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Assessment of genetic variation in zinc acquisition and transport to seed in diversified germplasm lines of rice (*Oryza sativa* L.)

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Zinc (Zn) deficiency being a major constraint to reduce the potential yield of rice, the major emphasis in this study was to examine the existing genotypic variability in leaf and seed Zn content, ultimately with an objective to identify the donor genotypes with high Zn. The results clearly demonstrated that genetic variability exists in leaf and seed Zn content. The observed variability suggested the possibility of identifying specific donor genotypes with high Zn efficiency and high Zn content. In spite of larged variation in leaf Zn, there was no relationship between leaf and seed Zn levels. It suggests that Zn acquisition and transport revealed the two independent traits.

Key words: Zinc acquisition, zinc transport, genetic variability

Introduction

Zinc (Zn) is a trace element found in all soils. It is an essential element for plants, animals and humans. As a component of proteins, Zn acts as a functional, structural and regulatory co factor of large number of enzymes. It is involved in many plants physiological processes, for instance carbohydrate metabolism, protein metabolism, membrane integrity, starch formation and seed maturation (Brown *et al*., 1993, Fageria, 2001). In addition Zn is of particular importance to human health. Zinc deficiency in human body can result in undesirable consequences including growth retardation, dermatitis and impaired immune functioning, child mortality and mid age depression (Welch, 1993).

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Zinc deficiency has been reported in various parts of the world (Cakmak, 2002). About 30% of the world's soils are also Zn deficient (Alloway, 2004). It is particularly acute in puddled soils. In the Indian context, more than 50 per cent of the agricultural soils is Zn deficient. Zinc deficiency being an important nutrient constraint, any approach to improve Zn uptake and its transport to grains has significant practical relevance. Plant breeding strategy appears to be the most sustainable and cost effective approach useful in improving Zn status of plants and also its concentrations in grains. Improving nutrient status by exploiting genetic variability has been well elucidated in number of studies.

A substantial genetic variability in tolerance to zinc deficiency was found in 35 common bean genotypes by Hacisalihoglu *et al.* (2004). Considerable genetic variation in rice genotypes was demonstrated in different rice genotypes, where 53% of the observed variability was associated with Zn uptake and translocation from roots and shoots (Wissuwa *et al.*, 2006). Mechanisms responsible for genotypic variation in zinc efficiency were thoroughly reviewed by Rengel (2001), Hacisalihoglu and Kochian (2003). The expression of high zinc efficiency in rice was related to enhanced uptake and translocation capacity of Zn into shoots and higher amounts of physiologically active Zn in leaf tissues (Cakmak and Marschner, 1998).

Plant breeding approach to improving the Zn acquisition and its transport has a greater significance and relevance compared to agronomical approaches in improving the rhizospheric Zn levels by fertilizer application. However, for improving Zn acquisition, one of the primary prerequisites is significant genetic variability in this trait. Such genotypic variations can be exploited in breeding programmes to produce genotypes with higher zinc efficiency. Improving Zn efficiency has recently become a major plant breeding task in several countries.

In view of this, we examined the genetic variability in Zn acquisition in diverse germplasm lines and few released varieties. Leaf Zn content considered as a measure of efficient Zn acquisition and its transport to shoot.

Materials and methods

To study the genetic variability in Zn acquisition and transport, 320 germplasm lines were selected during the year 2004. The experiment was laid out by randomized complete block design in two replications in sandy loam soil. The germplasm lines included IET/IVT entries, IRRON – A and IRRON - B module, locally adapted cultivars, wild relatives and aromatic rice varieties like Jeera rice, Basmati rice etc along with international check varieties like IR-50, a very early maturing, IR-72, early maturing PSBRC-2, medium maturing variety and national check, Jaya. Module –A is the regular IRRON set and Module – B is the set of 32 breeding lines of new plant material type. Module –A is composed of 112 test entries originating from breeding programmes of 15 countries and 14 international research centres (CIAT, IITA, IRRI and WARDA).

