Enrichment of ^{65}Zn in two contrasting rice genotypes under varying methods of zinc application

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ABSTRACT

Zinc (Zn) is an essential micronutrient for growth and development of almost all organisms and its deficiency severely affects the health of plants, animals and humans. In order to investigate the enrichment of Zn in cereals a pot experiment was performed in two contrasting rice varieties viz., PD16 (zinc efficient) and NDR359 (zinc inefficient) under different levels of zinc regimes 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 (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 (925 KBq of ^{65}Zn/pot). Both varieties markedly differ in ^{65}Zn accumulation and grain Zn content. NDR359 showed poor translocation efficiency and accumulated relatively less ^{65}Zn in all the plant parts. In both rice varieties, highest concentration of Zn in dehusked grains could be obtained with soil application of Zn + foliar spray of zinc sulphate. Though NDR359, a zinc inefficient variety exhibited poor zinc translocation efficiency yet, it contained more Zn content in grains with husk and dehusked grains than PD16.

Keywords: zinc uptake; translocation; accumulation; rice grains

Zinc (Zn) deficiency is a serious agricultural problem as around one half of the cereal-growing soils in the world are Zn deficient (Cakmak et al. 1999). The solubility and availability of Zn is limited by various factors like high $CaCO_3$, high pH, high clay content, low soil organic matter, low soil moisture, and high content of iron and aluminum oxides (Cakmak 2008). In low Zn soils, some plants mobilize more Zn by the exudation of low-molecular-weight compounds like malate and mugineic acid family phytosiderophores (Arnold et al. 2010). Zinc uptake across root plasma membrane is carrier-mediated. Zinc iron permeases (ZIP) family of metal transporters is the primary uptake system of plants, but channel proteins might also play role in Zn transport. Several Zn-transporter genes showed enhanced expression in rice roots under Zn deficiency i.e. OsZIP1, OsZIP3, OsZIP4, and OsZIP5 (Lee et al. 2010a,b) and OsZIP1, OsZIP3 and OsZIP4 in vascular bundles for Zn transport to shoot (Ishimaru et al. 2005).

In humans, Zn deficiency is widespread nutritional disorder which severely limits the physical and intellectual capacity of the people and adversely affects their health (Hambidge 2000, Bonsmann and Hurrell 2009). As per an estimate of the World Health Organization about 2 billion people (33%) are deprived of the US recommended dietary intake of Zn (WHO 2002). Around 1.2 billion (20%) people representing 20% of world population are at risk of inadequate Zn intakes (Hotz and Brown 2004). In case of Zn deficiency, the related adverse health results are mainly documented for infants and children like stunting, diarrhoea and pneumonia (Stein et al. 2005).

Dietary Zn content is very important for human health as the artificial supplementation of foods with essential minerals is often difficult to achieve, particularly in developing countries. Therefore, increased levels of Zn in the staple foods like rice may help reducing Zn deficiency. It is, therefore, essential to understand the molecular mechanism through which plants take up, mobilize, translocate and store Zn in the edible plant parts (Ishimaru et al. 2011). As genotypic variation for grain Zn and Fe concentration is relatively narrow and limited among different cereal crops, soil and foliar application of Zn is commonly practiced to augment Zn concentration in grains (Cakmak 2002, Pahlavan-Rad and Pessarakli 2009). In the present study, an attempt has been made to evaluate the translocation and enrichment of ^{65}Zn in two contrast rice varieties which varied in their Zn tolerance under different methods of zinc fertilizer application.

MATERIAL AND METHODS

A bulk surface (0–15 cm) soil (Typic Hapludoll) was collected from E1 plot of Norman E. Borlaug Crop Research Center, G.B. Pant University of Agriculture and Technology, Pantnagar, India. The soil sample was air-dried under shade and crushed with a wooden roller and passed through sieve having openings of 2 mm diameter. The experimental soil had sandy loam texture, 7.4 pH and 0.266 dS/m EC, 10.5 g organic C, 0.47 mg DTPA extractable Zn/kg soil and 25.3 mg DTPA extractable Fe/kg soil. The seeds of two contrasting genotypes of rice viz. NDR359 (Zn inefficient) and Pant Dhan16 (Zn efficient) were obtained from the Department of Genetics and Plant Breeding of the University. Seedlings of both rice varieties were raised in plastic trays filled with this soil.

