

Evaluation of Genetic Variability, Heritability and Genetic Advance for High Zinc in Grain in F₃ Population of Rice (*Oryza sativa* L.) under Aerobic Condition

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ABSTRACT

A study was carried out using progeny from a cross between 'Gopaldoddiga' and 'ARB6' cultivars. Analysis of variance for the traits evaluated showed highly significant differences ($P < 0.001$) among the genotypes for all traits. Higher phenotypic and genetic coefficients of variation for all the characters except brown rice zinc, and high heritability coupled with high genetic advance for most of the traits indicates strong additive genetic control of these traits. Zinc content and grain yield were high for hybrid progenies namely, line GA 214-132 and line GA 214-217 and therefore can be recommended for planting under suitable aerobic conditions. These zinc enriched high yielding lines can be effectively utilized in rice biofortification programs across the globe.

Keywords: Biofortification, GCV, PCV, zinc, rice (*Oryza sativa* L.)

RICE (*Oryza sativa* L.), one of the most important cereal grains in the world today, serves as a staple food source for more than half of the world's population, especially for people in Asia (Gross and Zhao, 2014). Rice nutrient content varies depending on the cultivar and production conditions in addition to the processing method (Rohman *et al.*, 2014).

Mukamuhirwa (2016) reported that rice contains mainly carbohydrates and contributes 20 per cent of the world's dietary energy. Calpe (2006) found that 100 g of raw white rice provides 361 kcal of energy and 6 g of proteins. Good proportions of thiamine, riboflavin, niacin and dietary fibers are found in rice. Some studies revealed that whole grains, especially from red and black rice are rich in polyphenols and insoluble fibers with nutraceutical and antioxidant benefits (Shao and Bao, 2015 and Zhang *et al.*, 2015) that play a role in preventing colorectal and intestinal cancer. Rice is considered as a potential food vehicle for the fortification of micronutrients since it rice is an essential part of daily diet for many. A study in Bangladeshi children and their care givers showed that rice was the main source of zinc intake, providing 49 per cent of dietary zinc to children and 69 per cent to women (Arsenault *et al.*, 2010). However, commercial varieties of rice are a poor source of essential micronutrients such as zinc (Zn) (Bouis and Welch, 2010).

In 2018, Harvest Plus reported Zn to be involved in more body functions than any other mineral. Zn is essential for survival normal growth and development, maintenance of body tissues, vision, immune system and functioning of more than 300 enzymes. Zn deficiency is reported to be associated with abnormal labor and fetal abnormalities in pregnant women, diarrhea, acute lower respiratory infections and cognitive capacity in children and delayed sexual maturity in adolescents. Harvest Plus (2018) estimated that over 17 per cent of the global population are at a risk of inadequate zinc intake and is as high as 25 per cent in Africa and 29 per cent in South Asia.

'Biologically fortified' or 'biofortified' refers to crops that have been nutritionally enhanced using agronomic practices, conventional breeding method and genetic modification (Garcia-Casal *et al.*, 2016). A fast and easy solution to prevent zinc deficiency in plants is through supplementation with fertilizers (Cakmak, 2008). However this method is limited by the costs involved in fertilizer applications and reduced availability of Zn to plants due to physical and chemical characteristics of soil. A promising and cost-effective alternative strategy is improving plant zinc use efficiency and grain zinc content by plant breeding or genetic engineering approaches (Henriques *et al.*, 2012). However, deregulation of genetically modified (GM) products for cultivation

is still a major challenge (Swamy *et al.*, 2016). Breeding efforts could increase the Zn level by 6–8 mg kg⁻¹ (Harvest Plus, 2014). Significant positive heterosis for grain Zn has been reported. In a line × tester analysis involving six lines and eight testers and a total of 48 hybrids, it was interesting to note that 14 out of 48 hybrids showed significant positive heterosis for grain Zn over the standard micronutrient check variety Chittimutyalu. Two crosses (PR116 × Chittimutyalu and Mandya Vijay × Jalamagna) showed more than 50 per cent heterosis for grain Zn (Babu *et al.*, 2012). Transgressive segregants were also observed for grain Zn (Stangoulis *et al.*, 2007).

