

ENRICHMENT OF WHEAT BY ZINC FERTILIZER, MYCORRHIZA AND PREHARVEST DROUGHT STRESS

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ABSTRACT

A field experiment was conducted to investigate the effects of Zn and mycorrhiza on enrichment possibility of wheat (*Triticum aestivum* L.) under preharvest drought stress condition. The treatments included irrigation (A₁: normal irrigation, A₂: preharvest drought stress) as the main plots and the combinations of zinc (Zn₁: 0 kg ha⁻¹ and Zn₂: 2 kg ha⁻¹) and mycorrhiza (Mc₁: 0 kg ha⁻¹ and Mc₂: 200 kg ha⁻¹) as the subplots. The results indicated that grain phosphorus, grain phytic acid, phytic acid to zinc ratio, grain yield and grain filling period decreased under drought stress condition. A significant reduction was observed in grain phosphorus, grain phytic acid, phytic acid (Phy) to zinc ratio and grain filling rate with application of Zn (2 kg ha⁻¹). Grain zinc, protein, yield and final grain weight were higher at 200 kg ha⁻¹ mycorrhiza than the control (0 kg ha⁻¹). Finally, considering drought crisis in some developing countries, the treatment of preharvest drought stress with Zn (2 kg ha⁻¹) saved 450 m³ha⁻¹ of water, produced the second highest grain yield, the highest protein content and the lowest phytic acid and Phy/Zn ratio. Therefore, this treatment can be recommended as a suitable and practical approach to improve wheat grain yield and quality.

Keywords: Foliar application, fortification, grain phosphorus, irrigation, micronutrient

INTRODUCTION

Burgeoning population together with low quantity and quality of the crops in the developing countries requires another Green Revolution (Huang et al., 2002). Wheat (*Triticum aestivum* L.) is an important grain crop, a permanent nutrient source in most developing countries and the third major cereal crop in the world, following maize and rice from production point of view (FAO, 2013). The nutritional value of wheat is extremely important as it takes an important place among the few crop species being extensively grown as staple food sources (Sramková et al., 2009). Improvement of wheat quality i.e. increasing grain micronutrients such as zinc and decreasing phytic acid content and phytic acid to zinc ratio, can play an important role in improving public health of the communities suffering from malnutrition. Wheat flour with high content of phytic acid which forms insoluble complexes with minerals such as Ca²⁺, Fe³⁺, Zn²⁺ and Mg²⁺ reduces their bioavailability (Sramková et al., 2009). The role of zinc in reduction of the phosphorous content and subsequently reduction of phytic acid and phytic acid to zinc ratio in wheat grain have been well documented (Zhang et al., 2012). According to WHO (1996), phytic acid to zinc ratio must be ≤12 for the

human diet. Zhang et al. (2012) reported that foliar application of Zn as ZnSO₄ increased wheat grain zinc concentration and decreased the phytic acid content. In a study by Jones et al. (2005), drought stress limited phosphorous accumulation and reduced phosphorous (P) translocation to the seed.

Arbuscular mycorrhiza (AM) fungi are widespread in soils and form symbiotic (and frequently mutualistic) associations with many plant species. Wheat responses to AM fungi in a highly calcareous soil differ from those of clover, and change with plant development and P supply (Li et al., 2005). Wheat also may show growth depressions in response to AM colonisation, at least during vegetative development (Graham and Abbott, 2000; Zhu et al., 2003; Li et al., 2005). Absorption of zinc, iron, nitrogen, potassium, phosphorus and copper in saline soil condition was improved by arbuscular mycorrhiza in wheat and tomato (Al-Karaki, 2006; Daei et al., 2009). Fortification of wheat grain (reduction of phytic acid, rising of zinc) naturally in field has received little attention. Therefore, the present study was undertaken with the aim of assessing the effects of zinc and mycorrhiza treatments on growth, yield and improvement of wheat grain quality under preharvest drought stress condition.

