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Research article

Effect of various application rates of phosphorus combined with different zinc rates and time of zinc application on phytic acid concentration and zinc bioavailability in wheat

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Abstract

Imbalance and improper time of application disturb the availability of zinc (Zn) to crops. Less Zn intake due to over-application of phosphorus (P) deteriorates cereal grain quality due to the accumulation of phytic acid. Phytic acid causes many human diseases, especially in children. Furthermore, impairments in linear growth, learning ability and improper immune functioning in humans are also major side effects of Zn deficiency. Phytic acid has been of interest regarding a balanced intake of Zn, but the influence of phosphatic fertilizers and the time of Zn application have not received considered attention. Therefore, the current experiment considered the hypothesis that the balanced use of P fertilizers and the time of Zn application would improve Zn availability for human consumption and reduce the phytic acid concentration. Two Zn rates (5 kg/ha, 10 kg/ha) were applied at the tillering stage (TS) and booting stage (BS) of wheat under three P rates (60 kg/ha, 90 kg/ha, 120 kg/ha). The results confirmed that relative to 5 kg/ha Zn, 10 kg/ha Zn application at TS significantly improved grain Zn concentration (22%) and grain yield (16%). The highest application of P (120 kg/ha) enhanced 33% and 27% grain phytic acid at TS and BS, respectively. The increased level of Zn and P decreased the grain concentration of Ca. It was concluded that 90 kg/ha P with 10 kg/ha Zn at TS was the best combination to achieve a sustainable wheat grain yield with a minimum phytic acid content.

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Introduction

Zinc (Zn) deficiency is one of the major issues for 30% of the world's population (Welch and Graham, 1999; Erdal et al., 2002; Alloway, 2008). More than 90 years ago, Zn was classified as an essential micronutrient (Sommer and Lipman, 1926). It serves in various biochemical processes in the rice plant, such as cytochrome and nucleotide synthesis, auxin metabolism, chlorophyll production, enzyme activation, and membrane integrity (Khan et al., 2012). Impairments in linear growth, learning ability, sexual maturation, the central nervous system and improper immune functioning in humans are some of the major side effects of Zn deficiency (Prasad, 1984; Tamura and Goldenberg, 1996). A Zn-enriched diet is recommended to address Zn deficiency in humans, especially in developing countries (Roohani et al., 2013). Cereals are considered one of the most abundant crop sources of Zn for humans (Cakmak, 2008). It has been observed that the demand of Zn for humans is in the range 40−60 mg/kg while the current status of Zn in cereals is in the range 13−23 mg/kg, which is below the required amount (Pfeiffer and McClafferty, 2007; Manzeke et al., 2014). Most cereals grains and oilseeds crops contain 1−3% phytic acid, which constitutes 50−80% of the total grain P in different forms (Graf, 1983). This phytic acid in higher concentrations significantly decreases Zn availability in cereal grains (Ferguson et al., 1989; Coulibaly et al., 2011). In addition, less Zn availability due to phytic acid decreases the availability of iron (Fe), magnesium (Mg) and calcium (Ca) (Hurrell et al., 2003; Bohn et al., 2004; Guttieri et al., 2006). The regular intake of a diet rich in phytic acid can cause weak growth of bones, rickets, short stature, anemia, tooth decay, narrow jaws and mental retardation in children (Hallberg et al., 1989). In plants, Zn deficiency disturbs the structure of roots, activation of enzymes and detoxification of free radicals in plants (Cakmak, 2008; Peck and McDonald, 2010). It has been documented that 50% of the cereal-producing area of the world is Zn deficient (Hamid and Ahmad, 2001). The presence of organic matter and the availability of water, clay contents and microbial activity in the rhizosphere directly play a vital role in the availability of Zn (Alloway, 2008). A high pH (Broadley et al., 2007) and calcareousness are significant contributors that decrease the bioavailability of Zn to plants (Imran and Rehim, 2017). In addition, a high concentration of P in the soil also reduces the bioavailability of Zn to the roots through the formation of a P-Zn complex (Zhang et al., 2012; Ova et al., 2015). Compared to $ZnSO_4$, this P-Zn complex is highly insoluble in water which reduces the efficiency of Zn uptake in plants (Ova et al., 2015). As much agricultural land is deficient in P and Zn in terms of their availability to plants, scientists have focused on developing an approach for the effective and simultaneous delivery of both P and Zn to plants without disturbing soil health (Watts-Williams et al., 2014). Wheat (*Triticum aestivum* L.) is a widely cultivated cereal crop due to its nutritional value (8−12% protein; 55% carbohydrates) which fulfil 20% of the daily intake in the human diet (Bos et al., 2005). The worldwide trade of one-fifth of all wheat produced makes it an important economic crop (Food and Agriculture Organization, 2003). The demand for wheat is expected to increase at the rate of 1.6% per annum up to 2020 (Ortiz et al., 2008). As limited literature is available on the timing of Zn and P application in relation to the phytic acid concentration in wheat and keeping in mind the importance of wheat and Zn for humans, the current study aimed to examine the optimum application rate of Zn and P fertilizer at different growth stages of wheat. It was hypothesized that changes in the time and stage of growth for Zn fertilizer application might be effective at improving the concentration of Zn and P in wheat grain.

