

# EXPLORING GENETIC VARIATION AND IDENTIFICATION OF DIVERGENT ZINC RICH DONORS IN A CORE RICE GERMPLASM

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## ABSTRACT

An experiment was carried out to explore high Zn donors and to study the extent of genetic divergence based on grain Zn content and agro-morphological traits including seed yield in a set of 47 core germplasm lines of rice. Grain zinc content ranged from 9.2-28.1ppm. The top Zn dense ( $\geq 24$  ppm) genotypes identified were Nagina 22(28.1ppm), BG 102(25.8ppm), Dudh Kandar (25.6ppm), R-RHZ-7 (24.7ppm) and IR 85850-AC157-1(24.0ppm). Among these, two breeding lines IR 85850-AC157-1(24.0ppm) and R-RHZ-7(24.7ppm) retained high grain zinc content along with moderately high seed yield potential. The set of genotypes were grouped into seven distinct clusters. The elite high Zn local land races "Nagina 22" and "Dudh Kandar" had poor yield potential but showed high genetic divergence. These may serve as valuable material for Zn biofortification breeding.

## INTRODUCTION

Rice (*Oryza sativa* L.) is the major staple food of more than half of the world population. Feeding hungry with nutritious rice seems to be a lasting solution to food and nutritional security. Zinc is a trace mineral and it serves as co-factor of more than 300 enzymes involved in cellular metabolism. In animals, Zn deficiency leads to loss of immunity to diseases, stunted growth, impaired learning ability, wound healing and reproduction; and increased risk of infection, DNA damage and cancer. Therefore, there is a need for Zn-biofortified rice in the food chain. In plants, Zn is needed for plant growth and resistance to biotic and abiotic stresses. Grain Zn content is a complex polygenic trait with high G x E interaction. There exists wide variation in zinc content (14.0-40.0ppm) (Martinez *et al.*, 2006) in brown rice suggesting tremendous scope for its enrichment in rice grains. Similarly, Liang *et al.*, (2007) revealed variation in Fe content (9.45 to 25.2ppm) and Zn content (13.0 to 39.0ppm) in rice grain of 56 Chinese rice varieties. Modern high yielding rice varieties are deficient Zn, while some of the land races (Roy and Sharma 2014), basmati types (Brar *et al.*, 2011) and wild rice (Banerjee *et al.*, 2010) retain high grain Zn content. Therefore, an attempt was undertaken to explore genetic variation and to identify divergent Zn rich donors in a core rice germplasm.

## MATERIALS AND METHODS

The experimental material includes 13 local land races, 23 improved biofortified breeding lines (IRRI), seven zinc rich

released varieties (BRR1 Dhan 62, BRR1 Dhan 64, BRR1 Dhan 72, DRR Dhan 45, DRR Dhan 48, DRR Dhan 49 and CGZR 1) and four high yielding ruling non-biofortified varieties (Swarna, MTU 1010, IR 64 and Sambamahsuri) of rice. These test entries were laid out in Randomized Block Design (RBD) with three replications to assess yield and ancillary traits in Kharif, 2017. Observations were recorded on nine agro-morphological traits along with seed yield and eight quality traits including grain Zn content. Dial micrometer was used to determine length and breadth of 10 grains and the respective kernels of each genotype. L/B ratios for grain and kernel were calculated taking respective mean values. Rice genotypes were classified into seven grain types e.g., Short slender(Score 1), Short bold(Score 2), Medium slender(Score 3), Medium bold(Score 3.5), Long bold(4), long slender (Score 5) and extra long slender (Score 6) as per Govindaswamy (1985) with minor modification.

For micronutrient analysis, fine ground samples of brown rice of the genotypes in three replicates were digested by di-acid mixture of nitric acid (HNO<sub>3</sub>): and perchloric acid (HClO<sub>4</sub>) in 3:2 ratio following the standard procedure of Jahan *et al.* (2013) with minor modification (i.e. 3:2 instead of 1:2 diacid ratio). Zinc content was estimated in the aliquot of seed extract by using Inductive Coupled Plasma-Optical Emission Spectrophotometer (ICP-OES) at 206.2nm wavelength at Central Instrumentation Facility (CIF), OUAT, Bhubaneswar. The variation in replications for each sample did not exceed  $\pm 1$ ppm. The mean of the three replicates were worked out to indicate Zn-content of each genotype.