Recommended NPK at the rate of 100:50:50 kg/ha was applied. The crop was raised as per the package of practices. Complete dose of nitrogen and phosphorus were applied at the time of transplanting and potassium was applied at two stages, 50% at the time of transplanting and remaining 50% was applied at anthesis. The seeds were sown in the nursery. Twenty four days old seedlings were transplanted to the field. The seedlings were planted 20cm between the rows and 10 cm between the plants. Zinc was estimated in the grains and leaf sample using Polarized Zeeman Atomic Absorption Spectrophotometer (AAS-2-6100) (Piper, 1966).

Results

There was a wide genetic variability observed with respect to leaf and seed Zn level. The range for seed Zn content was from 0.84 to 5.00 mg/100 g DW and for leaves it was from 1.26 to 14.88 mg/100g DW (Table 1).

Since significant variability was seen both in leaf and seed Zn, we analysed the relationship between these parameters. There was no relationship between leaf and seed Zn levels (Fig. 1). Therefore, 320 accessions were classified further into low, medium and high genotypes both for leaf and seed Zn, with an objective to see whether within the group leaf and seed Zn are related.

Table 1. Mean and range values for 320 germplasm lines.

	Range (mg/100g DW)	**Mean**	**SD**	**SEm**	**CD @5%**	**CV%**
Leaf Zn	1.26-14.88	7.30	3.85	0.215	1.02	0.536
Seed Zn	0.84-5.00	2.41	0.911	0.51	0.082	1.71

Low seed Zn types were in the range of 0.84 to 1.98 with a mean of 1.25, medium types, 2.15 to 2.99 with a mean of 2.62, high types 3.01 to 5 with a mean of 3.68 (Table 2). The data on leaf Zn indicated that their leaf zinc content in low types were in the range of 1.26 to 4.98 with a mean of 3.27. The range for medium and high types was 5.02 to 9.44 and 9.65 to 14.88 with a mean 6.85 and 12.20, respectively (Table 3). We assessed the relationship between leaf and seed Zn in these three different groups. Even within the group there was no significant relationship observed between leaf and seed Zn content (Figs. 2, 3 and 4).

In spite of large differences in leaf and seed Zn across the genotypes lack of relationship between leaf and seed Zn, suggests that Zn acquisition and subsequent transport of Zn to seed are independent traits. To identify contrasting genotypes differing in acquisition of Zn and transport to seed, Z-distribution analysis between leaf and seed Zn was made and based on distribution, the genotypes can be classified in to four distinct groups. Quadrant 'A' represents those with low leaf Zn but relatively high seed Zn. However, quadrant 'B' represent genotypes with high leaf and high seed Zn, similarly quadrant 'D' with low leaf and low seed Zn. Interestingly there are several genotypes in quadrant 'C' with low seed Zn despite high leaf Zn (Fig. 5). These groups may distinctly differ in their Zn acquisition and transport, despite high leaf Zn they lack transport of Zn to grains

Based on the Z-distribution data genotypes were selected and classified them as High Leaf High Seed Zn types (HLHS), Low Leaf High Seed Zn types (LLHS), Low Leaf Low Seed Zn types (LLLS) and High Leaf Low Seed Zn types (HLLS). The range for HLHS types is 7.77-14.88 and 2.37-5.00 mg/100g DW with a mean of 11.22 and 3.35 for leaf Zn and seed Zn, respectively. For LLHS Zn types, the range was from 1.26-7.57 and 0.84-3.94 (mg/100g DW) with a mean 4.49 and 2.36 respectively for leaf and seed Zn. Genotypes having low leaf and seed Zn (LLLS) had 1.40-4.94 and 0.84-3.03 mg/100g DW of range with a mean 3.11 and 1.97 respectively. In HLLS Zn types the range was from 7.74-14.08 in leaf Zn with a mean 10.89 and for seed Zn, 1.06-2.19 with a mean 1.6 (Table 4).

Table 2. Classification of genotypes based on leaf zinc content.