Materials and Methods

Site of experiment and soil characteristics

A field experiment on wheat was conducted in a research area of the Soil Chemistry Section (31°24'14.4"N 73°02'51.1"E), ISC & ES, Faisalabad, Pakistan. Soil texture was detemrined using the hydrometer method (Gee and Bauder, 1986). Organic matter in soil was analyzed according to Walkley (1935), while organic nitrogen was calculated using Equation 1:

$$\text{Organic N (\%)} = \text{Soil organic matter} / 20 \quad (1)$$

Soil extractable phosphorus and potassium were determined according to Olsen and Sommers (1982) and Nadeem et al. (2013), respectively.

Plot preparation and fertilizer application

In the experimental field, a plot of 5 m width and 7.5 m length with an area of 37.5 m^2 was made for each treatment. Macronutrients nitrogen (N) and potassium (K) were applied at the recommended rate of 120 kg/ha and 60 kg/ha, respectively. All K was applied in the form of sulfate of potash at the time sowing in a single dose. However, urea was applied in two doses (the first at the time of sowing and the second at the tillering stage) to fulfill the wheat requirement for N.

Experimental design and treatment plan

There were six treatments and three replications following two factorial arrangement using a randomized complete block design. The treatments consisted of: 60 kg P/ha + 5 kg Zn/ha (P60 + Zn5); 90 kg P/ha + 5 kg Zn/ha (P90 + Zn5); 120 kg P/ha + 5 kg Zn/ha (P120 + Zn5); 60 kg P/ha + 10 kg Zn/ha (P60 + Zn10); 90 kg P/ha + 10 kg Zn/ha (P90 + Zn10); and 120 kg P/ha + 10 kg Zn/ha (P120 + Zn10).

Zinc and phosphorus application rates

There were three application rates of P: low, 60 kg/ha; recommended, 90 kg/ha; and high, 120 kg/ha. Two application rates of Zn were applied at 5 kg/ha and 10 kg/ha.

Time of application of zinc

Zinc fertilizer was applied at the tillering stage (TS) and booting stage (BS) of wheat, each time in a single dose.

Seed collection and seed sowing

Seeds of Galaxy-2013 were collected from the Ayub Agriculture Research Institute Wheat Section. Seeds were sown at the rate of 100 kg/ha (Chauhdary et al., 2015). Damaged and weak seeds were initially screened out manually, followed by using a drill method to sow the seeds in each plot.

Irrigation

For wheat cultivation, four irrigation period were: 1st at 28 d (crown root initiation), 2nd at 59 d (tillering stage), 3rd at 90 d (heading stage) and 4th at 120 d (milky stage/soft dough).

Harvesting and sampling

For soil, wheat straw and grain analysis, harvesting was done at the time of maturity after 125 d of germination. Sampling for soil was carried out at five spots per plot in a zigzag manner. At each spot, three soil (0−15 cm depth) samples were collected to make a valid, representative, composite sample.

Sample preparation

All plant samples were threshed manually, after which the grain from each plot was collected for further analysis. After determining the grain yields, the straw and grain were ground in a wheat seed grinder and saved in plastic bottles for further analysis. The soil samples were oven-dried at 105 °C for 72 hr and then crushed. Finally, the soil samples were passed through a 2 mm sieve for further analysis.