The genetic biofortification strategy uses plant breeding techniques to produce staple food crops with higher micronutrient levels (Harvest Plus 2014). The world's first Zn enriched rice variety was released in 2013 by the Bangladesh Rice Research Institute (BRRI dhan 62), which is claimed to contain 20–22 mg Zn kg⁻¹ of brown rice. Nonetheless, this is short of the target of 30 mg Zn kg⁻¹ set by the Harvest Plus program (Shahzad *et al.*, 2014). With the aim of further enrichment of Zn in commercial cultivars, present study was undertaken to evaluate genetic variability, heritability and genetic advance for high zinc in grain in F₃ population of rice under aerobic condition.

MATERIAL AND METHODS

The experiment was carried out during *kharif* 2015 using augmented design (Federer, 1961) under aerobic condition at the field experimental plot of Department of Plant Biotechnology, University of Agricultural Sciences, GKVK, Bangalore. Ninety seven lines of F₃ population derived from *Gopaldoddiga* × ARB6 were sown in 10 blocks using *Gopaldoddiga*, ARB6, AM 143 and AM65 as checks under aerobic condition.

In all the plots, observations were recorded for days to 50 per cent flowering, days to maturity, plant height (cm), number of tillers per plant, number of productive tillers per plant, panicle length (cm) and grain yield per plant (g) at appropriate stages of the crop. Biomass of plant (g), harvest index (%), 100 grains weight (g) and brown rice Zn content (mg kg⁻¹) were recorded after harvest.

Grains of individual lines were harvested manually and hand threshed to avoid any contamination. The grains (0.5 g) were then manually dehusked. Unbroken, uniform grains were then washed in diluted hydrochloric acid followed by washing with double distilled water to remove any surface contaminants and dried in hot air oven at 70 °C for 72 hours. The Zn content in these grains was estimated using X-ray fluorescence (XRF) (Paltridge *et al.*, 2012). Five grams of brown rice from each plant was subjected to XRF and content in mg kg⁻¹ was calculated. The experimental data was compiled by taking mean values of three replications for each genotype.

The analysis of variance for different characters was carried out as suggested by Rana *et al.* (1991). Both phenotypic and genotypic co-efficient of variability (%) for all traits were estimated using the formulae of Burton and De Vane (1953). Heritability (%) and genetic advance (%) were worked out as per the method outlined by Hanson *et al.* (1956).

RESULTS AND DISCUSSION

Analysis of Variance (ANOVA)

Analysis of variance indicated highly significant differences among the genotypes for all the characters studied ($P < 0.001$) (Table I), suggesting genetic causes for the observed differences between various segregants. The effect of experimental blocks was insignificant for all traits (Table I), which suggested that environmental conditions were largely uniform in all 10 blocks. The performance of checks or F₃ population was influenced by two factors: the genetic properties it carries and the environment where it is cultivated; if the environment is uniform, the plant character will be influenced only by the genetic properties. Similar findings were reported earlier in rice by Bekele *et al.* (2013); Rashid *et al.* (2017) and Barokah *et al.* (2018).

Genetic variability parameters

Higher estimates (>15%) of GCV and PCV were observed for grain yield per plant, number of productive tillers, total number of tillers, harvest index, biomass per plant and 100 grains weight (Table II and Fig. 1). GCV and PCV estimates were moderate

TABLE I
Analysis of variance (mean sum of squares) for 10 different characters in F₃ population of Gopaldoddiga X ARB6 in kharif-2015

Source of Variation	Df	Mean Sum of Square				
		Day to 50 per cent Flowering	Plant Height	Total Number of Tillers	Number of Productive Tillers	Panicle Length
Blocks (eliminating check + var)	9	1.85	12.96	3.29	4.39	3.07
Progenies + Checks	103	208.14 ***	344.32 ***	114.07 ***	101.74 ***	23.26 ***
Checks	3	2154.00 ***	7213.78 ***	442.30 ***	397.29 ***	334.19 ***
Progenies	99	134.23 ***	111.10 ***	83.25 ***	75.96 ***	13.01 ***
Checks vs. Progenies	1	1465.65 ***	2124.92 ***	2088.86 ***	1689.63 ***	74.40 ***
Error	27	2.33	10.83	2.25	2.16	2.69

Source of Variation	Df	Mean Sum of Square				
		Biomass per plant	Grain yield per plant	Harvest index	100 grains weight	Brown rice zinc
Blocks (eliminating check + var)	9	3.16	1.48	0.002	0.04	2.60
Progenies + Checks	103	135.16 ***	147.18 ***	0.06 ***	0.32 ***	31.02 ***
Checks	3	1957.46 ***	347.79 ***	0.07 ***	1.06 ***	146.60 ***
Progenies	99	79.62 ***	98.16 ***	0.03 ***	0.26 ***	22.59 ***
Checks vs. Progenies	1	0.22	4251.41 ***	2.86 ***	4.45 ***	493.35 ***
Error	27	3.86	0.94	0.001	0.02	1.68

* Significant at 5%; ** Significant at 1%; *** Significant at 0.1%; Df: Degrees of freedom.

