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RESEARCH PAPER

Genetic diversity studies in rice (*Oryza sativa* L.) genotypes with high iron content in grains using microsatellite markers

D. SHUKLA[1], D. KULDEEP SINGH[1], C.N. NEERAJA, V. RAVINDRA BABU*, P. NAGESH[1] AND G. USHARANI[1]

Crop Improvement Division, Directorate of Rice Research (A.N.G.R.A.U.), Rajendranagar, HYDERABAD (A.P.) INDIA

(Email : rbvemuri1955@gmail.com)

Abstract : Rice is the most important staple food grain with regard to human nutrition and calorie intake providing more than one-fifth of the calories consumed worldwide by the humans. Biofortification of staple food crops has thus been considered a sustainable strategy to overcome the problem of micronutrient deficiencies prevalent in rice. The present study was conceptualized and executed with the prime objective of studying the genetic diversity of ninty six genotypes of rice with high iron content in grains using two SSR markers *viz*., SC 120 and SC 123 based on the yellow stripe like genes derived from the genomic regions associated with iron metabolism. No significant grouping based on the iron content in the grains could be obtained as the trait of iron accumulation in grains is controlled by many genes and the markers used in this study were of limited number, more markers preferably functional markers would elicit the genetic diversity of the characterized genotypes.

Key Words : Genetic diversity, Grain iron, SSRs

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INTRODUCTION

Rice (*Oryza sativa* L.) occupies an enviable prime place among the food crops cultivated around the world. It is the principal food for more than half of the world's population (Sasaki and Burr, 2000) and has been estimated that 50 per cent of the human population depends on rice as a main source of nutrition (White, 1994). Domestication of food crops and their subsequent genetic improvement resulting into an era of 'green revolution' was mainly aimed at increasing yields and tolerance to biotic and abiotic stresses, and as a consequence inadvertently sidelining the nutritional traits of crop plants.

Nutritional deficiencies account for almost two-third of the childhood death worldwide. Most of these afflicted are dependent on staple crops such as rice, wheat and maize for their sustenance. Those afflicted cannot afford the fortified food to meet out the micronutrient requirement. Although rice is not considered a major mineral source in the diet, any increase in its mineral concentration could significantly help reduce iron deficiency in humans because of the high levels of rice consumption among the poor in Asia. Thus, micronutrient enrichment of staple food crops has been considered a sustainable strategy to tackle the problem of micronutrient deficiencies.

The increase in iron content in plant staple foods can serve as a vector for iron ingestion in target populations, minimizing nutritional problems (Vansuyt *et al.,* 2000). In this regard, a better understanding of iron homeostasis, involving knowledge of the basic physiological processes of iron absorption, distribution and storage in plants, can serve as

* **Author for correspondence. (Present Address) :** Crop Improvement Section, Directorate of Rice Research (A.N.G.R.A.U.), Rajendranagar, HYDERABAD (A.P.)

[1]Department of Genetics and Plant Breeding, College of Agriculture (A.N.G.R.A.U.), Rajendranagar, HYDERABAD (A.P.) INDIA

starting point for the biotechnological manipulation of crops (Grusak, 2002; Grotz and Guerinot, 2002). Great strides have been made in recent years in identifying key genes and proteins involved in iron transport and in demonstrating the importance of transcriptional regulation of these genes in response to the iron status of the plants (Grusak and Pezeshgi, 1996).

Clearly, the potential exists for developing improved rice varieties with high iron content in the grain. The poor in South and Southeast Asia eat a lot of rice; this increased iron content in rice grain would have a meaningful impact on human nutrition and health, especially for anaemic women and children. Of late, an initiative in this direction has been taken up by the Department of Biotechnology, Government of India in collaboration with an international interdisciplinary research program, harvest plus wherein biofortification of major food crops like wheat, maize and rice has been proposed to be undertaken. Hence, the present study was undertaken to evaluate the extent of genetic diversity for iron content among different rice genotypes using microsatellite markers derived from the genomic regions associated with iron metabolism.

