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RESEARCH ARTICLE

Modulation of growth and key physiobiochemical attributes after foliar application of zinc sulphate ($ZnSO_4$) on wheat (*Triticum aestivum* L.) under cadmium (Cd) stress

Shagufta Perveen[1] · Muhammad Saeed[1] · Abida Parveen[1] · Muhammad Tariq Javed[1] · Sara Zafar[1] · Naeem Iqbal[1]

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Abstract A pot experiment was conducted to examine the effect of foliar application of various levels of $ZnSO_4$ on wheat (*Triticum aestivum* L.) under cadmium (Cd) stress. Seeds of two wheat varieties i.e., Ujala-2016 and Anaj-2017 were sown in sand filled plastic pots. Cadmium ($CdCl_2$) stress i.e., 0 and 0.5 mM $CdCl_2$ was applied in full strength Hoagland's nutrient solution after 4 weeks of seed germination. Foliar spray of varying $ZnSO_4$ levels i.e., 0, 2, 4, 6 and 8 mM was applied after 2 weeks of $CdCl_2$ stress induction (of 6 week old plants). After 3 weeks of foliar treatment leaf samples of 9 week old wheat plants were collected for the determination of changes in various growth and physiobiochemical attributes. Results obtained showed that cadmium stress (0.5 mM $CdCl_2$) significantly decreased shoot and root fresh and dry weights, shoot and root lengths, yield attributes, chlorophyll *a* contents and total phenolics, while increased hydrogen peroxide (H_2O_2), total soluble proteins, free proline, glycinebetaine (GB) contents, and activities of antioxidant enzymes i.e., catalase (CAT), ascorbate peroxidase (APX) and peroxidase (POD). Foliar application of varying $ZnSO_4$ levels significantly increased various growth attributes, chlorophyll *b* contents, H_2O_2, free proline, GB and activities of antioxidant enzymes i.e., CAT, POD and APX, while decreased relative water contents and total phenolics under Cd stress or non stress conditions. Furthermore, both wheat varieties showed differential response under Cd stress and towards foliar application of $ZnSO_4$ e.g., wheat var. Ujala-2016 was higher in shoot dry weight, root length, root fresh and dry weights, total leaf area per plant, 100 grains weight, number of tillers per plant, chlorophyll *b*, hydrogen peroxide (H_2O_2), activities of APX, POD, glycinebetaine and leaf free proline contents, while var. Anaj-2017 exhibited high shoot fresh weight, grain yield per plant, no. of grains per plant, chlorophyll contents, chlorophyll *a/b* ratio, total phenolics, MDA and total soluble protein contents under cadmium stress or non stress conditions.

Keywords Antioxidant enzymes · Cadmium stress · Glycinebetaine · Soluble proteins · Wheat plants

Introduction

Increased industrialization results in high heavy metal pollution level in environment that caused serious threat to human health and damages to agriculture (Fasahat 2015; Ghosh and Roy 2019). Cadmium (Cd) is one of the toxic heavy metals in soil that exerts adverse effects on growth and yield of cereal crops (Hussain et al. 2015; Ling et al. 2017; Hirzel et al. 2017). Major food crops which are used for the human diet includes wheat (*Triticum turgidum* L.), corn (*Zea mays* L.), rice (*Oryza sativa* L.), peas (*Pisum sativum* L.) and barley (*Hordeum vulgare* L.). These crops are more sensitive to Cd and uptake of heavy metal occur more in these crops (Vatehova et al. 2016; Ling et al. 2017; Hirzel et al. 2017; Hussain et al. 2018; Lu et al. 2020; Rehman et al. 2020). Cadmium caused a serious problem to the human health such as kidney problems when Cd ingestion in very small quantity in water, food and accumulation of air in the human body for long time (Hirzel et al. 2017; Guo et al. 2018; Rai et al. 2019). Cereal crops such as wheat are extra touchy to Cd and having the greater concentration of Cd in grains are present while grown in

✉ Shagufta Perveen
perveens1@yahoo.com

[1] Department of Botany, Government College University, Faisalabad 38000, Pakistan

the Cd contaminated soil and decrease the yield of the crop (Hussain et al. 2015, 2018). Toxic effects of Cd can damage the physiology, morphology, biochemistry of plants (Xuebin et al. 2020; Li et al. 2020; Hatamian et al. 2020).

Distribution and uptake of micronutrients is negatively affected under Cd stress in different plant species (Akhtar et al. 2017). Cadmium stress also reduce the uptake of essential mineral nutrients in plants because the permeability of plasma membrane was disturbed that results in reduced growth and yield (Ashraf et al. 2016; Rabelo et al. 2018; Loi et al., 2018; Ghosh and Roy, 2019; Song et al. 2019; Hatamian et al., 2020; Yang et al. 2020). Uptake of Cd in crop plant can affect the root and shoot length, relative water contents, germination, chlorophyll contents and alter the activities of antioxidant enzymes such as superoxide dismutase, peroxidase, ascorbate peroxidase and catalase (Cakmak et al. 1993; Ulusu et al. 2017).

Fertilization can minimize the uptake of Cd through plant and this approach help the wheat plants to grow within the Cd infected soil (Gomez-Coronado et al. 2016; Abbas et al. 2017; Sobolewska et al. 2020). Micronutrients are very essential for the crop plants (Wang et al. 2016). Its optimum concentrations can increase the plant growth and yield of food crop (Wu et al. 2020). Availability of micronutrient in less concentration leads to reduced growth and yield and quality of the plants resulting in serious problems to human health and animals which feed on these plants (Sohail et al. 2020; Wu et al. 2020). Different techniques are used to enhance the concentrations of these nutrients in crop plants such as biofortification to improve the bioavailability of these nutrients and foliar application of Zn and Fe can reduced the deficiency of nutrients in plants (Haider et al. 2018).

