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STUDY OF ALLELIC VARIATION AT GENOME WIDE SSR LOCI IN PARENTS OF MAPPING POPULATION FOR HIGH GRAIN ZINC IN RICE (*Oryza sativa* L.)

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ABSTRACT

Rice (*Oryza sativa* L.) is the source of energy and nutrition for more than half of the world's population hence it is a crop of global significance. Breeding of mineral dense rice varieties is the main target of biofortification strategy to address micronutrient malnutrition globally. Landraces and local genotypes are proven sources of novel alleles and are a promising donor for high grain mineral. A parental polymorphism survey is a prerequisite of any QTL mapping experiment. Present study consisting of SSR markers based parental polymorphism survey at IRRI South Asia Hub ICRISAT, Hyderabad. Parents of mapping population viz Rajendrakasturi (short grain aromatic rice variety with low grain zinc content) and URG-30 (a local genotype from Eastern Uttar Pradesh with high grain zinc content) were screened with 1013 microsatellite markers covering the entire length of all 12 chromosomes to study allelic variation at genome-wide SSR loci. The geographical diverse origin of parents is reflected in genotypic variations in terms of polymorphism. Out of 1013 whole genome wide SSR markers screened, 294 were found to be polymorphic which resulted in 29.02% polymorphism between the two parents. The highest polymorphism was observed with chromosome 4 (40.96%) whereas the lowest polymorphism was observed in chromosome 9 (16%). Based on the outcomes of the present study, a set of genome-wide polymorphic SSRs will be selected for genotyping of mapping population, preparation of linkage map and QTL analysis for high grain zinc content, iron content, grain quality and yield traits.

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1 Introduction

Rice is the world's most important crop and more than half of the global population is dependent on rice (Rao et al., 2020). Rice is the chief source of carbohydrate and is used as an integral part of a balanced diet for billions of people in the world (Das et al., 2020). So it is a crop of global significance. In the Indian context, rice has deeply embedded itself in Indian culture. Although the country has attained self-sufficiency in rice production (Ghose et al., 2013) but nutritional enrichment of rice with enhanced grain quality, without compromise on yield and consumer acceptance is a new challenge ahead for the plant breeders. Even though rice is rich in starch (80%) but it is deficient in major micronutrients such as zinc and iron. Zinc (Zn) is one of the essential micronutrients for human health which plays an important role in the normal functioning of the immune system, reproductive growth, nervous system development and behavior (Hotz & Brown, 2004). Iron (Fe) and Zinc (Zn) deficiencies are among the most prevalent micronutrient deficiencies in humans and one-third of the world's population are at the risk of zinc deficiency (HarvestPlus, 2014). Dietary deficiency of iron and zinc is identified as a major cause of malnutrition (Bouis & Saltzman, 2017). Micronutrient deficiencies have raised health risk in developing countries and also its existing spectrum has expanded to those strata of society who is dependent on the unbalanced diet of single staple food rice hence impacting global public health (Virk & Barry, 2009).

Biofortification is the process of enriching food grains for nutritive elements in crop plants using genetic approaches (Pradhan et al., 2020a). The biofortification strategy implemented with the aids of breeding will help to address the problem of micronutrient malnutrition in a more sustainable way (Bouis & Welch, 2010). The precision breeding approach is recommended for nutritional enhancement of high yielding varieties with major micronutrients which includes the use of potential donors, identification of QTLs for high grain mineral content, and identification of robust markers (Pradhan et al., 2020b). Dissecting genomic complexity by mapping quantitative trait loci (QTLs) for grain quality traits, for their deployment in marker-assisted selection and gene discovery is an effective forward genetic approach (Swamy et al., 2012). To design an effective breeding strategy of rice biofortification, identification of genomic regions associated with grain iron and zinc content is essential (Bollinedi et al., 2020). Improvement of any trait in rice has always benefited to a sizable proportion of the population.

In this context, plant breeders can play a major role in screening genetic variability in the trace mineral concentration, identification of genetic basis and genomic region of complex polygenic traits, perform target trait mapping and marker-assisted selection for the improvement of grain quality and nutritional traits. SSRs marker always remains the choice of plant breeders, due to their abundance, genome coverage, low analysis cost, co-dominant

nature, high reproducibility, high level of polymorphism and their high utility in marker assisted selection.

Although there are various markers systems available in rice, microsatellites or simple sequence repeats (SSRs) have high polymorphism and are technically simple for detection hence are widely used (McCouch et al., 2002). SSRs have been used widely in the majority of crop species including rice for which sequence information is available. These are mainly useful for gene tagging, genetic diversity studies, genotyping of mapping population, linkage map preparation, tracing marker-trait association, single-marker analysis and all types of QTL mapping analysis. Generally, there are two types of SSRs viz Class I and Class II. Class I SSRs also called hypervariable markers have longer repeats of ≥ 20 nucleotides in length hence are more polymorphic than Class II SSRs (Cho et al., 2000; Temnykh et al., 2000).

Parental polymorphism survey is a prerequisite for every QTL mapping experiment. Hence the present study was conducted to screen genome wide distributed large number of SSR markers between two parental genotypes of rice viz Rajendrakasturi and URG-30. The study was aimed to identify the polymorphic markers set for allelic variation between both parents, to study their distribution across the chromosomes and identify repeat motif. Based on these, a subset of SSR will be selected and utilized for performing QTL mapping in mapping population developed from the above cross between these two parents for various traits of interest like nutritional trait involving high grain zinc content, high grain iron content; grain quality traits and yield trait. As per phenotypic evaluation of parents a higher amount of variation was observed for enough traits like yield, yield-related traits, quality and nutritional traits. So, there is huge scope for QTL mapping analysis for the various number of traits in mapping population developed from the cross of both the parents.

2 Materials and Methods

2.1 Selection of Parents for Study

The two parents viz Rajendrakasturi and URG-30 were selected for this study, among these Rajendrakasturi is short grain aromatic variety which developed from Kasturi $\times$ Sungadha parentage by Dr. Rajendra Prasad Central Agricultural University, Pusa, Bihar. This was selected for its quality traits while URG-30 is a genotype from Eastern Uttar Pradesh collected by the Institute of Agricultural Sciences, Banaras Hindu University, Varanasi as local collection material and identified and considered by the Harvest Plus project as stable high zinc donor with high grain iron content. Both selected parents have contrasting features for desirable grain quality and nutritional traits. Details of all the traits of both the parents have been mentioned in table 1. The mapping population was developed by crossing both parents and advancing they confirm F_1 plant to raise F_2 population.

Table 1 Characteristics features of parental lines; two parents used for present study Rajendra kasturi and URG-30 used in the experiment

S.No.	Trait name	Rajendrakasturi	URG-30
1.	Source	Available in project material at Institute of Agricultural Sciences, BHU, Varanasi, U.P, India.	Upland rice genotype collected as Local germplasm by Institute of Agricultural Sciences, BHU, Varanasi, U.P, India.
2.	Parentage	Kasturi × Sungadha	Upland rice genotypes
3.	Release year and Centre	2004 by Dr. Rajendra Prasad Central Agricultural University, Pusa, Bihar	Included in Harvest Plus Project (High zinc donor)
4.	Yield	40-42q/ha	28-30q/ha
5.	Days to maturity	125-130 days	108-110 days
6.	Plant Height	105cm	101cm
7.	Brown rice Zinc content	17-19 ppm	30-33 ppm
8.	Aroma	Present	Absent
9.	Kernel length	4.0 mm	5.02 mm
10.	Kernel width	1.9 mm	2.4 mm
11.	Kernel color	White	Red
12.	Panicle type	Compact	Spreading
13.	Seed shattering	Absent	Present
14.	Awns	Absent	Present (small)

2.2 Field trial and leaf sample collection

Present experiment was conducted during *Rabi*-2018 at IRRI South Asia Hub, ICRISAT, Hyderabad. Both parents *viz* Rajendrakasturi and URG-30 were raised by following all recommended package of practices of the region. Leaf samples of both the parents were collected from 21 days old seedlings which were transplanted in the field later for evaluation. Collected leaf samples were preserved at -20°C up to further use. Both parents were evaluated for morphological and yield-related traits. Collected leaf samples were used for DNA isolation for the present study.