MATERIALS AND METHODS

The field experiment was taken for the diversity analysis regarding the difference in the iron content of ninty six rice genotypes. The genotypes used were grouped into three classes according to the iron content as very high iron (7.0 mg iron per kg dry weight), high iron content (5.0 mg iron per kg dry weight) and medium and low iron content (= 3.0 to 5.0 mg iron per kg of the dry weight). Iron content of grain samples was estimated by atomic absorption spectrophotometer as suggested by Lindsay and Norvell (1978). The details of genotypes used with iron content are given in Table A and classification of iron content in grain in Table B. The DNA was extracted from freshly germinated young seedlings using the method of Zheng *et al*. (1991). 30 well spot test plate available from Thomas Scientific, USA was used for DNA isolations. The purity and concentration of the isolated genomic DNA samples were estimated by UV- absorption spectrophotometer (Beckman DU 650 model) as per the procedure described by Sambrook and Russell (2001). Agarose gel electrophoresis (0.8%) was carried out for confirming the quality and quantity of the isolated DNA using a known concentration of DNA. The genomic DNA was subjected to PCR amplification as per the procedure described by Chen *et al*. (1997). DNA samples were amplified in 10µl reaction volumes containing 1X PCR buffer [10 mM Tris–HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 0.01% (v/v) gelatin] (Bangalore Genei, India), 0.2 mM of each dNTPs (Bangalore Genei, India), 10 pmol of each primer and 1 U of *Taq polymerase* (Bangalore Genei, India). PCR was carried out in a Thermal cycler (Perkin–Elmer-Gene Amp PCR System 9700, USA). A PCR profile consisting of 5 min of initial denaturation at 94°C, 35 cycles of

Table A : List of the genotypes used for diversity analysis

Sr. No.	Genotype	Iron content (mg kg^{-1})	Sr. No.	Genotype	Iron content (mg kg^{-1})
1.	NLR-145	5.89	49.	VRM-44-1	4.62
2.	BR-2655	5.70	50.	VRS-25	6.59
3.	NLR-33654	3.43	51.	VRS-19	5.83
4.	KRH-2	3.00	52.	VRS-7	6.97
5.	Chittimuthyalu	8.25	53.	VRS-4	5.34
6.	NDR-359	3.16	54.	VRS-3	5.47
7.	SGT-1	4.42	55.	VRM-3	8.03
8.	Yamini	--	56.	PR-111	4.85
9.	Swarna	4.07	57.	PR-113	3.11
10.	PR-116	3.68	58.	PR-114	6.48
11.	Vijetha	2.71	59.	PR-115	3.70
12.	DL-183	3.24	60.	PR-118	2.72
13.	Mandya Vijaya	2.69	61.	Sarla	5.34
14.	Basmati-370	2.70	62.	Savitri	6.91
15.	Pusa Basmati 1	3.32	63.	Gayatri	--
16.	IR-64	1.50	64.	Pooja	--
17.	Karjat5-3	2.08	65.	Durga	7.15
18.	Poornima	1.96	66.	Kanchana	4.65
19.	White Ponni	--	67.	Varsha	9.58
20.	BPT-5204	--	68.	Mattatriveni	2.69
21.	Jaya	3.35	69.	Harsha	3.08
22.	BPT-11711	5.81	70.	WGL-14	2.69
23.	ASG-4022	3.73	71.	Dharitri	--
24.	MTU-1001	3.12	72.	Aiswarya	1.92
25.	MTU-3626	6.74	73.	Jyothi	5.72
26.	GR-11	8.38	74.	Karjat-184	--
27.	Gurjari	4.29	75.	Karjat-2	5.23
28.	GR-104	7.19	76.	Karjat-4	4.13
29.	Jalmagna	5.27	77.	Sahyadri	2.50
30.	Giri	3.71	78.	Sahyadri-2	2.76
31.	PR-111	4.24	79.	Danteswari	3.53
32.	Vikas	3.16	80.	Indira	2.79
33.	Basmati-386	4.82	81.	Nagridubraj	3.43
34.	ADT-43	5.97	82.	R1027-2282-2-1	4.17
35.	PTB-39	4.80	83.	R979-1528-2-1	6.17
36.	Taroari Basmati	3.95	84.	MTU1010	3.66
37.	Pant Dhan-16	5.51	85.	Pant Dhan-12	8.05
38.	T-3	5.94	86.	Pant Dhan-10	4.10
39.	Ranbir Basmati	7.37	87.	Pant Sugandha-17	2.32
40.	Kranti	5.24	88.	Govind	3.70
41.	Shashi	--	89.	Parijat	1.81
42.	Suraksha	7.02	90.	Khandagiri	4.52
43.	IR30864	4.95	91.	Pathara	2.53
44.	Dandi	--	92.	Udayagiri	2.15
45.	Mahamaya	6.37	93.	Kashaur	2.29
46.	Dinesh	3.94	94.	Daya	5.78
47.	VRM-30	3.95	95.	Sabari	2.23
48.	VRM-31-1	3.75	96.	Lalat	2.98

