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ZINC REQUIREMENT FOR OPTIMUM GRAIN YIELD AND ZINC BIOFORTIFICATION DEPENDS ON PHOSPHORUS APPLICATION TO WHEAT CULTIVARS

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ABSTRACT

Human bioavailable zinc (Zn) in cereal grains depends on contents of Zn and phosphorus (P) in grains because these two nutrients interact for their concentration in wheat grains. In the present study, a pot experiment was conducted to investigate the effect of various levels of P (0, 60 and 120 mg kg^{-1} soil) and Zn (0 and 6 mg kg^{-1} soil) on yield and grain Zn contents of four wheat cultivars i.e. Shafaq-2006, Auqab-2000, Pasban-90 and Pak-81. Grain yield of all tested wheat cultivars was increased by the application of Zn and P, but at variable rate. Calculations of deficiency stress factors for P and Zn confirmed higher demand of one nutrient at a higher application rate of the other nutrient. Concentration ratio of P to Zn in wheat grains was influenced by Zn application especially at higher rates of P application. Results showed that Zn application aiming at optimum grain yields and grain Zn concentration depends on genotypic variation in wheat cultivars and rate of P.

Key words: calcareous soils, phosphorus, zinc biofortification, wheat grain.

INTRODUCTION

Zinc (Zn) is essential element required by humans and all higher plants. However, there is a widespread deficiency of this element in agricultural soils and in humans (Alloway, 2009).

Human Zn deficiency in Pakistan and other wheat consuming developing countries is thought to be due to the inherent concentrations of Zn in wheat grains lower than generally required to fulfill human requirements (Cakmak, 2008). The situation becomes much more complex when large quantities of P fertilizers are applied to soils that increase not only the grain yield but also the phytate concentration in wheat grains: high amounts of phytate in human diet decreases bioavailability of Zn and other metals for human absorption (Ryan et al., 2008; Bouis et al., 2011).

In Pakistan and many other countries, nutrient scarcity is one of the major factors which limit wheat production, due to large amount of calcium carbonate in soils (calcareousness of soils), high soil pH and low organic matter contents (Broadley et al., 2007; Singh et al., 2005). The most affected nutrients by these soil problems are Zn and P. Out of total P fertilizer applied to soil, only 10-15% is available for plants, while the remaining added P is adsorbed by soil particles or precipitated with calcium (Ca) or magnesium (Mg) compounds and becomes unavailable for plants (Rahmatullah et al., 1994). Plants have developed different mechanisms to survive under P deficiency. Modifications in length, morphology and architecture of roots (Dinkelaker et al., 1995; Lynch and Brown, 2001) along with exudation of proton organic acids (Neumann and Roemheld, 1999; Ryan and Delhaize,

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2001; Aziz et al., 2011a) are the key plant adaptations studied by scientists. The adaptations are related to higher nutrient use efficiencies. Researchers have also suggested nutrient efficient genotypes to economically deal with low nutrient status of soils. The efficiency of certain genotype for a particular nutrient depends on higher uptake from deficient soil, better translocation to growing parts and efficient utilization of absorbed nutrients within plant body (Welch and Graham, 2005).

Similar to P, genotypic differences in uptake and utilization efficiencies of Zn are also reported in different cultivars of crop plants (Irshad et al., 2004). Genotypes more resistant to Zn deficiency had higher Zn uptake rates and lower affinity for Zn than genotypes more susceptible to Zn deficiency (Clark, 1990). Efficient genotypes can increase Zn translocation to the shoot and regulate Ca, Cu, Fe, Mg, and P transport in order to maintain balanced nutrient ratios with respect to Zn (Cayton et al., 1985).

