



Relative grain zinc loading ability of cereals (rice, wheat, maize) and a grain legume (chickpea)

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ABSTRACT

Data from field experiments conducted at the ICAR-Indian Agricultural Research Institute, New Delhi, India during the last few years on Zn biofortification of the cereals, viz. wheat (*Triticum aestivum* L.), rice (*Oryza sativa* L.) and maize (*Zea mays* L.) and a grain legume (chickpea) were utilized in studying the comparative efficiency of these crops in grain Zn loading. Although Zn uptake per tonne of grain produced was the highest, the grain Zn loading was the lowest in rice. Zn concentration in grain (an intensity factor) was the highest in wheat at 46.8 mg/kg, closely followed by maize at 45.1 mg/kg, chickpea at 41.8 mg/kg and was the least for rice kernels at 21.2 mg/kg. ZnHI (a capacity factor for measuring grain Zn loading) was also the highest for wheat at 51%, followed by chickpea at 39.3%, maize at 35.9% and was the least for rice at 17.9%. Further, rice grain contains husk (about one-third by weight) and only kernel is consumed, for which ZnHI was only 6.3%; a major part of Zn loaded on grain in rice goes to the husk. Thus, from the Zn nutrition point of view rice is the poorest cereal. However, rice deserves utmost attention, because it is the staple food in South, Southeast and East Asia, where about 62.5% of the world's people live and hunger and malnutrition is rampant. Since most grain Zn loading in rice is from the Zn absorbed by the roots through xylem in contrast to other crops, where it is mostly achieved through mobilization of Zn in the vegetative tissue through phloem, it is suggested that rice plant types using grain Zn loading through phloem be developed. It is necessary, because applied Zn gets fixed or precipitated in soil through several mechanisms and its availability to rice plants gets reduced as it reaches grain filling stage.

Key words: Cereals, Grain Zn loading, Zn-biofortification of grain, Zn concentration in grain, Zn harvest index

Zinc (Zn) deficiency in soils and crops is widespread throughout the world (Alloway 2008), especially on high pH soils (Prasad 2007, Cakmak 2008). Maize (*Zea mays* L.) is well known as a Zn deficiency indicator plant (Camberato and Maloney 2012), while Nene (1966) reported that rice (*Oryza sativa* L.) is also very susceptible to Zn deficiency, which leads to *khaira* disease, indicated by patches of burnt brown plants in rice fields. A large number of field experiments with field crops including cereals have been conducted in India and good response to Zn fertilization has been reported (Patel 2011, Singh 2011). Most of these experiments provide data on grain yield and Zn uptake increases in crops due to Zn fertilization. Of late Zn deficiency in humans has received considerable attention and it is reported that more than one-third of the world's population suffers from Zn deficiency (Hotz and Brown 2004, Stein 2010). It has also been estimated that approximately 4% of the world's burden of morbidity and mortality in under 5-year children and a loss of nearly 16

million global disability adjusted life years in adults is due to poor Zn nutrition (Black *et al.* 2008, Walker 2009). Zinc deficiency in humans is more widespread in Asia, where cereals rice and/or wheat form the staple diet with only a small component of animal food product (Gibson 2005, Chatrath *et al.* 2007, FAO 2010, Pirzadeh 2010). The situation is worst in India, where a large majority of population is vegetarian.

Cereal grains, especially rice and wheat are considered low in concentration as well as bio-availability of Zn, particularly when grown on Zn deficient soils (Welch and Graham 2004, Cakmak 2010). Efforts are therefore underway for Zn biofortification of cereal grains using genetic tools (Bouis 2011, Graham *et al.* 2001) as well as agronomic practices (Cakmak 2008, Prasad *et al.* 2013, Prasad *et al.* 2014). We have conducted a large number of agronomic experiments at the ICAR-Indian Agricultural Research Institute, New Delhi on the effect of rates, sources, methods and timing of Zn application on rice, wheat (*Triticum aestivum* L.), maize and chickpea (*Cicer arietinum* L.) on Zn biofortification of grains of these crops and have showed that agronomic biofortification of food grains is the quickest and simplest way of achieving

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this (Shivay and Prasad 2014, Shivay *et al.* 2008, Shivay *et al.* 2013, Shivay *et al.* 2014, Shivay *et al.* 2015). Most of our publications have reported data on grain and straw yield and their Zn concentration and Zn uptake.

