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Evaluation of Elite Rice Accessions for Genetic Variability and Identification of Associated Markers for Iron and Zinc Content under Aerobic Condition

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Abstract

The objective of the present study was to evaluate genetic variability parameter and discern correlation for Iron (Fe) and Zinc (Zn) content in grains of 45 RPHP rice accessions grown in *Kharif*-2012 under aerobic condition. The study showed that grain yield per plant, culm biomass and Fe content showed higher genotypic and phenotypic co-efficient of variation. Significant positive correlation was observed for plant height with biomass of culm (r = 0.29), grain yield per plant (r= 0.41) and Fe content (r = 0.42). Number of tiller showed positive correlation with biomass of culm (r = 0.48) and grain yield per plant (0.49). Single marker analysis using 19 SSR markers showed that RM1089 and RM144 were associated with grain Feand Zn content respectively. The genetic information generated and molecular markers identified from this study may be used for future biofortification programme of rice.

Key words : **Aerobic rice, Biofortification, Micronutrient,SSR marker**

Introduction

Rice (*Oryza sativa* L.) is the most important cereal crop for more than half of the world's population and cultivated over an area of 150 million hectares.However, rice is a poor source of micronutrients such as Fe and Zn(Bouis and Welch, 2010). Micronutrients deficiency (hidden hunger) is a global health problem contributing to world's malnutrition which leads to high rate of mortality in women and children. It is estimated that more than 3.5 billion people in the world are deficient in vitamin A, Iodine (I), Fe and Zn (Pfeiffer and McClafferty, 2007).Fe and Zn are essential nutrients for increasing the immunity and it acts as cofactor for more than 300 enzymes. Fe is also essential for various cellular events in plants, such as photosynthetic electron transport, chlorophyll biosynthesis, respiration, (Bashir *et al.*, 2013).Zn is a vital micronutrient for all organisms, which plays important role in many reactions of the cellular metabolism and biological processes like carbohydrate metabolism,antioxidativedefense, auxin metabolism, protein synthesis, and stability of genetic materials (Broadley*et al.*,2007).To address these issues, Fe and Zn food fortification and supplementation approaches are beneficial, but these practices are not easy to implement in developing countries due to its higher cost(Howarth and Ross, 2010;Pfeiffer and McClafferty, 2007). Therefore, an alternative approach called "Fe and Zn biofortification" has been developed to enhance the nutrient content in staple food crops to reach resource poor rural society.

Most research in plant breeding was concentrated on breeding for high yields, resistance to biotic factor and tolerance to abiotic stresses. Recently, the trend has changed to incorporate desired quality parametersthrough 'breeding by design'in existing germplasm(Peleman and Roupppe van der Voort, 2003). The genetic basis of accumulation of micronutrients in the grains and mapping of the quantitative trait loci (QTL) will rapid the process of improving grain micronutrient content through marker-assisted selection and biotechnological approaches.Realizing the importance of biofortification, several studies have been conducted for the evaluation of germplasm and advance breeding lines for grain Fe and Zn content(Lu *et al.*, 2008). Ahmed *et al.* (1998) found a considerable variation in chemical composition of rice cultivars and Fe content varies from 1.5 to 5.0 mg 100 g^{-1} with an average value of 3.2 mg 100 g^{-1}.Nagarathana*et al.* (2010) reported the presence of wide genetic variability for rice grain Zn content which ranges from 8.4 to 50 ppm.

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As a trait, Fe and Zn content is a complex trait governed by a large number of genes (QTLs) located on different chromosomes with different phenotyping effects (Zhang *et al*.,2011; Banerjee *et al*., 2010; Lu *et al*., 2008). Various researchers have identified a number of QTLs for rice grain Fe and Zn content using different genetic backgrounds.Avendano(2000)identified a locus for Zn content in rice grains on chromosomes 5, located between *OSR*35 and RM267 in a study with RILs derived from IR26 and Modhukar. Lu *et al*.(2008)identified QTLs controlling Copper (Cu), Calcium (Ca), Zn, Manganese (Mn) and Fe content in rice grains. Garcia-Oliveira et al. [13] identified three QTLs (qZN-5, qZN-8 and qZN-12) for grain Zn concentration using 179 SSR markers. Thus considering the importance of single marker analysis approach, the present study has been applied to 45 RPHP rice accessions with 19 SSR markers to identify tightly linked marker associated with Fe and Zn content in rice grains.

