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Assessment of Genetic Variability for Iron Content in Grain of Rice (*Oryza sativa* L.) Germplasm Accessions and Identification of Transgressant Lines

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Iron and zinc deficiencies are serious global health problems among the people dependent on staple crops like rice and maize. Enrichment of nutrients in these crops can be achieved by adopting biofortification methods. The objective of this experiment was to identify high iron containing lines in rice by developing new mapping populations. Three mapping populations were developed based on grain iron content and yield viz., IRRI38 X Jeerigesanna, BI33 X Jeerigesanna and Azucena X Moromutant after the two seasons screening of 960 germplasm accessions under aerobic and wetland condition. Iron and zinc concentrations were estimated from RILs and the values ranged from 4.45 to 76 mg/kg and 7.73 to 146 mg/kg, respectively. Thirty two high iron and zinc containing genotypes were identified for all India trial in *Kharif* 2011; these genotypes were evaluated for iron and zinc content. AM127 and Karidaddi were found to have high iron content while AM65 and AM143 were found to be have high zinc content.

Key Words: Iron, Perls' Prussian blue, 2, 2-dipyridyl, RILs aerobic

Introduction

Rice (*Oryza sativa* L.) is India's pre-eminent crop, and is the staple food for people in the eastern and southern parts of the country. India is the second largest producer of rice, after China, accounting for 20% of world rice production. It is estimated that more than 3.5 billion people in the world are deficient in vitamin A, iodine, iron and zinc (Virk *et al.*, 2007). According to WHO (2011) approximately 1.62 billion people suffer from iron deficiency (anemia) and more than half of world population suffers from zinc deficiency.

Iron is a vital mineral required for all living organisms for their well being. But, iron deficiency is found to be the most prevalent nutritional deficiency worldwide. It is a major public health problem with adverse consequences (anemia) particularly among the women of reproductive age and for young children.

Mineral malnutrition is now considered to be one of the most serious global challenges to humankind and is unavoidable. This can be addressed by mineral supplementation, dietary diversification and food fortification. The first two approaches are not easy to adopt in developing countries where majority of people depend on a staple food like rice to suppress hunger. Hence crop biofortification is advocated. This method is cost effective, sustainable and will balance the micronutrient daily requirement in people of rural and urban areas where micronutrient malnutrition is prevalent.

Prussian blue staining is a rapid and cost effective screening method employed for localization of iron in large number of genotypes by scoring colour intensity in the embryo of cut and treated seeds with 2% Prussian blue through a stereomicroscope (Pearse, 1972). Use of 2, 2-dipyridyl for grain iron estimation is a simple, time saving and high throughput method (Snell and Snell, 1959). It involves the serial addition of a set of reagents to finely ground grains sample and finally measuring the intensity of coloured complex developed using UV-visible spectrophotometer.

These semi-quantitative methods have the potential to provide an easy screening technique for identification of genotypes with high Fe content in the grains, which can be further used for subsequent breeding programme. The genotypes selected for higher Fe and Zn in the grains can

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serve as targets for immediate utility as donors for the trait. The trait of high Fe and Zn containing genotypes needs to be combined with high yield potential, tolerance to biotic and abiotic stresses, and good grain quality (Martinez *et al*., 2007).

It is reported that aromatic rice varieties are associated with higher Fe content in the grains. This might be due to pleiotrophic effects or linkages of the traits (Graham *et al*., 2001). A gene controlling aroma in rice on chromosome 8 was identified (Ahn *et al*., 1992), which is located 4.5 cM away from RFLP marker RG28 in near isogenic lines (NILs). Similarly, three groups of genes associated with high Fe-trait were found to be located on chromosomes 7, 8 and 9 and three groups of genes associated with aroma were located on chromosomes 5, 7 and 8. Thus, the two chromosomes, common for the two traits show linkage between aroma and high Fe concentration (Gregorio, 2002).

