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Variability and Correlation between Yield and Its Component Traits in Mineral Rich (Fe & Zn) *Indica* Rice Genotypes

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ABSTRACT

In a population of 120 plants (HKR47 x BR4-10) a large variation was observed for all physio-morphological traits and Fe & Zn contents [Plant height(72.62-137.46cm); Effective No. of tillers/plant (8-14); Panicle length (13.35-30.59 cm); Grain yield/plant (9.84-24.51 g); 1000 grain wt. (17.14-27.93 g); L/B ratio of grain (2.14-4.01); Days to 50% flowering(85-109);Grains/panicle (68-161);days to maturity(129-134);Grain colour (1-4) Fe content (9.0-188.40 mg/kg); Zn content (19.80-96.10mg/kg)].

In F3 & F4 population of HKR47 x BR4-10 four plants identified will be used for further progenies [Plant No. 64(Fe 179.40 and Zn 95.50 mg/kg); 68 (Fe 180.80 and 68.30 mg/kg); 70 (Fe 183.10 mg/kg and 96.10 mg/kg); 71 (Fe 97.50 and 54.50mg/kg); HKR47 (Fe 54.3 and Zn 25.4mg/kg); BR4-10 (Fe 176.2 and Zn 23.2 mg/kg)]. Phenotypic correlation coefficient analysis of HKR 47 x BR4-10 population showed that Fe content is positively and significantly correlated with Zn content (0.0299, p=0.01). in this population grain yield/plant is positively correlated with plant height (0.186, p=0.05), effective numbers tillers/plant (0.217,p=0.01) and panicle length (0.342, p=0.01). plant height showed significant and positive correlation with effective numbers tillers/plant (0.217, p=0.01) and negative correlation with Zn content (-0.268, p=0.01). 1000 grain weight showed positive and significant correlation with Fe content (0.217, p=0.01) and negative correlation with Zn content (-0.251, p=0.01). Effective number of tillers/plant showed significant and positive correlation with panicle length (0.266, p=0.01). L:B ratio of grain showed negative correlation with Fe content (-0.153, p=0.05).

Key words: *Rice Genotypes, yield, Fe and Zn.*

INTRODUCTION

Rice (*Oryza sativa*), one of the most important food crops in the world, forms the staple diet of more than half of the world. It considered as a “model cereal crop” for genomic studies. Most of the staple foods including rice provide diets of low nutritional quality including vitamins, essential amino acids, iron, zinc, iodine, etc. Iron and zinc deficiencies are the two major factors for micronutrient malnutrition in the world, affecting an estimated 2 billion people.

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Biofortification, which refers to the breeding of staple plants/ foods products with high bioavailable micronutrient content has the potential to provide coverage for remote rural populations, where supplementation and fortification programs may not reach, and it inherently targets the poor especially women, infants and children who consume high levels of staple foods and little else. A lot of variability does exist for micronutrient (Fe, Zn, Vitamin A, etc) content and bioavailability in many crops including rice. Among the major micronutrients deficiencies commonly found in rice-consuming countries iron and zinc deficiencies are the most rampant. It is estimated that more than 3 billion people in the developing world are deficient in iron[8].

There are now several reports, which showed that mineral rich trait could be combined with superior agronomic and high yielding characteristics by breeding. IRRI (2002) identified an improved line (IR68144-3B-2-2-3) with high concentration of grain Fe derived from cross between IR72 and Zawa Bonday. Identification and linkage mapping of QTLs for micronutrient content in cereal grains can greatly accelerate the breeding of mineral-rich crops. Iron maintains metabolic regulation and organ function. It requires for synthesis of transport proteins, haeme and other Iron containing enzymes. Iron deficiency causes anemia. Zinc is an essential component of a large no. of enzymes, and plays a central role in cellular growth and differentiation in tissues. Zinc deficiency reduces growth, sexual maturity, and immune defense system.

Iron and zinc concentration in major crops ranges from 5µg/g to 150 µg/g [7]. In brown rice, iron, zinc, manganese and copper concentration was estimated at 22, 14, 11 and 2.4 µg/g respectively, which is not enough to meet all requirements for healthy body. Adequate diversity for micronutrient (iron and zinc) contents exists in rice, which can be used for developing micronutrient efficient crops[1,2,3,4,6].