Materials and Methods

Experimental Design

The experiment was carried out during *Kharif*- 2012 at aerobic rice research laboratory of department of Plant Biotechnology, UAS, GKVK, Bangalore. The study included samples collected from 10 locations over India by Directorate of Rice Research (DRR), Hyderabad, for high Fe and Zn Content, under the project name Rice Project Harvest Plus(RPHP). The experiment was laid out in augmented experimental designs which consist of 45 rice genotypes and two check varieties (Azzucena and Kalinga-3) **(Table 1)**.Seeds were sown directly in field by using standard agronomical practices. The data were recorded from five plants at random from middle rows for Plant height at maturity, Number of tillers per plant, Culm Biomass per plant (g), Grain yield per plant (g) and Grain Fe and Zn content (PPM).

Sample preparation and estimation of Fe and Zn content

Fe and Zn content of grain samples were estimated by Atomic Absorption Spectrophotometer (AAS). One gram of seed was taken and powdered it in the grinder (non metallic grinder). Powdered seed sample was digested in tri-acids (HNO_3+HCl+H_2SO_4) mixture (10:4:1) in micro-oven digester. The digested sample was cooled for 30 minutes and the volume was made up to 50ml with double distilled water. Then a known quantity of aliquot was used for subsequent analysis. Fe and Zn content were estimated in the aliquot of seed extract by using AAS at 213.86 nm for Zn and 248.33 nm for Fe.

Statistical analysis

The analysis of variance for all the qualitative traits was computed with AUGMENT 1 computer programme. Genetic parameters and correlation studies were analysed with GENRES statistical software package.

Genetic variability parameter and correlation studies

The extent of variability for any character is very important for the improvement of a crop through breeding. Genotypic and phenotypic component of variance were estimated by using the formulae specified by Cochran and Cox (1957). The phenotypic and genotypic variances were estimated according to Burton and Devane (1953).

Estimate of heritability and genetic advance as percent of mean

Broad sense heritability (H^2) and genetic advance (GA%) was carried out as given by Hansen *et al*. (1956) and Johnson *et al*. (1955).

Molecular analysis of RPHP rice accessions using SSR markers

DNA isolation was done by Cetyltrimethyl Ammoniac Bromide (CTAB) method from 21 day old leaves (Doyle and Doyle, 1990).The polymerase chain reaction (PCR) for SSR markers was performed in a total volume of 20 µl containing 1X PCR buffer (contains 10 mM Tris-HCl, pH 8.0 at 25^{0}C, 50 mM KCl, 1.5 mM $MgCl_2$), 0.25 µM of each forward and reverse primers, 50 ng rice genomic DNA, 0.5 mM dNTPs mix and 1.2 units of Taq polymerase (Bangalore Genei, India).The amplified product was resolved electrophoretically on a 2.5% agarose gel for 2-3 hrs, visualized under UV trans-illuminator and documented. Single marker analysis (SMA)was performed with SPSS16.0 (SPSS Inc.).

Results and discussion

Analysis of variance

In the present study, analysis of variance revealed significant differences existed among the rice genotypes for all the traits in a study in the season *Kharif* 2012.

Fe and Zn content in grains

RPHP accessions were cultivated under aerobic condition, which is a novel approach developed by crossing of lowland rice varieties with upland varieties to grow in rainfed, under non-flooded conditions (Bouman*et al*., 2007; Shashidhar, 2007). Atomic absorption spectrometry showed wide variation in grain Fe and Zn content among RPHP rice accessions. The Fe content in grains varies from 14.8 PPM (RPHP156) to 36.1 PPM (RPHP33) with an average value 25.71 PPM. Whereas, the Zn content varies from 27.45 PPM (RPHP53) to 43.90 PPM (RPHP16) with an average value 35.80 PPM under aerobic condition. The range of variation for grain yield from of 0.2g (RPHP42) to 16.40g (RPHP36) with an average yield of 5.1g per plant.