Table B : Iron concentration (mg kg^{-1}) of the genotypes studied for genetic diversity

Iron content	No. of genotypes	Genotypes
Very high (more than 7mg kg^{-1})	8	Durga, GR-104, Ranbir Basmati, Suraksha, Chittimuthyalu, VRM-3, GR-11 and Pant Dhan-12
High (more than 5mg kg^{-1})	23	Karjat-2, Kranti, Jalmagna, VRS-4, Sarla, VRS-3, Pant Dhan-16, BR-2655, Jyothi, Daya, BPT-11711, VRS-19, NLR-145, T-3, ADT-43, R979-1528-2-1, Mahamaya, PR-114, VRS-25, MTU-3626, Savitri, VRS-7 and Varsha
Medium (3 to5mg kg^{-1})	36	KRH-2, Harsha, PR-113, MTU-1001, Vikas, NDR-359, DL-183, Pusa Basmati 1, Jaya, NLR-33654, Nagridubraj, Danteswari, MTU1010, PR-116, PR-115, Govind, Giri, ASG-4022, VRM-31-1, Dinesh, VRM-30, Taroari Basmati, Swarna, Pant Dhan-10, Karjat-4, R1027-2282-2-1, PR-111, Gurjari, SGT-1, Khandagiri, VRM-44-1, Kanchana, PTB-39, Basmati-386, PR-111 and IR30864
Low (less than 3mg kg^{-1})	20	IR-64, Parijat, Aiswarya, Poornima, Karjat5-3, Udayagiri, Sabari, Kashaur, Pant Sugandha-17, Sahyadri, Pathara, Mattatriveni, WGL-14, Mandya Vijaya, Basmati-370, Vijetha, PR-118, Sahyadri-2 and Indira, Lalat

1 min of denaturation at 94°C, 1 min of annealing at 55°C, 2 min of extension at 72°C and 7 min of final extension at 72°C was followed. The amplified products were resolved on 3 per cent agarose gels, stained with ethidium bromide and visualized under UV in a gel documentation system (Alpha Innotech, USA). The allele sizes of each microsatellite marker for the given set of genotypes were noted and each allele was scored for the genotypes. Then the data were converted into presence (1) and absence (0) matrix. Indices of similarity were calculated using simple matching coefficient to estimate diversity between accessions. Cluster analyses were based on similarity matrices using unweighted pair group method with arithmetic mean using NTSYS-pc version 2.0 (Rohlf, 1994).

RESULTS AND DISCUSSION

Ninty six genotypes of rice were studied possessing varying quantities of iron in the grains with two polymorphic microsatellite markers, SC 120 and SC 123 located in the vicinity of the putative candidate genes involved in iron metabolism. SC 120 and SC 123 were based on yellow stripe like genes located on chromosome 4 which encode membrane protein involving iron uptake.SC indicates the microsatellite markers developed at Directorate of Rice Research.

Based on the cluster analysis, genotypes were divided into a major and a minor group at 43 per cent similarity. The major group has 89 genotypes and minor group has seven genotypes with iron content ranging from 2.50 to 8.03 with an average of 4.43 mg kg^{-1}. The major group was divided into two groups, group 1 and group 2 at 50 per cent similarity. Group 1 has a minor sub group A of seven genotypes with iron content ranging from 2.32 to 6.59 with an average of 4.35 mg kg^{-1} and major sub group B was divided into two clusters *i.e.* cluster 1 and cluster 2 at 68 per cent similarity. Cluster 2 has 28 genotypes with iron content ranging from 1.50 to 8.25 with an average of 4.75 mg kg^{-1}. Cluster 1 was divided into two sub clusters at 83 per cent similarity, sub cluster A and sub cluster B, with 10 and two genotypes respectively. In sub cluster A iron content is ranging from 2.69 to 8.05 with an average of 4.65 mg kg^{-1}. In sub cluster B iron content is ranging from 2.08 to 3.75 with an average of 2.92 mg kg^{-1}.

