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SSR Marker Studies on Genetic Diversity for Zinc Content in Different Genotypes of Rice (*Oryza sativa* L.)

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ABSTRACT

Genetic diversity underlies the improvement of crops by plant breeding. The present study was thus conceptualized and executed with the prime objective of assessing the genetic diversity of eighty three rice genotypes using nineteen SSR markers derived from the genomic regions associated with zinc metabolism. Three polymorphic markers generated 7 alleles in eighty three genotypes. The number of alleles for each marker ranged from 2 to 3 with an average value of 2.3. The amount of polymorphism exhibited by SC 116 was found to be high with the PIC value of 0.85, while the other two markers, SC 126 and SC 129, showed similar polymorphism by having the same PIC value of 0.73. Significant grouping of the rice genotypes based on the data generated by these three polymorphic markers was not possible, since the trait of zinc accumulation in grains is controlled by multiple genes and only three markers were used in the present investigation, which may not be able to reveal sufficient polymorphism. More markers, preferably functional molecular markers, would elicit the genetic diversity of the characterized germplasm.

Key words : Cluster analysis, Genetic diversity, Rice, SSR markers, Zinc content

Rice (*Oryza sativa* L.) is a cereal foodstuff which forms the daily bread of more than three billion people around the world. Zinc (Zn) deficiencies constitute a major public health problem in many countries, especially in regions where people rely on cereal-based food. The most recent and promising approach to combat micronutrient malnutrition is to improve zinc content in rice grains. Considerable efforts are undertaken to reach this goal through breeding, but understanding of the underlying processes is still partly lacking and would facilitate such breeding programmes (Clemens *et al*., 2002).

Knowledge of the genetic variability and identification of genotypes with higher zinc content in their grains over the best cultivated variety is a pre-requisite for choosing an appropriate breeding procedure for development of rice cultivars with high grain zinc content. Estimation of genetic diversity among the rice genotypes by DNA based markers will not only assist in identification of possible sources of genes to improve the performance of cultivars but also helps in grouping the genotypes according to their genetic relatedness. In rice, SSR markers have been used to assess the genetic diversity of both wild and cultivated species (Neeraja *et al*., 2005). Hence, the present study was undertaken to evaluate the extent of genetic diversity for zinc content among different rice genotypes using SSR markers derived from the genomic regions associated with zinc metabolism.

MATERIAL AND METHODS

Eighty three germplasm lines with ample variation for zinc content in their grains were tested at Directorate of Rice Research (DRR), Rajendranagar, Hyderabad. Zinc content of grain samples was estimated by Atomic Absorption Spectrophotometer as suggested by Lindsay and Norvell (1978). All the lines were categorized into three groups based on the zinc content in the grains as high (> 5 mg $100g^{-1}$), medium (3 to 5 mg $100g^{-1}$) and low (< 3 mg $100g^{-1}$) (Table 1). The genetic diversity among these rice germplasm lines was studied using nineteen SSR markers located in the vicinity of the putative candidate genes (Table 2) involved in the zinc metabolism designed at DRR based on the sequence information. The DNA was

Table 1. Genetic diversity of rice genotypes for zinc content .