2.3 DNA isolation

Parental genomic DNA isolation was done by a quick DNA extraction method (TPS Buffer) adopted from Dr. Yohie Koide for IRRI-JAPAN Collaborative Research Project and modified from Lenie A. Quiatchon (Kim et al., 2016). Micro Tubes of 1.1 ml were added with fresh leaf samples (around 100 mg), two steel beads and 600µl of TPS buffer (100mM Tris HCl pH 8.0, 10mM EDTA, 1M KCl, nano pure water for volume makeup). Micro Racks sealed properly and subjected to Geno Grinder with speed 900 rpm set for 3 min. (repeat this 3-4 times) and incubated in a water bath at 55°C for 30 min. Then samples were centrifuged for 30 min, at 3000 rpm and

the supernatant was collected in a fresh tube (96 Eppendorf deep well storage microplate). Chilled isopropanol @ 300 µl was added to each tube and kept in the refrigerator at -20°C for overnight. DNA pellet was collected by centrifuge at 3000 rpm for 30 min at 4°C and washed with 160µl 70% ethanol and centrifuge at 2000 rpm for 20 min. Pouring off ethanol, pellets were allowed to dry up completely and rehydrated with 100 µl of 1X TE buffer (1M Tris pH-8.0, 0.5M EDTA pH- 8.0).

2.4 Quantification of Genomic DNA and Agarose gel electrophoresis.

The quality of DNA was evaluated by running it on 0.8% agarose gel along with a standard ladder and compared with band intensity and thickness. 0.8% Agarose (800 mg) was added to 100 ml of 1X TAE buffer melted till it became transparent. The solution was cooled to about 50°C, added with 6 µl of ethidium bromide (0.5 µg mL⁻¹), and poured into the gel casting unit. 2µl of DNA samples added with gel loading dye were electrophoresed (GeNei horizontal mini gel unit) in agarose gel at 60 Volts in TAE buffer for 1 hr. After electrophoresis, the band intensity of genomic DNA was visualized on the Gel documentation unit (SYNGENE GBox, UK) (Yerva et al., 2018). These gels provided a visual measure of purity and integrity of DNA.

2.5 Use of Rice Microsatellite Markers for the parental polymorphism survey

A total of 1013 microsatellite markers (IDT[®]) covering the entire length of each of 12 chromosomes of rice were used for this study. In this study, it is ensured that marker distribution will be uniform with whole-genome coverage across all chromosomes. The details about McCouch Locus_ID, physical position (SSR start and SSR end), number of repeat motif, forward and reverse primer sequences, chromosome number, etc., were obtained from the Gramene markers database (<https://www.gramene.org/>).

2.6 Use of RAP-DB

For those markers whose information was not available in the Gramene database, it was obtained from RAP-DB (The Rice Annotation Project DataBase) by using its BLAST tool and submitting query sequence in FASTA format to run a search against DNA database (blastn) (<https://rapdb.dna.affrc.go.jp/tools/blast>).

2.7 PCR amplification of genomic DNA using SSR markers

DNA amplification using SSR markers was carried out with Polymerase chain reaction in Eppendorf nexus gradient master cycler. PCR reaction was done in 0.2ml 96 well PCR plates (Watson 137-174C) by adding 1µl *Taq* buffer [MgCl₂: 100mM Tris (pH 9.0) 500mM KCl, 15mM MgCl₂, and 0.1% gelatin], forward and reverse primer each 0.5µl (10 picomoles each), 3µl of genomic DNA, 0.5µl of dNTPs (2.5mM), 0.1µl *Taq* DNA polymerase 3U/µl (Bangalore GeNei Laboratories Private Limited) and 4.4µl of sterilized distilled water to make a final volume of 10µl for each reaction (Yerva et al., 2018). PCR plate kept in a thermal cycler and the reaction was set as 1st cycle initial denaturation at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing at 58°C (touch down -0.1°C/cycle) for 30 seconds and primer extension at 72°C for 1 minute. A final extension was given at 72°C for 5 min at the end of the cycle and samples were held at 4°C till retrieval. PCR product was analyzed on 3% agarose gel added with Ethidium bromide and standard DNA ladders of 50 bp and 100 bp was then visualized under the Gel Doc system. Band pattern was observed and polymorphic markers were recorded.

2.8 Use of GGT 2.0 (software for display of Graphical Genotypes)

Graphical representation of marker data was obtained with GGT 2.0 software (Van Berloo, 2008). GGT 2.0 deals with visualization and analyses that involve molecular marker scores (Van Berloo, 2008). Based on inputs of physical positions of markers given in row and column data matrix, visualization of the distribution of polymorphic markers across the length of chromosome according to their physical positions (Mb) was obtained by GGT represented in figure 1 (Van Berloo, 2007; Van Berloo, 2008).

3 Results and Discussion

In the present investigation, only those SSRs which showed clear polymorphism in terms of scorable band difference were considered (Figure 4). Out of 1013 SSR markers screened, 294 SSRs were polymorphic indicating overall polymorphism of 29.02% between the two parents used for the study. At the individual chromosome level, the highest polymorphism was observed with chromosome 4 (40.96%) followed by chromosome 6 (37.8%) whereas the lowest polymorphism was observed in chromosome 9 (16%). Details of polymorphism percentage of individual chromosomes are mentioned in table 2. Further 38 polymorphic markers were present on chromosome 1, followed by 34 polymorphic markers on chromosome 2 and 4 individually, the least number of polymorphic markers (12) were obtained for chromosome 12. A detailed list of all the polymorphic markers obtained on each chromosome is given in Table 3. Kumar et al. (2013) reported 31% polymorphism with 128 HRM primers which were screened between JGL 1798 and donor parents GPP 2 and NLR 145. Parental polymorphism survey between popular rice varieties of Andaman & Nicobar Islands viz C14-8 and CARI Dhan 5 with donor IRBB 60 showed 36 (18% polymorphism) and 48 (24% polymorphism) polymorphic markers out of 200 SSR markers screened and the highest polymorphism was of 53% reported for chromosome 4 (Gautam et al., 2015). Parental polymorphism survey between the two parents (N22 and Uma) revealed 20.82 percent polymorphism with 41 polymorphic markers out of 197 markers screened (Waghmare et al., 2018). Another study using parental lines PR122 and IR10M196 surveyed 647 SSR markers and found 108 polymorphic markers with 16.69% of polymorphism level between parents (Yerva et al., 2018). A polymorphism survey conducted by Challa & Kole (2019) using drought contrasting parents reported overall polymorphism of 13.17% in which the highest polymorphism was noticed in chromosome 5 (17.02%) and the lowest was noticed in chromosome 10 (5.36%). Parental polymorphism in iron and zinc rich rice varieties 52 polymorphic markers were obtained out of 171 total screened rice microsatellite markers (Shivani et al., 2020). In another study by Vishalakshi et al. (2020) also assess the polymorphism and reported that, out of 500 markers screened between Varalu and Vandana NIL, 150 polymorphic markers was reported which expressed 30% polymorphism. Out of this highest polymorphism was recorded for chromosome 12 (42.5%) and least for chromosome 8 (20%).

The necessity of combining different genetic backgrounds for QTL mapping experiment for grain zinc and iron content has been also highlighted by Lee et al. (2020). A higher level of polymorphism in the present study is a result of the diverse

Table 2 Details of study of chromosome wise polymorphism percentage of SSRs markers between the parents Rajendrakasturi and URG-30

Chromosome no.	Total No. of SSR marker run	No. of Polymorphic markers	Polymorphism (%)
1	138	38	27.53
2	96	34	35.41
3	107	32	29.90
4	83	34	40.96
5	82	26	31.70
6	82	31	37.80
7	57	14	24.56
8	81	24	29.62
9	96	15	16.00
10	54	15	27.77
11	88	19	21.59
12	49	12	24.48
Total	1013	294	29.02

Table 3 List of all the polymorphic markers obtained on 12 chromosomes with their details