Soil phosphorus and zinc

Extractable phosphorus was determined based on Olsen and Sommers (1982). However, extractable Zn in the soil was analyzed using diethylenetriaminepentaacetic acid extraction as described by Lindsay and Norvell (1978).

Phosphorus and calcium in grain

The determination of calcium (Ca) and phosphorus (P) in the wheat grain was based on digestion with H_2SO_4 as described by Jones et al. (1991). In the digested samples of wheat grain, Ca was analyzed using titration as described by Cheng and Bray (1951). The analysis of P in grain used a spectrophotometer at 420 nm wavelength, based on the yellow color method (Jones et al., 1991).

Zinc in grain

Grain samples were digested with a di-acid mixture of perchloric acid and nitric acid in a 1:2 ratio (Jones et al., 1991) for the determination of zinc (Zn) using an atomic absorption spectrophotometer (Mallick et al., 2013).

Phytic acid determination in grain

Seed samples were finely ground in a grinder and the level of phytic acid in each sample was determined using the ion-exchange method (Gargari et al., 2007) with minor modification. Initially, 5 g of dried and powdered sample were mixed with 40 ml HCl (2.4% V/V) for 3 hr at room temperature. After that, the solution was passed through Whatman No. 1 filter paper. The filtrate (1 ml) was pipetted into a 25 mL flask and 1 mL of NaOH (0.75 M)/Na2 ethylenediaminetetraacetic acid (0.11 M) solution was added. The final volume was made up to 25 mL. The phytic acid was washed with 15 mL of NaCl (0.7 M), while P was released using a concentrated HNO_3/H_2SO_4 (3.0 and 0.5 ml, respectively) mixture, which was mixed with molybdate solution (2.5% weight per volume ammonium molybdate in 1 N H_2SO_4). Absorbance was measured at 420 nm and the level of phytic acid was calculated using Equation 2:

$$\text{Phytic acid (mg/g)} = (\text{mean K} \times A \times 20) / 0.282 \times 1{,}000 \quad (2)$$

Where mean K is standard P-Phytate and A is absorbance and the phytic acid was presented as milligrams per gram of sample.

Statistical analysis

Statistical analysis of data used procedures according to Steel et al. (1997). A two-factorial analysis of variance was applied using the Statistix software (version 8.1; Statistix, Tallahassee, FL, USA) for calculation of the significance of treatments. All treatments were compared using Tukey's test at $p \leq 0.05$.

Results and Discussion

Application of 10 kg Zn/ha (Zn 10) differed significantly compared to Zn5 for the extractable soil P concentration at the TS and BS stages. It was noted that treatments P60 and P90 were not significantly different when applied at TS, but differed significantly at BS for extractable soil P concentration. Application of P120 was significantly the best at both TS and BS compared to P90 and P60 for the extractable soil P concentration. Compared to P60, the maximum increases in the extractable soil P concentration from applying P120 at TS and BS were 38% and 27%, respectively. Similarly, compared to Zn5, the maximum increases in the soil extractable phosphorus from applying Zn10 at TS and BS were 11% and 12%, respectively.

It was observed that the addition of Z10 was significantly better compared to Zn5 for extractable soil Zn concentration at both TS and BS. Application of P120 was significantly the best compared to P90 and P60 for extractable soil Zn concentration at TS. However, at BS, there was no significant difference between the application of P120 and P90, they did differ significantly compared to P60 for extractable soil Zn concentration. Compared to P60, the maximum increases in extractable soil Zn concentration from applying P120 at TS and BS were 50% and 46%, respectively. Similarly, compared to Zn5, the increases in soil extractable Zn from applying Zn10 were 39% and 14%, respectively.

It was noted that there was no significant difference between the application of P60 and P90 regarding grain Zn concentration at both TS and BS. A significant reduction in wheat grain Zn was noted when P120 was applied compared to P60 and P90 at both TS and BS. The application of Z10 was significantly better compared to Z5 for wheat grain Zn concentration at both TS and BS. Compared to P120, the maximum increases in wheat grain Zn from applying P60 at both TS and BS were 37% and 33%, respectively. Similarly at TS and BS, 22% and 15%, respectively, increases in grain Zn were observed when Zn10 was applied, compared to Zn5.