Routine statistical procedures were followed for analysis of

variance as per Singh and Choudhury (1985). Besides, the *inter se* varietal genetic distances between genotypes were estimated following SPSS software (Version 16) and dendrogram was constructed based on morpho-agronomic and quality traits to assess genotypic divergence among the test genotypes.

## RESULTS AND DISCUSSIONS

### Genetic variation for grain Zn content

Rice wild relatives, upland landraces and aromatic accessions, deep water rice and coloured rice are the best sources of high grain Zn (Mallikarjuna Swamy *et al.*, 2016). Wild species of rice e.g., *O. nivara*, *O. rufipogon*, *O. latifolia*, *O. officinalis* and *O. granulata* harbour about 2–3 fold higher grain Zn than cultivated rice. Besides, considerable variation for grain Zn content (14.5 to 35.3 ppm) exist in brown rice among local land races (Dikshit *et al.*, 2016 and Maganti *et al.* 2019). In the present study, a number of IRRI breeding lines developed from different high Zn donors were assessed for status of grain Zn content and morpho-agronomic traits compared to released varieties of Bangladesh (BRRI Dhan 62, BRRI Dhan 64, BRRI Dhan 72) and India (DRR Dhan 45, DRR Dhan 48, DRR Dhan 49, CGZR 1, CGZR 3, Hurz 1 and Hurz 3). It revealed a wide array of genetic variation in maturity duration, plant types, yield potentiality and quality features including grain Zn content. A quest for zinc dense genotype would help breeders to combine desirable specific morpho-agronomic features with high seed yield. Grain zinc content ranged from 9.2 ppm in IR 97443-11-2-1-1-1-B to as high as 28.1 ppm in Nagina 22 among 47 test genotypes including standard high yielding check varieties e.g., Swarna (17.3 ppm), MTU 1010 (15.1 ppm) and Sambamahsuri (12.2 ppm). The top Zn dense ( $\geq 20$  ppm) genotypes (Figure 1) identified in the present investigation were Nagina 22 (28.1 ppm) followed by BG 102 (25.8 ppm), Dudh Kandar (25.6 ppm), R-RHZ-7 (24.7 ppm), IR 85850-AC-157-1 (24.0 ppm), BRRI Dhan 64 (23.1 ppm), IR 95133: 1-B-16-14-10-GBS-P1-2-3 (23.1 ppm), IR 95133: 1-B-16-14-10-GBS-P1-2-2 (22.6 ppm), DRR Dhan 45 (22.1 ppm), IR 91143-AC-239-1 (22.0 ppm) and Sathi (21.0 ppm). The breeding target is 28ppm Zn in rice grain. Hence, Nagina 22 may serve as Zn dense donor for genetic biofortification breeding programme. Patil *et al.* (2015) evaluated grain zinc content of brown rice of 61 rice accessions including local land races and improved breeding lines which ranged from 14.03-31.94 ppm with an average of 24.3 ppm. Similarly, higher Zn content was shown to be associated with some of the aromatic rice (Gregorio 2002) and local upland rice (cv'Nam Roo': 31 ppm) (Jaksomsak *et al.*, 2015).

In the present study, IR 99642-57-1-1-1-B and BRRI Dhan 72 recorded high yield ( $\geq 46$ q/ha) followed by IR 97443-11-2-1-1-1-3-B, IR 97443-11-2-1-1-1-1-B, IR 91143-AC 290-1, IR 85850-AC157-1 and R-RHZ-7. The above first top four high yielding genotypes revealed very low to moderately low grain zinc content (14.4ppm, 17.9ppm, 12.7ppm and 9.2ppm respectively). While, the mid early maturing semi-dwarf breeding lines e.g., IR 85850-AC157-1 (24.0ppm) and R-RHZ-7 (24.7ppm) retained high grain zinc content along with moderately high seed yield potential (around 39.0qtl/ha).

Therefore, these elite genotypes have merit for commercial cultivation in irrigated and rainfed medium land ecosystem.