S. N	Name of SSR marker	Chr. No	Physical position (Mb)	Motif	Number of Repeats	Forward Primer Sequence	Reverse Primer Sequence
1	RM462	1	0.08	AG	12	CCGCGAATCCATTGAGACTGC	TCTAGGAGGAGATGGCGGAGTAGC
2	RM10009	1	0.19	AG	31	GATGCTCCGGAATAACTAGATTGG	GGAATTACAGCTGTCTTGAAGG
3	RM3252	1	0.3	AG	13	ATGCAAGCATCTGCTTATGG	GTTGGTAACCTTTGTTCCCATGC
4	RM10022	1	0.32	AAAG	5	CCTCCATAGAGTAAGGTTTGCATGG	CCTCCTCTCTGTCTTCTCTGTC
5	RM10047	1	0.74	AC	13	CAAGCAGTGATCATAAGCCTTCC	GCCATGGCTGAGAACAGAGAGC
6	RM84	1	3.95	TCT	10	TAAGGGTCCATCCACAAGATG	TTGCAATGCAGCTAGAGTAC
7	RM10252	1	4.34	ACAT	5	GGTGAGATACTTATTCGTGAGTGC	AACCGCTTACCATGATAGTACC
8	RM3233	1	5.05	AG	13	GAAATTCGAAATGGAGGGAGAGC	GGTGAGTAAACAGTGGTGGTGAAGC
9	RM151	1	5.56	AT	44	TGCTGATCAGTTACACGAATCAGAGC	GCGTACGTGCACAAATTAACAGACC
10	RM6138	1	6.99	CCG	9	GACTCGTTCAAGACGTGGTCAGG	ACTCTCGGCATCGACGAGTACG
11	RM1344	1	7.02	AG	23	AATGTTCTGCAATCCGAGTAGG	CACAGAATGTGAGCATTCACTCC
12	RM8098	1	7.25	AT	26	TTCGTATCCTGATACCGTTACGC	GGCCTTGATGAACCTTGATTTCG
13	RM5989	1	7.25	ACC	9	CTGCGAAGTAGGTGCCCTTTCG	TCCTTCAAACCTCATCACCATTTCG
14	RM6784	1	7.31	ACG	8	TACCACCTACCACCACGATGACG	GTAGACGACTAGCTCGCCCATCC
15	RM243	1	7.97	CT	18	GATCTGCAGACTGCAGTTGC	AGCTGCAACGATGTTGTCC
16	RM583	1	8.33	AAG	20	GTTGCGGTTTGTTCGTTCTTGC	TAGATCCCAGCAGACGGATCAGC
17	RM577	1	8.37	TA,CA	9,8	GCTTTCCCTCTAACCCTCT	GGATGTACCGCTGACATGAA
18	RM35	1	8.41	GA	19	TGGTTAATCGATCGGTCGCC	CGACGGCAGATATACACGG
19	RM578	1	8.41	GA	19	GGCGTCGTGTTTTCTCTCTC	CAAAAAGGAGGAGCAGATCG
20	RM10537	1	8.45	AAG	9	ATTGCGGTAACCTAAGTAGCATCC	CGACACTAGCTACCAACATGTCC

S. N	Name of SSR marker	Chr. No	Physical position (Mb)	Motif	Number of Repeats	Forward Primer Sequence	Reverse Primer Sequence
21	RM579	1	8.45	GA	25	TCCGAGTGGTTATGCAAATG	AATTGTGTCCAATGGGCTGT
22	RM10544	1	8.6	AGC	9	GGGCGAAACGGAAGAGTAGACG	TCCTACTACTCGTAGGCGAGAGG
23	RM572	1	9.87	AG	14	CGCGTTAATGTCATCTGATTGG	CCATACTTCGAGATCCAAGACTGACC
24	RM23	1	10.7	GA	15	CATTGGAGTGGAGGCTGG	GTCAGGCTTCTGCCATTCTC
25	RM3375	1	18.73	AG	16	TGCAAGGAAACTAGGAGGGAAGC	CTTGACCTCTCTCCACAACC
26	RM24	1	18.97	GA	29	GAAGTGTGATCACTGTAACC	TACAGTGGACGGCGAAGTCG
27	RM129	1	19.01	CGG	8	TCTCTCCGGAGCCAAGGCGAGG	CGAGCCACGACGCGATGTACCC
28	RM9	1	23.32	AG	14	GGCCCTCATCACCTTCGTAGC	CGTCTCCCTCTCCCTATCTCC
29	RM5	1	23.97	GA	14	TGCAACTTCTAGCTGCTCGA	GCATCCGATCTTGATGGG
30	RM306	1	24.44	GT,AT,CT,GT	18,8,1,6	CAAGGTCAAGAATGCAATGG	GCCACTTTAATCATTGCATC
31	RM488	1	24.8	AG	17	AACAACGAGCGTATGCGTTCTCG	CCCACGCGTTTGTAGGAAGAAGC
32	RM543	1	32.78	GCG	10	CTGCTGCAGACTCTACTGCG	AAATATTACCCATCCCCCCC
33	RM3825	1	36.46	GA	21	AAAGCCCCAAAAGCAGTAC	GTGAAACTCTGGGGTGTTCG
34	RM431	1	38.89	AG	16	GCTTGCTTGATCTGCATTGGTAGG	GGGATGATCCACTCTCTGTTTGG
35	RM12146	1	40.71	AG	11	AGTATGCCCTGCCACTACACTAGG	CAGCGAATGGCAAGAGCAACC
36	RM12276	1	43.04	TC	17	GTCGACGGCTTCTCAAGATTGG	TGAGACCTCTGTGAAGGCACTCG
37	RM12284	1	43.16	AAG	9	CAGCACGAACAGACAGGATGG	AGGAAGAAGAAGCAGCGATTCC
38	RM12292	1	43.22	AT	38	ATGAGACGATGAAAGCCTCAAGC	GTGGGACAAGCAAATTGAAACG
39	RM12300	2	0.05	AG	14	AGTCAGGCAGACAACACCATCG	TGCTGTTATCTGTGTCAGTCAGC
40	RM12308	2	0.12	AG	10	GTTTGCAGATGCATTAGTACC	GGATCGCCCTGCATATTTATACC
41	RM6842	2	0.12	AAG	21	CCGTGCATCTCGCTACCTAACC	TGCACACAACTTAGAGGAAGAAGG
42	RM12313	2	0.15	AATG	5	CCACAACGCATGCTACTCTAGC	ATGCAAGATGGTGGATGGATGG
43	RM109	2	0.18	AG	16	GCCGCCGAGAGGGAGAGAGAG	CCCCGACGGGATCTCCATCGTC
44	RM12317	2	0.2	AAAG	10	ATAATTGTGGTCCCGAGTCATCC	TGCTTCTTCAACTCAACTCTCAGC
45	RM12329	2	0.57	AG	15	AGGAAGAGGCGAAGGTAGATCG	CCAATCATGCTGTGTTTCAAGG
46	RM12433	2	2.07	AG	18	CTCTACACGCTTTCCTGTTTGG	TGATCAGGTTTCGAGATTGGGTATGG
47	RM236	2	2.11	CT	18	GCGCTGGTGGAAAATGAG	GGCATCCCTCTTTGATTCTCTC
48	RM12448	2	2.36	AT	28	CAATGGGCGGGTTTGACAGG	ACCACTTGTGCTTCACGCTCTCG
49	RM279	2	2.88	GA	16	GCGGGAGAGGGATCTCCT	GGCTAGGAGTTAACCTCGCG
50	RM423	2	3.83	TTC	9	AGCACCATGCCTTATGTTG	CCTTTTTCAGTAGCCCTCCC
51	RM174	2	7.01	AAG	7	ATAAGCGACGCCAAGACAAGTCG	GGAAGCAAGAAGGAAAGAGAGATGG
52	RM492	2	7.28	AG	21	CATTGACGTGGCACTTTGTTC	AGAGAGCACGCAATGGAGTATGC
53	RM12868	2	8.74	ATC	7	ACTCATCAACATCGCGAAACAGC	CTGACTCCGACAACAGCTATGAACC
54	RM71	2	8.76	ATT,T,ATT	10,1,4	CTAGAGGCGAAAACGAGATG	GGGTGGGCGAGGTAATAATG
55	RM452	2	9.56	ACG	9	GTGGACTTGGCGAGATGCTACG	GTTAAGGGCAGCCACCAGATCG
56	RM12949	2	10.07	AG	10	CTACGCCACCAAAACCACTCC	ATTTGATGCTGTGAGGCTGTGG
57	RM324	2	11.39	ATC	9	GATTCCACGTGAGGATCTTCTGG	GCTCACCAGTTGAGATTGAAAGG
58	RM424	2	11.39	CAT	9	TTTGTGGCTCACCAGTTGAG	TGGCGCATTCATGTCATC

