RESEARCH ARTICLE

Phenotyping and Association Analysis of Zinc Biofortified Rice Varieties for Grain Yield and Quality Traits

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Abstract

A set of 25 test genotypes including 11 promising high zinc landraces, four zinc biofortified released varieties, six Zn-dense advance breeding lines, one OUAT rice variety, one zinc dense check variety "Chittimatyalu" and three low zinc mega varieties of rice (Swarna and Sambamahsuri) were assessed for grain Zn content and inter-relationship among seed yield and quality traits. P44 selection revealed highest Zn content (52.7ppm) followed by Basudha, Manika, Nikipankhia, ORCZ 75-3-1 and Tikimashuri with Zn content more than 40 ppm. Grain yield showed a significant positive correlation with a number of ear-bearing tillers m^{-2} and grain number $panicle^{-1}$. Plant height exhibited a negative correlation with a number of effective bearing tillers m^{-2} and seed yield, but positively correlated with zinc content which seems to be the major hindrance for breeding semi-dwarf high yielding Zn-dense. The tall *indica* landraces such as Malliphulajhulli (43.8 ppm), Nikipankhia (42.8 ppm), Padmavati (31.3 ppm), Tikimahsuri (41.5 ppm), and Basudha (44.4 ppm) harbor high grain zinc content but revealed low yield potential. Transfer of zinc transporter genes available in the above tall *indica* types to semi-dwarf plant types may enrich the status of zinc content in grain.

Keywords: Association analysis, Biofortified rice, Grain yield, Phenotyping, Quality traits, Zinc content.

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Introduction

Most of the undernourished population in the world depend on cereal-based diets, and rice serves as the staple food for those millions of people in terms of food and nutritional security. Over 40% of the world's population, particularly women and children, are suffering from various nutritional deficiencies (Gearing, 2015). The mineral elements most commonly lacking in human diets are iron (Fe) and zinc (Zn) (White and Broadley, 2009; Stein, 2010). In fact, rice is deficient of the above mineral elements. Since rice serves as staple food, even a minimum increase in nutritive value of rice can significantly impact human health (Zeng *et al*., 2010; Chandel *et al*., 2010). The present-day mega varieties e.g., Swarna (11 mg/kg), MTU 1010 (5-7 mg/kg), Swarna Sub-1 (17.28 mg/kg), and IR 64 (16 mg/kg) harbor low grain Zn content, and it is not sufficient to meet the normal daily requirement of 12 mg/day for women, 4 mg/day for a 4-6-year-old child and 10 mg/day for an adult man. Therefore, biofortification breeding is stressed upon in many countries. Zn acts as catalytic or structural components of various enzymes involved in cellular metabolism. It promotes immunity, resistance to infections, growth, and development of the nervous system, production of antibodies against intestinal pathogens. With even 20% Zn absorption, and retention of 90 % in the blood serum, a daily ration of 422gm rice (having Zn conc. @ 28 mg Zn/kg kernel) can supplement at least 25% of daily requirement (Harvest Plus, 2014). Reports are indicating high correlations between Zn deficiency tolerance and high grain Zn in rice, and hence, cultivation of Zn biofortified rice seems to be rewarding in areas of Zn deficiency. Keeping in view the above facts, an attempt was undertaken to explore the status of grain Zn content in a set of selected rice genotypes and to assess its inter-relationship with agro-morphological and quality traits.