### Genetic divergence

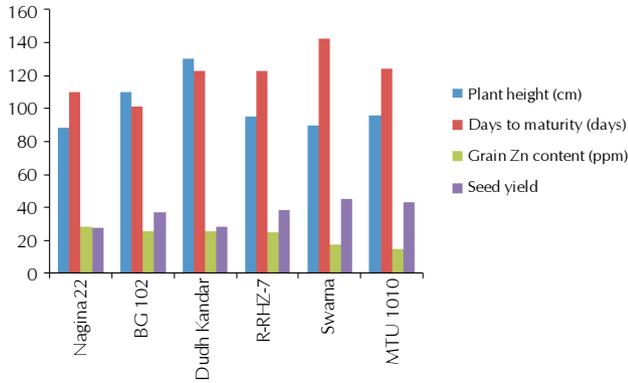
Genetic improvement mainly depends upon the amount of genetic variability present in the population. Therefore, assessment of genetic diversity in a set of breeding materials is a pre-requisite to distinguish the genotypes into genetically close and divergent types. The genotypes which are genetically distant enough are expected to generate wide range of genetic variation in recombination breeding and pave the way for greater scope for recovery of transgressive segregants (Zaman *et al.*, 2005 and Saxesena *et al.*, 2013). Therefore, an attempt has been made to assess the extent of genetic divergence in the present set rice genotypes.

### Clustering pattern

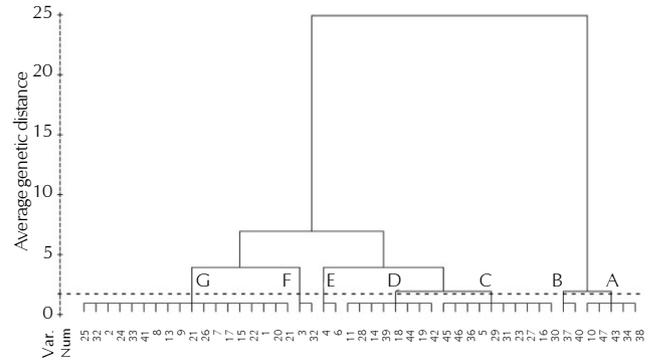
Grouping of test genotypes into different clusters was made based on Euclidian genetic distance between all possible pairs of genotypes. In the present investigation, the total 47 test genotypes including standard checks (Swarna) were grouped into seven distinct genetic clusters (Table 1, Figure 2). Further, the dendrogram constructed using SPSS software (version 16) showed clear hierarchical genetic relationship among 47 test genotypes. The cluster composition as per dendrogram at average genetic distance approximately 2.0 was shown to be exactly same to the grouping based on Euclidian genetic distance. Crosses involving parents belonging to the most divergent clusters would be expected to manifest maximum heterosis and wide variability of genetic architecture (Souroush *et al.*, 2004). Genotypes under Cluster-A and Cluster B were first separated from rest of the genotypes indicating their status of distinct divergence. This supports the earlier findings of Singh *et al.* (2018). Cluster A included five genotypes e.g., NAGINA-22, KARHANI, URG-1, URG-30 and Dudh Kandar while, Cluster B consisted of two genotypes e.g., M399 and Sathi. Local land races are reported to have considerable variation for grain Zn content (14.5 to 35.3ppm) (Maganti *et al.*, 2019). In the present study, two local land races, "Nagina 22" (28.1ppm) and "Dudh Kandar" (25.6ppm) have been identified as high Zn donors and hence, such elite genotypes belonging to the most divergent cluster (Cluster A) may serve as valuable material for Zn biofortification breeding. Besides, Sathi (21.0ppm) in Cluster B, URG-24 (21.9ppm) and BG 102 (25.8ppm) in cluster C and CGZR 1 (21.7) and BD 105 (20.0ppm) in Cluster E being high in grain Zn content, can serve the purpose. Rathod *et al.* (2017) revealed wide genetic diversity among high iron and zinc genotypes of rice.

Both Cluster E (CGZR -1 and BD 105) and Cluster F (IR 99642-57-1-1-1-B and BRRI Dhan 72) included two genotypes each. Separation of above genotypes into small clusters might be due to high *inter se* genetic distance owing to their specific features. Roy *et al.* (2002) revealed the nature and magnitude of genetic diversity in 50 high yielding varieties and traditional germplasm of rice. The genotypes were grouped into 10 clusters. Vivekanandan and Subramanian (1993) also assessed genetic divergence in twenty-eight genotypes of rain fed rice and identified few highly divergent rice genotypes suitable for upland rice breeding.

