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**Swapan K Tripathy**Department of Agricultural  
Biotechnology, College of  
Agriculture, OUAT,  
Bhubaneswar, Odisha, India**Suraj K Behera**Department of Agricultural  
Biotechnology, College of  
Agriculture, OUAT,  
Bhubaneswar, Odisha, India**Dibyabharati Sahu**Department of Agricultural  
Biotechnology, College of  
Agriculture, OUAT,  
Bhubaneswar, Odisha, India**Corresponding Author:****Swapan K Tripathy**Department of Agricultural  
Biotechnology, College of  
Agriculture, OUAT,  
Bhubaneswar, Odisha, India

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# Phenotyping and association analysis of grain zinc content with agro-morphological traits in a core rice germplasm

**Swapan K Tripathy, Suraj K Behera and Dibyabharati Sahu**

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### Abstract

A set of 47 biofortified core germplasm lines of rice were estimated for grain zinc (Zn) content to explore high Zn donors and to determine the degree of association of Zn content with agro-morphological traits including seed yield. The top Zn dense ( $\geq 20$ 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). R-RHZ-7 recorded maximum EBT/m<sup>2</sup> along with long panicle, while Nagina 22 maintained dwarf plant type with excellent grain fertility ( $\geq 92\%$ ), thin hull and known high degree of drought tolerance. Among these, IR 85850-AC157-1(24.0ppm) and R-RHZ-7 (24.7ppm) retained high grain zinc content along with moderately high seed yield potential. Grain number/panicle ( $r=0.757^{**}$ ), days to flowering, days to maturity and fertility percentage revealed strong positive correlation with seed yield. Grain Zn content had non-significant negative association with seed yield indicating genetic enhancement for Zn may not have much yield penalty. Interestingly, Zn content revealed positive significant relationship with grain breadth, but feeble negative association with grain length and kernel length indicating that selection for bold grain types would enrich Zn content. The above Zn rich elite genotypes with desirable ancillary traits may serve as potential donors for biofortification breeding programme.

**Keywords:** Association analysis, grain zinc content, seed yield, zinc biofortification, rice (*Oryza sativa* L.)

### Introduction

Malnutrition continues to be a crucial problem. Males aged between 15-74 years need ~12-15 mg of Zn daily while females aged between 12-74 years need ~68 mg of Zn (Sandstead, 1985) [27]. About half of world's population particularly from developing countries suffers from Zn deficiency and majority of them depend on rice as staple food. Rice grain is relatively low in zinc (Zn) compared to other cereals. It is cooked and consumed as whole grain or other forms by more than half of the world population. 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, quality considerations are much more important than for any other food crops (Hossain *et al.*, 2009) [16]. In India, rice is cultivated on 44.00 million hectares of area with a production of 103.00 million tons and productivity of 2.34 t/ha (Shivani *et al* 2019) [31]. India's current status of rice production need to be increased by 18.83% (122.4 mill tonnes) (Kumar *et al.* 2016) [20] to feed estimated 1.5 billion people by 2030. With the alarming situation of malnutrition affecting human health, breeding strategies have been reoriented in last two decades to include biofortification for micronutrients, vitamins and proteins in the mainstream breeding programme. Biofortification is a sustainable genetic approach (Bouis 2002) [8] which aims at genetic enrichment of food stuffs with vital nutrients required for human health. Grain zinc content is a complex trait accompanied by high G x E interaction which hinders progress in development of stable biofortified rice. There is wide variation in grain zinc concentration (14.3-31.94 ppm) (Patil *et al.* 2015) [25] in brown rice suggesting tremendous scope for enrichment of this micronutrient in rice grains. Study of genetic variation to explore truly exploitable nutrient dense stable donors as parents and assessment of the relationship of iron and zinc with morpho-agronomic traits including seed yield are the initial vital steps to start any biofortification programme. Therefore, an attempt was made to identify potential Zn-dense donors and to study the association of grain zinc content with seed yield and ancillary traits in a set of biofortified core germplasm of rice.

## Materials and Methods

### Plant materials and field plot technique

The experimental materials used in the present investigation comprised 47 test genotypes including 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 evaluated in a field trial at the Regional Research and Technology Transfer Station, Bhubaneswar following Randomized Block Design (RBD) with three replications to assess yield and ancillary traits. The crop was raised following recommended package of practices.