Most of the times, scientists have screened efficient crop genotypes based on only one nutrient (Fageria, 2001; Yaseen and Malhi, 2009; Maqsood et al., 2009). However, multiple nutrients disorder exists in soils; for example P and Zn deficiency is common feature of calcareous soils. The situation could be much more complex when the deficient nutrients interact with each other as nutrient interactions in crop plants are of significant importance for better crop yields. For example, P can stimulate lateral roots growth and Zn can influence P metabolism in the roots and other plant parts (Brady and Weil, 2008). Phosphorus and Zn interaction has been reported in the roots as they prohibit translocation of each other to the upper parts of the plant (Khan and Zende, 2004). In soil solutions and plants, P can bind to Zn thus forming insoluble zinc–phosphate complexes. This in turn would inhibit both the uptake of Zn by the root and the movement of Zn in the plant. Enhanced P fertilization can reduce Zn uptake but increased plant P uptake thus causing Zn deficiency (Zhao et al., 2007).

Widespread human Zn deficiency is thought to be related with low human bioavailability of Zn in wheat grains. Zinc in wheat grains compels with a P storing compound, phytate, which decreases its availability for intestinal absorption in humans. Therefore, variation in grain Zn concentration and concentration ratio of P: Zn in wheat grains is needed to be studied at various rates of Zn and P application. Present study was conducted to determine the genetic variation in grain yield and mineral (P and Zn) concentration in whole grains of four contrasting wheat cultivars supplied with different rates of Zn and P in a calcareous soil.

MATERIAL AND METHODS

Soil sample (0-15 cm depth) was collected from the Experimental Farm of University of Agriculture, Faisalabad. Soil, after passing through a 2-mm sieve, was then air-dried and well-grounded. Various soil properties were estimated in a representative sub sample of the soil. Hydrometer method (Gee and Bauder, 1986) was used to determine soil texture. Calomel glass electrode was used to evaluate the pH of saturated soil paste (pH_s 7.92). Saturated soil paste extract had electrical conductivity of 2.46 dS m^{-1}. Walkley-Black method (Nelson and Sommers, 1982) was used to determine soil organic matter (7.5 g kg^{-1} soil). Acid dissolution (Allison and Moodie, 1965) was used to estimate the free lime (calcium carbonate) in soil (5.0 g kg^{-1} soil). Plant available Zn from the soil was extracted by 0.005 M di-ethylenetriaminepentaacetic acid (DTPA) (Lindsay and Norvell, 1978). Zinc in the aliquot was measured by using an atomic absorption spectrophotometer (AAS) (*Perkin Elmer, 100 AAanalyst,* Waltham, USA), was 0.44 mg Zn kg^{-1} soil. Extractable P in the soil, 9.5 mg kg^{-1}, was measured on a spectrophotometer (Shimadzu, UV–1201, Kyoto, Japan) by following blue color method (Olsen and Sommers, 1982) after extracting with 0.5 M sodium bicarbonate solution (Olsen et al., 1954).

5 kg soil was filled in each of total 72 pots. Different treatments consisting of two Zn rates (0 and 6 mg Zn kg^{-1} soil), three P rates (0, 60 and 120 mg P kg^{-1} soil) and four wheat

cultivars (Shafaq-2006, Auqab-2000, Pasban-90 and Pak-81) were arranged according to 3-factorial CRD (Steel et al., 1997). Zinc as hydrated Zn sulfate and P as ammonium di-hydrogen phosphate were applied to soil in solution form. Basal uniform rates of 54 mg N and 60 mg K kg^{-1} soil were applied as $(NH_2)_2CO$ and K_2SO_4, respectively. Prior to sowing, basal nutrients and nutrient treatments, the soil was thoroughly mixed in each pot.

Ten pre-soaked seeds of each wheat cultivars were sown pot^{-1} at a depth of 2 cm. Twenty five days after the sowing, thinning had done to six seedlings pot^{-1}. Moisture content of soil was maintained by distilled water at field capacity (95 g kg^{-1} soil) in all the pots during whole experimental period. Another treatment of 30 mg N kg^{-1} soil was applied 30 days after sowing (DAS).

Plant samples were harvested at full maturity. Grain samples were separated by manual threshing of wheat spikes. Straw and grain samples were oven dried at 65°C for 72 hrs. Grain yield of the wheat cultivars was employed to calculate Zn and P deficiency stress factors (based on the ratio of grain yield achieved at control to that achieved at higher rate of respective nutrient).

