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Original article

Foliar application of zinc improves morpho-physiological and antioxidant defense mechanisms, and agronomic grain biofortification of wheat (*Triticum aestivum* L.) under water stress

Abdul Sattar [a,*], Xiukang Wang [b,*], Sami Ul-Allah [a], Ahmad Sher [a], Muhammad Ijaz [a], Muhammad Irfan [c], Tahira Abbas [a], Sajjad Hussain [d], Farukh Nawaz [a], Abdulrahman Al-Hashimi [e], Bandar M. Al Munqedhi [e], Milan Skalicky [f]

[a] *College of Agriculture, Bahauddin Zakariya University, Multan Bahadur Sub Campus Layyah, Punjab 31200, Pakistan*
[b] *College of Life Sciences, Yan'an University, Yan'an 716000, China*
[c] *Department of Agronomy, Faculty of Agricultural Sciences, Bahauddin Zakariya University, Multan, Pakistan*
[d] *Institute of Environment and Sustainable Development in Agriculture, Chinese Academy of Agricultural Sciences, Beijing 100081, China*
[e] *Department of Botany and Microbiology, College of Science, King Saud University, P.O. 2455, Riyadh 11451, Saudi Arabia*
[f] *Department of Botany and Plant Physiology, Faculty of Agrobiology, Food and Natural Resources, Czech University of Life Sciences, Prague, Czechia*

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ABSTRACT

Agronomic biofortification with zinc (Zn) may be engaged to improve the nutritious value of food crops along-with tolerance to water deficit conditions. The Zn may increase plant resistance to water stress by boosting physiological and enzymatic antioxidants defense mechanisms. Major objective of this study was to investigate the effect of foliar applied Zn on grain zin biofortification and drought tolerance in wheat. Treatments include application of Zinc at terminal growth phases (BBCH growth stage 49 and BBCH growth stage 65) with five levels: 0 (control-ck), water spray, 5, 10 and 15 mM under two levels of water regimes; well-watered (where 80% water holding capacity (WHC) was maintained in the soil) and water stress, (where 40% WHC was maintained in the soil). Results revealed that water stress significantly reduced relative water contents, gas exchange attributes, plant height, yield and yield related attributes of wheat. In contrast, hydrogen peroxide, free proline levels, activities of malondialdehyde, and concentration of soluble protein were markedly increased under water stress condition. Application of various levels of Zn significantly improved the CAT, SOD, POD and ASP activities at 40% WHC compared with control treatment. Foliarly applied 10 and 15 mM Zn predominantly reduced the damaging impact of water stress by improving the plant status in the form of plant height, RWC and gas exchange attributes. Likewise, wheat plant treated with 10 mM Zn under water stress condition increased the grain yield by improving number of grains per spike, 100 grain weight and biological yield compared with control. Moreover, increasing Zn levels also increased Zn concentration in grains and leaves. Overall, this study suggests that optimum level of Zn (10 mM) might be promising for alleviating the adverse impacts of water stress and enhance the grain biofortification in wheat.

1. Introduction

Water shortage is major challenge for sustaining global food security. Increasing population of world has increased the food demand which leads to a need of eight time more irrigated area as compared to previous century (Winter et al., 2017). Competition between water resources of different areas is increasing due to unexpected atmospheric changes therefore, there are chances of increasing the limitations and dangers for food security (Abdelkhalik et al., 2019). Deficiency of water causes the detrimen-

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* Corresponding authors at: College of Agriculture, Bahauddin Zakariya University, Multan, Bahadur Sub Campus Layyah, Punjab 31200, Pakistan (A. Sattar). College of Life Sciences, Yan'an University, Yan'an 716000, China (X. Wang).

E-mail addresses: abdulsattar04@gmail.com (A. Sattar), wangxiukang@yau.edu.cn (X. Wang).

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tal effects at all growth stages of wheat, most prominent affect was observed at reproductive stage particularly at grain filling stage that leads to less and reduced grain size in wheat (Yu et al.,2018). Water stress reduced the assimilate partitioning and also inhibited the activities of important enzymes which involved for the preparation of synthetic processes of sucrose and starch and leads to reduce grain filling (Shokat et al. 2020; Kapoor et al. 2020). Deficiency of water disrupted the nutrients relations in plant by reducing nutrients availability, uptake, transport and accumulation (Maghsoudi et al. 2019). Water stress induced the oxidative damage due to overproduction of (ROS) (Hasanuzzaman et al. 2020) like H_2O_2, OH^-, superoxide, and O^{2-} that can damage biological membranes through biochemical reactions (Mehlam et al. 2017; Hasanuzzaman et al. 2019). Plants have evolved physiological (like production of osmolytes and soluble sugars) and antioxidant defensive mechanisms (like ascorbate peroxidise (APX), superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD) to combat the toxicity of ROS (Sarker and Oba Catalase 2018).

