress (75% field capacity). Three levels of glutathione (1.5, 3.0, and 4.5 mM) were applied separately as well as in combination with 0.5% Zn as foliar spray. Water deficit imposed significant adverse effects on growth, biosynthesis of photosynthetic pigments in association with disturbed metabolic activities increased the lipid peroxidation regarding the contents of H<sub>2</sub>O<sub>2</sub> and malondialdehyde (MDA). Glutathione as alone or in combination with Zn alleviated the adverse impacts of water stress significantly of maize growth in association with positive boosting influences on biosynthesis of enzymatic and non-enzymatic antioxidants, photosynthetic pigments, accumulation of total soluble proteins (TSP), total soluble, reducing, and non-reducing sugars. Overall, the combined application of glutathione with Zn proved better as compared with alone. Moreover, 1.5 mM level of glutathione in combination with Zn was found the most efficient strategy to alleviate the adverse effects on plant in water deficit conditions and technology can be used to enhance the production in water stressed hit areas of the world.

**Keywords** Glutathione · Oxidative stress · Photosynthetic pigments · Water stress

## 1 Introduction

The extreme environmental factors including abiotic stress factors mainly the drought, flooding, salinity, heat and cold are the consequences of global climatic changes (Javed and Gao 2023) that lead to crop yield loss of economic important crops (Stevanovic et al. 2016). A sustainable human life needs the food security due to rapid increase in population (Myers et al. 2017). But it is not so easier to maintain the sustainable yield against the water stress (Ahmad et al. 2022a, b; Shabbir et al. 2022). Drought is an obstacle against the normal growth of all plants and final crop yield (Bukhari et al. 2022; Akhtar et al. 2022) because it interferes in physio-biochemical processes of plants (Yahaya and Shimelis 2022). It causes a cascade of oxidative changes and damage of membrane structures of the cell and macromolecules such as DNA and RNA (Parveen et al. 2020) and denaturing of proteins (Sofy et al. 2021). The drought factor cannot be exonerated from the growth and yield losses to prime crops overall the world.

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Excessive reactive oxygen species (ROS) under water deficit conditions can cause the oxidative damages which destroys the structure of internal plant organelles (Tiwari et al. 2021). The stress triggered over expressed ROS instigates the lipid peroxidation and damages the chloroplast and mitochondria that associated with poor photosynthesis and growth which in return lead to compromised crop production (Cui et al. 2017). The water stress reduced the photosynthetic activity and causes severe growth retardation along with damaged root morphology (Altaf et al. 2022). In contradiction, the plants possess well-developed natural water stress tolerant mechanisms adapted by them physiologically, biochemically and morphologically to counteract drought driven reverse impacts (Ghafar et al. 2021) because the plants generate antioxidants such as carotenoids, flavonoids, antioxidant enzymes such as catalase (CAT), peroxidase (POD), superoxide dismutase (SOD), and ascorbate peroxidase (APX) to respond unfavorable circumstances (Chai et al. 2016; Khatun et al. 2020). Accumulation of metabolites also plays a defensive role against water stress. The most common metabolites are proteins and total soluble sugars that are involve in maintaining the water balance at cellular level under water deficit regimes (Kabiri et al. 2018).

Maize is a cereal crop of vital importance all over the globe that having versatility in utilization as feed, fodder and biofuel. Maize has ancient origin about 8.7 hundred years old from Mexico (Piperno et al. 2009), and still, USA shares about 30% of total maize production to the world as the largest producer (<https://www.statista.com>). Besides that, crop production is highly sensitive to climatic and environmental variations, i.e., drought (Webber et al. 2018; Nguyen et al. 2021; Te et al. 2023). Therefore, requirements for effective sustainable methodologies and to induce the water stress tolerance in maize varieties for successful maize yield are pivotal.

Among the effective strategies, the exogenous application of micronutrients such as zinc (Zn) was found the most beneficial (Mannan et al. 2022) in loss reduction (Shahgholi et al. 2023) with water stress. Micro-nutrients application such as Zn is the most promising approach of biofortification that has role in better yield by enhancing the overall plants growth (Akbari et al. 2013; Shahgholi et al. 2023). Zn is an energetic element for plants growing and act as co-factor for certain enzymes (Ma et al. 2016) and directly involves in biosynthesis of growth regulators such as auxin and increases protein metabolism sexual fertility, cell division and fertilization (Shahid et al. 2022). Glutathione (GSH) is an important non-enzymatic antioxidant (Locato et al. 2017) that involved in water stress tolerant strategies. Therefore, exogenously application of GSH is the effective strategy to ameliorate water stress harms to plants (Sohag et al. 2020) with strong antioxidant defense system. It has

role as antioxidant agent in scavenging of the ROS species (Souri et al. 2021) under different abiotic stress. The GSH application increased the nutrient uptake in plants by endogenous GSH contents up-regulation (Qiu et al. 2013). It also involves in stress tolerance, increases the translocation and mobility of metal ions (Shankar et al. 2012).

In view of the above-mentioned information, it was hypothesized that exogenously applied GSH in combination with Zn might be more helpful for the induction of water stress tolerance irrespective of their individual application. Current study was aimed to elucidate the possible anti-stress role of GSH and Zn as foliar spray when applied separately in comparison with their combined application in modifying growth factors, physio-biochemicals attributes of maize to help the plants against water stress.

## 2 Materials and methods

**Experimental site** The research area of the Department of Botany at Government College University Faisalabad was chosen as the site for a pot experiment.

The complete randomized design (CRD) with four replicates of each treatment was arranged for the whole experiment. Experiment was comprised of total 72 pots (36 non-stressed ones and other 36 water stressed ones). The size of the plastic pots was 25 cm diameter × 28 cm height. The pots were filled with 10 kg of sandy loam soil. Before sowing of seeds, the soil in pots was saturated with canal water. After approaching the moisture contents of soil at field capacity, the seeds were hand sown. Ten healthy seeds in each pot were sown. The seeds were surface sterilized with 0.1% solution of HgCl<sub>2</sub> for 10 s, and then the seeds were washed vigorously with tap water. After 8 days of seedling emergence, four plants were kept in each pot by uprooting the extra plants to keep them at ideal distances.

After one week of seed germination, the seedlings in the set of pots corresponding to water stress treatment were exposed to water stress (75% field capacity) and the other set of pots were irrigated normally as per the irrigation requirements. Plants allocated to each treatment were sprayed with the respective solution of 1.5 mM GSH, 3 mM GSH, 4.5 mM of GSH, 1.5 mM GSH + 0.5% Zn, 3 mM GSH + 0.5% Zn, 4.5 mM GSH + 0.5% Zn, 0.5% Zn as ZnSO<sub>4</sub> and water spray as control treatment after 10th day of the start of water stress treatment. In each set of pots corresponding to water stress and non-stress, 4 pots were kept without any spray as control plants.