The soil was filled in plastic pots of 4 kg capacity. A basal dose of 22.3 mg N, 11.6 mg P and 18.5 mg K/kg soil using stock solutions of urea, KH_2PO_4 and KCl, respectively was applied to all the pots. The treatments tested in triplicate were: control (0 mg Zn/kg soil), soil application (5 mg Zn/kg soil, tagged with 3.7 MBq of ^{65}Zn/pot), foliar spray (0.5% $ZnSO_4$ + 0.25% lime at 30 (tillering), 60 (panicle initiation) and 90 (grain filling) 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 0.5% $ZnSO_4$ + 0.25% lime at 30 (tillering), 60 (panicle initiation) and 90 days (grain filling), tagged with 925 KBq of ^{65}Zn/pot) after transplanting. After soil application of Zn in the specified treatments, the soil of each pot was thoroughly mixed, flood irrigated and left for 3 days for equilibration. Five seedlings of rice varieties PD16 or NDR359 were planted in each pot. The solution used in each foliar spray was 10 mL/pot. Remaining nitrogen was top dressed in two equal splits through urea at 30 and 60 days after transplanting.

At maturity, the plants were harvested and the aerial parts of the plant were partitioned into leaves, pseudostems, grain with husk and grain without husk. Plant samples were washed sequentially with tap water, 0.1 mol/L HCl and distilled water to remove the surface contamination. Then the samples were dried in an electric oven at 60°C for 48 h and weighed. A portion of grains from each pot was manually dehulled to get grains without husk. One gram 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 digested sample was used to assay activities of ^{65}Zn on gamma ray spectrometer with NaI crystal (model GRS 101P, Mumbai, India). Activity was expressed as cpm (counts per min)/g dry weight of the plant sample. After recording the activities, final volume of this two mL aliquot was made up to 10 mL and the concentration of Zn in grains with husk and without husk was estimated by atomic absorption spectrophotometer (ECIL, Hyderabad, India) and expressed as μg/g dry weight of plant sample.

Statistical analysis. The statistical analysis of the data was done by the analysis of variance (ANOVA) using SPSS 16 (Bristol, UK). The means were tested for significance at $P \leq 0.05$.

RESULTS AND DISCUSSION

Grain, straw and total dry matter yield. Grain, straw and total dry matter yields of both rice varieties under different zinc regimes are presented in Table 1. Zinc efficient rice variety, PD16 produced higher straw yield than NDR359, a zinc inefficient rice variety. No significant effect of varieties was noted on grain and total dry matter yields. Among different zinc application methods, soil application of 5 mg Zn/kg soil + foliar application of Zn was the most efficacious as it gave nearly five-fold higher grain yield, more than two fold higher straw yield and about three fold higher total dry matter yield of rice over the control (no application of Zn). A

Table 1. Effect of different methods of zinc application on grain weight/pot (g), straw weight/pot (g) and total dry matter (TDM) of two rice genotypes

Treatments	Grain weight			Straw weight			TDM		
	PD16	NDR359	average	PD16	NDR359	average	PD16	NDR359	average
Control	2.60	2.46	2.53	4.60	5.19	4.89	7.21	7.65	7.43
Soil application	5.90	3.66	4.78	5.42	6.39	5.90	11.32	10.05	10.68
Foliar application	9.10	11.32	10.21	11.04	8.03	9.53	20.15	19.35	19.75
Soil + foliar application	11.98	12.62	12.30	11.60	10.22	10.91	22.58	22.84	22.71
Average	7.39	7.51	7.45	8.16	7.45	7.80	15.31	14.97	15.14
	SEM ±	$LSD_{0.05}$		SEM ±	$LSD_{0.05}$		SEM ±	$LSD_{0.05}$	
V (variety)	0.16	ns		0.16	0.47		0.24	ns	
T (treatment)	0.22	0.66		0.22	0.67		0.35	1.04	
V × T	0.31	0.93		0.32	0.95		0.49	ns	

SEM – standard error of mean; ns – nonsignificant

comparison of rice yields under rest of other two methods of Zn application revealed that foliar application of Zn was more effective in increasing grain, straw and total dry matter yields than soil application of 5 mg Zn/kg soil. The interaction effect of rice varieties and methods of Zn application influenced only the grain and straw yields significantly. In PD16, a Zn efficient rice variety, soil application of 5 mg Zn/kg soil, foliar application of Zn and combined soil and foliar spray of Zn increased grain yield by 126.9, 250.0 and 360.8% over the control, respectively. In NDR359, a Zn inefficient variety, soil application of 5 mg Zn/kg soil, foliar application of Zn and combined soil application of Zn + foliar application of Zn increased grain yield by 48.8, 380.2 and 413.0% over the control, respectively. Interestingly, the magnitude of yield increase under soil application of 5 mg Zn/kg soil was higher with PD16 than NDR359, a Zn inefficient variety while the magnitude of yield increase under foliar application of Zn was higher in NDR359 than PD16. The grain yield obtained under soil application of 5 mg Zn/kg soil + foliar application of Zn was statistically similar for both rice varieties. As regard the straw yields, in the case of PD16, a Zn efficient variety, only foliar application of Zn and combined soil application of 5 mg Zn/kg soil + foliar application of Zn increased straw yield by 140.0% and 152.2% over the control, respectively. In NDR359, soil application of 5 mg Zn/kg soil, foliar application of Zn and combined soil application of 5 mg Zn/kg soil + foliar application of Zn increased the straw yields significantly by 23.1, 54.7 and 96.9% over the control, respectively.