Under various levels of P, the addition of Zn5 significantly increased wheat grain Ca at both TS and BS. Application of P60 was significantly the best compared to P90 and P120 for grain Ca in wheat at both TS and BS. It was also noted at P90, the grain Ca was considerably higher compared to P120 at both TS and BS. Compared to P120, the maximum increases in grain Ca from applying P60 at both TS and BS were 18% and 19%, respectively. Similarly compared to Zn10, the increases in grain Ca from applying Zn5 at TS and BS were 13% and 11%, respectively.

There was no significant difference in in grain yield between the application of P120 and P90 at TS and BS. A significant reduction in wheat grains yield was noted when P60 was applied compared to P120 and P90 at TS and BS. The application of Z10 was significantly better than Z5 for wheat grain Zn concentration at TS. However, there was no significant difference between the addition of Z10 and Z5 regarding grain yield at BS. Compared to P60, the maximum increases in wheat grain yield from applying P120 at both TS and BS were 21% and 23%, respectively. Similarly, a 16% increase in grain yield was observed when Zn10 was applied compared to Zn5 at TS.

Under various levels of P, the addition of Zn5 significantly increased the level of wheat grain phytic acid at both TS and BS. Application of P120 was significantly the best at both TS and BS compared to P90 and P60 for wheat grain phytic acid. It was also noted that P90 significantly increased the grain phytic acid compared to P60 at both TS and BS. Compared to P60, the maximum increases in grain phytic acid from applying P120 at both TS and BS were 33% and 27%, respectively. Similarly compared to Zn10, the increases in grain phytic acid from applying Zn5 at both TS and BS were 18% and 18%, respectively.

The results of the current experiment confirmed that the application of P90 produced the maximum intake of Zn and a significant improvement in the yield of wheat. According to Mai et al. (2008), balanced nutrition of P improved crop yields, but over-fertilization of P usually resulted in restricted growth. Yang et al. (2011) suggested that a higher intake of P often decreased the shoot growth due to minimization of the vegetative growth period by shifting plants toward the reproductive phase when P becomes relatively available. Hussain et al. (2011) observed that the bioavailability of Zn decreased in Ca-rich soils, but the addition of Zn fertilizer significantly enhanced the availability of Zn to the grains which plays an essential role in the improvement of crop yield (Hussain et al., 2012). The findings of the current study were also consistent with the above arguments. In the current study, Ca had a negative correlation with Zn uptake in the plants. A proper human diet is categorized as low, medium and high Zn bioavailability to humans in terms of a phytate:Zn ratio of ≥ 15, 5–15 and ≤ 5, respectively (Brown et al., 2001). It has been observed that grains of wheat generally have a phytate:Zn ratio ≥ 25, while a ratio ≤ 15 is usually required to meet the requirements for better human nutrition (Weaver and Kannan, 2002). In the current study, the higher application rate of phosphorus (P120) significantly decreased the intake of Zn. It was also observed that the higher application of phosphorus (P120) also reduced the amount of grain Ca. This reduction in Ca and Zn might have been due to the antagonistic effect of P with Ca and Zn. In addition, the significant decrease in Zn due to increased P might have been a major cause of the substantial increase in phytic acid. Skoglund et al. (2009) documented that phytate (inositol hexaphosphate) is one of the major forms of P storage in plants. Their findings also supported the current study. They argued that a higher P concentration in the soil reduces the bioavailability of Zn, Ca and Mg to plants (Bohn et al., 2004; Phillippy, 2006). In the current study, changing the application time of Zn also positively contributed to a better uptake of Zn which might have been due to the change in reaction time or less fixation of Zn by the higher P level in the soil. According to Wang et al. (2015), increasing Zn application was significantly and positively related to improvement in Zn uptake. This improvement in uptake of Zn played an imperative role in the reduction of the phytic acid contents and fortification of Zn in plants. Phytic acid is major anti nutritional factor which is present is legumes and cereals in significant amounts (Kumar et al., 2017). A higher phytic acid concentration usually causes a significant reduction in Zn and Fe because of its strong binding ability for metal minerals (Kumar et al., 2017). Hussain et al. (2017) also pointed out that the factor of application time of Zn directly was affected by P fixation via a nutrient-nutrient interaction. The findings of the current study also showed that the phytic acid concentration was significantly increased with Zn deficiency. This increase in phytic acid was due to the binding capability of phytic acid with Zn and the antagonistic relationship of Zn and P in the soil. It was observed that phytic acid had a positive correlation with P, but a negative correlation with Zn. Higher Ca levels at the booting and tillering stages were positively correlated with phytic acid but negatively correlated with grain Zn concentration. Furthermore, the yield had a positive and significant correlation with Zn application at the booting and tillering stages. According to Liu et al. (2007), the higher intake of P in plants is usually stored (65–85%) in grain in the form of myo-inositol 1,2,3,4,5,6-hexakisphosphate (InsP6) phytic acid (Raboy et al., 2000). This phytic acid, when in contact with Zn^{+2} forms phytates that are insoluble. Although the limited availability of Zn indicated higher levels of phytic acid in wheat grains (Raboy et al., 2000), the results of the current study also showed that increasing the application rate of Zn fertilizer decreased the amount of P, which resulted in a level of phytic acid.