**Field capacity determination** At the stage of filling soil in pots, three soil samples of 200 g each were taken to calculate the field capacity. This collected soil was then kept in an oven for incubation (24 h) at 105 °C. Then, oven-dried

samples were weighed, and the average weight was taken to determine the total moisture contents at seed sowing stage. After that, the three soil samples were processed for estimation of saturation percentage. Hundred gram of each dried soil sample was weighed and made the past by using distilled water for the measuring the soil saturation percentage. After measuring the soil saturation percentage, the following formula was used for measuring the soil field capacity.

$$\text{Field Capacity} = \frac{\text{Saturation Percentage}}{2}$$

After determination of soil weight and moisture% of each pot, 75% of field capacity was maintained as water stress treatment. This field capacity was maintained during the whole period of the experiment by maintaining soil moisture on daily basis. On daily basis, the set of pots allocated to water stress were weighed on daily basis and maintained the weight of pots by adding water to pots.

**Texture of soil and physio-chemical properties** Soil contained (sandy clay) texture with (organic matter 0.9%), saturation percentage 30,  $\text{NO}_3\text{-N}$  6.1  $\text{mg kg}^{-1}$ ,  $\text{NH}_4\text{-N}$  2.96  $\text{mg kg}^{-1}$ , K 186  $\text{mg kg}^{-1}$ , Ca 108  $\text{mg kg}^{-1}$ , availability of P 5.13  $\text{mg kg}^{-1}$ , electrical conductivity (ECe) 2.5  $\text{dSm}^{-1}$  and pH 8.2. With the method of Jackson (1969), soil saturation, inorganic nutrients, soil pH and ECe were determined.

**Data collection** The data were collected regarding growth and physio-biochemical attributes after 15 days of foliar spray applications of glutathione and 0.5% Zn. Fresh leaf samples were stored for biochemical attributes at  $-80^\circ\text{C}$ .

**Growth and morphological attributes** The lengths of root and shoot samples were measured and weighed for fresh biomasses. After that, plants were dried in oven for 72 h at  $65^\circ\text{C}$  for weighing of dry biomass.

**Estimation of the photosynthetic pigments in leaves** Leaf total chlorophyll (T. Chl.), chlorophyll *b* (Chl. *b*), chlorophyll *a* (Chl. *a*) and carotenoid contents were measured using the method described by Arnon (1949). Briefly, fresh leaf material (0.1 g) two from each replicate was chopped into small pieces (1 cm) and leaf photosynthetic pigments were extracted using 10 mL of 80% acetone. The leaf material in acetone was then placed overnight and centrifuged at  $10,000 \times g$  for 15 min. The absorbance of the supernatant was then read at 663, 645 and 480 nm. Chl. *a* and Chl. *b* were calculated using the following formula.

$$\text{T. Chl} = [20.2 (\text{DA } 645) - 8.02 (\text{OD } 663)] \times V/1000 \times W$$

$$\text{Chl. } b = [22.9 (\text{OD } 645) - 4.68 (\text{OD } 663)] \times V/1000 \times W$$

$$\text{Chl. } a = [12.7 (\text{OD } 663) - 2.69 (\text{OD } 645)] \times V/1000 \times W$$

$V$  = volume of the extract (mL),  $W$  = weight of the fresh leaf tissue (g).

However, for the estimation of leaf carotenoid contents, the formula given by Kirk and Allen (1965) was used.

$$\text{Carotenoids (mg mL}^{-1}\text{)} = A \text{ car/Em } 100\% \times 1007$$

$$A \text{ Car (carotenoid)} = (\text{OD } 480) + 0.114(\text{OD } 663) - 0.638(\text{OD } 645)$$

$$\text{Em (Emission)} = \text{Em } 100\% = 2500$$

**Enzymatic and non-enzymatic antioxidants** *Enzymes assay.* Fresh leaf sample (0.5 g) was homogenized using a pestle and mortar in 10 mL of 50 mM pre-chilled phosphate buffer with pH 7.8. The homogenate was then centrifuged at  $10,000 \times g$  for 15 min at  $4^\circ\text{C}$ . The supernatant so collected was used for enzyme assays and total soluble protein.

*Determination of superoxide dismutase (SOD).* Activity of SOD was measured using the method as described by Giannopolitis and Ries (1977). For the preparation of reaction mixture, 50  $\mu\text{L}$  of enzyme extract was added to a prepared mixture (1 mL) consisting of 50  $\mu\text{M}$  NBT (NBT prepared solution in formamide), 13 mM methionine, 1.3  $\mu\text{M}$  riboflavin, 75 nM EDTA and 50 mM phosphate buffer (pH 7.8). The reaction mixture was placed under a 20 V bulb for 15 min in a chamber internally coated with aluminum foil. Riboflavin was added to the reaction mixture before placing the mixture under the light source. A blank sample was also prepared each time without adding any extract. The absorbance (Abs) of the reaction mixture was measured to be 560 nm using a spectrophotometer.

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*Determination of peroxidase (POD), catalase (CAT) and ascorbic peroxidase (APX).* Activity of CAT and POD was measured using the method as given by Chance and Maehly (1957). The reaction mixture (3 mL) contained 50 mM phosphate buffer (pH 7.8), 59 mM  $\text{H}_2\text{O}_2$ , and 0.1 mL enzyme extract. The enzyme extract (100  $\mu\text{L}$ ) was mixed in last to start the reaction. The change in Abs was recorded at 240 nm

for 120 s at intervals of 20 s. For the estimation of POD activity, the reaction mixture was prepared using 0.1 mL enzyme extract, 40 mM H<sub>2</sub>O<sub>2</sub>, 20 mM guaiacol and 50 mM phosphate buffer (pH 7.0). The change in the absorbance was measured at 470 nm for 120 s at intervals of 20 s. The APX activity was measured following the method described by Asada and Takahashi (1987). The decrease in the absorbance was measured at 290 nm for 120 s with intervals of 20 s. The reaction mixture was prepared by adding 2.1 mL phosphate buffer, 300 µL ascorbate (0.5 mM), 300 µL H<sub>2</sub>O<sub>2</sub> and 300 µL of enzyme sample.

**Total soluble proteins ((TSP).** The same supernatant used for the of antioxidant enzymes assay was utilized for TSP contents. Supernatant (0.1 mL) was treated with Bradford reagent (2 mL) at 595 nm reading which was taken by the method of Bradford (1976).