MATERIALS AND METHODS

Plant materials, treatments and experimental design

Two-year experiments (2014 and 2015) were carried out in research farm of Shahid Bahonar University of Kerman, Iran with average annual rainfall of 130 mm, and minimum and maximum annual temperatures of -8 °C and 40 °C, respectively. Evapotranspiration rates in 2014 and 2015 were reported 2421.8 mm and 2409.1 mm, respectively (measured with class A evaporation pan). Soil physical and chemical properties are shown in Table 1. To determine these properties a sample composed of 5 sub-samples was taken to a depth of 30 cm by a zig-zag

pattern providing a uniform distribution of sampling site. The soil texture was loam and considering the amount of organic carbon, soil water retention capacity was low to medium. Wheat seeds (400 seeds m⁻²), cv. Pishtaz were sown in 6 rows with a row distance of 20 cm and a row length of 6 m. The experiments were arranged as split factorial based on randomized complete block design with three replications. Two levels of irrigation (A₁: normal irrigation, A₂: preharvest drought stress in which irrigation withholds in the early milk development stage, Z.G.S.73) were considered as the main plots and the combinations of zinc (Zn₁: 0 kg ha⁻¹ and Zn₂: 2 kg ha⁻¹) and mycorrhiza (Mc₁: 0 kg ha⁻¹ and Mc₂: 200 kg ha⁻¹) as the subplots.

Table 1. Soil physical and chemical properties

Depth (cm)	clay	silt	sand	Fe	Zn	K	P	OC	PH	EC
	%			Plant available (mg kg ⁻¹)				%		(ds m ⁻¹)
0-30	16	34	50	1.4	0.46	340	14	0.40	8.1	3.9

Water used in each irrigation time measured by Parshall flume model SV: 530 m³ ha⁻¹ for the first irrigation due to soil dryness and 450 m³ ha⁻¹ for the subsequent ones. A number of irrigation times were 14 and 15 for preharvest drought stress and normal irrigation, respectively. Based on soil analysis there was no need to use P and K fertilizers, while 140 kg N ha⁻¹ was applied in two stages using urea fertilizer. Zinc was applied as foliar spray in two growth stages (at the beginning of stem elongation and a week later). *Arbuscular mycorrhiza* inoculums (specified for wheat) was incorporated into the soil just before sowing the seeds (200 kg ha⁻¹).

Measurements of zinc, phosphorus, phytic acid, phytic acid to zinc ratio, protein and the yield of grain

Plants were harvested at maturity and grain samples of 1.5 kg were taken from each plot. Grain samples were washed with distilled water to remove the surface contamination. Then the samples were ground and dried at 70 °C for 48 h. One gram of each sample was incubated for 12 hours in solution of HNO₃. After that, the samples were digested under heat in di-acid mixture of HNO₃: HClO₄ (10:1 v/v) (Pandey et al., 2012). Zinc concentration in grains was determined by atomic absorption spectrophotometer. Protein was determined through measuring of the total nitrogen content by Kjeldahl method. Then the specific conversion factor (5.7) was used to calculate the amount of protein (Jiao et al., 2007). The amount of phytate was measured based on ion exchange resin chromatography (Liang et al., 2007) and P content was determined colorimetrically as described by Kitson and Mellon (1944).

Grain filling process

In order to study the grain growth process, samples were taken at five different occasions from line 3 of each plot. At each sampling, 10 spikes, identified by color ribbon at the time of the appearance, were harvested and spikes 5-9, counting from the base, were plucked. From

each spike two of the seeds that were closest to the main axis were selected; 100 seeds in total were separated and weighed after drying. First sampling took place when growth stage was at ZGS70; four more samplings were followed over 4-day intervals; hence all samples were taken during the linear growth stage. The data was fitted using linear regression to plot grain-weight versus time, (Y= a + bx). Gradient of this regression line (b) was considered as the grain filling rate criterion. Grain filling duration was computed by dividing the final weight of the main spike, at the time of processing, to grain filling speed.

