



Research article

Adequate zinc nutrition improves the tolerance against drought and heat stresses in chickpea

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ABSTRACT

Two chickpea genotypes viz. Bhakar-2011 (*desi*) and Noor-2013 (*kabuli*) were sown in soil filled pots supplied with low (0.3 mg kg⁻¹) and high (3 mg kg⁻¹ soil) zinc (Zn) under control (70% water holding capacity and 25/20 °C day/night temperature), drought (35% water holding capacity) and heat (35/30 °C day/night temperature) stresses. Drought and heat stresses reduced rate of photosynthesis, photosystem II efficiency, plant growth and Zn uptake in chickpea. Low Zn supply exacerbated adverse effects of drought and heat stresses in chickpea, and caused reduction in plant biomass, carbon assimilation, antioxidant activity, impeded Zn uptake and enhanced oxidative damage. However, adequate Zn supply ameliorated adverse effect of drought and heat stresses in both chickpea types. The improvements were more in *desi* than *kabuli* type. Adequate Zn nutrition is crucial to augment growth of chickpea plants under high temperature and arid climatic conditions.

1. Introduction

Chickpea (*Cicer arietinum* L.) is a highly nutritious grain legume and is prone to abiotic stresses when grown under rainfed environment. Across the globe, drought stress causes 40–45% yield losses (Ahmad et al., 2005). Drought limits the vegetative growth of various legumes by restricting water uptake, reducing the tissue water contents, decreasing the gas exchange rate (Farooq et al., 2017a). The reduction in stomatal conductance increases leaf temperature (Sehgal et al., 2017) which causes wilting of leaf (Farooq et al., 2017a). Drought causes membrane damage (Awasthi et al., 2014), disruption of chlorophyll synthesis (Rahbarian et al., 2011), impact photosynthesis (Samarah et al., 2009), impairs mineral uptake (Gunes et al., 2006) and reduces nitrogen fixation in legumes (Serraj, 2003).

Over last several decades, there has been a rise in daily mean temperature. The global climatic models have simulated an increase of 4 °C in temperature (Intergovernmental Panel on Climate Change (IPCC) et al., 2013) by the end of this century that may induce severe losses in agricultural production. Winter legumes are more sensitive to high temperature stress (Hall, 2001), that have negative effects on the crop growth and development (Devasirvatham et al., 2012) and cause significant yield losses. Chickpea experiences a range of high

temperatures during the growing season (Devasirvatham et al., 2012); heat stress at sowing directly affects crop germination and establishment (Devasirvatham et al., 2012). Nodule size starts decreasing when temperature rises from 35 °C resulting in low nitrogen fixation and decreased activity of nitrogenase (Piha and Munns, 1987). In chickpea, temperature above 32/20 °C (day/night) cause reduction in RuBisCO activity (28–30%) and denaturation of RuBisCO subunits (SPS/SS; Kaushal et al., 2013) leading to reduced photosynthesis (Farooq et al., 2017b; Rehman et al., 2019).

Zinc is vital for all life forms and plays a crucial role in plant defense system due to its involvement in key enzymes activity, gene regulation (Lopez-Millan et al., 2005) and protein synthesis (Liu et al., 2015). It also helps maintain photosynthetic activities (Rehman et al., 2019), stabilize biological membranes (Cakmak, 2000), maintain CO₂ concentration in mesophyll cells and repair the PSII during photo-inhibition (Hansch and Mendel, 2009). Zinc deficiency increases sensitivity to heat (Rehman et al., 2019) and drought stresses and may result in drastic reductions in grain yield (Bagci et al., 2007).

In many chickpea growing countries, Zn is a common micronutrient which is deficient in the soils (Ahlawat et al., 2007; Ullah et al., 2019). Seeds derived from Zn deficient soils not only have less nutritional value for animal and humans but also result in poor crop stand,

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Table 1

Interactive effect of drought stress and Zn supply (Zn × D) on the growth, leaf photosynthesis, specific leaf area, leaf malondialdehyde contents, seedling dry weight, leaf free proline and shoot Zn concentration of chickpea types.

Chickpea types	Treatments	Mean (Zn. C.T)			Mean (Zn. C.T)		
		Control	Drought stress		Control	Drought stress	
		Leaf CO ₂ net assimilation rate (μmol s ⁻¹ m ⁻²)			Photosystem II efficiency (Fv/Fm)		
<i>Kabuli</i>	Low Zn	10.3 c	7.69 e	9.00 C	0.59 b	0.39 e	0.49 D
	Adequate Zn	11.9 b	8.59 de	10.2 B	0.63 b	0.47 d	0.55 C
<i>Desi</i>	Low Zn	12.3 b	8.98 d	10.6 B	0.77 a	0.52 c	0.65 B
	Adequate Zn	14.5 a	10.3 c	12.4 A	0.80 a	0.59 b	0.70 A
	Mean (stress)	12.2 A	8.88 B		0.70 A	0.49 B	
	LSD value	Zn. C.T = 0.72; Stress = 0.37; Zn. C.T × stress = 1.23			Zn. C.T = 0.023; Stress = 0.012; Zn. C.T × stress = 0.041		
		Relative leaf water contents (%)			Specific leaf area (cm ² g ⁻¹ leaf DM)		
<i>Kabuli</i>	Low Zn	90.2 d	79.6 h	84.9 D	14.6 d	9.43 f	12.0 C
	Adequate Zn	92.5 c	82.5 g	87.5 C	19.0 b	12.2 e	15.6 B
<i>Desi</i>	Low Zn	96.3 b	85.6 f	90.9 B	19.3 b	12.0 e	15.6 B
	Adequate Zn	97.5 a	88.5 e	93.0 A	25.2 a	15.8 c	20.5 A
	Mean (stress)	94.1 A	84.0 B		19.5 A	12.4 B	
	LSD value	Zn. C.T = 0.51; Stress = 0.26; Zn. C.T × stress = 0.87			Zn. C.T = 0.39; Stress = 0.20; Zn. C.T × stress = 0.67		
		Leaf malondialdehyde contents (μmol g ⁻¹ FW)			Seedling dry weight (g)		
<i>Kabuli</i>	Low Zn	19.6 c	28.9 a	24.2 A	9.28 c	5.81 e	7.55 C
	Adequate Zn	15.4 g	18.7 d	17.0 C	11.7 b	7.30 d	9.48 B
<i>Desi</i>	Low Zn	16.6 f	23.3 b	20.0 B	11.7 b	7.54 d	9.60 B
	Adequate Zn	13.9 h	17.8 e	15.8 D	14.6 a	9.60 c	12.1 A
	Mean (stress)	16.4 B	22.2 A		11.8 A	7.56 B	
	LSD value	Zn. C.T = 0.47; Stress = 0.24; Zn. C.T × stress = 0.81			Zn. C.T = 0.19; Stress = 0.10; Zn. C.T × stress = 0.33		
		Leaf free proline (μmol g ⁻¹ FW)			Shoot Zn concentration (mg kg ⁻¹)		
<i>Kabuli</i>	Low Zn	7.74 g	11.2 d	9.45 D	9.53 g	8.94 h	9.24 D
	Adequate Zn	9.80 e	14.2 b	12.0 B	16.8 d	20.6 b	18.7 B
<i>Desi</i>	Low Zn	8.81 f	13.4 c	11.1 C	11.8 e	11.2 f	11.5 C
	Adequate Zn	11.1 d	16.9 a	14.0 A	18.9 c	26.6 a	22.7 A
	Mean (stress)	9.36 B	13.9 A		14.3 B	16.8 A	
	LSD value	Zn. C.T = 0.39; Stress = 0.20; Zn. C.T × stress = 0.66			Zn. C.T = 0.29; Stress = 0.15; Zn. C.T × stress = 0.50		