Group 2 was divided into two subgroups A and B at 61 per cent similarity. Subgroup A was divided into two clusters namely cluster 1 and cluster 2 at 67 per cent similarity. Cluster 1 was divided into two sub clusters at 84 per cent similarity *i.e.* sub cluster A, which has 21 genotypes in it with iron content ranging from 2.23 to 9.58 with an average of 4.37 mg kg^{-1} and sub cluster B has only one genotype with 2.69 mg kg^{-1}. Subgroup B was divided into two clusters namely cluster 1 and cluster 2 at 68 per cent similarity. Cluster 1 has eight genotypes with iron content ranging from 1.95 to 5.82 with an average of 3.95 mg kg^{-1}. Cluster 2 was divided into two sub clusters at 84 per cent similarity *i.e.* sub cluster A has only one genotype with 2.70 mg kg^{-1} and sub cluster B has six genotypes with iron content ranging from 2.79 to 8.38 with an average of 4.65 mg kg^{-1}. Cluster analysis of 87 rice genotypes using UPGMA is given in Fig. 1.

Cluster analysis of 87 rice genotypes using SC 120 marker:

The analysis of ninty six genotypes of rice was studied with SC 120 microsatellite marker. Based on the cluster analysis, genotypes were divided into one major and one minor group at 35 per cent similarity. Minor group has 13 genotypes with iron content ranging from 2.32 to 8.38 with a mean of 4.47 mg kg^{-1}. Major group was divided into two groups group A and group B at 38 per cent similarity. Group A has two subgroups, subgroup 1 and 2 comprising of 25 and three genotypes at 66 per cent similarity, respectively. In subgroup 1 iron content is ranging from 1.92 to 8.05 with a mean of 4.42 mg kg^{-1}. In subgroup 2 iron content is ranging from 2.08 to 3.75 with a

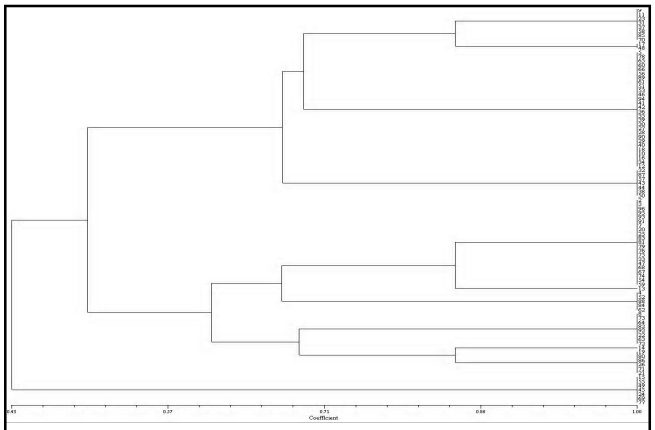

Fig. 1 : Cluster analysis of 96 rice genotypes using UPGMA

mean of 2.85 mg kg^{-1}. Group B has 55 genotypes with iron content ranging from 1.50 to 9.58 with a mean of 4.58 mg kg^{-1}. Cluster analysis of 96 rice genotypes using UPGMA with SC 120 marker is given in Fig. 2. Allelic diversity of SC 120 marker