Materials and Methods

The experimental materials used in the present investigation comprised of 25 test genotypes, including 11 promising landraces, four zinc biofortified released varieties (BRRI Dhan 64, BRRI Dhan 72, CGZR-1, and DRR Dhan 45), six Zn-dense advance breeding lines, one OUAT rice variety(Manika), one zinc dense check variety (Chittimuthyalu) and three low zinc mega varieties of rice (Swarna and Sambamahsuri). These test entries were evaluated in a field trial at the Regional Research and Technology Transfer Station, Bhubaneswar, during Kharif, 2018. The field experiment was laid out in randomized block design with three replications. Each genotype was transplanted in a single plant per hill in 4 rows of 2.5 meter length with a spacing of 20x10 cm. The recommended agronomic practices were followed to raise a good crop. A fertilizer dose of 60-30-30 N, P_2O_5, K_2O $kg.ha^{-1}$ was applied as per scheduled management practices. Observations were recorded on 10 randomly selected plants from the middle row of each plot for nine biometric traits except days to flowering, days to maturity, and 100-grain weight (g), which were recorded on a plot basis and from a random sample of plants of each plot respectively. The test genotypes were assessed for seven physical quality parameters, e.g., grain length (GL), grain breadth (GB), GL/GB, kernel length (KL), kernel breadth (KB), KL/KB, and grain type. Based on grain length and

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Length/Breadth ratio, rice varieties were classified into five-grain types (Govindaswamy, 1985).

For micronutrient analysis, fine grounded samples in three replicates were digested by a di-acid mixture of nitric acid (HNO_3): and perchloric acid ($HClO_4$) in a 3:2 ratio following the standard procedure of Jahan *et al*. (2013) with minor modification (i.e., 3:2 instead of 1:2 di-acid ratio). Zinc content was estimated in the aliquot of seed extract by using Inductive Coupled Plasma-Optical Emission Spectrophotometer (ICP-OES) at 206.2 nm at Central Instrumentation Facility (CIF), OUAT, Bhubaneswar. The variation in replications for each sample did not exceed ± 1 ppm. The mean of the three replicates were worked out to indicate Zn-content of each genotype.

The data recorded were subjected to statistical analysis to estimate critical difference (CD = √ 2EMS/r x t value) for each trait at error degree of freedom at a 5% level of significance. Correlation coefficients between each pair of characters were estimated as per Panse and Sukhateme (1985) to establish a genetic relationship among different characters.

Results and Discussion

Mean Performance of Seed Yield Contributing Traits

For zinc biofortification, medium plant height, plant types with 120–135 days duration and acceptable grain quality, and grain Zn content more than 22 ppm are the breeding targets in rice. A set of test genotypes was purposefully selected, including tall plant type high Zn donors, improved breeding line(s), released biofortified varieties with high Zinc content, and important Zn dense landraces popular in Odisha. These test genotypes were intended to compare with the popular mega variety "Swarna."

The mean performance of all 25 aromatic rice varieties for seed yield and its component traits have been shown in Table 1. Days to flowering and days to maturity ranged from 74–115 and 105–148 days, respectively. Sambamahsuri took 115 days to flower and matured at around 148 days. Similarly, Swarna revealed 112 days and 145 days for flowering and days to maturity, respectively. CGZR-1, Nagina 22, Chittimuthyalu, BG-102 and BRRI Dhan 64 flowered within 75–80 days and matured within 120 days. Nikipankhia is a long-duration Zn dense local landrace with maturity duration of around 148 days. The ORCZ75-3-1 and P44 selection breeding lines seem to have medium maturity duration (125-135days).

BG-102, Dudh Kandar, Katikalam, Tikimashuri, URG-24, Malliphulajhuli, and Padmavati exhibited tall plant type (>110 cm). In contrast, CGZR-1, BRRI Dhan 64, Nagina 22, IR 85850-AC157-1, IR 91143-AC239-1, and BRRI Dhan 72 exhibited dwarf plant type similar to the check varieties "Swarna" and "Sambamahsuri."

No. of effective bearing tillers m^{-2} (EBT/m^{-2}), grain number $panicle^{-1}$ and 100- grain weight along with fertility(%) directly determine yield potential of rice genotypes. Several effective bearing tillers ranged from 280 to 458 with a maximum in the standard check variety "Swarna" (458 tillers/m^2) followed by R-RHZ-7 (452), DRR Dhan 45, and P44 selection. Khush (1993) emphasized the importance of moderate tillering types with large panicles bearing high grain numbers for the realization of high grain yield in rice.