The present study revealed a favorable effect of zinc application on total dry matter accumulation, grains and straw yield of rice varieties. An increase in the grain weight and straw weight was observed in both the varieties, which could be ascribed to possible role of Zn in chlorophyll biosynthesis, maintaining chl *a*:*b* ratio, maintenance of photosynthetic machinery and biosynthesis of auxin, that determine the remobilization of carbohydrates from leaves to grains (Rehman et al. 2012). Statistically higher grain yield of PD16 as compared to NDR359 under soil application of 5 mg Zn/kg soil indicated much higher absorption and translocation ability of ^{65}Zn in PD16 (Zn efficient variety) as compared to NDR359, a Zn inefficient variety which responded better to foliar application of Zn. These results also signified that irrespective of Zn efficiency of rice variety, the highest grain and straw yields could be achieved with combined soil application of Zn and foliar spray of Zn (Karak and Das 2006).

Accumulation of ^{65}Zn in different plant parts. The cpm values of ^{65}Zn in different plant parts of rice varieties are shown in Figure 1. In general, irrespective of the method of Zn application, PD16; a Zn efficient variety accumulated more ^{65}Zn in leaves, stem and grains with husk than NDR359 (a Zn inefficient variety) however, the accumulation of ^{65}Zn in grains without husk of both the

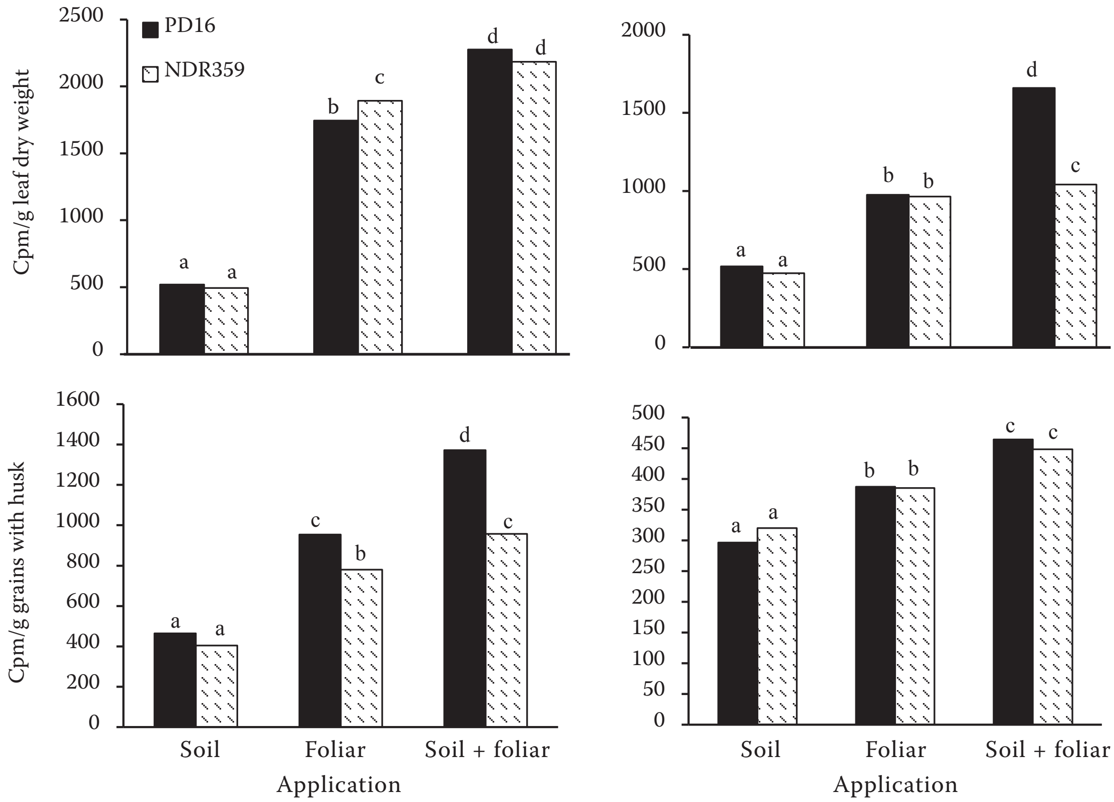

Figure 1. Enrichment of ^{65}Zn in different plant parts of two rice genotypes under different methods of Zn application. Dissimilar letters over histograms indicate statistically significant difference at $P \leq 0.05$

