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SSR based parental polymorphism survey for marker assisted backcross breeding in rice (*Oryza sativa* L.)

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ABSTRACT

The brown planthopper (BPH), *Nilaparvata lugens* (Stål), is a destructive and widespread pest in rice-growing regions across Asia and developing resistant varieties is considered to be the most effective solution. Marker assisted backcrossing (MABC) is a widely used approach for introgressing resistant genes using backcross breeding to the highly adapted varieties from donors with the help of molecular markers and the availability of polymorphic markers being a critical factor for its success. The present study was aimed to assess parental polymorphism per centage using SSR markers between the rice varieties BPT5204 and RP2068-18-3-5 (donor for BPH resistant gene). A total of 340 SSR markers covering 12 chromosomes were used for the survey and 96 markers were found to be polymorphic between the parents. The number of polymorphic markers per chromosome ranged from 6 to 11, with the highest number (11) observed on chromosomes 1 and 3. The percentage of polymorphism per chromosome ranged from 16.7% to 36.7%, with chromosome 3 showing the highest percentage. The average polymorphism rate per chromosome was 28.3%. The identified polymorphic markers will be useful for estimating the recurrent parent genome recovery per centage in marker-assisted background selection and for mapping QTLs associated with BPH resistance.

Key words: *Parental survey, Polymorphism, Rice and SSR markers*

Rice (*Oryza sativa* L.) is a staple food for more than 3.5 billion people worldwide. With a growing global population, rice production needs to be doubled by 2030 to meet the future demands. However, rice is highly susceptible to various diseases and pests throughout its growth and development, resulting in significant yield losses in many rice producing countries.

The brown planthopper (BPH) caused by *Nilaparvata lugens* (Stål), poses a significant threat to rice production globally. This pest causes substantial yield losses annually, making it a major concern for farmers and researchers. BPH inflicts damage on rice plants through direct feeding and by transmitting viral diseases. The pest's feeding behaviour involves sucking sap from the lower portions of rice plants, leading to a reduction in chlorophyll and protein content in leaves. This, in turn, decreases the rate of photosynthesis and results in leaf yellowing, reduced tiller number and the production of unfilled grains. In severe cases, BPH infestation can cause complete drying and plant death, a condition known as 'hopper burn' (Muduli *et al*., 2021).

Furthermore, BPH acts as a vector for various plant pathogens, transmitting viral diseases such as grassy stunt virus (RGSV) and ragged stunt virus (RRSV). These diseases indirectly harm rice crops, compounding the damage caused by direct feeding (Yan *et al*., 2023). In China, BPH outbreaks led to major yield losses, with about 3 million tons of rice destroyed during infestations from 2005 to 2008 (Hu *et al*., 2016). Comparable severe yield reductions occurred due to BPH invasions in Japan, Korea, Vietnam, Central Thailand and Indonesia in 2005, 2007, 2009 and 2011, respectively (Brar *et al.,* 2009 and Catindig *et al*., 2009). The most severe outbreak of BPH in India occurred in Kerala at the end of 1973 and early 1974 (Koya, 1974 and Nalinakumari and Mammen, 1975). In 2007, severe BPH infestations were reported in parts of the

Cauvery command area in Karnataka, and similar infestations were observed in Haryana, Punjab, and Delhi states in 2008 (Gowda, 2009).

Developing resistant cultivars using BPH resistance genes is regarded as the most promising strategy (Iswanto *et al*., 2020), rather than relying on pesticides, which are costly and contribute to environmental issues. To date, researchers have identified 40 BPH resistance genes from *indica* and wild *Oryza* species (Tan *et al*., 2021). Marker-assisted backcross breeding (MABB) is an efficient strategy for transferring a desired gene from a donor parent to a recurrent parent. This process involves selecting the target loci, minimizing the size of the introgressed fragment containing the target loci and maximizing the recovery of the recurrent parent genome through repeated backcrossing (Wang *et al*., 2019). A critical component of this approach is parental polymorphism survey, which is essential for marker-assisted background selection (MABS), a technique used to analyze the recovery of recurrent parent genome during gene introgression. Parental polymorphism refers to the genetic variation between parents used in breeding programs, and it is influenced by the specific combination of parents chosen. Among the molecular markers employed in polymorphism surveys, microsatellites (SSRs) are the most widely used due to their high polymorphism, co-dominance, wide genomic distribution and ease of amplification through polymerase chain reaction (PCR). SSR markers also enhance breeding efficiency by allowing precise transfer of specific genomic regions (Miah *et al*., 2013). In this context, the present investigation was aimed to assess parental polymorphism (%) between the rice varieties, BPT5204 and RP2068-18-3-5 (BPH donor parent) by using genome wide SSR markers.

