

Original Research Article

Parental Polymorphism Survey of economically important rice genotypes and Study of Allelic Variation for Grain Quality and Yield Traits using SSR Markers in Rice (*Oryza Sativa* L.)

(In this study the allelic variation is not studied, the outcomes mentioned in the manuscript are based on the previous reports/ study.)

ABSTRACT:

Improvement in rice grain quality traits particularly aroma ~~and~~, cooking, and eating quality is an important breeding objective in rice crop breeding. Identification of polymorphic markers is a prerequisite for ~~any~~ the QTL mapping experiment because if the parents are polymorphic for the traits of interest, then further selection of plants in the progenies becomes easy. Hence, the objective of the present study was to identify polymorphic markers for grain quality and yield related traits among the parental lines Improved Samba Mahsuri and Badshabhog. For this a total of 576 randomly selected microsatellite SSR markers distributed over all the 12 chromosomes of rice including 26 gene specific markers related to the aroma, cooking and eating quality, grain dimension, and yield related traits were used. Overall, 96 markers including 4 gene specific markers were found to be polymorphic between the two genotypes indicating a total polymorphism percentage of 16.67%. The highest polymorphism percentage was recorded on chromosome 6 (26.67%) followed by chromosome no 4 (21.43%) and the lowest polymorphism percentage was observed on chromosome 10 (8.93%). These markers will further be utilized in genotyping of $F_{2:3}$ population, linkage analysis and mapping QTL's for grain quality and yield traits.

Key words: Rice, aroma, cooking and eating quality, parental polymorphism, QTL, SSR.

1. INTRODUCTION:

Rice is an indispensable cereal to the world providing nutrition to about 21% of the global population and 2/3rd of the South East Asian population [24]. In 2019, global rice production was 755.47 million tons from 162.05 million ha of area. Among the 117 different rice producing countries, China ranks first with a production of 209.61 MT followed by India with 177.64 MT in 2019. Both these countries accounts for more than 50% of the total rice production. Asian countries contribute 89.6 % of the total world production (677.27 MT) followed by Africa (38.77 MT), America (35.32 MT), Europe (4.02 MT) and Oceania (0.76 MT) [2]. To meet the ever-increasing demand, several new varieties are breed every year to achieve the highest yield and are resistant to various biotic and abiotic stress also. But, the grain qualities in these varieties were less prioritized. Grain quality is very hard to define as it comprises of several different facets such as grain appearance quality, milling quality, nutritional quality, eating quality, and cooking quality [11,4]. The preferences and taste vary from one region to another, what may be preferable in one region may not necessarily be preferable in another region. [6]. Nearly 40% of people around the globe particularly in the USA, Canada, Europe, Middle East, and South East Asian countries desire to eat aromatic rice irrespective of grain types.

Most of the quality traits follow quantitative inheritance and have complex genetic architecture because of the considerable influence of genotype, environment, and their interactions [1,18]. Phenotype-based classical breeding approaches are time-consuming, laborious, and are inefficient in improving traits that are governed by quantitative trait loci (QTL), such as yield and other grain quality traits which show continuous phenotypic variation and lack discrete phenotypic segregation in the progeny [32,22]. However, several recent developments such as the use of potential donor parents, identification of QTLs regions associated with important grain qualities, and identification of robust marker have increased our understanding of the genes, pathways, and molecular mechanisms determining overall quality traits in rice [11,20]. Breeding programs should be directed in to develop rice varieties with better eating quality and to meet out the demands of the local consumers as well as emerging food processing industry.

Mapping of quantitative trait loci (QTLs) for grain quality traits in rice is an important forward genetic approach and using these dissected complex regions in marker-assisted selection MAS and gene discovery [27]. With the advent of several molecular techniques, the use of molecular markers has

increased to a large extent in the recent past. Among the PCR-based markers, the SSR markers have proved to be very effective tools and the first choice of breeders in the study of genetic diversity and organism relationships due to their several advantages such as they are co-dominant, highly polymorphic in nature, evenly distributed in the genome, efficient, less quantity of DNA is required, are highly cost-effective and transferability [15]. These micro-satelliteSSR (Keep microsatellite or SSR name throughout the manuscript) markers can be effectively used in studying the genetic polymorphism i.e., occurrence of multiple alleles at a single locus, where at least two alleles occur with a frequency greater than 1% [12]. Also, they can be used in population structure analysis, gene mapping and tagging, linkage map construction, tracing marker-trait association, Marker Assisted Selection (MAS), etc. [23]. So, developing rice varieties with desired grain quality Leviz., nutritional and cooking, and eating quality which arewith high yielding as well as resistancecet to various abiotic and biotic stresses with the help of by using molecular techniques and breeding tools will help to alleviate several malnutritional problems and will also fetch high price to farmers as well as food industries personnel. Thus, the present study was aimed to identify informative polymorphic SSR markers between two diverse parents, viz-Improved Samba Mahsuri and local rice germplasm Badshabhog for yield and quality traits in rice. The research was also planned to study the influence of the type of the repeat motif on polymorphism. (Write about the importance, ecology, and/ biological background of Improved Samba Mahsuri and Badshabhog in Introduction section)