S. No.	Genotypes	Zinc Content (mg 100 g^{-1})	S. No.	Genotypes	Zinc Content (mg 100 g^{-1})
With high (> 5 mg 100 g^{-1}) zinc content					
1.	Yamini	5.016	9.	CN-1230-12-2	7.580
2.	MTU-1010	5.373	10.	Sheshadri	7.882
3.	VRS-7	5.793	11.	Taroari Basmati 1	8.898
4.	Matta Triveni	6.198	12.	Taroari Basmati 2	8.898
5.	MO-4	6.361	13.	Pant dhan-12	9.130
6.	Ranjeet	7.158	14.	Varalu	9.232
7.	VRS-3	7.164	15.	BPT-11711	9.655
8.	MTU-1001	7.407	16.	Aishwarya	10.162
With medium (3 to 5 mg 100 g^{-1}) zinc content					
1.	SGT-1	3.103	6.	Pant dhan-16	3.832
2.	Kanchana	3.140	7.	VRM-31	4.515
3.	Kranthi	3.294	8.	Type-3	4.545
4.	VRM-3	3.700	9.	NDR-359	4.644
5.	Gouri	3.712			
With low (< 3 mg 100 g^{-1}) zinc content					
1.	Samba Mahsuri	1.030	30.	Basmati-386	2.157
2.	PR-115	1.946	31.	WGL-14	2.160
3.	Gajapathi	1.430	32.	Bhashana Rodi	2.168
4.	Suraksha	1.456	33.	Kavya	2.169
5.	Khanchan	1.699	34.	Pant dhan-10	2.179
6.	KKP-2	1.715	35.	IR-30864	2.185
7.	Phalguna	1.725	36.	Mandya Vijaya	2.193
8.	Amulya	1.736	37.	Nagri Dubraj	2.222
9.	Durga	1.743	38.	Gurjari	2.223
10.	Khithish	1.764	39.	Jalamagna 1	2.249
11.	Mounica	1.781	40.	Jalamagna 2	2.249
12.	Varsha	1.810	41.	Dhandi	2.255
13.	CN-1039-9	1.810	42.	Pothana	2.330
14.	PR-116	1.822	43.	Daya	2.333
15.	Champakala	1.877	44.	GR-11	2.462
16.	Dhuben	1.914	45.	PR-114	2.495
17.	AS-100	1.937	46.	Khandagiri	2.508
18.	PR-115	1.946	47.	Bas-370	2.688
19.	Jagabandu	1.959	48.	Harsha	2.709
20.	White ponni	1.973	49.	Giri	2.728
21.	PR-111	1.990	50.	Gayathri	2.763
22.	CN-1233-33-9	1.993	51.	Madhukar	2.771
23.	IR-70035	2.000	52.	GR-104	2.787
24.	Jyothi	2.065	53.	NLR-145	2.839
25.	Bhagirathi	2.071	54.	Jalanidhi	2.875
26.	Jaya 1	2.071	55.	Bhuban	2.908
27.	Jaya 2	2.071	56.	Pusa Basumati	2.921
28.	MTU-3626	2.084	57.	Mahamaya	2.933
29.	Dinesh	2.104	58.	Erramallelu	2.972

Table 2. Details of SSR markers used for evaluation of genetic diversity.

SSR markers	Nature of the targeted gene	Chromosomal Position
SC 100	*ZT*	1
SC 101	*ZT*	2
SC 102 and SC 103	*ZT*	3
SC 105	*ZT*	4
SC 112	*ZT*	7
SC 113, SC 114 and SC116	*ZT*	8
SC 118	*YSl*	2
SC 121	*YSl*	4
SC 126	*YSl*	8
SC 129	*ZIP*	3
SC 130	*ZIP*	6
SC 131	*ZIP*	4
SC 132	*ZIP*	8
SC 135	*ZIP*	5
SC 138	*NRAMP*	2
SC 139	*NRAMP*	7

SC denotes the SSR markers developed at Directorate of Rice Research, Hyderabad
ZT: Zinc Transport gene, YSl : Yellow Stripe like gene,
ZIP: Zrt/Irt related Protein gene, (Zrt/Irt: Zinc/Iron regulated transporter gene),
NRAMP: Natural Resistance-Associated Macrophage Protein gene

extracted from freshly germinated young seedlings of rice genotypes using the method of Zheng *et al*. (1991). 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 ëDNA. The genomic DNA was subjected to PCR amplification as per the procedure described by Chen *et al*. (1997). DNA samples were amplified in 10 µl 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). A PCR profile consisting of 5 min of initial denaturation at 94°C, 35 cycles of 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 in a Thermal cycler (Perkin–Elmer-Gene Amp PCR System 9700, USA). The amplified products were resolved on 3 % agarose gels, stained with ethidium bromide and visualized under UV in a gel documentation system (Alpha Innotech, USA). The allele sizes of each SSR marker for the given set of genotypes were noted and scored. Then the data was converted into presence (1) and absence (0) matrix. The Polymorphism Information Content (PIC) value for each SSR marker was used to refer the relative value of each marker with respect to the amount of polymorphism exhibited. The PIC value is related to the number of alleles and allele combinations and the PIC value was estimated as $PIC = 1 - \sum_{j=1}^{n} P^2_{ij,}$ where P_{ij} is the frequency of the j^{th} pattern for marker i and the summation extends over n parents (Anderson *et al*., 1993). The calculation was based on the number of alleles detected by a marker at a given locus and the relative frequency of each allele in 83 rice genotypes. The binary data matrix recorded was

subjected to cluster analysis. Sequential Agglomerative Hierarchial Non-overlapping (SAHN) clustering was performed on Squared Euclidean distance matrix and similarity matrix using Jacquard's Coefficient utilizing the Unweighted Pair Group Method with Arithmatic Averages (UPGMA) method. Data analysis was done using the software NTSYS-PC version 2.0 (Rohlf, 1994) and represented in the form of a dendrogram.

RESULTS AND DISCUSSION

Eighty three rice genotypes were categorized broadly into three groups based on the zinc content in their grains. Sixteen rice genotypes had high zinc content, while nine and fifty eight rice genotypes were found to have medium and low zinc contents, respectively in their grains.

