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PARENTAL POLYMORPHISM IN IRON- AND ZINC-RICH RICE VARIETIES (SWARNA AND TYPE 3) USING SSR MARKERS

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ABSTRACT

A microsatellite is a specific sequence of DNA bases which contains repeats. In present study, rice microsatellite (RM) markers were used to study the parental polymorphism between two parents. The experiment was conducted at Indian Institute of Rice Research, Rajendranagar Hyderabad (India). 'Swarna' and 'Type 3' rice varieties dense in grain iron and zinc were used to study the parental polymorphism. Out of 171 rice markers used for parental polymorphism, 52 markers showed polymorphism and 119 were monomorphic with expected base pair ranging from 84 to 383 bp. Therefore, these polymorphic rice microsatellite markers can be used in fine mapping of iron- and zinc-rich micronutrient genes to study the mapping populations of crosses obtained from these parents.

Keywords: Biofortification, iron, microsatellite markers, parental polymorphism, rice, zinc.

INTRODUCTION

Rice (*Oryza sativa* L.) is a "Global Grain" widely cultivated across the world. As humankind faces nutrient deficit in cultivated crops, malnutrition has been designed as the most serious challenge to humanity (Copenhagen Consensus, 2008) because two-third of the world's population is at risk of deficiency in one or more essential mineral elements (Cakmak, 2002; White and Broadley, 2009; Stein, 2010). Biofortification is a new approach that aims at biological and genetic enrichment of food stuffs with vital nutrients (vitamins, minerals and proteins) [Phattarakul *et al.*, 2012]. The rapid development of DNA marker technology provides great opportunities to enhance nutritive values of traditionally cultivated crops and grains.

Genetic polymorphism is defined as the simultaneous occurrence of a trait in the same population of two or more discontinuous variants or genotypes (Rahman *et al.*, 2007). DNA fingerprinting is used to describe the combined use of several single locus detection systems and is used as versatile tools for studying various aspects of plant genomes including the characterization of genetic variability, genome fingerprinting, genome mapping, gene localization, analysis of genome evolution, population genetics, etc. (Bouis, 2000). Molecular data provides a basis for better management and conservation of collection which could be used as reference for its enhanced use in breeding programs (Wang *et al*., 2005).

Marker-aided selection (MAS) techniques can increase the selection efficiency to as high as100% and permit simultaneous selection for a number of traits (Xu and Crouch, 2008). Cultivars are needed for developing the techniques that have been identified, but the DNA-level polymorphism between traditional cultivars and other yield improving types will determine the rate of progress. This can be done by microsatellites, or simple sequence repeats (SSRs) (referred to as a variable number of tandem repeats or VNTRs), at polymorphic loci present in nuclear and organellar DNA that consist of repeating units of 1-6 bp in length. They are highly polymorphic molecular markers, which have wide-ranging applications in the field of genetics (Cho *et al*., 2000).

Improved rice varieties with high nutrients like iron and zinc could also provide better nutrition to even the poor people. By increasing the iron and zinc content of food staples through plant breeding and biotechnology, the entire iron and zinc status distribution curve can be shifted to that smaller group of nutrient deficient persons which could become feasible in the developing countries. In next decade, it is expected that DNA markers will be available for finding micronutrient availability in a less expensive and less time-consuming procedure in rice crops (Chen *et al*., 1997). In view of above. the present work was undertaken to estimate the parental polymorphism in iron and zinc rich rice varieties Swarna and Type 3 using SSR markers.

MATERIALS AND METHODS

The experimental plant material for the study comprised of a donor parent 'Type 3', which is an aromatic rice variety with high iron content, and a recipient parent 'Swarna' (a high yielding variety adaptable long duration, medium resistant to bacterial leaf blight and sheath blight) and SSR markers were collected from IIRR (Indian Institute Of Rice Research), Hyderabad, India.