Zinc (Zn) is an imperative nutrient serving as a physical, basic, or regulatory cofactor for a variety of enzymatic functions (Marschner 1995) and regulates the growth and development. Zn supplementation reduced ROS production and protected cells from ROS damage. Zn deficiency can lead to high levels of ROS production and cell damage (Cakmak 2000). Under water stress condition, Zn deficiency became more prominent in wheat plant in Zn deficient soil (Bagci et al. 2007). Foliarly applied Zn regulates the nutrients balance and stomatal opening in maize to diminish the adversities of water deficit (Tabatabai et al. 2015). Adequate Zn fertilization imperatively improved the activities of POD, SOD, and CAT in response to water deficit conditions (Yavas and Unay 2016; Hassan et al. 2020a, 2020b). In another study, it was documented that optimum dose of Zn maintained the water status, stomatal regulation and adjustment of guard cells in chickpea under water stress (Khan et al. 2004). Furthermore, foliarly applied Zn improved the crop growth which ultimately increased the yield because it has ability to regulate stomatal openings, improving photosynthetic efficiency, increases in the chlorophyll formation and also increase in the leaf area of plant (Ma et al. 2017; Sultana et al. 2016; Karim et al. 2012).

The Zn deficiency is a severe threat around the world and about one third population of developing countries is facing Zn deficiency symptoms (Welch et al., 2013). Peoples of Pakistan, China, India and Turkey are facing Zn deficiency problems (Cakmak, 2008; Lyons, 2018; Zia et al., 2015; Phattarakul et al., 2012). The 43% population of these countries depends upon wheat nutrition; therefore, wheat bio-fortification with Zn would be beneficial for the human nutrition in these countries (FAOSTAT, 2016). Several agricultural practices like agronomic biofortification are considered as cost beneficial and sustainable approaches to increase the micronutrients especially Zn in the wheat grains to overcome Zn shortage symptoms in the humans (Bouis and Saltzman, 2017; Zou et al., 2012). Biofortification of Zn through these agricultural strategies increases grain Zn contents and diminishes the Zn deficiency symptoms in humans (Welch et al., 2013; Cakmak and Kutman, 2018). Several scientists have reported that foliarly applied Zn is an efficient agronomic strategy to achieve required Zn contents in wheat grains for human nutrition (Cakmak et al., 2017; Zou et al., 2012; Ram et al., 2016; Hussain et al., 2012).

Furthermore, various scientists have reported on efficiency of Zn in alleviation of negative impacts of water stress (Ma et al. 2017; Shemi et al. 2021). But there is a lack of knowledge on the effect of Zn to regulate the physiological and biochemical mechanism and Zn biofortification under water stress Therefore, major aim of this study was to investigate the potential of foliar applied Zn to regulate morpho-physiological, antioxidants defense mechanism and grain biofortification of wheat under terminal water stress for sustainable wheat yield.

2. Materials and methods

2.1. Experimental treatments and design

Experimental treatments comprised of two levels of irrigation regimes i.e., well-watered (where 80% water holding capacity (WHC) was maintained in the soil) and water stress, (where 40% WHC was maintained in the soil) from BBCH growth stage 49 to BBCH growth stage 65) and five levels of foliarly applied Zn i.e., 0 (control-ck), water spray, 5, 10 and 15 mM. These treatments were arranged according in a triplicated complete randomized design (CRD) with factorial arrangement. The Zn was applied as zinc sulphate ($ZnSO_4{\cdot}7H_2O$) and its application was done 7 days after the imposition of water stress. Water stress and well-watered conditions (40% WHC and 80% WHC) were maintained through tap water application in all the pots. Two levels of soil water holding capacity (WHC) were maintained through gravimetric basis (Reynolds, 1970) at reproductive stage of wheat. It was accomplished by measuring the soil water contents, which was assessed by weighing the targeted pot after each irrigation applied.

2.2. Experimental procedure

Seed of commercial wheat cultivar Bhakkar-2002 was collected from Ayyub Agricultural Research Institute, Faisalabad, Pakistan. The NPK fertilizers were applied 100, 90 and 60 mg kg^{-1} at the time of pot filling. Nitrogen, phosphorous and potassium were applied in the form of urea, diammonium phosphate and potassium sulphate respectively. Ten uniform size seeds were manually sown in each pot on the 15th of November 2019 at uniform depth of 3 cm. After one week of emergence five plants per pot were maintained for the subsequent studies.

2.3. Relative water contents and gas exchange attributes

The RWC contents were measured by the procedure reveled by (Schonfeld et al., 1988). Leaves were separated from the base of attachment and covered with polythene bag immediately and were brought to laboratory. Fresh weight of theses leaves was measured by using digital weighing balance. After those leaves were washed in distilled H_2O for 24 h at 25 °C temperature. Then measured the turgid weight of these leaves after the 18 h soaking. then the leaves were dried at 70 °C until constant weight. Following formula was used for calculation of RWC:

$$RWC = \frac{Freshweight - Dryweight}{Turgidweight - Dryweight} \times 100$$

Gas exchange traits were measured by using IRGA CI-340. Leaves of wheat plants were separated from each experimental treatment for the measurement of gas exchange attributes. Calibration of IRGA was done on daily basis throughout the whole measuring duration. Depending on the local weather conditions measurements were recorded from (08:30 am–10:30 am).

2.4. Enzymatic antioxidant activities

Homogenization of 0.5 g of wheat leaves was done in 500 Âµl of 0.15 M Tris-hydrochloric acid buffer (pH 7.5), containing 50 mg PVP (polyvinylpyrrolidone) on ice. Two times centrifugation was done at 4 °C temperature with 14000 rounds for 10 min. Giannopolitis and Reis (1977) was followed for SOD activity assay. SOD activity was noticed by inhibiting photochemical reduction of

NBT. Number of enzymes used for 50% inhibition of photochemical reduction of NBT at 560 nm wavelength was considered as SOD activity.