S. N	Name of SSR marker	Chr. No	Physical position (Mb)	Motif	Number of Repeats	Forward Primer Sequence	Reverse Primer Sequence
59	RM6374	2	15.29	AAG	16	TCACCAGACTCAACAAAGGATCG	TTCACCTTTCTTCTCCCTCATTC
60	RM561	2	18.76	GA	11	GAGCTGTTTGGACTACGGC	GAGTAGCTTTCTCCACCCC
61	RM475	2	20.4	TATC	8	CCTCACGATTTCTCCAAC	ACGGTGGGATTAGACTGTGC
62	RM262	2	20.79	CT	16	CATTCCGCTCTGGCTCAACT	CAGAGCAAGGTGGCTTGC
63	RM263	2	25.89	CT	34	CCCAGGCTAGCTCATGAACC	GCTACGTTTGAGCTACCACG
64	RM1367	2	26	AG	27	GTGTGTACGTAGGATCGGAG	TGCTACTCCTAGCTGCTACC
65	RM573	2	27.97	AG	11	TCATGTTGACGCACACATACACG	CTCTTCTCCCTGGACCACACC
66	RM525	2	28.29	AAG	12	GTTGGTATTCCTGATGAGTTTGC	GAGACAGAATCCTTACGCTGTGC
67	RM318	2	29.66	AC	11	GGCTACGCTCCAGTTAGTGAAGG	TGGTAGGAAGAAGGGAGAAGTTTAGG
68	RM530	2	30.56	AG	23	TTCTTTATTCCTCGCACTGACC	CAATGATGCCACAAACCGTAACC
69	RM250	2	32.8	AG	27	GTTCAAACCAAGCTGATCACAAGC	GGCGTCAGAGTCAGAGATGAAGG
70	RM166	2	34.35	T	12	GGTCTGGGTCAATAATTGGGTTACC	TTGCTGCATGATCCTAAACCGG
71	RM482	2	35.27	AT	9	TCTGAAAGCCTGACTCATCG	GTCAATTGCAGTGCCTTTTC
72	RM266	2	35.43	GA	19	TAGTTTAACCAAGACTCTC	GGTTGAACCCAAATCTGCA
73	RM3266	3	0.06	AG	13	CTTGGAAGAAGAGGCTGGAAGG	GGATGATATCCACCGGGATGC
74	RM14240	3	0.1	AGAT	11	GCTCAAAGATGACACCGATGC	CATGGTGAGTTTGGAGTGATTGG
75	RM7332	3	0.39	ACAT	12	ACACTGTACACCACATTCAGC	CACACCAAAGGAAATTAGG
76	RM14275	3	0.47	AT	32	GAAACAGTTGAGAGCGGTTACTGG	GAACCACTAGATGAATGAAGGCTAGG
77	RM14311	3	1.14	TA	37	AGCTTGACTTCACACCACTCG	GGACGACCTACCGATCAAGACG
78	RM523	3	1.3	AG	14	TGAATTCTTGACATGGTCAGC	TGGGAGGTTTGTAGGGTAATCC
79	RM81A	3	1.93	TCT	10	GAGTGCTTGTCGAAGATCCA	CTTCTTCACTCATGCAGTTC
80	RM231	3	2.45	CT	16	CCAGATTATTTCTGAGGTC	CACTTGATAGTTCTGCATTG
81	RM14412	3	3.15	AT	17	CCCAATCGGAATCAATCACTCC	CCATGAGGGCTCGACTGTGG
82	RM14417	3	3.19	AG	25	GCCGATAATTGAGAGAAGAGATGAGC	GTCTCTCGGAGTACAGCCACAGC
83	RM4352	3	4.3	AT	17	AATGCAAATGGCAACCAACAGC	GATTGACGCCACAACATGAACG
84	RM218	3	8.38	AG	24	TCAAACCAAGGTCCTTCAACTGC	TTTCTTCCACCGTCCATGTATCC
85	RM232	3	9.75	CT	24	CCGGTATCCTCGATATTGC	CCGACTTTTCTCTGACG
86	RM251	3	9.95	CT	29	GAATGGCAATGGCGCTAG	ATGCGGTTCAAGATTGATC
87	RM14780	3	10.11	AG	25	GCAACCTGTTCTCGCTGAAGTAGC	GCCATTGGAGGATCCAAGATAAGC
88	RM282	3	12.4	GA	15	CTGTGTCGAAAGGCTGCAC	CAGTCCTGTGTGCAGCAAG
89	RM6881	3	16.69	ACC	11	CGACTGATTGATTCCACAATGATCC	GCAGCTGGAAGCAGAGGAAGACG
90	RM15326	3	20.6	AT	29	TGAATCTACCGCTCTACTTGTGG	AAACAGTGCATCCTTCTGTGG
91	RM15339	3	20.87	AT	15	NA	NA
92	RM411	3	21.23	AAC	8	GTAGGAAATCTTCGCCAGATGC	CCGAGACTTGAACAATCTTAGGC
93	RM15539	3	24.39	AAT	24	CATGATGCATCGAATCTACCC	AAGCGTTGTCTACCAACATGG
94	RM15544	3	24.48	AGAT	5	GCTTGAGAGTGTGTAGGAGTGC	TTGGCAATGTACTCTGCTGTCC
95	RM135	3	27.22	CCG	11	TCCATGTCTTCAGCTTCTGG	GCTTCTACTGGAGGAGAGCAGAGG
96	RM426	3	27.4	AT	11	CATCGCCGAAATCCATCTTCC	AAGGCCCATTTATTGTAGAGTGC
97	RM168	3	27.9	AC	10	TGTCGTCGAGGATTGGAGATCG	GAATCAATCCACGGCACAGTCC