The parental lines used by Anuradha*et al*. (2012) had grain Zn concentration of 53.7 ppm (Madhukar) and 27.2 ppm (Swarna) and the RIL population generated from this cross showed Fe concentration in the range of 0.2 to 224 ppm and Zn in the range of 0.4 to 104 ppm. The study conducted by Bekele*et al*. (2013) on the estimation of genetic variability and correlation studies for grain Zn concentration in 64 rice genotypes revealed the range of variation for grain Zn concentration as 18.90 ppm to 36.90 ppm with an average value of 26.74 ppm. Wide variation was observed in Fe concentration among the population than Zn at both locations and similar results were obtained earlier (Anuradha*et al.*, 2012; Pandian *et al.*, 2011). There could be several reasons for these variations that may include effect of environment, genotype and environment interactions [14], soil properties like pH, organic matter content, Fe and Zn levels in the soil etc. (Pandian *et al.*, 2011; Chandel*et al.*,2010).

Genetic variability parameter and correlation analysis

Genetic variability parameters of RPHP accessions for all traits represented in Table 2. PCV and GCV were highest(>21%) for grain yield per plant (PCV=55.08, GCV=52.83), culm biomass per plant (PCV=49.14, GCV=51.05), number of tillers (PCV=24.68, GCV=31.00) and for plant height (PCV=23.94). Moderate value (10-20%) of PCV observed only for Zn content (PCV=14.34) and GCV for plant height (GCV=20.59), Fe content (GCV=19.70) and Zn content (GCV=13.40). The PCV and GCV were highest for plant height, number of tillers per plant, biomass of culm and grain yield per plant. These observations suggest that these characters were under the relatively greater influence of genetic factors and relatively less influenced by the environment. Hence, these characters can be relied upon and simple selection can be practiced for further improvement. These results were in conformity with Bisne*et al.* (2009)for grain yield per plant; Samak*et al.*(2011) for grain zinc content. High estimates of GCV and PCV was also reported for number of tillers per plant (Bisne*et al.*, 2009). High heritability associated with high genetic advances as percent mean were observed for almost all the traits plant height(H^2=73.96, GAM=31.38), number of tillers (H^2=63.38, GAM=32.05), culm biomass(H^2=92.65, GAM=93.75), grain yield (H^2=92.01, GAM=99.96), Fe (H^2=80.71, GAM=32.71and Zn (H^2=87.35, GAM=23.98) content(Table2). High heritability (>60%) coupled with high genetic advance as percent mean (>20%) were recorded for plant height, number of tillers per plant, biomass of culm, grain yield per plant, grain iron content and grain zinc content. This indicates that these traits were not much influenced by environmental factors. Hence, these traits are mostly controlled by additive and/or additive × additive gene interactions and expected to respond to direct selection for improvement. This observation is in accordance with Kumar *et al.* (2009) for plant height; Suman*et al.* (2006)for number of tillers per plant, productive tillers per plant and grain yield per plant;Akinwale*et al.* (2011)for grain yield per plant; Govindaraj*et al.* (2011)for grain Zn content and number of productive tillers.

Correlation coefficients

To study the effect of the environment on grain Fe and Zn content, correlation coefficient (r) was determined for all the data sets**.** Plant height showed highly significant positive correlation with biomass of culm (r = 0.30), grain yield per plant (r = 0.42) and Fe content (r = 0.43). The number of tillers showed highly significant positive correlation with biomass of culm (r = 0.44) and grain yield per plant (r = 0.50). The biomass of culm showed significant positive correlation with grain yield per plant (r = 0.56), whereas positive correlation with Fe content (r = 0.15) and Zn content in rice grains (r = 0.25) **(Table3)**. Highly significant and positive correlation was observed for plant height with grain yield per plant and iron content in rice grain. This was consistent with the reports of Rajeswari and Nadarajan(2004).Number of tillers showed highly positive significant correlation with biomass of culm and grain yield per plant. This result was in conformity with Nagarathna*et al.* (2010)and Nagesh*et al.* (2012). Hence, breeding for high grain Zncontent and enhancement of grain yield per plant has to be designed separately.