MATERIALS AND METHODS

Seed harvested from F2 plants of crosses between following genotype: HKR47 x BR4-10. Data were recorded on F3 and BC2 plants on the following physio-morphological traits and mineral content: Days to 50% flowering; Plant height (cm); Effective numbers of tiller/plant; Panicle length (cm); Grain/panicle; Grains yield/plant (g); L:B ratio (mm); 1000-grain weight(g); Days to maturity and Grain color.

To assess the mineral content (iron and zinc), 1 gm of oven dried, ground seeds were digested with 25 ml of diacid mixture (HNO3:HClO4; 5:1 v/v). The acid digested samples were used for the determination of iron and zinc by using Atomic Absorption Spectrophotometer.

DNA isolation: - DNA was isolated from the leaf tissues of the parental rice genotypes and F4 plants using CTAB DNA isolation procedure. DNA sample was subjected to amplification by the PCR using the method already standardized in the laboratory.

Molecular marker analysis: 60 SSR primers (available in the laboratory) were used for molecular marker analysis. Polyacrylamide gel electrophoresis (PAGE) was used for separation and detection of amplified products.

RESULTS

In a population of 120 plants (HKR47 x BR4-10) a large variation was observed for all physio-morphological traits and Fe & Zn contents [Plant height(72.62-137.46cm); Effective No. of tillers/plant (8-14); Panicle length (13.35-30.59 cm); Grain yield/plant (9.84-24.51 g); 1000 grain wt. (17.14-27.93 g); L/B ratio of grain (2.14-4.01); Days to 50% flowering(85-109);Grains/panicle(68-161);days to maturity(129-134);Grain color (1-4)Fe content (9.0-188.40µg /g); Zn content (19.80-96.10µg/g)]. (Table no. 1)

In F3 & F4 population of HKR47 x BR4-10 four plants identified will be used for further progenies [Plant No. 64(Fe 179.40 and

Zn 95.50µg /g); 68 (Fe 180.80 and 68.30 µg/g); 70 (Fe 183.10µg /g and 96.10µg /g); 71 (Fe 97.50 and 54.50µg/g); HKR47 (Fe 54.3 and Zn 25.4µg/g); BR4-10 (Fe 176.2 and Zn 23.2µg /g)]. 18 SSR markers were polymorphic regarding to variability for F4 plant population. (Table no. 2)

Table .1: Pooled data of Various Agronomic Traits in HKR47 x BR4-10 Populations (F_3 & F_4)

S.NO	PARAMETER	RANGE	HKR47	BR4-10
1.	Plant Height (cm)	72.6-137.4	80.6 ± 1.11	152.3 ± 0.98
2.	Effective No. of Tillers	8-14	15 ± 2	14 ± 1
3.	Total Grain Yield/Plant (g)	9.84-24.51	58.50 ± 0.99	31.09 ± 0.78
4.	1000 grain weight (g)	17.14-27.93	23.71 ± 0.26	25.82 ± 0.36
5.	Panicle length (cm)	13.35-30.59	26.40 ± 0.41	21.70 ± 1.37
6.	Length/Breadth ratio	2.70-4.41	3.69 ± 0.07	2.92 ± 0.08
7.	Grain /Panicle	68-161	154 ± 5.0	136 ± 2.0
8.	Days to 50% flowering	85-109	84 ± 88	93 ± 96
9.	Days to maturity	129-134	128-135	120 ± 128
10.	Grain colour	1-4	1-3	1-4
11.	Fe Content (µg/g)	9.0-188.40	54.3 ± 0.51	176.2 ± 0.52
12.	Zn Content (µg/g)	11.40-96.10	25.4 ± 0.40	23.2 ± 0.43

Table: 2 Value of mineral content of four promising HKR 47 × BR 4-10 F_4 population

Plants No.	Iron (Fe) (µg/g)	Zn (µg/g)
64	179.40	95.50
68	180.80	68.30
70	183.10	96.10
71	97.50	54.50
HKR-47	54.3	25.4
BR 4-10	176.2	23.2

Correlation coefficient analysis

Phenotypic correlation coefficient analysis of HKR 47 x BR4-10 population showed that Fe content is positively and significantly correlated with Zn content (0.0299, p=0.01). In this population grain yield/plant is positively correlated with plant height (0.186, p=0.05), effective numbers tillers/plant (0.217, p=0.01) and panicle length (0.342, p=0.01). Plant height showed significant and positive correlation with effective numbers tillers/plant (0.217, p=0.01) and negative correlation with Zn content (-0.268, p=0.01). 1000 grain weight showed positive and significant correlation with Fe content (0.217, p=0.01) and negative correlation with Zn content (-0.251, p=0.01). Effective number of tillers/plant showed significant and positive correlation with panicle length (0.266, p=0.01). L:B ratio of grain showed negative correlation with Fe content (-0.153, p=0.05).