Statistical analysis

Combined analysis of variance (ANOVA) was performed using SAS (ver. 9.2, 2010). The means of treatments were compared by the protected least significant difference (LSD) procedure and Duncan's Multiple Range Test both at *P* < 0.05.

RESULTS

Grain zinc content

The combined analysis of two years experimental data showed that the effects of zinc and mycorrhiza treatments on grain zinc were significant (*p* < 0.01; Table 2). The effects of irrigation and year were non-significant on this trait (Table 2). The highest grain zinc concentration was obtained from Zn treatment, 33.9 mg kg⁻¹ (Table 2). Zinc application resulted in increasing 22.83% of grain zinc compared with Zn absence treatment (Table 2). Application of 200 kg ha⁻¹ of mycorrhiza also caused increase in the zinc concentration of wheat grain (Table 2), but to a lesser extent compared with Zn application. Interactions of irrigation × Zn (Table 3) and Zn × mycorrhiza (Table 5) affected this trait significantly while irrigation × mycorrhiza (Table 4) did not. Zinc content of grain in preharvest drought stress with the application of Zn (A₂Zn₂) increased by 19.67%, compared with drought stress treatment without application of Zn (A₂Zn₁) (Table

3). Significant increase in zinc content was observed with application of mycorrhiza together with Zn (Z₂Mc₂) compared with the control (Zn₁Mc₁) (Table 5).

Grain phosphorous content

Simple effects of experimental treatments (irrigation, zinc and mycorrhiza) on grain phosphorous content were significant ($p < 0.01$; Table 2). Drought stress caused a significant reduction in phosphorous content (Table 2). The lowest and highest values of phosphorous content were obtained from application of 2 and 0 kg ha⁻¹ of Zn, respectively (Table 2). The same results were observed for

mycorrhiza treatment (Table 2). Zinc applications under both normal irrigation (A₁Zn₂) and preharvest drought stress (A₂Zn₂) conditions resulted in reduction of grain phosphorous content (Table 3). The least amount of grain phosphorous content (0.18%) was recorded for A₂Zn₂ that was 45.5% lower than A₂Zn₁. Mycorrhiza application under drought stress reduced grain phosphorous content to 0.21%. The amount of reduction was 27.6% compared with absence of mycorrhiza under normal irrigation (0.29%; Table 4). Application of mycorrhiza together with zinc also resulted in the lowest phosphorus content (0.21; Table 5).

Table 2. Effects of Drought stress, zinc and mycorrhiza on yield and quality traits of wheat grain

Experiment factors		Zn content (mg kg ⁻¹)	Phosphorus (%)	Phytic acid (g kg ⁻¹)	Phy/Zn	Protein (%)	Grain filling rate (mg day ⁻¹)	Grain filling period (day)	Final grain weight (mg)	Grain yield (kg ha ⁻¹)
Irrigation (A)	Control	31 a	0.29 a	10.55 a	36.96 a	12.4 a	0.588 b	74.31 a	43.92 a	6215 a
	Drought stress	30 a	0.22 b	8.00 b	27.84 b	12.2 a	0.756 a	54.01 b	41.91 a	5202 b
	L.S.	ns	**	**	**	ns	**	**	ns	**
Zinc (Zn)	0 kg ha ⁻¹	27.6 b	0.32 a	10.7 a	41.27 a	11.53 b	0.689 a	62.64 b	39.85 b	5170 b
	2 kg ha ⁻¹	33.9 a	0.22 b	7.9 b	23.57 b	13.18 a	0.654 b	65.96 a	45.99 a	6247 a
	L.S.	**	**	**	**	**	*	*	*	**
Mycorrhiza (Mc)	0 kg ha ⁻¹	29.8 b	0.28 a	2.34 a	34 a	11 b	0.693 a	63.87 a	41.25 b	5076 b
	200 kg ha ⁻¹	31.7 a	0.25 b	2.36 a	30 b	13 a	0.650 b	64.46 a	44.85 a	6340 a
	L.S.	**	**	ns	**	**	*	ns	*	**
Year	2014	30.95	0.262	9.33	31.85	12.42	0.672	64.18	42.92	5727
	2015	30.56	0.263	9.28	32.96	12.28	0.672	64.15	42.91	5689
	L.S.	ns	ns	ns	ns	ns	ns	ns	ns	ns