Among different clusters, Cluster-G was the largest cluster



**Figure 1: Grain Zn content and morpho-agronomic performance of elite Zn-rich genotypes compared to mega varieties of rice (Swarna and MTU 1010)**



**Figure 2: Dendrogram showing hierarchical genetic relationship of test genotypes based on morpho-economic and quality traits including grain Zn content**

**Table 1: Cluster composition of different clusters for 47 rice genotypes**

Cluster No.	No. of genotypes	Name of the genotypes
A	5	NAGINA-22 (38), KARHANI (34), URG-1 (43), URG-30 (47), Dudh Kandar (10)
B	2	M399 (37), SATHI (40)
C	10	IR 15M 1689 (30), IR 95133: 1-B-16-14-10-GBS-P1-2-2 (16), IR 96248-16-3-3-2-B (27), IR 99704-24-2-1 (23), IR 15M 1633 (31), IR 15M 1546 (29), BG 102 (5), M48 (36), URG-24 (46), URG-22 (45)
D	8	SAMBAMAHSURI (42), IR 95133: 1-B-16-14-10-GBS-P5-2-3 (19), URG-19 (44), IR 95133: 1-B-16-14-10-GBS-P5-1-3 (18), R-RHZ-7 (39), IR 85850-AC157-1 (14), IR 15M 1537 (28), IR 91143-AC239-1 (11), CGZR -1(6) and BD 105 (4)
E	2	CGZR -1(6) and BD 105 (4)
F	2	IR 99642-57-1-1-1-B (32) and BRR1 Dhan 72 (3)
G	18	IR 95044: 8-B-5-22-19-GBS (21), IR 95133: 1-B-16-14-10-GBS-P6-1-5 (20), BRR1 Dhan 62(1), IR 84847-RIL-195-1-1-1-1 (22), IR 82475-110-2-2-1-2 (15), IR 95133: 1-B-16-14-10-GBS-P1-2-3 (17), DRR Dhan 45 (7), IR 97443-11-2-1-1-1-3-B (26), IR 91143-AC290-1 (12), DRR Dhan 49 (9), IR 91143-AC293-1 (13), DRR Dhan 48 (8), SWARNA (41), IR 64 (33), IR 99647-109-1-1 (24), BRR1 Dhan 64 (2), MTU 1010 (35), IR 97443-11-2-1-1-1-1-B (25)

**Table 2: Inter-cluster distances among different clusters for 47 rice genotypes**

Clusters	Cluster-A	Cluster-B	Cluster-C	Cluster-D	Cluster-E	Cluster-F
Cluster-B	262.33					
Cluster-C	776.90	1038.00				
Cluster-D	1101.00	1362.00	324.28			
Cluster-E	461.14	722.11	316.97	640.85		
Cluster-F	1782.0	2043.0	1007.0	685.63	1321.0	
Cluster-G	1446.20	1707.0	669.09	345.20	985.54	346.17

**Table 3: Cluster means for different characters in a set of 47 rice genotypes**

Characters	Cluster-A	Cluster-B	Cluster-C	Cluster-D	Cluster-E	Cluster-F	Cluster-G
DF	76.6	66.5	81.3	90.4	77.0	86.3	87.6
DM	110	96.0	110.5	120.2	106.0	115.6	117.4
PHT	96.4	107.5	104.0	97.2	90.0	94.6	93.5
Tillers/m <sup>2</sup>	316.6	298	352.9	379.9	322.5	326.0	397.1
PL	27.5	18.0	26.8	29.2	23.0	28.3	27.7
G/P	78.6	75.0	6.6	97.6	82.5	135.6	109.1
100-GW	2.32	1.97	2.35	2.34	2.27	1.96	2.3
F%	7.10	70.4	71.34	75.2	68.5	86.9	83.6
GL	7.54	8.75	8.97	8.8	8.0	9.0	8.5
GB	2.68	2.75	2.67	2.57	2.8	2.6	2.5
GL/GB	2.83	3.25	3.40	3.45	2.85	3.51	3.4
Grain type	4.20	4.5	4.2	4.1	4.5	4.33	3.9
KL	6.30	7.00	7.6	7.47	6.75	7.66	7.2
KB	2.16	2.4	2.26	2.22	2.65	2.1	2.2
KL/KB	2.96	3.07	3.46	3.4	2.57	3.76	3.3
Zn content	20.8	19.25	18.26	19.0	20.8	15.0	17.1
Seed yield	2780.4	2520.0	3556.4	3879.1	3241.3	4562.5	4223.7