S. N	Name of SSR marker	Chr. No	Physical position (Mb)	Motif	Number of Repeats	Forward Primer Sequence	Reverse Primer Sequence
98	RM6817	3	31.72	AAG	9	AAGAAGAAGCGAGAGCGAGAGG	TCCTCTCTTGACGTGTTGAGC
99	RM16011	3	32.14	AG	23	CACCTTCGATTTCTTCTCATCG	CGAGTTGTACTCTGGTTGCTTCC
100	RM16019	3	32.36	AT	44	GGTTTGCTCTTTACACTCATGC	GTTAGGATTGGACAGCAGAAGG
101	RM16049	3	32.9	AC	12	TGGAATTATGCGCAGTACATCAGC	GGAGCCCATACTCCATGTAGAACC
102	RM130	3	33.18	AG	10	CGAGAAGAAGGAGCCCATCTCG	GCGAAGCGAAGAGGAGCAAGG
103	RM570	3	35.38	AG	15	AGAAATGGTGAAAGATGGTGCTACCG	CTGAATGTTCTTCACTCCAGTGC
104	RM85	3	36.34	TGG, TCT	5,12	CCAAAGATGAAACCTGGATTG	GCACAAGGTGAGCAGTCC
105	RM551	4	0.17	AG	18	CTTACTCCATTGGGCTGGAACC	TGTAGGGTGGTAAGAGATCCACTCC
106	RM16284	4	0.52	AT	28	ACACTGCTCGGGAGTTACTGACC	CAATAAGCAGGGCTACAACATGC
107	RM518	4	2.03	AG	15	AAGACACAAGCAAACAGCTCAACC	AAGCTTGCTTGGTTCAAGAGAGG
108	RM16367	4	2.4	AT	21	GTTCCCAACCGGAACAGTACG	CCCTTAGGCTGTGTTTGACATCC
109	RM16430	4	4.44	AGG	8	CCACTCCTCCCATCTCTACTCC	GCCACCGCACTTGTCTATGC
110	RM8213	4	4.44	AG	19	TGTTGGGTGGGTAAAGTAGATGC	CCCAAGTATACAAAGATGAGTTGG
111	RM16431	4	4.45	AGG	8	CCACTCCTCCCATCTCTACTCC	GCCACCGCACTTGTCTATGC
112	RM16556	4	8.83	AT	13	TTGGACCAGGAGATCAATGAAGG	GTGCGCACACTCTTCTATGTGC
113	RM5953	4	9.37	CAC	6	AAACTTTCTGTGATGGTATC	ATCCTTGCTAGAATTGACA
114	RM16705	4	15.26	AT	22	GGTTAAGCACTAATGGTGGTAGG	TAAGTAGTCATCTCTGCTGCAACC
115	RM5687	4	15.93	AAT	17	AGAGAAGAAGGGAAGGAGGAAGG	AGTGACTTGTGGGTGACTTGTGG
116	RM16739	4	16.65	AG	21	TGCATAGTCCACCTAAGAGTTCAAGG	TTTCTGCAGGCTGATTAGTGTAGG
117	RM3643	4	20.13	AG	14	GCTAAGCTAATCTGACCGGATCTACG	GATGGGCCGATTAAACAAATTC
118	RM5749	4	20.13	ACT	7	GCTCGTTTCTCTCGATCACTCG	GCAAGGTGGATCAGTCATTTCG
119	RM1388	4	25.2	AG	46	GATCGTTGCTGCTTTCAATGAGG	AGTGTTCACCTTGACCCATGC
120	RM252	4	25.36	CT	19	TTCGCTGACGTGATAGGTTG	ATGACTTGATCCCGAGAACC
121	RM241	4	27.02	CT	31	GAGCCAAATAAGATCGCTGA	TGCAAGCAGCAGATTAGTG
122	RM2441	4	28.02	AT	27	CGCTGCCATCATCTACAACAGG	CAACAAATGCTACCTCACCATGC
123	RM470	4	28.25	AAG	14	CCCTCCCGTAGACCTTGTACCC	CCACAGCTAACCAATCCTTCTCC
124	RM451	4	28.54	GAT	8	GATCCCTCCGTCAAACAC	CCCTTCTCCTTCTCAACC
125	RM317	4	29.22	GC,GT	4,18	CATACTTACCAGTTCACCGCC	CTGGAGAGTGCAGCTAGTTGA
126	RM17418	4	30.05	ACG	10	GCATCCAGCATCAGAGATAACC	CATTCTTGC GTGAGAACCAACC
127	RM6748	4	30.9	AAT	26	AGAGAAGCAGCTGGTGATTAGCC	CAACGATGTACCAGTTGAATACCC
128	RM255	4	30.99	AG	19	GAGGAGGAGGAGGAGATCAGG	AACGAAACCGCTCAGTTCAACC
129	RM5709	4	32.09	AAT	24	TTTGGGATAAATGGGAGAGG	TCAGCTGGTTATTATGGAGAGG
130	RM348	4	32.65	CAG	7	CCGCTACTAATAGCAGAGAG	GGAGCTTTGTTCTTGCGAAC
131	RM3648	4	33.34	AG	14	CGAGAAGCCGAAGAGGAAGAGAGC	CCAACAGATCCATCTCAACCAACTCC
132	RM17623	4	34.3	AG	12	GTTCTTGGGTTGGTGAGCTTCC	TAGGGCGCATGTGATTCTTCTCC
133	RM131	4	34.64	AG	15	GGAGCAGCTTCTCGAGCATGG	CCAATCTCGCTCGTTTAGCC
134	RM127	4	34.75	AGG	8	GTGGGATAGTCGCTCGCTCG	AGGCCAGGGTGTGGCATGCTG
135	RM567	4	34.75	AG	23	GCATACCGTAATGTTGGTGAAGC	AATAGCAACTGGGAGGAGGTAAGG
136	RM280	4	35.21	AG	11	GTGCTCTCCATGTCGGATTATGC	CAAGGCAACAAGATTGGTTAGTGG

S. N	Name of SSR marker	Chr. No	Physical position (Mb)	Motif	Number of Repeats	Forward Primer Sequence	Reverse Primer Sequence
137	RM559	4	35.37	AAAC	6	AGAGCGATGGGTGTCAGTTTGC	CGTACGTACACTTGGCCCTATGC
138	RM3531	4	35.43	AG	12	TATCGCCACTCGTCCATTCTCC	CAGCTACCAACACCTCCATACCC
139	RM17713	5	0.04	AG	20	GGGCACCATTAACTTAACAACG	GCAATGGTACAAATAGCCAAGC
140	RM17722	5	0.12	AGGC	5	TGCAGCTGAAGAAGAAATGAGC	ATGATGGATCTTCCAACAGTGC
141	RM17724	5	0.14	AT	30	TGCTCTCTTGAGTACCACTGTCC	TTTGAGACGACCACGTAGAGC
142	RM153	5	0.16	AAG	9	CCTCGAGCATCATCATCAGTAGG	TCCTCTTGTGCTTGTCTTCTCC
143	RM17798	5	1.09	AT	29	TAATAGGAAGAGTGCCTCAGAGC	TCGACCAGTGATAACCAGTAACC
144	RM2010	5	1.12	AT	19	GCAATACACGCCAATCTTCTAGG	TTGGGATGGAGGTAGTATTGTGC
145	RM5374	5	1.2	AG	13	TTAGGGTTAGGTCGTGTGTACTGG	CGCTGGATCACAACACTGTAGC
146	RM17819	5	1.55	AAG	18	TTGCCTCATGTTTGCTTCATCC	AGCTGACAACGACGACACTGC
147	RM17823	5	1.59	AT	31	ACATGGTCTCGTAGATCTTTCC	CTTCTCCATGATAAGCAAAGC
148	RM17847	5	1.81	AG	19	TTCTTCTCCCTCTTACTCTCC	CTCTAGTCTCTTCTGCAAGTTCC
149	RM5579	5	1.86	AC	22	CAACAGAACCTGCCAACTCTTGC	TGCCTCATGGTAATAAGCCAAGC
150	RM7302	5	1.92	AATT	6	GGGAGAGAGGAAGGAGGAGCTTCG	CGGGACCGGAACCTTTCAACG
151	RM13	5	2.01	GA	16	TCCAACATGGCAAGAGAGAG	GGTGGCATTGATTCCAG
152	RM17867	5	2.16	AT	33	CATCGATAGAGCCAGATAGATGC	TTTCTACAGGAAGTCAGGAACG
153	RM592	5	2.74	AAT	26	ACCTCACCCGAATTACTGTGATATGC	GTTGAATTGCACGCGACTCTGG
154	RM548	5	2.81	CT	12	TCGGTGAGAACTGAGAGTACG	AAGGAGGCCATCTCAATGTG
155	RM267	5	2.88	GA	21	TGCAGACATAGAGAAGGAAGTG	AGCAACAGCACAACTTGATG
156	RM574	5	3.45	AG	11	AAACTAGCCACGGTTTGGTAGGG	AGGGTGGCAGGGATGTAATTTCC
157	RM289	5	7.8	G,GA	11,16	TTCCATGGCACACAAGCC	CTGTGCACGAACCTCCAAAG
158	RM249	5	10.77	AG,A,A G	5,2,14	GGCGTAAAGGTTTTGCATGT	ATGATGCCATGAAGGTCAGC
159	RM430	5	18.61	AG	25	GTCCCTGATCAGAAACGAGATGG	TAGGGTTGGAAGAATGCAAGACC
160	RM440	5	19.83	AAG	22	GGTAGGCACCAAGAGTTTGACG	GGCATCACCTTATCCAATCACC
161	RM459	5	20.1	ATCC	6	CCTCCAGTATCGATCACCAAAGC	CGAAAGAAAGTCATAGCACGATGG
162	RM421	5	23.89	AGAT	6	GCATAGCTATCCAGAATCCATTGACC	AGACGTAGCTCAGGTGAAACATCC
163	RM274	5	26.84	GA,CG G	15-7,5	CCTCGCTTATGAGAGCTTCG	CTTCTCCATCACTCCCATGG
164	RM31	5	28.45	AG	20	CGCTCTCCACTCTTCTCTACC	CGTGCAGAAAGTCCATTACTCTCC
165	RM7158	6	0.22	ACAT	12	CATGGACTTGTAGCAGCGTACC	ATTTTCATCGATGGCCTCTACACC
166	RM133	6	0.23	CT	8	TTGGATTGTTTTGCTGGCTCGC	GGAACACGGGGTCGGAAGCGAC
167	RM540	6	0.38	AG	16	GCCTTCTGGCTCATTTATGC	CTAGGCCTGCCAGATTGAAC
168	RM508	6	0.44	AG	17	AGAAGCCGGTTCATAGTTCATGC	ACCCGTGAACCACAAAGAACG
169	RM19288	6	1.17	CCG	8	CGGAGCTGTTGCCGTTCTGC	CGATGTGCCATGTGAGGATGACC
170	RM589	6	1.38	AC	24	GTGGCTTAACCACATGAGAACTACC	TCACATCATTAGGTGGCAATCG
171	RM19311	6	1.46	AC	10	TGCGGTGCTGTTACCTACTATCG	GCACTGAAGCTGGTGCAATCG
172	RM586	6	1.48	AG	23	TGCCATCTCATAAACCCACTAACC	CTGAGATACGCCAACGAGATACC
173	RM1369	6	1.56	AG	27	CATCGATTAGCTTACATGGCAACG	ACTAGTGCAGCCGTCTTCAATGG
174	RM588	6	1.61	AGC	9	TCTTGCTGTGCTGTTAGTGTACG	GCAGGACATAAATACTAGGCATGG