varieties was statistically similar. Further, as regards the method of Zn application, the highest accumulation of ^{65}Zn in different plant parts was noted under combined soil application + foliar application, followed by foliar application and the least accumulation of ^{65}Zn was recorded with soil application. The interaction effect of variety and the method of application of Zn fertilizer on ^{65}Zn accumulation in different plant parts of rice varieties indicated that with the foliar application of Zn PD16 grains with husk accumulated significantly higher activity of ^{65}Zn as compared to NDR359 even though the leaves of the latter variety had significantly higher activity of ^{65}Zn than those of PD16, indicating poor Zn translocation ability of NDR359. With combined soil application and foliar application of Zn, PD16 stem and grains with husk accumulated significantly higher activity of ^{65}Zn than NDR359.

Regarding the accumulation of ^{65}Zn in different plant parts of both the varieties i.e., PD16 (efficient) and NDR359 (inefficient), showed a marked variation. Irrespective of the method of Zn application, PD16 showed a higher accumulation of ^{65}Zn in different plant parts as compared to NDR359 which is attributed to a better translocation efficiency of PD16. Similar genotypic variation in translocation and accumulation of ^{65}Zn was also reported earlier by Shankhdhar et al. (2000). Translocation efficiency depends on expression of the efflux transporter genes of nicotianamine (ENA1 and ENA2) which are likely to play role in Zn uptake and transport inside plants (Nozoye et al. 2011). Increased amounts of nicotianamine and deoxymugineic acid are involved in the efficient translocation of Zn and Fe into rice grains (Kobayashi and Nishizawa 2012).

Zinc concentration. The data on the effect of different methods of Zn application on Zn concentration in grains with husk and grains without husk are presented in Table 2. The main effect of variety indicated that NDR359 grains with husk had significantly higher Zn concentration than those of PD16; however, reverse was true in case of grains without husk. As regards the methods of Zn application, combined soil application + foliar application of Zn was most effective in increasing Zn concentration in both grains with husk and

Table 2. Effect of different methods of zinc application on Zn concentration (μg/g) in grains with husk of two rice genotypes

Treatments	Grains with husk			Grains without husk		
	PD16	NDR359	average	PD16	NDR359	average
Control	15.80	18.63	17.21	12.90	11.80	12.35
Soil application	24.75	37.09	30.92	19.16	20.74	19.95
Foliar application	42.24	54.42	48.33	35.98	29.16	32.57
Soil + foliar application	44.28	69.31	56.79	40.18	39.52	39.85
Average	31.76	44.86	38.31	27.05	25.30	26.18
	SEM ±	$LSD_{0.05}$		SEM ±	$LSD_{0.05}$	
V (variety)	0.55	1.66		0.33	0.99	
T (treatment)	0.78	2.35		0.47	1.40	
V × T	1.11	3.32		0.66	1.98	

SEM – standard error of mean

without husk followed by foliar application of Zn and the lowest increase was observed with soil application of Zn. The interaction effect of variety and method of Zn application indicated that NDR359 grains with husk accumulated more Zn concentration than husked grains of PD16 under all methods of Zn application except control. In the case of grains without husk, the interaction effect of rice variety and method of Zn application revealed that with foliar spray of Zn, PD16 variety maintained significantly higher Zn concentration in dehusked grains compared to NDR359. The differences in Zn concentration of dehusked grains of both varieties were statistically not significant under control, soil application of Zn or combined soil application + foliar spray of Zn.

Zinc concentration in rice grains increased with Zn application and the highest Zn concentration was recorded with combined soil application + foliar spray of Zn in both the varieties. In NDR359, the grains with husk accumulated higher content of Zn than PD16 but in dehulled grains PD16 acquired higher Zn concentration than NDR359 and this indicated that more Zn concentrated in husk part of NDR359 grains. Genotypic variations in grain Zn concentration have been attributed to the difference in physiological processes (Gao et al. 2012). Recently, the results of Bharti et al. (2013) also showed the highest Zn content in wheat grains under the treatment receiving soil application of 20 kg Zn/ha along with foliar spray of 0.5% of zinc sulphate solution.

In conclusion, irrespective of Zn efficiency of rice varieties, the highest yield potential of rice can be obtained with combined application of 5 mg Zn/kg soil and three foliar sprays of Zn. NDR359 showed relatively poor translocation efficiency of Zn and accumulated relatively higher concentration of Zn in husk part as compared to PD16, a Zn efficient variety. The highest concentration of Zn in dehusked grains could be obtained by soil application of 5 mg Zn/kg soil + foliar sprays of 0.5% zinc sulphate.

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