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Parental polymorphic survey for high Zn and Fe content in grains of rice (*Oryza sativa*. L) using SSR markers

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Abstract
To begin marker assisted selection or marker assisted back cross breeding for introgression of a desirable trait into a variety from the donor parent, study of parental polymorphism is a pre-requisite. Unless the parents are polymorphic for the traits of interest, the further selection of plants carrying the traits of interest is not possible in the progenies. The experiment was conducted in Banaras Hindu University, Varanasi. In the present study Rice microsatellite (RM) markers were used to study the parental polymorphism between the selected two parents PR122 widely grown high yielding but low level of zinc in rice grain (15.90mg kg^{-1}) and IR10M196, pre breeding line found with higher Zn content (30.39mg kg^{-1}) from IRRI, Philippines. The two parents were screened for parental polymorphism using 647 SSR markers, of which 108 markers exhibited polymorphism. The overall polymorphism level for the surveyed SSR markers was 16.69% across the 12 chromosomes. Construction of a Linkage Map could be ensued procuring the generated genotyping data which could further avail QTL analysis and identification of markers linked to zinc content.

Keywords: rice, parental polymorphism, simple sequence repeats (SSR), zinc

Introduction
The availability of large genetic variability in micronutrient concentration in grains of rice and its huge preference as a staple food by large populations, particularly resource poor people in the world, made it the best candidate for biofortification of food grains to enrich with crucial micronutrients (Graham, *et al.,* 1999) [5]. Biofortification is a genetic approach which aims at biological and genetic enrichment of food stuffs with vital nutrients like vitamins, minerals and proteins (Bouis, 2002) [2]. Ideally, once rice is bio-fortified with vital nutrients, the farmer can grow indefinitely without any additional input to produce nutrient packed rice grains in a sustainable way. This is also the only feasible way of reaching the malnourished population in India (Nagesh, *et al*., 2012) [10]. Thus enhancing the availability of grain iron and zinc by biofortification strategy involving molecular breeding tools will help to reduce the problem of global micronutrient malnutrition in mankind. Earlier studies were mostly confined to the production of high yielding varieties, but currently the focus has shifted to enrichment of micronutrients in staple food crops like rice and wheat which helps in ameliorating the problems of micronutrient deficiency in the form of hidden hunger in the human population. Also, in the past two decades, the major effort in breeding has changed from traditional phenotypic-pedigree based selection systems to molecular genetics with emphasis on QTL identification and Marker Assisted Selection (MAS). Breeding efforts to improve the Zn content in the existing cultivars is done with introgression of those genes/Qtl's are responsible for Zn enhancement. There is scope for increasing at least 8-10mg (Harvest Plus 2014) [7]. Screening large no. of cultivars for complex traits such as zinc and iron is able to identify a better entry with high amount of zinc, further it can be used to develop mapping populations for identifying novel Qtl's associated with the trait of interest. Malnutrition in children's is widespread prevalence in the Africa and Asian continent. As per the Harvest plus Assumption, about 35- 40 percent of daily requirement available if we are able to increase the concentration of Zn to the level 30mg Kg^{-1} through biofortification (Saltzman *et al*., 2013) [11]. Rice is the staple food for more than half of the world and enriching zinc in the directly benefits the millions of undernourished children in economically poorer countries (Sharma *et al*. 2013) [12]. Major detrimental effect in enriching Zn is largely affected by soil conditions due to varied levels of Zn content present in it (Cakmak 2008a) [3]. So molecular breeding with precise

phenotypic data is gives reliable results. Rural people who are not affordable to nourished food would get more benefit from the developing Zn rich rice varieties (Bouis *et al*., 2011b) [6].
The term microsatellite was given by Litt and Luty. The microsatellites were found on both coding and non-coding regions. These markers have lower level of mutation rate (10^{-2} and 10^{-4}) per generation Microsatellites are generally 1 to 10 nucleotides long tandem repetitive regions evenly spread throughout the genome. (Gemayel *et al* 2013) [4]. SSRs are most widely used markers over the last two decades for genotyping plants because they are codominant, reproducible and the same markers are usable in related crops (Mason 2015) [9]. The studies of population structure, genetic mapping, and evolutionary processes in crop plants are easily processed with SSR markers. Those SSR markers with higher value of phenotypic information content (PIC) suitable for MAS study. However, significant drawbacks do exist with respect to using microsatellite-based methods, including relatively high development costs and technical challenges during the construction of enriched libraries and species-specific primers