Plant samples were finely ground for wet digestion. A di-acid (HNO_3:$HClO_4$ ratio of 2:1) mixture (Jones and Case, 1990) was used to digest a sub sample plant material (Jones and Case, 1990). AAS (*Perkin Elmer, 100 AAanalyst*, Waltham, USA) was used to measure the concentration of Zn in the digest while P was analyzed on a spectrophotometer by ammonium-molybdate yellow color (Chapman and Pratt, 1961).

Nutrient content in grains were calculated by multiplying the grain yield achieved to the concentration of respective nutrient in grains. Concentration ratio of P to Zn was also calculated in grains of wheat cultivars.

The data obtained were analyzed using Microsoft Excel 2007® (Microsoft Cooperation, USA) and Statistix 9® (Analytical Software, Tallahassee, USA). Significantly different means were separated using Tukey's HSD test (Steel et al., 1997).

RESULTS

Plant growth and grain yield

The straw yield of wheat cultivars was significantly (P≤0.01) influenced by main and interaction effects of Zn and P applications (Table 1). On average, application of both Zn and P significantly increased straw yield; maximum straw yield was achieved when 6 mg Zn kg^{-1} soil was combined with 60 or 120 mg P kg^{-1}. However, cultivars also differed significantly (P≤0.01) not only on average bases but also in their response to P application. Pak-81 and Pasban-90 produced straw yield of respectively 32 and 18% greater at 120 mg P kg^{-1} soil when compared to control. However, straw yield of all the four cultivars was not significantly different at 60 and 120 mg P kg^{-1} soil.

Table 1. Straw and grain yield of four wheat cultivars supplied with different rates of Zn and P. There were six plants per pot with three replications.

Cultivars	Zn application (mg kg^{-1} soil)	Straw yield (g pot^{-1})			Grain yield (g pot^{-1})		
		P application (mg kg^{-1} soil)					
		0	60	120	0	60	120
Shafaq-2006	0	20.4	27.3	29.5	16.4	24.4	25.6
	6	21.0	29.3	29.1	16.4	27.4	32.5
Auqab-2000	0	26.9	29.7	28.2	18.0	20.8	24.6
	6	28.0	33.9	31.6	18.6	22.3	28.8
Pasban-90	0	23.9	26.5	26.0	15.5	21.3	19.6
	6	23.4	31.8	29.7	17.1	26.5	25.9
Pak-81	0	24.3	29.8	29.8	15.1	20.7	18.5
	6	22.8	28.9	33.6	16.8	25.2	24.5
$HSD_{0.05}$ for 3-way interaction		4.9			3.3		

There were significant (P≤0.01) main and second order interaction effects of cultivar, Zn application and P application on grain yield of wheat cultivars (Table 1). Similar to straw yield, application of both Zn and P significantly increased grain yield of all the tested cultivars. Averaged over cultivars, maximum grain yield was achieved by applying 6 mg Zn and 120 mg P kg^{-1} soil. However, Zn application at 0 mg P kg^{-1} soil only not significantly influenced grain yield.

On average, Zn application resulted in maximum increase of 23% over control in Pasban-90. Shafaq-2006 and Auqab-2000 achieved maximum grain yields at 120 mg P kg^{-1} soil while Pasban-90 and Pak-81 achieved maximum grain yields at 60 mg P kg^{-1} soil. However, grain yield of later two was not significantly different at 60 and 120 mg P kg^{-1} soil.

Deficiency Stress Factor of Zn and P

Zinc stress factor of the wheat cultivars was significantly (P≤0.01) affected by main effects of cultivar and P application (Figure 1a). Values of Zn stress factor for Pak-81 and Pasban-90 were significantly greater than for other two cultivars. However, values of Zn stress factor for all the cultivars were progressively decreased with incremental P applications.

Main effects of cultivar and Zn application along with interaction effect of cultivar × P application significantly (P≤0.01) affected P stress factor of wheat cultivars (Figure 1b). On average, P stress factor was 12 % decreased with Zn application. Phosphorus application significantly influenced P stress factor only in Auqab-2000 which possessed greatest P stress factor at 60 mg P kg^{-1} soil. At both 60 and 120 mg P kg^{-1} soil, P stress factor was least in Shafaq-2006.