Molecular analysis of rice accession with SSR markers

Fe and Zn accumulation in rice grains is a complex trait which is governed by number of QTLs located on different chromosomes (Lu et al., 2008; Garcia-Olivera et al., 2009; Zhang et al., 2011). Out of 19 SSR markers, 9 SSR markers were found polymorphic for the RPHP lines**(Table 4)**. The segregation pattern of all RPHP rice accession with a represented primer RM144 is depicted in fig.1. The range of phenotypic variance (R^2 %) for these 9 SSR markers was 0.2 to 22.0%. Among these 9 markers, RM1089 and RM144 werefound to be associated with Fe and Zn content in grains respectively.RM1089 which is associated with Fe content,is located on chromosome 5, with phenotyping variation of 15.3%. In earlier reports, Poli*et al.* (2013)reported that RM1089 was associated with number of tillers and yield/plant. Cho *et al.* (2012)identified RM1089 flanked a QTL for culm length and days to flowering. RM144 is located on chromosome 11, exhibits association with Zn content by showing 22% of phenotyping variation under aerobic condition. RM144 was previously reported to be associated with plant height and yield per plant (Brar*et al.*, 2015). Thus, these markers can be used for marker-assisted selection or genomic assisted breeding for Fe and Zn biofortification programme for future scrutiny.

Conclusion

In the present study, 45 RPHP lines obtained from different location of India were evaluated for grain Fe, Zn content and other grain yield related traits, grown during *Kharif* 2012. Assessment of the relationship between grain Fe and Zn content and grain yield per plant in 45 rice accession using linear regression showed a linear relationship between Fe and Zn content with increase in grain yield. Using single-marker analysis in RPHP rice varieties, it was found that one of the SSR marker (RM144) showed significant association with grain Zn content while another SSR marker (RM1086) showed significant association with grain Fe content.

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Author contribution

RakeshK. Prajapat had designed and executed the experimental procedures and recorded the biometric parameters. The statisticalanalysis of the obtained results was done by Deepak Pawar. Shashidhar.H.E and VijayakumarSwamy were the Principal Investigator and Co-Principal Investigator respectively, who helped in monitoring the work and conducting the experiments smoothly.

Table 1 . : Iron and Zinc content of selected 47 RPHP rice accession under aerobic condition

Sl. No.	DRR code	Variety name / Cross	Iron content in rice grains (ppm)	Zinc content in rice grains (ppm)
1	RPHP-7	AM-72	29.3	42.4
2	RPHP-9	BJ-23	33.4	41.15
3	RPHP-10	AM-27	29.8	40.95
4	RPHP-11	AM-143	24.3	43.75
5	RPHP-16	AM-65	33.8	43.9
6	RPHP-21	AM-94B	27.2	42.35
7	RPHP-27	AZUCENA	32	40.15
8	RPHP-29	BJ-5	32.3	36.05
9	RPHP-33	BJ-21	36.1	41.1
10	RPHP-36	TKM-9	21.5	37.25
11	RPHP-37	Mainpuri	31	32.35
12	RPHP-42	Shalimar Rice -1	25.7	30.9
13	RPHP-44	BR- 2655	19.3	28.15
14	RPHP-45	Panvel -3	24.1	37.95
15	RPHP-47	Pathara	22.5	31.15
16	RPHP-48	Bindli	28.2	35.6
17	RPHP-51	Vandana	25.4	42
18	RPHP-52	Sebati	26	39.95
19	RPHP-53	PB-164	24.7	27.45
20	RPHP-55	Kalinga -3	28.2	36.75
21	RPHP-56	IRRI-44	27.8	29.3
22	RPHP-68	Subhdra	20.9	34.05
23	RPHP-80	24(K)	29.6	41.55
24	RPHP-87	140(M)	25.5	31.3
25	RPHP-90	182(M)	25.1	36.85
26	RPHP-91	185(M)	30	36.95
27	RPHP-92	196(M)	26.3	34.95
28	RPHP-93	Type-3	32.4	34.55
29	RPHP-102	Kanchana	16.1	41
30	RPHP-104	Kasturi	24.4	33.5
31	RPHP-105	Moirangphou	24.3	28.85
32	RPHP-106	Akutphou	23.8	34.65
33	RPHP-107	Improved ChittiMutyalu	23.5	27.7
34	RPHP-108	High Iron Rice	28.9	31.25