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2. MATERIALS AND METHOD:

2.1 Plant Material used in the study:

The experimental plant material for this study comprised of a highly contrasting rice lines-parents for having desirable grain quality and yield traits. The first parent is short grain aromatic landrace 'Badshabhog', a popular and preferred line among the aromatic rice breeders and second variety is 'Improved Samba Mahsuri' a fine grain, medium slender non-aromatic rice, popular specially for grain quality. Details of all the traits of both the parents is-are presented in table 1. These two are popular varieties and being used as a source for desirable grain traits in rice breeding. Both the parents were raised by following all recommended package of practices as per recommendation of this???? zone. Twenty-one days old seedlings of Improved Samba Mahsuri and donor Badshabhog were transplanted in

the [main](#)-field for evaluation. Leaf samples were collected 14 days after transplanting and were preserved at -20°C for parental polymorphism survey after DNA extraction.

Table 1. Details of the parents used in parental polymorphism experiment:

Sr.no	Trait name	Recipient	Donor
1	Name	Improved Samba Mahsuri	Badshabhog
2	Type	Released variety in 2008 jointly by ICAR-IIRR and CSIR-CCMB Hyderabad, India	Local landrace collected by Institute of Agricultural Sciences, BHU, Varanasi, U.P, India
3	Parentage	Samba Mahsuri*4/SS1113	Local landrace
4	Yield	4.75-5.0 tonnes ha ⁻¹	2.5-3 tonnes ha ⁻¹
5	Days to 50 % flowering	100-103 days	120-123 days
6	Days to maturity	130-134 days	150-154 days
7	Plant height	100-103 cm	155-160 cm
8	Grain length	8.15 mm	5.53 mm
9	Grain breadth	2.01 mm	2.11 mm
10	Kernel length	5.32 mm	3.83 mm
11	Kernel breadth	1.78 mm	1.85 mm
12	Aroma	Non aromatic	Highly aromatic
13	Amylose content	23.84 unit ???	18.46 unit??

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2.2 Genomic DNA Isolation and quality check:

The genomic DNA of both the parents were extracted by the CTAB method [16], with some modifications. 100 mg of young leaves was weighed and the genomic DNA was extracted [with DNA by using](#) extraction buffer (10% CTAB, 1 M Tris HCl (pH 8.0), 0.5M EDTA (pH 8.0), 5 M NaCl, and 0.2 % β-mercaptoethanol) preheated at 60° C. The quality and quantity of extracted DNA was [judged-estimated](#) on 0.8 % agarose gel [electrophoresis](#) along with a standard ladder and compared with band intensity and thickness. DNA quantification and purity were checked by measuring the O.D values at 260 and 280 nm using a NanoDrop ND100 spectrophotometer. The ratio of UV absorbance at A_{260}/A_{280} ranged between 1.89-1.96, and this ratio indicates good-quality DNA. The quantity of DNA in the isolated samples ranged from 1288.50 to 1701.30 ng/μl. After quantification, the DNA samples were diluted with TE buffer, [so as](#) to make final concentration of the DNA as 50 ng / μl stored at 4° C.

2.3 Details of SSR markers used in the study:

A total of 576 randomly selected [microsatellite-SSR](#) markers including 26 gene specific markers [covering the entire length of each of distributed on](#) 12 chromosomes of rice were used to identify

polymorphism between Improved Samba Mahsuri and Badshabhog. The ~~information regarding~~ chromosomal location, sequences of primers (forward and reverse), physical position (SSR start and SSR end), ~~number and number~~ of repeat motifs were obtained from Gramene markers database (<https://www.gramene.org/>). RAP-DB (The Rice Annotation Project Data Base) was also used for those markers whose information was not available in the Gramene 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>).