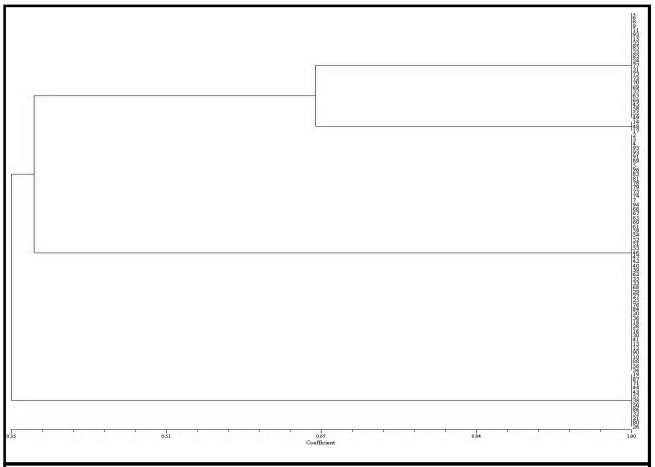

Fig. 2 : Cluster analysis of 96 rice genotypes using UPGMA with SC 120

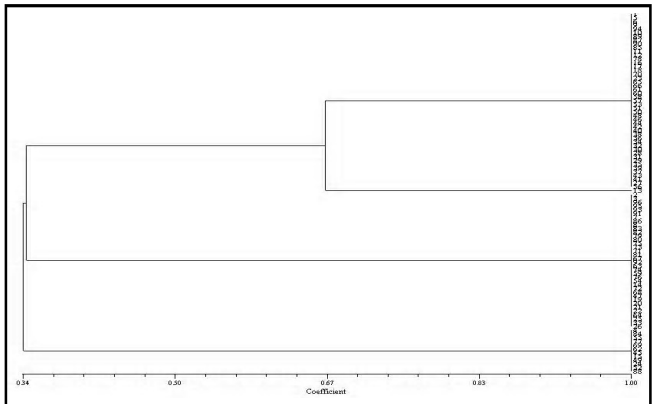

Fig. 3 : Cluster analysis of 96 rice genotypes using UPGMA with SC 123

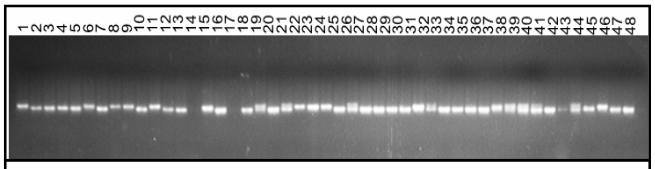

Fig. 4 (a) :Allelic diversity of SC 120 in 96 rice genotypes (Note: order of the genotypes is as per given in Table A)

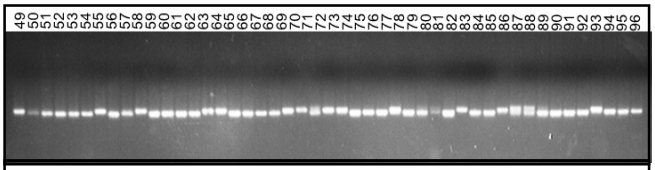

Fig. 4 (b) :Allelic diversity of SC 120 in 96 rice genotypes (Note: order of the genotypes is as per given in Table A)

in 96 rice genotypes is given in Fig. 4(a) and 4(b).

Cluster analysis of 87 rice genotypes by using SC 123 marker:

Based on the cluster analysis of 96 rice genotypes with SC 123 marker, genotypes were divided into one major and one minor group at 34 per cent similarity. Minor group has 12 genotypes with iron content ranging from 2.50 to 8.03 and mean value of 4.61 mg kg^{-1}. Major group was divided into two groups namely group A and group B at 35 per cent similarity. Group was divided into subgroups at 67 per cent similarity, subgroup 1 and 2 comprises of 47 and one genotype (iron content 2.69 mg kg^{-1}), respectively. Subgroup 1 has 47 genotypes with iron content ranging from 1.50 to 8.25 with a mean of 4.59 mg kg^{-1}. Group B is a big group which has 36 genotypes with iron content ranging from 1.92 to 9.58 and a mean of 4.28 mg kg^{-1}. Cluster analysis of 96 rice genotypes using UPGMA with SC 123 marker is given in Fig. 3. Thus, the findings from the present study indicated that much more markers preferably functional markers would elicit the genetic diversity of the characterized genotypes associated with high iron content in the grains.

Conclusion:

The biotechnological manipulation of plant crops in a way to increase their capacity to adequate the iron content may result in yield improvement. The present day, biotechnology combined with conventional plant breeding is gaining importance in early selection of desirable genotypes. With molecular characterization combined with plant breeding one can overcome cost, time and efficiency involved in developing a line / variety. These identified diverse genotypes are useful in incorporating genes in popular varieties through proper molecular breeding. In the present investigation, the used markers were less and much more markers relating to

iron metabolism identified by Gross *et al.* (2003) can be used for further investigation.

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