Means with different letters for each trait denoted statistically difference between treatments according to LSD test ($P < 0.05$). L.S.: level of significance, * $P < 0.05$, ** $P < 0.01$, ns: non-significant,

Table 3. Interactions of irrigation × zinc on yield and quality traits of wheat grain

Experiment factors	Zn content (mg kg ⁻¹)	Phosphorus (%)	Phytic acid (g kg ⁻¹)	Phy/Zn ratio	Protein (%)	Grain filling rate (mg day ⁻¹)	Grain filling duration (day)	Final grain weight (mg)	Grain yield (kg ha ⁻¹)
A ₁ Zn ₁	27.55 b	0.33 a	11.78 a	46.62 a	11.17 c	0.572 b	72.78 a	41.11 ab	5579 ab
A ₁ Zn ₂	34.52 a	0.26 b	9.32 b	27.30 bc	12.93 ab	0.603 b	75.85 a	46.73 a	6850 a
A ₂ Zn ₁	27.75 b	0.27 b	9.63 b	35.91 b	11.88 bc	0.736 a	52.50 b	38.58 b	4760 b
A ₂ Zn ₂	33.21 a	0.18 c	6.42 c	19.77 c	13.42 a	0.775 a	55.53 b	45.25 ab	5643 ab

Means with different letters in each column denoted statistically difference between treatments according to Duncan multiple range test ($P < 0.05$). A₁: normal irrigation A₂: Preharvest drought stress, Zn₁: Zinc not used, Zn₂: Zinc used.

Table 4. Interactions of irrigation × mycorrhiza on yield and quality traits of wheat grain

Experiment factors	Zn content (mg kg ⁻¹)	Phosphorus (%)	Phytic acid (g kg ⁻¹)	Phy/Zn ratio	Protein (%)	Grain filling rate (mg day ⁻¹)	Grain filling duration (day)	Final grain weight (mg)	Grain yield (kg ha ⁻¹)
A ₁ Mc ₁	30.33 a	0.29 a	10.45 a	37.36 a	11.54 b	0.575 c	73.19 a	41.29 a	5634 ab
A ₁ Mc ₂	31.75 a	0.30 a	10.65 a	36.57 a	12.57 ab	0.600 c	75.44 a	46.56 a	6795 a
A ₂ Mc ₁	29.29 a	0.24 b	8.65 b	30.69 ab	12.07 ab	0.726 b	54.55 b	41.22 a	4518 b
A ₂ Mc ₂	31.67 a	0.21 b	7.47 b	24.99 b	13.23 a	0.786 a	53.48 b	42.61 a	5885 a

Means with different letters in each column denoted statistically difference between treatments according to Duncan multiple range test ($P < 0.05$). A₁: Normal irrigation A₂: Preharvest drought stress, Mc₁: Mycorrhiza fungi absence & Mc₂: Mycorrhiza fungi presence

Grain phytic acid content

Effects of irrigation and zinc treatments on grain phytic acid content were significant statistically (Table 2). Mean comparison of irrigation levels showed that phytic acid content decreased by 24.17% at drought stress

condition (Table 2). Phytic acid content decreased from 10.7 g kg⁻¹ to 7.9 g kg⁻¹ with application of zinc (2 kg ha⁻¹; Table 2). Zinc application under both normal and drought stress conditions decreased grain phytic acid and the lowest content belonged to Zn application under preharvest drought stress treatment (Table 3). Mycorrhiza

inoculation caused a reduction of 28.52% in grain phytic acid when it was applied under drought stress condition compared with control (Table 4). Treatment of mycorrhiza with zinc in comparison with no mycorrhiza and no zinc reduced grain phytic acid by 30% while the comparison of former treatment with zinc application alone (Zn_2Mc_1) reduced it by 5.9% (Table 5).