Materials and methods

Before developing a mapping population for mapping of Qtl's for Zn content, initially parental polymorphic survey was conducted using 647 SSR markers to assess the extent of diversity between the parents. For this study, random rice microsatellite (RM) markers were used. The details of parents chosen for the study given in Table 1.

Table 1: Details of parents used for polymorphism study

S. No.	Character	PR122	IR10M196
1.	Parentage	---	IR75862-206-2-8-3-B-B-B/IR69428-6-1-1-3-3
2.	Year of release	2003	Pre Breeding line
3.	Place	Punjab and Western Uttar Pradesh	---
4.	Ecosystem	Irrigated Ecology	Rainfed, Low land
5.	Grain type	Medium bold	Short bold, light brown husk
6.	Height	Tall	Semi-dwarf
7.	Daysto maturity	155 days	130-140 days
8.	Yield (t ha-1)	6.5 t/ha	5.4t/ha
9	Zn Content	15.30mg^{-1}/kg or PPM	30.70mg^{-1}/kg or PPM

***Source*:** http://www.rkmp.co.in

Collection and preservation of leaf material

The leaf samples of ninety six genotypes were collected from 15-20 days old seedlings of selected genotypes from the field during early hours (9am) and stored at -20^{0}C for further use.

DNA extraction

Genomic DNA was extracted from each selected genotypes by TPS method (Table 2) for genotyping. TPS method as compared with CTAB, shortened by about 80-90% and reduces costs of about 90%. For the TPS method, ≈2-cm lengths of rice leaf tips were harvested and ground by using a Geno Grinder 2010 in TPS buffer [100 mM Tris·HCl (pH 8.0), 1 M KCl, 10 mM EDTA]. After centrifugation, the supernatant was recovered, and an equal volume of isopropyl alcohol was added. Isopropyl alcohol-insoluble material was recovered by centrifugation, and the pellet was washed with 70% ethanol. The pellet was then dried and dissolved in TE [10 mM Tris·HCl (pH 8.0), 1 mM EDTA]. The purified DNA samples were then genotyped by using SSR markers.

Table 2: TPS buffer composition

Component	Stock concentration	Working concentration	Volume/quantity of stock
Tris-HCl (pH 8.0)	1M	100 mM	100 ml
EDTA (pH 8.0)	0.5M	10 mM	20 ml
KCl	1M	1.4 M	74.55(g)
Nano pure water	To make the volume		
Total volume	1000 ml		

*mM- milli-molar, g- Gram

DNA extraction from leaf sample

Fresh leaf samples (around 100mg) chopped into small pieces and put it in 2ml micro-centrifuge tube. Steel balls in each well have been added, 600µl of TPS buffer added in the each tube and Grinded them using the Geno Grinder. After grinding, plates were kept in water-bath at 60°C for 30 minutes, then centrifuged at 3000rpm, at 24°C for 30 minutes. The supernatant was transferred into fresh DNA tubes immediately after centrifugation. Equal volume of chilled isopropanol to the each well and keep it at 4°C for overnight incubation to remove the supernatant and add once again 150µl of ethanol, Centrifuge at 4°C, 3000 rpm for 30 minutes. Remove the supernatant and dried the pellet in incubator for 1 hour Add 150µl of TE buffer and incubated at 55°C for 5 minutes and Stored the DNA @ 4°C for immediate usage.

