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Zinc enrichment in wheat genotypes under various methods of zinc application

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ABSTRACT

Around half of the cereal growing soil in the world are zinc (Zn)-deficient and it severally affects the health of plants, animals and humans. In order to investigate the enrichment of Zn in cereals a pot experiment was conducted in two contrasting wheat genotypes viz., UP2628 (Zn efficient) and UP262 (Zn inefficient) under different methods of Zn application such as control (0 Zn), soil application (5 mg Zn/kg soil tagged with 3.7 MBq of ^{65}Zn/pot), foliar spray of 0.5% $ZnSO_4$ at 30, 60 and 90 days (tagged with 925 KBq of ^{65}Zn/pot), soil application (5 mg Zn/kg soil tagged with 3.7 MBq of ^{65}Zn/pot) + foliar spray of 0.5% $ZnSO_4$ at 30, 60 and 90 days (tagged with 925 KBq of ^{65}Zn/pot). Cultivars showed marked difference in ^{65}Zn accumulation and grain Zn content. In both contrasting genotypes the highest Zn content in grains was recorded under soil application + foliar spray of Zn fertilizers. Both UP262 and UP2628 showed similar accumulation of ^{65}Zn in leaves however, UP2628 exhibited better translocation efficiency and accumulated higher ^{65}Zn in stem and grains than UP262.

Keywords: *Triticum aestivum* L.; zinc deficiency; zinc availability; micronutrient; nutritional quality

Wheat (*Triticum aestivum* L.) is an important staple food crop all over the world. Average yield of wheat is severely affected by deficiency of nitrogen and phosphorus followed by zinc (Zn), a major cause of yield reduction (Hotz and Braun 2004). Nearly 50% soil of cereal-growing areas in the world has low Zn availability. The problem is most serious in areas where soil pH and $CaCO_3$ content are high and soil organic matter content is low (Mirzapour and Khoshgoftar 2006). Zinc deficiency affects one third of the world's population. The problem is most serious in developing countries, where about 50–70% of the daily calories are derived from cereal grains (Cakmak 2008). The effects of Zn deficiency on human health are significant and leading to growth retardation, reduced immunity, learning deficits, mental retardation, DNA damage etc. (Prasad 2007).

There are many factors that lead to the Zn deficiency in agricultural soils. The main soil factors are low total content of Zn, soil pH, high contents of calcite, high concentrations of bicarbonate ions and salts, high levels of available phosphorus and interaction with other nutrient element (Alloway 2009). Zinc fertilization has been widely used to alleviate Zn deficiency in crops in recent years. Application methods include direct soil amendment, pre-sowing seed soaking and foliar spray. Zinc is easily immobilized in the soil solution due to high soil pH; therefore, foliar Zn application is generally the most effective means for increasing grain Zn concentrations (Wen et al. 2011). Application of micronutrient fertilizers to the crop foliage increased micronutrient concentrations in grain. The most effective method for increasing zinc in grain was the soil + foliar application method. When a high concentration of grain zinc is aimed in addition to a high grain yield, combined soil and foliar application is recommended (Bharti et al. 2013).

Therefore, an attempt has been made to evaluate the translocation and enrichment of ^{65}Zn in

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two contrasting wheat genotypes under various methods of Zn application.

MATERIAL AND METHODS

A bulk of surface soil (0–15 cm) was collected from E1 plot of the Norman E. Borlauge Crop Research Center, G.B. Pant University of Agriculture and Technology, Pantnagar, India. The experimental soil had sandy loam texture, 7.4 pH and 0.266 dS/m electical conductivity, 10.5 g organic C, 7.9 mg Olsen's extractable P/kg soil, 42.7 mg ammonium acetate extractable K/mg soil, 218 mg ammonium acetate extractable Ca/kg soil, 36 mg ammonium acetate extractable Mg/kg soil, 0.47 mg DTPA extractable Zn/kg soil and 25.3 mg DTPA extractable Fe/kg soil. Soil was low in K supply, medium in P supply and high in Ca and Mg supply. Two contrasting wheat genotypes viz. UP262 (Zn inefficient) and UP2628 (Zn efficient) were used for the study and the seeds of both the genotypes were obtained from the Department of Genetics and Plant Breeding of the University. Seeds were allowed to germinate before sowing.

