



Phenotyping of Elite Zinc Rich Rice Varieties for Agronomic Traits and Grain Zinc Content

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Abstract

An experiment was carried out to explore high zinc(Zn) donors to be amenable for use as parents in zinc biofortification programme. A semi-dwarf rice breeding line IR 128773-4-4-2-2-B revealed high tillering ability, slender kernel feature ($KL \geq 8.0$ and $KL/KB > 4.0$), acceptable grain filling (fertility%) with maturity duration 120-125 days. It had shown significantly higher yield than Swarna (Check), while BRRI Dhan 72 and DRR Dhan 45 were at par to this check variety. IR 128773-4-4-2-2-B and few local land races (Mal-liphulajhuli, Nikipankhia and Tikimahsuri) exceeded 40 ppm grain zinc content. Among these, IR 128773-4-4-2-2-B seem to have merit for use as zinc dense donor parent owing to its excellent agronomic features coupled with high grain zinc content.

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Introduction

Rice is the single most important food crop for more than 50% of the world population. Feeding hungry with nutritious rice seems to be a lasting solution to the chronic problem of malnutrition. Zn is a trace mineral and it serves as co-factor of several enzymes associated with cellular metabolism [1]. In animals, Zn deficiency leads to sensitivity to diseases, dwarfism, loss of mental ability, wound repair and reproduction; and higher chance of infection, damage of DNA and possibility of cancer. Hence, there is a need for Zn-biofortified rice in the food chain. In plants, Zn is needed for growth of plants, maintenance

of chloroplast structure and function, and induced tolerance to biotic and abiotic stresses. Rice grains usually harbour very minimum amount of Zn (12-15mg/kg) as compared to the target fixed (Zn: 28-30ppm) to meet the recommended daily allowance (RDA) of 10-12mg Zn/day [2, 3]. There is wide variation in zinc content (14.0-40.0ppm) [4] in brown rice. Grain zinc content of brown rice ranged from 16.5-33.0ppm in North East Land Races (NELR) of rice using ED-XRF[5]. Therefore, an initiative was undertaken to identify agronomically suitable zinc rich rice genotypes in a set of 26 selected germplasm lines.



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Materials and methods

The present study dealt with 26 test genotypes including 11 promising land races, four zinc biofortified released varieties (BRRI Dhan 64, BRRI Dhan 72, CGZR-1 and DRR Dhan 45), nine Zn-dense advance breeding lines, one zinc dense check variety (Chittimuthyalu) and one low zinc mega variety of rice (Swarna) as yield check. The genotypes under study were field tested at the Regional Research and Technology Transfer Station, Bhubaneswar in Kharif, 2019. These test entries were laid put in Randomized Block Design (RBD) with three replications to assess yield and ancillary traits. 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 [6] with minor modification.

Fine ground samples of brown rice of each of the genotypes in three replicates were digested by di-acid mixture of nitric acid (HNO₃): and perchloric acid (HClO₄) in 3:2 ratio following the standard procedure of Jahan et al.[7] with minor modification (i.e. 3:2 instead of 1:2 diacid ratio). Zn content were estimated in the aliquot of seed extract by using Inductive Coupled Plasma-Optical Emission Spectrophotometer (ICP-OES) at 206.2nm wavelength (Figure 1) at Central Instrumentation Facility (CIF), OUAT, Bhubaneswar. The variation in replications for each sample did not exceed ± 1ppm. The mean of the three replicates were worked out to indicate Zn-content of each genotype.

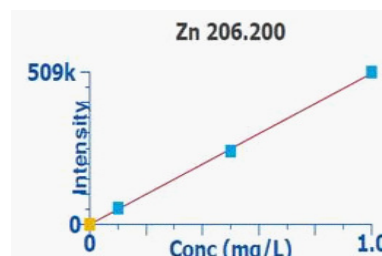


Figure 1: Calibration of standard curve for grain Fe and Zn content using ICP-OES.

Table 1: Mean performance of a set of 26 Zn biofortified rice genotypes.