### Agro-morphological characterization

Observations were recorded on 5 randomly selected plants from middle row of each plot for plant height(cm), number of ear bearing tillers/hill, panicle length(cm), grain fertility(%) and grains per panicle, while days to 50% flowering, days to maturity and seed yield (q/ha) were recorded on plot basis. For 100-grain weight (g), observation was taken from random sample of seeds of each plot. 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. Basing on grain length and L/B ratio, rice genotypes were classified into seven grain types as per Govindaswamy (1985) [12] with minor modification as mentioned below (Table 1).

**Table 1.** Classification of rice genotypes based on grain type.

Descriptor	Grain length(mm)	Grain Length/Breadth	Score
Short slender(SS)	< 6.0	≥ 3.0	1
Short bold(SB)	< 6.0	< 2.5	2
Medium slender(MS)	< 6.0	2.5 to 3.0	3
Medium bold(MB)	6.0-7.0	2.0-2.5	3.5
Long bold(LB)	≥ 6.0	2.5 to 3.0	4
Long slender(LS)	≥ 6.0	≥ 3.0	5
Extra long slender(ELS)	≥ 9.0	≥ 4.0	6

### Micronutrient analysis

Fine ground samples of each 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) [18] with minor modification (i.e. 3:2 instead of 1:2 diacid ratio). It is based on the principle that atoms of Zn which normally remain in ground state, under flame condition absorb energy and subjected to radiation is proportional to the specific wavelength. The absorption of radiation is proportional to the concentration of zinc. 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 ± 1ppm. The mean of the three replicates were worked out to indicate Zn-content of each genotype.

### Statistical analysis

Routine statistical procedures were followed for analysis of variance and covariance as per Singh and Choudhury (1985) [32] using sample means of various traits under study. Estimates of the correlation coefficient for each pair of characters were computed following Al-Jibouri *et al.* (1958)

[3] to establish inter-relationship among different characters and the significance of correlation coefficients was tested by 't'- test at n-2 degrees of freedom.

## Results and Discussion

### Mean performance of morpho-agronomic traits in relation to 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) [21]. 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 35.3ppm) exist in brown rice among local land races (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 (BRR1 Dhan 62, BRR1 Dhan 64, BRR1 Dhan 72) and India (DRR Dhan 45, DRR Dhan 48, DRR Dhan 49, CGZR 1, CGZR 3, Hurz 1 and Hurz 3) (Table 2). 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 9.2ppm in IR 97443-11-2-1-1-1-B to as high as 28.1ppm in Nagina 22 among 47 test genotypes including standard high yielding check varieties e.g., Swarna (17.3ppm), MTU 1010 (15.1ppm) and Sambamahsuri (12.2ppm). The top Zn dense (≥20ppm) genotypes identified in the present investigation were Nagina 22(28.1ppm) followed by BG 102(25.8ppm), Dudh Kandar (25.6ppm), R-RHZ-7 (24.7ppm), IR 85850-AC-157-1(24.0ppm), BRR1 Dhan 64(23.1ppm), IR 95133: 1-B-16-14-10-GBS-P1-2-3(23.1ppm), IR 95133: 1-B-16-14-10-GBS-P1-2-2(22.6ppm), DRR Dhan 45 (22.1ppm), IR 91143-AC-239-1(22.0ppm) and Sathi (21.0ppm). The breeding target is 28ppm Zn in rice grain. Hence, Nagina 22 may serve as Zn dense donor for genetic biofortification breeding programme. Similarly, higher Zn content was shown to be associated with some of the aromatic rice (Gregorio 2002) [13] and local upland rice (cv'Nam Roo': 31ppm) (Jaksomsak *et al.* 2015) [19].

Farmers are sceptic to reap more produce from each penny invested to suffice their food requirement and earning profit/unit area. Therefore, a biofortified rice variety must retain high yield potential besides being rich in micronutrient. Zn biofortification is targeted to breed mid-early (110-125days) Zn rich rice genotypes. Number of effective bearing tillers (EBT), grain number/panicle, panicle length, test weight and seed fertility status determine yield potential of a test genotype. In the present investigation, R-RHZ-7 recorded maximum EBT/m<sup>2</sup> along with long panicle. In contrast, the top zinc rich donors "Dudh Kandar" and "BG 102" had shown tall and low tillering ability. Genotypes with good grain filling offers substantial contribution towards seed yield. BG 102 and Dudh Kandar recorded 100-grain weight even more than 3.0g. Fertility percentage was extremely low(54-58%) in IR 15M 1546, Sathi and BD 102 while, few of the test genotypes maintained high fertility status (≥92%) as in case of N22 and IR 95044: 8-B-5-22-19-GBS.