S. N	Name of SSR marker	Chr. No	Physical position (Mb)	Motif	Number of Repeats	Forward Primer Sequence	Reverse Primer Sequence
175	RM197	6	3.09	ACC	8	ATCCTGCTGCTGCTGTGTCG	CTTCGTTTAGCGTGAAGCAACG
176	RM585	6	3.16	AG	45	CTAGCTAGCCATGCTCTCGTACC	CTGTGACTGACTTGGTCATAGGG
177	RM204	6	3.17	CT	44	GTGACTGACTTGGTCATAGGG	GCTAGCCATGCTCTCGTACC
178	RM217	6	4.23	CT	20	ATCGCAGCAATGCCTCGT	GGGTGTGAACAAAGACAC
179	RM314	6	4.84	GT,CG,GT	8,3,5	CTAGCAGGAACCTCTTCAGG	AACATTCCACACACACACGC
180	RM253	6	5.44	AG	22	CCATCTCTGCCTCTGACTCACC	TCCTTCAATGGTCGTATCTTCTCC
181	RM19771	6	9.04	AT	47	AACCAATGCACACTTCTTGTGTC	CAACTGTAGAGTTGGAATGATCTGC
182	RM8226	6	9.32	AAG	14	GCGTGTTCAAGAAATAGGATACGG	GATCTCGCCACGTAATTGTGTGC
183	RM564	6	11.61	AC	14	TCCTTCTGCATCAATTCTCTCG	CTCCATAGCCTTGTAAAGTGATGAGC
184	RM20185	6	20.31	AT	44	AAACCGCTCAACTTTAGCTAGG	ACCTCTACTCGTTTGTGTCTGG
185	RM20242	6	20.97	AG	23	CTAGCCAACTCACTTGTCATCC	GATATGACTGATTGGGACAGTGG
186	RM162	6	23.65	AC	20	TTGTTCCAGTTCAGGTCTTGTGC	CCCTACAAACACCATAAGAAGCAACC
187	RM20476	6	25.76	AAT	18	ATGGACTGAGAACCCTCAACACG	GCTGTGTATTCTTGGTTGATGC
188	RM528	6	26.17	AGAT	9	GGAGGAGAACCATATTTGGCAAGG	AGCTTGACCCTTCAACACTAAGC
189	RM30	6	26.87	AG	13	GTCACATTCCGTTTCCCATCTTCC	CCTCACCTCACCACACGACACG
190	RM400	6	28.05	AAT	63	TTACACCAGGCTACCCAACTCG	TTGCTGAGTTCCTCGTCTATCC
191	RM340	6	28.22	AAG	10	GGGTAATGGACAATCCTATGG	ACCCTATTCTGGAGTTCATCTGG
192	RM20632	6	28.22	AAG	10	GGGTAATGGACAATCCTATGG	ACCCTATTCTGGAGTTCATCTGG
193	RM20633	6	28.23	AG	10	TTACCTCTCTCTCTCTCTATCC	TCAACCACAGTGCAGTGACTTCG
194	RM439	6	29.11	AAT	13	CTGGGTCTAATCTCGTCTAAATTGC	CGCCTCTCATAACAGTCCACTCC
195	RM176	6	29.75	CCG	7	TACGAGCTCTCCAGCCTCATCC	CGCTAATCAATCAGAGCGATGC
196	RM20779	7	0.10	AT	31	TGCTTCTCCTAGGTTGTGTGATCG	TCTTGGCTCTACATGCTCCTACG
197	RM20834	7	0.59	AC	43	GATATGGTTCCACTTCACCATGC	TTAGAACTCGCCTTCAGAAGTGC
198	RM481	7	2.88	CAA	12	TAGCTAGCCGATTGAATGGC	CTCCACCTCTATGTTGTTG
199	RM5499	7	10.02	AG	25	GGACGAAAGGGTATTGATTGG	CCTCAAGGTGGTCTCCTTCTCC
200	RM542	7	12.66	AG	22	CGCTCCTTCTAGTCCATCTCC	AACTGCAACGAGTAAGGCAGAGG
201	RM320	7	18.64	AT	18	TACAGCAGGGTGGAATATCGACAAGC	TCGTTGTAACGCGTGGATATTTGG
202	RM560	7	19.53	AG	12	ATTTGGATGTCATCGCCAAGG	CCTGATTGCGTGAACACATAAAGC
203	RM346	7	21.05	CTT	18	CGAGAGAGCCATAACTACG	ACAAGACGACGAGGAGGGAC
204	RM336	7	21.82	AAG	18	GTATCTTACAGAGAAACGGCATCG	GGTTTGTTCAGGTTCTCTATCC
205	RM10	7	22.19	GA	15	TTGTCAAGAGGAGGCATCG	CAGAATGGGAAATGGGTCC
206	RM70	7	22.78	ATT	33	GTGGACTTCATTTCAACTCG	GATGTATAAGATAGTCCC
207	RM234	7	25.42	AG	13	TTCAGCCAAGAACAGAACAGTGG	CTTCTCTCATCTCTCTCTTGG
208	RM5455	7	26.41	AG	18	GATCAACGAACCCACCACACC	CGCGTCTGTATATGCACTTGATCC
209	RM172	7	29.56	AGG	6	TGCAGCTGCCACAGCCATAG	CAACCACGACCCGCGGTGTG
210	RM5911	8	0.07	ATT	22	CCCTCTTTTAAAGTCTGGGG	GGTGCCTCTTTCAAAGTTG
211	RM6369	8	0.12	AAG	14	AGCTAGCTTACCTACCTACCTCACC	ATGGTCATGCTGTTGGTTTGC
212	RM22212	8	0.18	AGC	8	TCCTCTGAATCTTCACAGTTGG	GAAGAAGAACTCGAAGCATGG
213	RM1019	8	0.2	AC	13	AGGACTTGTCTATAACCTCCAAGC	AATGCAAGTATCCACCTCTGTCC