Means sharing different letters within columns and rows, for each trait, are statistically different from each other at 5% probability level; Mean Zn = zinc; C.T = Chickpea types; control = 70% water holding capacity; drought stress = 35% water holding capacity; low Zn = 0.3 mg kg⁻¹; adequate Zn = 3 mg kg⁻¹ soil.

particularly in stressful growing conditions (Candan et al., 2018; Faran et al., 2019). In many plants with an associated antioxidative response, increase in Zn supply can up regulate antioxidant enzymes activity to reduce the oxidative damages (Cakmak, 2000). Therefore, adequate Zn fertilization is essential for sustainable crop production and mitigation of drastic effects of various abiotic stresses (Rehman et al., 2018, 2019).

The combined effect of abiotic stresses (drought and heat) and Zn deficiency on the photosynthesis, water relation, enzymes activity, plant biomass and shoot Zn concentration of chickpea has been rarely studied. Therefore, the present study is unique that here, We investigated the interactive effects of low Zn supply with drought stress and supra-optimal temperature using two chickpea types (*desi* and *kabuli*) on plant photosynthetic assessment, antioxidant activities and biomass production. The specific objective of this study was to improve the drought and heat tolerance in chickpea types through sufficient Zn supply at early growth stages.

2. Materials and methods

2.1. Plant material

Seeds of chickpea types '*desi* (cv. Bakhar-2011)' and *kabuli* (cv. Noor-2013) were received from the 'Ayub Agricultural Research Institute', Faisalabad, Pakistan.

2.2. Experimental description

The current study comprising of two independent experiments was

conducted at department of Crop Sciences, College of Agricultural and Marine Sciences, Sultan Qaboos University, Oman during 2018. The experimental soil was sandy loam with pH 7.9, electrical conductivity 0.14 dS m⁻¹, organic matter 0.56%, nitrogen 380 mg kg⁻¹, phosphorus 09 mg kg⁻¹, potassium 102 mg kg⁻¹ soil, and diethylene triamine penta acetic acid (DTPA) extractable Zn 0.63 mg kg⁻¹ soil. The extraction of soil, analysis and nutrient status was performed using protocol of George et al. (2013). The experiments were executed in a completely randomized design with factorial arrangement and repeated thrice.

2.2.1. Experiment 1

Chickpea seeds of both types (*desi* and *kabuli*) (6 seeds per pot) were sown in soil filled plastic pots (5 kg), maintained at 70% water holding capacity (WHC) (control) and 35% WHC (drought stress). After emergence (5 days after sowing), the pots were thinned to three plants per pot and were kept in a growth chamber with a day/night temperature (18/15 °C), a light/dark photoperiod (16/8 h).

The all 24 pots (2 water levels × 2 Zn levels × 2 chickpea cultivars × 3 replications) were well-watered (70% WHC) by weighing and adding water on daily basis (w/v) till imposing drought. Half pots were maintained at 70% WHC during whole experimental duration as control while remaining half pots were subjected to drought stress after one week of emergence by maintaining 35% WHC. The WHC was determined following the protocol as detailed in Faran et al. (2019) by taking 100 g of dry soil on a filter paper and saturating it with 100 mL of water and saturated weight was recorded after the drainage stopped. Using the formula the 100% WHC of the experimental soil was