Sl. No.	Genotype	DF (Days)	DM (Days)	PHT (cm)	Till-ers/ m ²	PL (cm)	GN/P	100- GW (g)	Ferti- lity %	GL (mm)	GB (mm)	GL /GB	Grain Type & Score	KL (mm)	KB (mm)	KL /KB	Zn ppm	Yield (q/ha)
1	BG 102	75	106	115	328	27.5	86	3.08	88.3	9.1	3.2	2.8	LB (4)	7.6	2.8	2.7	25.8	30.60
2	BRRI Dhan 64	82	114	98	410	22.8	130	2.42	90.9	6.9	3.2	2.2	MB (3.5)	6.3	2.8	2.3	23.1	42.16
3	BRRI Dhan 72	95	125	96	390	27.0	148	2.48	82.5	9.1	3.1	2.9	LB (4)	8.2	2.9	2.8	17.9	45.22
4	CHITTIMATYALLU	81	112	85	434	26.8	138	2.36	93.8	5.7	2.3	2.5	SB (2)	5.2	1.8	2.9	22.0	40.08
5	Dudh Kandar	95	125	135	310	29.3	88	3.00	83.4	8.7	3.2	2.7	LB (4)	7.9	2.7	2.9	25.6	28.60
6	DRR Dhan 45	98	129	99	450	24.4	130	2.48	82.3	8.1	2.3	3.5	LS (5)	7.2	2.0	3.6	22.1	44.20
7	IR 95133: 1-B-16-14-10-GBS-P1-2-3	95	125	110	398	33.6	126	2.50	86.5	10.2	2.3	4.3	ELS (6)	9.0	1.9	4.7	23.1	39.06
8	IR 85850-AC157-1	97	128	99	423	25.5	95	2.20	88.8	10.1	3.1	3.2	LS (5)	9.0	2.8	3.2	24.0	31.10
9	IR 91143-AC239-1	84	114	85	408	23.8	102	2.70	82.1	9.1	2.8	3.3	LS (5)	7.8	2.3	3.4	22.0	37.20
10	KALANAMAK	105	136	103	380	28.9	125	2.12	87.5	8.2	2.2	3.7	LS (5)	7.4	2.0	3.7	21.1	34.80
11	CGZR -1	83	112	98	430	23.6	105	2.72	86.6	9.1	2.9	3.1	LS (5)	7.9	2.5	3.2	21.7	38.40
12	NAGINA-22	85	116	99	432	23.2	92	2.50	92.9	5.7	2.6	2.2	SB (2)	5.0	2.0	2.5	28.1	34.50
13	SWARNA	115	145	98	462	25.3	140	2.18	88.0	5.9	2.1	2.8	MS (3)	5.3	1.8	2.9	11.3	41.80
14	R-RHZ-7	98	128	104	450	30.9	128	2.24	92.5	9.1	2.5	3.6	LS (5)	8.2	2.1	3.9	24.7	39.90
15	NIKIPANKHIA	112	145	125	398	27.0	115	2.91	86.0	7.7	2.5	3.1	LS (5)	6.2	2.0	3.1	42.8	39.20
16	TIKIMAHSURI	110	140	120	335	27.5	105	2.36	84.0	8.3	3.0	2.8	LB (4)	6.9	2.7	2.6	41.5	30.40
17	URG-24	98	128	124	320	26.2	106	2.42	89.0	7.5	3.0	2.5	LB (4)	6.2	2.7	2.3	21.9	29.10
18	Bishnupriya	108	140	128	358	25.8	112	2.30	83.8	8.3	2.3	3.6	LS (5)	6.8	1.9	3.6	21.6	31.80
19	CR 2829-PLN-37	95	126	95	415	23.4	122	2.30	84.5	9.0	2.3	3.9	LS (5)	8.0	2.0	4.0	22.1	38.44
20	Hundar	105	138	98	380	23.0	108	2.45	80.7	7.1	2.8	2.5	LB (4)	6.3	2.5	2.5	20.5	35.80
21	IR 128773-4-4-2-2-B	90	122	90	462	23.1	129	2.35	85.2	9.0	2.4	3.8	LS (5)	8.3	2.1	4.0	41.3	46.91
22	Karhani	82	110	92	300	25.0	101	2.18	66.7	7.8	2.8	2.8	LB (4)	6.5	2.5	2.6	21.6	26.08
23	MI 127	95	126	84	382	23.8	115	2.21	86.5	8.5	2.4	3.5	LS (5)	7.8	2.0	3.9	21.5	34.55
24	MALLIPHULAJHULI	101	131	128	290	25.6	126	2.20	83.5	9.1	2.5	3.6	LS (5)	8.3	2.0	4.2	43.8	28.50
25	PADMAVATI	108	139	115	389	26.1	104	2.25	83.5	8.0	2.8	2.8	LB (4)	7.3	2.3	3.2	31.3	32.20