Procedure for quality and quantity estimation of DNA

Quantification of DNA was done by analyzing the purified DNA on 0.8 percent agarose gel using diluted 100 bp ladder DNA as standard. Based on the intensity and thickness of genomic DNA bands when compared to DNA, the concentration and quality of DNA in individual samples was determined. The gel was prepared by taking 0.8 g of agarose in 100 ml of 1X TAE buffer. The agarose was melted in a microwave oven until a clear, transparent solution was obtained. Appropriate gel tray was fixed into the gel cast and the combs were placed on the gel tray. Melted agarose was allowed to cool for 5 minutes to room temperature and ethidium bromide was added, mixed well by swirling and poured into the gel tray with prefixed combs, carefully avoiding the formation of air bubbles. The gel was allowed to solidify at room temperature for 1 hr. The solidified gel was

transferred to an electrophoresis unit containing the running buffer (1X TAE). The DNA samples were mixed with 1/3rd volume of gel loading cum tracking dye (0.25 % sucrose: 0.25 % Bromophenol blue) and loaded onto the gel. Electrophoresis was carried out at 100 volts at room temperature for about 1hr after which, the gel was visualized in a UV light transmitted gel documentation system (BIORAD, USA). After checking the concentration of the DNA, based on the band intensity, the DNA samples were diluted to 50ng l-1 for further PCR analysis.

Genomic DNA amplification by PCR using SSR-markers

The PCR was carried out using a programmable thermocycler (Eppendorf, Germany). The PCR plates were taken and 2 ml of 50 ng template DNA was pipette out into each PCR tube after proper labeling and kept in the PCR plate at 4oC. The master mix was prepared by taking 0.5 ml of 10 pmol each of both forward and reverse primers, 0.75 ml of 2.5 mM deoxy ribonucleotides (dNTPS), 1 ml of 10X assay buffer (Banglore Genei Private Limited, Bangalore), 10 mM Tris-Cl (pH 8.3), 50 mM KCl, 1.5 mM MgCl2, 0.01% gelatin, 0.2 ml of 3 U/ml Taq DNA polymerase (Banglore Genei Private Limited, Bangalore) and 5.05 ml of sterile distilled water to make up the volume to 10 ml. The quantity of master mix to be prepared depends on the number of samples that are set for PCR. The master mix was centrifuged for a short duration of about 10 sec for thorough mixing of the components. 8 ml of master mix was added to each PCR tube having 2 ml of template DNA to make the final volume to 10 ml. The PCR plate was covered and kept in a thermal cycler which was set to a thermal profile given below: STEP-I Initial denaturation 94oC for 5 minutes, Denaturation 94oC for 30 seconds, STEP-II Primer annealing 55oC for 30 seconds 35 cycles Elongation 72oC for 1 minute STEP-III Final elongation 72oC for 7 minutes. The temperature profile including primer annealing temperatures was the same for all primers used under this study.

Agarose gel electrophoresis

The PCR products were analyzed by electrophoresis using a 3 % agarose gel using a gel Electrophoresis Unit (CBS Scientific, USA). About 9.0 g of agarose was weighed and transferred to a reagent bottle containing 300 ml of 1X TAE buffer and mixed well. The contents were boiled gently in a microwave oven with intermittent mixing. The process was followed until complete melting of agarose was achieved and the solution became crystal clear. The gel-casting tray was washed with water and wiped with ethanol. The boiled agarose was cooled to room temperature, 2 ml of ethidium bromide (10 mg ml-1) was added to the melted agarose, mixed thoroughly and poured into the gel cast tray prefixed with the appropriate gel combs and was allowed to solidify at room temperature for 20-30 minutes. The gel was transferred to the Electrophoresis Unit containing 1X TAE buffer. Before loading, PCR amplified products were mixed with 1/6th volume of gel loading dye (40 % sucrose and 0.25 % bromophenol blue) and loaded into the wells. 100 bp ladder was added in one well to determine the size of amplified fragments. The DNA fragments were visualized under UV-transilluminator and documented using gel documentation system.