Genomic DNA was extracted from both the parents as per the protocol described by Sambrook (1989). The extracted DNA was checked for quality and quantity by spectrophotometer. PCR amplification was performed in 96 well plate for 171 RM markers. The PCR plates were labeled with respective sample number and 4 µL (i.e. 50-100 ng) of template DNA was added to the respective wells. The master mix consisted of 1.0 µL forward primer (2.5 pmoles), 1.0 µL reverse primer (2.5 pmoles), 0.5 µL dNTP's (2.5 mM), 0.3 µL Taq DNA polymerase (3U μL^{-1}; Bangalore Genei Pvt. Ltd.), 1.0 µL of 10 x PCR buffer (Tris with 1.5 mM $MgCl_2$, Bangalore Genei Pvt. Ltd.) and 2.2 µL of sterile distilled water was added to make up the volume to 10 µL. Then the master mix (6.0 µL) was dispensed to the PCR plate with template DNA. The PCR plate was covered with a sealing mat. It was then placed in a programmable thermal cycle (M/s Applied Biosystem, USA) for DNA amplification.

PCR amplification

PCR reactions were performed on each DNA sample in a 10 µL reaction mix containing 1 µL of 10x AmpliTaq polymerase buffer, 2 µL of 10 µM primer, 1 µL of 250 µM dNTPs, 1 unit of AmpliTaq DNA polymerase (Bangalore Genei, India) and 75 ng of genomic DNA and a suitable amount of sterile deionized water. DNA amplification was performed in an oil-free thermal cycler (Master Cycler Gradient, Eppendorf). The reaction mix was preheated at 94ºC for 3 min followed by 40 cycles of 1 min denaturation at 94ºC, 1 min annealing at 54ºC and elongation or extension at 72ºC for 2 min. After the last cycle, a final extension of 7 min at 72ºC was added.

Agarose gel electrophoresis

Amplified products from each sample were separated electrophoretically on 1.4% agarose gel (Fisher Biotech, USA) containing ethidium bromide in 1XTAE buffer at 120 V for 1½ h. To determine molecular weight a DNA marker (Ø X 174 DNA/Hae III digest and /or 100 bp DNA ladder) was electrophoresed alongside RAPD products. DNA bands were observed on UV-transilluminator and photographed by a Gel Cam Polaroid camera.

RESULTS AND DISCUSSION

The parental polymorphism was performed by using 171 rice markers (RM) distributed across the 12 chromosomes. Of these, 119 were monomorphic and 52 SSR markers were found to be polymorphic across the 12 chromosomes as given in Table 2 and Fig. 1. Similar results on parental polymorphism were reported by Kiranmayi *et al*. (2014) using 71 gene specific markers, of these only 13 (18.3%) were polymorphic while Gangaprasad Chowdary *et al.* (2013) used 64 rice SSR markers and of these 52 markers showed polymorphism. Similarly, Shankar Ilango and Sarla (2010) used 112 RM markers and found 33 RM primers to be polymorphic. The distribution of 52 polymorphic markers across 12 chromosomes is given in Table 2. Some of the representative polymorphic markers have been shown in gel pictures (Plate 1 & 2). Among all the chromosomes higher number of polymorphic primers i.e. nine were observed on chromosome 6.

Table 1: Distribution of 52 polymorphic markers across 12 chromosomes

Chromosome No.	No. of markers	Markers names
1	7	RM11744, RM11743, RM11741, RM10361, RM11307, RM 243 and RM10018
2	3	RM13347, RM14181 and RM3515
3	5	RM16097, RM15630, RM 282, RM517 and RM232
4	6	RM348, RM261, RM 17201, RM241, RM413 and RM16427
5	5	RM 169, RM142, RM3486, RM413 and RM164
6	9	RM340, RM19341, RM19620, RM3431, RM253, R111, RM276, RM402 and RM190
7	2	RM20844, RM11
8	2	RM22885, RM22524
9	5	RM3912, RM257, RM 24035, RM24085 and RM296
10	1	RM5095
11	3	RM27289, RM206 and RM27962
12	4	RM27962, RM 28607, RM235 and RM5313