2.4 PCR amplification:

PCR ~~reaction~~ was carried out in ~~in~~ Eppendorf thermal cycler with a final reaction volume of 15 μ l containing 1.2 μ l of genomic DNA, 1.5 μ l of 10 X Taq assay buffer containing 15 mM MgCl₂, forward and reverse primer each 1 μ l (10 pmol/ μ l), 0.2 μ l of dNTPs (3 mM), 0.2 μ l Taq DNA polymerase (GeNei) and 9.9 μ l of HPLC water. The PCR reaction was performed under the following conditions: Initial denaturation at 94° C for 5 min, followed by 35 cycles of denaturation at 94° C for 45 seconds, annealing at the temperature specific to primer for 30 seconds, extension at 72° C for 30 seconds, followed by the final extension at 72° C for 5 min and hold at 4° C for ∞ . After completion of amplification, PCR products were stored at -20° C

2.5 Agarose gel electrophoresis:

The ~~final~~ amplified products were ~~analyzed by resolved on electrophoresis using~~ 2.5% agarose gels in 1X TAE Buffer at 65V for initial 30 minutes followed by 90V for 1.5 hours. Gel was stained by Ethidium bromide (10 mg/ml) @ 2.5 μ l/1000 ml 1X TAE Buffer. The DNA fragments were then visualized under Gel documentation system (Gel Doc TM XR+, BIO-RAD, USA) and the banding pattern was observed and recorded for ~~further use~~ [further analysis](#).

3. RESULTS AND DISCUSSION:

In the present study, the parental polymorphism survey between the parents, Improved Samba Mahsuri and Badshabhog indicated a clear polymorphism and it is very important to understand this variation at molecular level. ~~A total of 576 randomly selected SSR primers including 26 gene specific markers distributed all over 12 chromosomes were used for testing polymorphism between two parents.~~ This was

confirmed and tested by using graphical genotypes (GGTs) software package 2.0.[29] [\(Give the fig of GGT having high dpi\)](#). Fig. 1 depicts the distribution of all the identified polymorphic markers across the length of 12 rice chromosome, according to their physical positions (Mb) on a genetic map. ~~Of the~~Among 576 SSR markers used, 96 markers including 4 gene specific markers were found to be polymorphic between the two genotypes indicating a total polymorphism percentage of 16.67%. [The polymorphic banding pattern of S](#)some of the identified ~~polymorphic~~ markers are ~~displayed shown~~ in gel picture (Fig. 2). ~~Of all the chromosomes~~The highest polymorphism percentage was recorded on chromosome 6 (26.67%) followed by chromosome no 4 (21.43%) and the lowest polymorphism percentage was observed on chromosome 10 (8.93%) [\(Table 2\)](#). ~~The chromosome wise polymorphism percentage is stated in table 2~~. For QTL mapping genetically diverse parents are required to map the traits of interest [9] and a high percentage of polymorphism between the two parents used in the study shows that the two parents are genetically diverse. The identified polymorphic markers can further be effectively used in QTL mapping experiment for preparation of linkage map and identification of QTL's related to grain quality and yield traits in mapping population derived from the ~~above cross~~both the parents.

Table 2. Chromosomal wise polymorphism percentage of SSR markers between the parents Improved Samba Mahsuri and Badshabhog

Chromosome no.	Total No. of SSR marker used	No. of Polymorphic markers obtained	Polymorphism (%)
Chromosome 1	51	10	19.61
Chromosome 2	47	7	14.89
Chromosome 3	55	8	14.55
Chromosome 4	42	9	21.43
Chromosome 5	52	8	15.38
Chromosome 6	30	8	26.67 (Highest)
Chromosome 7	45	8	17.78
Chromosome 8	53	11	20.75
Chromosome 9	48	8	16.67
Chromosome 10	56	5	8.93 (Lowest)
Chromosome 11	45	7	15.56
Chromosome 12	52	7	13.46
Total	576	96	16.67