Longer panicle with high grain density (more grains $panicle^{-1}$) usually contributes significantly to seed yield. Among the test genotypes, panicle length ranged from 21.6–33.0 cm with the maximum being revealed in IR95133:1-B-16-14-10-GBS-P-1-2-3 (33.0) followed by P44 selection and R-RHZ-7 (more than 30 cm). Among the test genotypes grains, $panicle^{-1}$ was maximum in ORCZ75-3- 1 followed by P44 selection. Both the varieties exhibited longer panicles (27–30 cm). The same is the case in BRRI Dhan 72 with 142 grains $panicle^{-1}$ in a panicle of 27.5cm length as compared to 128 grains in Swarna. The grain weight based on 100- grains in each genotype ranged from 1.42–3.05 gm. BG-102 bore very bold grains (3.05 gm). Similarly, Dudh Kandar exhibited very aggressive grains with a 100-grain weight equivalent to 3.00 gm as compared to Swarna (2.15 gm) (Table 1).

Grain filling depends upon effective translocation of photosynthates to the sink (seed). The activity of a number of enzymes related to the transport of photosynthates is needed for healthy and plumpy grains at physiological maturity. 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 (Tripathy and Bal, 2021). In the present investigation, the genotypes were selected based on high Zn content and compared with the standard check mega variety "Swarna" and a qualitatively improved variety Sambamahsuri. The fertility % in almost all the test genotypes was higher than 80% except IR91143-AC239-1 (62.11). BG-102, Chittimuthyalu, and Nagina 22, having high grain Zn content exhibited more field grains per panicle (>90%) assessed at physiological maturity. Zinc serves as a cofactor for more than 300 enzymes in plant system resulting increase in activity of enzymes. Higher fertility % in the present set of genotypes can therefore be related to the selection history of the test genotypes.

Mean Performance for Quality Traits

Grain length and grain breath determine grain type of rice genotypes. Grain type score ranged from 2.0 to 5.0 (short bold to long slender grain type). Grain length/grain breadth ratio indicates proportionate length-wise grain dimension per unit grain breath. It ranged from 2.3-4.3, with highest being revealed in Malliphulajhuli and IR95133:1-B-16-14-10-GBS-P-1-2-3 followed by R-RHZ-7 as compared to "Swarna" (2.72) (Table 1). Kernel dimension is a primary consideration for consumers' preference. Usually, medium slender to long slender kernel types fetch better price in the market and are also suitable for table rice. Malliphulajhuli and IR 95133:1-B-16-14-10-GBS-P-1-2-3, ORCZ 75-3-1, P44 selection, and R-RHZ-7 had revealed proportionally longer kernel per unit kernel breadth with KL/KB value of more than 4.0. These could be considered for consumers' preferences.

Usually, Zn content varies from 5.0-50 ppm depending upon the set of germplasm as test materials. Local landraces and wild rice, *Oryza nivara* are usually rich in grain Zn content. Patil *et al*. (2015) studied 61 rice accessions, including landraces and improved lines for grain (brown rice) Zn content. The variation in grain Zn content ranged from 14.03 to 31.94 pm with an average of 24.3 ppm. Mallikarjuna Swamy *et al.* (2016) observed significant variation for grain Zn in IRRI rice germplasm. In the present investigation,22 genotypes including Swarna and

Table 1: Mean performance of a set of 25 Zn biofortified rice genotypes.