Results and Discussion

The parental polymorphism survey indicated that a clear polymorphism was observed between the parents. A total of 647 SSR primer pairs mapped on all the 12 chromosomes were used for testing polymorphism between two parents. 108 SSR primer pairs exhibited polymorphism between recipient parent PR122 and donor parent IR10M196 and remaining 539 were monomorphic. Percentage of polymorphism highest (Table 4) on chromosome 8 (34.78) and least on chromosome 10 (7.69). The average per cent polymorphism on all the chromosomes was 17.05. The lack of detectable polymorphism between two parents could be due to the fact that both parents, PR122 and IR10M196 are indica lines. Lack of molecular marker polymorphism between indica genotypes has been earlier noticed in studies by Xu. (2002) [13] and Biradar (2004) [1]. Polymerase Chain Reaction was done to check each sample for parental polymorphism using specific Rice Microsatellite (RM) primers as shown in fig. 1. Out of 647 primers used the parents showed polymorphic only to 108 RM primers listed in Table 3. The percentage of polymorphism was calculated using the formula:

$$= \frac{\text{RM Primers Showing Polymorphism}}{\text{The total number of RM primers use}} \times 100$$

Table 3: Microsatellite markers exhibiting polymorphism between PR122 X IR10M196

S. No.	Marker Name	Chr No.	Forward Primer	Reverse Primer
1	RM212	1	CCACTTTCAGCTACTACCAG	CACCCATTTGTCTCTCATTATG
2	RM220	1	GGAAGGTAACTGTTTCCAAC	GAAATGCTTCCCACATGTCT
3	RM243	1	GATCTGCAGACTGCAGTTGC	AGCTGCAACGATGTTGTCC
4	RM248	1	TCCTTGTGAAATCTGGTCCC	GTAGCCTAGCATGGTGCATG
5	RM297	1	TCTTTGGAGGCGAGCTGAG	CGAAGGGTACATCTGCTTAG
6	RM302	1	TCATGTCATCTACCATCACAC	ATGGAGAAGATGGAATACTTGC
7	RM443	1	GATGGTTTTCATCGGCTACG	AGTCCCAGAATGTCGTTTCG
8	RM488	1	CAGCTAGGGTTTTGAGGCTG	TAGCAACAACCAGCGTATGC
9	RM493	1	TAGCTCCAACAGGATCGACC	GTACGTAAACGCGGAAGGTG
10	RM562	1	CACAACCCACAAACAGCAAG	CTTCCCCCAAAGTTTTAGCC
11	RM594	1	GCCACCAGTAAAAGCAATAC	TTGATCTGCTAGTGAGACCC
12	RM12433	2	CTCTACACGCTTTGCCTGTTTGG	TGATCAGGTTCGAGATTGGGTATGG
13	RM1367	2	GTGTGTACGTAGGATCGGAG	TGCTACTCCTAGCTGCTACC
14	RM145	2	CCGGTAGGCGCCCTGCAGTTTC	CAAGGACCCCATCCTCGGCGTC
15	RM154	2	ACCCTCTCCGCCTCGCCTCCTC	CGATCTGCGAGAAACCCTCTCC
16	RM240	2	CCTTAATGGGTAGTGTGCAC	TGTAACCATTCCTTCCATCC
17	RM250	2	GGTTCAAACCAAGCTGATCA	GATGAAGGCCTTCCACGCAG
18	RM263	2	CCCAGGCTAGCTCATGAACC	GCTACGTTTGAGCTACCACG
19	RM290	2	ACCCTTATTCCTGCTCTCCTC	GTGCTGTAGATGGAAGGGAG