After the processing, soil was filled in plastic pots of 4 kg capacity. A basal dose of nitrogen, phosphorus and potassium was applied to all pots at the rate of 22.3 mg N, 11.6 mg P and 18.5 mg K per kg soil using stock solutions of urea, KH_2PO_4 and KCl, respectively. The treatments applied were control (0 Zn), soil application (5 mg Zn/kg soil tagged with 3.7 MBq of ^{65}Zn/pot), foliar application of 0.5% $ZnSO_4$ (tagged with 925 KBq of ^{65}Zn/pot) at 30, 60 and 90 days after planting, soil application (5 mg Zn/kg soil tagged with 3.7 MBq of ^{65}Zn/pot) + foliar spray of 0.5% $ZnSO_4$ (tagged with 925 KBq of ^{65}Zn/pot) at 30, 60 and 90 days after planting in triplicate. The experimental design was two factorial completely randomized designs.

Plants were harvested at maturity and the aerial parts were partitioned into leaves, psuedostems, and grains. Plant samples were washed with tap water, 0.1 mol/L HCl and subsequently with distilled water to remove the surface contamination. Then the samples were dried and 1 g of each plant part was digested in di-acid (HNO_3:$HClO_4$ in 4:1 v/v) and final volume was made up to 14 mL with double distilled water. Two mL aliquot of each digested sample was used to record activities of ^{65}Zn on gamma ray spectrometer with NaI crystal (model GRS 101P, Mumbai, India). Activity was expressed as cpm (counts per min) per g dry weight of plant sample. After recording the activities of ^{65}Zn, concentration of Zn was estimated in grains by atomic absorption spectrophotometer (ECIL, Hyderabad, India) and expressed as μg/g dry weight of plant sample. The statistical analysis of all the parameters was done by using the analysis of variance (ANOVA) using SPSS 16 (Bristrol, UK). The means were tested at $P \leq 0.05$ level of significance.

RESULTS AND DISCUSSION

Grain, straw and total dry matter. Grain yield, straw yield and total dry matter of both the wheat cultivars under different methods of Zn application are presented in Table 1. UP2628 produced higher grain yield as compared to UP262 and effect of cultivars was noted significant. Surprisingly, UP262, produced higher straw yield and total dry matter as compared to UP2628. Among different Zn application methods, soil application of 5 mg Zn/kg of soil along with foliar spray of 0.5% zinc sulphate solution was the most effective as it increased grain yield, straw yield and total dry matter by 39.8, 90.9 and 66.2%, respectively as compared to control. Soil application of 5 mg Zn/kg soil along with foliar application of 0.5% zinc sulphate solution increased the grain yield, straw yield and total dry matter by 24.4, 50.9 and 40.9%, respectively, as compared to soil application of 5 mg Zn/kg soil alone whereas the increment was 5.8, 18.0 and 12.4%, respectively, in comparison to foliar spray of 0.5% zinc sulphate solution. The interaction effect of wheat cultivars and different methods of Zn application significantly affected the grain and straw yield while the effect was noted non-significant for total dry matter. In UP2628, only foliar spray of Zn solution and combined soil and foliar application of Zn increased grain yield by 36.9% and 50.2% over control, respectively. Likewise in UP262, foliar spray of Zn and combined soil and foliar application of Zn increased the grain yield by 27.2% and 29.8% over control, respectively. As regard the straw yield, in UP2628, only foliar spray of Zn solution and both soil application of 5 mg Zn/kg soil and foliar application of Zn increased the straw yield by 51.7% and 85.5%, respectively. In case of UP262, soil application of 5 mg Zn/kg soil, foliar spray of Zn solution and combined soil and

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Table 1. Effect of different methods of zinc application on grain weight/pot (g), straw weight/pot (g) and total dry matter/pot (g) of two contrasting wheat genotypes (UP2628 – Zn efficient, UP262 – Zn inefficient)

Treatment	Grain weight/pot			Straw weight/pot			Total dry matter/pot		
	UP2628	UP262	average	UP2628	UP262	average	UP2628	UP262	average
Control	1.95	1.91	1.93	2.01	1.97	1.99	3.95	3.88	3.91
Soil application	2.16	2.19	2.17	2.29	2.57	2.43	4.46	4.76	4.61
Foliar application	2.67	2.44	2.55	3.05	3.40	3.22	5.73	5.84	5.78
Soil + foliar application	2.93	2.48	2.70	3.73	3.88	3.80	6.65	6.36	6.50
Average	2.42	2.25	2.33	2.77	2.95	2.86	5.19	5.21	5.20
	SEM ±	CD at 5%		SEM ±	CD at 5%		SEM ±	CD at 5%	
Cultivar (V)	0.06	0.19		0.08	0.24		0.16	0.34	
Treatment (T)	0.09	0.28		0.10	0.34		0.11	0.49	
V × T	0.13	0.39		0.10	0.48		0.23	ns	

SEM – standard error of mean; ns – non significant; CD – critical difference

foliar application of Zn increased the straw yield by 30.4, 72.5 and 96.9% over control, respectively.