26	R-RHZ- IH -82	92	123	80	399	24.5	110	2.24	82.5	8.6	2.3	3.7	LS (5)	7.5	2.0	3.8	24.2	34.70
Mean		96	126	104	390	25.9	115	2.43	85.5	8.22	2.65	3.13	4.4	7.23	2.27	3.25	25.6	35.90
Range		75-115	106-145	80-135	290-462	22.8-33.6	86-148	2.12-3.08	66.7-93.8	5.7-10.2	2.1-3.2	2.2-4.3	2.0-6.0	5.0-9.5	1.8-2.9	2.3-4.7	11.3-43.8	26.08-46.91
C.D. _{0.05}		6.5	10.5	15.2	45.2	2.9	22.8	0.05	7.3	1.7	0.25	0.65	0.43	1.98	0.23	0.57	5.20	4.02

Results and discussion

There exists an appreciable genetic variability in maturity duration, plant habit, yield performance and grain quality traits. Exploring agronomical superior high zinc rice donors would immensely help plant breeders to breed zinc bio-fortified rice varieties.

Mean performance for agro-economic traits

Characterization of morphological traits is the priori for varietal identification. A systematic and meticulous effort was therefore, undertaken to assess all important agro-morphological traits. Mean performance of all 26 rice varieties for seed yield and its component traits have been shown in Table 1.

The average performance of genotypes clearly envisaged the wide scope for selection of better genotypes. Appreciably large range variation in morpho-economic traits further confirms the possibility of effective selection pressure for desirable plant types. The elite genotypes expressing specific important trait(s) may be utilized as donors in ongoing genetic improvement programme.

Usually, Zn biofortification in rice is targeted for maturity duration 125-130 days. In the present set of materials, days required to attain flowering and maturity ranged from 75-115 days and 106-145 days with an average of 96 days and 126 days respectively. BG 102, Karhani, Chittimuthyalu, CGZR 1, IR 91143-AC 239-1 and Nagina 22 took around 75-85 days to attain 50% flowering and these matured as early as 105-116 days. While, Nikipankhia, Tikimahsuri, Bishnupriya and Padmavati matured at around 140-145 days compared to Swarna which matured at 145days. Rest of the test genotypes recorded medium duration.

Semi-dwarf plant type (90-100cm) with moderate to high tillering and heavy panicle at maturity are always preferred as agronomically important for enhancing yield potential[9]. Besides, semi-dwarf to intermediate plant types are needed to prevent the crop from lodging and to retain physical quality features. In this context, BRRI Dhan 64, BRRI Dhan 72, DRR Dhan 45, IR 85850-AC 157-1, CGZR-1, Nagina 22, Swarna, CR 2829-PLN-37, IR 128773-4-4-2-2-B, Karhani and Hundar exhibited semi-dwarf plant type. Among these, BRRI Dhan 64, DRR Dhan 45, IR 85850 AC-157-1, CGZR 1, Nagina 22, CR 2829-PLN-37, Swarna and IR 128773-4-4-2-2-B revealed higher tiller number (more than 400/m²) with maximum being recorded in last two test genotypes (462 /m²).