20	RM324	2	CTGATTCCACACACTTGTGC	GATTCCACGTCAGGATCTTC
21	RM341	2	CAAGAAACCTCAATCCGAGC	CTCCTCCCGATCCCAATC
22	RM424	2	TTTGTGGCTCACCAGTTGAG	TGGCGCATTCATGTCATC
23	RM425	2	CCAACGAAGATTCGAAGCTC	CAGCACCATGAAGTCGCC
24	RM475	2	CCTCACGATTTTCCTCCAAC	ACGGTGGGATTAGACTGTGC
25	RM485	2	CACACTTTCCAGTCCTCTCC	CATCTTCCTCTCTTCGGCAC
26	RM525	2	GGCCCGTCCAAGAAATATTG	GAGACAGAATCCTTACGCTGTGC
27	RM573	2	CCAGCCTTTGCTCCAAGTAC	TCTTCTTCCCTGGACCACAC
28	RM6374	2	TGAGGACGCTGATTGTCAAC	GCTGCCCCTATTATTTCACC
29	RM6	2	GTCCCCTCCACCCAATTC	TCGTCTACTGTTGGCTGCAC
30	RM3212	2	GACCCGAGCTAGACGACAAACACC	ACGCAGCCTCGCACTGTACTCG
31	RM29	2	CAGGGACCCACCTGTCATAC	AACGTTGGTCATATCGGTGG
32	RM130	3	TGTTGCTTGCCCTCACGCGAAG	GGTCGCGTGCTTGGTTTGGTTC
33	RM15924	3	GGCTCAATCCCTATGATCACAATCC	TTCCACAACAGCCCTTCAAATCC
34	RM16030	3	GCGAACTATGAGCATGCCAACC	GGATTACCTGGTGTGTGCAGTGTCC
35	RM168_IR	3	GATGGTTTGGAGGATCGGGT	AACGAATCAATCCACGGCAC
36	RM231	3	CCAGATTATTTCCTGAGGTC	CACTTGCATAGTTCTGCATTG
37	RM251	3	GAATGGCAATGGCGCTAG	ATGCGGTTCAAGATTCGATC
38	RM347	3	CACCTCAAACTTTTAACCGCAC	TCCGGCAAGGGATACGGCGG
39	RM411	3	ACACCAACTCTTGCCTGCAT	TGAAGCAAAAACATGGCTAGG
40	RM426	3	ATGAGATGAGTTCAAGGCCC	AACTCTGTACCTCCATCGCC
41	RM7	3	TTCGCCATGAAGTCTCTCG	CCTCCCATCATTTCGTTGTT
42	RM81B	3	GAGTGCTTGTGCAAGATCCA	CTTCTTCACTCATGCAGTTC
43	RM127	4	GTGGGATAGCTGCGTCGCGTCG	AGGCCAGGGTGTTGGCATGCTG
44	RM16556	4	TTGGACCAGGAGATCAATGAAGG	GTGCGCACACTCTTCTATGTGC
45	RM17620	4	ACCATCTCGTATTTGGCTCATCC	AACATGCACTGGATGATCTCTCG
46	RM252	4	TTCGCTGACGTGATAGGTTG	ATGACTTGATCCCGAGAACG
47	RM303	4	GCATGGCCAAATATTAAAGG	GGTTGGAAATAGAAGTTCGGT
48	RM307	4	GTACTACCGACCTACCGTTCAC	CTGCTATGCATGAACTGCTC
49	RM348	4	CCGCTACTAATAGCAGAGAG	GGAGCTTTGTTCTTGCGAAC
50	RM5687	4	GATCGCTGGCGATTGATC	GACTTGTGGGGTGGTTTTTG
51	RM122	5	GAGTCGATGTAATGTCATCAGTGC	GAAGGAGGTATCGCTTTGTTGGAC
52	RM161	5	TGCAGATGAGAAGCGGCGCCTC	TGTGTCATCAGACGGCGCTCCG
53	RM267	5	TGCAGACATAGAGAAGGAAGTG	AGCAACAGCACAACTTGATG
54	RM31	5	GATCACGATCCACTGGAGCT	AAGTCCATTACTCTCCTCCC