Catalase enzyme activity (CAT) was measured by procedure evaluated by Aebi (1984). 100 Âµl of the enzyme extract, 0.1 millimolar phosphate buffer pH 7, 0.1 millimolar Ethylenediaminetetraacetic acid (EDTA), and 0.3% hydrogen peroxide were mixed to form assay solution. By measuring the reduction in optical density at 240 nm, CAT activity was recorded. POD activity was measured by following the procedure explored by Thomas et al. (1982) and it was recorded at 436 nm. Nakano and Asada (1981) revealed the determination of APX activity. It was recorded by recoding the absorbance of drop at 290 nm for three minutes.

2.5. Determination of H_2O_2

The hydrogen peroxide was measured according to the method of Velikova et al. (2000). Homogenization of 300 mg leaf sample was done with 3 ml of 0.1% (w/v) TCA under cool environment. Centrifugation was done for 15 mins at 21000 × g. 1 mL of 1 M potassium iodate, 500 Âµl of 10 millimolar K_2PO_4 buffer (pH 7) were mixed with 500 µL of the supernatant. The absorbance is measured at 390 nm.

2.6. Measurement of MDA

Malondialdehyde concentration was measured by the method of Rao and Sresty (2000). Homogenization of frozen wheat leaves (300 mg) was done in 0.1% trichloroacetic acid (TCA) on ice. Centrifugation of homogenized solution was made at 4 °C temperature for 10 mins at 10,000*g*, and the precipitate was extracted twice with the same solvent. 0.5 ml of supernatant was mixed with 1.5 ml of 20% TCA, 0.5% thiobarbituric acid was added, heated at 95 °C for twenty five minutes, the mixture was cooled to room temperature (RT), and then centrifuged at room temperature for 10 min. The sample is measured at 532 nm and corrected by non-specific absorption at 600 nm.

2.7. Proline and protein contents

Bates et al. (1973) method was used to measure the proline contents. In short, for twenty minutes at 85 °C temperature 20 mg of ground fresh leaves were added with 400 Âµl ethanol. Centrifugation was done with 14,000 rounds for five minutes at 25 °C temperature. The supernatant (50 Âµl) was added with 100 Âµl of the reaction mixture (ninhydrin 1% (w/v), acetic acid 60% (v/v), ethanol 20% (v/v)), and then heated at 95-degree Celsius temperature in the heater for 20 min. Centrifugation was made for one minute about 2500 rounds per minute after cooling at 25 °C temperature and the optical density of the solution was notices at 520 nm. Similarly protein concentration was measured by following the method described by Bradford (1976).

2.8. Morphological and yield attributes

At maturing, plant height and spike length of randomly selected five wheat plants from each experimental treatment was measured with help of meter rod. After measuring the plant height and spike length these plants were harvested with the help of scissors at the soil level and then weighed to measure biological yield of each experimental treatment. After the determination biological yield, spikes were separated from wheat plants and their grains were threshed manually and number of grains per spike was counted via digital seed counter. Weight of 100 grains and total grain yield was noticed by using digital weighing balance and averaged. All the plant and yield parameters were recorded by following the standard procedures as described by Ul-Allah et al. (2018).

2.9. Zn concentration in shoot and grains

Zn concentration in shoots and grains was measured according to (Rashid (1986)) who revealed that wet ashing of shoots and grains harvested at final stage was done. Samples were ground through grinder and then weighed after drying in an oven at 70 °C for 24 h. Digestion was done on digestion plate (Heidolph, USA model, MR3003) in a di-acid ($HClO_4$: HNO_3 at 3:10 v/v ratio). Zn concentration in grains and shoots was measured by using the atomic absorption spectrophotometer (Shimadzu, UV-1201, Kyoto, Japan).

2.10. Statistical analysis

Using Fisher's Analysis of Variance technique, all the data of the experiment was analyzed considering a two-factor complete factorial completely randomized design (Steel et al. 1997). For the comparison of means, post-hoc test (students' LSD) was carried out by using statistical software StatistiX 8.1. Moreover, figures were prepared by using Microsoft Excel ©365.

3. Results

3.1. Gas exchange attributes and relative water contents

Application of Zn under both irrigation conditions more water and water stress significantly elevated the net photosynthetic rate (*pn*), stomatal conductance (*gs*) and transpiration rate (*E*) but the effect was more prominent under water stress conditions, than control. Maximum improvement in *pn* (34%), *E* (47) and *gs* (52%) relative to control were observed under water stress conditions at 15 mM Zn application (Fig. 1). Likewise, RWC were suggestively enhanced by exogenous application Zn and plants maintained the leaf water status under both well-watered and water stress with Zn foliar fertilization. respectively.

3.2. Production of osmolytes and enzymatic antioxidant activities

Plant produces osmoprotectants and antioxidants in response to abiotic stresses, especially water stress and salt stress. Proline and Protein contents were imperatively increased by exogenously applied zinc under water stress conditions, but effect of Zn was non-significant under well-watered conditions (Fig. 2). Correspondingly, application of zinc (15 mM) improved proline and protein contents (34% and 38% respectively) under water stress conditions, and it was statistically at par where zinc was applied at 10 mM concentration.