**Leaf total phenolic content (TPC) and total flavonoid content (TFC) estimation.** The total phenolic contents (TPC) for seed were determined by Julkenen-Titto (1985) protocol. Using 10 mL of 80% acetone to fresh leaf (0.1 g) was homogenized. To obtain the supernatant, it was centrifuged at 4 °C at 10,000×g for 10 min. The supernatant containing 20% Na<sub>2</sub>CO<sub>3</sub> was treated with the Folin-Ciocalteu phenol reagent, and the mixture's absorbance was determined at 750 nm. The total flavonoid content (TFC) was also measured according to Karadeniz et al. (2005). One gram of plant part of maize was taken and ground with 20 mL of 80% aqueous methanol with help of mortar and pestle. Then filtration was carried out to obtain the filtrate. To the 0.5 mL filtrate, addition of 3 mL of distilled water with 0.3 mL of 5% NaNO<sub>2</sub> was carried out. After mixing well, the solution was allowed to stand under room temperature. After that, 0.6 mL of 10% AlCl<sub>3</sub> was mixed. After 6 min, 2 mL of 1 M NaOH was also added. The volume of solution was maintained up-to 10 mL with distilled water. At 510 nm, the absorbance was read.

**Total anthocyanin determination (TAC).** The described method was adapted to measure the seedling TAC content by Mirecki and Teramura (1984). 0.2 g of fresh leaves was homogenized in 2 mL of (1%) methanol (acidified). Readings at 530 and 657 nm of the supernatant were collected after centrifuging the mixture at 12,000 g for 20 min.

**Total free amino acids (TFAA) quantification.** These contents were measured by (Hamilton et al. 1943) method. In the enzyme assay, 1 mL of the extract was mixed with 2 mL of each (10% pyridine and 2% ninhydrin). Before measuring absorbance at 570 nm, for 30 min solution was heated at 95 °C.

**Total soluble sugars (TSS) and total reducing sugars (TRS).** The Dubois et al. (1956) method was adapted for TSS determination. For fresh leaf sample, anthrone reagent was used and then measured the absorbance at 490 nm. The

Henson and Stone (1988) technique was used to determine the quantity of RS.

Estimation of non-reducing sugars (NRS) was via the following formula as given by Lakho et al. (2017):

$$\text{Nonreducing sugars} = \text{Total soluble sugars} - \text{reducing sugars}$$

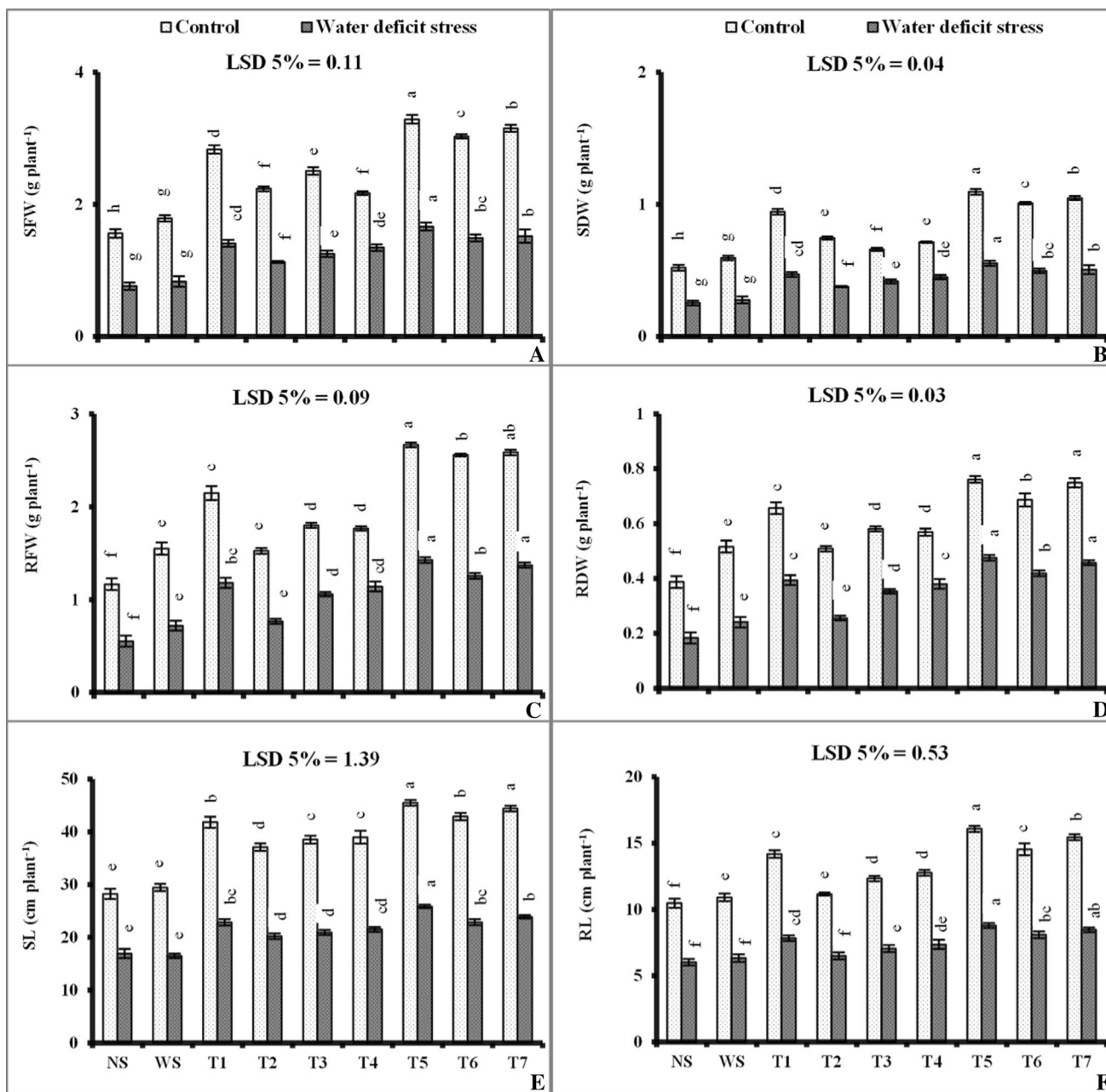
**Ascorbic acid (AsA) estimation.** Leaf AsA was determined by adapting the Mukherjee and Choudhuri (1983) developed method. Fresh leaf material (0.25 g) was mixed gently in 5 mL of 6% TCA solution, before being centrifuged at 10,000 g for 20 min. 4 mL of leaf extract additionally added 10% thiourea drop and 2 mL of 2% dinitrophenyl hydrazine solution (prepared in an acidic broth). At 95 °C in a water bath, heating the sample for 20 min, 80% H<sub>2</sub>SO<sub>4</sub> (5 mL) (v/v) was added. A UV-visible spectrophotometer was used to calculate the absorbance at 530 nm.