55	RM334	5	GTTCAGTGTTCAGTGCCACC	CACTTTGATCTTTGGTGGACG
56	RM459	5	CTGCAATGCTGCATGACC	CACTTTCTCTGCAGCACCAG
57	RM548	5	TCGGTGAGAAACTGAGAGTACG	AAGGAGGCCATCTCAATGTG
58	RM19771	6	AACCAATGCACACTTCTTCTGTGC	CAACTGTAGAGGTTGGAATGATCTGC
59	RM204	6	GTGACTGACTTGGTCATAGGG	GCTAGCCATGCTCTCGTACC
60	RM340	6	GGTAAATGGACAATCCTATGGC	GACAAATATAAGGGCAGTGTGC
61	RM510	6	AACCGGATTAGTTTCTCGCC	TGAGGACGACGAGCAGATTC
62	RM527	6	GGCTCGATCTAGAAAATCCG	TTGCACAGGTTGCGATAGAG
63	RM585	6	CAGTCTTGCTCCGTTTGTTG	CTGTGACTGACTTGGTCATAGG
64	RM5850	6	TTATACACAGATGACGCACACG	TGGGTTAAGGGACACACTTAGG
65	RM587	6	ACGCGAACAAATTAACAGCC	CTTTGCTACCAGTAGATCCAGC
66	RM225	6	TGCCCATATGGTCTGGATG	GAAAGTGGATCAGGAAGGC
67	RM10	7	TTGTCAAGAGGAGGCATCG	CAGAATGGGAAATGGGTCC
68	RM134	7	TGTTGCTTGCCCTCACGCGAAG	GGTCGCGTGCTTGGTTTGGTTC
69	RM336	7	CTTACAGAGAAACGGCATCG	GCTGGTTTGTTTCAGGTTCG
70	RM351	7	CCATCCTCCACCGCCTCTCG	TGGAGGAAGGAAAGGGGACG
71	RM418	7	TCGCGTATCGTCATGCATAG	GAGCACATATGCCACGTACG
72	RM505	7	AGAGTTATGAGCCGGGTGTG	GATTTGGCGATCTTAGCAGC
73	RM11	7	CACAACCTTTGAGCACCGGGTC	ACGCCTGCAGCTTGATCACCGG
74	RM248	7	AGAGAGCAAGTTTGAAGCGAAGC	ACCAAGAGGGTAGCCTAGCATGG
75	GM4 DRR	8	CGAGAGAAGACAAGAAGGTAGAA	TCTCAGAAAGTACCGGCAGT
76	RM137	8	GACATCGCCACCAGCCCACCAC	CGGGTGGTCCCCGAGGATCTTG
77	RM149	8	GCTGACCAACGAACCTAGGCCG	GTTGGAAGCCTTTCCTCGTAACACG
78	RM210	8	TCACATTCGGTGGCATTG	CGAGGATGGTTGTTCACTTG
79	RM22711	8	CGACTACGTACTTCCTTCCA	ATGAACGGAGGTGTACTTGT
80	RM264	8	GTTGCGTCCTACTGCTACTTC	GATCCGTGTCGATGATTAGC
81	RM281	8	ACCAAGCATCCAGTGACCAG	GTTCTTCATACAGTCCACATG
82	RM310	8	CCAAAACATTTAAAATATCATG	GCTTGTTGGTCATTACCATTC
83	RM337	8	GTAGGAAAGGAAGGGCAGAG	CGATAGATAGCTAGATGTGGCC
84	RM342A	8	CCATCCTCCTACTTCAATGAAG	ACTATGCAGTGGTGTCACCC
85	RM408	8	CAACGAGCTAACTTCCGTCC	ACTGCTACTTGGGTAGCTGACC
86	RM515	8	TAGGACGACCAAAGGGTGAG	TGGCCTGCTCTCTCTCTCTC
87	RM547	8	TTGTCAAGATCATCCTCGTAGC	GTCAAGATCATCCTCGTAGCG
88	RM556	8	ACTCCAAACCTCACTGCACC	TAGCACACTGAACAGCTGGC