Fageria and Baligar (2005) suggested a term 'Zn harvest index (ZnHI)', which expresses Zn uptake by grains as a part of total Zn uptake by a crop and therefore provides a simple measure of grain Zn loading of a crop. We have therefore re-examined our data on agronomic experiments from the ZnHI view point across four crops and the same is reported in this paper. The need for this paper also arose, because most Geneticists, Plant Breeders and Agronomists confine their research to a single crop and comparisons across crops are generally not made and such information is not available.

MATERIALS AND METHODS

The experiments on Zn biofortification of grains in wheat, maize and chickpea were conducted at the research farm of the ICAR-Indian Agricultural Research Institute (IARI), New Delhi, while those on rice were conducted on the farmer's fields in the adjoining state of Uttar Pradesh at Aligarh and Meerut. The soils at all the locations were Typic Ustochrepts and sandy loam or sandy clay loam in texture. At ICAR-IARI fields, the soil pH ranged from 8.1 to 8.3 (1:2.5 soil to solution ratio) and DTPA-extractable (available) Zn (Lindsay and Norwell 1978) varied from 0.36 to 0.69 mg/kg soil. The soil at the farm in Aligarh had a pH of 8.5 and DTPA-extractable Zn was 0.33 mg/kg soil, while that at Meerut had a pH of 8.2 and DTPA-extractable Zn was 0.35 mg/kg soil. Thus, soils of all the fields where these experiments were conducted were alkaline and DTPA-extractable Zn was within the critical limit of 0.4-0.8 mg/kg soil as suggested by Takkar (1996) and crop response to Zn was expected.

All the four crops were grown as per the standard recommended package of practices however zinc treatments were applied in each crop as per the treatment details given in the tables of the results of the respective crops. All the crops were harvested at physiological maturity. At harvest, grain yields of respective crops have been recorded for

each plot and finally converted into tonnes/ha. The rice grain yield was recorded at 14% moisture, whereas wheat, maize and chickpea yields were recorded at 12% moisture.

Grain and straw/stover samples were collected at harvesting and dried in a hot air oven at 60±2 °C for 6 hr. The oven dried samples were sieved by passing through 40 mesh sieve in a Macro-Wiley Mill. From each replication, 0.5 g dry matter samples of rice, wheat, maize and chickpea grain and straw/stover samples were taken for chemical analysis to determine the Zn concentration in these crops grain and straw/stover. The Zn concentration in dry matter was determined by wet-digestion (di-acid digestion) procedure described by Prasad *et al.* (2006) using Atomic Absorption Spectrophotometry (AAS). The Zn concentration was expressed as mg/kg. The Zn uptake was computed by multiplying with respective Zn concentration in grain and straw/stover with yields (grain and straw/stover) of the respective crop and was expressed as Zn uptake in g/ha. Fageria and Baligar (2005) suggested a term 'Zn harvest index' (ZnHI) which can be calculated by the following formula:

$$\text{ZnHI} = \frac{\text{Zn uptake by grain at harvest (g/ha)}}{\text{Zn uptake by whole crop (grain + straw) at harvest (g/ha)}} \times 100$$

RESULTS AND DISCUSSION

The main thrust of this research paper is focused to make the comparison between different crops for zinc loading ability including a grain legume. The few general observations are made and some basic data is presented in Tables 1-5 for the ready reference to the readers. In all crops studied a significant response to Zn fertilization was recorded up to the highest rate (5 or 7.5 kg Zn/ha) studied. In rice (Tables 1 and 2) and maize (Table 4) application of 1 kg Zn/ha through foliage in conjunction with soil application of 5 kg Zn/ha increased grain yield and total Zn uptake by the crop, however, application of 1 kg Zn/ha through foliage alone was no better than soil application of 5 kg Zn/ha. As regards sources of Zn, 2% Zn oxide or Zn sulphate were equally effective and superior to 1%