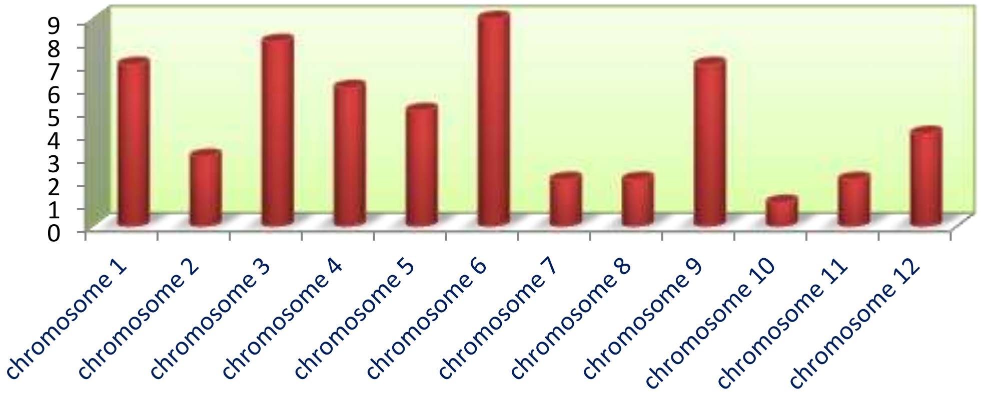

Fig. 1: Parental polymorphic SSR markers across 12 chromosomes

Table 2: Details of parental polymorphic SSR markers

S. No.	Markers	Chr No.	Position (bp)	Forward sequence	Reverse sequence
1	RM11744	1	33,162,055-33,162,203	CCACCCGTATAGGACCAGTCG	TAGAGTCTCCAGGCAGTCTCACC
2	RM 11743	1	33047896-33047923	AAGGTCAAGGAAACAGGGACTGG	AGCCACGAATTCCACTTTCAGC
3	RM 11741	1	32981599 -32981624	TGCAGGTAGAAACTTGAAGC	AGTGGATGTTAGGTGTAACAGG
4	RM 10361	1	6155048-6155081	CCTTGATCGGAAGTAGCTCAACG	GCCCTCAGAGCAGTAAGGAGAGG
5	RM10018	1	272,515-272,662	ACTAGTACACCTCAACTTCACTCC	CCTTTAGTTTGCTTGTGACC
6	RM11307	1	23,929,580-23,929,727	AAAGCTCTGCAATCTTCTCTCC	GAATACGACATCAGAACAGTGC
7	RM243	1	7,970,722-7,970,836	CAGACTGCAGTTGCACGATACTACG	GAAAGCTGCAACGATGTTGTCC
8	RM14181	2	35160202-35160225	AGTACCACCACCATTCTCTGCAAGC	TCGATTGGCCATGAGTTCTCG
9	RM13347	2	19374071-19374103	TCCTTCTCCTTGAACAGCGACAGC	AGAGGCGAGAGGCATGGAGTGC
10	RM3515	2	24,016,552-24,016,748	GGAAAGAAGATATGCCATGC	AGAGAGAATCAGAAACACCAAC
11	RM16097	3	33589253-33589300	CGCCTCGTAAGGTTGAGATCG	TGCCCTGTTCTTTCCATCTTGC
12	RM15630	3	26,073,971-26,074,256	AACTCGAAGGATCTCGCCCAACC	ACCCACCTCCTCACGCTGTACG
13	RM282	3	12,407,382-12,407,510	CTGTGTCGAAAGGCTGCAC	CAGTCCTGTGTTGCAGCAAG
14	RM232	3	76,720,300-76,721,100	CCGGTATCCTTCGATATTGC	CCGACTTTTCCTCCTGACG
15	RM517	3	6,165,992-6,166,181	CAGCTCCTTCCTATCCGTCTCC	TCAGATCTAGCCGAGAAATCAAGG
16	RM261	4	6,574,396-6,574,518	CTACTTCTCCCCTTGTGTCG	TGTACCATCGCCAAATCTCC
17	RM 17201	4	25274544-25274570	GATCGTTGCTGCTTTCAATGAGG	AGTGTTTCACCTTGGACCCATGC
18	RM13482	4	172000000	CCCTGCTGATGCAACTACGG	GCGGATTAGGAGCGTTTGTAGG
19	RM241	4	26,857,374-26,857,637	GTTCATTTCGTGATCTCTGAGC	GCAGATTTACAGGTTTGCTAGG
20	RM413	4	2,212,736-2,212,814	CCAATCTTGTCTTCCGGATCTTGC	AGATAGCCATGGGCGATTCTTGG
21	RM16427	4	4,354,814-4,355,105	CTCCTCATGTCGCTGATTCTTGG	CCGAGATCTACCTCTTGCTGTCC
22	RM142	5	20,518,899-20,519,086	TCTTCCTCTCCACTTCCATTTCC	AAGAAGCTCGGGATCTTCACC
23	RM3486	5	25,908,305-25,908,660	GGAGGTCGGCACGTAGTAGAGG	GTCGGTACTATTCCTGCCATCG
24	RM413	5	2,212,736-2,212,814	CCAATCTTGTCTTCCGGATCTTGC	AGATAGCCATGGGCGATTCTTGG
25	RM164	5	19,196,472-19,196,736	TCTTGCCCGTCACTGCAGATATCC	GCAGCCCTAATGCTACAATTCTTC
26	RM169	5	7,497,918-7,498,084	TGGCTGGCTCCGTGGGTAGCTG	TCCCGTTGCCGTTCATCCCTCC