Zinc (Zn) is a micronutrient and its optimum concentration is necessary for the growth of plants. Zn play a major role in the biomass production of crop plants (Haider et al. 2018). Zn can also enhance the protein production in plants and production of oil contents in plants (Hussain et al. 2018). Deficiency of Zn caused the nutritional disorder in plants (Jiao et al. 2020). Zinc deficiency can be reduced by the application of Zn fertilizer (Sarwar et al. 2014; Sazawal et al. 2018). Deficiency of Zn is major problem in plants. In soybean plants application of Zn may show the positive or negative effects while in wheat crop application of Zn fertilizer showed positive effect and increased the yield (Saifullah et al. 2016; Sobolewska et al. 2020). By the use of foliar application of Zn can enhanced the nutrients in the edible parts of the crop plants and this is the less expensive techniques to enhance the concentrations of these nutrients in plants (Saifullah et al. 2016).

Several exogenous substances can be used to mitigate the poisonous effects of Cd in crop plants. Zinc (Zn) is one of the crucial plant micronutrients and is used in several physiological functions in plants. Zn may additionally alleviate Cd toxicity in plants because of the chemical similarity of Zn with Cd. Published reports confirmed that Zn can alleviate poisonous effects of Cd in flowers by means of increasing plant growth, regulating Cd uptake, increasing photosynthesis and reducing oxidative strain.

Materials and methods

A pot experiment was conducted in the Botanical Garden of Government College University Faisalabad, to find the role of zinc sulphate ($ZnSO_4$) treatment as a foliar spray on wheat (*Triticum aestivum* L.) under the cadmium (Cd) stress. In this experiment different growth and physiobiochemical parameters were determined under two levels of cadmium (i.e., 0 and 0.5 mM $CdCl_2$) stress and five levels of $ZnSO_4$ (0, 2, 4, 6, and 8 mM) application. The seeds of two wheat varieties i.e., Ujala-2016 and Anaj-2017 were collected from Ayub Agricultural Research Institute (AARI) Faisalabad, Pakistan and sown in sand filled plastic pots. The design of experiment was completely randomized (CRD) with three replicates. Plants were germinated after 1 week and treated with full strength of Hoagland's nutrient solution. Hoagland's nutrient solution was given to the plants at regular intervals of weeks. Cadmium chloride stress (i.e., 0 and 0.5 mM $CdCl_2$) was applied after four of weeks of seed germination. After 6 weeks of seed germination wheat plant were foliarly supplied with different zinc sulphate levels i.e., 0, 2, 4, 6, and 8 mM $ZnSO_4$. After 9 weeks of seed germination, leaf sample were collected for the determination of various growth and physiobiochemical attributes. In each pot two plants were left for yield attributes.

Measuring growth and yield parameters

Two plants from each pot were uprooted, washed with distilled water and measured shoot and root fresh weights. Same plants were oven-dried at 65 °C and dry weights of shoot and root were measured with the electric balance. At maturity, yield attributes were determined.

Determination of chlorophyll contents

Arnon (1949) method was used for the determination of total chlorophyll contents. In this protocol, 0.5 g leaf tissue were ground in 10 ml of 80% acetone. After that samples were centrifuged at 10,000×*g* for 10 min. After that absorbance of supernatant were taken at various wavelengths such as 663, 645 and 480 nm using a spectrophotometer.

Total phenolic contents

The total phenolic contents were determined by the method of Julkenen-Titto (1985). Fresh leaf (0.5 g) was finely homogenized in 2 ml of 80% acetone. Then solutions were centrifuges at 1200 rpm for 10 min, after centrifugation the supernatant was separated and kept in test tube. To 100 µl leaf extract, 2.0 ml distilled water was added in a test tube. To this mixture, added 0.5 ml Folin-Ciocalteu reagent and 2 ml of Na_2CO_3 (20%) and maintained the volume up to 5 ml with distilled water. This mixture was vortexed for 5–10 s and absorbance of samples was taken at 750 nm using a spectrophotometer.

Total soluble proteins

Total contents of total soluble proteins were determined by the Bradford 1976) protocol. Fresh leaf tissue (0.5 g) were finely homogenized in 10 ml of phosphate buffer (50 mM). The samples were centrifuged at 12,000 rpm for 10 min at 4 °C. After that in the supernatant Bradford reagent was added and the absorbance was taken at 595 nm using a spectrophotometer.

Determination of glycinebetaine contents

Glycinebetaine contents were determined following the method of Grieve and Grattan (1983). Leaf samples (0.5 g) were finely homogenized in distilled water (10 ml). Then homogenate was filtered by the use of Whatman No.2 filter paper. To 1 ml of filtrate, 1 ml of $2NH_2SO_4$ was added. To 0.5 ml of above mixture, 0.2 ml KI_3 added and solution was kept in an ice bath for 90 min. In this cold mixture, distilled water (2.8 ml) and 1–2 dichloromethane (6 ml) were added. Two distinct layers were observed and absorbance of colored layer was read at the wavelength of 365 nm using a spectrophotometer.