**Estimation of MDA and H<sub>2</sub>O<sub>2</sub> contents** The 0.25 g maize sample (leaf) grinded with 5 mL of trichloroacetic acid (6%). For 10 min, sample was centrifuged at 10,000×g. Then supernatant (0.5 mL) was mixed with thiobarbituric acid (5%) and the mixture temperature was maintained at 95 °C in water bath. By using the method as described by Cakmak and Horst (1991), the reading was taken at 532 and 600 nm with help of spectrophotometer. For H<sub>2</sub>O<sub>2</sub>, 50 mM potassium phosphate buffer (pH 7.5) and KI (1 M) were collective with the supernatant (0.5 mL), and the solution was incubated for 50 min. A measurement at 390 nm was then made using a method developed by Velikova et al. (2000).

**Statistical analysis** The CoStat Computer Program (version 6.303, PMB 320, Monterey, CA, 93940 USA) was utilized to analyze the significant variation among treatments for the studied attributes. The least significant difference (LSD) test at a 5% significance level was employed to identify significant differences among the means of the studied attributes. Furthermore, heat maps and correlations were generated using the Corrplot package in R-studio (version 4.2.2) for the aforementioned attributes.

### 3 Results

**Growth attributes** Data presented in (Fig. 1A–F; Table 1) show 42.40%, 40.04%, 52.63%, 67.06%, 50.98% and 51.08%, significantly ( $P \leq 0.001$ ) decrease in growth attributes, respectively, of RL (root length), SL (shoot length), RDW (root dry weight), RFW (root fresh weight), SDW (shoot dry weight), and SFW (shoot fresh weight), under water stressed conditions (75% FC). The significant limitations noted in growth related characteristics refers the



**Fig. 1** Different growth attributes of maize foliary-supplied with different levels of reduced glutathione applied separately or in combination with 0.5% Zn when grown under non-stressed and water stressed conditions (Mean  $\pm$  SE;  $n=4$ ). Bars with same alphabets specified for water stressed and non-stressed conditions do not differ significantly. SFW =shoot fresh weight; SDW =shoot dry weight; RFW =root fresh weight; RDW =root dry weight; SL =shoot length; RL =root length

clear indication of water stress related negative impacts on growing plant physiology. The biomass and growth are the consequences of the induced water stress also depicted in this study. The exogenously applied GSH along with Zn or alone eliminates the adverse impact of water stress on all studied growth attributes. Comparatively, the most increase

was presented by plants that were grown with combined spray of GSH and Zn than their separate applications. In case of GSH application in combination with Zn, 1.5 mM level imparts the maximum improvements under non-stressed and stressed situations. These applied substances suggested the better quality and ability of maize against the

**Table 1** Mean squares from analysis of variance of studied attributes of maize plants when grown under non-stressed and water stressed conditions after foliary-supplied with different levels of GSH separately and in combination with 0.5% Zn

SOV	df	SFW	SDW	RFW	RDW	SL	RL	MDA	H <sub>2</sub> O <sub>2</sub>
Stress (S)	1	27.581***	2.763***	15.232***	1.131***	5360.150***	587.235***	14,156***	2961.6***
Treatment (T)	8	1.657***	0.191***	1.395***	0.099***	169.500***	18.407***	250.98***	54.04***
S × T	8	0.221***	0.031***	0.145***	0.002*	20.285***	2.319***	203.13***	26.30***
Error	54	0.012	0.001	0.007	0.001	1.926	0.278	19.32	1.09
SOV	df	ASA	Chl. a	Chl. b	T. Chl	Chl. a/b	Car	TAC	TFC
Stress (S)	1	25,440***	4.196***	0.746***	8.483***	18.219***	0.006***	4.270***	1192.83***
Treatment (T)	8	215.37***	0.174***	0.040***	0.383***	0.763***	3.126e-4***	0.082***	55.213***
S × T	8	81.68**	0.004 ns	0.001 ns	0.003 ns	0.013 ns	2.509e-6 ns	0.035***	3.120***
Error	54	20.75	0.003	0.001	0.002	0.016	1.118e-5	0.003	0.469
SOV	df	SOD	POD	CAT	APX	TSP	TSS	RS	NRS
Stress (S)	1	7137.57***	17,631.51***	820.22***	42.98***	1,067,304***	58,110***	1792.5***	39,069***
Treatment (T)	8	328.17***	347.69***	41.52***	1.295***	44,124***	1571.2***	60.80***	998***
S × T	8	17.29 ns	56.33***	0.765 ns	0.018 ns	4880.80***	55.39 ns	0.221 ns	42.84 ns
Error	54	22.64	11.94	0.437	0.021	173.11	34.81	1.223	24.28
SOV	df	TPC	TFAA						
Stress (S)	1	15,156.4***	134.33***						
Treatment (T)	8	925.33***	6.872***						
S × T	8	227.15***	3.968***						
Error	54	26.90	0.264						

ns nonsignificant, RFW root fresh weight, RDW root dry weight, SFW shoot fresh weight, SDW shoot dry weight, SL shoot length, RL root length, Car carotenoids, Chlorophyll a=chlorophyll a; Chlorophyll b=chlorophyll b; T.Chlorophyll total chlorophyll, Chl a/b ratio of chlorophyll a/chlorophyll b, TSS total soluble sugar, TSP total soluble protein, TFAA total free amino acid, MDA malondialdehyde, H<sub>2</sub>O<sub>2</sub> hydrogen peroxide, AsA ascorbic acid, TAC total anthocyanin content, TFC total flavonoid content, SOD superoxide dismutase, POD peroxide dismutase, CAT catalase, APX ascorbate peroxidase, RS reducing sugar, NRS non-reducing sugar, TPC total phenolic content

\*, \*\*, \*\*\* significant at 0.05, 0.01 and 0.001 levels, respectively

water shortage regime. The foliar spray of 1.5 mM GSH along with Zn under non-stressed conditions increased SFW by 117.56%, SDW 158.18%, RFW 116.53%, RDW 162.29%, SL 52.89% and RL 45.84% of maize plants while 110.90%, 128.2%, 113.72%, 94.87%, 61.30% and 53.48% increase, respectively, was noticed under water stressed conditions (Fig. 1A–F; Table 1).