In the present study, IR 99642-57-1-1-1-B and BRR1 Dhan 72 recorded high yield (≥ 46q/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 test genotypes 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.

**Table 2:** Mean performance of germplasm lines for grain Zinc content and morpho-agronomic traits.

No.	Genotype	DF (Days)	DM (days)	PHT (cm)	Tille rs/m <sup>2</sup>	PL (cm.)	GN/P	100-GW	F%	GL	GB	GL/GB	Grain Type Score	KL	KB	KL/KB	Zn (ppm)	Yield (kg/ha)
1	BRR1 Dhan 62	78	108	75	425	26.5	120	2.36	88.88	10.0	2.9	3.44	5	8.2	2.5	3.28	16.0	4102.6
2	BRR1 Dhan 64	82	112	90	300	22.0	130	2.40	85.92	7.9	3.0	2.63	4	6.5	2.8	2.23	23.1	4310.2
3	BRR1 Dhan 72	91	120	92	310	27.0	132	2.46	87.05	9.0	3.1	2.90	4	8.0	2.7	2.96	17.9	4656.5
4	BD 105	76	106	90	290	23.5	75	1.88	58.82	7.0	2.8	2.50	4	6.0	2.8	2.14	20.0	3202.6
5	BG 102	70	101	110	300	28.8	78	3.04	89.28	9.0	3.0	3.00	4	7.5	2.8	2.67	25.8	3680.2
6	CGZR -1	78	106	90	355	22.5	90	2.67	78.26	9.0	2.8	3.21	5	7.5	2.5	3.00	21.7	3280.0
7	DRR Dhan 45	90	129	96	423	24.0	110	2.47	74.13	8.0	2.4	3.33	5	7.0	2.0	3.50	22.1	4020.4
8	DRR Dhan 48	97	127	92	420	26.0	99	1.75	81.59	6.0	2.4	2.50	3	5.5	2.0	2.75	20.0	4250.3
9	DRR Dhan 49	97	126	92	412	24.6	102	1.65	81.65	6.0	2.4	2.50	3	5.5	1.8	3.00	19.0	4280.1
10	Dudh Kandar	74	123	130	294	29.3	70	2.97	72.64	8.9	3.2	2.78	4	7.8	2.2	3.54	25.6	2850.4
11	IR 91143-AC239-1	81	109	78	423	22.7	85	2.71	61.11	9.0	2.7	3.33	5	7.2	2.1	3.42	22.0	3920.0
12	IR 91143-AC290-1	85	113	95	428	28.2	90	2.04	83.29	9.8	2.8	3.50	5	7.5	2.5	3.00	20.5	4320.8
13	IR 91143-AC293-1	84	112	98	410	31.6	92	2.38	89.76	8.0	2.2	3.63	5	7.0	1.9	3.68	20.1	4260.4
14	IR 85850-AC157-1	98	127	93	412	25.4	68	2.18	74.28	10.0	3.0	3.33	5	8.8	2.8	3.14	24.0	3902.8
15	IR 82475-110-2-2-1-2	94	124	92	440	28.2	105	2.91	80.59	11.0	3.0	3.66	5	9.0	2.8	3.16	16.7	4130.3
16	IR 95133: 1-B-16-14-10-GBS-P1-2-2	94	123	100	342	28.2	98	2.49	63.29	10.0	2.6	3.84	5	9.1	2.1	4.33	22.6	3520.6
17	IR 95133: 1-B-16-14-10-GBS-P1-2-3	91	120	98	329	33.0	102	2.50	82.35	10.0	2.3	4.30	5	8.0	1.9	4.21	23.1	4008.4
18	IR 95133: 1-B-16-14-10-GBS-P5-1-3	94	124	101	286	30.6	105	2.72	83.56	10.0	2.4	4.16	5	8.0	2.0	4.00	17.8	3780.7
19	IR 95133: 1-B-16-14-10-GBS-P5-2-3	93	122	108	348	35.8	108	2.80	80.53	10.0	2.3	4.30	5	8.0	1.9	4.21	11.1	3820.4
20	IR 95133: 1-B-16-14-10-GBS-P6-1-5	86	114	102	362	29.7	103	2.56	81.25	11.0	2.5	4.40	5	9.5	2.0	4.75	14.8	4160.0
21	IR 95044: 8-B-5-22-19-GBS	79	108	95	323	30.5	106	2.34	93.69	7.0	2.2	3.18	5	6.0	2.0	3.00	16.6	4160.6
22	IR 84847-RIL-195-1-1-1-1	85	113	98	424	26.6	100	2.80	87.35	10.0	2.7	3.70	5	8.2	2.5	3.28	15.9	4120.4