Determination of free proline contents

Free proline contents were determined by the method developed by Bates et al. (1973). Fresh leaf sample (0.5 g) were ground in 10 ml of sulfosalicylic acid and filtrate was derived using Whatman No. 2 filter paper. To 2 ml of filtrate, 2 ml each of acid ninhydrin and glacial acetic acid were added and heated the samples at 100 °C in water bath for 30 min. After that, samples were cooled at normal temperature and in the reaction mixture added the toluene (4 ml). Two different layers were observed and organic layer was taken out and reading was taken at 520 nm using a UV–VIS spectrophotometer.

Determination of hydrogen peroxide (H_2O_2) contents

The method used for the determination of hydrogen peroxide contents was developed by the Velikova et al. (2000). Fresh leaf (0.5 g) was finely homogenized with 5 ml of 0.1% trichloroacetic acid (TCA) with pestle and mortar in an ice bath. Then the homogenate was centrifuged at 12,000 rpm, then the supernatant was separated. To 0.5 ml supernatant, 0.5 ml buffer of phosphate (pH 7.0) and 1 ml of potassium iodide were added. Then reading was taken at the wavelength of 390 nm using a spectrophotometer.

Determination of malondialdehyde (MDA) contents

The protocol used for the determination of malondialdehyde contents were developed by Carmak and Horst (1991). To 0.5 g finely ground leaf tissue 0.1% (w/v) trichloroacetic acid (10 ml) were added. Then solution was centrifuged for 10 min at 12,000×g. To 1 ml of supernatant added 4.5 ml (0.5%) thiobarbituric acid (prepared in 20% TCA was added. Then reaction mixture was kept in a water bath at 95 °C for 30 min. After that, samples were cooled in an ice bath and absorbance of the samples was read at two wavelengths 532 nm and 600 nm using a spectrophotometer.

Analysis of antioxidant enzymes activity

Fresh leaf tissue (0.5 g) were finely homogenized on the ice bath in 10 ml of 50 mM phosphate buffer (pH 7.8). Then homogenate was centrifuged at 12,000×g at 40 °C for 20 min. The supernatant was kept at − 20 °C in refrigerator for the determination of antioxidant enzymes activities.

Measuring the catalase (CAT) and peroxidase (POD) activities

Catalase (CAT) and peroxidase activities were determined by the protocol developed by Chance and Maehly (1955). For determination of CAT activity reaction mixture consists of 1 ml (5.9 mM) of hydrogen peroxide, 1.9 ml (50 mM, pH 7.0) of phosphate buffer and 0.1 ml of enzyme extract and reading were taken with in every 20 s for 2 min at 240 nm. For determination of POD activity reaction mixture consists of 1.9 µl (50 mM) of phosphate buffer, 100 µl (20 mM) of guaiacol, 100 µl (40 mM) of hydrogen peroxide and enzyme extract (100 µl) and reading was taken at 470 nm after every 20 s for 2 min. Enzyme activity was determined on protein basis by

considering one unit each of CAT and POD equivalents to 0.01 units per min change in absorbance.

Determination of ascorbate peroxidase (APX) activity

For the determination of ascorbate peroxidase (APX) activity method of Krivosheeva et al. (1996) was used. A 3 ml reaction mixture was prepared by adding 1.9 µl (50 mM) phosphate buffer, 0.5 mM hydrogen peroxide, 0.5 mM of ascorbic acid and 0.1 ml of enzyme extract and absorbance of samples were taken at 290 nm using a spectrophotometer.

Statistical analysis

A three way analysis of variance (ANOVA) of data for all attributes was used using a COSTAT computer program (version v6.303) and least significant difference (LSD) was used to compare mean values of all treatments (Snedecor and Cochran, 1980).

Results

Shoot fresh and dry weight significantly ($P \leq 0.001$) decreased in wheat (*Triticum aestivum* L.) varieties (var) (Anaj-2017 and Ujala-2016) under cadmium (Cd) stress (0.5 mM). While foliar application of $ZnSO_4$ (0, 2, 4, 6 and 8 mM) significantly ($P \leq 0.01$) enhanced shoots fresh weight. In wheat var. Anaj-2017 and Ujala-2016 control plants and plants treated with $ZnSO_4$ have more shoot fresh and dry weight as compared to the Cd stressed plants. Wheat var. Anaj-2017 was better tolerant to Cd stress as compared to Ujala-2016. In both wheat varieties 4 mM $ZnSO_4$ exerted more positive effect on shoot fresh weight than other levels (Table 1; Fig. 1a, b).

Cadmium stress (0.5 mM) significantly ($P \leq 0.001$) decreased root fresh and dry weight. Foliar treatment of varying $ZnSO_4$ (0, 2, 4, 6 and 8 mM $ZnSO_4$) levels significantly ($P \leq 0.001$) enhanced roots fresh and dry weight of both wheat varieties (var. Anaj-2017 and Ujala-2016). In var. Anaj-2017 8 mM $ZnSO_4$ exerted more positive effect on root fresh weight than other levels, while in var. Ujala-2016 6 mM $ZnSO_4$ showed more positive effective. Overall, in var. Ujala-2016 root fresh and dry weight increased than var. Anaj-2017. Wheat var. Ujala-2016 was higher in root fresh and dry weight than that of var. Anaj-2017 under Cd stress (Table 1; Fig. 1c, d).