In the present investigation, panicle length ranged from 22.8cm to 33.6cm with an average of 25.9cm and the longest panicle being revealed in IR 95133: 1-B-16-14-10-BS-P 1-2-3 (33.6cm) followed by R-RHZ-7 (30.9cm) with moderate grain number and Dudh Kandar (29.3cm) with very low number of grains/panicle.

Panicle weight is determined by number of fertile grains per panicle and grain size of the genotype. Thus, genotypes with bold grains and having more number of grains/ panicle would contribute towards high yield potential. In this regard, compact

panicle feature with increased fertile grain number may offer greater dividend than increasing the panicle length as mostly, longer panicle length is often associated with loose panicle feature. Similarly, bold grain type genotypes usually bore less number of grains/panicle and reverse is also a reality. Thus, multiplicative product of grain weight and grain number can have direct bearing on panicle weight. For instance, Swarna and Chittimuthyalu exhibited significantly higher number of grains per panicle (138-140 grains /panicle) with moderately low grain weight (2.18g and 2.6g), but were characterized dense panicle leading to contribute towards high yield potential. The local land races, BG 102 and Dudh Kandar bore bold grain and had shown maximum 100-grain weight ($\geq 3.0g$), but grain number was extremely low resulting in low seed yield (Table 1).

Genotypes with good grain filling offers substantial contribution towards seed yield. Genotypes having efficient Zn transport to shoot and grain are expected to offer better crop stand with reduced spikelet sterility and follow-up satisfactory grain filling. Panicle with high fertility status and higher number of fertile grains per panicle lead to heavy panicle. In the present study, fertility percentage ranged from 66.7% in Karhani to as high as 93.8% in Chittimuthyalu followed by Nagina 22(92.9%), R-RHZ -7(92.5%) and BRRI Dhan 64 (90.9%). Among the high fertility genotypes, Nagina 22 had short bold grain with short panicle; BRRI Dhan had medium bold grains and moderate panicle length with loose panicle feature, while R-RHZ-7 bore long slender grains and moderate panicle length with moderate grain density.

Farmers are sceptic for realization of more produce from each penny invested so as to earn more profit per unit area of cultivation and to suffice their food requirement. Therefore, a bio-fortified rice genotype must qualify high yield potential in addition to harbouring high status of essential mineral element(s) [9]. In the present study, IR 128773-4-4-2-2-B, BRRI 72, DRR Dhan 45, Chittimuthyalu and Swarna recorded higher seed yield (>40.0q/ha) as most of these had moderately high tillering capacity associated with heavy panicle feature. Swarna served as yield check and Chittimuthyalu was included as quality check. IR 128773-4-4-2-2-B had recorded significantly higher yield than Swarna, while BRRI Dhan 72 and DRR Dhan 45 were shown to have comparable yield potential to such mega variety (Swarna) (Table 1). In this context, Tripathy et al. [10] reported two mid early maturing semi-dwarf breeding lines e.g., IR 85850-AC157-1 (24.0ppm) and R-RHZ- 7 (24.7ppm) to have high grain zinc content along with moderately high seed yield potential (around 39.0qtl/ha).

Physical quality traits

Grain and kernel size are observed on the basis of their length, breadth and L/B ratio. Grain length and corresponding kernel length varied from 5.7-11.2mm and 5.0-9.5mm respectively with maximum being recorded in IR 95133: 1B-16-14-10-GBS-P1-2-3 (Table 1). Bold grain feature (GB >3.0) was associated with BG 102, BRRI Dhan 64, BRRI Dhan 72, Dudh Kandar