Based on the current results, it was concluded that a higher application of phosphorus decreases the intake of Ca and Zn in wheat grains. A reduced intake of Zn due to increased amounts of P significantly increased the phytic acid concentration in what grains. However, the application of P at 90 kg/ha with Zn at 10 kg/ha at the tillering stage was the best combination to improve the grain Zn concentration (by 22% and 15% at the tillering and booting stages, respectively) and to decrease the phytic acid concentration without any significant loss in wheat grain yield.

Conflict of Interest

The authors declare that there are no conflicts of interest.

Table 1 Two factorial analysis of variance

Variable	Effect	df	SS	MS	F test	p-Value
Soil phosphorus at tillering stage	P application	2	45.9871	22.9935	18.84	0.0002**
	Zn application	1	6.0089	6.0089	4.92	0.0465**
	P × Zn application	2	1.5611	0.7805	0.64	0.5446ns
	Error	12	14.6449	1.2204		
Soil phosphorus at booting stage	P application	2	15.5401	7.77005	19.10	0.0002**
	Zn application	1	5.3792	5.37920	13.22	0.0034**
	P × Zn application	2	0.0754	0.03772	0.09	0.9121ns
	Error	12	4.8815	0.40679		
Soil zinc at tillering stage	P application	2	0.42334	0.21167	21.43	0.0001**
	Zn application	1	0.41709	0.41709	42.22	0.0000**
	P × Zn application	2	0.02108	0.01054	1.07	0.3746ns
	Error	12	0.11853	0.00988		
Soil zinc at booting stage	P application	2	0.05493	0.02747	24.72	0.0001**
	Zn application	1	0.01176	0.01176	10.58	0.0069**
	P × Zn application	2	0.00058	0.00029	0.26	0.7753ns
	Error	12	0.01333	0.00111		
Grain zinc at tillering stage	P application	2	298.070	149.035	19.23	0.0002**
	Zn application	1	182.469	182.469	23.55	0.0004**
	P × Zn application	2	3.951	1.976	0.25	0.7791ns
	Error	12	92.984	7.749		
Grain zinc at booting stage	P application	2	247.190	123.595	15.08	0.0005**
	Zn application	1	82.347	82.347	10.04	0.0081**
	P × Zn application	2	2.221	1.111	0.14	0.8746ns
	Error	12	98.374	8.198		
Grain calcium at tillering stage	P application	2	2828.44	1414.22	17.26	0.0003**
	Zn application	1	2450.00	2450.00	29.90	0.0001**
	P × Zn application	2	37.33	18.67	0.23	0.7997ns
	Error	12	983.33	81.94		
Grain calcium at booting stage	P application	2	3346.78	1673.39	19.88	0.0002**
	Zn application	1	1820.06	1820.06	21.62	0.0006**
	P × Zn application	2	10.78	5.39	0.06	0.9383ns
	Error	12	1010.00	84.17	19.88	
Grain yield at tillering stage	P application	2	1.09714	0.54857	17.46	0.0003**
	Zn application	1	0.94761	0.94761	30.15	0.0001**
	P × Zn application	2	0.01028	0.00514	0.16	0.8510ns
	Error	12	0.37713	0.03143		
Grain yield at booting stage	P application	2	1.56263	0.78132	13.53	0.0008**
	Zn application	1	0.24734	0.24734	4.28	0.0608ns
	P × Zn application	2	0.02008	0.01004	0.17	0.8426ns
	Error	12	0.69320	0.05777		
Grain phytic acid at tillering stage	P application	2	20.6511	10.3255	26.01	0.0000**
	Zn application	1	10.3209	10.3209	25.99	0.0003**
	P × Zn application	2	0.3411	0.1706	0.43	0.6604ns
	Error	12	4.7645	0.3970		
Grain phytic acid at booting stage	P application	2	12.0605	6.03027	20.46	0.0001**
	Zn application	1	8.4050	8.40500	28.52	0.0002**
	P × Zn application	2	0.5904	0.29522	1.00	0.3960ns
	Error	12	3.5363	0.29469		