MATERIAL AND METHODS

The present study was conducted at Agricultural Research Station, Bapatla, Andhra Pradesh, India during *rabi*, 2021-22. Parental polymorphism survey was performed at Central Instrumentation Cell, Agricultural College, Bapatla. The genotypes in the study were BPT5204 (recurrent parent) which is a high yielding but susceptible to brown planthopper, whereas RP2068-18-3-5 (donor parent) is a brown planthopper resistant variety and contains *Bph33(t)* gene on chromosome 1.

DNA extraction and Quality check

DNA was extracted from leaf samples collected from 20 to 25 days old seedling for parental polymorphism survey using the CTAB method described by Doyle and Doyle (1990). The finely chopped leaf samples were ground using mortar and pestle with 500 ìL of CTAB extraction buffer (2% CTAB, 100 mM Tris, pH 8.0, EDTA pH 8, 1.4 M NaCl). An additional 300 ìL of extraction buffer was added to each homogenized sample in an Eppendorf tube, followed by heating the samples in a water bath at 65°C for 45 minutes. The tubes were centrifuged at 13,000 rpm for 20 minutes at 4°C and collected the supernatant into fresh centrifuge tubes. After collecting the supernatant, an equal volume of Chloroform: Isoamyl alcohol (24:1) was added, and the tubes were vortexed for 10 minutes. The mixture was centrifuged at 13,000 rpm for 20 minutes at 4°C and the supernatant was transferred to a fresh sterile tube. An equal volume of cold isopropanol was added, and the tubes were stored overnight at -20°C. The next day, the samples were centrifuged at 10,000 rpm for 15 minutes at 4°C, and the supernatant was carefully discarded without disturbing the DNA pellet. The pellet was washed with 200 ìL of 70% ethanol and centrifuged again at 10,000 rpm for 10 minutes at 24°C. The pellet was then air-dried at room temperature overnight. Depending on the pellet size, 100 µL molecular grade water was added to dissolve the DNA. The purity of the extracted DNA was assessed using the Nanodrop.

Polymerase chain reaction using SSR markers

In the present study, a set of 340 SSR markers were utilized to conduct parental polymorphism survey across all 12 chromosomes of rice. The polymerase chain reaction (PCR) was performed in a total volume of 10 ìL, which included 2 ìL of template DNA. Master mix was prepared by taking each 1 ìL of 10 pmol marker (both forward and reverse markers), 0.5 ìL of 2.5 mM deoxy ribonucleotides (dNTPs), 2 µL of 10 X Hi-buffer with 0.5 µL of 50 mM $MgCl_2$ and 0.5 U (0.1 ìL) of 5U/iL Taq DNA polymerase and 3.9 ìL of molecular grade water was added to make up the volume to 10 ìL. After centrifuging the PCR mixture at 1000 rpm for one minute, it was placed in a 96-well PCR thermal cycler. The protocol began with a 5 minute denaturation step at 94ºC, followed by 35 cycles

consisting of 45 seconds at 94ºC of denaturation, 45 seconds at 56ºC for primer annealing, 1 minute at 72ºC for extension, and concluded with a final extension at 72ºC for 10 minutes.

Agarose gel electrophoresis and gel image documentation

The PCR products were analyzed by electrophoresis using 3% agarose gel in a gel electrophoresis unit. 3.0 g of agarose was weighed and transferred to a conical flask, to which 100 ml of 1X TAE buffer was added and mixed thoroughly. The mixture was then boiled slowly while stirring intermittently in a microwave until the agarose was completely melted, resulting in a transparent solution. To clean the gel-casting tray, it was soaked in water and wiped down with ethanol. Once the agarose was cooled to room temperature, 2 ìL of EBr (10 mg/ml) was added to the molten agarose, which was then poured into a gel casting tray fitted with the necessary gel combs and allowed to set for 20 to 30 minutes. The gel was then transferred to the electrophoresis unit containing 1X TAE buffer.

Before loading, the 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. A 100 bp DNA ladder was included in one well to determine the sizes of the amplified fragments. The DNA fragments were visualized under a gel documentation system.

Parental polymorphism per centage

$$\text{Polymorphism (\%)} = \frac{\text{Markers showing polymorphism}}{\text{Total number of markers used}} \times 100$$

The polymorphism per centage was calculated using the above formula

where markers showing polymorphism is the molecular markers that showed different alleles between the two parents (donor and recurrent parents) total number of markers used is the total number of SSR markers that were analyzed between the two parents

Graphical genotyping

To visualize the marker data, the GGT 2.0 program was employed (Berloo, 2008). GGT 2.0 focuses on the visualization and analysis of molecular marker scores. It was used to map the distribution of polymorphic markers along the length of the chromosome based on their physical positions in megabases (Mb). The visualization was generated from the input of physical marker positions in a row-and-column data matrix.