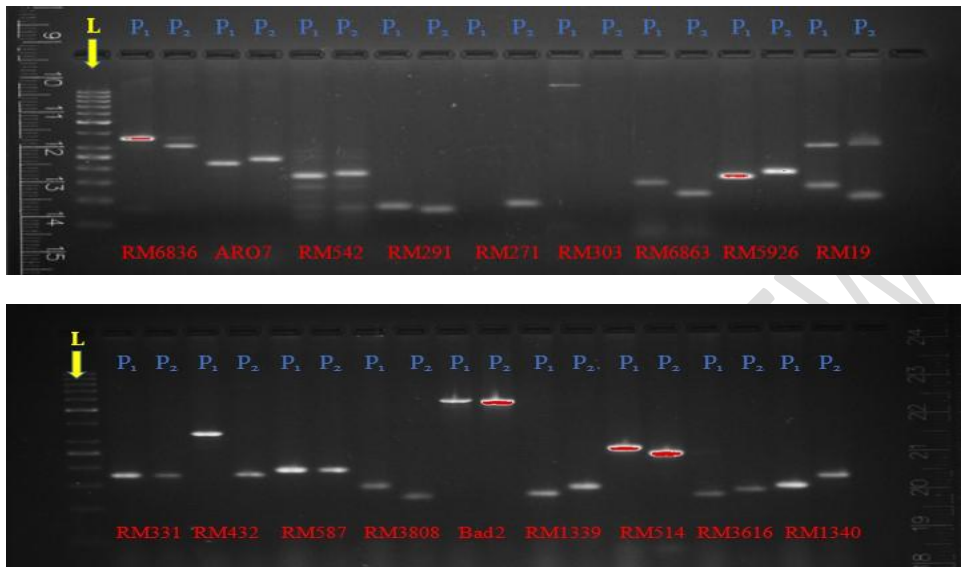


Fig. 2. Gel picture showing parental polymorphism survey with SSR markers among the parents. P1- Improved Samba Mahsuri, P2- Badshahhog, L- Ladder 50 bp.

The recent past studies on parental polymorphism also ~~Similar results were reported-revealed the by other authors also comparable reports.~~ The polymorphism percentage highest for chromosome 6 and lowest on chromosome 10 was also reported by (Write the name)[23]. Similarly, [13] (Write the name of author) reported polymorphism percentage of 29.02% between the two parents Rajendrakasturi and URG-30. The highest polymorphism was reported on chromosome 4 (40.96%) whereas the lowest polymorphism was observed in chromosome 9 (16%). (Write the name)[7] found a total polymorphism percentage of 20.75% by using 800 SSR markers between BPT5204 and *O. rufipogon* WR119. A polymorphic percentage of 6.93 was reported by [14] (Write the name of author) using 1,904 genomic SSRs markers among the parents IR58025A and KMR-3R.

In the present study a total of 96 polymorphic markers were identified, out of which 20 SSR markers were reported to be associated with QTLs for different quality traits and the 4 gene specific markers were associated with genes of aroma and eating and cooking quality traits of rice from previous studies. ~~Of the~~Among 4 gene specific markers 3 markers were found to tightly linked with the genes

associated with aroma content in rice *viz.*, ARO7 [26], BADEX7_5 [21], nksbad2 [1], and one marker SSI [3] was found to be associated with cooking and eating quality traits. The gene specific primer ARO7 is linked with *fgr* gene encoding BAD2 which is associated in the expression of aroma in rice, while BADEX 7_5 and nksbad2 is linked to badh2 gene, with 8-bp deletion in the exon 7 of badh2 gene implicates with rice aroma. The gene specific primer SSI is linked to gene encoding granule-bound starch synthase I. The SSR markers RM19, RM217, RM219 flank the QTL associated with cooking and eating quality traits such as amylose content and alkali digestion value and RM19 was also linked with the trait kernel length after cooking [5]. [31] [Write the author name](#) found that RM488, RM562, RM432 were linked with grain dimension traits such as grain length and grain breadth while RM432 was also found to be associated with amylose content in rice. [10] reported that RM7180, RM3616, RM6836, RM3183 were significantly associated with grain dimension traits such as grain length and grain breadth. Three SSR markers CHR3_14, CHR3_20, CHR8_4 was found to be associated with aroma content in rice [25]. Two SSR markers RM404, RM547 were found to be linked with aroma content and RM17 with amylose content in rice in a study conducted by [author ??? Make a correction at all places](#) [30]. [1] reported that RM80 and RM252 was found to be associated with aroma content in rice and RM217 and RM432 with cooking quality traits such as amylose content and alkali digestion value and grain dimension traits such as grain length and grain breadth. One SSR marker RM42 of chromosome8 was found to be linked with grain aroma content in two studies conducted by [8]. While [27] reported that RM5688 was found to be associated with amylose content and other cooking quality traits on chromosome 9.

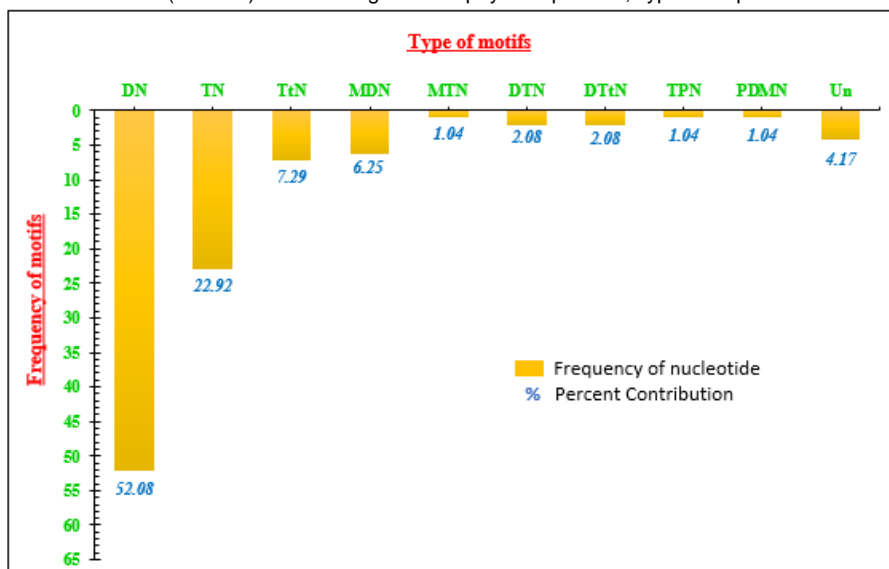
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Effect of the nature and form of the motif on polymorphism detection:

~~Within the set of~~The 96 [identified](#) polymorphic marker, most consisted of dinucleotide motifs (52.08%), followed by trinucleotide motifs (22.92%), subsequently by tetranucleotide motifs (7.29%). Similarly group of either mono-dinucleotide (6.25%), mono-trinucleotide (1.04%), di-trinucleotide (2.08%), di-tetranucleotide (2.08%), tri-pentanucleotide (1.04%), and penta-di-mononucleotide (1.04%) were also found (Fig. 3). These findings are in accordance with the results of [????](#)[17,19]. The dinucleotide motifs showed a much larger polymorphism level than the trinucleotide, tetranucleotide and the other motifs classes. However, the number of repeat motifs does not affect the level of polymorphism rate. Among the most frequent motif *viz.*, dinucleotide motifs, (GA)_n repeats were more frequent (39.34%), followed by

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(CT)_n with 27.87% and least by (TA)_n with 1.64%. Among the trinucleotide motifs (CCT)_n repeats were maximum. (ATAG)_n repeats characterized 30% of the tetranucleotide motifs followed by (AGAT)_n 20% whereas the other repeat classes (CATC)_n, (GAGT)_n, (GATG)_n, (TAAT)_n, (TCTT)_n each represented 10%. On the other hand, the number of repeats ranged from 5 to 46 for the different polymorphic microsatellite markers (Table 3). The findings of the physical position, type of repeat motif and no. of



repeats of the polymorphic SSR markers in the present investigation will aid in formation of set of polymorphic SSR's which can further be used in genotyping of mapping population for QTL mapping and marker-assisted selection in breeding.

Fig. 3. Frequency distribution of different types of SSR repeat motifs. (DN: Dinucleotide, TN: Trinucleotide, TtN: Tetranucleotide, MDN: Mono-Dinucleotide, MTN: Mono-Trinucleotide, DTN: Di-Trinucleotide, DTtN: Di-Tetranucleotide, TPN: Tri-Pentanucleotide, PDMN: Penta-Di-Mononucleotide, Un: Unknown)

CONCLUSION:

Thus, in the present study, a total of 96 SSR markers including 4 gene specific markers were found to be polymorphic among-across the two parents indicating a total polymorphism percentage of 16.67%.

Dinucleotide repeats were in much larger number as compared with other classes of nucleotides. The suitable polymorphic markers depending upon the nucleotide repeats will further be selected and utilized

in genotyping of $F_{2:3}$ population and identification of QTLs for grain quality and yield traits- [results in development of improve rice cultivars having a more economic value.](#)

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Table 3: Identified polymorphic markers between the two parents along with their complete details is mentioned in table below:

Sr. no.	Name	Chr no.	Temp	Forward sequence	Reverse sequence	Map Distance	Motif	No. of repeats
1.	RM3252	1	57.4	GGTAACTTTGTTCCCATGC	GGTCAATCATGCATGCAC	0.3	CT	13
2.	RM220	1	53.1	GGAAGGTAACGTGTTCCAC	GAAATGCTTCCCACATGTCT	4.42	CT	17
3.	RM259	1	56.6	TGGAGTTTGAGAGGAGGG	CTTGTTCATGGTGCCATGT	7.44	CT	17
4.	RM562	1	55.3	CACAACCCACAAACAGCAAG	CTTCCCCAAAGTTTATGCC	14.62	AAG	13
5.	RM24	1	58.2	GAAGTGTGATCACTGTAAAC	TACAGTGGACGGCGAAGTCG	18.97	GA	29
6.	RM9	1	58.2	GGTGCCATTGTGCTCCTC	ACGGCCCTCATCACCTTC	23.32	GA, GT, GA	15, 1, 2
7.	RM543	1	59.3	CTGCTGCAGACTCTACTGCG	AAATATTACCCATCCCCC	32.78	GCG	10
8.	RM7180	1	48.3	GTGTTTATAGGGGTGCCAC	TGTTGGTGGTGCAGGTAAAG	34.1	ATAG	6
9.	RM1339	1	56.3	ATCAAAGCATGTAAACCAAC	CGTAAGATCTCCCTACC	38.19	AG	22
10.	RM14	1	55.7	CCGAGGAGAGGAGTTCGAC	GTGCCAATTCCTCGAAA	41.36	GA	18
11.	RM279	2	57.3	GCGGGAGAGGGATCTCCT	GGCTAGGAGTTAACCTCGCG	2.88	GA	16
12.	RM71	2	58.3	CTAGAGGGCGAAAACGAGATG	GGGTGGGCGAGGTAATAATG	8.76	ATT, T, ATT	10, 1, 4
13.	RM452	2	58.3	CGATCGAGAGCGTTAAGG	GGGATCAAACCACGTTTCTG	9.56	GTC	9
14.	RM324	2	57.3	CTGATTCCACACACTTGTGC	GATTCCACGTCAGGATCTTC	11.38	CAT	21
15.	RM263	2	60.4	CCCAGGCTAGCTCATGAAAC	GCTACGTTTGAGCTACCACG	25.86	CT	34
16.	RM526	2	57.5	CCCAAGCAATACGTCCCTAG	ACCTGGTCATGACAAGGAGG	26.66	TAAT	5
17.	RM14220	2	61.5	TCTCCACACAAAATTGGAACACG	GTCTGTATTGTGGGTGCAAGAGG	35.67	AC	14
18.	CHR3_14	3	52.6	GCCTACTCGTCTACCAACA	GGCACTTATCCATTTCAG	4.97	AT	n/a
19.	CHR3_20	3	55.3	ATTGACACGAAGAGGACA	GTGCCCGAGGTGAGTGAATG	5.95	GTA	n/a
20.	RM36	3	58.3	CAACTATGCACCATTGTGCG	GTACTCCACAAGACCGTACC	7.12	GA	23
21.	RM16	3	57.4	CGCTAGGGCAGCATCTAA	AACACAGCAGGTACGCGC	23.12	TCG, GA	5, 16
22.	RM3513	3	61.5	TACTCCTATCCTGCCATGG	TGTAGTAGACGAGAGGCCCGG	25.11	CT	28
23.	RM4796	3	57.4	CCACGGTAGTTTTGGTCTAC	AGAGGGGAAGAGTGAGAGAG	27.47	TA	26
24.	RM520	3	55.3	AGGAGCAAGAAAAGTTCC	GCCAATGTGTGACGCAATAG	30.91	AG	10

25.	RM51 4	3	56.3	AGATTGATCTCCATTCCC C	CACGAGCATATTACTAG TGG	35.28	AC	12
26.	RM33 5	4	59.8	GTACACACCCACATCGAG AAG	GCTCTATGCGAGTATCC ATGG	0.68	CTT	25
27.	RM26 1	4	58.3	CTACTTCTCCCCTTGTGTC G	TGTACCATCGCCAAATC TCC	6.57	C, CT	9, 8
28.	RM11 42	4	57.1	AAGCACACGTAAAACGGA GG	CGTCACTCTCACCACCA CC	21.39	AG	12
29.	RM13 88	4	54	TTCAATGAGGCAAAGGTA AG	ATTGTAGCTTGGACTAG GGG	25.2	AG	46
30.	RM31 7	4	59.8	CATACTTACCAGTTCACCG CC	CTGGAGAGTGTACAGCTA GTTGA	29.06	GC, GT	4, 18
31.	RM67 48	4	52.7	ATTGGGTTTCTCATATTAT G	CCAACACTCCTAACTAG TTC	30.9	TAT	18
32.	RM38 43	4	61.5	ACCCTACTCCCAACAGTCC C	GGGGTCGTACGCTCATG TC	31.49	GA	23
33.	RM11 13	4	55.3	GGGCGCATGTGTATTTCTT C	TGGGGAAAAACCACAA GCC	34.08	AG	12
34.	RM28 0	4	57.4	ACACGATCCACTTTGCGC G	TGTGTCTTGAGCAGCCA GG	34.98	GA	16
35.	RM12 2	5	61.5	GAGTCGATGTAATGTCATC AGTGC	GAAGGAGGTATCGCTTT GTTGGAC	0.31	GA, A, GA, A, GA	7, 1, 2, 1, 11
36.	RM13	5	56.6	TCCAACATGGCAAGAGAG AG	GGTGGCATTTCGATTCCA G	2.01	GA	16
37.	RM54 8	5	56.4	TCGGTGAGAACTGAGAG TACC	AAGGAGGCCATCTCAAT GTG	2.81	CT	12
38.	RM26 7	5	56.8	TGCAGACATAGAAAGGA AGTG	AGCAACAGCACAACCTG ATG	2.88	GA	21
39.	RM28 9	5	56.6	TTCCATGGCACACAAGCC	CTGTGCAGAACTTCCA AAG	7.8	G, GA	11, 16
40.	RM43 0	5	55	AAACAACGACGTCCCTGA TC	GTGCCTCCGTGGTTATG AAC	18.69	GA	25
41.	RM16 3	5	60.4	ATCCATGTGCGCCTTTATG AGGA	CGCTACCTCCTCACTTA CTAGT	19.18	GGAGA, GA, C, GA	4, 11, 1, 20
42.	RM36 16	5	56.3	GTGCGGATTTCCTCTCT C	TGCCGGTCCATTCTAG AAG	26.28	GA	13
43.	SSI	6	57.4	GATCCGTTTTTGCTGTGCC C	CCTCCTCTCCGCCGATC CTG	3.09	n/a	
44.	RM21 7	6	54.4	ATCGCAGCAATGCCTCGT	GGGTGTGAACAAAGAC AC	4.23	CT	20
45.	RM68 36	6	53.9	TGTTGCATATGGTGCTATT TGA	GATACGGCTTCTAGGCC AAA	9.3	TCT	14
46.	RM56 4	6	54.3	TCCTTCTGCATCAATTCCT CTCG	CTCCATAGCCTTGTTAA GTGATGAGC	11.61	GT	14
47.	RM31 83	6	56.6	GCTCCACAGAAAAGCAAA GC	TGCAACAGTAGCTGTAG CCG	12.44	CT	12
48.	RM13 40	6	56.3	TCCAACTAGTGGGAACG C	CTCAACGCCATGAACCT C	23.34	AG	22
49.	RM52 8	6	57.3	GGCATCCAATTTACCCCT C	AAATGGAGCATGGAGGT CAC	26.55	AGAT	9
50.	RM30	6	59.8	GGTTAGGCATCGTCACGG	TCACCTCACCACACGAC ACG	26.87	AG, A, GA	9, 1, 12
51.	RM69 65	7	53.2	TCATTTGGATCATAAGCTG G	TTGGATGAGATAACCAA TGC	2.8	TTC	11