First response of water stress is induction of ROS which include H_2O_2 and MDA etc. Under normal conditions, there were no variations in productions of ROS among the treatments. Under water stressed environments, synthesis of ROS increases, but application of Zn reduce the ROS production, probably due to more production of antioxidants. Application of Zn (15 mM) under moisture deficit conditions reduced the production of H_2O_2 and MDA by 38% and 31% relative to control treatment (Fig. 2).

Antioxidants are produced to save plant from ROS damage. Zn treatments don't affect antioxidant enzymatic activities under normal irrigation conditions. But under water stress conditions, antioxidant enzymatic activities increased with application of Zn. Application of Zn (15 mM) enhanced the SOD, POD, CAT and APX activities by 18%, 22%, 30% and 25% respectively, relative to control treatment. POD activities at 10 mM were statistically at par with

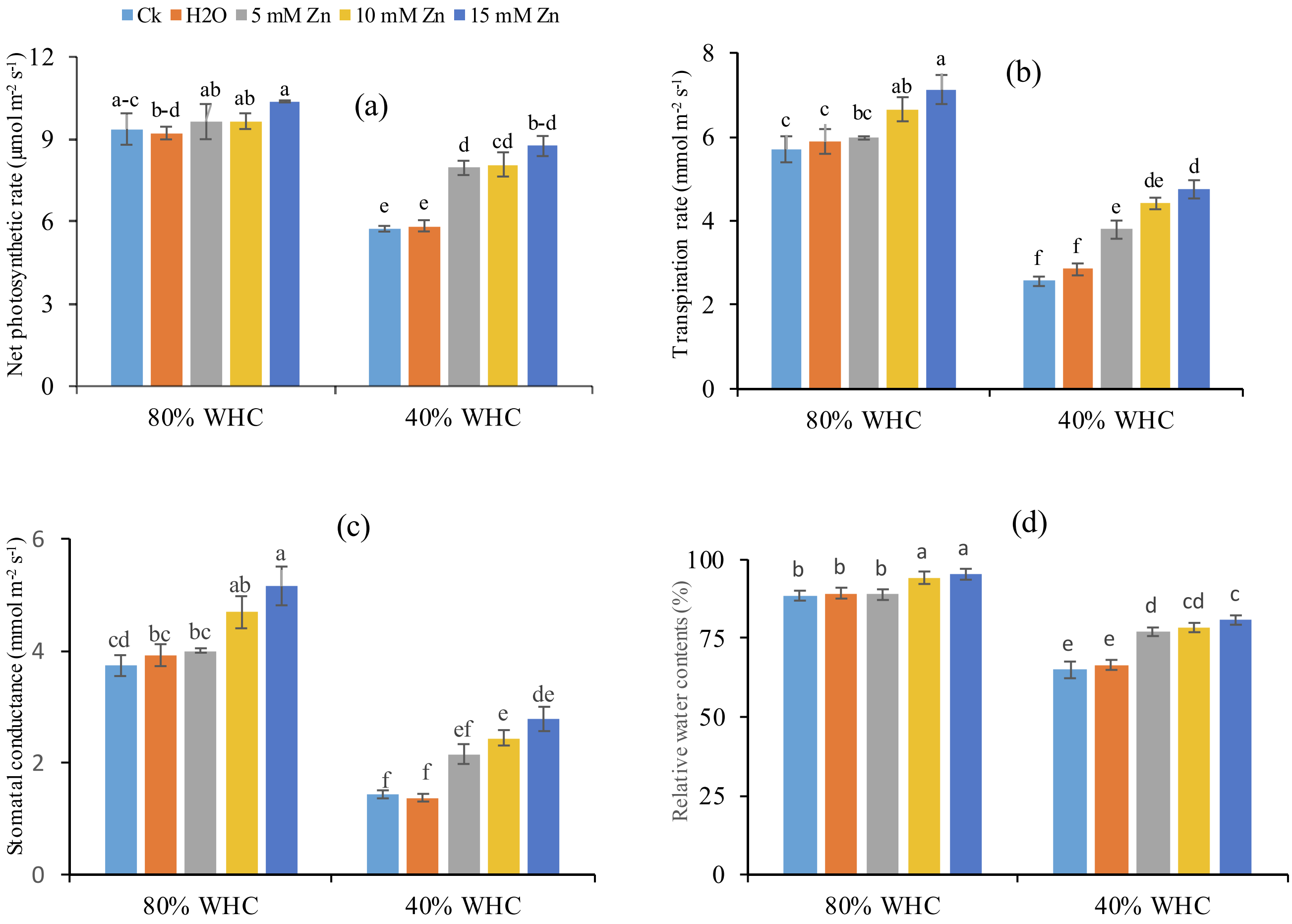

Fig. 1. Influence of foliar application of Zn on net photosynthetic rate (a), transpiration rate (b), stomatal conductance (c) and relative water contents (d) of wheat leaf under drought stress condition. Values are mean SE of three replicates. Bars marked with different letters are significantly different by LSD ($p \leq 0.05$).