TABLE II
Estimate of genetic parameters for different traits in F₃ population of Gopaldoddiga X ARB6 in Kharif-2015

Plant characters	Min.	Max.	Mean ± S.E	GCV(%)	PCV(%)	h ² (%)	GAM(%)
Day to 50% flowering	70.00	107.00	87.21 ± 1.18	11.45	11.58	97.71	23.32
Plant height (cm)	75.00	116.00	89.27 ± 1.07	9.75	10.43	87.50	18.79
Total number of tillers	8.00	39.00	25.94 ± 0.93	30.17	30.72	96.46	78.22
Number of productive tillers	7.00	38.00	24.30 ± 0.88	30.74	31.33	96.27	62.13
Panicle length (cm)	15.00	28.67	22.27 ± 0.37	12.54	14.54	74.37	22.27
Biomass per plant (g)	19.30	59.40	37.83 ± 0.91	20.01	20.67	93.68	39.89
Grain yield per plant(g)	8.40	40.10	22.60 ± 0.88	32.63	32.84	98.73	66.79
Harvest index (%)	23.23	79.42	59.01 ± 0.02	22.46	23.16	94.08	44.88
100 grains weight (g)	1.25	3.63	2.65 ± 0.05	15.78	16.78	88.49	30.58
Brown rice zinc (mg kg ⁻¹)	20.10	37.60	28.32 ± 0.48	14.04	14.76	90.37	27.49

* Significant at 5%; PCV = Phenotypic Coefficient of variation; GCV= Genotypic Coefficient of variation; h² % = Heritability percentage in broad sense; GAM: Genetic advancement as percentage of mean.

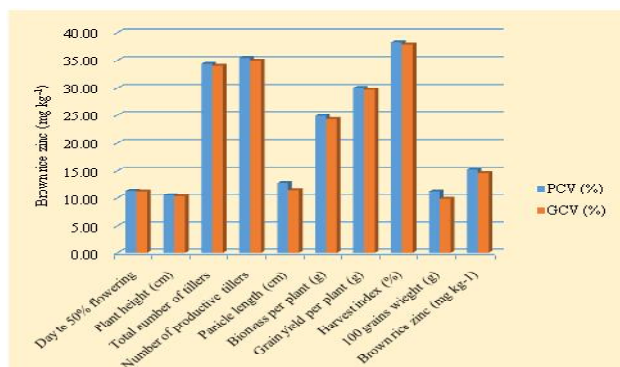


Fig. 1 : Graphical representation of phenotypic (PCV) and genotypic (GCV) coefficients of variation

(<15%) for brown rice zinc, panicle length, day to 50 per cent flowering and plant height. PCV values were only slightly higher than GCV values under investigation for all the traits suggesting that these traits were under the strong influence of genetic control and less influence of the environment. Thus individual plant selection can be practiced for these characters. Similar results were reported in rice by Bisne *et al.* (2009); Akinwale *et al.* (2011); Govintharaj *et al.* (2016); Revathi *et al.* (2016);

Yugandhar *et al.* (2017); Prasad *et al.* (2017); Abebe *et al.* (2017); Rajpoot *et al.* (2017); Ajmera *et al.* (2017); Nandeshwar *et al.* (2010); Shet *et al.* (2012); Kiran *et al.* (2013); Bekele *et al.* (2013); Tuhina-Khatun *et al.* (2015) and Mamata *et al.* (2018).

Moderate PCV and GCV values were observed for grain zinc content and are in agreement with previous reports; Purusothaman *et al.* (2010); Samak *et al.* (2011); Shashidhara *et al.* (2013), Sala *et al.* (2014), Anjali (2017), Ajmera *et al.* (2017); Madhubabu *et al.* (2017) and Shashidhara *et al.* (2017).