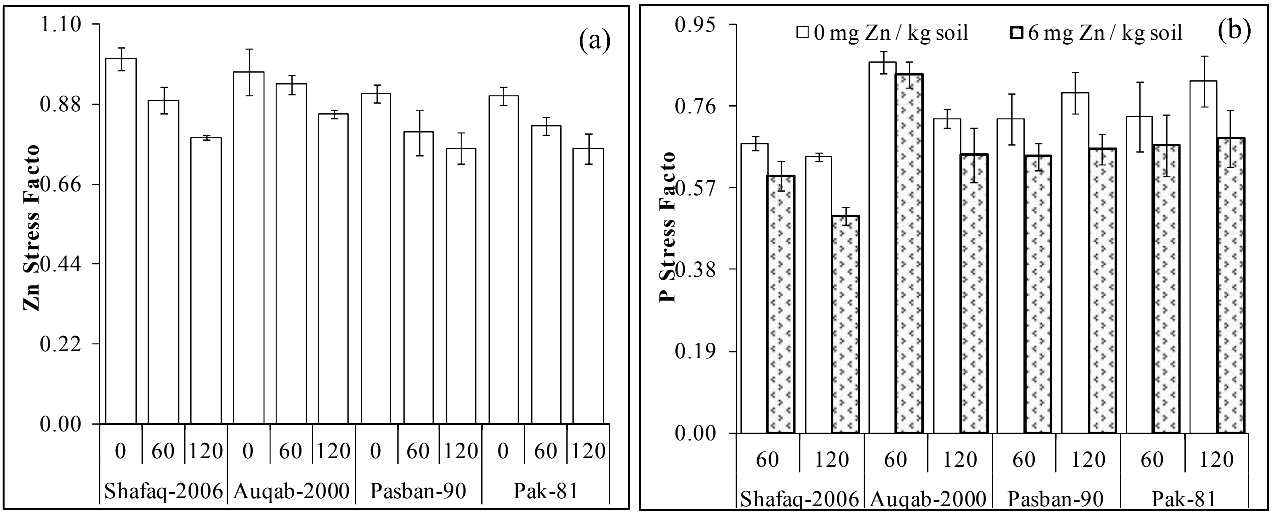

Figure 1. (a) Zinc deficiency stress factor (based on the ratio of grain yield achieved at control to that achieved at 6 mg Zn kg^{-1} soil) and (b) P deficiency stress factor (based on the ratio of grain yield achieved at control to that achieved at 60 and 120 mg P kg^{-1} soil) of wheat four cultivars (Tuckey $HSD_{0.05}$: interaction effect for P stress factor, 0.17; for Zn stress factor, 4.4)

Grain Zn accumulation

Grain Zn concentration in various wheat cultivars ranged from 16 to 40 mg kg^{-1} at various rates of Zn and P. All the main effects (cultivar, Zn application and P application), cultivar × Zn application interaction and Zn × P application interaction significantly (P≤0.01) affected grain Zn concentration (Figure 2). Various cultivars showed significant difference in grain Zn only at control level of Zn application. Zn concentration was significantly increased by application of Zn in grains of all wheat cultivar and at all rates of P application. At 6 mg Zn kg^{-1} soil, incremental P rates progressively decreased the grain Zn concentration. Grain Zn contents were significantly influenced by all main and interactive effects (Table 2). Grain Zn content in various cultivars was highest at 6 mg Zn kg^{-1} soil combined with 60 or 120 mg P kg^{-1} soil.