S. No.	Markers	Chr No.	Position (bp)	Forward sequence	Reverse sequence
27	RM19341	6	1764661-1764696	GCTACAAATAGCCACCCACACC	CAACACAAGCAGAGAAGTGAAGC
28	RM340	6	28,599,181-28,599,319	GGTAAATGGACAATCCTATGGC	GACAAATATAAGGGCAGTGTGC
29	RM253	6	5,425,408-5,425,602	TCCTTCAAGAGTGCAAAACC	GCATTGTCATGTCGAAGCC
30	RM111	6	5,096,744-5,096,867	CACAACCTTTGAGCACCGGGTC	ACGCCTGCAGCTTGATCACCGG
31	RM276	6	6,230,045-6,230,185	CTCAACGTTGACACCTCGTG	TCCTCCATCGAGCAGTATCA
32	RM190	6	1,764,586-1,764,729	GCTACAAATAGCCACCCACACC	CAACACAAGCAGAGAAGTGAAGC
33	RM19620	6	6,212,764-6,212,931	GCGACGAGGAAGAAGATTAGTTCG	GCGGCACTTCGAGCAGTACG
34	RM3431	6	8,744,950-8,745,144	AAGGGAACATTCTGGAAGACACG	ACACATTGCGTGTAGTGTGAAGC
35	RM402	6	6,399,680-6,399,813	CATCTCTGCTAGGTGGTGAATGG	CTCAGCTGGCCTATGACAATGG
36	RM20844	7	653350-653383	GAGAGGGAAGGAGTTTCTTAGC	TAGTTTACACGTACCCATGTGC
37	RM11	7	19,256,914-19,257,039	TCTCCTCTTCCCCCGATC	ATAGCGGGCGAGGCTTAG
38	RM22885	8	14355643-14355666	ACTGGGTGTGATCCTTTCTGATGC	GTGATCCCAGATACACGATGTAGGG
39	RM22524	8	5110450-5110493	GACTTGTGGTTGTTGCTTGTTGG	ACTGCCATATGCATTTCCCTAGC
40	RM3912	9	10,826,210-10,826,472	CACTCAGATTTGGCCGATCC	GCTGATCCAGATCTACCTGACACC
41	RM296	9	46135020-46138300	CACATGGCACCAACCTCC	GCCAAGTCATTCACTACTCTGG
42	RM257	9	17,719,660-17,719,823	CAGTTCCGAGCAAGAGTACTC	GGATCGGACGTGGCATATG
43	RM24035	9	9,969,092-9,969,322	GCTCCAGTTTCTAGTGGGCTTGC	ATGCGGCAGTCAATCAACAGG
44	RM24085	9	10,811,135-10,811,264	CGACGAACTCCTCTACCGTTTACC	CTGCGTGTATCCAATCCCAAGG
45	RM5095	10	51,785-51,964	CTATATGACTATGCGAATGG	ACAAATGCAACTAAGGTAGA
46	RM27962	11	12,079,770-12,079,904	GGGAGTCGTGGATTCTGAGACG	ATCCCACGCCAGGAGATAATAAGG
47	RM 27289	11	26796502-26796522	ATCGATCGATCTTCACGAGG	TGCTATAAAAGGCATTCGGG
48	RM206	11	22,014,679-22,014,851	CCCATGCGTTTAACTATTCT	CGTTCCATCGATCCGTATGG
49	RM28607	12	24,766,329-24,766,528	AGCATTACAGTGTCCAGGTAGGG	CCTCCCTTCTTATATGCCTTTCC
50	RM5313	12	27,139,375-27,139,563	TCTTCTACTCCTCGTCTTCGTTCG	CATGCAGAGCAGAGACTTCTTGG
51	RM235	12	26,107,904-26,108,004	AGAAGCTAGGGCTAACGAAC	TCACCTGGTCAGCCTCTTTCT
52	RM27962	12	12,079,770-12,079,904	GGGAGTCGTGGATTCTGAGACG	ATCCCACGCCAGGAGATAATAAGG