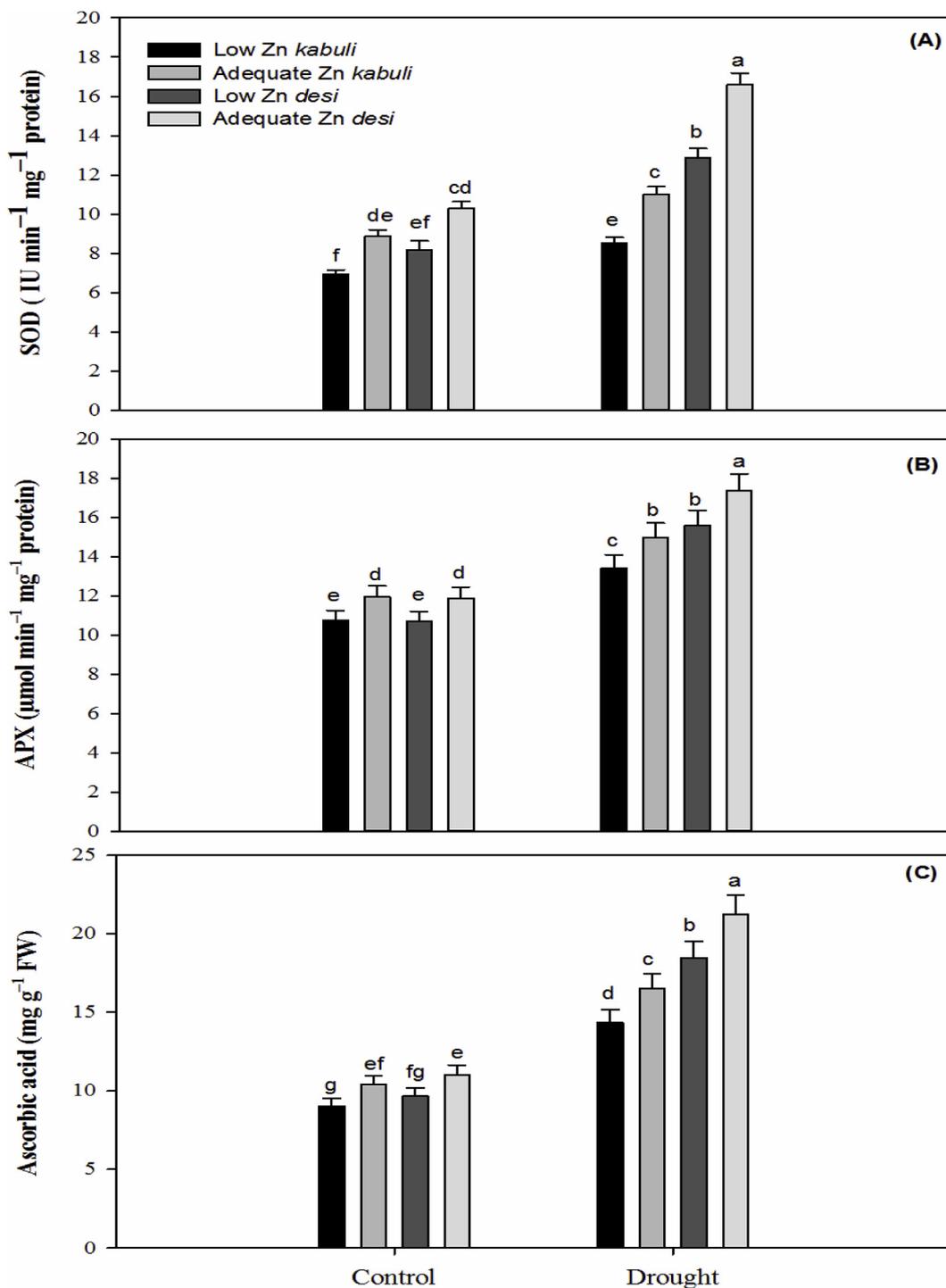


Fig. 1. Interactive effect of drought stress and Zn supply (D × Zn) on the ascorbic acid contents and enzyme activities of chickpea types.

calculated;

$$WHC = \frac{\text{Weight of saturated drained soil} - \text{weight of dry soil}}{\text{weight of dry soil}}$$

To maintain 70 and 35% WHC, the amount of water was calculated on alternate days using the soil weight per pot.

2.2.2. Experiment 2

Chickpea seeds of both types (*desi* and *kabuli*) (6 seeds per pot) were sown in soil filled plastic pots (5 kg). After emergence (5 days after sowing), the pots were thinned to three plants per pot and were kept in

a growth chamber with a day/night temperature (18/15 °C), a light/dark photoperiod (16/8 h) and the photosynthetic active photon flux (350 μmol s⁻¹ m⁻²) at the surface of pots measured with Ceptometer AccuPAR LP-80 Decagon Devices, Pullman, WA, USA and temperature was maintained as (25/20 °C day/night) normal condition, and (35/30 °C day/night) as heat stress.

To impose heat stress, five days after emergence, half of the pots were kept in a climate chamber with an optimal temperature (25/20 °C day/night) (control), and remaining half of the pots having one week old seedlings were subjected to heat stress (35/30 °C day/night). In both experiments, Zn was applied as high Zn (3 mg kg⁻¹) and low Zn as (0.3 mg kg⁻¹ soil) using ZnSO₄·7H₂O as source. The plants in both

Table 2

Interactive effect of heat stress and Zn supply (Zn × H) on the growth, leaf photosynthesis, specific leaf area, leaf malondialdehyde contents, seedling dry weight, leaf free proline and shoot Zn concentration of chickpea types.

Chickpea types	Treatments	Control	Heat stress	Mean (Zn. C.T)	Control	Heat stress	Mean (Zn. C.T)
		Leaf CO ₂ net assimilation rate (μmol s ⁻¹ m ⁻²)				PSII efficiency (Fv/Fm)	
Kabuli	Low Zn	10.1 c	7.71 e	8.91 C	0.58 b	0.38 e	0.48 D
	Adequate Zn	12.0 b	8.65 d	10.3 B	0.61 b	0.46 d	0.54 C
Desi	Low Zn	12.0 b	8.94 d	10.5 B	0.79 a	0.51 c	0.65 B
	Adequate Zn	14.5 a	10.6 c	12.5 A	0.82 a	0.59 b	0.70 A
	Mean (stress)	12.1 A	8.97 B		0.70 A	0.49 B	
	LSD value	Zn. C.T = 0.34; Stress = 0.17; Zn. C.T × stress = 0.58			Zn. C.T = 0.022; Stress = 0.011; Zn. C.T × stress = 0.038		
Relative leaf water contents (%)				Specific leaf area (cm ² g ⁻¹ leaf DM)			
Kabuli	Low Zn	93.0 c	78.1 g	85.5 D	15.0 e	9.82 g	12.4 D
	Adequate Zn	95.4 a	80.9 f	88.2 C	19.6 b	12.8 f	16.2 B
Desi	Low Zn	94.4 b	84.0 e	89.2 B	17.7 c	12.5 f	15.1 C
	Adequate Zn	95.9 a	86.8 d	91.4 A	23.1 a	16.3 d	19.7 A
	Mean (stress)	94.7 A	82.4 B		18.9 A	12.9 B	
	LSD value	Zn. C.T = 0.54; Stress = 0.28; Zn. C.T × stress = 0.93			Zn. C.T = 0.28; Stress = 0.15; Zn. C.T × stress = 0.48		
Leaf malondialdehyde contents (μmol g ⁻¹ FW)				Seedling dry weight (g)			
Kabuli	Low Zn	17.5 d	28.7 a	23.1 A	9.11 e	6.10 h	7.60 D
	Adequate Zn	13.7 f	18.8 c	16.2 C	11.4 c	7.63 g	9.53 C
Desi	Low Zn	15.3 e	23.3 b	19.3 B	12.0 b	8.05 f	10.0 B
	Adequate Zn	12.7 g	17.8 d	15.3 D	15.1 a	10.1 d	12.6 A
	Mean (stress)	14.8 B	22.1 A		11.9 A	7.97 B	
	LSD value	Zn. C.T = 0.49; Stress = 0.26; Zn. C.T × stress = 0.85			Zn. C.T = 0.10; Stress = 0.05; Zn. C.T × stress = 0.17		
Leaf free proline (μmol g ⁻¹ FW)				Shoot Zn concentration (mg kg ⁻¹)			
Kabuli	Low Zn	7.60 g	11.3 d	9.43 D	10.2 g	9.74 h	9.95 D
	Adequate Zn	9.61 e	14.2 b	11.9 B	17.3 d	21.1 b	19.2 B
Desi	Low Zn	8.65 f	13.4 c	11.0 C	12.4 e	11.9 f	12.1 C
	Adequate Zn	10.9 d	16.9 a	13.9 A	19.3 c	26.9 a	23.1 A
	Mean (stress)	9.19 B	13.9 A		14.8 B	17.4 A	
	LSD value	Zn. C.T = 0.36; Stress = 0.19; Zn. C.T × stress = 0.63			Zn. C.T = 0.24; Stress = 0.12; Zn. C.T × stress = 0.41		