df = degree of freedom; SS = sum of square; MS = mean square * = $p \le 0.05$; ** = $p \le 0.01$; ns = non-significant

Table 2 Effect of phosphorus levels in combination with various rates (5 and 10 kg/ha) and times (tillering and booting) of zinc fertilizer application on soil extractable phosphorus and zinc concentration.

Zn application rate	Extractable soil P (µg/g)							
	Zn application stage							
	Tillering				Booting			
	Level of phosphorus (kg/ha)							
	60	90	120	ME (P)	60	90	120	ME (P)
	IE (Zn × P)				IE (Zn × P)			
5 kg/ha	8.68 ± 0.51	9.49 ± 0.61	11.9 ± 0.85	10.0^{B}	7.63 ± 0.30	8.62 ± 0.28	9.97 ± 0.42	8.74^{B}
10 kg/ha	9.89 ± 0.36	9.90 ± 0.81	13.8 ± 0.54	11.2^{A}	8.71 ± 0.25	9.88 ± 0.43	10.9 ± 0.47	9.83^{A}
ME (Zn)	9.29^{B}	9.69^{B}	12.86^{A}		8.17^{C}	9.25^{B}	10.44^{A}	
	Extractable Soil Zinc (µg/g)							
5 kg/ha	0.62 ± 0.05	0.85 ± 0.03	0.94 ± 0.05	0.80^{B}	0.28 ± 0.02	0.36 ± 0.02	0.42 ± 0.01	0.35^{B}
10 kg/ha	0.90 ± 0.10	1.09 ± 0.04	1.34 ± 0.06	1.11^{A}	0.33 ± 0.01	0.42 ± 0.02	0.46 ± 0.01	0.40^{A}
ME (Zn)	0.76^{C}	0.97^{B}	1.14^{A}		0.30^{B}	0.39^{A}	0.44^{A}	

ME = main effect; IE = interactive effect.
Mean values (± SE) in the same row with different uppercase superscripts are significantly ($p \leq 0.05$) different.

Table 3 Effect of phosphorus levels in combination with various rates and times (tillering and booting) of zinc fertilizer application on grains phosphorus and grain calcium concentrations

Zn application rate	Grain Zn (µg/g)							
	Zn application stage							
	Tillering				Booting			
	Level of P (kg/ha)							
	60	90	120	ME (P)	60	90	120	ME (P)
	IE (Zn × P)				IE (Zn × P)			
5 kg/ha	32.3 ± 2.01	29.9 ± 1.50	23.2 ± 1.46	28.5^{B}	32.63 ± 1.46	30.40 ± 1.13	24.67 ± 1.24	29.2^{B}
10 kg/ha	39.9 ± 1.41	35.3 ± 1.65	29.3 ± 1.53	34.8^{A}	37.64 ± 2.34	34.90 ± 1.50	28.00 ± 1.93	33.5^{A}
ME (Zn)	36.07^{A}	32.61^{A}	26.25^{B}		35.14^{A}	32.65^{A}	26.33^{B}	
	Grains Calcium (mg/g)							
5 kg/ha	214 ± 5.03	197 ± 4.75	180 ± 5.29	197^{A}	218 ± 5.03	201 ± 4.75	186 ± 5.29	202^{A}
10 kg/ha	188 ± 5.69	172 ± 5.13	161 ± 5.81	174^{B}	200 ± 7.00	180 ± 5.78	165 ± 2.91	182^{B}
ME (Zn)	201^{A}	184^{B}	170^{C}		209^{A}	190^{B}	176^{C}	

ME = main effect; IE = interactive effect.
Mean values (± SE) in the same row with different uppercase superscripts are significantly ($p \leq 0.05$) different.