52.	RM48 1	7	57.3	TAGCTAGCCGATTGAATG GC	CTCCACCTCCTATGTTGT TG	2.88	CAA	12
53.	RM54 2	7	55	TGAATCAAGCCCCTCACTA C	CTGCAACGAGTAAGGCA GAG	12.71	CT	22
54.	RM43 2	7	55.5	TTCTGTCTCACGCTGGATT G	AGCTGCGTACGTGATGA ATG	18.95	CATC	9
55.	RM35 1	7	58.2	CCATCCTCCACCGCCTCTC G	TGGAGGAAGGAAAGGG GACG	23.92	CCG, CGAAG	9, 4
56.	RM11 32	7	61.5	ATCACCTGAGAAACATCC GG	CTCCTCCCACGTCAAGG TC	23.98	AG	12
57.	RM23 4	7	55	ACAGTATCCAAGGCCCTG G	CACGTGAGACAAAGAC GGAG	25.47	CT	25
58.	RM35 55	7	57.4	TGGAAGTTTCTGGCGATA G	TGGTTGGACTGAAAAGT CCC	27.89	GA	12
59.	RM40 8	8	61.5	CAACGAGCTAACTTCCGTC C	ACTGCTACTTGGGTAGC TGACC	0.12	CT	13
60.	RM68 63	8	54.4	GCTGCAGAATTAAGGAGA AC	TGCTCAAATAATCAGC TCC	2.01	TGC	9
61.	RM56 47	8	58	ACTCCGACTGCAGTTTTTG C	AACTTGGTCGTGGACAG TGC	2.89	AAG	16
62.	RM54 7	8	58.5	TAGGTTGGCAGACCTTTTC G	GTCAAGATCATCCTCGT AGCG	5.59	ATT	19
63.	CHR8 4	8	56.9	GATTGAAAGAGAAAGGTG GTT	CTGTGTAACCGAGTTAC GTTT	9.95	ATAG	n/a
64.	RM40 4	8	57.3	CCAATCATTAACCCCTGAG C	GCCTTCATGCTTCAGAA GAC	15.43	GA	33
65.	RM42	8	58.3	ATCCTACCGCTGACCATGA G	TTTGGTCTACGTGGCGT ACA	20.09	AG, AG, T, GA	6-2, 1, 5
66.	ARO7	8	54.4	ATTTGCCTCCTGAGTCTG	GAGGATGGGGAAGATA AA	20.26	n/a	
67.	BADE X7-5	8	53.1	TGTTTTCTGTTAGGTTGCA TT	ATCCACAGAAATTTGGA AAC	20.32	n/a	
68.	nksbad 2	8	61	GGTTGCATTTACTGGGAGT TATG	TCCACAGAAATTTGGAA ACAAAC	20.38	n/a	
69.	RM80	8	57.7	TTGAAGGCGCTGAAGGAG	CATCAACCTCGTCTTCA CCG	24.47	TCT	25
70.	RM31 6	9	56.7	CTAGTTGGGCATACGATG GC	ACGCTTATATGTTACGT CAAC	1.1	GT, TG, TTTG, TG	8-9, 4, 4
71.	RM56 88	9	56.3	GCAGTGTCCAACCATCTGT G	ATCTGGTCACCCTTTGCT TG	1.71	AAT	17
72.	RM21 9	9	54.6	CGTCGGATGATGTAAAGC CT	CATATCGGCATTCGCCT G	7.9	CT	17
73.	RM73 87	9	54.6	GCAGTAGGGAGCATGGAA AG	AAACGAGTCTCTTCAG GGG	10.13	GAGT	6
74.	RM10 5	9	67.7	GTCGTCGACCCATCGGAG CCAC	TGGTCGAGGTGGGGATC GGGTC	12.6	CCT	6
75.	RM32 8	9	57.5	CATAGTGGAGTATGCAGC TGC	CCTTCTCCAGTCGTATC TG	19.72	CAT	5
76.	RM16 0	9	66.7	CCCAAATCAGGAAAGTTT CTCAGC	AGTCATCCTTGGCTACC AGATGC	19.78	GAA	23
77.	RM38 08	9	55.6	CGTTAGCGAAACGAACAG TG	CAGTGGCTCGGTAATCG C	20.54	GA	20
78.	RM47 4	10	55.3	AAGATGTACGGGTGGCAT TC	TATGAGCTGGTGAGCAA TGG	1.81	AT	13