The results of the present study revealed that the Zn application methods had a favorable effect on grain yield, straw yield and total dry matter accumulation of both contrasting wheat cultivars. An increase in the grain weight, straw weight and total dry matter was observed with increasing regimes of Zn in both the cultivars. UP2628 produced more grain yield as compared to UP262 while the straw yield was measured higher in UP262 in comparison to UP2628. This clearly showed a better remobilization of carbohydrates from leaves to grains of UP2628. The most probable reason of these results might be due to the role of Zn in chlorophyll biosynthesis, maintaining chl *a:b* ratio, maintenance of photosynthetic machinery and biosynthesis of auxin, which regulate the remobilization of carbohydrates to the grains (Rehman et al. 2012). The highest grain and straw yields of both the wheat cultivars could be achieved with combined soil and foliar application of Zn (Karak and Das 2006). Similarly, increased in grain yield (15.85% higher that control) and biological yield was recorded by the combined application of soil and foliar spray of zinc sulphate (20 kg Zn/ha) as compared to control in wheat cultivars differing in their Zn efficiency (Bharti et al. 2013). Gomaa et al. (2015) also reported increased grain yield with foliar application of micronutrient as compared to soil application.

Accumulation of ^{65}Zn in different plant parts. Counts per min of ^{65}Zn in leaves, stem and grains of both wheat cultivars are presented in Figure 1. Irrespective of the Zn application method, UP2628 accumulated more ^{65}Zn in stem and grains than UP262; however, the accumulation of ^{65}Zn in leaves of both the cultivars was statistically similar. Regarding the methods of Zn application, the highest accumulation of ^{65}Zn was recorded with combined soil and foliar application of Zn followed by foliar application and least accumulation was noted with soil application of Zn. Interaction effect of cultivar and methods of Zn application showed that UP2628 accumulated higher ^{65}Zn in all the plant parts as compared to UP262 when Zn was applied through soil and foliar spray, individually. With combined soil and foliar application of Zn fertilizer UP2628 and UP262 showed similar accumulation of Zn, however, UP2628 showed better translocation efficiency than UP262 and accumulated higher ^{65}Zn in stem and grains.

The data regarding the ^{65}Zn accumulation clearly revealed that UP2628 accumulated more ^{65}Zn in leaves as well as in stem and grains. This showed a better uptake and translocation efficiency of UP2628 as compared to UP262 which accumulated comparatively lesser amount of ^{65}Zn in all the plant parts. Efflux transporter genes of nicotianamine (ENA1 and ENA2) play a very important role in Zn uptake and transport in the plants and thus determine translocation efficiency of the plant (Nozoye et al. 2012). Increased amounts of NA and DMA (de-oxymugeinic acid) are involved in the efficient translocation of zinc and iron into rice grains (Kobayashi and Nishizawa 2012). Shankhdhar et

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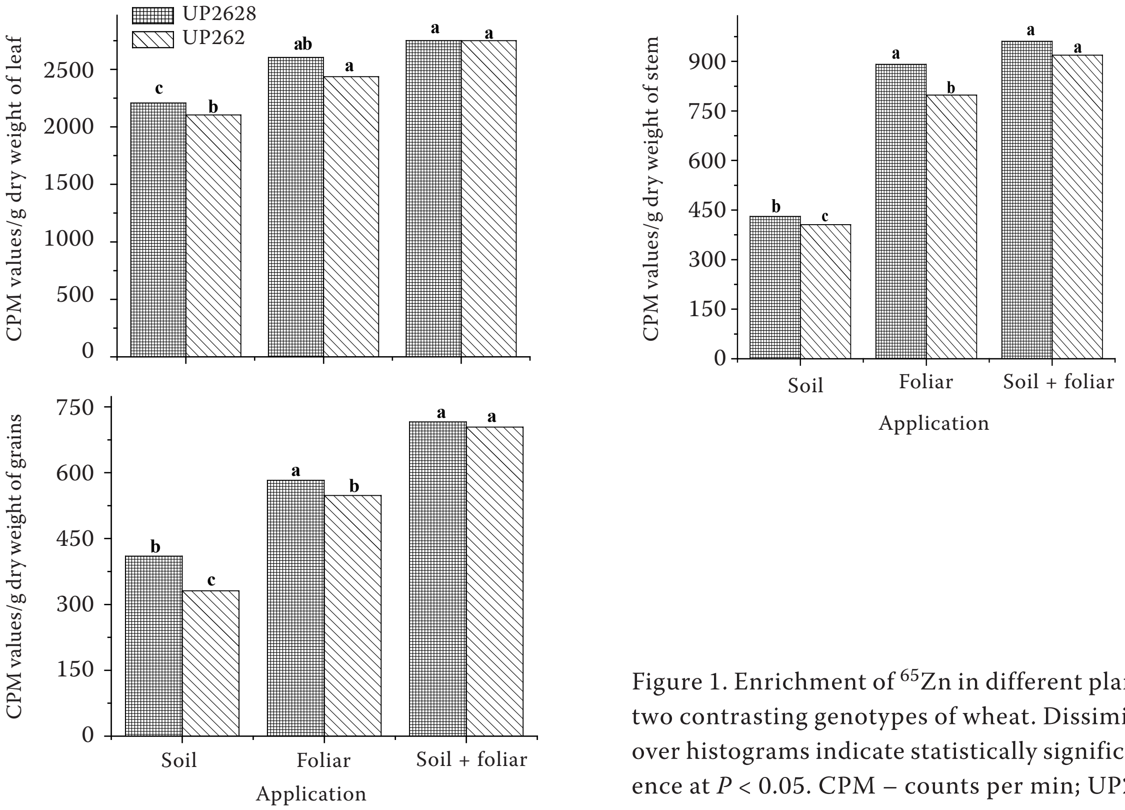