which accommodated 18 genotypes followed by Cluster-C and Cluster-D which included 10 and 8 genotypes respectively indicating genetic proximity of the test genotypes grouped in these clusters. Cluster G contained BRR1 Dhan 62, BRR1 Dhan 64, DRR Dhan 45, DRR Dhan 48, DRR Dhan 49, ten IRR1 breeding lines and popular varieties e.g., IR 64, MTU 1010, SWARNA. Grouping of large number of genotypes in Cluster-G might have resulted due to similar selection pressure favouring identical expression of characters influencing grain yield during the development of the breeding material. Further, it is interesting to note that the above large cluster contains two popular mega varieties e.g., Swarna and MTU 1010 indicating their proximity with other genotypes included in this cluster.

#### Inter cluster distance

Inter cluster distance among seven genetic groups (Table 2) ranged from around 262.33 (between Cluster A & Cluster B) to as high as 2043.0 (between Cluster F & Cluster B). Cluster B emerged as the highly divergent group which was farthest from Cluster F followed by Cluster G and cluster D. In contrast, Cluster F (IR 99642-57-1-1-B and BRR1 Dhan 72) and Cluster B (M399 and Sathi) showed least *inter se* genetic distance indicating high homology among genotypes included in such clusters. Besides, Cluster G with Cluster F and Cluster D; and Cluster C with Cluster D and Cluster E maintained considerably high homology as revealed from comparatively negligible *inter se* genetic distance of around 345.20-346.17; and 316.97-324.28 respectively. Crosses involving parents belonging to the most divergent clusters would be expected to manifest maximum heterosis and recovery of transgressive variants (Souroush *et al.*, 2004, Zaman *et al.*, 2005 and Saxesena *et al.*, 2013). for yield traits with enriched grain Zn content.

#### Characteristic features of clusters

In a set of test genotypes, some may have common features and therefore are clubbed into single cluster. Hence, common feature is the basis for clustering. Each of the cluster reflects specific features. In the present investigation, Cluster B (Sl. 37 and 40) exhibited moderately tall plant stature with early flowering and maturity (Table 3). In contrast, Cluster E (Sl. 4 and 6) revealed characteristic dwarf plant type. Tillers/m<sup>2</sup> was maximum (397.1) in case of Cluster G indicating profuse tillering ability, while genotypes included under Cluster B exhibited bit shy tillering habit. Among the seven genotypic groups, Cluster D had shown highest panicle length (29.2 cm) followed by Cluster F. Grain number/panicle, grain weight and fertility percentage are usually considered as major determinant of seed yield. In the present investigation, Cluster F (Sl. 3, 26 and 32) revealed maximum grain number/panicle (135.6) with highest fertility percentage (86.9%) for which such cluster recorded highest mean seed yield (4562.5kg/ha). Similarly, Cluster G with moderately higher number of grains/panicle, grain-weight and fertility percentage, recorded high seed yield (4223.7kg/ha).

Cluster F exhibited highest grain and kernel length as well as highest grain length/breadth and kernel length/breadth ratio (Table 3). Such characteristic features associated with high yield potential. However, the said genetic cluster revealed low grain Zn content possibly due to inverse relationship of seed

yield with grain Zn content. In this context, Cluster A and Cluster E recorded grain Zn content more than 20ppm and such genotypic groups included erstwhile mentioned important genotypes i.e., Nagina 22, Dudh Kandar, BD 105,CGZR-1 (Chattisgarh Zinc Rich Rice-1) nutritionally rich in grain Zn content.

## CONCLUSIONS

Rice is highly deficient in grain zinc content. However, a few local land races, aromatic varieties and wild rice are the best source of Zn. With the alarming situation of Zn deficiency affecting human health and premature death, a number of research centres have geared up for high Zn product development in rice. The high Zn rice varieties so far developed are still to compete the present day mega varieties of rice (Swarna and MTU 1010). The major bottlenecks lie with the identification of stable Zn dense donors and handling the breeding materials for recovery of a truly widely adaptable Zn rich genotype. The elite highly divergent Zn dense donors identified in this investigation can serve as ideal starting materials for Zn biofortification breeding.

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