Table 1 Effect of different rates, sources and method of Zn application on grain yield, total Zn uptake, Zn concentration in grain and ZnHI in aromatic rice variety Pusa Basmati 1 at Aligarh, Uttar Pradesh

Treatment	Grain yield (tonnes/ha)	Total Zn uptake (g/ha)	Zn concentration in kernel (mg/kg)	ZnHI (%)		
				Kernel	Husk	Grain
5 kg Zn/ha (soil)	3.93	970 (246.8)	21.3	5.7	17.4	23.1
1 kg Zn/ha (foliar)	3.80	979 (257.6)	22.0	5.7	18.8	24.5
5 kg Zn/ha (soil) + 1 kg Zn/ha (foliar)	4.52	1207 (267.0)	25.0	6.3	21.7	28.0
2.83 kg Zn/ha as Zn coated urea (soil)	4.10	1096 (267.3)	23.8	6.0	20.9	26.9
Mean	3.99	1013 (253.1)	22.4	5.9	19.4	25.3
SEm±	0.03	9.27	0.03			
LSD (P=0.05)	0.08	26.3	0.86			

Source: Data other than ZnHI from Shivay *et al.* (2015). ZnHI values were computed from the data on Zn uptake by different components for which SE± and LSD values are available in original paper referred. ¹g Zn/tonne grain.

Table 2 Effect of different rates, sources and method of Zn application on grain yield, total Zn uptake, Zn concentration in grain and ZnHI in aromatic rice variety Pusa Sugandh 5 at Meerut, Uttar Pradesh

Treatment	Grain yield (tonnes/ha)	Total Zn uptake (g/ha)	Zn concentration in kernel (mg/kg)	ZnHI (%)		
				Kernel	Husk	Grain
Check (no Zn)	5.40	1934 (191.5) ¹	18.0	6.3	3.0	9.3
5 kg Zn/ha (soil)	5.70	1157 (202.9)	18.7	6.2	3.8	10.0
1 kg Zn/ha (foliar)	5.60	1220 (217.8)	20.2	6.2	5.3	11.5
5 kg Zn/ha (soil) + 1 kg Zn/ha (foliar)	6.00	1319 (219.8)	21.2	6.4	5.6	12.0
2.83 kg Zn/ha as Zn coated urea (soil)	5.70	1256 (220.3)	22.2	6.7	3.8	10.5
Mean	5.68	1197 (210.5)	20.1	6.6	4.3	10.6
Mean over 2 rice experiments ²	4.84	1105 (231.7)	21.2	6.3	11.8	17.9
SEm±	0.19	15.76	0.28			
LSD (P=0.05)	0.27	44.65	0.78			

Source: Data other than ZnHI from Shivay *et al.* (2015). ZnHI values were computed from the data on Zn uptake by different components for which SE± and LSD values are available in original paper referred. ¹g Zn/tonne grain, ²Averaged over data in Table 1 and 2.

Zn-coated with Zn oxide or Zn sulphate in wheat (Table 3). For rice and maize, where Zn-coated urea (ZnCU) was tested it was found to be fairly good, considering the fact that only 60% of Zn was applied as ZnCU as compared to application of 5 kg Zn/ha as Zn sulphate.

Average total Zn uptake by crop was the highest for rice at 1105 g/ha, followed by maize at 577 g/ha, wheat at 392 g/ha and was the least by chickpea at 214 g/ha. Since

Table 3 Effect of different rates and sources of Zn application on grain yield, total Zn uptake, Zn concentration in grain and ZnHI in wheat variety HD 2643 at New Delhi

Treatment	Grain yield (tonnes/ha)	Total Zn uptake (g/ha)	Zn concentration in grain (mg/kg)	ZnHI (%)
Check (no Zn)	3.72	269 (72.3) ¹	39.5	54.6
1% Zn as ZnO-enriched urea	4.65	368 (79.1)	45.6	51.3
1% Zn as ZnSO ₄ -enriched urea	4.80	443 (92.3)	49.0	47.2
2% Zn as ZnO-enriched urea	5.07	423 (83.4)	48.9	51.1
2% Zn as ZnSO ₄ -enriched urea	5.26	455 (86.5)	51.1	50.8
Mean	4.70	392 (82.7)	46.8	51.0
SE±	0.12	6.8	0.93	-
LSD (P=0.05)	0.37	20.5	2.80	-