Chr = Chromosome

The screening of markers for parental polymorphism among the rice cultivars forms the basis for tagging of the desired resistance gene, fine mapping of the gene in rice chromosome and in subsequent marker assisted selection (MAS) programmes. The polymorphic rice markers can be used in fine mapping for iron and zinc rich micronutrient genes and to study the mapping population of crosses obtained from these parents (Welch and Graham, 2004). The present work gives the first approach in understanding the biofortification of micronutrient such as iron and zinc in cereals like rice. Because trace minerals are important not only for human nutrition but also for plant nutrition.

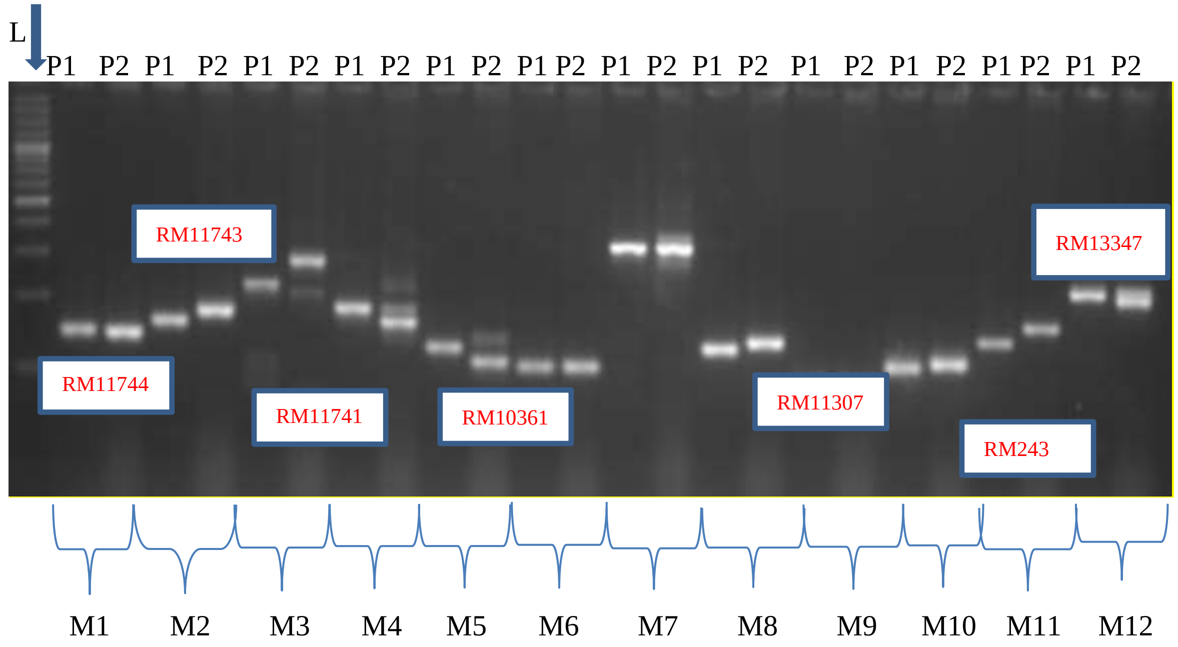

Plate 1: Representative gel picture of parental polymorphism survey with SSR markers

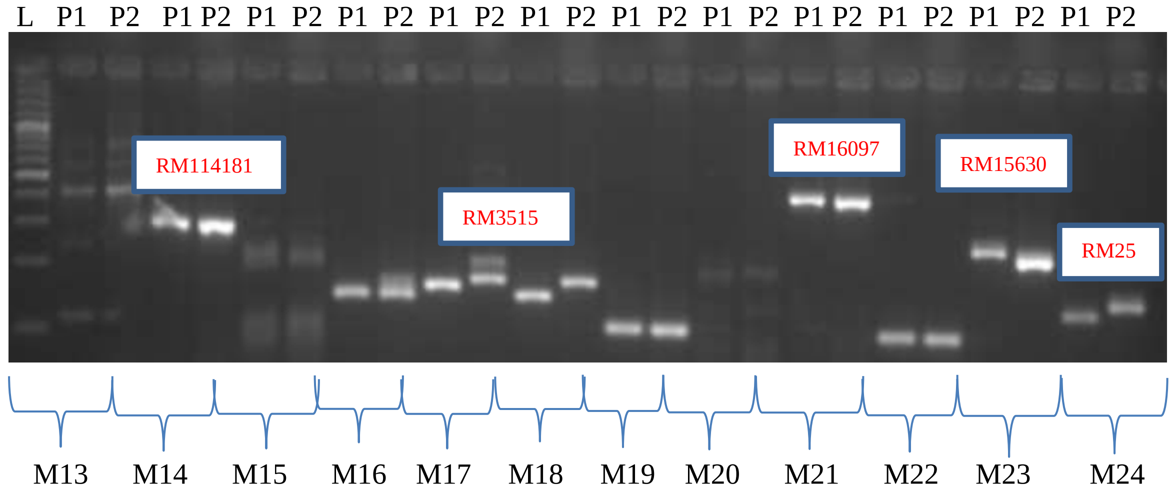

Plate 2: Representative gel picture of parental polymorphism survey with SSR markers L-Ladder (100 bp), P1 – Parent 1 (Swarna), P2 – Parent 2 (Type 3)

Plant breeding with MAS holds great promise for making a significant, low-cost and sustainable contribution to reducing deficiencies of micronutrients, particularly of minerals, in humans and may have important spin-off effects in increasing farm productivity in developing countries in a way that is environmentally beneficial.

Conclusion: The present study provides a strategy that holds great promise in significantly reducing the recurrent expenditures required for high-cost long-run programmes. The present biofortification strategy may significantly reduce the numbers of people requiring treatment, because staple foods are eaten in large quantities every day by the malnourished poor people. The delivery of enriched staple foods (fortified by the plants themselves during growth) can rely on existing consumer behaviours.

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