Figure 1. Enrichment of ^{65}Zn in different plant parts of two contrasting genotypes of wheat. Dissimilar letters over histograms indicate statistically significant difference at $P < 0.05$. CPM – counts per min; UP2628 – Zn efficient; UP262 – Zn inefficient

al. (2000) also reported similar genotypic variation in translocation and accumulation of ^{65}Zn in rice. Similarly, uptake of Zn was found to be increased under increasing regimes of Zn (Shehu and Jamala 2010). Accumulation of Zn in grains of wheat was also found to be higher when Zn was applied through both soil and foliar application (Zhao et al. 2011) and through foliar application alone (Zhang et al. 2012) as compared to no application of Zn.

Zinc content. The effect of different methods of Zn application on Zn concentration in grains of both contrasting wheat cultivars is presented in Figure 2. The main effect of cultivar showed that grains of UP2628 had significantly higher Zn concentration than UP262. The main effect of methods of Zn application indicated that combined soil application + foliar spray of Zn fertilizer was most effective in increasing the grain Zn concentration followed by foliar application alone and the lowest increase was noted with soil application of Zn. Interaction effect of cultivar and method of Zn application indicated that UP2628 had higher grain Zn concentration than those of UP262, with both combined soil + foliar application and with foliar spray of Zn fertilizers. Surprisingly, UP262 had higher Zn concentration in grains as compared to UP2628 with soil application of Zn.

Among the different methods of Zn application soil + foliar application was found to be most effective in increasing Zn concentration in grains of both the cultivars. Irrespective of the Zn application methods, UP2628 contain higher concentration of zinc as compared to UP262. Khan et al.

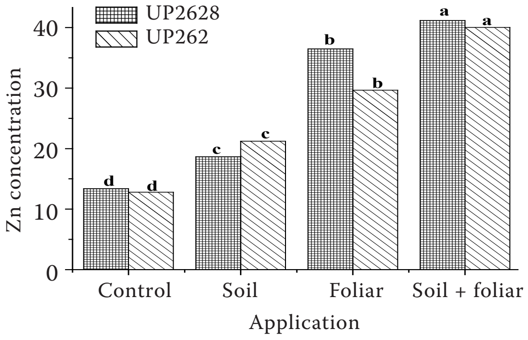

Figure 2. Effect of different methods of zinc application on zinc concentration (μg/g) in grains of two contrasting wheat genotypes. Dissimilar letters over histograms indicate statistically significant difference at $P < 0.05$. UP2628 – Zn efficient; UP262 – Zn inefficient

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(2012), reported a percent increase of 130.8% in Zn content in rice grains by the application of 15 kg Zn/ha as compared to control. Maximum Zn content was reported in wheat grains under soil applied 20 kg Zn/ha along with foliar spray of 0.5% of zinc sulphate (Bharti et al. 2013). Recently, the highest Zn content was reported in wheat grains under foliar application of Zn (Shivay et al. 2015).

In conclusion, irrespective of the Zn efficiency, the highest yield was obtained by combined soil (5 mg Zn/kg soil) and foliar application of Zn (0.5% zinc sulphate) fertilizers. Regarding the Zn efficiency, UP2628 showed better translocation efficiency, thus accumulated more Zn in grains than UP262. The highest concentration of Zn in grains could be obtained by soil application of 5 mg Zn/kg soil + foliar sprays of 0.5% zinc sulphate.