	Range (mg/100g DW)	Mean	SD	SEm	CD (p=0.05)	CV %
Low Zn types	1.26-4.98	3.269	1.074	0.017	0.0481	0.7886
Medium Zn types	5.02-9.44	6.852	1.18	0.03	0.0867	0.6199
High Zn types	9.65-14.88	12.2	1.25	0.054	0.876	0.6025

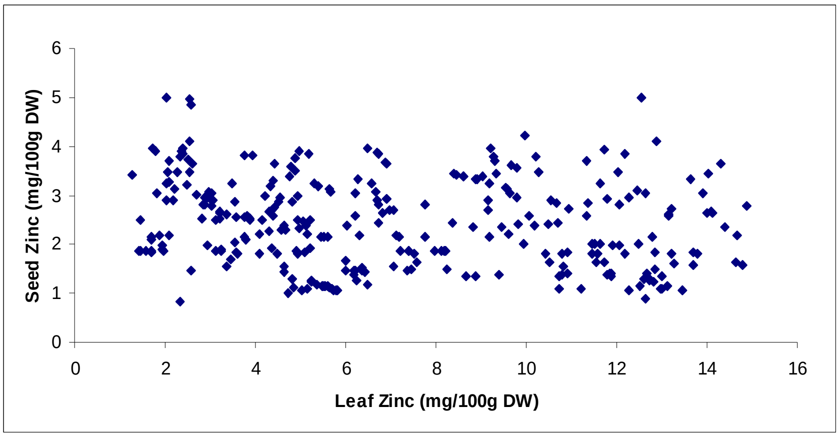

Fig. 1. Relationship between leaf zinc and seed zinc of 320 germplasm lines.

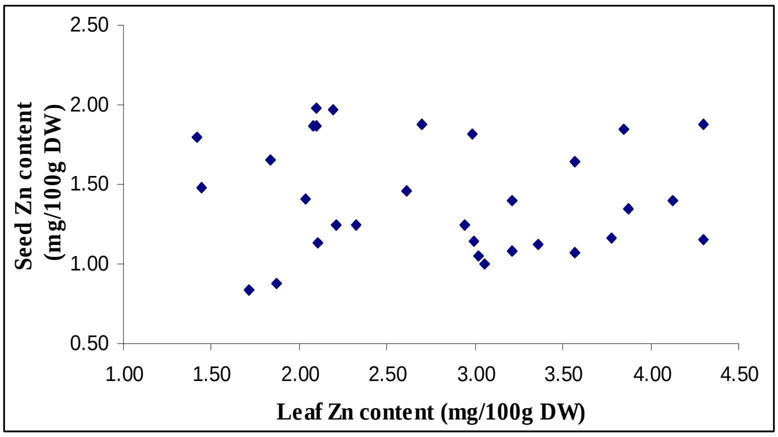

Fig. 2. Relationship between leaf and seed Zn content of low Zn type.

Table 3. Classification of genotypes based on seed zinc content**.**

	Range	Mean	SD	SEm	CD (p=0.05)	CV %
Low Zn types	0.84-1.98	1.25	0.35	0.014	0.039	1.360
Medium Zn types	2.15-2.99	2.62	0.25	0.032	0.093	1.693
High Zn types	3.01-5.00	3.68	0.43	0.024	0.069	0.917

Table 4. Mean and range values for four contrast groups.

Quadrants	Zn types	Leaf zinc (mg/100g DW)			Seed zinc (mg/100g DW)		
		Range	Mean	SD	Range	Mean	SD
A	LLHS	1.26-7.57	4.49	1.81	0.84-3.95	2.36	1.79
B	HLHS	7.77-14.88	11.22	1.813	2.37-5.00	3.35	0.66
C	HLLS	7.74-14.08	10.89	2.02	1.06-2.19	1.6	0.37
D	LLLS	1.40-4.94	3.11	1.01	0.84-3.03	1.97	0.72

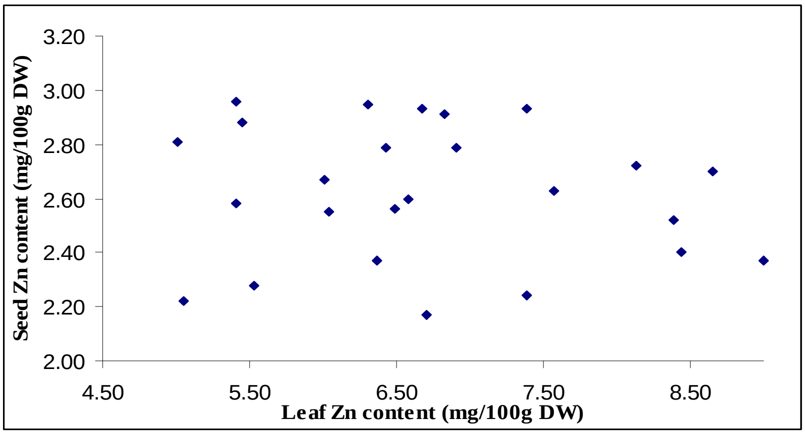

Fig. 3. Relationship between leaf and seed Zn content of medium Zn types.