Sl. No.	Genotype	DF (Days)	DM (days)	PHT	Tillers/ m^2	PL (cm.)	GN/P	100-GW	F%	GL	GB	GL /GB	Grain Type Score	KL	KB	KL /KB	Zn ppm	Yield (q/ha)
1	BASUDHA	102	133	115	308	21.6	135	2.15	80.00	7.0	3.0	2.33	4	6.6	2.8	2.35	44.4	28.10
2	BRRI Dhan 64	83	115	93	398	22.2	133	2.40	86.92	7.9	3.0	2.63	4	6.5	2.8	2.23	23.1	44.16
	BRRI Dhan 72	93	123	90	388	27.5	142	2.45	88.05	9.0	3.1	2.90	4	8.0	2.7	2.96	17.9	45.26
4	BG 102	74	105	112	325	28.6	80	3.05	90.28	9.0	3.0	3.00	4	7.5	2.8	2.67	25.8	35.60
5	CHITTIMATYALLU	79	110	78	428	26.4	128	2.35	92.88	10.0	2.9	3.44	5	8.2	2.5	3.28	22.0	40.05
6	CGZR -1	80	109	93	418	22.6	98	2.70	82.26	9.0	2.8	3.21	5	7.5	2.5	3.00	21.7	40.50
7	DRR Dhan 45	96	129	95	453	24.0	115	2.45	80.13	8.0	2.4	3.33	5	7.0	2.0	3.50	22.1	40.28
8	Dudh Kandar	92	123	132	294	29.5	78	2.98	80.64	8.9	3.2	2.78	4	7.8	2.2	3.54	25.6	28.60
9	IR 91143-AC239-1	82	111	80	423	22.8	98	2.72	62.11	9.0	2.7	3.33	5	7.2	2.1	3.42	22.0	39.20
10	IR 85850-AC157-1	98	128	95	412	25.6	80	2.19	80.28	10.0	3.0	3.33	5	8.8	2.8	3.14	24.0	38.10
11	IR 95133: 1-B-16-14-10-GBS-P1-2-3	93	123	100	389	33.0	106	2.52	82.35	10.0	2.3	4.30	5	8.0	1.9	4.21	23.1	40.00
12	KALANAMAK	102	134	98	368	28.5	118	2.10	87.75	8.0	2.3	3.47	5	7.2	2.0	3.60	21.1	38.80
13	KATIKALAM	106	136	125	350	26.3	108	1.70	83.98	8.0	2.2	3.63	5	6.5	1.7	3.82	39.1	30.20
14	MANIKA	103	142	92	368	22.0	105	2.22	80.90	7.5	3.0	2.50	4	6.0	2.8	2.14	42.7	35.80
15	MALLIPHULAJHULI	99	131	125	280	24.4	120	1.80	83.59	10.0	2.3	4.30	5	8.0	1.9	4.21	43.8	38.80
16	NAGINA-22	82	110	92	420	26.2	80	2.50	92.80	5.8	2.5	2.32	2	5.2	1.9	2.88	28.1	28.50
17	NIKIPANKHIA	114	148	120	380	28.0	110	2.90	83.50	11.0	3.0	3.66	5	9.0	2.8	3.16	42.8	35.30
18	ORCZ 75-3-1	93	123	108	428	27.0	145	1.40	85.29	9.0	2.3	3.90	5	7.0	1.7	4.11	45.2	44.10
19	P 44 -SELECTION	102	132	110	449	30.3	142	2.21	86.60	9.0	2.3	3.9	5	8.0	2.0	4.00	52.7	45.08
20	PADMAVATI	107	138	118	370	29.1	98	2.15	87.50	9.0	3.0	3.00	4	8.0	2.5	3.20	31.3	35.20
21	R-RHZ-7	96	125	98	452	30.5	112	2.14	82.05	10.0	2.4	4.16	5	8.2	2.0	4.10	24.7	38.80
22	SWARNA	112	145	92	458	29.3	128	2.15	81.00	6.0	2.2	2.72	4	5.5	2.0	2.75	17.3	40.20
23	SAMBAMAHSURI	115	148	88	418	29.5	123	1.44	84.70	6.0	2.4	2.50	3	7.0	2.2	3.18	12.2	38.80
24	TIKIMAHSURI	105	134	117	302	29.6	95	2.35	80.50	8.0	3.0	2.66	4	6.5	2.8	2.23	41.5	35.20
25	URG-24	95	125	125	298	25.4	95	2.38	88.50	7.5	3.0	2.50	4	6.0	2.8	2.14	**21.9**	36.80
Mean		96.1	127.2	103.6	383.1	26.8	111	2.3	83.8	8.5	2.7	3.2	4.4	7.2	2.3	3.2	29.4	37.7
Range		74-115	105-148	78-132	280-458	21.6-33.0	78-145	1.4-3.1	62.1-92.9	5.8-11.0	2.2-3.2	2.3-4.3	2-5.0	5.2-9.0	1.7-2.8	2.1-4.2	12.2-52.7	28.1-45.3
$C.D._{0.05}$		7.5	12.2	14.6	58.2	2.8	20.5	0.05	6.8	1.8	0.06	0.5	0.8	2.1	0.23	0.6	5.2	7.8

Table 2: Character association of agro-economic traits with grain Zinc content.