Source: Data other than ZnHI from Shivay *et al.* (2008). ZnHI values were computed from the data on Zn uptake by different components for which SE± and LSD values are available in original paper referred. ¹g Zn/tonne grain.

the grain yields of different crops varied, total Zn uptake per tonne of grain was worked out. Again the highest value was for rice at 231.7 g/tonne grain, followed by maize at 124.6 g/tonne grain, chickpea at 102.6 g/tonne grain and was the least for wheat at 82.7 g/tonne grain (Fig 1). These results would suggest that rice is a highly efficient cereal in respect of Zn absorption and uptake. However, as regards grain Zn loading, Zn concentration in grain (intensity factor) was the highest in wheat at 46.8 mg/kg, closely followed by maize at 45.1 mg/kg, chickpea at 41.8 mg/kg and was the least for rice at 17.9 mg/kg (Fig 2). Thus, from the Zn nutrition point of view rice is the poorest cereal. As regards ZnHI (capacity factor) it was also the highest for wheat at 51%, followed by chickpea at 39.3%, maize at 35.9% and was the least for rice at 17.9%. Further, rice grain contains husk (about one-third by weight) and only kernel is consumed, for which ZnHI was only 6.3% (average over 2 rice experiments). Thus, a part of Zn loaded on grain in rice goes to the husk. In our studies, ZnHI for husk was 4.3% for Pusa Sugandh 5 at Meerut and 19.4% (nearly one-fifth of the total Zn uptake by crop) for Pusa Basmati 1 at Aligarh, suggesting that rice varieties could greatly differ in respect of Zn distribution between kernel and husk. Of course soil conditions could also be partly responsible for this difference.

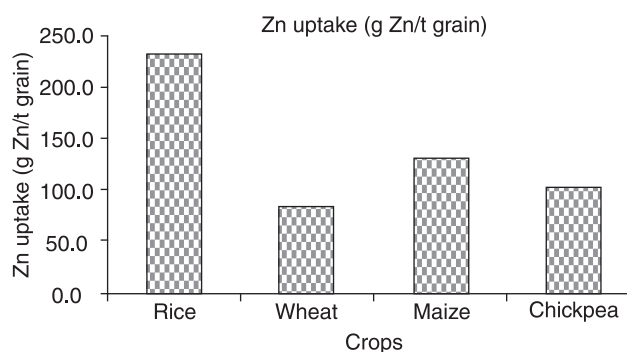


Fig 1 Average Zn uptake (g/tonne) by rice, wheat, maize and chickpea. For rice, the value is average of two experiments

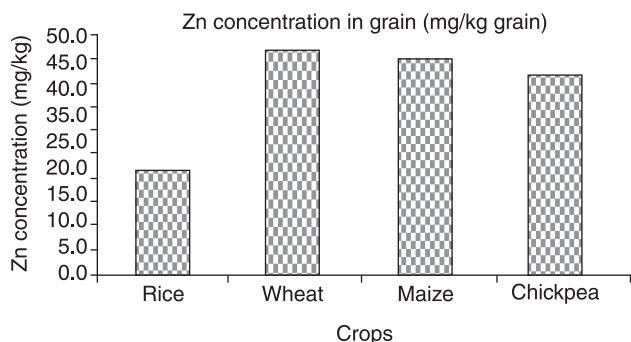


Fig 2 Average Zn concentration (mg/kg grain) in different crops. For rice, the value is average of two experiments

Soil Scientists use the terms ‘intensity’ and ‘quantity’ in relation to availability of plant nutrients in soil, for example, in the case of phosphorus to indicate readily available P and total P in soil (Raven and Hossner 1994, Anderson and Wu 2001). Similarly terms ‘active’ and ‘potential’ are used in respect of soil acidity; pH of soil solution representing active acidity and exchangeable acidity as potential acidity (Prasad and Power 1997). Taking a lead from this terminology we suggest that Zn concentration in grain could be considered as a measure of Zn intensity, while Zn harvest index (ZnHI) could be taken as a measure of total quantity of Zn removed