S. N	Name of SSR marker	Chr. No	Physical position (Mb)	Motif	Number of Repeats	Forward Primer Sequence	Reverse Primer Sequence
214	RM310	8	5.11	GT	19	CCAAAACATTTAAATATCATG	GCTTGTGGTCATTACCATTC
215	RM547	8	5.59	ATT	19	TAGGTTGGCAGACCTTTTCG	GTCAAGATCATCTCGTAGCG
216	RM22565	8	5.79	ACAT	15	TCCACGCGTTGTCGTAGAAATTTAGC	AGCCCAGCACCATGAAACACC
217	RM22679	8	8.57	AG	22	CCTCATCATCGTCTCCGACTGC	TCTCAAACCTCAACAATGTCTCG
218	RM22685	8	8.8	AGG	10	ATGGGCTTCCAGGCTCAATCTCG	CCCACTCTCAGCTCCTCTCTTCC
219	RM22688	8	8.85	AAT	28	CTTCGATCTCTGCCTGGATACC	CCATTTCTGTGGTTTCAGTCC
220	RM22695	8	8.98	AAT	16	CAAGGGATTCACTAACGGTTCCTTCC	CACATTGAACCGTTGCTTTGTGC
221	RM483	8	11.92	AT	26	CTTCCACCATAAAACCGGAG	ACACCGGTGATCTTGATGCC
222	RM22865	8	13.74	AT	32	NA	NA
223	RM404	8	15.43	AG	29	GGAGCAGCTAAGGCAGATAAGAGG	GCCTTCATGCTTCAGAAGACAGC
224	RM339	8	17.94	CTT, CCT, CTT	8,1,5	GTAATCGATGCTGTGGGAAG	GAGTCATGTGATAGCCGATATG
225	RM515	8	20.28	AG	11	CGAGATACGGTGAATGACACACG	ACCAATCAGAGAGCACACACAGG
226	RM223	8	20.65	AG	20	GCTGGGCCTATATGGACTTTTCG	AGGCAAGCTTGGCACTGTAAGC
227	RM284	8	21.14	AG	12	ACTGCATGATCCTCCTCAGATCC	CCCTCTGATCTCTGATACTCCATCC
228	RM256	8	24.27	CT	21	GACAGGGAGTGATTGAAGGC	GTTGATTTCGCCAAGGGC
229	RM80	8	24.47	TCT	25	TTGAAGGCGCTGAAGGAG	CATCAACCTCGTCTTCACCG
230	RM23408	8	25.08	AT	47	CCATCTCAACTCCTTCGTTTACTGC	TCGACTGTTTCTTGGAATAGGC
231	RM419	8	25.82	AG	12	TTCTTCCAGTGATTCTCTCC	CCCTTCTCCTCTTGGTATGC
232	RM458	8	27.35	TAG	8	GGTGATCTGCATTGTCAACG	TGCAATGGATCTAGCGACTG
233	RM281	8	27.89	GA	21	ACCAAGCATCCAGTGACCAG	GTTCTTCATACAGTCCACATG
234	RM23668	9	0.60	ACG	10	TGCATAGCATATCAACTAGCCCTACC	GCTGAAACAGAATGAAAGCACAGC
235	RM23679	9	0.82	AAAG	10	TCACAGCTTAGTGCATGTTGAGC	GATTACCTGGCAATGAGAACG
236	RM316	9	1.02	GT, TG, TTTG, TG	8-9,4,4	CTAGTTGGGCATACGATGGC	ACGCTTATATGTTACGTCAAC
237	RM6196	9	5.4	CCG	8	GGGCACGGACTACCAAAGGAAGC	ATCAGGCCAGCACCCAAACC
238	RM23914	9	7.19	AT	23	GAGGATCCTTACCATCAAACCTTCG	CCAAGAACCTGCATTCTTCAAGG
239	RM7390	9	10.42	AGAT	20	TGAGAGCTCGTAGGAAGTGTC	CAGAGTCAGCAATCGCTAAGG
240	RM3912	9	10.77	AC	22	CACTCAGATTGGCCGATCC	GCTGATCCAGATCTACCTGACACC
241	RM24166	9	12.34	AGCG	5	CTCTTCGCTGACCTCCTGTGG	GTCGGTGGTGATACATCTGCTTCG
242	RM24188	9	12.59	AC	18	GAAAGCGACACCTGATAGATACAGC	CAAAGTGGATGTCAGTTGTGATGG
243	RM24263	9	14.13	AT	30	CTCATCGGCGACATATCACAGC	ATGGGATGTACACAGCCAAACG
244	RM3700	9	15.37	AG	15	CCTTTGCCGCTTCTCTTGG	ACGAGTTCGCCGTTAACCTTACG
245	RM24353	9	15.45	AG	31	GTTCTTCCTACTGTGAACATGG	GTGAAATATCCCTATGCTCTCACC
246	RM24355	9	15.49	AG	11	CGCCAAGGACAATCCAAGAACG	GTGTGTAATGCGTGTGAAACTGATGG
247	RM24357	9	15.5	CCG	7	ACGCTTGCTTCTCATCGTCTCG	CTGGAACGTCGCCACGTACTGC
248	RM160	9	19.49	AAG	23	CCCAAATCAGGAAAGTTTCTCAGC	AGTCATCCTTGGCTACCAGATGC
249	RM24856	10	0.10	ACGG	5	CCTTCACGTGTGTTATCACGTTCC	AGAAATGGACAAGAGCATGGTAGC
250	RM24875	10	0.28	ACAT	11	GATGAGCGAGGACAAGAATTAAGC	GCTAGCTGATTGATCCCATCG

S. N	Name of SSR marker	Chr. No	Physical position (Mb)	Motif	Number of Repeats	Forward Primer Sequence	Reverse Primer Sequence
251	RM474	10	1.8	AT	13	TACACGAGGGAGTACTCGAATGG	CATGGAGGTATAGAAGAGCATTGG
252	RM216	10	4.99	AG	12	GATGGTAAAGGAAGACGTGTGC	CACTCATAGACGCATCACATAGCC
253	RM467	10	13.04	AG	21	TGTTGTACATGAGATGGCTATGC	GCTGACCTTGTGAGACGTTTAGACC
254	RM258	10	17.57	AG	11	CTCCCTGGCCTTTAAAGCTGTCG	GACGAACAGCAGCAGAAGAGAAGC
255	RM6100	10	18.37	ACG	8	TTCCTGCAAGATTCTAGCTACACC	TGTTCTGTCGACCAAGAACTCAGG
256	RM25735	10	19.56	AAG	25	AGGCAGGCAAGCAGTAGTTTCG	ATCAAGATCAGGAGCCGCAAGG
257	RM25745	10	19.68	AT	11	TGGAGATGAGTTCACGCTACTTCC	CGTGTGTCTTCTGTTGGTATGG
258	RM25766	10	20.1	AG	15	CTGGGTAGTTGTTTCTCATGTGC	AGTGCTATTGCCATTGTACTGC
259	RM5352	10	20.67	AG	13	CATGAAGAGGAGATCAATGTAGCC	CTAAACATGGTGCAGGTATGAGG
260	RM496	10	21.98	AG	24	GACATGCGAACAACGACATCC	CTATAGTTGTTGCACATGCGATCC
261	RM25893	10	22.11	AAG	7	GAAGAAGATGGCGATGAAACTTGG	CAGAGCGGAGATCCAGATTGACC
262	RM25895	10	22.14	ACG	7	CTCTGTCTGCTCACTCCAGATTCC	GTCGAATTGAGAGGGATTGATGG
263	RM25898	10	22.18	AT	30	GCAGCCAATCAGAAATCATAGC	AAATATGTCCGTGTCCCAATGC
264	RM26018	11	1.22	AT	16	GGCGAGGACACCAGTAGACAGC	GCCAGCCACTAGATCCACATGC
265	RM26092	11	2.69	ATC	7	GTCATCGTCGTGGTCGAGTCC	TCTGGAGTGGCGTTGACTGTTGC
266	RM26097	11	2.74	AGG	7	ATGGTTCAAATGTGGAGGAGAGG	GAACCTTCCAGAACAATCCAGTATCC
267	RM26105	11	2.83	AG	10	AGCATCAATTCAGCTTGCTTGC	TTCTGGTTCTTTGAGAGAGTGTGC
268	RM26168	11	3.91	CCG	7	AGGTTGCGCCGCTTGACACC	CTTCGTCCACGACCACGAGAGC
269	RM167	11	4.06	AG	19	CTCCGAGTCCGACCACAAGG	TCCAGCCCTTCATCATATTGC
270	RM26185	11	4.27	AGAT	5	CTGAGAATTTCTATCCTCCGATGC	GCAAATTATGGCAGCAAGATCC
271	RM552	11	4.82	AAT	13	CGCAGTTGTGGATTTCAGTGC	TCATGCTCAACGTTTGACTGTCC
272	RM202	11	8.91	AG	24	TGGAACACCCATAGACAAACAGC	TGGCAAGTGGTATTCTTCTCTCC
273	RM26463	11	10.13	AT	38	CGGATTTCCGAATCGTTCAGAGC	AACCTGCAACCAAGGCCATAGG
274	RM287	11	16.61	AG	15	GGCTACACCTACACGCGAGAACC	AGATGCATGGAATGCCTGTTTGG
275	RM209	11	17.8	CT	18	ATATGAGTTGCTGTCGTGCG	CAACTGTCATCTCCCTCC
276	RM206	11	21.63	AG	33	ATCGATCCGTATGGGTTCTAGC	GTCCATGTAGCCAATCTTATGTGG
277	RM254	11	24.23	TC,AT T,CT	6,1,11	AGCCCCGAATAAATCCACCT	CTGGAGGAGCATTTGGTAGC
278	RM27147	11	24.26	AT	14	CACTGGCAAAGTCAGTAGTCCTTCC	GGAGGCTAGCTCCGTAATCTGG
279	RM27150	11	24.32	AG	10	ATTCAGGCTCGTTACCATCTCC	CCTCTGCTGTCCAAATCACC
280	RM224	11	26.8	AAG,AG	8,13	ATCGATCGATCTTCACGAGG	TGCTATAAAAGGCATTGCGG
281	RM27336	11	27.76	AT	19	AGCAGTGATTCATCGCTCTATCG	GTGATGCTGTGCCGATAATATTCC
282	RM5926	11	28.33	AAT	30	TAGGTCCATCCAAATCTCGATCC	TGGCAGAGGAGATTAGAGTAATACGG
283	RM5367	12	0.17	AG	13	ATTCCTCACGCCCACTGTACC	AGTGATATCCAACCCGAAGTGC
284	RM6371	12	1.08	AAG	14	ACGACCACCGTGTACGAAAGC	GATGAAGACGGATGAGGTGTTGC
285	RM19	12	2.43	ATC	10	CAAAAACAGAGCAGATGAC	CTCAAGATGGACGCCAAGA
286	RM247	12	3.19	CT	16	TAGTGCCGATCGATGTAACG	CATATGGTTTTGACAAAGCG
287	RM491	12	3.58	AT	14	CACATGATGCGTAGCGAGTTGC	TTATGCCTCTCCCTCCCAATTCC
288	RM511	12	17.4	ACG	7	AACGAAAGCGAAGCTGTCTCC	ATTTGTTCCCTTCTTCGATCC
289	RM1261	12	17.58	AG	16	ATGGTAGAGACACAAGTCCATGC	GACAAATTGGTGTAGGTGAAGG