23	IR 99704-24-2-1	57	88	102	410	29.6	78	2.14	77.55	9.0	3.0	3.00	4	8.0	2.5	3.20	16.8	3500.2
24	IR 99647-109-1-1	82	110	102	445	30.0	102	2.22	80.68	9.0	2.3	3.9	5	8.0	2.0	4.00	17.9	4208.8
25	IR 97443-11-2-1-1-1-1-B	80	119	95	360	26.4	118	1.67	83.91	8.0	2.2	3.63	5	6.5	1.7	3.82	9.2	4320.2
26	IR 97443-11-2-1-1-1-3-B	80	110	90	420	27.0	135	1.37	85.23	9.0	2.3	3.90	5	7.0	1.7	4.11	12.7	4410.6
27	IR 96248-16-3-3-2-B	85	114	90	430	24.8	82	2.10	65.27	10.0	2.5	4.00	5	8.2	1.8	4.55	11.9	3510.2
28	IR 15M 1537	76	105	98	420	31.6	98	2.16	78.42	7.0	2.8	2.50	4	6.0	2.5	2.40	12.1	3900.0
29	IR 15M 1546	88	116	85	398	16.0	90	2.24	54.83	9.0	2.3	3.90	5	7.8	1.9	4.10	19.7	3460.8
30	IR 15M 1689	88	117	90	405	26.4	80	2.21	60.00	9.1	2.4	3.80	5	7.8	2.0	3.90	13.5	3580.6
31	IR 15M 1633	87	115	92	380	34.0	86	2.79	68.42	10.0	2.5	4.00	5	8.2	2.2	3.72	12.5	3460.5
32	IR 99642-57-1-1-1-B	88	117	102	348	31.0	140	2.07	88.57	9.0	2.4	3.75	5	8.0	1.9	4.21	14.4	4620.4
33	IR 64(Check)	81	109	90	420	28.4	130	2.88	73.43	10.0	2.8	3.57	5	8.5	2.5	3.40	14.5	4190.0
34	KARHANI	82	110	92	300	25.0	72	2.17	66.07	7.5	2.9	2.58	4	6.0	2.5	2.40	17.2	2680.4
35	MTU 1010(Check)	96	124	96	368	28.0	120	2.11	82.75	8.0	2.3	3.47	5	7.2	2.0	3.60	15.1	4320.6
36	M48	76	104	115	294	33.2	89	2.68	82.43	8.1	2.9	2.79	4	7.5	2.5	3.00	17.0	3620.0
37	M399(Awned)	60	89	105	294	20.0	70	1.87	81.96	7.5	3.0	2.50	4	6.0	2.8	2.14	17.5	2580.0
38	NAGINA-22	80	110	88	374	26.0	76	2.49	92.85	5.8	2.5	2.32	2	5.2	1.9	2.88	28.1	2740.2
39	R-RHZ-7	95	123	95	450	30.0	98	2.11	70.00	10.0	2.4	4.16	5	8.2	2.0	4.10	24.7	3850.5
40	SATHI	73	103	110	302	16.0	80	2.07	58.82	10.1	2.5	4.02	5	8.0	2.0	4.00	21.0	2460.0
41	SWARNA(Check)	108	142	90	420	29.0	120	2.12	80.00	6.0	2.2	2.72	4	5.5	2.0	2.75	17.3	4220.9
42	SAMBAMAHSURI(Check)	101	130	80	410	29.8	110	1.41	64.77	6.0	2.4	2.50	3	7.0	2.2	3.18	12.2	3780.0
43	URG-1	73	103	70	340	30.1	90	1.79	79.62	8.5	2.3	3.70	5	7.0	2.0	3.50	18.0	2830.8
44	URG-19	85	113	125	298	29.0	92	2.34	82.53	8.0	3.0	2.66	4	6.5	2.8	2.23	20.9	3808.2
45	URG-22	85	116	128	290	22.0	95	1.52	70.74	8.0	2.5	3.20	5	6.0	2.0	3.00	20.9	3610.0
46	URG-24	83	111	128	280	25.0	90	2.36	81.57	7.5	3.0	2.50	4	6.0	2.8	2.14	21.9	3620.6
47	URG-30	74	104	102	275	27.0	85	2.21	79.54	7.0	2.5	2.80	4	5.5	2.2	2.50	15.0	2800.2
	Mean	84.1	114	97.3	363	27.2	97.7	2.3	77.6	8.6	2.6	3.3	4.1	7.2	2.2	3.3	18.3	3762.2
	Range	57-103	88-132	70-130	275-450	16-35.8	68-140	1.4-3.0	54.8-93.7	5.8-11	2.2-3.2	2.3-4.4	2-5	5.2-9.5	1.7-2.8	2.1-4.7	9.2-28.1	2460-4656.5
	C.D. <sub>0.05</sub>	8.6	7.2	5.8	36.3	4.9	10.8	0.4	8.2	2.6	0.16	0.21	0.56	0.52	0.09	0.23	2.1	210.8