RESULTS AND DISCUSSION

Parental polymorphism data is essential for marker-assisted breeding programs. In this study, genome-wide microsatellite (SSR) markers were used to evaluate parental polymorphism between two contrasting rice varieties, BPT5204 and RP2068-18-3-5 for identifying informative polymorphic SSR markers.

A total of 340 SSR markers were used for parental polymorphism survey across 12 chromosomes using PCR, following the standard rice microsatellite protocol. The survey revealed significant variation between the parental lines, with 96 markers being polymorphic and 244 monomorphic. Details on the number of markers tested, polymorphic markers identified and percentage of polymorphism per chromosome are presented in Table 1.

The number of SSR markers screened for polymorphism across the 12 chromosomes ranged from 21 to 36, with chromosome 10 having the highest markers screened (36). Chromosome 1 was screened with 31 markers, followed by chromosomes 2 and 3 with 30 each, chromosome 7 with 29, chromosomes 5 and 12 with 28, chromosomes 6, 8 and 11 with 27, chromosome 9 with 26 and chromosome 4 with 21 markers.

The results showed that the number of polymorphic markers ranged from 6 to 11, with the highest number (11) observed on chromosomes 1 and 3. Chromosome 2 showed 10 polymorphic markers, followed by chromosome 7 with 9, chromosomes 5 and 12 with 8, chromosomes 6, 8 and 11 with 7 and chromosomes 4, 9 and 10 with 6 polymorphic markers each. The lower levels of polymorphism on certain chromosomes may be due to genetic similarities between the parental lines (Marri *et al*., 2005). The frequency distribution of polymorphic SSR markers across 12 chromosomes is presented in Fig.1 and the representative gel image of the marker, polymorphism survey is presented in Fig. 2.

Table 1. Details of SSR Markers polymorphic between BPT5204 and RP2068-18-3-5

S.No.	Chro.No.	Total no. of markers screened/ chromosome	No. of polymorphic markers/ chromosome	No. of monomorphic markers/ chromosome	Percentage of polymorphism per chromosome
1	1	31	11	20	35.5
2	2	30	10	20	33.3
3	3	30	11	19	36.7
4	4	21	6	15	28.6
5	5	28	8	20	28.6
6	6	27	7	20	25.9
7	7	29	9	20	31
8	8	27	7	20	25.9
9	9	26	6	20	23.1
10	10	36	6	30	16.7
11	11	27	7	20	25.9
12	12	28	8	20	28.6
	Total	**340**	**96**	**244**	
	Average percent of polymorphism				**28.3**