Shoot and root lengths were significantly ($P \leq 0.001$) decreased as a result of Cd stress (0.5 mM). Foliar treatment of varying $ZnSO_4$ (0, 2, 4, 6 and 8 mM $ZnSO_4$) levels significantly ($P \leq 0.001$) enhanced shoots and root length of both wheat varieties i.e., var. Anaj-2017 and var. Ujala-2016. In var. Anaj-2017 2 mM $ZnSO_4$ exerted more positive effect on shoot length than other levels, while in var. Ujala-2016 variety 2 mM $ZnSO_4$ level was effective. Foliar application of 2 mM $ZnSO_4$ increased the shoot length in var. Anaj-2017 under normal conditions, while in var. Ujala-2016 the same level 2 mM $ZnSO_4$ increased shoot length under Cd stress. Overall, var. Ujala-2016 showed higher shoot and root lengths than that of var. Anaj-2017 (Table 1; Fig. 1e, f).

Yield parameters i.e., grains yield per plant, number of tillers and 100 grain weight significantly ($P \leq 0.001$) decreased as a result of Cd (0.5 mM) stress. Foliar application of varying $ZnSO_4$ (0, 2, 4, 6 and 8 mM $ZnSO_4$) levels significantly ($P \leq 0.001$) enhanced yield parameters of both wheat varieties (i.e., Anaj-2017 and Ujala-2016). In var. Anaj-2017 4 mM $ZnSO_4$ exerted more positive effect on grains yield per plant than other levels, while in var. Ujala-2016 2 mM $ZnSO_4$ level was proved more effective. Wheat var. Anaj-2017 was better tolerant to Cd stress as compared to var. Ujala-2016 (Table 1; Figs. 1g, h, 2a).

Relative water contents (RWC) significantly ($P \leq 0.001$) decreased under Cd stress (0.5 mM). Foliar treatment of varying $ZnSO_4$ (0, 2, 4, 6 and 8 mM)) levels significantly ($P \leq 0.001$) increased the RWC of both wheat varieties (Anaj-2017 and Ujala-2016). In wheat var. Ujala-2016 foliar application of 6 mM $ZnSO_4$ level showed more effect. Overall, RWC increased in var. Anaj-2017 as compared to var. Ujala-2016 (Table 1; Fig. 2b).

Chlorophyll *a* and *b* contents significantly ($P \leq 0.001$) decreased as a result of Cd stress (0.5 mM). While foliar application of $ZnSO_4$ (0, 2, 4, 6 and 8 mM) significantly ($P \leq 0.001$) increased chlorophyll *a* and *b* contents of both wheat varieties (Anaj-2017 and Ujala-2016). In Anaj-2017 foliar application of 4 mM $ZnSO_4$ exerted positive effect on chlorophyll *a* than other levels, while in Ujala-2016 foliar application of 6 mM $ZnSO_4$ level showed more effect. Overall, in wheat var. Anaj-2017 chlorophyll *a* and *b* increased than var. Ujala-2016. Wheat var. Anaj-2017 showed higher growth and chlorophyll contents than var. Ujala-2016 under Cd stress or non stress conditions (Table 1; Fig. 2c, d).

Malondialdehyde (MDA) and hydrogen peroxide (H_2O_2) contents significantly ($P \leq 0.001$) increased due to Cd (0.5 mM) stress. Foliar treatment of $ZnSO_4$ (0, 2, 4, 6 and 8 mM) levels significantly ($P \leq 0.001$) decreased the MDA and H_2O_2 in both wheat varieties (i.e., var. Anaj-2017 and var. Ujala-2016). Foliar application of 2 mM $ZnSO_4$ exerted positive effect on MDA than other levels in var. Anaj-2017, while in Ujala-2016 foliar application of 4 mM $ZnSO_4$ level showed more effect. Overall, var. Anaj-2017 showed higher growth than Ujala-2016 under Cd stress (Table 1; Fig. 2e, f).

Table 1 Analysis of variance of the parameters of growth, relative water content (%), chlorophyll, hydrogen peroxide, malondialdehyde, total soluble proteins, total phenolics, activities of antioxidant enzymes, free proline and glycinebetaine contents of wheat (*Triticum aestivum* L.) plants foliarly sprayed with varying levels of $ZnSO_4$ under Cd stress and non-stress conditions