Means sharing different letters within columns and rows, for each trait, are statistically different from each other at 5% probability level; Mean Zn = zinc; C.T = Chickpea types; control = (25/20 °C); heat stress = (35/30 °C); low Zn = 0.3 mg kg⁻¹; adequate Zn = 3 mg kg⁻¹ soil.

experiments were harvested six weeks after planting.

2.3. Observations and measurements

2.3.1. Leaf photosynthesis

Leaf net CO₂ assimilation rate was measured with a portable LI-6400 infrared gas analyzer (LiCor, Inc., Lincoln, NE, USA) between 09:00 to 11:00 a.m. The leaf maximum efficiency of photosystem II (Fv/Fm) was determined using Handy PEA (Plant Efficiency Analyzer, Hansatech, Norfolk, UK) having excitation light energy of 3000 μmol m⁻¹ s⁻¹.

2.3.2. Plant growth

For recording the seedling dry weight (SDW), seedlings were harvested six weeks after planting and dried in an electric oven at 70 ± 2 °C. Before drying, the leaves were separated from harvested plants and leaf area was recorded with a leaf area meter (DT Area meter, Model MK2; Delta-T Devices, Cambridge, UK). The same leaves were dried in an electric oven at 70 ± 2 °C for 96 h, and specific leaf area (SLA) recorded as the ratio of leaf area to leaf dry weight.

2.3.3. Lipid peroxidation and free leaf proline

Lipid peroxidation was measured in terms of malondialdehyde contents (MDA). Leaf samples of 1 g were homogenized in 10 mL of 0.1% trichloroacetic acid and MDA contents estimated following the protocol of Heath and Packer (1968). For free leaf proline, leaf samples were homogenized with 10 mL of aqueous sulphosalicylic acid (w/v) and then filtered. The filtrate (2 mL) was mixed with 2 mL each of

glacial acetic acid and acid ninhydrin and incubated at 100 °C in a water bath for 1 h. After vortexing for 20 s the reaction was terminated in an ice bath and 4 mL of toluene. Following Bates et al. (1973) the chromophore containing free proline was measured.

2.3.4. Enzymatic analysis

The superoxide dismutase (SOD) was estimated following the method of Giannopolitis and Ries (1977). In this method, the inhibition of photochemical reduction of *p*-nitro blue tetrazolium chloride (NBT) is noted spectroscopically at 560 nm. The reaction mixture contained 2.9 mL potassium-phosphate buffer (K-P buffer), 0.5 mL 50 mM Na₂CO₃, 0.5 mL 50 mM 12 mM l-methionine, 0.5 mL 75 μM NBT and 500 μl 2 μM riboflavin with 100 μl supernatants. After addition of Riboflavin, the tubes containing reaction mixture were placed under light in a growth room for 8 min and absorbance was taken at 560 nm.

The ascorbate peroxidase (APX) activity was estimated using the protocol of Nakano and Asada (1981) by observing the decline in absorbance at 290 nm. For APX, the reaction mixture contained 50 mM K-P 650–800 μl K-P buffer (pH 7.6) containing 0.1 mM Na-EDTA, 100 μl of 12 mM H₂O₂, 50–150 μl sample and 100 μl of 0.25 mM ascorbic acid. The ascorbic acid was measured following the protocol of Pisoschi et al. (2008).

2.3.5. Plant water relations (%)

To determine relative leaf water content (RLWC), fresh leaves (W_f) were weighed and then soaked in water for 4 h to record saturated weight (W_s) at 5 °C. To record the dry weight (W_d) the same leaves were then oven-dried at 80 °C till constant weight. The RLWC (%) was

