

## Improving nutritional quality of wheat through soil and foliar zinc application

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### ABSTRACT

A field study was conducted to ascertain the effect of three zinc (Zn) levels: 0, 20 kg ZnSO<sub>4</sub>/ha and 20 kg ZnSO<sub>4</sub>/ha + foliar spray of 0.5% ZnSO<sub>4</sub>, on wheat grain Zn content and factors contributing to or hindering in its bioavailability. Increasing Zn levels were established as serviceable in improving the nutritional status of genotypes. Soil application + foliar spray proved to be paramount for all the traits leading to an 80% increase in grain Zn content, 61.3% in methionine content and a decrease of 23.2% in phytic acid as an average of all genotypes and both years. The genotype UP 2382 was found more suited to Zn fertilization in allocating Zn and maintaining a lower phytate to Zn molar ratio.

**Keywords:** phytic acid; zinc content; methionine; ascorbic acid; phytate to zinc molar ratio

Zinc (Zn) is discerned as an indispensable micro-nutrient for plants and animals. It is a component of over 300 plant enzymes and vital proteins like Zn-finger DNA binding proteins, rarely interesting new genes (RING) fingers, and LIM (Lin11, Isl-1, Mec-3) proteins (Rhodes and Klug 1993, Vallee and Falchuk 1993). Not surprisingly, its inadequacy in plants may lead to malfunctioning of the enzymes and proteins disturbing the metabolism, growth and development of plants. Zn deficiency causes a number of complications in humans e.g. stunted growth, sexual immaturity, immune dysfunction, impairment in learning ability etc. Unfortunately, micronutrient deficiencies including that of Zn effectuate about 3 billion people around the world. It is found to account for about 450 000 deaths in children under five years of age. Number of individuals suffering from Zn deficiency is the highest in Southeast Asia (33.1%), Sub Saharan

Africa (28.2%) and South Asia (33.1%), Sub Saharan America and the Carribean (24.8%) (Wuehler et al. 2005). The root cause of Zn deficiency at this magnitude is its dearth at soil level rendering 50% of India's agricultural land to be deficit in Zn. A number of factors emanates in low availability and content of zinc in soil including low organic matter, high level of phosphorus, calcareous soils, cool soil temperature, a continuous nitrogen fertilization etc. (Lindsay 1972, Losak et al. 2011). As a result, plants are unable to absorb sufficient Zn from the deficient soils. It is a common constraint in cereal crops posing a serious threat in countries consuming cereal-rich diets; it becomes one of the major reasons for Zn deficiency in humans. Wheat, a staple and planetary cereal crop, is particularly subjected to Zn insufficiency. The world estimates for wheat production and consumption for the year 2012 are 684 and 677 million tonnes,

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respectively, India being its second largest producer in the world. Wheat not only falls short of grain Zn concentration but also endows with substances such as polyphenols and phytic acid, hampering thus the bioavailability or Zn absorption in the human gut (Welch and Graham 2004). Phytic acid despite of being a store of phosphorus in grains has been distinguished as the key chelator of Zn, promoting the insolubility of Zn in diet and reducing its retention in human body. What makes the problem more complicated? A large proportion of Zn is present in the embryo and aleurone layer (Ozturk et al. 2006) which are also opulent in phytic acid (Lott and Spitzer 1980), enhancing the probability of Zn exhaustion to form Zn-phytate. For its imperative role in seed germination and seedling vigour, endeavour is made to maintain a balanced ratio of phytate and Zn by enriching the seeds with Zn. In recent years sulphur containing amino-acids was converging the attention of many researchers to elevating Zn absorption rate in animal gut (Ashmead 1992). Ascorbic acid is another component that was found to prevent the formation of phytic acid complexes with Fe. It is thought to promote the absorption of Zn as well but demands further clarification.

Such a scenario of Zn deficient soils points out towards the idea of supplying them with supplemental Zn at judicious rates. Since there are several absorption associated problems through soil, Zn should also be applied through foliage to the crop. The approach would be more logical if genotypes that are more suitable to the Zn application were screened out. This work was thus carried out to fulfil the above objectives by evaluating the potential of 20 kg ZnSO<sub>4</sub>/ha soil application with and without foliar spray of 0.5% ZnSO<sub>4</sub> solution in different genotypes of wheat in order to improve their nutritional value.