10 mM Zn application, while for others, antioxidant enzymatic activities were statistically less than 15 mM (Fig. 3).

3.3. Morphological and yield attributes

Different levels of Zn (5, 10 and 15 mM) imperatively upgraded the morphology attributes of wheat plants under both irrigation regimes (well-watered and water stress). Wheat plants subjected to exogenous Zn under both water regimes 80% and 40% WHC increased the plant height and spike length at different concentrations (Table 1). Interaction of water stress and Zn fertilization was statistically significant. Overall, water stress reduced the value of morphological traits of wheat, and exogenous application of Zn treatment improved the vale. Impact of Zn fertilization was more prominent under moisture deficit conditions than under normal conditions. For all the morphological traits, best results were observed at 15 mM Zn fertilization under well-watered and water stressed conditions, and for both conditions vales of morphological traits at 15 mM were statistically at par with 10 mM (Table 1). Likewise, grain yield, biological yield and harvest index reduced under water stress conditions and were improved by the application Zn. Maximum grain yield, biological yield and harvest index were observed at 10 mM Zn application, but the value was statistically at par with 15 mM Zn application.

3.4. Zn concentration in shoot and grains

Zinc contents in shoots were significantly enhanced by exogenously applied Zn under both irrigation conditions water stress and well- watered. Under water stress environment zinc contents were increased about (63.6%) by 15 mM Zn application as compared to control treatment where Zn was not applied. Similarly, under well-watered conditions (80% WHC) 15 mM Zn increased up to 59% zinc contents in shoots as compared to control treatment (Fig. 4). Furthermore, zinc contents in grains were also enhanced at 10 mM and 15 mM Zn application about (34.3% and 37.6%) respectively as compared to control treatment under well-watered circumstances (80% WHC) and they were statistically similar with each other. Meanwhile, under water stress environment (40% WHC) Zn application at 15 mM increased the zinc contents in grains about 36.9% as compared to control treatment where Zn was not applied (Fig. 4).

4. Discussion

Abiotic stresses are the greatest threat to food security in climate change scenario which affects the growth and development of crop plants by disturbing the physiological and biochemical process in the plant. Zn is important micronutrients which is a part of many metabolic processes and regulate the plant growth and