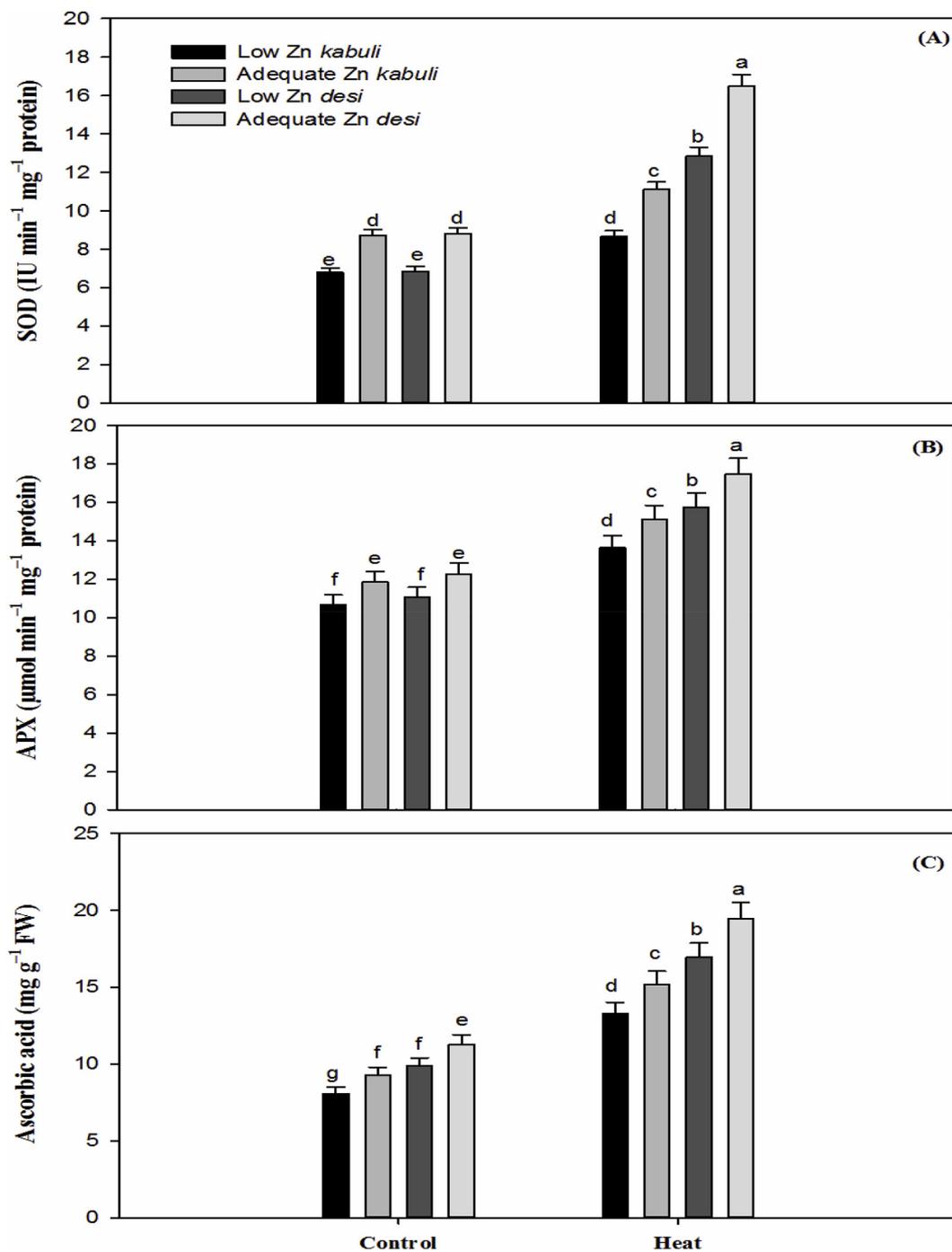


Fig. 2. Interactive effect of heat stress and Zn supply (H × Zn) on the ascorbic acid contents and enzyme activities of chickpea types.

calculated as described by Barr and Weatherley (1962);

$$RLWC = (W_f - W_d) / (W_s - W_d) \times 100$$

2.3.6. Zinc determination

To determine the shoot Zn concentration, inductively coupled plasma (ICP) atomic absorption with a PerkinElmer Optima 5300 DV optical emission spectrometer (OES-Shelton, CT, USA) was used. The samples were digested using concentrated H₂O₂ and HNO₃ in a closed microwave digestion system (MarsExpress, CEM Corp., Matthews, NC, USA).

2.4. Statistical analysis

The collected data were statistically analyzed with analysis of variance using statistical software CO-Stat (CoHort, Berkeley, CA, USA) and Least Significant Difference (LSD) test at 5% probability level was used for mean separation (Steel et al., 1997). The graphs of experimental data were developed using Sigma Plot 12.5 version.

3. Results

3.1. Drought stress experiment

3.1.1. Plant growth and leaf photosynthesis

Drought stress reduced the gas exchange traits, leaf area and biomass accumulation in chickpea. This decline in plant photosynthetic assessment traits and biomass production was further reduced under low Zn supply. However, adequate Zn supply enhanced the chickpea growth and leaf photosynthesis under optimal and drought stress condition. In this regard, the highest leaf CO₂ net assimilation rate (22%), PSII efficiency (27%), SLA (33%) and SDW (25%) was recorded under normal condition with adequate Zn application in *desi* chickpea compared to *kabuli* (Table 1).

3.1.2. Relative leaf water content (RLWC)

Drought stress drastically reduced the leaf water content and this decrease was further worsen by Zn deficiency. Nevertheless, application of adequate Zn in soil improved the RLWC of both chickpea types; however, *desi* chickpea type showed more improvement in RLWC with Zn application. The maximum RLWC (5.40 and 7.27%) was recorded in *desi* chickpea with adequate Zn application under normal and drought stress conditions respectively (Table 1).

3.1.3. Lipid peroxidation and free leaf proline

Water deficit condition increased the lipid peroxidation and reduced the leaf proline accumulation which was further accelerated in Zn deficient plants. Nevertheless, addition of adequate Zn in soil reduced the lipid peroxidation and augmented the leaf free proline accumulation in chickpea. Minimum leaf MDA contents were recorded in *desi* chickpea with adequate Zn application under normal and drought stress conditions (Table 1). Whereas, maximum leaf free proline contents (26%) were recorded under drought stress with adequate Zn supply in *desi* chickpea (Table 1).

3.1.4. Enzymes and shoot Zn concentration

Shoot Zn concentration was reduced in plants grown in soil receiving low Zn and the decrease in shoot Zn was further reduced under drought stress condition. Adequate Zn supply enhanced the tissue Zn level in both chickpea types under both drought and well watered condition. Maximum shoot Zn concentration was recorded in *desi* chickpea (29%) with adequate Zn application under drought stress compared to *kabuli* (Table 1). The antioxidant enzyme activities were increased under drought stress in chickpea and the greater response was recorded with adequate Zn supply compared to low Zn. Moreover, application of adequate Zn increased the SOD, APX activities and ascorbic acid level under normal and drought stress conditions in both chickpea types. However, the highest activities of SOD (51%), APX (16%) and ascorbic acid contents (29%) were recorded in drought stressed plants of *desi* chickpea supplied with adequate soil Zn compared to *kabuli* (Fig. 1).