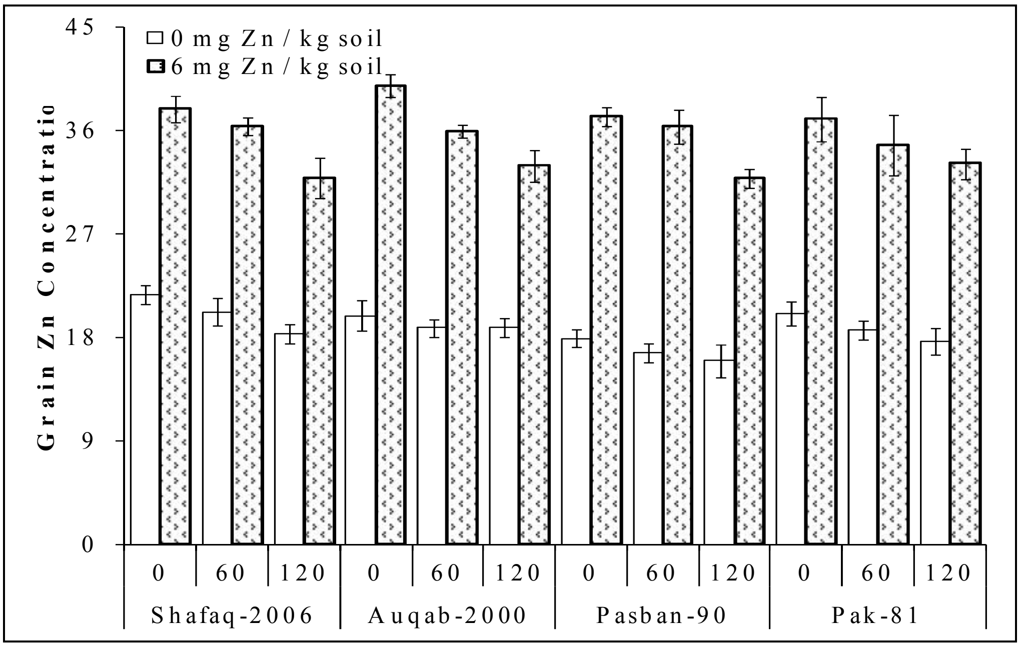

Figure 2. Concentration of Zn in grains of four wheat cultivars supplied with different rates of Zn (0 or 6 mg kg^{-1} soil) and P (0, 60 or 120 mg kg^{-1} soil) (Tuckey $HSD_{0.05}$ for 3-way interaction, 4.4)

*Table 2***.** Content of Zn and P in grains of four wheat cultivars supplied with different rates of Zn and P. There were six plants per pot with three replications.

Cultivars	Zn application (mg kg^{-1} soil)	Grain Zn content (μg pot^{-1})			Grain P content (mg pot^{-1})		
		P application (mg kg^{-1} soil)					
		0	60	120	0	60	120
Shafaq-2006	0	357	493	468	68	119	150
	6	620	997	1036	67	131	159
Auqab-2000	0	357	392	463	72	122	147
	6	741	801	949	70	124	156
Pasban-90	0	277	354	313	65	119	120
	6	635	964	823	70	141	149
Pak-81	0	304	386	327	67	125	130
	6	623	873	812	68	133	151
$HSD_{0.05}$ for 3–way interaction		139			20		

Grain P accumulation

Grain P concentration of wheat cultivars was significantly (P≤0.01) influenced by main and interaction effects of cultivar, Zn application and P application (Figure 3). In general, application of P increased while application of Zn decreased the grain P concentration which ranged from 3.8 (in Auqab-2000 supplied with 6 mg Zn and 0 mg P g^{-1} soil) to 7.0 mg g^{-1} (in Pak-81 supplied with 0 mg Zn and 120 mg P g^{-1} soil). Application of P significantly increased grain P concentration at both levels of Zn application. While Zn application was effective in decreasing grain P concentration of only Pasban-90 and Shafaq-2006 at 120 mg P g^{-1} soil and of Pak-81 at 60 mg P g^{-1} soil.

There were significant (P≤0.01) main and interaction effects of Zn and P applications on grain P content (Table 2). Application of P progressively and significantly ($P \leq 0.01$) increased grain P concentration at both levels of Zn application. Application of Zn also increased grain P content at 60 and 120 mg P kg^{-1} soil. However, cultivars differed significantly in their response to application of both Zn and P. Cultivars differed in grain P content only at control level of Zn where Pasban-90 accumulated significantly less P than other cultivars. At control and 60 mg P kg^{-1} soil different cultivars were not different with respect to grain P content. At 120 mg P g^{-1} soil, however, Shafaq-2006 accumulated higher P content than other cultivars.