**Leaf photosynthetic pigments and metabolites** Data presented in (Fig. 2A–E; Table 1) showed 51.16%, 59.52%, 54.28%, 61.53% significantly ( $P \leq 0.001$ ) decrease, respectively, in (Chl. a/b, T. Chl., Chl. b and Chl. a) of the maize plants while 123.52% significantly ( $P \leq 0.001$ ) increase in leaf Car. ontent was shown when maize plants grown in water deficit conditions (75% FC). The chlorophyll degradation is the main indicator of water tress negative consequences on growth. The better chlorophyll under poor water supply showed better tolerance capability to maintain normal growth. In contrast to water stress, foliar spray treatment with GSH, Zn as well as in combination increased the contents of leaf (Chl. a, Chl. b, T. Chl., Caro. and Chl. a/b ratio) significantly. However, variations in r increase extent

were GSH level specific when applied separately or in combination with Zn. Comparatively more increase in (Chl. a/b, leaf Car., T. Chl., Chl. a, Chl. b) was found in plants grown from plants treated with GSH in combination with Zn, and the seed treated with 1.5 mM GSH shows maximum increased (Fig. 2A–F; Table 1). The chlorophyll pigment retention against water stress with foliar spray of GSH and Zn confirmed their role in imposed water stress tolerance improvements. The foliar spray of 1.5 mM GSH along with Zn under controlled conditions increased Chl. a by 39.56%, Chl. b 60%, T. Chl. 45.23%, Car. 111.76%, and Chl. a/b 42.32% of maize plants while 137.14%, 112.5%, 129.41%, 42.1% and 91.42% increase, respectively, was noticed under water stressed conditions (Fig. 2A–E; Table 1).

Significant ( $P \leq 0.001$ ) increase was recorded in the contents of TAC, TSP, TSS, RS and NRS by 322.1%, 56.21%, 134.31%, 115.93% and 130.76%, respectively, of maize plants under poor water availability (75% FC). Foliar spray with different levels of GSH separately and in combination with 0.5% Zn further significantly increased the contents of TAC, TSP, TSS, RS and NRS under water stressed conditions. The maximum enhancements in the levels of

TAC, TSP, TSS, RS and NRS by 13.96%, 60.03%, 35.37%, 45.43% and 33.30% were found due to foliar spray with 1.5 mM GSH in combination with 0.5% Zn under stressed conditions while 269.47%, 53.33%, 101.14%, 97.87% and 95.27%, respectively, increased under controlled conditions. (Fig. 2F–J; Table 1).

**Enzymatic antioxidants** Enzymatic antioxidant activities (SOD, POD, CAT, and APX) significant ( $P \leq 0.001$ ) increased by 49.03%, 214.57%, 60.02% and 105.37%, respectively, under water stress (75% FC). The enzymatic antioxidant system in respond to ROS species and upgraded itself to increase activities of enzymes. The better enzymatic activities were considered as the efficient antioxidant system to scavenges ROS. Foliar spray with various levels of GSH alone or co-application with 0.5% Zn found helpful to further increase the activities of all studied antioxidant enzymes. The further upgradation of enzymatic antioxidants clarified the role of GSH and Zn in water stress resistance with strong antioxidant system. The increased enzymatic antioxidant activities with foliar spray of GSH alone or in combination with 0.5% was also recorded under controlled conditions. The maximum 77.48%, 67.06%, 43.52% and 40.19% increase in SOD, POD, CAT, and APX activities, respectively, due to foliar spray was recorded in plants grown from foliar spray with 1.5 mM GSH + 0.5% Zn treatment under stressed conditions while 46.41%, 114.57%, 58.9% and 73.82% increase, respectively, was detected under controlled conditions (Fig. 3A–D; Table 1).

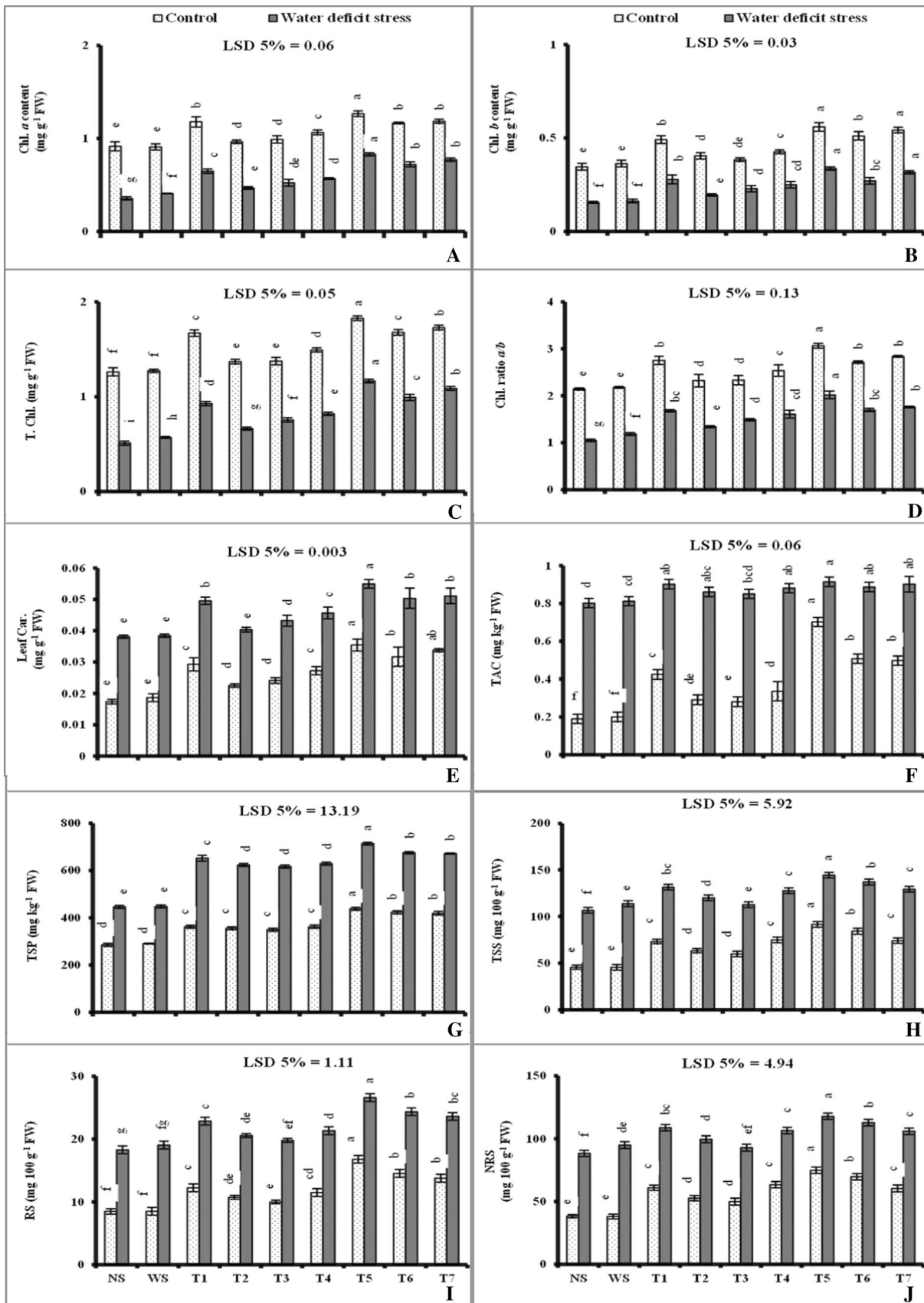
**Non-enzymatic antioxidants** Leaf TPC, TFC and AsA contents as presented in (Fig. 3E–G; Table 1) significant ( $P \leq 0.001$ ) increased by 57.31%, 180% and 79.51%, respectively, under water stress (75% FC). Foliar spray with different levels of GSH separately or in combination with 0.5% Zn further significantly increased the contents of TAC and TFC under water stress (75% FC). This increase in TPC, TFC and AsA of maize seedlings due to different GSH treatments was also recorded under controlled conditions. The maximum increase by 104.94%, 40% and 39.76% in TPC, TFC and AsA was detected in maize plants grown from foliar spray with 1.5 mM GSH in combination with 0.5% Zn under stressed conditions while 48.57%, 168.57% and 14.24% increase, respectively, was recorded under non-stressed situations as compared to other treatments. Significant ( $P \leq 0.001$ ) increase was recorded in the contents TFAA by 58.64% of maize plants under poor water availability (75% FC). The TFAA contents decreased maximally under both stressed and controlled conditions by exogenously applied of 1.5 mM GSH alone or combination with 0.5% Zn as compared with other treatments (Fig. 3H; Table 1).