Heritability and Genetic Advance

The estimates of heritability act as predictive instrument in expressing the reliability of phenotypic selection, aiding in effective selection for a particular character. In this study, high estimates of broad sense heritability along with high genetic advance (expressed as per cent of mean) was observed for days to 50 per cent flowering, total number of tillers, number of productive tillers, panicle length, biomass

TABLE III
Transgressant lines selected from F_3 population of Gopaldoddiga X ARB6 based on grain zinc content, grown in kharif-2015

Superior plants	Day to 50 % flowering	Plant height (cm)	Total number of tillers	Number of productive tillers	Panicle length (cm)	Biomass per plant (g)	Grain yield per plant (g)	Harvest index (%)	100 grains weight (g)	Brown rice zinc (mg kg ⁻¹)
GA 240-450	99	78.2	34	31	21.67	30.2	8.4	27.81	2.95	37.6
GA 247-93	98	79	32	31	21	38.2	14.4	37.70	2.15	37.4
GA 214-132	78	93.6	20	18	18.33	41.6	23.9	57.45	2.96	37.1
GA 247-280	105	75	39	35	26	43	26.5	61.63	2.36	37.1
GA 191-209	73	104	16	14	27	40.1	30.7	76.56	3.21	36.7
GA 214-217	80	91.8	21	20	19.33	46.8	30.7	65.60	2.84	36.6
GA 254-9	96	80.4	32	30	20.67	42.2	24.6	58.29	2.3	36.6
GA 254-173	102	82.4	38	35	28.67	31.5	20.6	65.40	2.51	36.6
GA 254-19	100	77.2	36	32	23.33	43.5	28.7	65.98	2.84	36.6
GA 287-63	71	102.4	9	9	18.33	41.5	22.4	53.98	3.36	36.2
Gopaldoddiga (Parent-1)	72.5	101.1	8.3	8	24.12	26.26	6.74	25.67	2.72	29.71
ARB 6 (Parent-2)	99.7	80.6	21.2	20.1	18.68	27.84	12.54	45.04	1.96	22.3
AM 143 (Check-1)	102.1	75.4	23.3	22.3	20.88	41.23	16.07	38.98	2.12	21.12
AM 65 (Check-2)	103.3	134.62	16.6	15.9	31.89	56.32	20.73	36.81	2.23	23.47

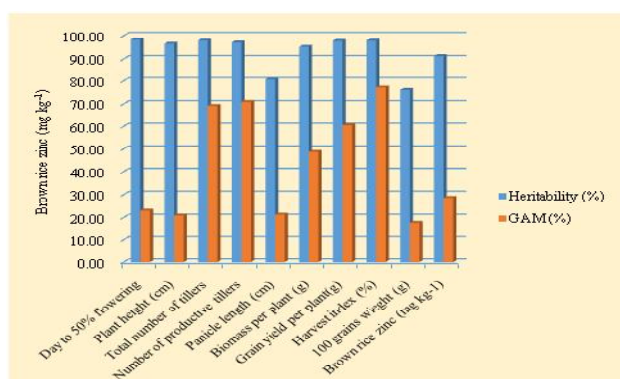


Fig. 2 : Graphical representation of heritability and genetic advancement as percentage of mean (GAM)

per plant, grain yield per plant, harvest index, 100 grains weight and brown rice zinc (Table II and Fig. 2). This indicates the presence of strong additive gene effects and potential for genetic improvement of these traits in future breeding programmes. From the results of the present study, it can be concluded that single plant selection could be effectively practiced as environment does not have any significant influence in the variation of these traits. High heritability and genetic advance as per cent of mean was earlier reported in rice by Babu *et al.* (2012), Bekele *et al.* (2013), Sadimantara *et al.* (2014); Soman *et al.* (2015); Limbani *et al.* (2017); Yadav *et al.* (2017); Sumanth *et al.* (2017) and Shamim *et al.* (2017).

Selected superior segregants in F₃ segregating generations

Ten progenies with high zinc content in grain, yield and yield *component* traits such as day to 50 per cent flowering, plant height, total number of tillers, number of productive tillers, panicle length, biomass per plant, grain yield per plant, harvest index and 100 grains weight, were selected from F₃ segregating populations (Table III). From the selection it was observed that high yielding progenies have higher brown rice zinc content per plant. As these progenies were still segregating, more generations need to be tested before releasing for multi-location trial.

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