## MATERIAL AND METHODS

The field study was conducted at the N.E. Borlaug Crop Research Centre during the rabi season of 2009–2010 and 2010–2011 and the related laboratory experiments were conducted in the Department of Plant Physiology, College of Basic Sciences and Humanities, Govind Ballabh Pant University of Agriculture and Technology, Pantnagar, India. The experiment was laid out in split plot design. The experimental plot had a loam texture, 7.0 pH and

0.278 dS/m EC at 25°C, 10.3 g organic C/kg, 149.44 alkaline KMnO<sub>4</sub> hydrolyzable N mg/kg soil, 10.09 mg Olsen's P/kg soil, 115.5 mg ammonium acetate extractable K/kg soil and 0.42 mg DTPA extractable Zn mg/kg soil (Singh et al. 2005). Ten wheat genotypes namely UP 262, UP 2338, UP 2382, UP 2572, UP 2554, UP 2584, PBW 343, PBW 550, PBW 175 and PBW 590 were used in the given experiment. Three treatments were given in the field namely 0 kg ZnSO<sub>4</sub>/ha (Zn<sub>0</sub>), 20 kg ZnSO<sub>4</sub>/ha (Zn<sub>20</sub>) and 20 kg ZnSO<sub>4</sub>/ha + foliar spray of 0.5% solution of ZnSO<sub>4</sub> (Zn<sub>20+F</sub>). Foliar spray was given at maximum tillering stage and one week after flowering. Each treatment was replicated thrice. Phytic acid was estimated in wheat grains using a method described by Harland and Oberleas (1977). Zn concentration was determined in wheat grains with modifications in the methodology outlined by Ereifej and Gharaibeh (1993). The molar ratio between phytate and Zn was obtained after dividing the mole of phytate with the mole of Zn (phytate: 660.04 g/mol; Zn: 65.4 g/mol). Ascorbic acid was estimated in wheat grains according to Thimmaiah (2009). Methionine was estimated in wheat grains according to Sadasivam and Manickam (1991). The statistical analysis of data for all the parameters was carried out with analysis of variance for split plot design. The means were tested at  $P > 0.05$  using the STPR software designed at the Department of Mathematics, Statistics and Computer Science, CBSH, G.B. Pant University of Agriculture and Technology, Pantnagar, India.

## RESULTS AND DISCUSSION

**Phytic acid (PA).** The phytic acid content in different wheat genotypes decreased with increasing levels of Zn with the lowest values obtained when nourished with 20 kg ZnSO<sub>4</sub>/ha + foliar spray (Zn<sub>20+F</sub>). Relative to control Zn<sub>20+F</sub> brought about a decrement of 35.6% in 2009 and 10.8% in 2010 as an average of all genotypes. The genotypes PBW 590, UP 2554, UP 262 in 2009 and UP 262, UP 2338, UP 2554 in 2010 were found to have the lowest PA of all at utilization of Zn<sub>20+F</sub> (Table 1). Zn content observed in the present study seemed not to be bioavailable due to high content of PA. Phytic acid is a primary phosphate storage compound in seeds forming 70% of its reserves and therefore a greater P uptake might be a reason for an antagonistic relationship between PA and Zn nutrition. These

Table 1. Effect of different zinc levels on grain Zn content (mg/kg dry weight) and phytic acid (PA) (mg/g dry weight) in different genotypes of wheat (2009–2010 and 2010–2011)

Genotypes	Grain Zn content						PA					
	2009			2010			2009			2010		
	Zn <sub>0</sub>	Zn <sub>20</sub>	Zn <sub>20 + F</sub>	Zn <sub>0</sub>	Zn <sub>20</sub>	Zn <sub>20 + F</sub>	Zn <sub>0</sub>	Zn <sub>20</sub>	Zn <sub>20 + F</sub>	Zn <sub>0</sub>	Zn <sub>20</sub>	Zn <sub>20 + F</sub>
UP 262	17.23	20.0	30.77	18.57	19.07	34.37	22.58	21.43	13.13	23.98	19.60	19.19
UP 2338	20.03	22.7	32.83	18.97	24.43	36.90	19.04	23.07	13.20	22.42	21.95	19.26
UP 2382	18.37	31.27	36.90	22.87	26.53	37.57	22.23	20.66	13.60	22.71	20.76	19.64
UP 2572	10.53	18.97	28.97	22.57	22.47	40.47	22.12	19.62	14.71	21.63	21.63	20.20
UP 2554	25.10	24.13	42.57	23.37	19.73	30.93	22.01	19.26	12.70	21.15	17.59	19.45
UP 2584	24.87	15.40	25.87	20.57	24.03	32.23	23.68	21.76	15.61	23.27	18.65	20.38
PBW 343	11.57	18.97	25.13	22.00	21.23	34.73	24.13	21.50	13.52	23.03	22.49	20.74
PBW 550	16.20	25.10	36.17	22.27	25.77	38.07	20.85	25.20	16.43	21.76	18.91	20.64
PBW 175	16.17	17.70	26.23	24.23	26.83	33.57	24.93	14.53	20.81	22.30	21.15	20.68
PBW 590	14.63	25.40	36.40	20.20	21.93	35.70	24.99	18.15	11.77	22.40	19.60	19.94
	SeM ±	CD		SeM ±	CD		SeM ±	CD		SeM ±	CD	
T	0.34	1.31		0.70	2.75		0.80	3.12		0.31	1.20	
V	0.81	2.29		0.52	1.47		0.86	2.44		0.43	1.23	
V within T		3.97			2.55			4.23			2.13	
V across T		3.98			3.61			5.03			2.33	