### Mean performance of physical quality traits in relation to grain Zn content

The top zinc dense donor Nagina 22 is a short bold type upland rice, while most of the other promising zinc dense genotypes (BG 102 :25.8ppm, Dudh Kandar :25.6ppm and IR 85850-AC157-1:24.0ppm) were characterized by long bold grains except R-RHZ-7(24.7ppm) which is a long slender type (Table 2). L/B ratio determines the grain and kernel dimension as well as grain density. Improved grain density refers to the degree of compactness of starch grains in the kernel and complete development of kernel, leaving no space between the kernel and hull. Besides, it seems to have direct bearing on improved head rice recovery (HRR). Interestingly, maximum increase in L/B ratio upon hulling was revealed in some zinc rich donors e.g., Dudh Kandar followed by Nagina 22; and it was comparable to standard quality check cv. Sambamahsuri indicating their characteristic thin and more compact hull during grain development. Besides, L/B ratio was observed to be almost equal in zinc rich rice genotypes e.g., Sathi(21.0ppm), R-RHZ-7(24.7ppm) and IR 91143-AC293-1(20.1ppm) indicating proportionate minimum decrease in length and breadth after hulling leading to maintain high HRR.

### Association analysis

#### Inter-relationship of morpho-economic traits with seed yield

Days to flowering, days to maturity, number of effective bearing tillers/m<sup>2</sup>, panicle length, grain number/panicle and fertility percentage had shown positive significant correlation with seed yield. Among these, the strength of association was much higher in case of grain number/panicle ( $r=0.757^{**}$ ) followed by days to flowering, days to maturity, fertility percentage with seed yield at even 1% level of significance (Table 3). Thus, direct selection based on these component

traits may offer a substantial dividend for augmentation of seed yield. Grain yield is reported to be significantly correlated with number of productive tillers/plant (Shashidhar *et al.*, 2005, Girish *et al.*, 2006, Monalisa *et al.* 2006 and Bekele *et al.* 2013) [29, 10], number of grains per panicle (Monalisa *et al.*, 2006, Suman *et al.*, 2006, Nagesh *et al.* 2012,2013) [34, 24], panicle weight (Akinwale *et al.* 2011) [2], grain fertility (Goswami *et al.* 2000) [11], test weight (Nagesh *et al.* 2012) [24], harvest index and biological yield (Satish *et al.* 2003) [28].

In the present investigation, plant height inversely correlated ( $r=-0.526^{**}$ ) with number of effective bearing tillers/ m<sup>2</sup> at even 1% level of significance. This is due to the fact that majority of the genotypes in the present set of materials exhibited semi-dwarf plant types with high tillering ability. Besides, the number of effective bearing tillers had shown positive significant relationship with days to flowering indicating selection of semi-dwarf plant types with medium maturity duration may increase seed yield. Grain number revealed positive significant relationship with days to maturity and fertility percentage. Fertility percentage is an important component trait for realization high seed yield. It correlated positively with both panicle length and grain number as also reported by Gupta *et al.* (1999) [15] and Sri Devi *et al.* (2019) [33]. This suggests that the genotypes with longer panicle can accommodate more number of fertile grains/panicle and thus contribute to high seed yield. This corroborates the findings of Veni *et al.* (2013) [35]. Besides, it is interesting to note that panicle length and fertility percentage which individually correlated positively with seed yield; had also significant *inter se* high positive correlation (0.413<sup>\*\*</sup>). Therefore, selection for any one of these characters automatically selects the other trait and thus, together could result recovery of high grain yield.