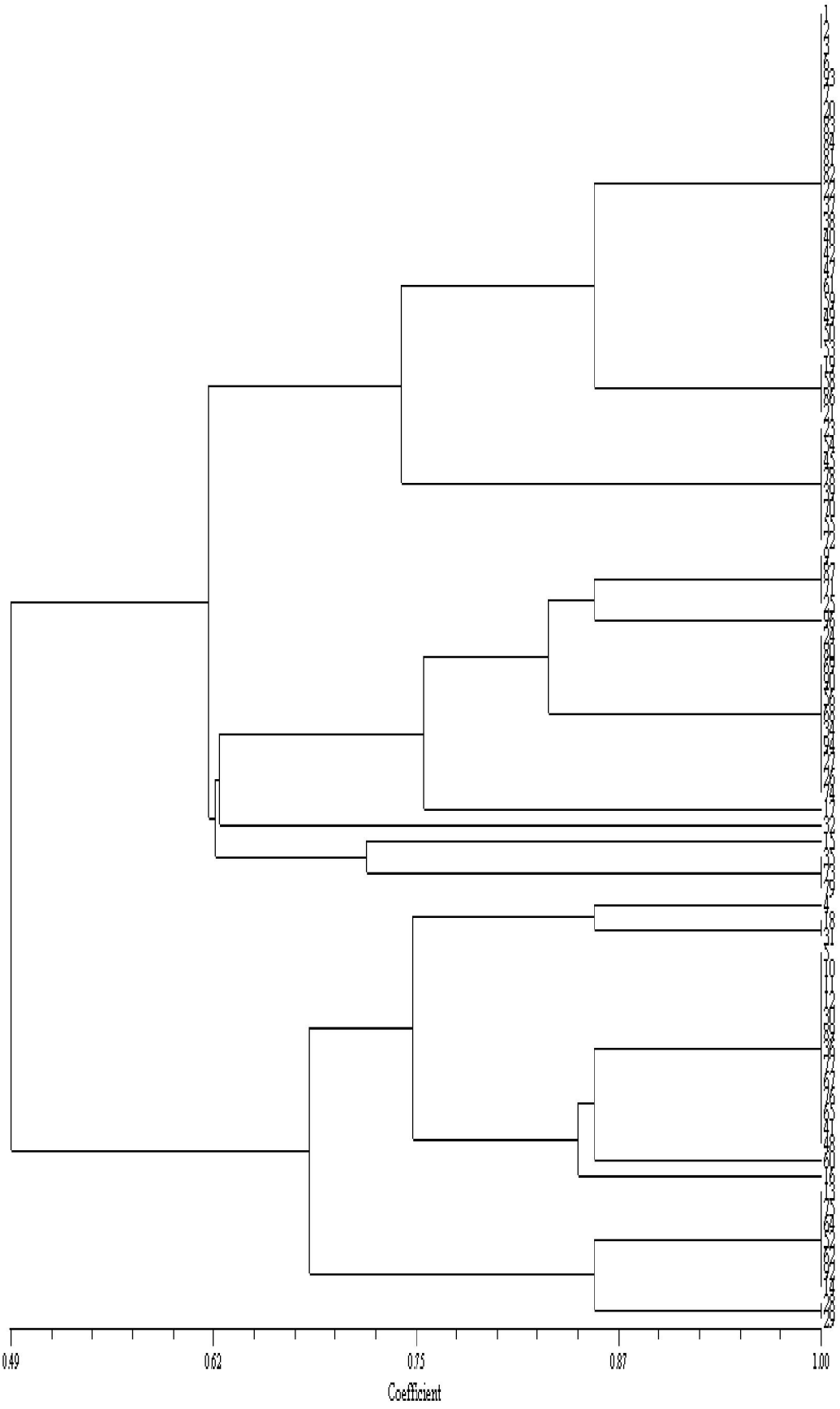
Genetic diversity of eighty three genotypes for zinc content in grains was studied using nineteen SSR markers derived from genomic regions associated with zinc metabolism. Among them, thirteen (68.42 %) markers (SC 101, SC 103, SC 105, SC 112, SC 113, SC 116, SC 126, SC 129, SC 131, SC 132, SC 135, SC 138 and SC 139) showed polymorphism, four markers (SC 114, SC 118, SC 121 and SC 130) were monomorphic and two markers (SC 100 and SC 102) were not amplified. Only the clear and unambiguous bands of SSR markers were scored. Of the thirteen polymorphic markers, only three markers whose banding pattern could be properly scored were selected to detect polymorphism. The data obtained with three SSR markers *viz.,* SC 116 marker based on *Zinc Transport* (*ZT*) gene and SC 126 based on *Yellow stripe like (Ysl)* gene located on chromosome 8 and SC 129 based on *ZIP (Zrt/Irt related protein)* gene present on chromosome 3 were employed for cluster analysis. These three polymorphic markers generated 7 alleles in eighty three genotypes. The number of alleles for each marker ranged from 2 to 3 with an average value of 2.3. The SC 116 primer located on chromosome 8 produced 3 alleles, with an allelic frequency of 30 %, 59 % and 7 %, while the SC 126 primer located on chromosome 8 produced 2 alleles with an allelic frequency of 12 % and 72 % and SC 129 primer located on chromosome 3 produced 2 alleles, with an allelic frequency of 66 % and 31 %. The amount of polymorphism exhibited by SC 116 was found to be high with the PIC value of 0.85, while the other two markers, SC 126 and SC 129 showed similar polymorphism by having the same PIC value of 0.73.