T – treatment; V – variety; CD – critical difference

findings are in conformity with those of previous researchers (Cakmak et al. 1999, Yang et al. 2011). The greater P uptake under reduced supply of Zn or reduced P uptake under increased Zn nutrition may be presumably due to reduced competition generating between both the elements for the same absorption site in roots. Also Zn deficiency causes an increase in the expression of P transporter genes (Huang et al. 2000).

**Grain Zn content.** Grain Zn content behaved unlike to PA at application of different Zn rates. During both years 2009 and 2010, it increased progressively with increasing levels of the micro-nutrient with Zn<sub>20 + F</sub>, proving to be uttermost and outdoing the zero Zn level by 94.4% in 2009 and 65.5% in 2010. Overall a higher content was observed in UP 2554, UP 2382, PBW 590 in 2009 and UP 2572, PBW 550, UP 2382 in 2010 when supplied with Zn<sub>20 + F</sub> (Table 1). The Zn content in grains should undoubtedly increase with increasing the Zn levels, which occurred in this nutritional work. Increasing Zn nutrition enables the plants to uptake greater amount of Zn and to accumulate it in different tissues, especially leaves; it is then remobilized and gets allocated in grains (Jiang et al.

2008). The present study draws support from that of Muthukumararaja and Sriramachandrasekharan (2012), in which the highest grain zinc content was observed at the utmost Zn rate of 7.5 mg/kg soil application. Similarly, in their study Yilmaz et al. (1997) showed that soil along with foliar Zn application could improve it by 3 to 4 fold.

**Phytate to Zn molar ratio (PA:Zn).** The phytate to Zn molar ratio exhibited a progressive reduction with increasing levels of zinc with Zn<sub>20 + F</sub> proving to be the best zinc rate in its reduction. It showed about 39.5% to 73.4% and 30.3% to 56.6% reduced ratio when compared to control in 2009 and 2010, respectively. Overall it gave lower ratios in PBW 590, UP 2338, UP 2382 in 2009 and UP 2572, UP 2338, UP 2382 in 2010. It is apparent for PA:Zn to follow a declining trend as it is attributable to the reducing PA and enhancing grain Zn content with Zn application. In the present study PA:Zn ratios are reported to be very high on native soils and wide differences were observed at applying Zn. The solution to the problem was also sketched out earlier by establishing an inverse relationship between PA:Zn and Zn fertilization (Kaya et al. 2000, Malakouti et al. 2007).