Source of variation	Varieties (Var)	Cadmim (Cd)	Zinc sulphate (Zns)	Var × Cd	Var × Zns	Cd × Zns	Var × Cd × Zns	Error
Shoot f. wt.	0.117 ns	7.986***	2.175**	0.019 ns	0.460 ns	0.726 ns	0.804 ns	0.114
Shoot dry wt.	0.047 ns	0.788***	0.0166 ns	0.0232 ns	0.010 ns	0.022 ns	0.009 ns	0.012
Root f. wt.	0.115***	0.159***	0.007***	2.016 ns	0.001 ns	0.006***	0.001 ns	8.9
Root dry wt.	0.004***	0.022***	0.001***	0.002***	4.541 ns	0.001 ns	3.525 ns	2.283
Shoot length	191.53***	102.96**	24.326***	2.016 ns	23.08***	13.090***	2.878 ns	1.772
Root length	2.0535 ns	13.31***	7.847*	3.220 ns	4.249 ns	3.550 ns	1.605 ns	2.517
RWC (%)	430.63 ns	11.32 ns	1404.65*	111.97 ns	109.10 ns	760.19 ns	113.10 ns	530.85
100 grains weight (g)	1.069*	2.997***	0.436 ns	0.042 ns	0.490*	0.089 ns	0.327 ns	0.175
No. of tillers plant^{-1}	1.066 ns	19.26***	6.025***	0.066 ns	1.608*	0.558 ns	0.441 ns	0.516
Grain yield	27.439**	23.96***	18.359**	3.370 ns	33.93***	5.613 ns	4.921 ns	3.477
Chl. *a*	0.014 ns	0.427***	0.006 ns	0.003 ns	0.067*	0.022 ns	0.055*	0.019
Chl. *b*	0.001 ns	0.027 ns	0.026*	0.003 ns	0.024*	0.002 ns	0.009 ns	0.007
H_2O_2	13.659***	28.25***	6.376***	0.907 ns	0.522 ns	0.616 ns	0.678 ns	0.598
MDA	3.654*	8.831***	0.544 ns	1.237 ns	0.741 ns	0.114 ns	0.246 ns	0.560
Total soluble proteins	275.69**	54.57***	2268.93 ns	9086.31 ns	412.11 ns	870.97 ns	4416.20 ns	281.06
Total phenolics	35.761 ns	46.86***	102.88***	171.87 ns	445.59 ns	211.81 ns	187.39 ns	116.09
CAT	3.86**	4.35**	2.22**	1.90 ns	2.33**	0.64 ns	0.60 ns	0.51
POD	75.49***	53.29***	20.80***	35.99***	20.89***	26.39***	30.53***	0.56
APX	2.40***	1.56***	0.13**	0.004 ns	0.21***	0.12*	0.14**	0.03
GB	65.086***	159.325***	13.548***	7.029 ns	30.620***	22.321***	4.300 ns	1.975
Free proline	15.07*	22.15***	14.343*	31.98 ns	13.58 ns	7.912 ns	18.301 ns	31.37
Df	1	1	4	1	4	4	4	40

df = degrees of freedom; ns = non-significant; RWC (%) = relative water content; chl. *a* = chlorophyll *a*; chl. *b* = chlorophyll *b*; H_2O_2 = hydrogen peroxide; MDA = malondialdehyde; CAT = catalase; POD = peroxidase; APX = ascorbate peroxidase; GB = glycinebetaine

*, **, ***Significant at 0.05, 0.01, and 0.001 levels, respectively

In this study, total soluble proteins increased, while total phenolic contents were decreased under Cd (0.5 mM) stress. Foliar application of $ZnSO_4$ significantly ($P \leq 0.001$) decreased the total soluble proteins and total phenolic in both wheat varieties (Anaj-2017 and Ujala-2016). In var. Anaj-2017 foliar application of 6 mM $ZnSO_4$ showed more positive effect on total soluble proteins and total phenolic as compared to other levels, while in Ujala-2016 foliar application of 4 mM $ZnSO_4$ level showed more effect. Wheat var. Anaj-2017 showed higher growth than Ujala-2016 under Cd stress (Table 1; Fig. 2g, h).

Proline and glycinebetaine (GB) contents were significantly ($P \leq 0.001$) increased as a result of Cd stress (0.5 mM). Foliar application of varying $ZnSO_4$ levels significantly ($P \leq 0.001$) decreased the proline and (GB) contents of both wheat varieties (Anaj-2017 and Ujala-2016). In wheat var. Ujala-2016 foliar application of 4 mM $ZnSO_4$ showed more positive effect on proline contents than other levels. Overall, proline and GB contents were higher in var. Ujala-2016 than var. Anaj-2017. In var. Anaj-2017 4 mM $ZnSO_4$ increased proline contents under Cd stress or non stress conditions (Table 1; Fig. 3a, b).

Cadmium stress (0.5 mM) significantly ($P \leq 0.001$) increased ascorbate peroxidase (APX), peroxidase (POD) and catalase (CAT) activity. Foliar application of $ZnSO_4$ significantly ($P \leq 0.001$) decreased the APX and CAT in both wheat verities (Anaj-2017 and Ujala-2016). Foliar application of 4 mM $ZnSO_4$ exerted more positive effect on APX and CAT in Ujala-2016 than other levels. Foliar application of 6 mM $ZnSO_4$ exerted positive effect on catalase (CAT) activity in wheat var. Ujala-2016. Var. Ujala-2016 showed higher APX contents than var. Anaj-2017 under Cd stress or non stress conditions (Table 1; Fig. 3c–e).