Table 4 Effect of different rates, sources and method of Zn application on grain yield, total Zn uptake, Zn concentration in grain and ZnHI in High Quality Maize Hybrid 1 at New Delhi

Treatment	Grain yield (tonnes/ha)	Total Zn uptake (g/ha)	Zn concentration in grain (mg/kg)	ZnHI (%)
Check (no Zn)	4.00	435 (108.7) ¹	40.2	36.9
5 kg Zn/ha (soil)	4.70	536 (114.0)	44.2	36.9
1 kg Zn/ha (foliar)	4.42	588 (133.0)	46.0	34.5
5 kg Zn/ha (soil) + 1 kg Zn/ha (foliar)	5.10	704 (138.0)	49.2	35.6
2.83 kg Zn/ha as Zn coated urea (soil)	4.80	621 (129.4)	45.8	35.4
Mean	4.60	576.8 (124.6)	45.1	35.9
SEm±	0.12	3.8	0.6	0.6
LSD (P=0.05)	0.38	12.5	2.0	2.1

Source: Data from Shivay and Prasad (2014.) ZnHI values were computed from the data on Zn uptake by different components for which SE± and LSD values are available in original paper referred. ¹g Zn/tonne grain.

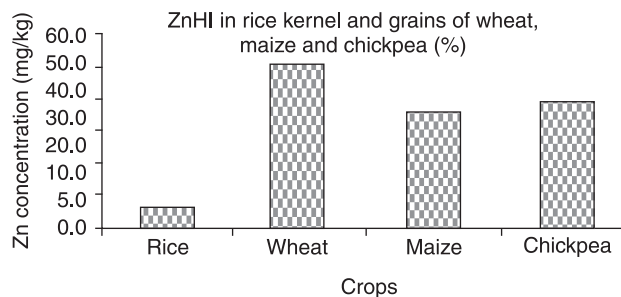


Fig 3 The ZnHI (%) in rice kernel and grains of different crops. For rice, the value is average of two experiments

in the grains of a crop.

As regards total Zn uptake by crops, rice recorded the highest and was superior to wheat, maize or chickpea, however, in respect to Zn concentration (intensity) in grain and ZnHI (capacity), it was inferior to all other three crops, suggesting problems in grain Zn loading in rice. There are two mechanisms for grain Zn loading in crops: i) Direct

Table 5 Effect of different varieties and rates of Zn application on grain yield, total Zn uptake, Zn concentration in grain and ZnHI in chickpea at New Delhi

Treatment	Grain yield (tonnes/ha)	Total Zn uptake (g/ha)	Zn concentration in grain (mg/kg)	ZnHI (%)
<i>Varieties</i>				
Pusa 2024	2.11	205.3 (97.3) ¹	39.9	37.7
Pusa 5028	1.99	207.6 (104.3)	42.8	41.1
Pusa 372	2.13	228.9 (107.5)	42.8	38.5
Mean	2.07	213.9 (103.0)	41.8	39.1
SE±	0.007	0.29	0.02	
LSD (P=0.05)	0.026	1.16	0.06	
<i>Rates of Zn application</i>				
Check (no Zn)	1.89	155.9 (82.5)	36.1	41.4
2.5 kg Zn/ha	2.02	196.6 (97.3)	40.5	39.2
5.0 kg Zn/ha	2.15	232.6 (108.2)	43.5	38.7
7.5 kg Zn/ha	2.24	271.1 (121.0)	47.1	38.3
Mean	2.07	214.0 (102.6)	41.8	39.3
SE±	0.038	3.74	0.51	
LSD (P=0.05)	0.114	12.90	1.48	

Source: Data other than ZnHI from Shivay et al. (2014.) ZnHI values were computed from the data on Zn uptake by different components for which SE± and LSD values are available in original paper referred. ¹g Zn/tonne grain.