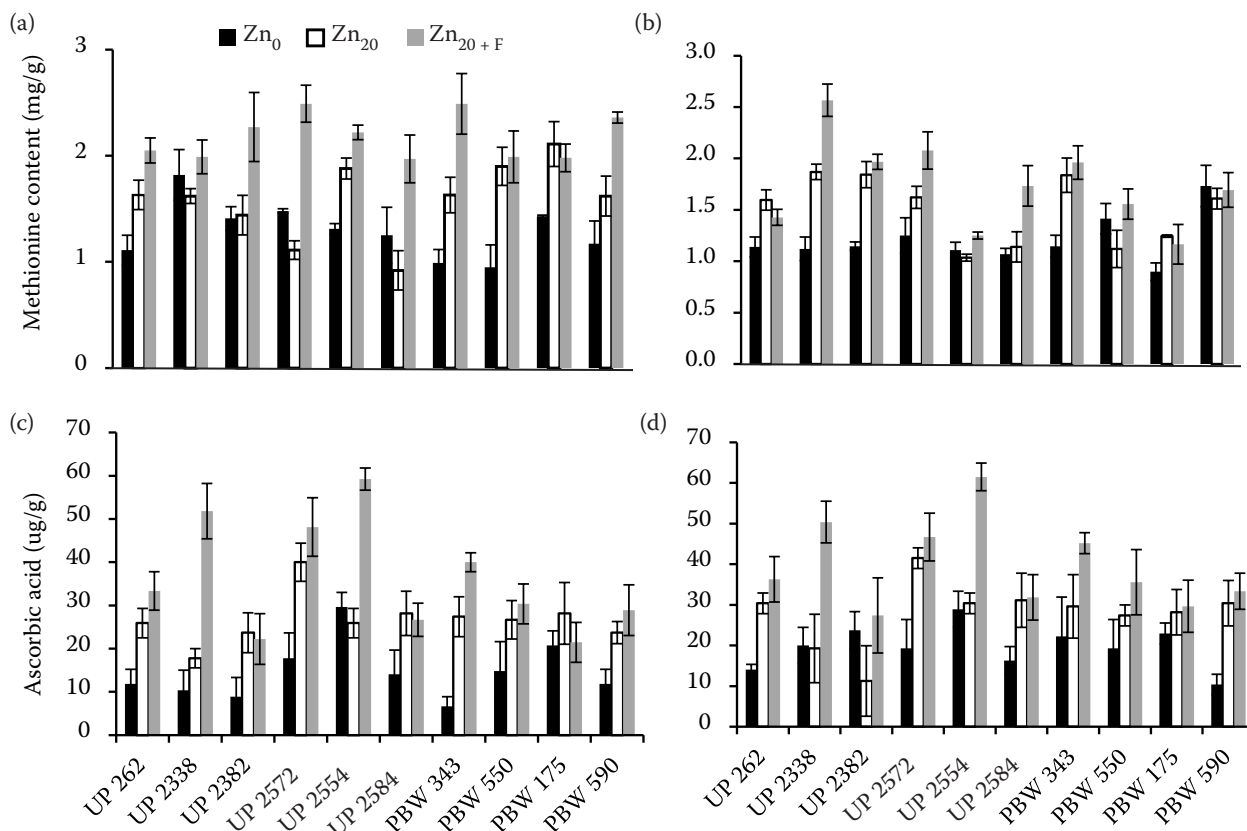


Figure 1. Effect of different Zn levels on methionine [2009–2010 (a) and 2010–2011 (b)] and ascorbic acid content [2009–2010 (c) and 2010–2011 (d)] in dry weight of grains of different genotypes of wheat (vertical bars indicate  $\pm$  SD)

**Methionine content (Met).** Methionine content increased with the progressive application of Zn with the highest values gained at  $Zn_{20+F}$  in both years. The greater the Zn rate, the greater the Met content for most of the genotypes.  $Zn_{20+F}$  surpassed  $Zn_0$  by 75% in 2009 and 47.6% in 2010. A higher content was observed in UP 2572, PBW 343, PBW 590 in 2009 and UP 2338, UP 2572, UP 2382 in 2010 at application of  $Zn_{20+F}$  (Figure 1a,b). It is well documented that Zn is an important cofactor of RNA polymerase facilitating the polymerisation of mRNA encoding amino acids including the S-containing ones such as methionine. A direct rise in Met content was observed earlier in crops at Zn fertilization (Juliano et al. 1987, Misra and Abidi 2010).

**Ascorbic acid (AA).** Ascorbic acid content increased gradually with increasing rates of Zn in both the crop seasons. The highest values for AA in all the genotypes were achieved at  $Zn_{20+F}$ . It gave 184.4% and 111.4% higher AA relative to control in 2009 and 2010, respectively. It gave higher AA values in UP 2554, UP 2338 and UP 2572 in 2009 and UP 2554, UP 2338, UP 2572 in

2010 (Figure 1c,d). In the present study a slight amount of AA was detected and is found to increase with increasing levels of Zn. To our knowledge not much literature is available at the presence of AA in wheat grains. Therefore these observations find support from the findings in which an increase in AA content was observed at application of Zn in leaves of wheat genotypes under salt stress (Sairam et al. 2005). A little amount of AA was detected in red sorghum, finger millet and maize fortified with micronutrients (Towo et al. 2006). A correlation between Zn and AA was also highlighted in wheat seedlings under salt stress in which an increase in Zn and Fe content was observed at application of AA (Chen et al. 2011).

In conclusion, undoubtedly, application of Zn in soil was beneficial in ameliorating the nutritional status of wheat grains but soil application + foliar spray outperformed all other treatments. All the genotypes proclaimed a genetic variance responding distinctly to different Zn approaches. As set forth herein the different genotypes conferred different response for varying traits. It is noteworthy as well that the genotypes differ in

their performance on Zn fertilization. As evincible from the two-year study, the genotype UP 2382 can be suggested as capable of both accumulating greater Zn content and maintaining lower PA:Zn at Zn fertilization. UP 2572 was revealed as the one high in AA and Met under similar conditions. UP 2554A was manifested as genotype high in AA and low in PA at the same time.

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