Table: 3 Phenotypic correlation coefficients between yield, yield components and mineral contents in HKR 47 x BR 4-10

	Grain yield/p.	**Plant height (cm)**	**Effective no. tillers/ pl.**	**Panicle length (cm)**	**1000 gra in wt**	**L:B ratio**	**Fe content (µg/g)**	**Zn content (µg/g)**
Grain yield/plant	1							
Plant height(cm)	0.186*	1						
Effective numbers of tillers/plant	0.755**	0.217**	1					
Panicle length	0.342**	0.093	0.266**	1				
1000 grain weight (g)	0.144	0.054	-0.011	0.138	1			
L:B ratio	0.049	0.080	-0.055	0.122	-0.041	1		
Fe content(µg/g)	0.117	-0.149	-0.001	0.007	0.217**	-0.153*	1	
Zn content(µg/g)	0.150	-0.268**	0.057	0.064	-0.251**	0.008	0.299**	1

*significant at 5%,**significant at 1% level

Variability for iron and zinc content

Researchers at IRRI (Graham *et al*., 1999[4] and Gregorio *et al*., 1999[5]) evaluated the genetic variability of Fe and Zn concentration in rice grain and they reported a wide range of Fe and Zn concentrations in brown rice within the eight sets of genotypes (n = 1,138). The range was 6.3-24.4 µg/g for Fe and 13.5-58.4 µg/g for Zn. Thus, within those genotypes tested, there was approximately a fourfold difference in Fe and Zn concentrations, suggesting some genetic potential to increase the concentrations of these micronutrients in rice grain. Notably, highest grain-Fe (18-22 µg/g) and grain-Zn (24-35 µg/g) concentrations were found in several aromatic rice varieties, such as Jalmagna, Zuchem and Xua Bue Nuo.

A large variation was observed for mineral content (iron and zinc) in HKR47 x BR 4-10 F_3 plants. Iron content differed significantly in F_3 population ranging from 9.0-188.4 µg/g. The grain iron content of HKR47 and BR 4-10, were 54.3 and 176.2 µg/g respectively. Out of 120 plants, of F_3 population 42 plants had iron content above and 78 plants had low iron content from the mean value of 56.17 µg/g. The frequency for iron content is given in (Figure 2). Maximum numbers of F_3 plants come in the range of 0-60 µg/g for iron content.

Zinc content in dehusked rice grains also varied significantly ranging between 11.4 -96.10 µg/g with a mean value of 33.70 µg/g (HKR47-25.4 µg/g and BR 4-10-23.2 µg/g). Out of 120 plants, 47 plants had zinc content above the mean value of 23.90 µg/g and 73 plants had zinc content low from mean value. The frequency for zinc content is given in Figure 4. Maximum numbers of F_3 plants come in the range of 0-35 µg/g for zinc content.