79.	RM311	10	50.7	TGGTAGTATAGGFACTAA ACAT	TCCTATACACATACAAA CATAAC	9.74	GT, GTAT, GT	3, 8, 5
80.	RM258	10	58	TGCTGTATGTAGCTCGCAC C	TGGCCTTTAAAGCTGTC GC	18.01	GA, GGA	21, 3
81.	RM171	10	58.7	AACGCGAGGACACGTA TACT	ACGAGATACGTACGCCT TTG	19.04	GATG	5
82.	RM590	10	58.5	CATCTCCGCTCTCCATGC	GGAGTTGGGGTCTTGT TCG	23.04	TCT	10
83.	RM286	11	56.9	GGCTTCATCTTTGGCGAC	CCGGATTCACGAGATAA ACTC	0.38	GA	16
84.	RM202	11	55.6	CAGATTGGAGATGAAGTC CTCC	CCAGCAAGCATGTCAAT GTA	9	CT	30
85.	RM229	11	57.4	CACTCACACGAACGACTG AC	CGCAGGTCTTTGTGAAA TGT	18.4	TC, CT, C, CT	11, 5, 3, 5
86.	RM21	11	60.4	ACAGTATCCGTAGGCAC GG	GTCCTATGAGGGTGGTA GAG	19.6	GA	18
87.	RM206	11	56.3	CCCATGCGTTAACTATTC T	CGTCCATCGATCCGTA TGG	22.01	CT	21
88.	RM1233	11	53.2	TTCGTTTTCTTGGTTAGT	ATTGGCTCCTGAAGAAG G	26.53	AG	15
89.	RM5926	11	54.4	ATATACTGTAGGTCCATCC A	AGATAGTATAGCGTAGC AGC	28.43	ATT	21
90.	RM20	12	57.8	ATCTTGTCCTGCAGGTCA T	GAAACAGAGGCACATTT CATTG	0.97	ATT	14
91.	RM19	12	54.4	CAAAAACAGAGCAGATGA C	CTCAAGATGGACGCCAA GA	2.43	ATC	10
92.	RM1337	12	55.3	GTGCAATGCTGAGGAGTA TC	CTGAGAACTGGAGTGC TTG	11.93	AG	21
93.	RM28305	12	56.5	GTCATCTTCGCAAATGGTG ATGG	GGTCGTCGTGGTGTAT TCTTGG	19.92	GA	31
94.	RM28367	12	62	CGTATCTCCACCTCCCAG AAGC	GCCAAATCTCACGGATC GAAGC	21.17	AG	21
95.	RM28556	12	61.1	CTAGTAGTGCCACTTAACC GTGTTTCG	GGATCCAAACACCACCT TAGCC	23.98	TC	14
96.	RM17	12	59.3	TGCCCTGTTATTTTCTTCTC TC	GGTGATCCTTTCCCATTT CA	26.95	GA	21

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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