**Table 3:** Inter-relationship between character pairs in a set of zn rich rice genotypes.

Character	DF (Days)	DM (days)	PHT	Tillers/m <sup>2</sup>	PL (cm.)	GN/P	1000-GW	F%	GL	GB	GL/GB	Grain Type Score	KL	KB	KL/KB	Zn (ppm)
DM	0															
PHT	-0.19	-0.08														
Tillers/m <sup>2</sup>	0.31*	0.26	-0.53**													
PL	0.18	0.17	0.09	0.06												
G/P	0.39**	0.37*	-0.22	0.11	0.22											
100-GW	-0.06	-0.03	0.18	-0.08	0.27	-0.11										
F%	-0.09	-0.09	0.11	-0.11	0.41**	0.39**	0.13									
GL	0.01	-0.02	0.03	0.14	0.13	0.05	0.48**	-0.14								
GB	-0.39**	-0.34*	0.29*	-0.25	-0.16	-0.30	0.37**	0.02	0.15							
GL/GB	0.21	0.16	-0.12	0.23	0.21	0.21	0.20	-0.14	0.81**	-0.46**						
Grain type	-0.47**	-0.42**	0.36*	-0.49**	-0.01	-0.02	0.24	-0.06	0.21	0.55**	-0.10					
KL	0.12	0.09	-0.04	0.20	0.22	0.09	0.44**	-0.19	0.90**	0.10	0.75**	0.10				
KB	-0.30*	-0.35*	0.18	-0.24	-0.11	-0.23	0.31*	0.08	0.04	0.88**	-0.49**	0.55**	-0.01			
KL/KB	0.27	0.29*	-0.13	0.27	0.21	0.22	0.07	-0.20	0.61**	-0.55**	0.88**	-0.29	0.69**	-0.7**		
Zn content	-0.04	0.02	0.22	-0.17	-0.29*	-0.38**	0.24	0.01	-0.07	0.32*	-0.24	-0.03	-0.09	0.24	-0.24	
SeedYield	0.50**	0.45**	-0.15	0.38**	0.33*	0.76**	-0.02	0.42**	0.12	-0.21	0.21	-0.17	0.18	-0.12	0.19	-0.25

### Inter se relationship with physical quality traits

Physical quality traits determine consumers preference. Medium to long slender kernel types fit for export and most preferred by the consumers. None of the physical quality traits exhibited significant positive correlation with seed yield indicating the fact that quality traits under study had no significant contribution for realization of high seed yield. Besides, such traits revealed no significant negative correlation with seed yield, suggesting no adverse affect on seed yield while improving physical quality traits. It is

expected that increase in dimension of grain (grain length and grain breadth) and kernel (kernel length and kernel breadth) would result more 100-grain weight as revealed from their inter se significant positive correlation. Grain length and kernel length had significant inter se positive correlation as also reported by Vivekanandan and Giridharan (1998) [36] and these also maintained similar relationships with grain length/breadth and kernel length/breadth respectively. Besides, as expected grain breadth and kernel breadth had shown negative significant relationship with GL/GB and

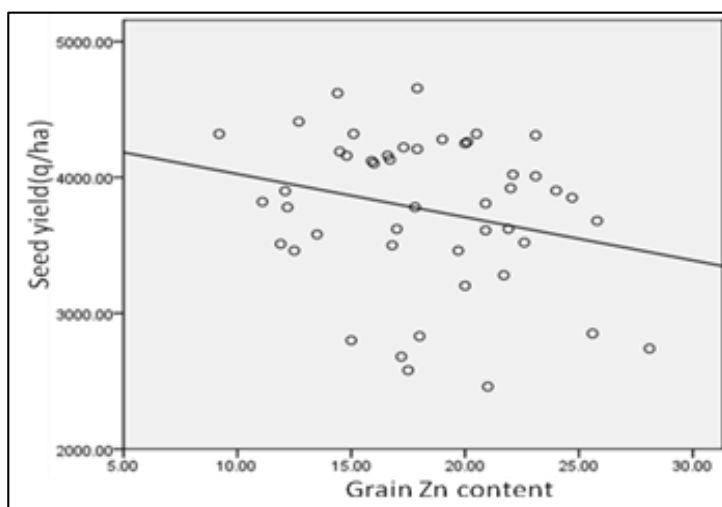
KL/KB respectively indicating that bold grain and bold kernel genotypes would have less length-wise dimension. Chouhan (1996)<sup>[9]</sup> noted similar strong association for kernel traits in some crosses of aromatic x non-aromatic varieties. Further, we noted significant positive correlation of grain length and kernel length with grain type score. This means, the long grain types inherently showed slender grain and kernel characteristics which would have relevance for export value and suitability for consumption.