89	RM72	8	CCGGCGATAAAACAATGAG	GCATCGGTCCTAACTAAGG
90	RM80	8	TTGAAGGCGCTGAAGGAG	CATCAACCTCGTCTTCACCG
91	RM219	9	CGTCGGATGATGTAAAGCCT	CATATCGGCATTCGCCTG
92	RM23679	9	TCACAGCTTAGTGCATGTTGAGC	GATTCACCTGGCAATGAGAACG
93	RM23865	9	TCATCCCATTCTCTTCCTCACC	CATACGGCCATACAAATGAACC
94	RM242	9	GGCCAACGTGTGTATGTCTC	TATATGCCAAGACGGATGGG
95	RM278	9	GTAGTGAGCCTAACAATAATC	TCAACTCAGCATCTCTGTCC
96	RM434	9	GCCTCATCCCTCTAACCCTC	CAAGAAAGATCAGTGCGTGG
97	RM444	9	GCTCCACCTGCTTAAGCATC	TGAAGACCATGTTCTGCAGG
98	RM464	9	GAAGCAGGAAACAAGAAGAGAAGG	AACGGGCACATTCTGTCTTC
99	RM566	9	ACCCAACTACGATCAGCTCG	CTCCAGGAACACGCTCTTTC
100	RM474	10	AAGATGTACGGGTGGCATTC	TATGAGCTGGTGAGCAATGG
101	RM224	11	ATCGATCGATCTTCACGAGG	TGCTATAAAAGGCATTCGGG
102	RM27149	11	TTTGTGGATGGAGGACTAGAACG	CATGACCAAACAGACAGCTAATACCC
103	RM27290	11	ACGTGCTTCGTTTCTCTTTGACG	CGAACTGTGGACTTCTCACTTGTCC
104	RM27296	11	GGGTCTTTGTACACATTCTTGTGG	CTTGAAGGATGAGCAGTATCTCG
105	RM4A	11	TTGACGAGGTCAGCACTGAC	AGGGTGTATCCGACTCATCG
106	RM7228	11	CTCGTATTGGGTTCTTGTATGG	AGACTGCTGTTCAGGTGTAATCC
107	RM17	12	TGCCCTGTTATTTTCTTCTCTC	GGTGATCCTTTCCCATTTCA
108	RM28166	12	TGCTTGCAAACATTGCTTCTGG	ACTGATGTACTGAACACGGGAAGG