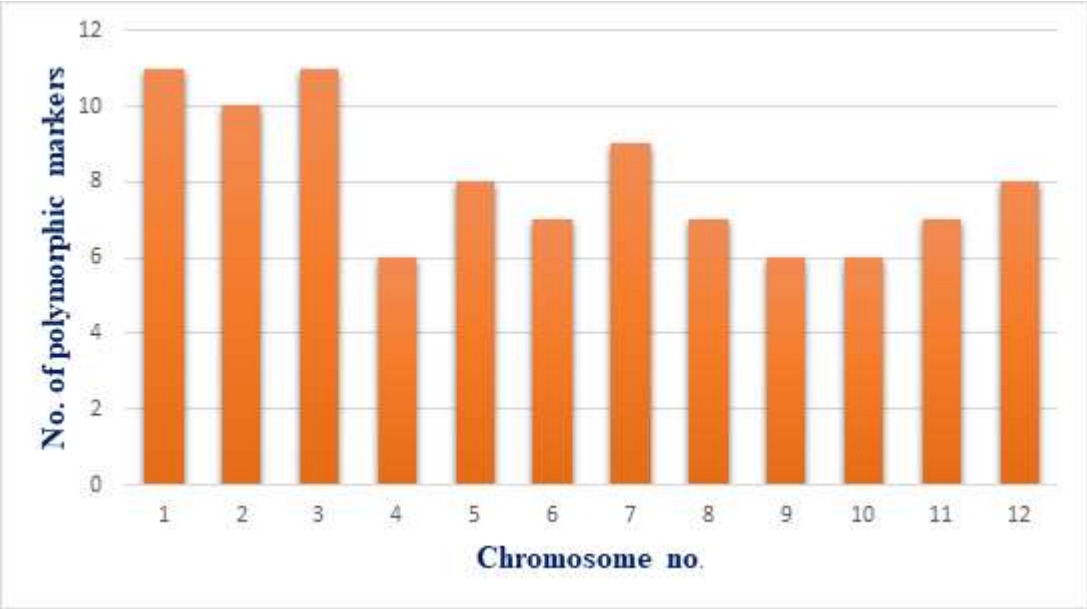
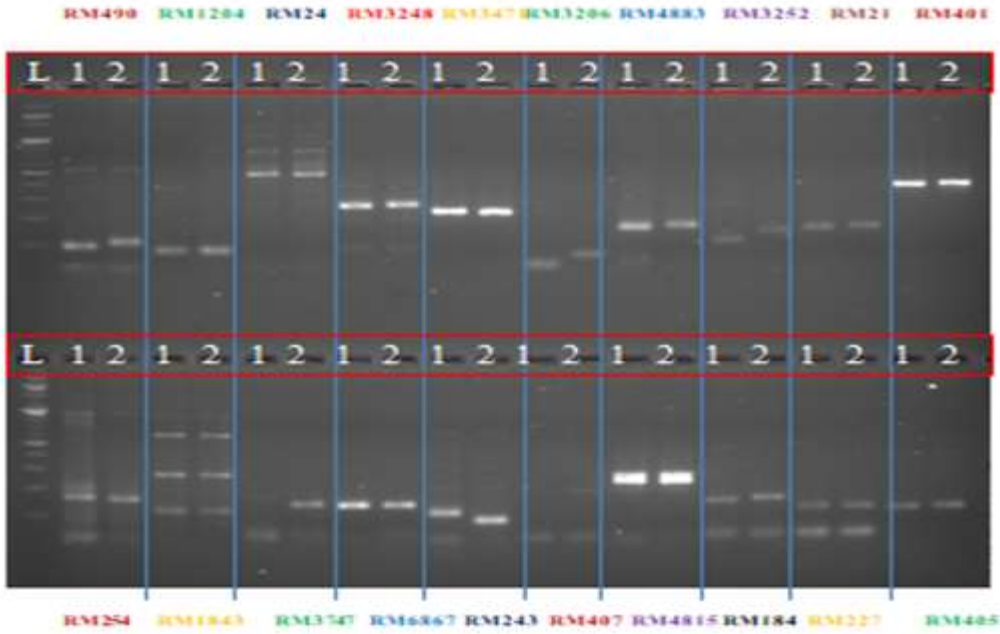


Figure 1. Polymorphic SSR markers on each chromosome between BPT5204 and RP2068-18-3-5

The percentage of polymorphism varied across the chromosomes, ranging from 16.7% to 36.7%, with chromosome 3 showing the highest per centage of polymorphism (36.7%) while the chromosome 10 recorded the lowest (16.7%).

Chromosome 1 exhibited 35.5% polymorphism, followed by chromosome 2 with 33.3%, chromosome 7 with 31%, chromosomes 4, 5 and 12 with 28.6% and chromosomes 6, 8 and 11 with 25.9% and chromosome 9 with 23.1%. The average per centage of polymorphism was 28.3%, indicating substantial genetic variability between the parents BPT5204 and RP2068-18-3-5. In a parental polymorphism survey, a higher percentage of polymorphism on a chromosome indicates greater genetic diversity between the parents for that particular chromosome. This suggests that the parents possess more contrasting alleles or loci on that chromosome. In the present study, chromosome 3 showed the higher percentage of polymorphism.

These results are consistent with Jairin *et al.* (2009) who identified 75 polymorphic markers out of 120 SSR markers screened in a parental polymorphism survey between Rathu Heenathi and



L- 100 bp Ladder; **1**- BPT5204; **2**-RP2068-18-3-5
Polymorphic markers- RM490, RM3206, RM3252, RM254, RM3747, RM243 and RM184
Fig 2. Parental polymorphism survey between BPT5204 and RP2068-18-3-5 with SSR markers

KDML105 of rice. Similarly, Suh *et al.* (2011) used 260 SSR markers in a parental polymorphism survey and reported an average polymorphism rate of 84.4%. Lakshmi *et al.* (2021) detected 87 polymorphic markers (17.1%) out of 494 SSR markers exploited in a parental polymorphism survey between a BPH-resistant line (M-229) and a susceptible line (RNR 15048), while Bhargava *et al*. (2023) utilized 816 SSR markers in a parental polymorphism survey and identified 97 as polymorphic with an average polymorphism rate of 12.5 %.