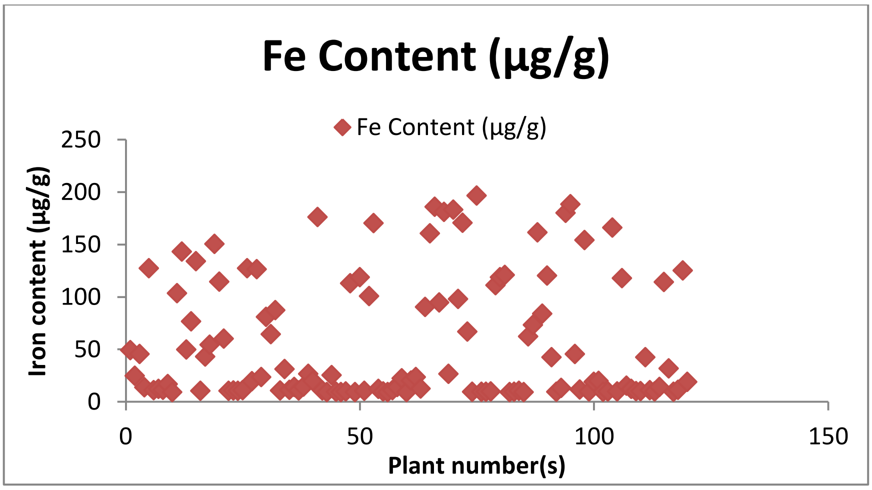

Fig. 1: Variation for Fe content in HKR47xBR 4-10 F_3 population

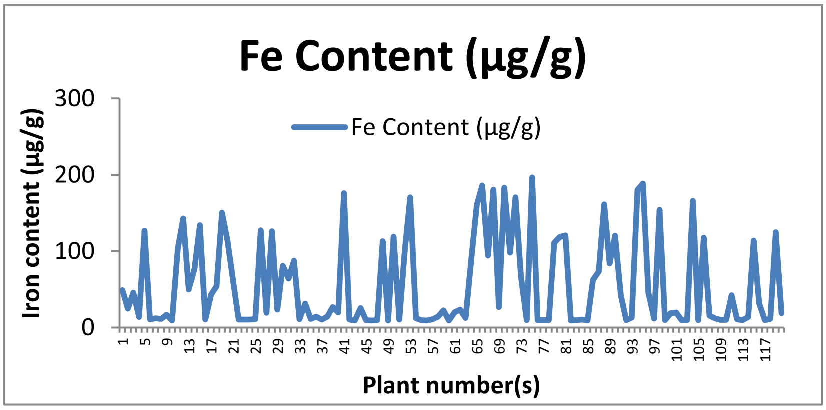

Fig. 2: Frequency distribution for Fe content in HKR47xBR 4-10 F_3 population

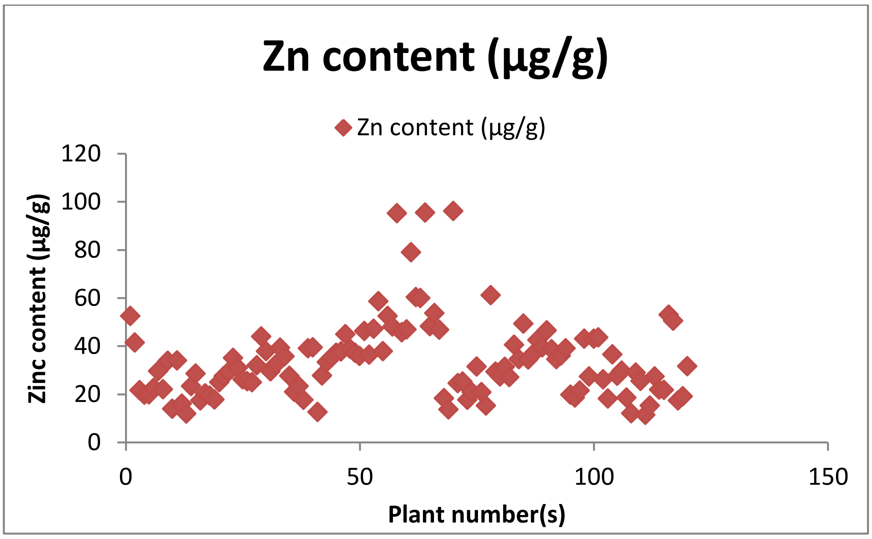

Fig. 3: Variation for Zn content in HKR47xBR 4-10 F_3 population

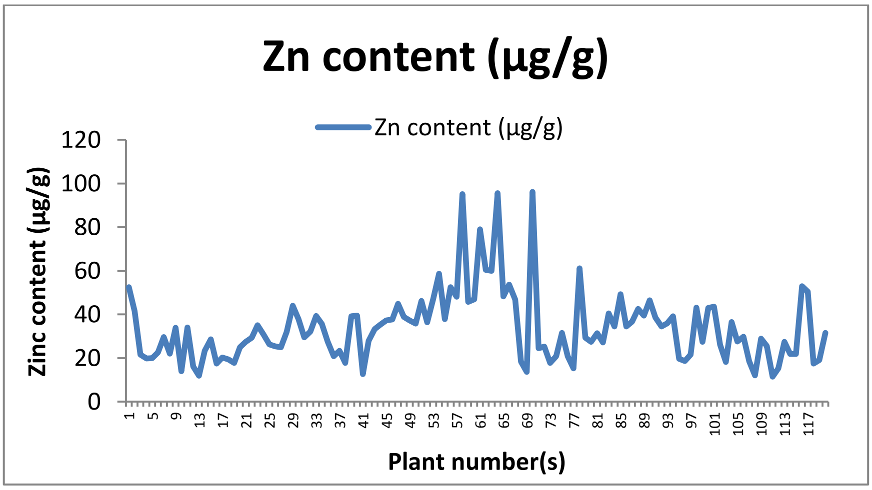

Fig. 4: Frequency Distribution for Zn content in HKR47xBR 4-10 F_3 population

Table: 4 Variation for plant height, yield and yield attribute components, L:B ratio and mineral content in (HKR 47 x BR 4-10) x HKR 47 BC_2 plants

Back cross plant no.	Plant height (cm)	Panicle length (cm)	Effective number tillers/ plant	Grain yield/plant	1000 grain weight (g)	L/B ratio of grain	Fe content (µg/g)	Zn content (µg/g)
HKR 47	80.6	26.40	15	58.50	23.71	3.69	54.3	25.4
BR4-10	152.3	21.70	14	31.09	25.82	2.92	176.4	23.2
11	78.2	22.60	27	109.63	22.35	3.78	74.3	95.6
32	75.5	21.70	12	30.68	18.69	3.63	69.4	89.7
33	80.8	19.56	8	19.82	21.38	3.40	79.4	33.8
54	77.1	20.40	15	62.05	20.42	3.43	70.6	27.9
59	82.3	21.16	14	49.33	20.74	3.61	86.1	21.5
60	78.1	20.96	16	53.70	19.64	3.97	74.5	73.6
77	76.9	21.66	10	19.51	20.82	3.53	72.5	61.8
80	77.3	21.16	15	53.23	20.86	3.47	80.6	69.7
Mean	78.28	21.15	14.62	49.74	20.61	3.60	75.92	59.20
Range	75.5-82.3	19.56-22.60	8-27	19.51-109.63	18.69-22.35	3.40-3.97	69.4-86.1	21.5-95.6
CD		0.905			1.519	0.095	0.811	0.737
SE(D)		0.418			0.701	0.044	0.374	0.340