Around 75% of Asia's rice is produced in irrigated lowland fields where irrigation requirements can often be high, while irrigation water is becoming an increasingly limited resource (Bouman *et al*. 2007). Therefore, water-saving production systems such as aerobic rice may provide viable adaptation strategies for farmers who want to continue growing rice under water-deficit conditions. Generally, rice is grown under flooded condition, where availability of Fe^{2+} is more as compared with aerobic and well drained soils (Ponnamperuma, 1972; Shashidhar, 2007). But in some cases availability of grain iron content is more in well drained condition (Beyrouty, 1994). Thus, in this study an effort is made to identify genotypes with higher Fe concentration in the grains of rice grown under aerobic condition.

Materials and Methods

Genetic Material and Evaluation Methods

Experiment was conducted in *Kharif* 2005 using 751 local germplasm and 209 advance stage breeding lines collected from IRRI, in farmer's field at Shettigere, outskirts of Bangalore, Karnataka, India (130 10'47"N, 770 39'56"E) in augmented experimental design with two checks (Rasi and BI 48) under aerobic condition.

At crop harvesting stage plant height, number of tillers, grain yield and straw yield were recorded per plant. The iron content was analyzed by using Perls' Prussian Blue Method (Perls *et al*,.1867, Doucet and Viel, 2002) from the dehusked grains.

Out of 960 genotypes used for this experiment, initially 68 genotypes were selected during *Kharif* 2005, based on high iron content in grains and high grain yield. In *Rabi* 2006, the related 68 genotypes were grown both in irrigated and aerobic conditions. Iron content of dehusked grains was estimated using 2, 2-dipyridyl method in both conditions.

Population Development

Five superior genotypes were selected from 68 genotypes to serve as potential parents and three mapping population (IRRI 38 X Jeerigesasnna, B I33 X Jeerigesanna and Azucena X Moro mutant) were developed during *Kharif* 2006 (Table 1).

In *Kharif* 2007, these three mapping population were evaluated for aroma in F_1 and F_2 generation using the method suggested by Sood and Siddiq (1978) and these population were advanced till F_7 generation to develop recombinant inbred lines by continuous selfing.

Table 1. Salient features of the parents used for the development of mapping populations

Accession	Origin / pedigree	Plant height	Maturity (days)	Grain type	Yield (t ha^{-1})	Fe content (mg kg^{-1})	Salient features
IRRI 38	Line from IRRI, Philippines	Semi-dwarf	100-105	Medium slender	3.5	8.71	Drought tolerant
Jeerigesanna	Traditional	Tall	130-140	Short bold	2.5	28.02	Non basmati aromatic
B I33	Budda X IR64	Semi-dwarf	116-122	Long slender	4.5	14.7	Drought tolerant
Azucena	Japonica	Tall	131-145	Medium bold	3.2	19.8	Non basmati aromatic
Moro mutant	Mutant from Moroberekan	Semi-dwarf	115-124	Medium bold	4.2	13.25	Drought tolerant

In *Kharif* 2010, these populations were evaluated for iron and zinc using ICP-OES.

Results and Discussion

Breeding for high micronutrient content depends on exploitation of new genotypes from existing germplasm accessions. Once donor genotypes for Fe content in grains are identified, this trait can be combined with high yield and tolerance to biotic and abiotic stresses (Welch, 1999; Pfeiffer and McClatterty, 2007).

Genetic Differences for Iron Content and Morphological Characters

In this study 960 genotypes were categorized based on grain iron content using Perls' Prussian blue method and also grain iron content was compared against phenotypic characters. Among 960 genotypes, it was found that iron content in the grain was high in 18 genotypes, medium in 72 genotypes and low in 870 genotypes (Table 2). The genotypes with high iron content in grains exhibited less number of tillers, low grain yield and high straw yield when compared to medium and low iron containing genotypes. The genotypes showing medium iron content in grains showed high number of tillers, highest yield and low straw yield when compared to high and low iron containing genotypes. The genotypes showing low iron content in grains showed less number of tillers, medium grain yield and straw yield when compared to high and medium iron containing genotypes. This indicates that high iron content was negatively correlated with grain yield. Similar results were reported by Velu *et al.* (2006) in pearl millet using Perls' Prussian blue method. In transgenic rice grains, Perls' Prussian blue staining showed distribution of iron in aleurone and sub layers of aleurone and also in the central layers of endosperm, whereas in non-transgenic grains it was shown only in aleurone layer (Sivaprakash *et al.*, 2006). Thus, Prussian blue staining was effective in differentiating genotypes with high and low Fe content.