Information regarding 96 SSR markers, such as chromosomal location, forward and reverse primer sequences and physical positions (start and end of the SSR), was curated from the Gramene Markers Database and the details of 96 polymorphic SSR markers are presented in Table 2 and the distribution of polymorphic SSR markers across all the 12 chromosomes, created using Graphical Genotyping 2.0 software are presented in Figure 3.

CONCLUSION

The 96 identified polymorphic SSR markers can be utilized in marker-assisted background selection to estimate the percentage of recurrent parent genome recovery. Identifying polymorphic markers between parents is crucial for mapping genomic regions that influence key traits, particularly BPH resistance. This study highlights the potential of polymorphic marker for effective quantitative trait loci (QTL) mapping and marker-assisted selection (MAS) in rice breeding. The identified polymorphic markers offer valuable tools for marker-assisted breeding strategies, though further research is needed to overcome limitations and broaden the application to other traits and diverse rice germplasm.

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Table 2. Details of 96 SSR markers polymorphic between BPT5204 and RP2068-18-3-5

S.No.	Mc Couch Locus_ ID	Position (cM)	Chro. No.	Forward sequence	Reverse sequence	PCR product size	Annealing temp (^{O}C)
1	RM009	19.4	1	GGTGCCATTGTCGTCCTC	ACGGCCCTCATCACCTTC	136	55
2	RM243	57.3	1	GATCTGCAGACTGCAGTTGC	AGCTGCAACGATGTTGTCC	116	55
3	RM246	115.2	1	GAGCTCCATCAGCCATTCAG	CTGAGTGCTGCTGCGACT	116	55
4	RM490	51	1	ATCTGCACACTGCAAACACC	AGCAAGCAGTGCTTTCAGAG	101	55
5	RM493	79.7	1	TAGCTCCAACAGGATCGACC	GTACGTAAACGCGGAAGGTG	211	55
6	RM580	68.2	1	GATGAACTCGAATTTGCATCC	CACTCCCATGTTTGGCTCC	221	55
7	RM583	43.2	1	AGATCCATCCCTGTGGAGAG	GCGAACTCGCGTTGTAATC	192	55
8	RM6407	176.3	1	TGAAATGGTGGAGTCCAAGG	ACGGAGCCACTGACAGGTC	145	50
9	RM1297	121.6	1	GTGCCTTACAACTCAACGAC	CACTCCCAGTTCAGTACGTC	168	55
10	RM1349	103.7	1	CGTTCCAATATTCAGACACAG	TTTCCATCTCGAGAAGCTC	160	55
11	RM3252	0.3	1	GGTAACTTTGTTCCCATGCC	GGTCAATCATGCATGCAAGC	172	55
12	RM263	127.5	2	CCCAGGCTAGCTCATGAACC	GCTACGTTTGAGCTACCACG	199	55
13	RM279	17.3	2	GCGGGAGAGGGATCTCCT	GGCTAGGAGTTAACCTCGCG	174	55
14	RM482	187.5	2	TCTGAAAGCCTGACTCATCG	GTCAATTGCAGTGCCCTTTC	188	55
15	RM492	53	2	CCAAAAATAGCGCGAGAGAG	AAGACGTACATGGGTCAGGC	224	55