Phytic acid to zinc ratio

Simple effects of experimental treatments (irrigation, zinc and mycorrhiza) on phytic acid to Zn ratio of wheat grain were significant ($p < 0.01$; Table 2). Mean comparison of irrigation levels showed that phytic acid to Zn ratio decreased by 24.67% under drought stress

condition (Table 2). This ratio decreased by 42.88% and 11.76% with application of zinc (2 kg ha^{-1}) and mycorrhiza (200 kg ha^{-1}) treatments, respectively, (Table 2). The lowest value of grain Phytic acid to zinc (19.77) was obtained from application of 2 kg ha^{-1} zinc under drought stress condition (A_2Zn_2). The second lowest of this trait was recorded for application of zinc under normal condition (27.3) (Table 3). Application of mycorrhiza under both irrigation conditions resulted in decreasing of this ratio, while this reduction was only significant for A_2Mc_2 (Table 4). Mycorrhiza reduced this ratio by 9.2% and 48.5% when applied without and with zinc, respectively (Table 5).

Table 5. Interaction of zinc \times mycorrhiza on yield and quality traits of wheat grain

Experiment factors	Zn content (mg kg ⁻¹)	Phosphorus (%)	Phytic acid (g kg ⁻¹)	Phy/Zn ratio	Protein (%)	Grain filling rate (mg day ⁻¹)	Grain filling duration (day)	Final grain weight (mg)	Grain yield (kg ha ⁻¹)
Zn_1Mc_1	26.58 b	0.30 a	10.95 a	43.26 a	10.93 c	0.710 b	61.00 a	37.78 b	4567 b
Zn_1Mc_2	28.72 b	0.29 a	10.45 a	39.27 a	12.12 bc	0.975 a	64.28 a	41.91 ab	5772 ab
Zn_2Mc_1	33.40 a	0.23 b	8.15 b	24.78 b	12.67 ab	0.669 bc	66.74 a	44.73 a	5585 b
Zn_2Mc_2	34.69 a	0.21 b	7.67 b	22.29 b	13.68 a	0.632 c	64.65 a	47.25 a	6909 a

Means with different letters in each column denoted statistically difference between treatments according to Duncan multiple range test ($P < 0.05$). Zn_1 : Zinc not used, Zn_2 : Zinc used, Mc_1 : Mycorrhiza fungi absence & Mc_2 : Mycorrhiza fungi presence.

Grain protein content

Application of zinc (2 kg ha^{-1}) and mycorrhiza (200 kg ha^{-1}) caused significant increase in the grain protein content (Table 2). Effect of irrigation treatment was not significant for this trait (Table 2). The highest value of this trait was obtained under drought stress condition with application of 2 kg ha^{-1} of zinc (Table 3). The same result was observed for mycorrhiza application under the same condition (Table 4). Protein production in zinc \times mycorrhiza treatment was the highest (Table 5). Comparing it with the treatment of no zinc and mycorrhiza, it increased by 25.16%.

Grain filling rate (mg day⁻¹)

The results showed that preharvest drought stress caused a significant increase (28.57 %) in grain filling rate, compared with normal irrigation treatment (Table 2). As presented in Table 2, grain filling rate decreased by 5.35 % and 6.62 % when zinc (2 kg ha^{-1}) and mycorrhiza (200 kg ha^{-1}) were applied, respectively. Zinc application led to a significant increase in the grain filling rate when applied under drought stress condition (Table 3). This trend was also observed for mycorrhiza application (Table 4). Mycorrhiza application led to the increasing of grain filling rate in the absence of zinc, but it decreased grain filling rate when applied with zinc (Table 5).