Table 2. Genotypes selected for high iron content in the grains

Sl.no	Genotype	Sl.no	Genotype
1	CTH3	10	RSK2-4
2	HP44	11	IR80312-6-B-3-2-B
3	IET13693	12	IR80021-B-86-3-4
4	IET13796	13	IR79913-B-102-B-3
5	IET16346	14	IET5784
6	IET16666	15	IET7191
7	IET18757	16	IET8794
8	IET18814	17	IET8957
9	Karidaddi	18	IET13142

Analysis of variance revealed significant differences among the genotypes for all the traits. Rice genotypes in this study showed high GCV (21.62, 19.85) and PCV (36.65, 36.08) for straw yield and grain yield, respectively. Similar results were reported for biological yield (Pandey *et al*,. 2012) and grain yield (Bisne *et al.*, 2009; Akinwale *et al.*, 2011) indicating these characters to be genetically controlled. Moderate GCV (16.05) and high PCV (23.47) was observed for number of tillers per plant. This coefficient values indicated considerable amount of variability for this characters. High GCV and PCV for number of tillers were reported (Kole *et al.*, 2008 and Bisne *et al.*, 2009). Moderate PCV and GCV were observed for plant height (Bisne *et al.*, 2009). High heritability (75.00) coupled with high genetic advance as per cent mean (28.69) was observed for plant height; moderate heritability with high genetic advance as percent mean were observed for number of tillers per plant, straw yield and grain yield (Kumar *et al.*, 2001, Suman *et al.*, 2005, Bisne *et al.*, 2009 and Akinwale *et al.*, 2011). This indicated that these traits were not much influenced by environmental factors. Hence, these traits can be improved by direct selection.

Correlation Studies

Highly significant and positive correlation was observed for grain Fe content with grain yield and number of tillers per plant at genotypic level whereas, it had significant negative correlation with plant height and straw yield (Table 3). Similarly, significant positive correlation for iron content with grain yield is reported in soybean (Achakzai, 2004). However, non-significant correlation between iron content with grain yield was also reported (Govindaraj *et al.*, 2009, Nagesh *et al.*, 2012).

Grain yield per plant showed significant positive correlation with straw yield per plant at genotypic and phenotypic level. Grain yield also showed significant positive correlation with number of tillers at genotypic level but showed negative correlation at phenotypic level. Significant correlation between grain yield and number of tillers per plant (Shashidhar *et al*. 2005, Nagesh *et al.*, 2012) and also between biomass and grain yield was reported (Chandrababu *et al*., 2003).

Table 3. Genotypic and phenotypic correlation coefficient matrix

S.No	Character	Plant height	No of tillers	Grain yield	Straw yield	Fe content
1	Plant height	1	-0.20**	-0.14**	0.15**	-0.11**
2	No of tillers	-0.09**	1	0.26**	0.22**	0.29**
3	Grain yield	0.01	0.31**	1	0.27**	0.42**
4	Straw yield	0.17**	0.30**	0.24**	1	-0.69**
5	Fe content	-0.05	0.05	0.04	0	1

*Significant at 0.05 % level, ** Significant at 0.01 % level

Comparison of rice genotypes for grain Fe content grown under wetland and aerobic condition

In the present study, Fe content in the grains of rice was compared among genotypes grown under wetland and aerobic conditions. It has been observed that Fe content was higher in genotypes grown under aerobic condition than those grown under wetland conditions. Similar results were reported for genotypes grown under dry land and wetland conditions Ponnamperuma, 1972. Biomass and grain yield was higher for those genotypes grown under wetland condition compared to aerobic condition (Table 4). The probable reason for higher Fe content in aerobic condition compared to wetland condition can be ascribed to the iron absorbed from the soil is distributed to all the grains and biomass of the individual plant. Similarly, in the wetland condition absorbed iron is distributed to more grains and biomass. Grain Fe content has inverse relationship with grain yield and biomass under aerobic condition.