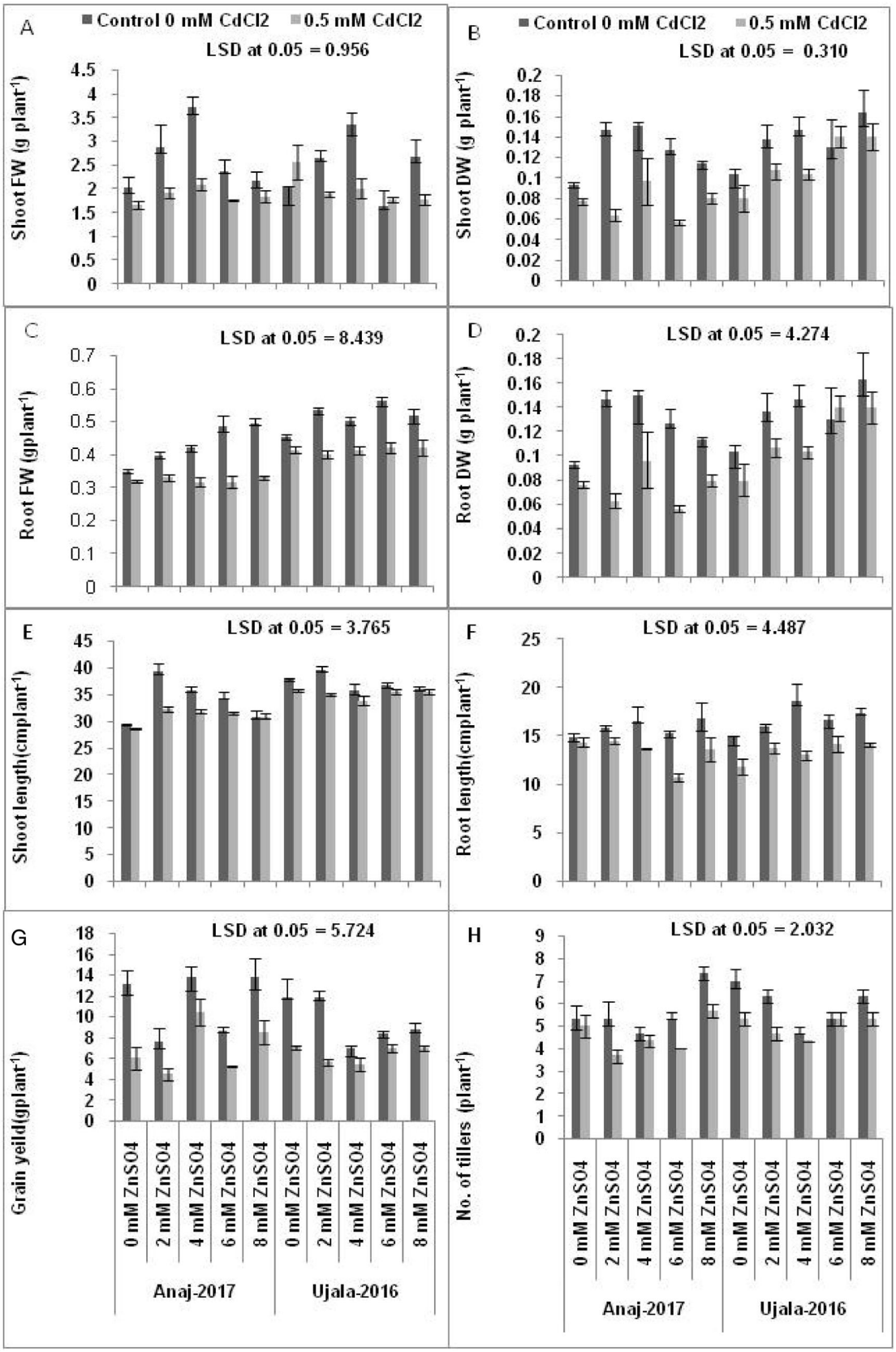

Fig. 1 **a–h** Shoot and root fresh and dry weights, shoot and root lengths, and relative water content of wheat (*Triticum aestivum* L.) plants foliarly-sprayed with $ZnSO_4$ under cadmium-stress and non-stress conditions