16	RM497	150.8	2	TCCTCTTCACCTATGGGTGG	GCCAGTGCTAGGAGAGTTGG	213	55
17	RM530	158	2	GCACTGACCACGACTGTTTG	ACCGTAACCCGGATCTATCC	161	55
18	RM555	34.7	2	TTGGATCAGCCAAAGGAGAC	CAGCATTGTGGCATGGATAC	223	55
19	RM573	143.7	2	CCAGCCTTTGCTCCAAGTAC	TCTTCTTCCCTGGACCACAC	201	55
20	RM5430	91.5	2	TAAAAACTGAGCCGTGAGCC	ACCATGGGGAGCTGCTTC	181	61
21	RM6933	123.9	2	TGTAGCAGAAACCAATGCTC	GTCACTCCACTTCGCTTATC	215	55
22	RM168	171.2	3	TGCTGCTTGCCTGCTTCCTTT	GAAACGAATCAATCCACGGC	116	55
23	RM251	79.3	3	GAATGGCAATGGCGCTAG	ATGCGGTTCAAGATTCGATC	147	55
24	RM338	108.4	3	CACAGGAGCAGGAGAAGAGC	GGCAAACCGATCACTCAGTC	183	55
25	RM347	131.5	3	CACCTCAAACTTTTAACCGCAC	TCCGGCAAGGGATACGGCGG	207	55
26	RM422	205.4	3	TTCAACCTGCATCCGCTC	CCATCCAAATCAGCAACAGC	385	55
27	RM442	224.2	3	CTTAAGCCGATGCATGAAGG	ATCCTATCGACGAATGCACC	257	55
28	RM520	191.6	3	AGGAGCAAGAAAAGTTCCCC	GCCAATGTGTGACGCAATAG	247	55
29	RM85	231	3	CCAAAGATGAAACCTGGATTG	GCACAAGGTGAGCAGTCC	107	55
30	RM3206	28.2	3	GCGCCTCTCTTCTTCCTCTC	GAAAATCGAATCACGGCGAC	112	55
31	RM5924	31.3	3	CTCCCAAGAAACTGAACCAG	AGGATTCGTCGTTGCTCAAC	209	55
32	RM6283	83.3	3	TGGAGACTGAGCTGATGCC	TCAGGTGGTCGGTTCCTTAC	93	55
33	RM131	148.8	4	TCCTCCCTCCCTTCGCCCACTG	CGATGTTCGCCATGGCTGCTCC	215	61
34	RM335	21.5	4	GTACACACCCACATCGAGAAG	GCTCTATGCGAGTATCCATGG	104	55
35	RM451	115.5	4	GATCCCCTCCGTCAAACAC	CCCTTCTCCTTTCCTCAACC	207	55
36	RM471	53.8	4	ACGCACAAGCAGATGATGAG	GGGAGAAGACGAATGTTTGC	106	55
37	RM5709	109.9	4	CTGAATTTATTATAGGACGGAAG	CATAGTATTGGATTGGACACG	163	55
38	RM8213	10.7	4	AGCCCAGTGATACAAAGATG	GCGAGGAGATACCAAGAAAG	177	55
39	RM163	78.7	5	ATCCATGTGCGCCTTTATGAGGA	CGCTACCTCCTTCACTTACTAGT	124	55
40	RM334	141.8	5	GTTCAGTGTTCAGTGCCACC	GACTTTGATCTTTGGTGGACG	182	55
41	RM465	68.3	5	GTGCCTCCATCATCATCATC	TAGGACAAGCGAAGAAACCG	212	55
42	RM480	130.6	5	GCTCAAGCATTCTGCAGTTG	GCGCTTCTGCTTATTGGAAG	225	55
43	RM574	41	5	GGCGAATTCTTTGCACTTGG	ACGGTTTGGTAGGGTGTCAC	155	55
44	RM2010	12	5	ATCTTCTAGGAAATCGAGGA	GTTGGCAACTTGTAGTCTTG	117	55
45	RM6024	67.5	5	ACATTCGTCCAGGGATTCAC	TTGTGGTTGCTCACCTCTTG	178	50
46	RM7446	103.9	5	TGAAGGCAGTTTCACTGACG	AGCCAAGAAGAAGAAAGGGG	188	55
47	RM340	133.5	6	GGTAAATGGACAATCCTATGGC	GACAAATATAAGGGCAGTGTGC	163	55
48	RM402	40.3	6	GAGCCATGGAAAGATGCATG	TCAGCTGGCCTATGACAATG	133	55
49	RM510	20.8	6	AACCGGATTAGTTTCTCGCC	TGAGGACGACGAGCAGATTC	122	55
50	RM527	61.2	6	GCTCGTACGGTGGGTGAATCC	GATGCGTCCTTCTTAGGTTGAAAG	273	55