Sl. No.	DRR code	Variety name / Cross	Iron content in rice grains (ppm)	Zinc content in rice grains (ppm)
35	RPHP-125	NDR 2026	20.1	40.5
36	RPHP-129	Kamad	28.1	29.4
37	RPHP-130	China 1007	25.7	35
38	RPHP-134	NJA VORA	17.3	35.3
39	RPHP-135	KADAMAKUDY POKKALI	17.5	32.3
40	RPHP-138	EDAVANKUDI POKKALI	22.2	41.6
41	RPHP-140	VYTILLA ANAKONDAN	19.3	34.45
42	RPHP-156(ABCDE)	BPT 5204 /ChittiMutyalu-SB	14.8	34.1
43	RPHP-157 (ABCDE)	BPT 5204 /ChittiMutyalu-MS	28	42.05
44	RPHP- 161	ChampaKhushi	31.5	31.8
45	RPHP-163	Seeta sail	24.7	38.45
46	RPHP -165	Tilakkachari	34.3	34.85
47	RPHP-166	NC 365	20.3	31.75

Table 2. : Genetic variability parameters for different quantitative traits among selected rice accessions

Character	Min	Max	Mean	GCV	PCV	H^2%	GAM
Plant Height (cm)	32.3	103	60.1	20.59	23.94	73.96	31.38
Number of Tillers	4.0	35.8	13.4	24.68	31.00	63.38	32.05
Culm Biomass (g)	2.8	47.2	17.4	49.14	51.05	92.65	93.75
Grain Yield (g)	0.2	16.4	5.1	52.83	55.08	92.01	99.96
Fe (PPM)	14.8	36.1	25.7	19.70	21.93	80.71	32.71
Zn (PPM)	27.45	43.90	35.8	13.40	14.34	87.35	23.98

GCV : Genotypic Coefficient of Variation, PCV: Phenotypic Coefficient of Variation, H^2%: Heritability percentage in Broad sense, GAM: Genetic Advance as Percent Mean, Fe: Grain Fe content (ppm), Zn: Grain Zn content (ppm)

Table 3. : Phenotypic correlation coefficient for all quantitative traits

	PHT	NT	BOC	GYP	Fe	Zn
PHT	1	-.196	.299*	.410**	.422**	.158
NT		1	.437**	.491**	-.095	-.003
BOC			1	.565**	.154	.250
GYP				1	.233	.154
Fe					1	.217
Zn						1

*,**=Correlation significant at the 5% and 1% level of significance respectively,

PTH = Plant Height (cm), NT = Number of Tillers, CBP = Biomass of Culm per plant (g), GYP = Grain Yield pre plant (g), Fe = Fe content in rice grain (PPM), Zn = Zinc content in rice grain (PPM).