**Oxidative stress markers** Data presented in (Fig. 3I, J; Table 1) showed 112.13% and 111.39% significant ( $P \leq 0.001$ ) increase in  $H_2O_2$  and malondialdehyde (MDA) contents, respectively, of maize under water deficiency (75% FC). The increased contents of lipid peroxidation were due to water stress administered superoxide radicals. Both these biomolecules' enhancements against water stress depicted their role as stress indicators. Foliar spray with different levels of GSH separately or in combination with 0.5% Zn decreased significantly,  $H_2O_2$ , and MDA under both water regimes. However, the extent of decrease in MDA and  $H_2O_2$  was the GSH dose specific when applied separately or in combination with 0.5% Zn under both water regimes. The decreased contents of both biomolecules manifested the strong antioxidant system that associated with efficient scavenging mechanism of ROS. The maximum decrease in MDA and  $H_2O_2$  by 36.53% and 89.44%, respectively, was found in maize grown from foliar spray with 1.5 mM level of GSH when applied combination with 0.5% Zn as compared to alone treatments while under controlled conditions, 1.5 mM GSH along with Zn reduced MDA and  $H_2O_2$  by 5.49% and 34.56%, respectively.

**Heat map** Histogram correlation analysis was carried out to depict relationships among morpho-physio-biochemical attributes of maize foliar-supplied with different levels of reduced glutathione applied separately or in combination with 0.5% Zn at both conditions (non-stressed and water stressed conditions) when seed were grown (Fig. 4). The significantly variations were detected in antioxidative defense mechanism, growth and photosynthetic studied parameters with different glutathione treatments in combination with Zn while heat map showing significant results with all other studied parameters under water stress when foliar applied with different levels of glutathione alone or in combination with Zn 0.5%. Though red color is showing strong negative correlation differences within the treatments, purple and blue colors depict a significant positive correlation differences in the histogram study. This histogram is showing a clear difference of water stress those with glutathione application in combination with Zn 0.5%.

## 4 Discussion

The water stress hinders the plant growth that led to yield loss in all crops (Kamali et al. 2022). The most vulnerable to water stress is considered the germination and growth of plants (Anjum et al. 2017). Hence, the water stress exposure at seedling stage reduced the overall biomass of plants (Yang et al. 2022) and especially extended the developmental period of maize (Ahmad et al. 2022a, b). However, such circumstances of abiotic stressed situations, GSH positively





**Fig. 2** Leaf Chl. a, Chl. b, T. Chl., Chl a/b, Caro., TAC, TSP, TSS, RS and NRS contents of maize foliary-supplied with different levels of reduced glutathione applied separately or in combination with 0.5% Zn when grown under non-stressed and water stressed conditions (Mean  $\pm$  SE;  $n=4$ ). Bars with same alphabets specified for water stressed and non-stressed conditions do not differ significantly. Chl. a=chlorophyll a; Chl. b=chlorophyll b; T. Chl.=total chlorophyll; Car.=carotenoids; TAC=total anthocyanin content; TSP=total soluble proteins; TSS=total soluble sugars; RS=reducing sugars; NRS=non-reducing sugars