Zn uptake from roots through xylem; and ii) Mobilization of shoot Zn through phloem (Li *et al.* 2013). In wheat and barley, the xylem discontinues at the base of the grain, so Zn has to enter the grain through phloem (Palmgren 2008), while there is no discontinuation of xylem in rice and Zn can enter the grain directly through the xylem (Stomph 2009). Therefore, in wheat and barley, a large portion of grain Zn loading takes place through mobilization of tissue Zn from leaves throughout the grain filling stage (Zee and O'Brien 1970). Because Zn does not diffuse readily through cell membranes (Kambe *et al.* 2004) special Zn transporters are employed by plants for this purpose including those belonging to P_{1B}-ATPase, zinc-regulated transporter (ZRT), iron-regulated transporter (IRT) -like proteins (ZIP), natural resistance-associated macrophage protein (NRAMP) and cation diffusion facilitator (CDF) families (Colangelo and Guirenot 2006). Recently Li *et al.* (2013) suggested that ZmZIP genes encode functional Zn or Fe transporters that may be responsible for the uptake, translocation, detoxification and storage of divalent metal ions in plant cells, especially in maize.

In rice, on the other hand, grain Zn loading depends mainly from the roots through xylem (Jiang *et al.* 2007, Impa *et al.* 2013) and is therefore very much influenced by soil conditions, especially pH (Rupa and Tomar 1999) and redox potential (Impa and Johnson-Beebout 2012). Carroll and Loneragan (1969) suggested that the critical level of Zn for plant growth in continuous flow culture is 10⁻⁷ to 10⁻⁸ M, while Lindsay (1991) reported that in alkaline soils Zn concentration tends to be between 10⁻⁸ to 10⁻¹⁰ M. Thus, Zn availability to crop plants is considerably reduced in alkaline soils. Rupa and Tomar (1999) reported that above pH 6.75 in soil solution all Zn was sorbed on soil particles and was thus less available to plants. In the present study, experiments were conducted on high pH (above 8.0) soils, which adversely affected Zn uptake by roots resulting in poor grain Zn loading in rice. Zinc applied to soil also gets fixed in soil by a number of mechanisms including complex formation with organic matter, diffusion into microspores and inter-particle places (McLaughlin 2001, Tye *et al.* 2003), effects of bicarbonates in calcareous soils (Yang *et al.* 1993), co-precipitation with other metals, such as franklinite (ZnFe₂O₄) (Sajwan 1985) and precipitation as Zn carbonate in calcareous soil (Bostick 2001). All these soil factors reduce Zn availability to crop plants in alkaline soils.

Other factor that adversely affects grain Zn loading in rice is poor root system that taps only a few centimeter thick surface soil layers. Importance of a good root system in Zn nutrition of crop plants is well known (Broadley *et al.* 2007) and rice has received special attention (Teo *et al.* 1995, Rose *et al.* 2013), however, an ideal root system for increased Zn uptake is yet to be evolved.

Since soil factors are difficult to control in rice due to heavy rains occasionally leading to flooding during growing season in several areas. Therefore, genetic improvement of rice plant for improved grain Zn loading is urgently needed. Wu *et al.* (2010) have reported mobilization of Zn

from vegetative tissue to grain in rice in some genotypes and this area of research needs to be intensively pursued to develop more efficient grain Zn loading plant types in rice. Research on identifying Zn transporters in rice could be helpful.

It may be mentioned that about 90% of rice is grown and consumed in South, Southeast and East Asia, where about 62.5% of the world's hungry people reside (FAO 2010) and it is estimated that 60-70% of population in this region could be at risk of low Zn deficiency intake (Gibson 2005).

Our studies show that Zn concentration in rice kernels as well as ZnHI is lower than wheat, maize and chickpea. This appears due to the fact that during grain filling most grain Zn loading in rice is due to absorption of Zn by roots and its transmission to grain via xylem as compared to other crops, where most grain Zn loading is probably due to mobilization of Zn from vegetative tissue and its translocation to grain. Better rice plant types involving the latter mechanism need to be developed, because Zn in soil gets sorbed on soil particles or gets fixed and is not readily available to plants, especially at grain filling stage.

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