### Correlation with grain Zn content

Grain zinc content determines the success of biofortification in rice. Zinc is one of the essential micronutrients, which serves as the co-factor for more than 300 enzymes involved in the metabolism of carbohydrates, lipids, proteins, and nucleic acids; hence its importance in normal growth and development of plants and animals (Roohani *et al.* 2013)<sup>[26]</sup>. It negatively correlated with seed yield, though not significant at even 5% level of significance indicating that genetic enhancement for Zn content would have less yield penalty. The same trend was also revealed from its regression on seed yield (fig 1). Such a feeble inverse relationship with seed yield may be ascribed to its negative relationship with panicle length and grains/panicle. Therefore, a compromise for panicle length and grain number must be taken into account while selection of plants for high grain Zn content. Ajmera *et al.* (2017)<sup>[1]</sup> observed feeble non-significant positive association of grain Zn content with seed yield in a set of 37 rice varieties. In contrast, a number of researchers reported significant negative correlation of grain Zn content with seed

yield in rice (Negesh *et al.* 2012, Naesh *et al.* 2013, Shivani *et al.* 2019<sup>[31]</sup> and Inabangan-Asilo *et al.* (2019) except Sri Devi *et al.* (2019)<sup>[33]</sup> who observed significant positive association between these traits in coloured rice. Genetic enhancement for grain Zn content was initially argued to have no/feeble yield penalty (Ashok Kumar *et al.* 2009)<sup>[6]</sup>. But, subsequently it was realized that over accumulation beyond 40µg Zn/g of rice grain (in os-NAS/IR 64 transgenic progenies) was associated with significant reductions in a number of agro-morphological traits affecting grain yield (Moreno-Moyano *et al.* 2016)<sup>[22]</sup>.

It was interesting to note that grain Zn content revealed positive significant relationship with grain breadth (0.315), but feeble negative association with grain length and kernel length indicating that bold grain types may harbour more Zn in the kernel. This means that the varieties which have efficient transport of zinc would have higher loading of the same to the grain resulting better grain filling and increased sink size. A set of 126 germplasm lines revealed strong negative association of Zn concentration with grain elongation (Anuradha *et al.* 2012)<sup>[5]</sup>. This was also the case in traditional short bold land races (Anandan *et al.*, 2011)<sup>[4]</sup> and wild rice (Gregorio *et al.* 2000)<sup>[13]</sup> which harbour high grain zinc content. Overall, it suggests that there is possibility of increasing Zn content through selection of medium length panicle containing moderate number of bold grains. A typical example is the BRRI 64 which retains similar panicle features with heavy bold grain and high grain zinc content. However, Bekele *et al.* (2013)<sup>[7]</sup> showed positive relationship of grain zinc concentration with grain weight coupled with grain length.



**Fig 1:** Relationship of grain zinc content (ppm) with seed yield (kg/ha).

### Conclusion

Malnutrition continues to be a chronic health problem and even led to premature death. Rice being the major staple food, it is targeted by many researchers for Zn biofortification to achieve food and nutritional security. Grain Zn content is a highly complex trait and has high G x E interaction. Exploring a stable high Zn donor and study of the nature of association of grain Zn content with seed yield and related traits are priori to biofortification programme. The high Zn donors with desirable morpho-agronomic traits identified in this investigation can serve as source of valuable QTLs and/or important candidate genes (Zn-transporters) for genetic enhancement of grain Zn content in rice. Grain number/panicle, days to flowering, days to maturity and fertility percentage had strong positive correlation with seed

yield. Grain Zn content had significant positive association with grain breadth, but feeble negative association with grain length and kernel length. Besides, grain Zn content had non-significant negative association with seed yield indicating genetic enhancement for Zn may not have much yield penalty. The overall association analysis suggests that selection of semi-dwarf medium maturity plant types with more number of fertile bold grains/panicle would enrich Zn content in rice.

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