S. N	Name of SSR marker	Chr. No	Physical position (Mb)	Motif	Number of Repeats	Forward Primer Sequence	Reverse Primer Sequence
290	RM28166	12	17.65	AG	12	TGCTTGCAAACATTGCTTCTGG	ACTGATGTACTGAACACGGGAAGG
291	RM519	12	19.97	AAG	8	AATTTCCGCGAAATCAGCATCC	TCATCTGGACAGTCGAGGTACGC
292	RM313	12	20.87	GT,CA,CG,GT	6,1,5-6,8	TGCTACAAGTGTTCTTCAGGAC	GCTCACCTTTTGTGTTCCAC
293	RM309	12	21.52	AC	14	CACGCACCTTTCTGGCTTTCAGC	AGCAACCTCCGACGGGAGAAGG
294	RM463	12	22.16	AAAT	5	GAGGATTAATTAGCGTGTGACC	GTCGTGACATCTACTCAAATGG

background of the two selected parents. The selection of genotypically diverse parents is imperative for any QTL mapping program. The geographical diverse origin of parents reflects in genotypic variations in terms of polymorphism. Mutual relation between the geographic distribution of germplasm and polymorphism of molecular markers has been also noticed by He et al. (2004). Also, a significant correlation between genetic relationship and geographical place of *indica* local rice varieties has been indicated (Zhang, 2005).

As SSR based polymorphism is characterized by repeat motifs (di, tri, tetra); in the current study also it was observed that SSR repeat motifs with dinucleotide repeats were more polymorphic than others. Out of 294 polymorphic markers obtained from screening 1013 SSRs, 1 marker possesses a single nucleotide repeat, 182 contained dinucleotide repeats, 87 showed trinucleotide repeats and 23 were having tetranucleotide repeats. Out of the 182, dinucleotide repeats AG motif appeared more polymorphic than others, which constitutes 49.45% of dinucleotide repeats, followed by AT repeats (21.97%). The abundance of (AG)_n and (AT)_n dinucleotide repeat has been noticed by earlier studies (Grover et al., 2007; Kumar et al., 2013). Out of 87 trinucleotides, AAG repeated 23 times representing 26.43% of its total, followed by AAT 12 times (13.79%). It was also observed that GC rich trinucleotide repeats viz., ACG, AGC, CGG, CCG are at a variable frequency (Figure 2). The occurrence of 80% trinucleotide GC rich repeats in predicted exons and their frequent association with genes (or ESTs) and (AC)_n/(AT)_n dinucleotides and tetranucleotide repeat motif in the non-coding intergenomic region has been reported (Temnykh et al., 2001). Out of 23 tetranucleotide repeats, AGAT appeared 6 times (26.08%). In a comparative study across the genome of two subspecies of *Oryza sativa* viz ssp. *indica* and ssp. *japonica* abundance of (AGAT)_n repeats has been observed (Grover et al., 2007).

In the present study tetra nucleotides were found to be frequent for chromosomes 5 and 9 and least frequent in chromosome 8, 11, 12, and 4. Tri nucleotides were found to be highest for chromosome 4 and less frequent in chromosomes 9, 12, and 7 (Figure.3). Similarly long trinucleotides repeats have been reported on

chromosome 1, 4, and 12 (Grover et al., 2007). Dinucleotides were most frequent for chromosome 1 and less frequent in chromosome 12 and 9. The richness of (AT)_n dinucleotide repeat motif has been explained by the presence of micron transposable sequences that particularly target (AT)_n repeats (Temnykh et al., 2001; Akagi et al., 2001; Grover et al., 2007). Out of 840 hypervariable rice microsatellite (hvRM) markers identified, markers with dinucleotides repeat motifs were more in number followed by markers with tri and tetranucleotides repeat motifs (Narshimulu et al., 2011). In present study number of repeats ranged from 5 to 63. Details of repeat motif, length of repeats, and physical position for all the polymorphic microsatellites in the present study will assist in selection for set of polymorphic microsatellites to be used in genotyping of mapping population for QTL mapping and marker-assisted selection in breeding.

Distribution of polymorphic markers based on their physical position (Gramene database) is almost uniform for chromosome 2, 3, and 9 (gap between polymorphic markers is less than 5Mb) but in chromosome 12, 10, 6, 1, and 7 longer stretches of DNA found to be monomorphic indicating conserved sequences distributed in the two parents selected for the study. The importance of evenly distributed markers for analysis and to generate few but highly informative data points either for mapping or threshold recovery of a recurrent parent in marker assisted backcross breeding has been highlighted (Prigge et al, 2009; Servin & Hospital, 2002). Non-random and non-uniform distribution of microsatellite markers in plant genome have also been reported (Morgante et al., 2002). Also, preferential positioning of the microsatellite in the rice genome was revealed (Grover et al., 2007). The reason behind this kind of positioning of markers has been explained as DNA replication and repair mechanisms during the synthesis of new repeats and elimination of existing repeats (Schlotterer, 2000). Low polymorphic regions can be recovered by screening more SSR markers for a given region based on available resources and published literature on the genome map of rice.

The identified polymorphic markers from this investigation will be utilized for the mapping of major QTLs/ Candidate genes associated with different micro-nutrients and quality-related traits

Figure 2 Frequency distribution of different types of SSR repeat motifs (di, tri, tetra)

Figure 3 Number of different types of repeat motifs across the 12 chromosomes of Rice

Figure 4 Parental polymorphism profile of parents; Rajendrakasturi (P_1) and URG-30 (P_2); here 50bp- DNA ladder, P_1 -Parent 1 (Rajendrakasturi), P_2 -Parent 2 (URG-30)

in rice. Once identified QTL region defined by marker interval can be narrowed down further by fine-mapping approaches. In detail scanning of genome-wide polymorphic SSR resources for a given biparental mapping population, provides way forward for studying 'Causal polymorphism in terms of SNP variant' in a genomic region defined by QTL interval (Gupta et al., 2019). Recent advancement in sequencing technologies has strengthened this possibility by high-throughput genotyping and NGS-led QTL-seq assay. QTL-seq analysis and differential expression profiling studies were performed by Daware et al. (2016) to identify a potential candidate and allelic variants for grain weight trait in rice is an example of successful integrated use of SSR and SNP markers data.

Conclusion

Breeding mineral dense rice variety is one of the sustainable approaches for ensuring the nutritional security of the nation. Local genotypes and landraces are a proven source of higher mineral content and the present study attempts to make use of local genotype to identify new genomic regions for high grain zinc and

iron content. Identified markers showing allelic variant for whole genome wide SSRs covering the whole genome of rice in the present parental polymorphism study will be utilized for genotyping of whole mapping population developed from the above cross and better understanding of mapping QTLs/genes for high grain zinc, iron, grain quality, and yield traits. It can significantly contribute towards the development of mineral dense rice variety using marker assisted selection and marker assisted backcross approaches.

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Conflicts of interest

The authors declare that there is no conflict of interest.

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