3.2. Heat stress experiment

3.2.1. Plant growth and leaf photosynthesis

Heat stress reduced the growth, leaf photosynthesis and biomass accumulation in chickpea. The decrease in plant photosynthetic assessment traits and biomass production was further accelerated under Zn deficiency. The chickpea plants receiving adequate Zn supply exhibited improved growth and leaf photosynthesis under normal and heat stressed conditions. In this regard, the highest leaf CO₂ net assimilation rate (21 and 23%), PSII efficiency (34 and 28%), SLA (18 and 27%) and SDW (33 and 32%) was recorded under normal as well as heat stress conditions with adequate Zn supply in *desi* chickpea compared to *kabuli* (Table 2).

3.2.2. Relative leaf water content (RLWC)

Heat stress disturbed the plant RLWC and the decline in RLWC was greater in chickpea plants supplied with low Zn. Nevertheless, the application of Zn improved the RLWC of chickpea types; however, *desi* chickpea had more improvement than *kabuli*. The maximum RLWC was recorded in *desi* chickpea (1.58 and 3.33%) with adequate Zn application under normal and heat stressed conditions (Table 2).

3.2.3. Lipid peroxidation and free leaf proline

Heat stress accelerated the lipid peroxidation and reduced the leaf free proline accumulation in chickpea. Zinc deficiency further accelerated the lipid peroxidation and reduced the leaf proline accumulation. Nevertheless, addition of adequate Zn in soil reduced the lipid peroxidation and augmented the leaf free proline accumulation in both chickpea types. In this regard, minimum leaf MDA contents were recorded in *desi* chickpea with adequate Zn application under normal and heat stress conditions (Table 2). Whereas, the maximum leaf free proline contents (19%) were recorded under heat stress with adequate Zn supply in *desi* chickpea compared to *kabuli* (Table 2).

3.2.4. Enzymes and shoot Zn concentration

Heat stress limits the Zn accumulation in leaves of chickpea. However, adequate Zn supply enhanced the tissue Zn level in both chickpea types under both normal and heat stressed conditions. Nevertheless, the maximum shoot Zn concentration (27%) was recorded in *desi* chickpea with adequate Zn application under heat stress (Table 2). The antioxidant enzyme activities were increased under heat stress in chickpea and the greater response was recorded with adequate Zn supply compared to low Zn. Moreover, the application of adequate Zn increased the activities of SOD and APX with high ascorbic acid level under optimal and heat stress conditions in both chickpea types. The maximum SOD (48%), APX activities (15%) and ascorbic acid contents (28%) were recorded under heat stress condition with adequate Zn supply in *desi* chickpea (Fig. 2).

4. Discussion

Crop productivity is largely dependent upon plant nutritional status and environmental stresses. This study demonstrated that drought and heat stresses exacerbate the low Zn supply on photosynthesis of chickpea (Tables 1 and 2). Under drought and heat stresses, the growth and yield of grain legumes is negatively affected due to reduced nutrient supply and biosynthesis of carbohydrates (Farooq et al., 2017a, b). Furthermore, Zn deficiency coupled with drought and heat stress causes growth and yield losses in chickpea by net reduction in photosynthesis (Tables 1 and 2; Khan et al., 2004), PSII function, leaf chlorophyll contents and damage biological membranes (Awasthi et al., 2017; Hussain et al., 2019). Nevertheless, the harmful effects of abiotic stresses on plant photosynthetic traits were counteracted by enhanced Zn supply as chickpea plants receiving adequate Zn exhibited an increase in leaf CO₂ net assimilation rate and photosystem II efficiency under sub-optimal conditions (Tables 1 and 2; Rehman et al., 2019). Under Zn deficiency, the decrease in photosynthetic traits was due to reduction in chlorophyll biosynthesis (Rehman et al., 2019) along with disruption of its biosynthetic pathway and involvement of Zn in chloroplast functioning and development (Hansch and Mendel, 2009). Both chickpea types differ for CO₂ assimilation in response to Zn supply as higher rate of photosynthesis was recorded in *desi* chickpea compared to *kabuli* as *desi* chickpea was more efficient in Zn uptake (Tables 1 and 2), which have resulted in better chloroplast functioning and chlorophyll biosynthesis by involvement of Zn in these processes (Hansch and Mendel, 2009; Rehman et al., 2019).

Under Zn deficiency, Drought and heat stresses decreased the growth of chickpea by decreasing SLA, SDW and plant water relations (Tables 1 and 2) due to disruption of water from xylem to elongating cells and ceased cell division (Farooq et al., 2009, 2017a, b). However,

application of adequate Zn supply increased the SLA, SDW with better RWLC of chickpea under drought and heat stress (Tables 1 and 2) owing to involvement of Zn in chloroplast functioning and development (Hansch and Mendel, 2009). Adequate Zn supply improved the plant biomass and SLA on the account of enhanced leaf photosynthesis, RLWC, shoot Zn concentration and antioxidant enzymes activities under sub-optimal conditions (Tables 1 and 2; Figs. 1–2; Rehman et al., 2019; Faran et al., 2019). In case of interaction of Zn supply and chickpea types, a better response was recorded in *desi* chickpea under both sub-optimal and optimal conditions compared with *kabuli* as *desi* chickpea was more efficient in Zn uptake which helped in better photosynthetic rate, biomass production and leaf expansion under normal and stressful growth conditions (Tables 1 and 2; Rehman et al., 2019; Faran et al., 2019).

Deficiency of Zn along with abiotic stresses (drought and heat) damage the lipid membranes as was evident from enhanced leaf malondialdehyde (MDA) contents under abiotic stresses coupled with Zn deficiency (Tables 1 and 2). Under stressful growth conditions, the heat and drought stress cause over-production of reactive oxygen species (ROS), damage cellular membranes (Faran et al., 2019; Hussain et al., 2019; Rehman et al., 2019) and water relations (Sehgal et al., 2017). Abiotic stresses (drought and heat) increase the activity of antioxidant enzymes and in the present study, SOD activity was substantially increased in both chickpea types. However, SOD is a Zn dependent enzyme and its activity was low in plants with limited Zn supply (Figs. 1 and 2; Rehman et al., 2019). Likewise, sufficient Zn supply increased the APX activities, ascorbic acid and free proline level in chickpea under sub-optimal conditions (Figs. 1 and 2) as enhanced Zn supply increased the activities of APX (Li et al., 2013). Furthermore, increased activities of antioxidant enzymes and secondary metabolites in adequate Zn plants of chickpea were due to involvement of Zn in enzyme activation, protein synthesis and gene regulation (Rehman et al., 2018). Among the chickpea types, *kabuli* chickpea is more vulnerable to ROS damage compared with *desi* as *desi* chickpea was more efficient in Zn uptake and accumulation of free proline under sub-optimal conditions which helped in better antioxidant activities and membrane stability (Tables 1 and 2).