Table 4. : SMA of polymorphic SSR marker used in RPHP for iron and zinc content in rice grains in *Kharif*-2012

Sr. No.	Marker	Chr. No.	Trait	P value	R^2 (%)
1	RM513	1	Iron	0.068	0.072
			Zinc	0.391	0.016
2	RM148	3	Iron	0.304	0.053
			Zinc	0.409	0.040
3	RM131	4	Iron	0.097	0.101
			Zinc	0.403	0.043
4	RM1089	5	Iron	0.026*	0.153
			Zinc	0.243	0.063
5	RM204	6	Iron	0.860	0.007
			Zinc	0.897	0.005
6	RM264	8	Iron	0.532	0.028
			Zinc	0.941	0.003
7	RM144	11	Iron	0.953	0.002
			Zinc	0.004*	0.220
8	RM167	11	Iron	0.800	0.010
			Zinc	0.379	0.044
9	RM3331	12	Iron	0.435	0.037
			Zinc	0.130	0.089

* Significant at 5%

References

Ahmed, S. A., I. Baraua, and D. Das. 1998. Chemical composition of scented rice. Oryza, 35(2), 167-169.

Akinwale, M. G., G. Gregorio, F. Nwilene, B. O. Akinyele, S. A. Ogunbayo and Odiyi. 2011. Heritability and correlation coefficient analysis for yield and its components in rice (*oryzasativa*L.). Afr. J. Plant sci., 5 (3): 207 - 212.

Anuradha K., A. Surekha, R. Y. Venkateswara, K. V. Rao, B. C. Viraktamath and N. Sarla. 2012. Mapping QTLs and candidate genes for iron and zinc concentrations in unpolished rice of madhukar x swarna RILs. Gene, 508: 233-240.

Bashir K., T. Nozoye, Y. Ishimaru, H. Nakanishi and N. K.Nishizawa.2013. Exploiting new tools for iron bio-fortification of rice. Biotechnology advances, 31:1624-1633.

Bekele B. D., S. Rakhi, G. K. Naveen, P. J. Kundur and H. E.Shashidhar. 2013. Estimation of genetic variability and correlation studies for grain zinc concentrations and yield related traits in selected rice (*oryzasativa* L.) genotypes. Asian j. Exp. Biol. Sci., 4: 345-351.

Bisne R., A. K. Sarawgi and S. B.Verulkar. 2009. Study of heritability, genetic advance and variability for yield contributing characters in rice. Bangladesh j. Agril. Res., 34 (2): 175-179.

Bouman B. A. M., R. M. Lampayan and T. P.Tuong. 2007. Water management in irrigated rice: coping with water scarcity. Int. Rice res. Inst., Los Banos, Philippines, isbn: 9789712202193, pages: 54.

Brar B., R. K. Jain and S. Jain. 2015. Correlation of molecular marker allele size with physio-morphological and micronutrient (Zn, Fe) traits among rice genotypes. Int. J. Curr. Sci., 15: e 42-50

Broadley M., P. J.White, J. P.Hammond, I. Zelko, A. Lux.2007. Zinc in plants. New phytol.,173: 677-702..

Burton G. K. and E. M. Devane. 1953. Estimating heritability in tall fescue (*festucaarundinaceae*) from replicated clonal material. Agronomy journal, 45: 478-481.

Chandel G., S. Banerjee, S. See, R. Meena, D. J.Sharma, S. B.Verulkar. 2010. Effects of different nitrogen fertilizer levels and native soil properties on rice grain fe, zn and protein contents. Rice sci., 17: 213-227.

Cho J. H., H. K.Upreti, Y. C.Song, W. G. Ha, S. P.Khatiwada, J. H.Lee, J. Y.Lee, U. S. Yeo, D. S. Park and H. W.Kang. 2012. QTLs analysis of agronomic traits based on cultivation of low and high altitude locations in rice (*oryzasativa* L.). Kor j breed sci., 44(2):110–120.

Cochran W. G. and G. M. Cox. 1957. Experimental designs. Wiley, New York.

Doyle J. J. and J. L.Doyle. 1990. A rapid total DNA preparation procedure for fresh plant tissue. Focus, 12: 13-15.

Gholipoor M., H. Zeinali and M. A. Rostami. 1998. Study of correlation between yield and some important agronomic traits using path analysis in rice. Iranian j. Agric. Sci., 29(3): 627 - 638.