Cluster analysis:

All the eighty three genotypes were grouped into two clusters, one major and one minor cluster at 49 % similarity (Fig. 1). The major cluster had two sub clusters at 62 % similarity with an average value of 3.56. The sub cluster 1 was further divided into sub cluster 1A and sub cluster 1B at 75 % similarity. Sub cluster 1A was again divided into two groups at 86 % similarity. The group 1 had twenty-two genotypes with an average value of 3.77, having the maximum value for Varalu (9.232) and minimum value for Phalguna (1.725). In group 2 there were four genotypes with an average value of 3.57, with a maximum value of 7.58 for CN-1230-12-2 and minimum value for Bhagirathi (2.071). Sub cluster 1B possessed eight genotypes with an average value of 4.25, having the maximum value for Taroari Basmati (8.898) and minimum value for AS-100 (1.937). The sub cluster 2 was further divided into sub cluster 2A and sub cluster 2B at 62.1 % similarity. Sub cluster 2A was again divided into two groups at 62.2 % similarity. The group 1 was divided into two sub groups at 75 % similarity. The sub group 1 was divided into 1A and 1B at 85 % similarity. The 1A was divided into two at 87 % similarity into 1Aa and 1Ab. 1Aa had four genotypes with an average value being 2.86 and having the maximum value for VRM-31 (4.515) and minimum value for CN-1039-9 (1.81). 1Ab had one genotype, PR-111(1.99). The 1B had eleven genotypes with an average value of 2.92, with the maximum value of 10.162 for Aishwarya and minimum value for Suraksha (1.456). The sub group 2 had one genotype, NLR-145 (2.839). The group 2 had one genotype, Sheshadri (7.882). The Sub cluster 2B was divided into two groups at 73 % similarity. The group 1 had one genotype, Yamini (5.016). The group 2 had three genotypes with an average value of 2.272, with the maximum value for GR-11 (2.462) and minimum value for Kavya (2.169).

The minor cluster had two sub clusters at 68 % similarity with an average value of 3.15. The sub cluster 1 was further divided into sub cluster

Fig. 1. Clustering of rice genotypes with SSR markers SC 116, SC 126 and SC 129.



1A and sub cluster 1B at 75 % similarity. Sub cluster 1A was again divided into two groups at 86 % similarity. The group 1 had one genotype, Khanchan (1.699). The group 2 had two genotypes with an average value of 2.362, having the maximum value for Kranthi (3.294) and minimum value for Gajapathi (1.43). The sub cluster 1B was divided into two groups at 86 % similarity. The group 1 was divided into two sub groups. The sub group 1 had thirteen genotypes with an average value of 3.356, having the maximum value for BPT-11711 (9.655) and minimum value for KKP-2 (1.715). The sub group 2 had one genotype, Jaya (2.071). The group 2 had one genotype, Pant dhan-16 (3.832). The sub cluster 2 was further divided into sub cluster 2A and sub cluster 2B at 86 % similarity. Sub cluster 2A had seven genotypes with an average value of 3.61, having the maximum value for MTU-1001 (7.407) and minimum value for Durga (1.743). Sub cluster 2B had two genotypes with an average value of 1.996, having the maximum value for IR-70035 (2.000) and minimum value for CN-1233-33-9 (1.993). The data generated by the three polymorphic markers was insufficient for grouping of all the 83 rice genotypes properly, since the trait of zinc accumulation in grains is controlled by multiple genes.

Knowledge regarding the amount of genetic variation in germplasm accessions and genetic relationships between genotypes are important considerations for designing effective breeding programs. The genetic modifications offer good opportunities to develop improved rice varieties with higher zinc content in grains.

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