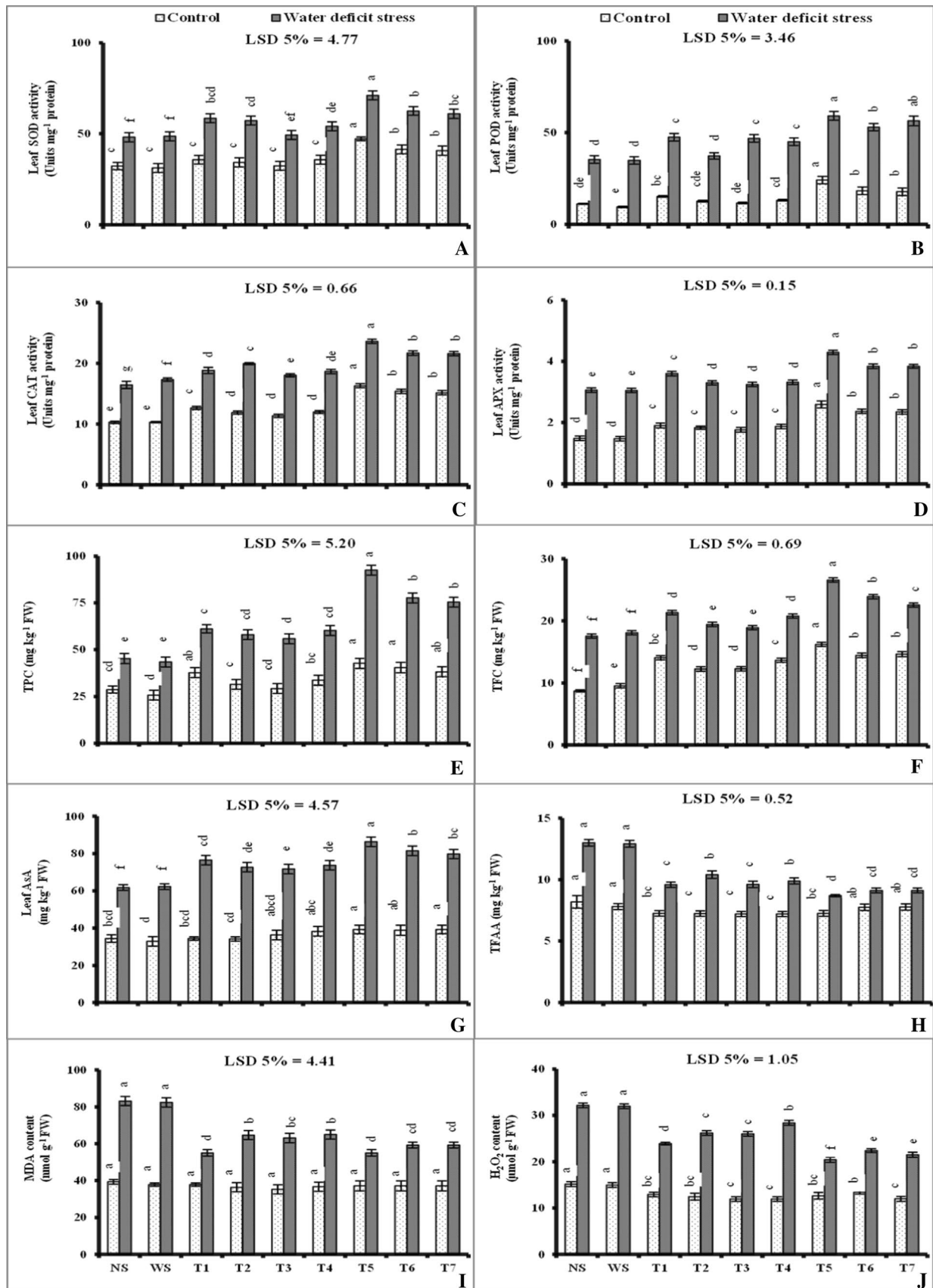
interferes in different physiological and chemical responses in plants and combat to enhance antioxidant machinery, biochemical processes, photosynthesis and plant's growth (Gul et al. 2022). The growth and biomass (SFW, RFW, SDW, RDW, RL, SL) of maize plants decreased under water stress were enhanced with exogenously applied GSH along with Zn because GSH involved in meristem zone cell division of roots and resulted in root elongation (Vernoux et al. 2000). Same trend of growth enhancements under abiotic condition with exogenic treatments of GSH has already investigated in soybeans (Cheng et al. 2015), mung bean (Nahar et al. 2015) and Arabidopsis (Akram et al. 2017). Similar way, Zn may also increase the crop growth potential under water stress and minimized oxidative damages stress (Jan et al. 2022). The chlorophyll contents showed a remarkable decrease under water deficiency (Fig. 2) and are the common indicators of abiotic stress (Singh and Gautam 2013) and retarded the overall growth of plants. Chlorophyll pigments also decreased under water stress due to accumulation of ROS that directly harm the chloroplast which are the most susceptible to oxidative stress (Yamauchi et al. 2018). Contradictory, current study showed increase in carotenoids under water stress (Fig. 2) indicated the role of it as antioxidant to protect chloroplast as result of oxidative injuries (Emiliani et al. 2021). The exogenous applied GSH with Zn increased the photosynthesis under water stress could be due to that GSH controls and protect the various activities of enzymes involving in photosynthesis (Schurmann and Jacquo 2000; Gul et al. 2022). The GSH also have significant role in avoiding the active sites from inhibitor binding sites that might stop the photosynthetic process (Pietrini et al. 2003).

When plants face unfavorable conditions, they cause over production of ROS that cause increased lipid peroxidation (MDA) by changing the cellular membrane lipids (Gill and Tuteja 2010). The ROS scavenging is the major function of GSH among the other functions. It maintains the antioxidants in their active form followed by scavenging the ROS (Hasanuzzaman et al. 2022) and inserted the oxidative stress tolerance in plants. The membrane damages and

H<sub>2</sub>O<sub>2</sub> contents were found minimum in current study with application of GSH that has also been reported by (Gul et al. 2022). Application of Zn also mitigates the H<sub>2</sub>O<sub>2</sub> contents and MDA and relieved oxidative injuries of plants (Jan et al. 2022).

Plants are adapted to upregulate the antioxidant enzymes by closing the stomata via guard cells and maintain the water status of plants (Lou et al. 2018; Nguyen et al. 2018). Similarly, the enzymes expression (SOD, APX, CAT and POD) is responsible to scavenge ROS under water stressed condition. Increased catalase (CAT) and POD activity is correlated with root elongation and enhancing growth of seedlings (Harman 2011). Similarly, SOD imparts the oxidative stress resistance in rice plants (Ali et al. 2017). Same way, non-enzymatic antioxidants such as phenolics and flavonoids scavenge the harmful radicals in response to stress (Sarwat et al. 2016). The present study manifested the deposition of these plant defense related substances (Fig. 3) that may be involved in free oxygen radical scavenging and avoiding cell membrane damages under abiotic stress (Javed and Gao 2023) while, under stress, Zn along with GSH exogenously enhanced the ROS scavenging abilities of various antioxidants (Pei et al. 2019). Exogenic application of Zn might be involved in mitigation of induced water stress negative effects caused by ROS on crop plants because it is the component of Cu/Zn-SOD that accelerates the SOD production (Mousavi et al. 2013; Farooq et al. 2021). Zinc also involves in reduction of lipid peroxidation with regulation of enzyme activities (Kausar et al. 2023) that ultimately enhances the antioxidant defense system under water stress (Lou et al. 2018).