Grain filling period (day)

The results of combined analysis of variance showed that only the effects of irrigation ($p < 0.01$) and zinc ($p < 0.05$) treatments were significant on the grain filling period (Table 2). Drought stress caused a significant reduction (27.35%) in this trait compared with the normal irrigation (Table 2). Grain filling period significantly

increased from 62.64 to 65.96 day with application of zinc (2 kg ha^{-1} ; Table 2). Application of zinc under both normal irrigation and drought stress increased grain filling period by 3 days which was insignificant when compared with their respective control, while A_2Zn_2 reduced this trait by 17 days that was significant in comparison with A_1Zn_1 (Table 3). The minimum grain filling period was obtained by mycorrhiza application with preharvest drought stress (Table 4).

Final grain weight (mg)

Application of zinc (2 kg ha^{-1}) and mycorrhiza (200 kg ha^{-1}) significantly caused an increase in the final grain weight (Table 2). Effect of irrigation treatment was not significant for this trait (Table 2). Applying zinc under both normal and drought stress conditions increased final grain weight. The amount of increase in final grain weight under drought stress condition was higher than the normal condition (Table 3). Increase in final grain weight due to mycorrhiza application was only 12.8 % under normal irrigation condition that was not significant (Table 4). Application of mycorrhiza together with zinc produced the highest final grain weight (47.25 mg). The comparison of Zn_2Mc_2 with no application of zinc and mycorrhiza showed that the final grain weight increased by 25.1% (Table 5).

Grain yield

The results showed that effects of irrigation, zinc and mycorrhiza treatments on grain yield were significant (Table 2). Drought stress caused a significant decrease (16.3%) in this trait, compared with the normal irrigation (Table 2). Grain yield increased by 20.85 kg ha^{-1} and 24.9 kg ha^{-1} when zinc (2 kg ha^{-1}) and mycorrhiza (200 kg ha^{-1}) were applied, respectively (Table 2). The interactions of

irrigation \times zinc, irrigation \times mycorrhiza, and zinc \times mycorrhiza were significant for grain yield (Tables 3, 4 and 5). The highest grain yield (6850 kg ha⁻¹) was obtained with the application of 2 kg ha⁻¹ zinc with normal irrigation (A₁Zn₂; Table 3). Zinc application under drought stress condition also caused an increase in the grain yield compared with its respective control (A₂Zn₁; Table 3). Mycorrhiza applied under both irrigation conditions increased grain yield but the increase was significant only when they were compared with drought stress condition without mycorrhiza (Table 4). Grain yield increased when Zn applied together with mycorrhiza (Table 5). Comparing this latter treatment with no application of mycorrhiza and zinc treatment, the amount of increase was 51.3% (Table 5).

DISCUSSION

Wheat is staple food of many parts of the world. Studies have shown that the use of fertilizers containing iron and zinc improves Fe and Zn concentrations in wheat grain (Zhang et al., 2012). Foliar application of Zn significantly caused an increase of zinc content in the wheat grain (Table 2). Similar results have been reported on wheat in other researches (Cakmak et al., 2010; Yadav et al., 2011; Zhang et al., 2012). Enriching wheat grain by zinc can eliminate problem of zinc deficiency that is rising in the societies of developing countries (Jorge et al., 2008). The majority of inorganic phosphorus (40-80%) in the seeds of higher plants like oilseeds, cereal grains and legumes is stored as phytic acid or phytate which forms some complexes with minerals such as Ca²⁺, Fe³⁺, Zn²⁺ and Mg²⁺ reducing their bioavailability (Šramková et al., 2009). Thus, reducing the amount of seed phosphorus can result in reduction of phytic acid. In this study, A₂Zn₂ and A₂Mc₂ reduced the amount of phosphorus in wheat grain significantly (Table 3 and 4). This reduction was previously reported in barley (Jones et al., 2005). Under drought stress, phosphorus accumulation in roots of pea has also been reported (Murray and Collier, 1977). This accumulation is associated with the increase of phosphatase concentration. Phosphate can be co-transported along H⁺ gradient and the result is an osmotic potential inside the cell (Sakano et al., 1992). This process can increase phosphorus accumulation in roots and prevents its transfer to seeds and subsequently reduces the production of phytic acid. Application of zinc or mycorrhiza in the preharvest drought stress treatment reduced the content of grain phosphorus more than the preharvest drought stress alone (Table 2, 3 and 4). Enhancing zinc uptake in wheat by mycorrhiza application reduced P uptake and disrupted its accumulation in seeds (Daei et al., 2009). This can be due to the increased of roots surface area and the number of lateral roots. Reduction of the grain phosphorus resulted in decreased grain phytic acid concentrations. For preharvest drought stress treatments with Zn and mycorrhiza, grain phytic acid reduced to 6.42, 7.47 g kg⁻¹, respectively (Table 3 and 4). Since phytic acid declines bioavailability of some nutrients, it seems essential to reduce its concentration in wheat grains. Irrigation after seed formation enhances