51	RM589	3.2	6	ATCATGGTCGGTGGCTTAAC	CAGGTTCCAACCAGACACTG	186	55
52	RM1130	105.1	6	AGATCGGATTGGGATGGC	ACCCAACCAATTAGTGCCAC	129	55
53	RM810	8.2	6	CACTGACATAGCTAAGGTCTCATGTCTTAT	TGGTTAACTCGCTATTATAATGAGTTCG	273	55
54	RM234	88.2	7	ACAGTATCCAAGGCCCTGG	CACGTGAGACAAAGACGGAG	156	55
55	RM295	0	7	CGAGACGAGCATCGGATAAG	GATCTGGTGGAGGGGAGG	180	55
56	RM320	36.1	7	CAACGTGATCGAGGATAGATC	GGATTTGCTTACCACAGCTC	167	55
57	RM342	78.4	7	CCATCCTCCTACTTCAATGAAG	ACTATGCAGTGGTGTCACCC	141	55
58	RM1048	70.8	7	CAAGCCTATAATGTGAATTG	AATTTTTAGTTTGGGGTAGA	138	55
59	RM1335	105.7	7	GCATGCATGAATATGATGG	AGATCGAACAAGAAGAGTGG	168	55
60	RM3753	81.05	7	GAATGAGCTAAGAACACGCC	CTGATGGCCCAAGACTTTTG	119	50
61	RM5508	81.05	7	TCGCACACTAGCTCGATCAG	TGGTCCTCTTCTCCATCCAG	177	50
62	RM5711	24.2	7	GTCCATGCATCCATCTCTAG	ACGGAAGGAATACGTCTGTA	145	55
63	RM407	5.7	8	GATTGAGGAGACGAGCCATC	CTTTTTCAGATCTGCGCTCC	172	55
64	RM230	112.2	8	GCCAGACCGTGGATGTTC	CACCGCAGTCACTTTTCAAG	257	55
65	RM264	128.6	8	GTTGCGTCCTACTGCTACTTC	GATCCGTGTCGATGATTAGC	178	55
66	RM281	128.6	8	ACCAAGCATCCAGTGACCAG	GTTCTTCATACAGTCCACATG	138	55
67	RM325	69	8	GACGATGAATCAGGAGAACG	GGCATGCATCTGAGTAATGG	201	55
68	RM331	69	8	GAACCAGAGGACAAAAATGC	CATCATACATTTGCAGCCAG	176	55
69	RM404	60.9	8	CCAATCATTAACCCCTGAGC	GCCTTCATGCTTCAGAAGAC	236	55
70	RM105	32.1	9	GTCGTCGACCCATCGGAGCCAC	TGGTCGAGGTGGGGATCGGGTC	134	55
71	RM215	99.4	9	CAAAATGGAGCAGCAAGAGC	TGAGCACCTCCTTCTCTGTAG	148	55
72	RM1896	36	9	GGACAGGGTAAAGTGTTAGA	CCTAAGACCTATCAACTCCA	108	55
73	RM242	72.3	9	GGCCAACGTGTGTATGTCTC	TATATGCCAAGACGGATGGG	225	55
74	RM288	74.6	9	CCGGTCAGTTCAAGCTCTG	ACGTACGGACGTGACGAC	125	55
75	RM316	1.8	9	CTAGTTGGGCATACGATGGC	ACGCTTATATGTTACGTCAAC	192	55
76	RM147	99.8	10	TACGGCTTCGGCGGCTGATTCC	CCCCCGAATCCCATCGAAACCC	97	55
77	RM184	58.3	10	ATCCCATTCGCCAAAACCGGCC	TGACACTTGGAGAGCGGTGTGG	219	55
78	RM484	97.3	10	TCTCCCTCCTCACCATTGTC	TGCTGCCCTCTCTCTCTCTC	259	55
79	RM1108	55.3	10	GCTCGCGAATCAATCCAC	CTGGATCCTGGACAGACGAG	124	55
80	RM5271	3.9	10	CGGTGTAGATTGTAGGTACA	GTAGTTTAGTTATTGCGCAC	184	55
81	RM5352	71.4	10	GGAACTAAACATGGTGCAAG	ACCAGATCACATGAAGAGGA	125	50
82	RM116	41.7	11	TCACGCACAGCGTGCCGTTCTC	CAAGATCAAGCCATGAAAGGAGGG	258	55
83	RM139	121.3	11	GAGAGGGAGGAAGGGAGGCGGC	CTGCCATGGCAGAGAAGGGGCC	386	55
84	RM144	123.2	11	TGCCCTGGCGCAAATTTGATCC	GCTAGAGGAGATCAGATGGTAGTGCATG	237	55
85	RM215	85.7	11	CAAAATGGAGCAGCAAGAGC	TGAGCACCTCCTTCTCTGTAG	148	55
86	RM254	110	11	AGCCCCGAATAAATCCACCT	CTGGAGGAGCATTTGGTAGC	165	55
87	RM552	40.6	11	CGCAGTTGTGGATTTCAGTG	TGCTCAACGTTTGACTGTCC	195	55
88	RM3747	8.6	11	AGCAATGCACTCCCTTGATC	TGTCTTCCTCCTTGGTTTGG	143	55
89	RM235	91.3	12	AGAAGCTAGGGCTAACGAAC	TCACCTGGTCAGCCTCTTTC	124	55
90	RM247	32.3	12	TAGTGCCGATCGATGTAACG	CATATGGTTTTGACAAAGCG	131	55
91	RM313	65.5	12	TGCTACAAGTGTTCTTCAGGAC	GCTCACCTTTTGTGTTCCAC	111	55
92	RM1880	9.4	12	ACCACTAAATAAGCACATAC	GGCATCATACATTAAAATAC	128	55
93	RM2529	79.1	12	CATTAAAATCAGTGGGACTG	AGGCATTTCCTGATATGATC	134	55
94	RM 297	65.3	12	GAGCCAATATGTTGTCTTGA	GTTCAGATCATGATGCCTAC	159	55
95	RM3331	89.5	12	CCTCCTCCATGAGCTAATGC	AGGAGGAGCGGATTTCTCTC	129	50
96	RM7102	71.85	12	TTGAGAGCGTTTTTAGGATG	TCGGTTTACTTGGTTACTCG	169	55