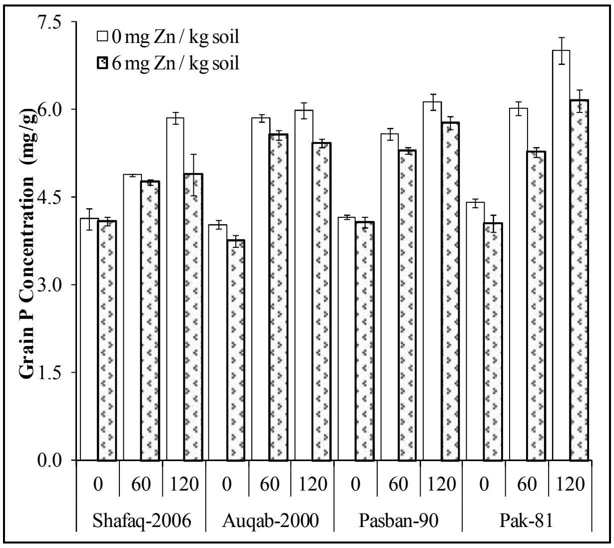

Figure 3. Concentration of P in grains of wheat four cultivars supplied with different rates of Zn (0 or 6 mg kg^{-1} soil) and P (0, 60 or 120 mg kg^{-1} soil) (Tuckey $HSD_{0.05}$ for 3-way interaction, 0.5)

Grain P:Zn concentration ratio

There were significant ($P \leq 0.01$) main and second order interaction effects of cultivar, Zn application and P application on P:Zn concentration ratio in grains of wheat cultivars (Figure 4). On average, Zn application significantly decreased P:Zn concentration ratio at various P application rates while P application progressively and significantly increased P:Zn concentration ratio at both levels of Zn application. However, cultivars differed at control rate of Zn application, with Pasban-90 and Pak-81 having 17-20% greater P:Zn concentration ratio in grains than other cultivars. As compared to other cultivars, Pasban-90 and Pak-81 also had greater P:Zn concentration ratio in grains (390 and 400) at 120 mg P g^{-1} soil. At 60 mg P g^{-1} soil, however, only Shafaq-2006 had significantly less P:Zn concentration compared to other cultivars.

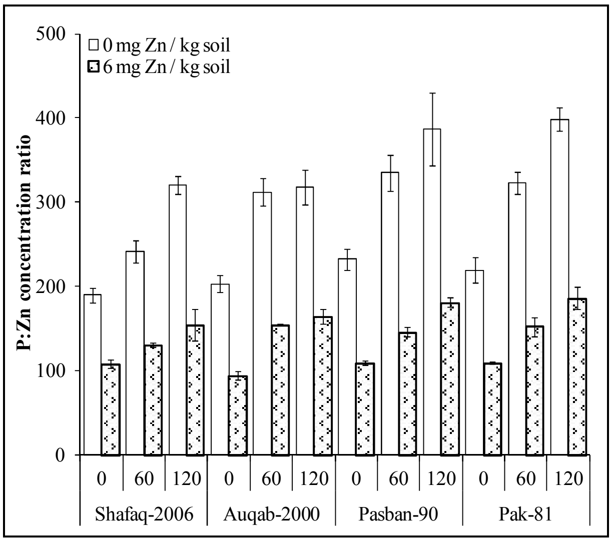

Figure 4. Concentration ratio of P to Zn (P:Zn ratio) in grains of wheat four cultivars supplied with different rates of Zn (0 or 6 mg kg^{-1} soil) and P (0, 60 or 120 mg kg^{-1} soil) (Tuckey $HSD_{0.05}$ for 3-way interaction, 52)

DISCUSSION

The calcareous soils are characterized by low availability of Zn and P to plants because of high fixation and low contents of organic matter (Kizilgoz and Sakin, 2010; Hussain et al., 2011; Maqsood et al., 2011; Ahmad et al., 2012). Therefore, application of Zn and P improved plant growth and grain yield (Sattar et al., 2011; Hussain et al., 2012a, 2012b); a response similar to present study (Table 1). In most cases, application of Zn was much more effective in increasing straw and grain yield at higher levels of P application. This could be due to P induced Zn deficiency and higher Zn demand at higher P application. Moreover, effects of nutrient application were much more obvious in grain yield as compared to straw yield.