Genetic difference for aroma in F_2 mapping population

The aroma evaluation in leaves of three mapping population at F_1 generation showed that all are non-aromatic. Similar results were reported in grains and leaves of F_1 population (Tsuzuki and Shimokwa, 1990). In F_2 generation out of 347 plants of IRRI38 X Jeerigesanna cross, 256 were found to be non-aromatic and 91 were aromatic, in BI33 X Jeerigesanna cross out of 280 population, 213 plants were found to be non-aromatic and 67 plants were aromatic and in Azucena X Moromutant cross out of 378 populations, 283 were non- aromatic and 95 were found to be aromatic. In all the three crosses, segregation of aroma was not significantly deviating from the monogenic ratio of 3:1, as revealed by the chi-square test. However, F_2 generation followed standard mendelian ratio of 3:1. This indicated that the inheritance of aroma in rice is possibly due to a single recessive gene (Sun *et al*., 2008).

The results of ICP-OES showed that the iron and zinc content in brown rice ranged from 4.45 to76.0 mg/kg and 7.73 to 146.0 mg/kg respectively. From these results 26 lines of mapping population and six local genotypes with high iron and zinc content were nominated for all India trial in *Kharif* 2011 (Table 5).

From this study three mapping populations were developed from parental lines selected for high iron content and yield parameters. These mapping populations were advanced to develop RILs under aerobic condition for further evaluation of iron and zinc content in brown rice and selection of transgressant lines using marker assisted selection.

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Table 4. Comparison of genotypes for iron content grown under wetland and aerobic condition (n= 68)

	Fe content (mg kg^{-1})		Grain yield (t ha^{-1})		Straw yield (t ha^{-1})	
	Wetland	Aerobic	Wetland	Aerobic	Wetland	Aerobic
Mean ± S.E	10.48 ± 0.52	17 ± 0.81	4.38 ± 0.29	3.08 ± 0.38	8.37 ± 0.46	5.34 ± 0.57
Variance	18.71	44.29	7.14	3.42	22.73	13.71
t α = 0.05	6.67		4.07		3.24	

Table 5. Iron and zinc content of genotype nominated for all India trial in *Kharif* 2011

S.No.	Genotype	Iron (mg/Kg)	Zinc (mg/Kg)	Sl.no.	Genotype	Iron (mg/Kg)	Zinc (mg/Kg)
1	AM-158	23.6	38.2	17	AM-79	33.9	37.7
2	Karna	23.2	30.0	18	AM-36	32.0	34.4
3	Pushpaka	20.3	23.8	19	AM-132B	32.5	39.3
4	BJ-22	24.5	41.1	20	BJ-18	25.7	25.7
5	AM-81C	24.1	33.5	21	AM-94B	27.2	42.4
6	BJ-6	30.9	38.0	22	AM-127B	29.5	38.3
7	AM-72	29.3	42.4	23	AM-144	27.8	36.2
8	AM-127	34.7	40.7	24	KHP-2	29.7	31.7
9	BJ-23	33.4	41.2	25	BJ-17	24.2	34.2
10	AM-27	29.8	41.0	26	BJ-16	31.2	33.5
11	AM-143	24.3	43.8	27	AZUCENA	32.0	40.2
12	AM-1	31.7	42.3	28	Karidaddi	34.3	32.5
13	BJ-2	30.9	36.2	29	BJ-5	32.3	36.1
14	AM-132	28.1	33.0	30	IVT(SHW) /91/10608	31.1	33.4
15	AM-81	33.2	41.4	31	Jeerigesanna	28.3	33.9
16	AM-65	33.8	43.9	32	BJ-66	25.8	33.0

Note: **AM** (Azucena X Moromutant), BJ (BI33 X Jeerigesanna)

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