and IR 85850-AC-157-1. Grain length /breadth ratio is the relative measure of the length of grain per unit measure in grain breadth and it gives a clear picture of grain dimension. In this context, IR 95133: 1B-16-14-10-GBS-P1-2-3 (GL/GB=4.4) followed by IR 128773-4-4-2-2-B (GL/GB=3.8) and Kalanamak (GL/GB=3.7) revealed slender grain characteristics which is the most preferred criteria for consumers' preference. As per Govindaswamy [6], these test genotypes scored 6.0, 5.0 and 5.0 respectively. Kernel length, kernel breadth and kernel L/B ratio are the determining factors for suitability of rice for consumption. Mostly, the slender kernel types fetch consumers' acceptance and therefore, have more market price [9]. In the present investigation, IR 95133: 1B-16-14-10-GBS-P1-2-3 and IR 128773-4-4-2-2-B revealed slender kernel feature ($KL \geq 8.0$ and $KL/KB > 4.0$) along with high grain zinc content and high yield performance. Hence, these may be preferred for general cultivation and suitable as table rice.

In general, L/B ratio of kernel either reduced or bit increased due to varied reduction in length and breadth after dehusking during milling process, L/B ratio of grain and kernel L/B ratio varied widely among the genotypes under study. Hull thickness and differential degree of air space between husk and kernel directly contribute to the grain density in rice. Grain density in rice reflects the extent of compactness of starch grains in the kernel and complete development of kernel without any space between the kernel and hull. Kalanamak revealed nearly equal kernel and grain L/B ratio due to proportionately almost equal decrease in length and breadth after hulling. Similar was the case in IR 85850-AC 157-1, Kalanamak, Nikipankhia, Bishnupriya and Hundar.

Grain Zn content

In Zn biofortification programme, seed yield along with acceptable physiochemical quality features with high grain Zn content are the prime target traits in rice. Brown rice is an important source of vitamins and minerals [11]. Endosperm harbours 57% Zn content while bran and embryo contain 34% and 9% Zn in brown rice kernel. About 40% of zinc in rice is lost while milling, polishing and washing before cooking. Besides, 25% of Zn intake is usually bio-available and it goes to blood serum. Estimated Av. Requirement (EAR) of Zn is 12mg/day for women and 4mg/day for 4-6 year child, 10mg/day for adult man. Presently, EAR met by feeding 400gm of non-biofortified rice (15mg/kg) is 6.0mg Zn/day. Hence, breeding target is fixed at 28-30mg/kg i.e. double than the present status (15mg/kg of rice) which seems to be a challenging task [9].

Wild forms of rice, local landraces adapted to upland conditions, scented rice germplasms, deep water rice and black/purple rice are the good sources of high grain Zn [12]. *O. nivara*, *O. rufipogon*, *O. granulata*, *O. latifolia* and *O. officinalis* harbour about two to three times higher zinc content in grains than cultivated rice. Brown rice of local land races showed appreciable variation for status of grain zinc (14.5 to 35.3ppm) [13].

In this study, the average of the three replicates was considered as the status of grain zinc content of each genotype. A set of 26 selected rice genotypes including local land races, advanced breeding lines and a number of improved varieties were estimated for grain Zn content and listed in Table 1. Among 26 selected test genotypes, grain Zn content of brown rice ranged from 11.3 ppm in Swarna to as high as 43.8ppm Zn in a local land race 'Malliphulajhuli' with average grain Zn content of 25.60ppm (Table 1). Other high Zn test genotypes

exceeding 40ppm for grain Zn content were IR 128773-4-4-2-2-B, Nikipankhia and Tikimahsuri, while Dudh Kandar, BG 102 and Nagina 22 were shown to have grain Zn content 25-30ppm in brown rice. Among these, IR 128773-4-4-2-2-B had recorded significantly high grain yield potential (46.91q/ha). Besides, a local land race Nikipankhi and a high Zn advance breeding line R-RHZ-7 with grain Zn content 24.7ppm recorded higher seed yield (39.20-39.90q/ha) and these were comparable to Swarna (yield check). Hence, these three elite genotypes seem to have merit to serve as donors for Zn biofortification programme in rice. More or less, grain Zn content in some of the aromatic rice [14] and local upland rice (cv'Nam Roo': 31ppm) [15] was reported to be higher than general high yielding rice varieties.

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