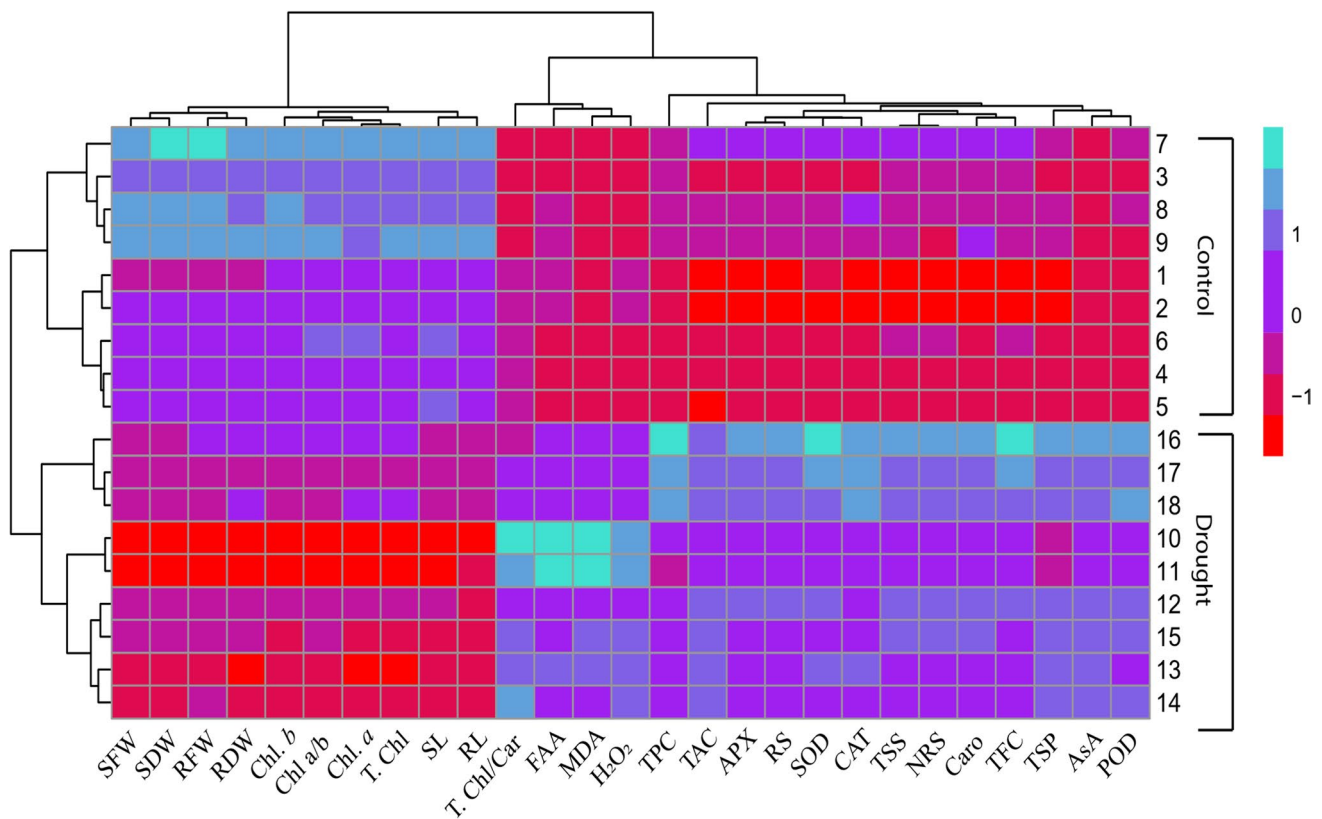
Anthocyanin deposition, as another defensive strategy adapted by plants to detoxify ROS under water stress situations (Huang et al. 2019). Greater anthocyanin contents are considered (Nguyen et al. 2021) as water stress tolerance indicator of plants. Similarly, soluble sugars and proteins are involved in maintaining the water balance (Kabiri et al. 2018) at cellular level under water shortage terms. The current plant also depicted the increased contents of these substances under water stressed condition. The application of GSH improved the contents of these secondary metabolites and non-enzymatic antioxidants under water stress that might be due to that GSH improves the activities of other antioxidants and is itself acted as non-enzymatic antioxidant (Hasanuzzaman et al. 2017). Coordinately, protein synthesis and carbohydrate metabolism are also regulated with involvement of Zn (Mousavi et al. 2013).



**Fig. 3** Activities of SOD, POD, CAT, APX and the contents of TPC, TFC, AsA, TFAA, MDA and H<sub>2</sub>O<sub>2</sub> of maize foliary-supplied with different levels of reduced glutathione applied separately or in combination with 0.5% Zn when grown under non-stressed and water stressed conditions (Mean ± SE; n=4). Bars with same alphabets specified for water stressed and non-stressed conditions do not differ significantly. SOD=superoxide dismutase; POD=peroxidase; CAT=catalase; APX=ascorbate peroxidase; TPC=total phenolic content; TFC=total flavonoid content; AsA=ascorbic acid; TFAA=total free amino acids; MDA=malondialdehyde; H<sub>2</sub>O<sub>2</sub>=hydrogen peroxide

In conclusion, it is resulted that glutathione applied improvement in growth, photosynthetic pigment and antioxidative defense mechanisms when applied alone or in combination with Zn is associated with its role in increasing the metabolic activities in terms of conversion of larger metabolites to simpler ones that reflects its role in hydrolyzing enzymes triggering. Under water shortage conditions,

the co-application of glutathione and Zn to maize plants resulted in higher development, and this was linked to better maintenance of the photosynthetic pigments on the leaves and the improvement in the antioxidative defense mechanism with an alternate decrease in lipid peroxidation. The better effects of glutathione were found in combination with Zn might be the dual impact of both of glutathione and Zn that shows their beneficial interaction in amelioration of the water stress. As literature depicts that studies are not available regarding the combine application of glutathione and Zn for boosting up the biomass of maize plant under poor water regimes and the findings of this study show that using them together rather than separately has more positive impacts. So, it can be recommended that the combinatorial application of Zn and glutathione as foliar spray is more helpful in combating the adverse effect, of water stress that will be helpful for better and uniform crop stands and will lead to better production under water deficit conditions.



**Fig. 4** Heatmap histogram correlation between different studied attributes of maize foliary-supplied with different levels of reduced glutathione applied separately or in combination with 0.5% Zn when grown under non-stressed [non soaking (1), water soaking (2), 1.5 mM GSH (3), 3 mM GSH (4), 4.5 mM GSH (5), 0.5% Zn (6), 1.5 mM GSH+0.5% Zn (7), 3 mM+0.5% Zn (8), 4.5 mM+0.5%Zn (9)] and water stressed conditions [non soaking (10), water soaking (11), 1.5 mM GSH (12), 3 mM GSH (13), 4.5 mM GSH (14), 0.5% Zn (15), 1.5 mM GSH+0.5% Zn (16), 3 mM+0.5% Zn (17), 4.5 mM+0.5%Zn (18)]. 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; TSS=total soluble sugar; TSP=total soluble protein; TFAA=total free amino acid; MDA=malondialdehyde; H<sub>2</sub>O<sub>2</sub>=hydrogen peroxide; AsA=ascorbic acid; TAC=total anthocyanin content; TFC=total flavonoid content; SOD=superoxide dismutase; POD=peroxide dismutase; CAT=catalase; APX=ascorbate peroxidase; RS=reducing sugar; NRS=non-reducing sugar; TPC=total phenolic content

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**Data Availability** The data that support the findings of the current study is available within the manuscript.

## Declarations

**Conflict of interest** Authors have no conflict of interest.

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