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REFERENCES

Alloway B.J. (2009): Soil factors associated with zinc deficiency in crops and humans. Environmental Geochemistry and Health, 31: 537–548.

Bharti K., Pandey N., Shankhdhar D., Srivastava P.C., Shankhdhar S.C. (2013): Evaluation of some promising wheat genotypes (*Triticum aestivum* L.) at different zinc regimes for crop production. Cereal Research Communications, 41: 539–549.

Bharti K., Pandey N., Shankhdhar D., Srivastava P.C., Shankhdhar S.C. (2013): Improving nutritional quality of wheat through soil and foliar zinc application. Plant, Soil and Environment, 59: 348–352.

Cakmak I. (2008): Enrichment of cereal grains with zinc: Agronomic or genetic biofortification. Plant and Soil, 302: 1–17.

Gomaa M.A., Radwan F.I., Kandil E.E., Seham M.A., El-Zweek (2015): Effect of some macro and micronutrients application methods on productivity and quality of wheat (*Triticum aestivum*, L.). Middle East Journal of Agriculture Research, 4: 1–11.

Hotz C., Braun K.H. (2004): Assessment of the risk of zinc deficiency in populations and options for its control. Journal of Food Nutrition Bulletin, 2: 94–204.

Karak T., Das D. (2006): Effect of foliar application of different sources of Zn application on the changes in Zn content, uptake and yield of rice (*Oryza sativa* L.). In: Proceedings of the 18th World Congress of Soil Science, July 9–15, Philadelphia.

Khan P., Memon M.Y., Imtiaz M., Depar N., Aslam M., Memon M.S., Shah J.A. (2012): Determining the zinc requirements of rice genotype Sarshar evolved at NIA, Tandojam. Sarhad Journal of Agriculture, 28: 232–240.

Kobayashi T., Nishizawa N.K. (2012): Iron uptake, translocation, and regulation in higher plants. Annual Review of Plant Biology, 63: 131–152.

Mirzapour M.H., Khoshgoftar A.H. (2006): Zinc application effects on yield and seed oil content of sunflower grown on a saline calcareous soil. Journal of Plant Nutrition, 29: 1719–1727.

Nozoye T., Nagasaka S., Kobayashi T., Takahashi M., Sato Y., Sato Y., Uozumi N., Kobayashi T., Nishizawa N.K. (2012): Iron uptake, translocation, and regulation in higher plants. Annual Review of Plant Biology, 63: 131–152.

Prasad A.S. (2007): Zinc: Mechanisms of host defense. Journal of Nutrition, 137: 1345–1349.

Rehman H., Aziz T., Farooq M., Wakeel A., Rengel Z. (2012): Zinc nutrition in rice production systems: A review. Plant and Soil, 361: 203–226.

Shankhdhar S.C., Shankhdhar D., Sharma H.C., Mani S.C., Pant R.C. (2000): Genotypic variation of zinc-65 uptake and distribution in rice *(Oryza sativa* L.). Journal of Plant Biology, 27: 253–257.

Shehu H.E., Jamala G.Y. (2010): Available Zn distribution, response and uptake of rice (*Oriza sativa*) to applied Zn along a toposequence of lake Gerio Fadama soils at Yola, North-eastern Nigeria. Journal of American Science, 6*:* 1013–1016.

Shivay Y.S., Prasad R., Singh R.K., Pal M. (2015): Relative efficiency of zinc-coated urea and soil and foliar application of zinc sulphate on yield, nitrogen, phosphorus, potassium, zinc and iron biofortification in grains and uptake by basmati rice (*Oryza sativa* L.). Journal of Agricultural Science. doi: 10.5539/jas.v7np161.

Wen Y.X., Hong T.X., Chun L.X., William G., Xian C.Y. (2011): Foliar zinc fertilization improves the zinc nutritional value of wheat (*Triticum aestivum* L.) grain. African Journal of Biotechnology, 10: 14778–14785.

Zhang Y.Q., Sun Y.X., Ye Y.L., Karim M.R., Xue Y.F., Yan P., Meng Q.F., Cui Z.L., Cakmak I., Hang F.S., Zou C.Q. (2012): Zinc biofortification of wheat through fertilizer applications in different locations of China. Field Crops Research, 125: 1–7.

Zhao A., Lu X.C., Chen Z.H., Tian X.H., Yang X.W. (2011): Zinc fertilization methods on zinc absorption and translocation in wheat. Journal of Agricultural Science, 3: 28–35.

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