SE(E)		0.296			0.496	0.031	0.265	0.241
CV		2.420			4.16	1.492	0.604	0.703

Table: 5 Variability for iron and zinc content in 120 Plants (F3 population) of HKR47 x BR 4-10

Plant no.	Fe Content (µg/g)	Zn content (µg/g)	Plant no.	Fe Content (µg/g)	Zn content (µg/g)	Plant no.	Fe Content (µg/g)	Zn content (µg/g)
1	49.10	52.50	41	176.00	12.60	81	120.80	31.40
2	24.70	41.50	42	10.50	27.80	82	9.20	27.10
3	45.50	21.60	43	9.20	33.30	83	9.30	40.50
4	13.50	19.80	44	25.30	35.30	84	10.40	34.50
5	127.10	19.90	45	9.40	37.20	85	9.20	49.30
6	10.90	22.60	46	9.00	37.70	86	62.30	34.40
7	12.00	29.60	47	9.60	44.90	87	73.20	36.60
8	11.20	22.00	48	112.9	38.80	88	161.40	42.50
9	16.80	33.90	49	9.20	37.20	89	83.80	39.40
10	**9.00**	13.90	50	118.8	35.80	90	120.30	46.50
11	103.50	34.00	51	10.3	46.20	91	42.40	38.60
12	143.0	16.10	52	100.80	36.40	92	9.40	34.50
13	49.60	11.90	53	170.40	47.20	93	13.00	36.00
14	76.50	23.30	54	12.00	58.60	94	180.00	39.10
15	134.0	28.60	55	9.60	37.90	95	**188.40**	19.70
16	10.20	17.40	56	9.10	52.50	96	45.40	18.60
17	42.90	20.30	57	10.90	48.10	97	11.50	21.50
18	54.10	19.40	58	14.10	95.20	98	154.00	43.10
19	150.40	17.80	59	22.30	45.70	99	9.70	27.40
20	114.50	24.90	60	9.20	46.90	100	18.9	43.00
21	60.10	27.50	61	20.10	79.00	101	19.7	43.60
22	10.20	29.30	62	23.30	60.40	102	9.40	26.10
23	10.50	35.00	63	12.30	60.00	103	9.70	18.20
24	10.30	31.00	64	90.40	95.50	104	166.10	36.50
25	10.80	26.20	65	160.60	48.20	105	9.70	27.60