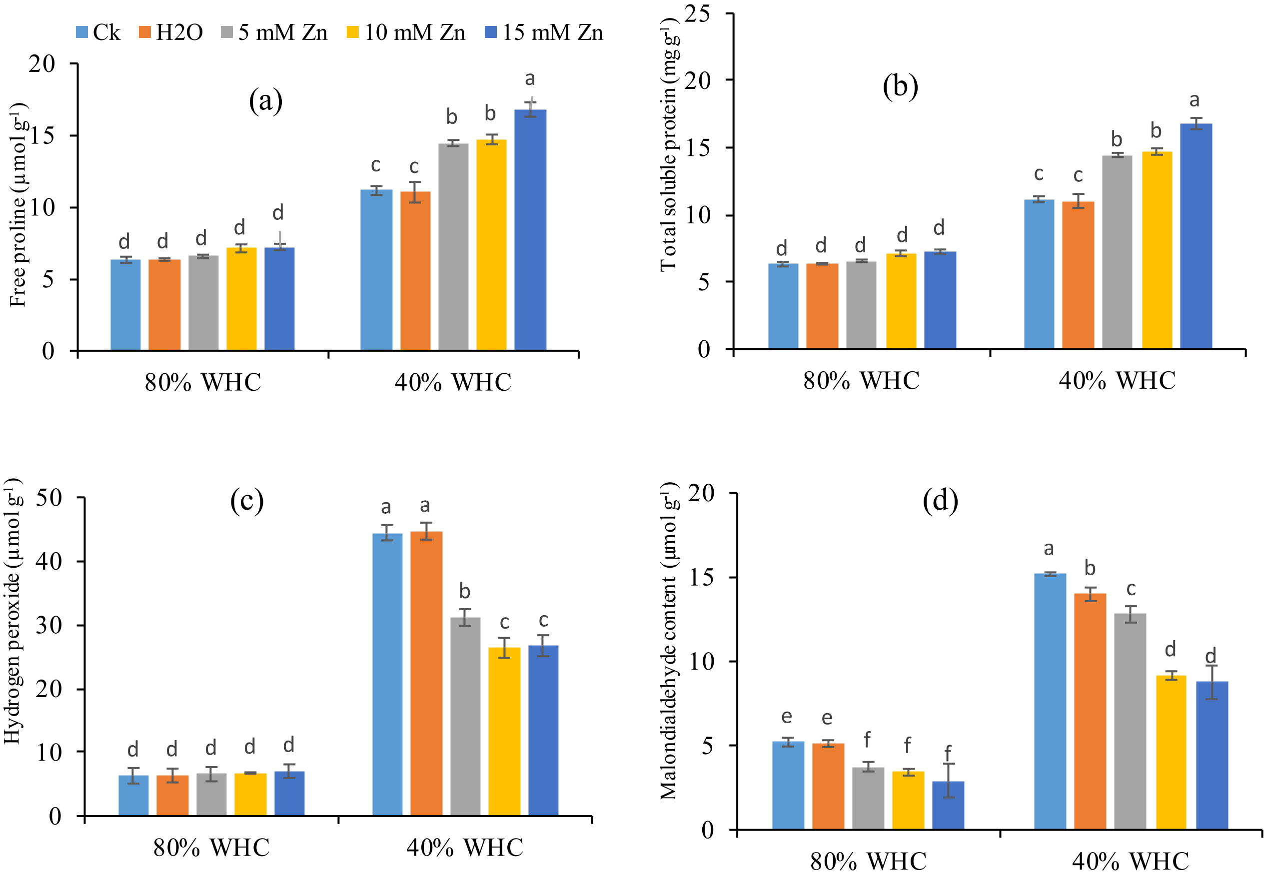

Fig. 2. Influence of foliar application of Zn on free proline (a), total soluble protein (b), hydrogen peroxide (c) and malondialdehyde contents (d) of wheat leaf under drought stress condition. Values are mean SE of three replicates. Bars marked with different letters are significantly different by LSD ($p \leq 0.05$).

development even under stress (Barrameda-Medina et al. 2017; Khatun et al., 2018; Sun et al., 2021a, 2021b). In current study, water stress affected various physiological and biochemical processes i.e., reduces the photosynthetic rate, RWC and transpiration which all are linked with stomatal conductance. But application of Zn improved these parameters and alleviates the drought effects. This improvement attributed to different homeostasis of different physiological and metabolic process by the Zn. Huang et al. (2009) reported that a zinc finger protein, DST, cause cell homeostasis by regulating the closing and opening of stomata under drought and salt tolerance and imparts the tolerance in rice and this homeostasis is linked with production of H_2O_2. In current study, production of H_2O_2also regulated in Zn applied plants (Fig. 2). Sun et al. (2021a, 2021b) reported that ZnO applied as non-particles improves the water relations, UDP-glucose pyrophosphorylase, reduces the degradation of green pigments and regulate the stomatal opening to improve the photosynthetic efficiency in maize under water stress which leads to better growth and development. Likewise, Barrameda and Medina et al. (2017) reported that alleviation of water stress and improved photosynthetic efficiency attributed to enhanced nitrogen metabolism due to Zn fertilization. Thus, regulation in photosynthetic rate, water relation and transpiration rate are attributed to the role of Zn in different metabolic and physiological processes.

In plant homeostasis of role of osmolytes cannot be denied. Under water stress, concentration of osmolytes (proline and proteins) decreased which led to higher production of reactive oxygen species i.e., H_2O_2 and MDA (ROS) (Fig. 2). But application Zn improved the production of osmolytes under water stress and reduced the production of ROS. MDA is a measure of oxidative lipids injury of membranes due to abiotic stress and it severely affects plant growth and development. Zn acts like a ROS scavenger by improving the antioxidant activities like SOD, POD and APX and regulate the function of bio membranes smooth (Fig. 3; Weisany et al., 2012; Khatun et al., 2018; Sun et al., 2021a, 2021b). Sun et al. (2021a, 2021b) reported that application Zn improves the production of osmolytes and antioxidant enzymatic activities which sustain the cell functioning even under stress conditions. Wu et al. (2015) reported that Zn application up regulate the expression of Zn finger protein which led to higher antioxidant activities and reduced ROS production also evident from current study (Fig. 3). In conclusion, improved antioxidant enzymatic activities and osmolytes production scavenges ROS and save wheat plants from injury under water stress.

Morphological traits predict apparent growth and development of plant. In current study, Application of Zn improved morphological and yield traits both under drought and normal conditions. This improvement in morphological and yield traits attributed to improvement in physiological and biochemical parameters due to Zn application. Due to better photosynthesis, more assimilates and produced and stored in the grain which led to improved grain size and higher grain yield and harvest index (Table 1, 2). Moreover, higher yield with higher Zn contents in necessary for food security and to combat Zn malnutrition. Abd El-Hady Effect (2007) reported that Zn application improves the growth and development of barely plant by improving the uptake of N, P and K under salinity stress. It reported that upto 23% increase in uptake of N, P and K, Zn and 50% decrease in Na uptake under different salinity levels by application on 30 mg Zn Kg^{-1} od soil. Improvements in uptake of nutrients and Zn by application of Zn in other crops have also been reported