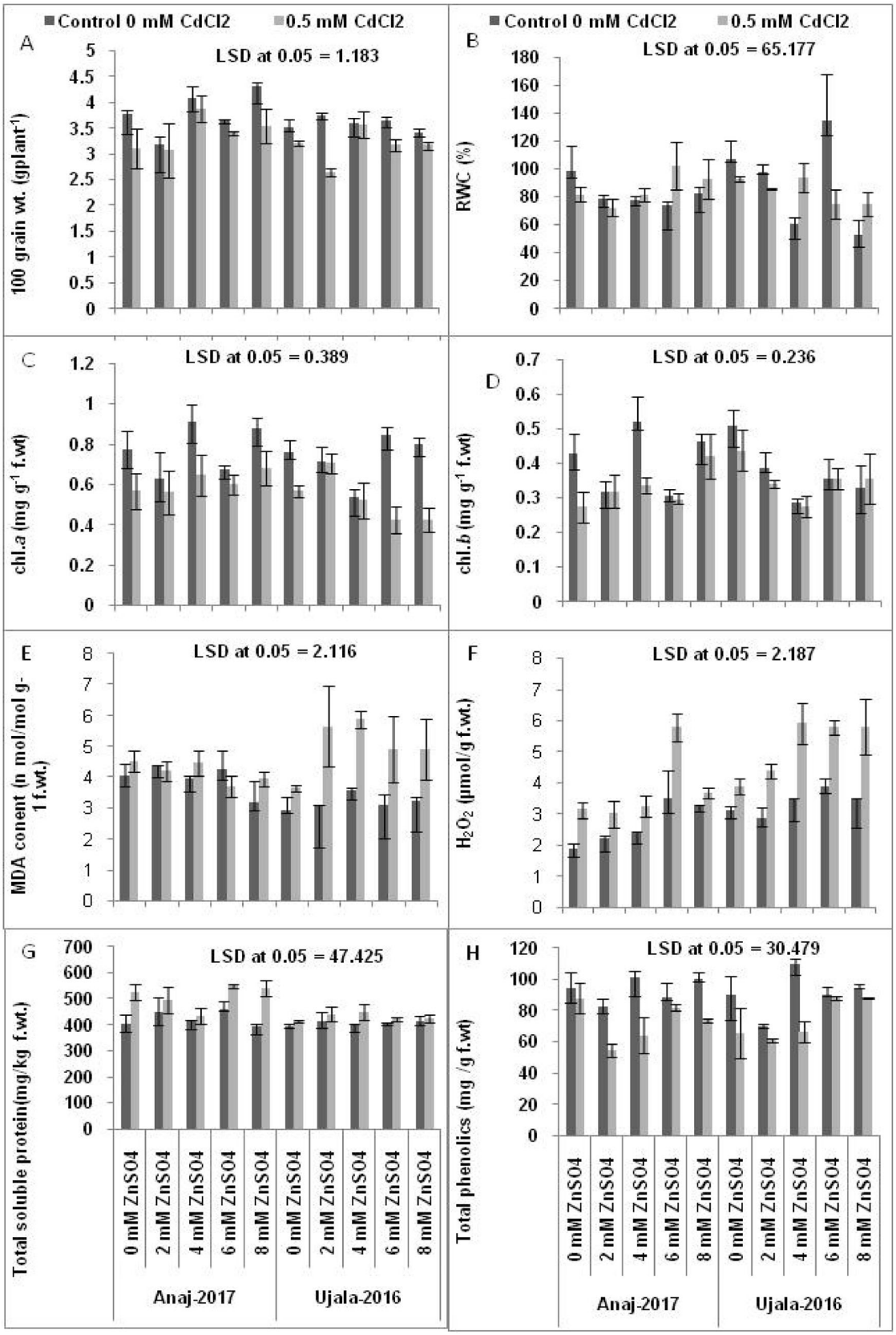

Fig. 2 100 grains weight, relative water contents, chlorophyll *a*, chlorophyll *b*, malondialdehyde, hydrogen peroxide, total soluble protein and total phenolics content in wheat (*Triticum aestivum* L.) plants foliarly-sprayed with $ZnSO_4$ under Cd-stress and non-stress conditions

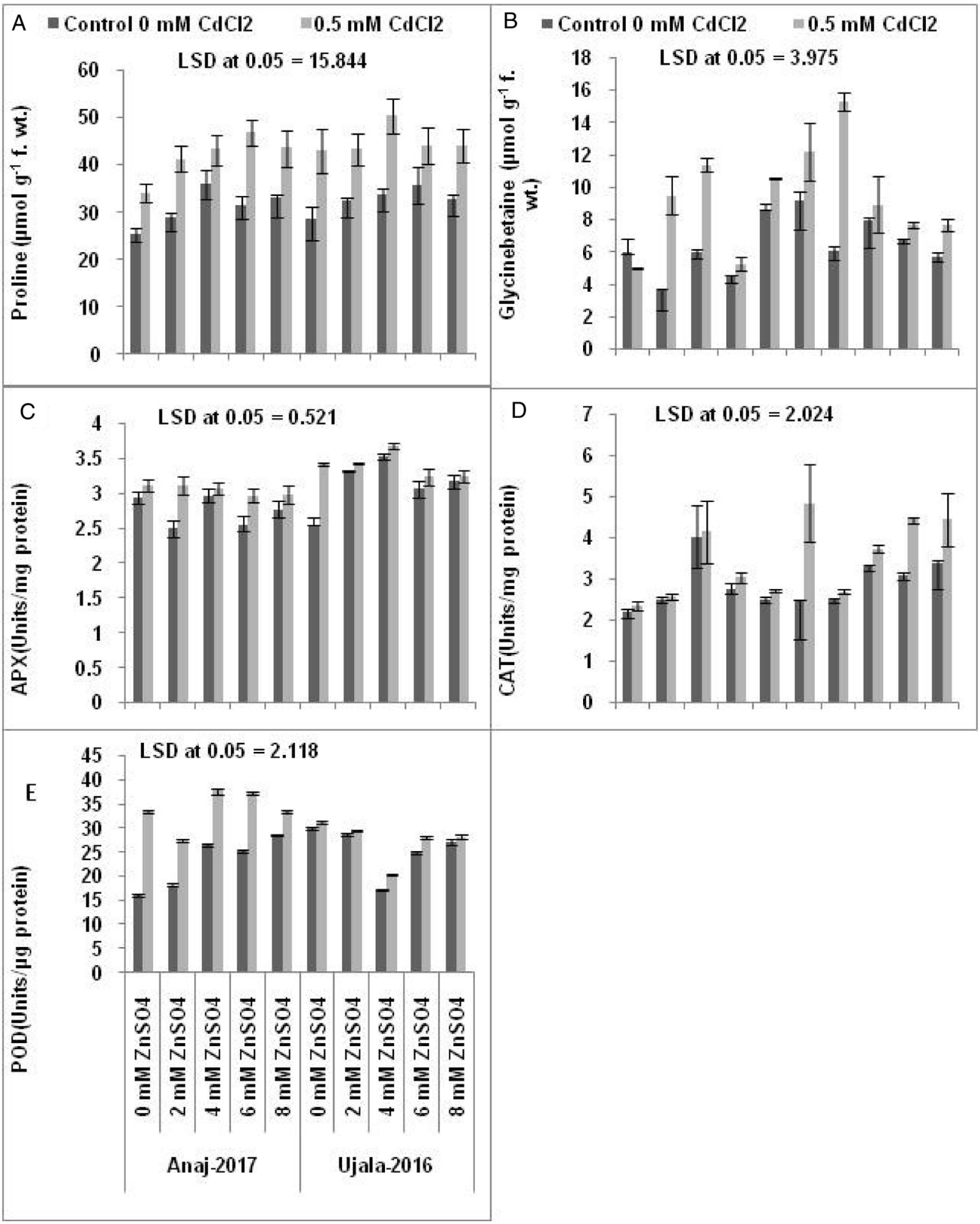

Fig. 3 Free proline, glycinebetaine contents, activities of ascorbate peroxidase, catalase and peroxidase of wheat (*Triticum aestivum* L.) plants foliarly-sprayed with cysteine under drought-stress and non-stress conditions