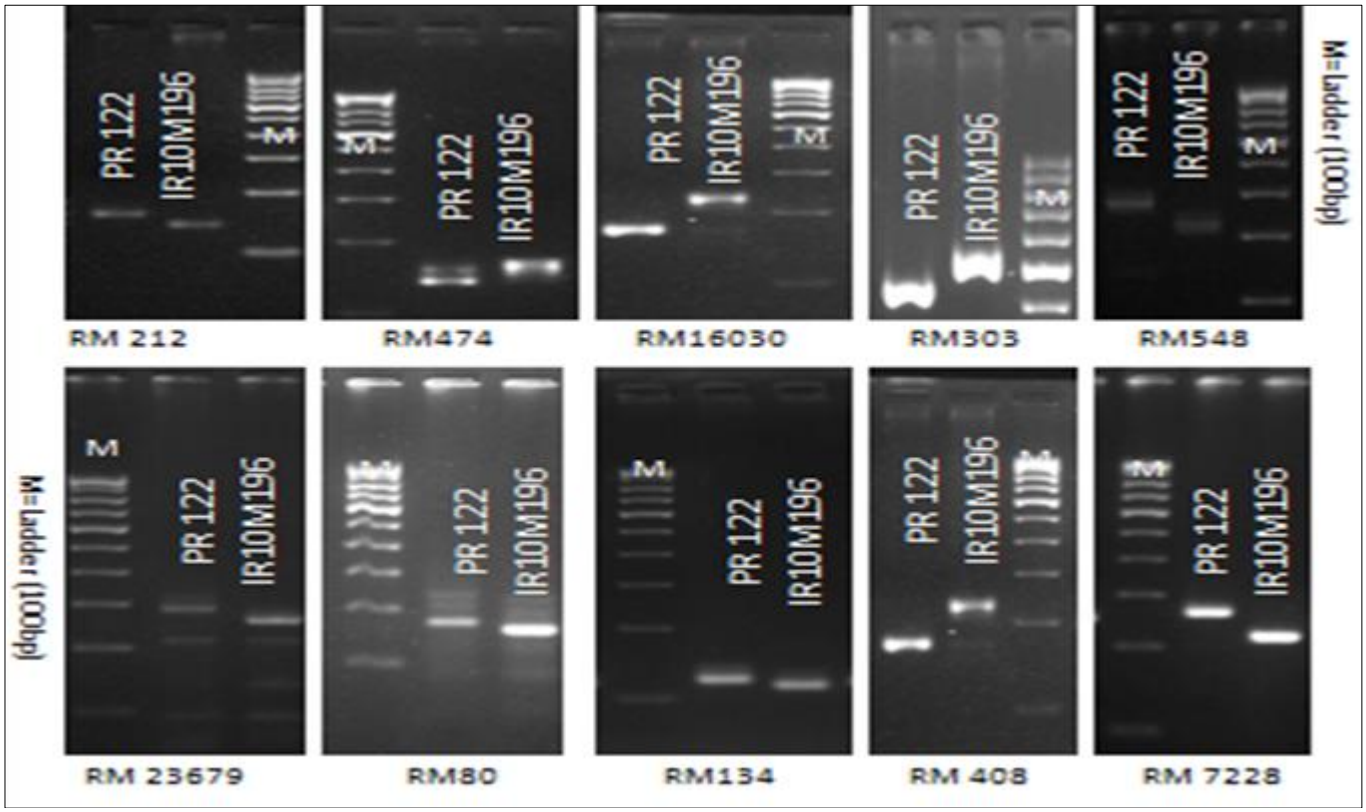

Fig 1: Polymorphism between recipient parent PR122 and donor parent IR10M196 using various SSR's

Table 4: Chromosome wise percentage of primers showing polymorphism between parents, PR122 and IR10M196

Chr No.	Total Number markers screened for each chromosome	No. of Polymorphic markers on each chromosme	No. of monomorphic primers on each chromosome	Perecentage of polymorphism on each chromosome
1	85	11	74	12.94
2	97	22	75	22.68
3	53	11	42	20.75
4	68	8	60	11.76
5	54	7	47	12.96
6	45	9	36	20.00
7	62	8	54	12.90
8	46	16	30	34.78
9	33	9	24	27.27
10	13	1	12	7.69
11	46	6	40	13.04
12	15	2	13	13.33

The usage of molecular marker technology in breeding programs has greatly increased its efficiency and fastens the transfer of desirable genes among varieties and to introgress novel genes from related wild species. Polygenic markers which were previously difficult to analyze using traditional breeding methods, would now be easily tagged using molecular markers. The screening of markers for parental polymorphism among the rice cultivars forms the basis for tagging of the desired gene, fine mapping of the gene in the rice chromosome and in the subsequent Marker Assisted Selection (MAS) programmes. The polymorphic RM markers can be used in the fine mapping of Zinc trait and to study the mapping populations of crosses obtained from these parents.

Conclusion

The outcome of the present study represent the main approach in understanding the quality traits to the extent of how much complexity in diversity and its inheritance in rice breeding and how it can be materialized to improve micronutrient availability (Zn and FE). A little improvement in Zn concentration directly benefits the people of the country.

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