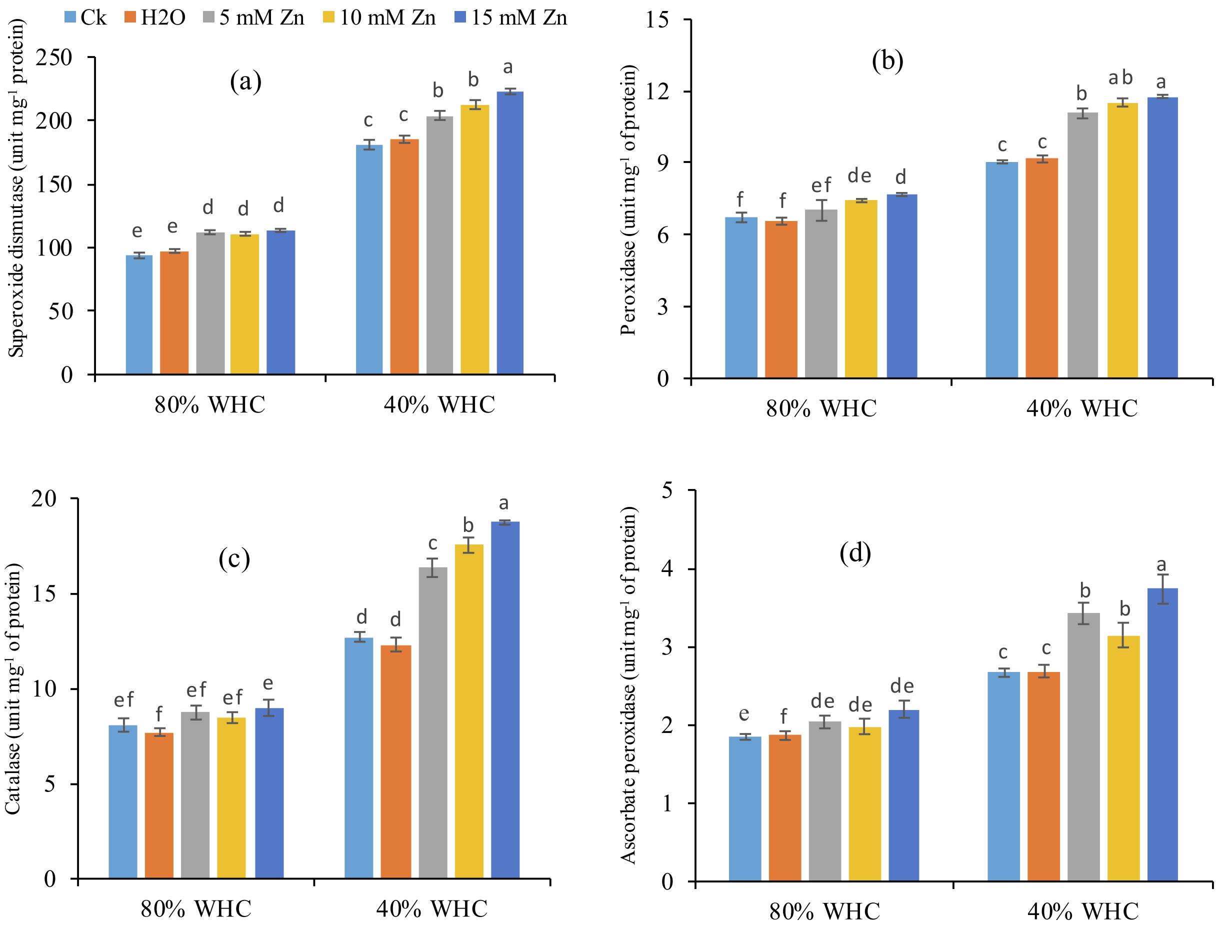

Fig. 3. Influence of foliar application of Zn on superoxidase dismutase (a), peroxidase (b) catalase (c), and ascorbate peroxidase (d) of wheat under water stress conditions. Values are mean SE of three replicates. Bars marked with different letters are significantly different by LSD ($p \le 0.05$).

Table 1
Influence of foliar application of Zn on plant height, spike length, grains per spike, 100-grain weight of wheat under water stress conditions.

WHC	Zn application (mM)	Plant height (cm)	Spike length (cm)	Grains per spike	100-grain weight (g)
80%	Control	93.5 ± 2.4 a	12.2 ± 0.23b	45.7 ± 0.84b	5.0 ± 0.03c
	H_2O	95.5 ± 0.8 a	12.1 ± 0.22b	45.4 ± 0.53b	5.0 ± 0.06c
	5 mM	97.8 ± 0.6 a	12.7 ± 0.23b	52.5 ± 0.38 a	5.7 ± 0.03 ab
	10 mM	96.5 ± 1.8 a	12.2 ± 0.33b	55.1 ± 0.19 a	5.5 ± 0.02b
	15 mM	97.0 ± 0.3 a	14.4 ± 0.16 a	54.6 ± 1.04 a	6.0 ± 0.12 a
40%	Control	75.0 ± 0.9c	8.7 ± 0.20c	32.8 ± 0.31 d	3.6 ± 0.05 e
	H_2O	77.2 ± 0.5 bc	8.8 ± 0.05c	32.9 ± 0.21 d	3.7 ± 0.04 e
	5 mM	81.8 ± 1.8 bc	9.6 ± 0.13c	41.9 ± 0.41 bc	4.3 ± 0.03 d
	10 mM	83.9 ± 0.6b	9.8 ± 0.12c	43.7 ± 0.25 bc	4.1 ± 0.03 d
	15 mM	84.3 ± 0.4b	8.9 ± 0.02c	40.1 ± 0.32c	4.0 ± 0.03 d
LSD $\le$ 0.01		8.5	1.14	3.93	0.32

Means sharing similar causing digits did not be at variance significantly $p \le 0.05$.