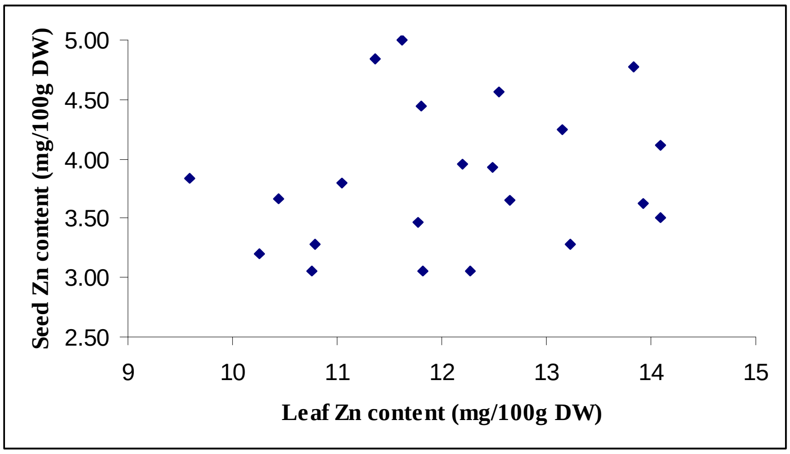

Fig. 4. Relationship between leaf and seed Zn content of high Zn types**.**

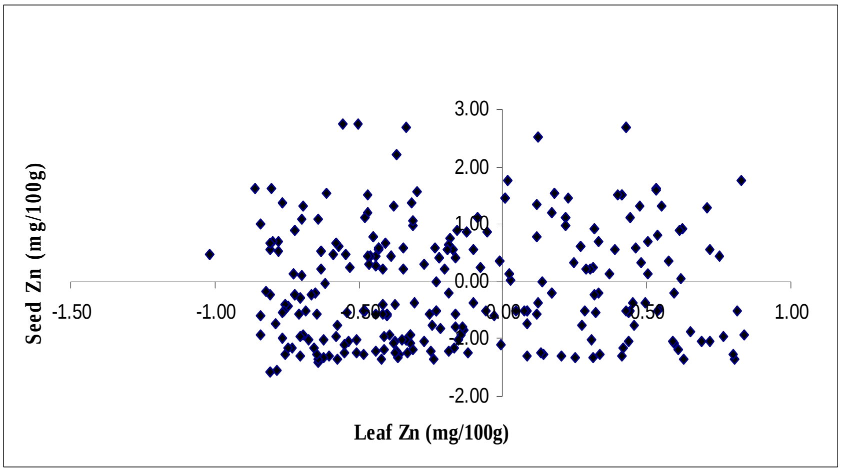

Fig. 5. Classification of genotypes into four groups by Z-distribution analysis based on leaf and seed Zn content from the year 2004 data.

Discussion

Besides Zn as nutrient, genotypes with high seed Zn has phenomenal relevance in human nutrition. One of the objectives is to analyse whether the Zn acquisition (leaf Zn levels) is related to seed Zn content. However, our analysis showed that leaf and seed Zn levels were not related. There were genotypes which show relatively high seed Zn despite low leaf Zn levels and also vice versa. In general, it was assumed that shoot Zn levels and seed Zn levels are related. Since there was no relationship in our study, we classified the genotypes into three different groups based on Zn acquisition and examined the relationship between Zn status and seed Zn content. Even within the groups also there was no relationship suggesting that Zn acquisition and transport to shoot Zn are independent traits. Classification of genotypes as high leaf high seed (HLHS), high leaf low seed (HLLS), low leaf high seed (LLHS) and low leaf low seed (LLLS) Zn types, can provide the leads in understanding the basic mechanisms in acquisition and the factors associated with variability in seed Zn content in seeds. The observed variability helps in identifying specific donor genotypes with high Zn efficiency and high Zn content.

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