Characters	DF (Days)	DM (days)	PHT	Tillers/ m^2	PL (cm.)	GN/P	100-GW	F%	GL	GB	GL/GB	Grain Type Score	KL	KB	KL /KB	Zn (ppm)
DM (days)	0.99**															
PHT(cm.)	0.31	0.28														
Tillers/m^2	-0.06	-0.07	-0.73**													
PL (cm.)	0.29	0.22	0.19	0.09												
GN/P	0.25	0.25	-0.21	0.32	-0.05											
100-Grain Wt.	-0.49*	-0.46*	0.01	-0.14	-0.02	-0.52**										
F%	-0.11	-0.10	0.12	-0.07	0.26	0.12	-0.11									
GL	-0.19	-0.19	0.16	-0.03	0.18	-0.04	0.27	-0.09								
GB	-0.24	-0.20	0.13	-0.43*	-0.28	-0.34	0.56	0.03	0.17							
GL/GB	0.00	-0.03	0.10	0.21	0.33	0.21	-0.17	-0.09	0.74**	-0.53**						
Grain Type Score	0.00	0.00	0.06	0.14	-0.01	0.23	-0.03	-0.32	0.73**	-0.22	0.79**					
KL	-0.01	-0.02	0.09	0.05	0.30	0.02	0.19	-0.03	0.89**	0.17	0.63**	0.59**				
KB	-0.08	-0.03	0.02	-0.35	-0.31	-0.19	0.43*	0.09	0.08	0.88**	-0.53**	-0.19	0.11			
KL/KB	0.05	0.00	0.08	0.27	0.43*	0.19	-0.29	-0.09	0.49*	-0.65**	0.87**	0.55**	0.51**	-0.79**		
Zn (ppm)	0.27	0.27	0.54**	-0.29	-0.10	0.20	-0.20	-0.01	0.20	-0.05	0.24	0.21	0.05	-0.03	0.12	
Yield(q/ha)	-0.11	-0.10	-0.47*	0.53**	0.08	0.59**	-0.18	0.02	0.27	-0.24	0.39	0.41*	0.27	-0.08	0.23	-0.18

Sambamahsuri were estimated for grain Zn content using ICP-OES. Grain Zn content ranged from 12.22 to 52.70 ppm among the test genotypes. P44 selection revealed the highest Zn content (52.7 ppm) followed by Basudha, Manika, Nikipankhia, ORCZ 75-3-1 and Tikimashuri with Zn content more than 40 ppm. Genotype with Zn content above the threshold limit of 22 ppm is considered as a Zn biofortified rice variety. The popular Zn biofortified rice varieties, such as CGZR-1, BRRI Dhan-64, and Kalanamak, revealed grain Zn content around 22 ppm. The high Zn rice varieties (more than 40 ppm) revealed in this investigation may serve as a potential donor for Zn biofortification.

Mean Performance for Seed Yield

High seed yield along with grain Zn content more than 22 ppm is the prime consideration for breeding towards Zn biofortification in rice (Tripathy *et al*., 2020). Seed yield ranged from 28.1–45.3 q. ha^{-1}. Among the test genotypes; BRRI Dhan 64, BRRI Dhan 72, CGZ-R-1, DRR Dhan 45, IR95133:1-B-16-14-10-GBS-P 1-2-3, ORCZ 75-3-1, and P44 selection revealed high yield potential (more than 40 q.ha^{-1}), and those are more or less equivalent to or higher seed yield than the standard mega-variety Swarna (40.20 q.ha^{-1}). Considering both grain Zn content and high yield potential, ORCZ75-3-1 and P44 selection are the best Zn biofortified product with grain yield around 44-45 q.ha^{-1} and grain Zn content more than 45.0 ppm.