The activity of these antioxidants were impeded under low Zn supply (Figs. 1 and 2), showing the enhanced sensitivity of chickpea to drought and heat stress due to low capacity to detoxify ROS. Under Zn deficiency, the activity of SOD-ZnCu decreases and the production of free radicals increases which induces damage to the plant tissues (Marschner, 1995; Rehman et al., 2019). Sufficient Zn supply improved the shoot Zn concentration in chickpea under sub-optimal conditions (Tables 1 and 2) as adequate supply enhanced the Zn uptake and its translocation towards root and shoot (Rehman et al., 2019). Moreover, high intrinsic Zn in stressed plants augmented the antioxidant enzymes activity particularly SOD which helped in reducing the ROS damage (Rehman et al., 2019) as was observed in this study. Moreover, increase in free leaf proline with adequate Zn supply under abiotic stresses (Tables 1 and 2) protects the plants against ROS damage (Farooq et al., 2018a, b) and improved tolerance in chickpea against heat and drought stresses by maintaining SLA (Tables 1 and 2), CO₂ assimilation and efficiency of PSII (Tables 1 and 2) by involvement of Zn in chlorophyll biosynthesis and carbonic anhydrase activity (Cakmak, 2000).

5. Conclusions

Drought and heat along with Zn deficiency reduced the chickpea growth by limiting the photosynthesis, water relation, specific leaf area, biomass production and enzymes activity. However, sufficient Zn supply under abiotic stresses improved the chickpea growth, and photosynthesis by regulating the PSII efficiency, improving the water relations, leaf free proline, antioxidant enzymes activity (SOD, and APX) and leaf Zn concentration. *Desi* chickpea was more efficient in Zn uptake and accumulation of free proline and performed better than *kabuli*

under both optimal and sub-optimal conditions.

Contribution

A. Ullah and L. Romdhane conducted the experiment and collected data. A. Ullah and A. Rehman prepared the first draft of manuscript. M. Farooq planned the study and finalized the final draft of manuscript.

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References

- Ahlawat, I., Gangaiah, B., Zahid, M.A., 2007. Nutrient management in chickpea. In: Yadav, S.S., Redden, R., Chen, W., Sharma, B. (Eds.), *Chickpea Breeding and Management*. CAB International, Wallingford, Oxon, United Kingdom, pp. 213–232.
- Ahmad, F., Gaur, P., Croser, J., 2005. Chickpea (*Cicer arietinum* L.). In: Singh, R., Jauhar, P. (Eds.), *Genetic Resources, Chromosome Engineering and Crop Improvement-Grains Legumes*. CRC Press, USA, pp. 185–214.
- Awasthi, R., Kaushal, N., Vadez, V., Turner, N.C., Berger, J., Siddique, K.H.M., Nayyar, H., 2014. Individual and combined effects of transient drought and heat stress on carbon assimilation and seed filling in chickpea. *Funct. Plant Biol.* 41, 1148–1167.
- Awasthi, R., Gaur, P., Turner, N.C., Vadez, V., Siddique, K.H.M., Nayyar, H., 2017. Effects of individual and combined heat and drought stress during seed filling on the oxidative metabolism and yield of chickpea (*Cicer arietinum*) genotypes differing in heat and drought tolerance. *Crop Pasture Sci.* 68, 823–841.
- Bagci, S.A., Ekiz, H., Yilmaz, A., Cakmak, I., 2007. Effects of zinc deficiency and drought on grain yield of field-grown wheat cultivars in Central Anatolia. *J. Agron. Crop Sci.* 193, 198–206.
- Barr, H.D., Weatherley, P.E., 1962. A re-examination of the relative turgidity technique for estimating water deficit in leaves. *Aust. J. Biol. Sci.* 15, 413–428.
- Bates, L.S., Waldre, R.P., Teare, I.D., 1973. Rapid determination of free proline for water stress studies. *Plant Soil* 39, 205–208.
- Cakmak, I., 2000. Possible roles of zinc in protecting plant cells from damage by reactive oxygen species. *New Phytol.* 146, 185–205.
- Candan, N., Cakmak, I., Ozturk, L., 2018. Zinc-biofortified seeds improved seedling growth under zinc deficiency and drought stress in durum wheat. *J. Plant Nutr. Soil Sci.* 181, 388–395.
- Devasirvatham, V., Gaur, P.M., Mallikarjuna, N., Tokachichu, R.N., Trethowan, R.M., Tan, D.K.Y., 2012. Effect of high temperature on the reproductive development of chickpea genotypes under controlled environments. *Funct. Plant Biol.* 39, 1009–1018.
- Farooq, M., Ullah, A., Lee, D.J., Alghamdi, S.S., Siddique, K.H.M., 2018a. *Desi* chickpea genotypes tolerate drought stress better than *kabuli* types by modulating germination metabolism, trehalose accumulation, and carbon assimilation. *Plant Physiol. Biochem.* 126, 47–54.
- Farooq, M., Ullah, A., Lee, D.J., Alghamdi, S.S., 2018b. Terminal drought-priming improves the drought tolerance in *desi* and *kabuli* chickpea. *Int. J. Agric. Biol.* 20, 1129–1136.
- Farooq, M., Gogoi, N., Barthakur, S., Baroowa, B., Bharadwaj, N., Alghamdi, S.S., Siddique, K.H.M., 2017a. Drought stress in grain legumes during reproduction and grain filling. *J. Agron. Crop Sci.* 203, 81–102.
- Farooq, M., Nadeem, F., Gogoi, N., Ullah, A., Alghamdi, S.S., Nayyar, H., Siddique, K.H.M., 2017b. Heat stress in grain legumes during reproductive and grain-filling phases. *Crop Pasture Sci.* 68, 985–1005.
- Farooq, M., Wahid, A., Kobayashi, N., Fujita, D., Basra, S.M.A., 2009. Plant drought stress: effects, mechanisms and management. *Agron. Sustain. Dev.* 29, 153–188.
- Faran, M., Farooq, M., Rehman, A., Nawaz, A., Saleem, M.K., Ali, N., Siddique, K.H.M., 2019. High intrinsic seed Zn concentration improves abiotic stress tolerance in wheat. *Plant Soil* 437, 195–213.
- George, E., Rolf, S., John, R., 2013. *Methods of Soil, Plant, and Water Analysis. A Manual for the West Asia and North Africa Region*. International Center for Agricultural Research in the Dry Areas (ICARDA), pp. 244.
- Giannopolitis, N., Ries, S.K., 1977. Superoxide dismutase. I. Occurrence in higher plants. *Plant Physiol.* 59, 309–314.
- Gunes, A., Cicek, N., Inal, A., Alpaslan, M., Eraslan, F., Guneri, E., Guzelordu, T., 2006. Genotypic response of chickpea (*Cicer arietinum* L.) cultivars to drought stress implemented at pre- and post-anthesis stages and its relations with nutrient uptake and efficiency. *Plant Soil Environ.* 52, 368–376.
- Hall, A.E., 2001. *Crop Responses to Environment*. CRC Press, Boca Raton, FL.
- Hansch, R., Mendel, R.R., 2009. Physiological functions of mineral micronutrients (Cu, Zn, Mn, Fe, Ni, Mo, B, Cl). *Curr. Opin. Plant Biol.* 12, 259–266.
- Heath, R.L., Packer, L., 1968. Photoperoxidation in isolated chloroplast. I. Kinetics and stoichiometry of fatty acid peroxidation. *Arch. Biochem. Biophys.* 125, 189–198.
- Hussain, H.A., Men, S., Hussain, S., Chen, Y., Ali, S., Zhang, S., Zhang, K., Li, Y., Xu, Q., Liao, C., Wang, L., 2019. Interactive effects of drought and heat stresses on morpho-physiological attributes, yield, nutrient uptake and oxidative status in maize hybrids. *Sci. Rep.* 9, 3890. <https://doi.org/10.1038/s41598-019-40362-7>.