Discussion

Cadmium (Cd) is a toxic metal that exerts hazardous effects on plants growth, yield and development of food crops (Rehman et al. 2017, 2018, 2020; Rai et al. 2019; Yang et al. 2020; Xuebin et al. 2020). The reduction in root growth is the first toxic effect of Cd due to its direct contact with roots in the growth medium (Al-Qurainy et al. 2017; Li et al. 2020). Cadmium accumulation in plants can inhibit the synthesis and activity of enzymes, reduce the growth and development of plants and reduced the mineral nutrition (Ashraf et al. 2016; Xuebin et al. 2020). Cd can affect the production of grain, yield of the plant and also affect the morphology of the plants (Hirzel et al. 2017). Cadmium can reduced the growth of plant, change the relation of water, photosynthesis of plants, change the metabolism ions and can also effects the activities of enzymes and free radicles (Liu et al. 2016; Xuebin et al. 2020; Yang et al. 2020). However, foliar application of zinc has been reported to increase plant growth and significantly reduced the cadmium concentration in wheat plants (Wu et al. 2020).

Zinc sulphate ($ZnSO_4$) can decrease the negative effect of Cd and decrease the grains that were contaminated with the cadmium (Saifullah et al. 2016). Foliar application of Zn increased the increased the seed yield and grain biofortification in mungbean (Haider et al. 2018) and wheat (Abbas et al. 2017). In this study it was determined that cadmium stress of 0.5 mM adversely effects the growth and yield attributes of wheat (shoot fresh and dry weight, root fresh and dry weight, shoot and root lengths, 100 grains weight and grain yield per plant. However, foliar application of $ZnSO_4$ at varying level (0, 2, 4, 6 and 8 mM $ZnSO_4$) significantly enhanced the growth attributes of wheat under cadmium (cd) stress or non stress conditions. It has been reported by Al-Qurainy et al. (2017) that root and shoot fresh weight reduced under cadmium stress i*n Phoenix dactylifera* L.

Cadmium stress increase the oxidative stress and damaged nucleus in *Brassica juncea* L. plants (Kapoor and Bhardwaj 2020). Chlorophyll *a* and chlorophyll *b* contents reduced under cadmium stress in corn plants (Ling et al. 2017). Imposition of cadmium can reduce growth due to impairment of chloroplast, high level of H_2O_2 and MDA production in different plant species (Guo et al. 2016; Li et al. 2020). In soybean, plant growth and pigment concentration decreased, while hydrogen peroxide and lipid peroxidation increased under cadmium stress (Hashem 2014). In this study, cadmium stress (0.5 mM) enhanced H_2O_2 and MDA contents in wheat plants.

In this study, non enzymatic antioxidant such as total phenolic contents decreased under both cadmium stress and by foliar application of varying levels of $ZnSO_4$. It has been reported that under cadmium stress total phenolics increased in *Gynura procumbens* (Ibrahim et al. 2017). It has been reported that free proline and glycine contents enhanced under cadmium stress condition (Ling et al. 2017). In the current study, proline and glycinebetaine contents increased and improved by the foliar application $ZnSO_4$ under Cd stress condition (0.5 mM).

Cadmium stress badly affected growth, physiological parameters, pigment contents like chlorophyll and increased the antioxidants activity and osmoprotectants in wheat (Xuebin et al. 2020). In this study, activities of antioxidant enzymes (e.g., catalase, ascorbate peroxidase and peroxidase) enhanced under both cadmium stress (0.5 mM) and by the application of varying $ZnSO_4$ levels in both wheat varieties i.e., Ujala-2016 and Anaj-2017.

Conclusion

In conclusion, cadmium (0.5 mM) stress significantly decreased shoot and root fresh and dry weights, shoot and root lengths, yield attributes, chlorophyll *a* contents and total phenolics, while increased hydrogen peroxide (H_2O_2), total soluble proteins, free proline, glycinebetaine (GB) contents, and activities of antioxidant enzymes i.e., catalase (CAT), ascorbate peroxidase (APX) and peroxidase (POD). Foliar application of varying $ZnSO_4$ levels significantly increased various growth attributes, chlorophyll *b* contents, H_2O_2, free proline, GB and activities of antioxidant enzymes i.e., CAT, POD and APX, while decreased relative water contents and total phenolics under Cd stress or non stress conditions. Both wheat varieties showed differential response under Cd stress and towards foliar application of $ZnSO_4$ e.g., wheat var. Ujala-2016 was higher in shoot dry weight, root length, root fresh and dry weights, total leaf area per plant, 100 grains weight, number of tillers per plant, chlorophyll *b*, hydrogen peroxide (H_2O_2), activities of APX, POD, glycinebetaine and leaf free proline contents, while var. Anaj-2017 exhibited high shoot fresh weight, grain yield per plant, no. of grains per plant, chlorophyll contents, chlorophyll *a/b* ratio, total phenolics, MDA and total soluble protein contents under cadmium stress or non stress conditions.

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