(Sathisha et al., 2020; Hassan et al. 2020a, 2020b; Xue et al., 2019; Rehman et al., 2018). Sufficient supply of crop nutrient leads to improvement in crop growth, yield and nutritional quality. In current study higher grain yield and grain Zn contents were observed with application of Zn which is supported by the finding of other researcher who reported improvement in grain yield with Zn fertilization and Zn biofortification (Ma et al., 2017; Rehman et al., 2018; Faran et al., 2019; Asif et al., 2019). Asif et al. (2019) reported that climate change effects like increased CO_2, temperature and water stress decreases the duration of growth stages of wheat which results in lower carbohydrate assimilates and resultantly small spike, short grain and lower grain yield. But application of Zn and N improves the spike traits and improved overall grain yield and grain ZN contents. Ma et al. (2017) reported that application of Zn under alleviates water stress by improving the green pigments and photosynthetic efficiency along-with transcription of antioxidant related genes involved in ascorbate–glutathione cycle and flavonoid biosynthesis. Moreover, they have reported improvement in grain yield and grain Zn contents by 10 and 15% respectively under normal irrigated wheat plants and 28 and 32% respectively under water stressed wheat plants by Zn fertilization which strengthen our findings. Thus, for improvement in grain yield and Zn biofortification under water stress conditions, optimum dose of Zn much be combined with NPK.

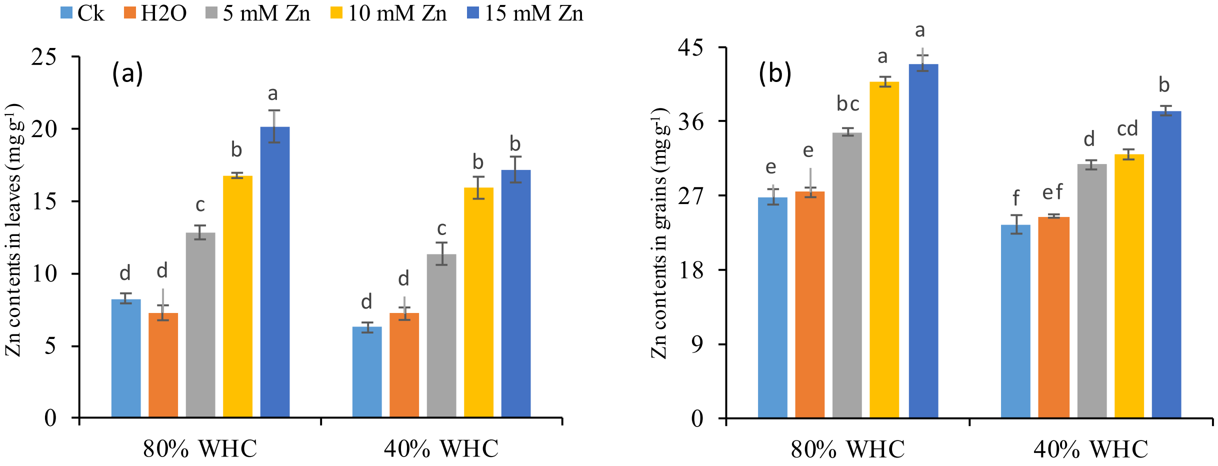

Fig. 4. Influence of foliar application of Zn on Zn contents in leaves (a), and Zn contents in grain (b) of wheat under water stress conditions. Values are mean SE of three replicates. Bars marked with different letters are significantly different by LSD ($p \leq 0.05$).

Table 2
Influence of foliar application of Zn on grain yield, biological yield, and harvest index of wheat under water stress conditions.

WHC	Zn application	Grain yield (g)	Biological yield (g)	Harvest index (%)
80%	Control	4.9 ± 0.08 bc	17.2 ± 0.40b	28.8 ± 0.20 ab
	H_2O	4.9 ± 0.09 bc	17.1 ± 0.33b	29.0 ± 0.15 ab
	5 mM	5.1 ± 0.25 ab	21.8 ± 0.48 a	23.8 ± 2.76 ab
	10 mM	5.8 ± 0.19 a	23.9 ± 1.12 a	24.3 ± 1.05 ab
	15 mM	5.1 ± 0.01 ab	23.4 ± 0.52 a	22.0 ± 0.65b
40%	Control	3.5 ± 0.03 d	12.2 ± 0.15c	28.7 ± 1.14 ab
	H_2O	3.5 ± 0.06 d	12.3 ± 0.08c	28.4 ± 0.66 ab
	5 mM	4.3 ± 0.04c	15.3 ± 0.42 bc	28.2 ± 0.98 ab
	10 mM	4.7 ± 0.14 bc	16.2 ± 0.39b	29.4 ± 2.11 a
	15 mM	4.3 ± 0.08c	14.9 ± 0.41 bc	28.9 ± 1.29 ab
LSD ≤ 0.01		0.76	3.78	7.13

Means sharing similar digits did not be at variance significantly $p \leq 0.05$.

5. Conclusion

Water stress severely affected the growth and development of wheat plant by production of ROS and results in lower yield. Application of Zn improves antioxidant enzymatic activities which scavenge the ROS. Moreover, by the application Zn, concentration of osmolytes increased which regulates the stomatal conductance and, photosynthesis and improved the growth and development of wheat plants under drought conditions. Although improvement in spike related traits, grain yield and grain Zn contents was observed in normal irrigated plants due to Zn fertilization, but the improvement in the traits was relatively more prominent under water stress conditions. In most of the cases, results of 10 mM Zn and 15 mM Zn were statistically at par. Based on the results, an optimum dose of Zn (10 mM) is suggested along with NPK fertilization for alleviation of water stress and grain biofortification.

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.

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