seed phosphorus content and therefore, it raises the accumulation of phytic acid in seeds. Thus it is illustrated why all the treatments under preharvest drought stress had lower phytic acid than their respective treatments under normal irrigation. The consumption of Zn with drought stress increased grain Zn and decreased its P content and phytic acid concentration in wheat and corn grain, respectively (Jones et al., 2005). By reducing phytic acid and increasing Zn concentration in seed, phytic acid to Zn ratio decreased substantially under both irrigation condition (Table 2, 3 and 4). This ratio is one of the criteria used to evaluate the quality and enrichment of wheat grain. The results showed that A₂Zn₂ reduced phytic acid to Zn ratio to 19.77 (Table 3). The obtained ratio is almost equal to 20, the value set for human diet reported by Weaver and Kannan (2002). Enriched wheat along with low level of phosphorus and phytic acid eliminates the need to separate bran. Another quality assessment index is grain protein content that has a significant correlation with Zn and mycorrhiza bioavailability. Zn and mycorrhiza bioavailability and uptake improve grain protein content. In this study, the addition of Zn and mycorrhiza increased grain protein significantly (Table 2). It has been reported that Zn addition increased the protein content while Zn deficiency had a reverse effect (Römheld and Marschner, 1991). The positive effect of mycorrhiza inoculation on protein content of wheat grain (14.83%) was reported under salinity and drought stresses by Daei et al. (2009). Their results were explained by the expansion of surface area of the plant root system, nutrient bioavailability and uptake by mycorrhiza. In present study, Zn₁Mc₁ increased grain yield to 51.3% compared to the control (Table 5). This result may be due to the increased chlorophyll, indole acetic acid, photosynthesis rate and finally the increased dry matter weight (Khan et al., 2010). Final grain weight as a yield component of grain in wheat is determined by rate and duration of grain filling. Time to maturity is determined by the length of grain filling period. A thorough understanding of the grain filling process and its influence on grain weight and maturity may help breeders to increase grain yield and decrease time to maturity in wheat. Final grain weight is a function of both the rate and duration of grain filling (Van Sanford and Mackown, 1985; Bruckner and Froberg, 1987). In this study, drought stress substantially increased grain filling rate, decreased grain filling duration and insignificantly decreased final grain weight. During grain filling, drought stress counterbalances the diminished duration of grain filling, by increasing the filling rate. Under drought stress condition the end of grain filling took place earlier, resulting in a 20-day reduction in its grain filling duration (Table 2) in comparison to normal irrigation. It was found that wheat exposure to zinc under both normal and drought conditions increased grain filling rate and final grain weight and hastened physiological maturity (shortened the grain filling period). Grewal and Williams (2000) showed that plants supplied with adequate Zn nutrition were better able to cope with both water stress and excessive soil moisture. Although application of zinc under normal irrigation (A₁Zn₂)

produced the highest yield, considering the fact that cultivation of wheat in arid and semi-arid regions of the world is facing the terminal stresses such as drought, the application of zinc under drought stress (A_2Zn_2 treatment) was recognized as the most suitable treatment. It is due to the production of second highest yield, the highest protein, the lowest P content, phytic acid and phytic acid to Zn ratio (as quantitative and qualitative criteria) and notably saving $450\text{ m}^3\text{ha}^{-1}$ of precious water.

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