Govindaraj M., B. Selvi, S. Rajarathinam and P. Sumathi, 2011. Genetic variability and heritability of grain yield components and grain mineral concentration in india's pearl millet (*Pennisetumglaucum* (L) R. Br.) Accessions.Afr. J. Food agric. Nut. Dev., 11(3): 4758 - 4771.

Hanson C. H., H. F.Robinson, R. E.Comstock, 1956. Biometrical studies of yield in segregating population of koreanhespidiza. Agronomy journal, 48:268-272.

Johnson R. W., H. F.Robinson, R. E.Comstock. 1955. Genotypic and phenotypic correlations in soybean and their implication in selection. Agronomy journal, 47: 477-483.

Kumar A. A., B. V. S. Reddy, B. Ramiah, P. S.Reddy, K. L. Sahrawatand Upadhyaya. 2009. Genetic variability and plant character association of grain Fe and Zn in selected core collection accessions of sorghum germplasm and breeding lines. Sat ejournal, 7: 1 - 4.

Lu K., L. Li, X. Zheng, Z. Zhang, T. Mou and Z. Hu. 2008. Quantitative trait loci controlling Cu, Ca, Zn, Mn and Fe content in rice grains. J. Genet., 87: 305–310.

Martinez C. P., J. Borrero, S. J.Carabali, Y. Sanabria, O. X. Giraldo, M. C. Duque, J. Silva, J. Barrona, and J. Tohme. 2007. Breeding strategies to increase the content of iron and zinc in the rice grain. Annual report. Executive summary, sba-4: rice, 2007.

Nagarathna T. K., A. G. Shankar and M. Udayakumar. 2010. Assessment of genetic variation in zinc acquisition and transport to seed in diversified germplasm lines of rice (*oryzasativa*L.). J. Agri. Technol., 6: 171 - 178.

Nagesh, V. Ravindrababu, G. Usharani and T. D. Reddy. 2012. Grain iron and zinc association studies in rice (*oryzasativa*L.) F1 progenies.Arch. Appl. Sci. Res., 4 (1): 696 – 702.

Pandian S. S., S. Robin, K. K. Vinod, S. Rajeswari, S. Manonmani, K. S. Subramanian, R. Saraswathi and A. P. M. Kirubhakaran. 2011. Influence of intrinsic soil factors on genotype-by-environment interactions governing micronutrient content of milled rice grains. Australian j. Crop sci., 5: 1737-1744

Peleman J. D., J. Roupppe Van der Voort. 2003. Breeding by design. Trends in plant science, 8: 330-334.

Pfeiffer W. H. and B.McClafferty. 2007. Harvestplus: breeding crops for better nutrition. Crop science, 47: s88-s105.

Poli, Y., R. K. **Basava**, M. **Panigrahy, V. P. Vinukonda,** N. R. **Dokula,** , **S. R. Voleti, S. Desiraju,** and S. **Neelamraju**.2013. Characterization of a nagina22 rice mutant for heat tolerance and mapping of yield traits. Rice, 6:36.

Rajeshwari S. and N. Nadarajan. 2004. Correlation between yield and yield components in rice (*oryzasativa* L.). Agric. Sci. Digest, 24: 280 - 282.

Samak N. R. A., S. Hittalmani, N. Shashidhar, and H. Biradar. 2011. Exploratory studies on genetic variability and genetic control for protein and micronutrient content in f4 and f5 generation of rice (*oryzasativa*L.). Asian j. Plant sci., 10: 376 - 379.

Shashidhar H. E. 2007, Aerobic rice: an efficient water management strategy for rice production. Food and water security in developing countries.Chapter 12. Pp. 131–139.

Suman A., N. Sreedhar and I. V. SubbaRao. 2006. Correlation and path analysis of yield and its components in rice (*oryzasativa* L.). Int. J. Trop. Agric., 24:49-53.

Zhang X., G. Zhang, L. Guo, H. Wang, D. Zeng, G. Dong, Q. Qian and D. Xue. 2011. Identification of quantitative trait loci for Cd and Zn concentrations of brown rice grown in cd-polluted soils. Euphytica, 180: 173 – 179.