- Intergovernmental Panel on Climate Change (IPCC), 2013. Climate change the physical science basis. In: Collins, M., Knutti, R. (Eds.), Contribution of IPCC Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, UK, pp. 1–27.
- Kaushal, N., Awasthi, R., Gupta, K., Gaur, P., Siddique, K.H.M., Nayyar, H., 2013. Heat-stress induced reproductive failures in chickpea (*Cicer arietinum* L.) are associated with impaired sucrose metabolism in leaves and anthers. *Funct. Plant Biol.* 40, 1334–1349.
- Khan, H., McDonald, G., Rengel, Z., 2004. Zinc fertilization and water stress affects plant water relations, stomatal conductance and osmotic adjustment in chickpea (*Cicer arietinum* L.). *Plant Soil* 267, 271–284.
- Li, M., Yang, X.W., Tian, X.H., Wang, S.X., Chen, Y.L., 2013. Effect of nitrogen fertilizer and foliar zinc application at different growth stages on zinc translocation and utilization efficiency in winter wheat. *Cereal Res. Commun.* 42, 81–90.
- Liu, H.E., Wang, Q.Y., Rengel, Z., Zhao, P., 2015. Zinc fertilization alters flour protein composition of winter wheat genotypes varying in gluten content. *Plant Soil Environ.* 61, 195–200.
- Lopez-Millan, A.F., Ellis, D.R., Grusak, M.A., 2005. Effect of zinc and manganese supply on the activities of superoxide dismutase and carbonic anhydrase in *Medicago truncatula* wild type and raz mutant plants. *Plant Sci.* 168, 1015–1022.
- Marschner, H., 1995. Mineral Nutrition of Higher Plants. Academic Press London.
- Nakano, Y., Asada, K., 1981. Hydrogen peroxide is scavenged by ascorbate specific peroxidase in spinach chloroplasts. *Plant Cell Physiol.* 22, 867–880.
- Piha, M.L., Munns, D.N., 1987. Sensitivity of the common bean (*Phaseolus vulgaris* L.) symbiosis to high soil temperature. *Plant Soil* 98, 183–194.
- Pisoschi, A.M., Danet, A.F., Kalinowski, S., 2008. Ascorbic acid determination in commercial fruit juice samples by cyclic voltammetry. *J. Anal. Methods Chem.* <https://doi.org/10.1155/2008/937651>.
- Rahbarian, R., Khavari-Nejad, R., Ganjeali, A., Bagheri, A., Najafi, F., 2011. Drought stress effects on photosynthesis, chlorophyll fluorescence and water relations in tolerant and susceptible chickpea genotypes. *Acta Biol. Cracov. Bot.* 53, 47–56.
- Rehman, A., Farooq, M., Asif, M., Ozturk, L., 2019. Supra-optimal growth temperature exacerbates adverse effects of low Zn supply in wheat. *J. Plant Nutr. Soil Sci.* <https://doi.org/10.1002/jpln.201800654>.
- Rehman, A., Farooq, M., Ozturk, L., Asif, M., Siddique, K.H.M., 2018. Zinc nutrition in wheat-based cropping systems. *Plant Soil* 422, 283–315.
- Samarah, N.H., Alqudah, A.M., Amayreh, J.A., McAndrews, G.M., 2009. The effect of late-terminal drought stress on yield components of four barley cultivars. *J. Agron. Crop Sci.* 195, 427–441.
- Sehgal, A., Sita, K., Kumar, J., Kumar, S., Singh, S., Siddique, K.H.M., Nayyar, H., 2017. Effects of drought, heat and their interaction on the growth, yield and photosynthetic function of lentil (*Lens culinaris* Medikus) genotypes varying in heat and drought sensitivity. *Front. Plant Sci.* 8, 1776.
- Serraj, R., 2003. Effects of drought stress on legume symbiotic nitrogen fixation: physiological mechanisms. *Indian J. Exp. Biol.* 41, 1136–1141.
- Steel, R.G.D., Torrie, J.H., Dicky, D.A., 1997. Principles and Procedures of Statistics, a Biometrical Approach, third ed. McGraw Hill, Inc. Book Co, N.Y, pp. 352–358.
- Ullah, A., Farooq, M., Hussain, M., 2019. Improving the productivity, profitability and grain quality of kabuli chickpea with co-application of zinc and endophyte bacteria enterobacter sp. strain MN17. *Arch. Agron Soil Sci.* <https://doi.org/10.1080/03650340.2019.1644501>.