Nutrient deficiency stress factor helped to understand the relative plant demand of a nutrient at varying rate of the other nutrient. Application of either nutrient (Zn or P) significantly decreased stress factor of the other, indicating higher requirement of a nutrient at a higher rates of the other nutrient. Farmers in Pakistan and other developing countries are only using macronutrient (e.g. N, P and K) fertilizers while micronutrient application has been ignored till now (Hussain et al., 2008; Aziz et al., 2011). Present study clearly indicated that ignoring Zn application, especially at optimum to excessive rates of P application, resulted in lower than optimum grain yields.

Grain Zn concentration in various cultivars ranged from 16 to 40 mg kg^{-1} at various rates of Zn and P application (Figure 2). Cakmak (2008) suggested the average concentration of Zn in whole grain of wheat (20 to 35 mg kg^{-1}) should be increased at least by approximately 10 mg kg^{-1} for a measurable biological impact on human health. Yang et al. (2011) revealed that a medium rate of P increased grain Zn concentration. Many other authors, however, have reported significant decrease in grain Zn concentration with the application of P to root medium (Zhao et al., 2007). In the present study, grain Zn concentration was significantly decreased by P application in wheat cultivars, excepting Auqab-2000. Therefore, variation in grain Zn concentration under P applications is mediated by genetic variations. This decrease in Zn concentration may be attributed to growth dilution effect as application of P increased grain yield; a general response to P application in P deficient soils. Grain Zn concentration was mainly influenced by Zn application. Whereas, application of P increased grain Zn content particularly because of higher grain yields.

Low Zn supply combined with a high P supply markedly enhanced P concentration in plant tissues and resulted in appearance of Zn deficiency symptoms (Loneragan et al., 1979, 1982; Webb and Loneragan, 1988, 1990; Cakmak and Marschner, 2006). Grains are the most important tissue for the storages of P and higher rate P significantly increased grain P concentration (Figure 3). The decreased P concentration with Zn application might be related to retarded translocation of each other to the upper parts of the plant (Khan and Zende, 2004). Similarly, increased P fertilization increased dry matter and P concentration in the plant (Li et al., 2003).

Most of P in grains is in the form of phytate (Stangoulis et al., 2007), which is an anti-nutritional factor and reduces the availability of Zn, Fe, Ca and other metals to human beings. As most of the P in cereals grains is in phytate form and is strongly related with grain phytate concentration, a lower P in grains means a higher bioavailability of Zn for humans (Erdal et al., 2002). The P:Zn ratio is recently reported as a bioavailability trait in cereals (Šimić et al., 2012). At 120 mg P kg^{-1} soil, greater P:Zn ratio (106-151) indicated Zn deficiency hazard for plant growth and for the human population. The P:Zn ratio in grains was decreased by 1.8 to 2.3 folds with Zn application to various cultivars supplied with various P levels. This happens due to a significant increase in grain Zn concentration and a significant decrease in grain P concentration with the application of Zn.

CONCLUSIONS

Application of both Zn and P increased grain yield of all the four wheat cultivars. Calculations of deficiency stress factors for P and Zn confirmed higher demand of a nutrient at a higher rate of the other. The content of Zn and P also increased with the application of both Zn and P. Zinc application decreased grain P concentration in all cultivars. However, only the highest rate of P application significantly decreased grain Zn concentration in Shafaq-2006, Pasban-90 and Pak-81. Concentration of Zn and P : Zn concentration ratio were much more dependent on Zn application to wheat cultivars, especially at higher rates of P application. Therefore, Zn requirement for optimum grain yield and grain Zn concentration depends on P application to soil and genetic variation in wheat cultivars.

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