26	127.20	25.40	66	185.90	53.70	106	117.90	29.80
27	19.30	24.90	67	94.40	46.80	107	15.30	18.50
28	126.30	32.00	68	180.80	18.30	108	12.10	12.00
29	23.30	44.00	69	26.50	13.70	109	10.10	28.90
30	80.80	37.90	70	183.10	**96.10**	110	9.90	25.50
31	64.20	29.50	71	97.90	24.50	111	42.40	**11.40**
32	87.30	32.10	72	170.50	25.20	112	10.80	15.20
33	10.60	39.30	73	66.90	17.70	113	9.60	27.40
34	31.10	35.70	74	9.30	20.80	114	13.90	21.90
35	11.10	27.60	75	196.60	31.50	115	114.00	21.80
36	14.00	20.90	76	9.30	20.80	116	31.70	53.00
37	10.50	23.40	77	9.40	15.20	117	9.30	50.50
38	14.20	17.70	78	9.70	61.20	118	11.30	17.40
39	26.60	39.10	79	111.10	29.30	119	125.00	19.10
40	19.70	39.40	80	118.70	27.40	120	18.70	31.60

Conclusion and summary

The study was carried out to evaluate F3 populations (HKR 47 x BR 4-10) derived from crosses between iron rich (BR 4-10) and high yielding indica rice variety (HKR 47) for various agronomic traits and mineral content. Phenotypic correlation coefficient was calculated to assess the association between various traits. Additionally, 8 BC2 plants [(HKR 47 x BR 4-10) x HKR 47] were evaluated for various agronomic traits and mineral content.

•Field evaluation of F3 population (HKR 47 x BR 4-10) showed large variation for days to 50% flowering (85-109), plant height (72.6-137.4 cm), effective number of tillers/plant (8-14), panicle length (13.35-30.59 cm), grain yield/ plant (9.84-24.51 g), grains/panicle (68-161), graincolour (1-4), days to maturity (129-134 days), 1000 - grain weight (l7.l4-27.93 g) and grain L/B ratio (2.70-4.41). In the F3 population, grain yield per plant showed significant positive correlation with plant height, effective number of tillers/plant and panicle length.

•F3 population displayed large variation in grain iron content ranging from 9.0-188.40 µg/g. (HKR 47, 54.3 µg/g and BR 4-10, 176.2 µg/g).

• Zinc content also varied significantly in F3 population from 11.40-96.10 µg/g (HKR 47, 25.4 µg/g and BR 4-10, 23.2µg/g). Notably, four of HKR 47x BR 4-10 F3 plants had higher (>50 µg/g) zinc content than the parental rice genotypes, plant number 64, 68, 70 and 71 with high zinc content. Thus, transgressive segregation for grain iron & zinc contents was apparently visible in F3 individuals of the cross.

•In the F3 population, grain iron content showed significant positive correlation (r=O.523 and 0.299) with grain zinc content. Apparently, no significant correlation was observed between grain iron and zinc contents and grain yield/plant in the F3 population. The 1000- grain weight showed positive correlation with grain iron content and negative correlation with grain zinc content. Thus, it should be plausible to improve Fe and Zn levels simultaneously in rice grain through plant breeding.

•Eight BC2 (HKR 47 x BR4-10) of plants also differed significantly for iron (69.4 - 86.1 µg/g) and zinc contents (21.5 -95.6 µg/g). The average iron content was maximum in BC2 plants (75.9 µg/g), followed by HKR 47 x BR 4-10 population (54.3µg/g). Conspicuously, the average zinc content was also maximum in BC2 plants (59.2-µg/g), followed by HKR 47 x BR 4-10 F3 (25.4µg/g) population.

•It is surprising that F3 population derived from cross between the two parental rice genotypes, HKR 47 (Zn, 25.4 µg/g) and BR 4-10 (Zn, 23.2 µg/g), which did not differ much for grain zinc content, displayed huge variation (11.40-96.10 µg/g) for grain zinc content. It could be possible that some favorable combinations of genes/QTLs from two rice genotypes have been generated via segregation and recombination during meiosis promoting accumulation of both iron and zinc in grain.

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