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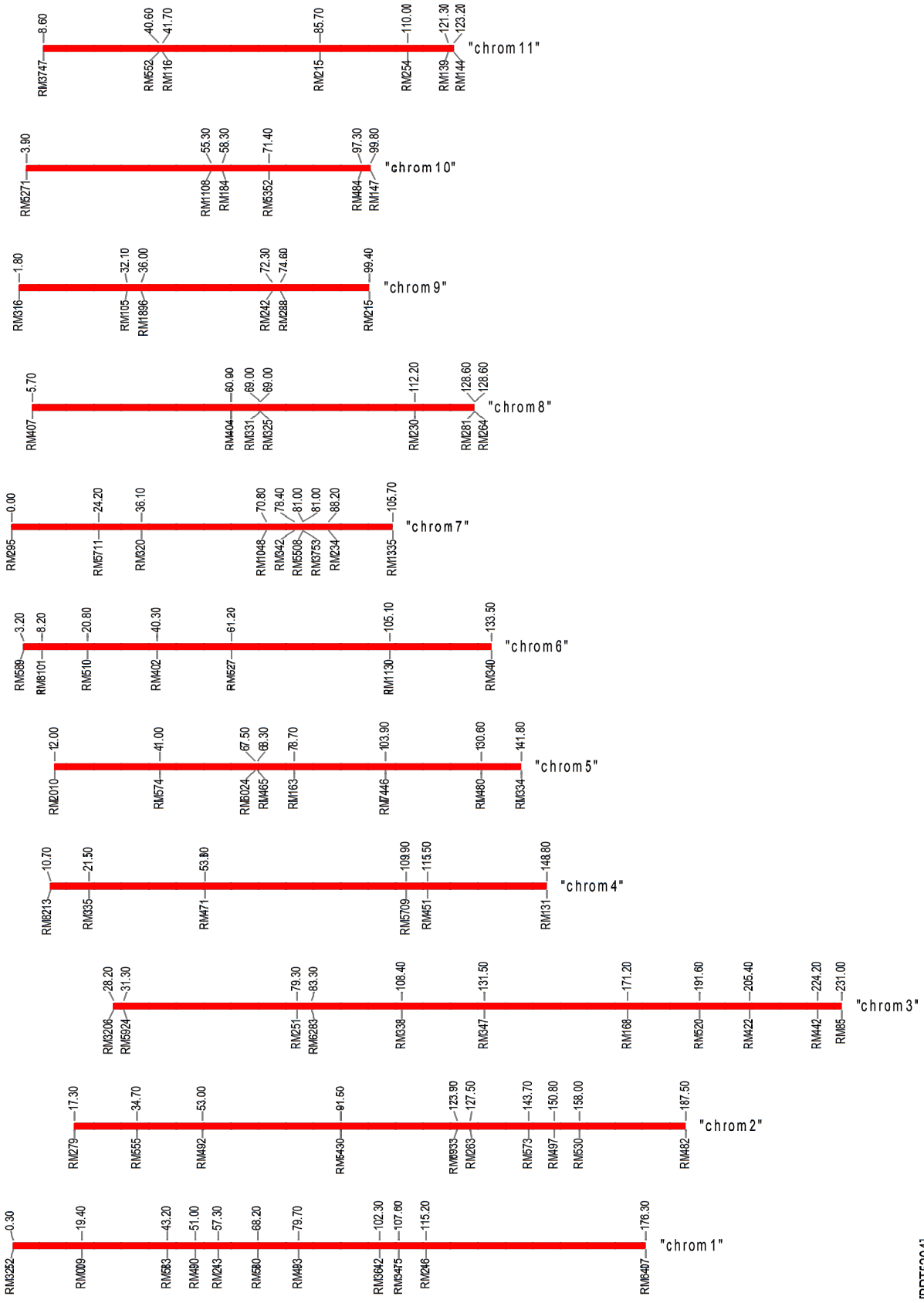

Fig 3. Position of 96 polymorphic SSR markers across the 12 chromosomes

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