Table 4 Effect of phosphorus levels in combination with various rates and times (tillering and booting) of zinc fertilizer application on yield and phytic acid concentration of grain

Zn application rate	Grain yield (t/ha)							
	Zn application stage							
	Tillering				Booting			
	Level of P (kg/ha)							
	60	90	120	ME (P)	60	90	120	ME (P)
	IE (Zn × P)				IE (Zn × P)			
5 kg/ha	2.48 ± 0.16	2.91 ± 0.13	3.08 ± 0.07	2.82^{B}	2.72 ± 0.06	3.36 ± 0.18	3.43 ± 0.13	3.17^{A}
10 kg/ha	2.94 ± 0.05	3.43 ± 0.12	3.48 ± 0.03	3.28^{A}	3.03 ± 0.12	3.51 ± 0.18	3.67 ± 0.13	3.41^{A}
ME (Zn)	2.71^{B}	3.17^{A}	3.28^{A}		2.88^{B}	3.44^{A}	3.55^{A}	
	Grains Phytic acid (mg/g)							
5 kg/ha	8.81 ± 0.32	10.2 ± 0.39	11.1 ± 0.34	10.03^{A}	7.83 ± 0.38	9.25 ± 0.32	10.2 ± 0.28	9.09^{A}
10 kg/ha	6.97 ± 0.35	8.68 ± 0.40	9.91 ± 0.39	8.52^{B}	6.97 ± 0.32	7.58 ± 0.23	8.62 ± 0.33	7.72^{B}
ME (Zn)	7.89^{C}	9.45^{B}	10.49^{A}		7.40^{C}	8.42^{B}	9.41^{A}	

ME = main effect; IE = interactive effect.
Mean values (± SE) in the same row with different uppercase superscripts are significantly ($p \leq 0.05$) different.

Table 5 Pearson correlation among soil and grain nutrients

Subject	Attribute	Crop	Treatment		Soil				Grains						
		Stage	Nutrients		P		Zn		Phytic acid		Zn		Ca		Yield
			P	Zn	Booting	Tiller	Booting	Tiller	Booting	Tiller	Booting	Tiller	Booting	Tiller	Booting
Soil	P	Booting	0.7746*	0.4559^{ns}											
		Tiller	0.7498*	0.2968^{ns}	0.7179*										
	Zn	Booting	0.8135*	0.3819^{ns}	0.7917*	0.5889*									
		Tiller	0.6561*	0.6524*	0.7508*	0.7486*	0.8174*								
Grain	Phytic acid	Booting	0.7003*	-0.5846*	0.1903^{ns}	0.3388^{ns}	0.3208^{ns}	0.0902^{ns}							
		Tiller	0.7517*	-0.5349*	0.3376^{ns}	0.3810^{ns}	0.4351^{ns}	0.1394^{ns}	0.8403*						
	Zn	Booting	-0.7352*	0.4375^{ns}	-0.1961^{ns}	-0.4710*	-0.3770^{ns}	-0.2036^{ns}	-0.8583*	-0.7487*					
		Tiller	-0.7082*	0.5621*	-0.3311^{ns}	-0.3541^{ns}	-0.4060^{ns}	-0.1050^{ns}	-0.7018*	-0.8129*	0.7191*				
	Ca	Booting	0.7340*	-0.5424*	0.3352^{ns}	0.4656^{ns}	0.4175^{ns}	0.1785^{ns}	0.7451*	0.8489*	-0.7164*	-0.9429*			
		Tiller	0.6692*	-0.6237^{ns}	0.2313^{ns}	0.3636^{ns}	0.3366^{ns}	0.0602^{ns}	0.7742*	0.8652*	-0.7039*	-0.9217*	0.9747*		
	Yield	Booting	0.7342*	0.3131^{ns}	0.7464^{ns}	0.4083^{ns}	0.7808*	0.6887*	0.4011^{ns}	0.3857^{ns}	-0.3380^{ns}	-0.2566^{ns}	0.2683^{ns}	0.2209^{ns}	
		Tiller	0.6349*	0.6242^{ns}	0.7796*	0.5867*	0.7875*	0.8192*	0.0220^{ns}	0.0943^{ns}	-0.1698^{ns}	-0.1343^{ns}	0.1354^{ns}	0.0511^{ns}	0.6872*

* = significant at $p \leq 0.05$; ns = not significant at $p > 0.05$.

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