Association Analysis

Grain yield showed significant positive correlation with number of ear bearing tillers m^{-2} (r = 0.53), grain number per panicle (r = 0.59) and grain type score (r = 0.41), but exhibited negative correlation with plant height (r = 0.47) (Table 2). This corroborates the findings of Nagesh *et al*. (2013) and Bekele *et al*. (2013). Days to flowering and days to maturity are intimately related with each other, with the highest correlation value of 0.99. Both the traits revealed a negative correlation with 100-grain weight. The rest of the traits seem to have no significant *inter-se* correlation with days to flowering and days to maturity, indicating negligible merit of the latter two traits in improvement of grain yield through correlated response with agro-economic traits. The negative correlation of grain weight with a duration of the crop (r = 0.49* and -0.46*) has direct relevance with the effective rate of translocation of photosynthates to the grain. Short duration semi-dwarf plant types of rice seem to have higher efficiency of translocation of photosynthates as compared to tall *indica* plant type, and therefore these posses high yield potential.

Plant height exhibited a negative correlation with the number of effective bearing tillers per m^2 and seed yield, but positively correlated with zinc content. This envisaged that tall plant types have inherent high potential of zinc transport to the grains as compared to semi-dwarf plant types. Such an undesirable linkage often hinders zinc biofortification breeding programs in rice. A number of tillers is also negatively correlated with grain breadth, although it has a strong correlation with seed yield. Grain number per panicle revealed a strong negative correlation with 100-grain weight but correlated positively with seed yield. This indicates that a genotype with higher number of slender grains $panicle^{-1}$ would pave the way for higher yield potential. Fertility percentage is an important trait that determines the panicle weight and contributes considerably to seed yield. However, the present investigation revealed no significant correlation of fertility percentage with any yield contributing traits as well as seed yield *per se*.

Grain length revealed a close association with kernel length (r=0.89) followed by GL/GB, grain type, and KL/KB, as Vivekanandan and Giridharan (1998) reported. Chouhan (1995) reported a significant positive association of kernel length and kernel L/B ratio but the negative association with kernel breadth in some crosses of aromatic x non-aromatic varieties. Grain breadth negatively correlated with tillers per m^2, GL/GB, and KL/KB. This indicates that low tillering plant types (tall *indica* types) usually have bold grains. Grain breadth positively correlated with kernel breadth with a high correlation coefficient value of 0.88, indicating close correspondence between the two traits. Grain type score is determined by length and breadth. Grain type correlated significantly with grain length (0.73**), GL/GB (0.79**), kernel length (0.59**) and KL/KB (0.55**) resulting significant correlation with seed yield (r=0.41*).

Grain zinc content significantly correlated with plant height. No other agro-economic traits, including seed yield, seem to significantly associate with grain Zn content. The erstwhile mentioned tall indica landraces such as Malliphulajhulli (43.8 ppm), Nikipankhia (42.8 ppm) Padmavati (31.3 ppm), Tikimahsuri (41.5 ppm) and Basudha (44.4 ppm) harbor high grain zinc content but revealed low yield potential. Transfer of zinc transporter genes available in above tall *indica* types to semi-dwarf plant types may enrich the status of zinc content in grain.

Conclusion

Selection becomes ineffective unless the presence of wide genetic variability of the trait of interest is ensured. Therefore, identification of donors for grain Zn content in rice is a prerequisite for biofortification breeding. In this context, a few Zn dense genotypes selected in this study can be used as parents for rice breeding. Besides, association analysis revealed a significant correlation of number of tillers m^{-2} and grain number per panicle with seed yield. Plant height maintained an inverse relationship with the number of tillers m^{-2} and seed yield but positively correlated with the grain zinc content. Besides, grain breadth negatively correlated with tillers per m^2. Therefore, the selection of semi-dwarf plant types with high tillering ability and compact panicles containing medium slender grains may be rewarding for Zn biofortification breeding.

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