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Parental Polymorphism Survey for Identifying Genes/QTLs for BPH Resistance in Assam Land Race 10-3

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ABSTRACT

The current research was taken up at Institute of Biotechnology (IBT), PJTSAU, Hyderabad Telangana, India in *kharif* from August to November, 2021 to access the parental polymorphism between 10-3 and Telangana Sona. 10-3, an Assam landrace, is a bold grain rice variety with high BPH resistance to biotype-4. Whereas, Telangana Sona (RNR 15048) is a short and slender grain variety of rice that is popular among farmers and consumers across Telangana and other Indian states due to its high yield and low glycemic index. Despite being blast resistant, Telangana Sona is susceptible to the insect pest Brown Plant hopper (BPH) *Nilaparvata lugens* (Stål). The present study aimed to characterize the parental polymorphism between 10–3 and Telangana sona to facilitate mapping of genetic loci linked with BPH resistance in the 10–3 and to optimize the retention of favourable alleles from Telangana sona during marker assisted backcross breeding. Eight hundred and sixteen highly variable Simple Sequence Repeats (SSRs) including thirty-five BPH linked markers covering 12 chromosomes were used to assess DNA polymorphism between 10-3 and Telangana Sona. Out of 816 markers screened, 97 (11.89%) were found to be polymorphic between 10–3 and Telangana Sona of which, 82 were genomic SSRs and 15 were BPH linked markers. Chromosome 6 exhibited highest polymorphism percentage with a value of 30%, indicating that this chromosome is useful in studying parental variation and lowest polymorphism percentage found on chromosome 5 (3.49%). These 97 polymorphic markers will help in construction of linkage map for identifying QTLs linked with BPH resistance and for marker assisted backcrossing breeding in Telangana Sona.

KEYWORDS: Brown plant hopper (BPH), parental polymorphism, simple sequence repeats (SSRs)

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Data Availability Statement: Legal restrictions are imposed on the public sharing of raw data. However, authors have full right to transfer or share the data in raw form upon request subject to either meeting the conditions of the original consents and the original research study. Further, access of data needs to meet whether the user complies with the ethical and legal obligations as data controllers to allow for secondary use of the data outside of the original study.

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1. INTRODUCTION

Rice (*Oryza sativa* L.) is a crucial staple food crop (Sarao and Mangat, 2014) that plays a significant role in global agricultural production. However, rice productivity faces severe constraints due to rapid climate change and various biotic and abiotic stresses. The brown plant hopper *Nilaparvata lugens* (Stål), has been a major destructive pest of rice for several decades (Jena et al., 2018), causing significant damage to rice plants by feeding on phloem sap, resulting in 'hopper burn' (Elanchezhyan et al., 2020). It also transmits ragged stunt virus (RRSV) and grassy stunt virus (RGSV), resulting in substantial yield losses (Zheng et al., 2020). Win et al. (2011) reported that a yield loss of approximately 10–40% can occur due to this viruses. To address the challenges of pest management and reduce the negative impacts of pesticides such as environmental pollution, pest resurgence, high costs and stronger pathogen strain virulence (Frisvold, 2019), developing resistant cultivars by utilizing BPH resistance genes is considered the most promising strategy (Horgan et al., 2018, Iswanto et al., 2020).

Till date, researchers have identified 40 BPH resistance genes from *indica* and wild species of *oryza* (Tan et al., 2021) and these genes are typically clustered on chromosomes 3, 4, 6, and 12 (Du et al., 2020, Tan et al., 2021, Kim et al., 2022). A map-based cloning strategy has enabled the isolation and characterization of several BPH resistance genes including Bph14, Bph3/Bph15, Bph26/bph2, bph29, bph7/Bph9/Bph10/Bph21, Bph18/Bph1, Bph32, Bph6, Bph30/Bph40, and Bph37 (Cheng et al., 2013, Tamura et al., 2014, Wang et al., 2015, Ji et al., 2016, Ren et al., 2016, Zhao et al., 2016, Jing et al., 2017, Guo et al., 2018, Shi et al., 2021, Zhou et al., 2021). However, despite the presence of these genes, only a few BPH-resistant varieties are typically grown due to the pest's ability to quickly adapt and overcome plant resistance, coupled with limited sources of resistance. Thus, it is imperative to identify new and appropriate resistance sources to effectively combat this insect and develop varieties resistant to different biotypes.

The work done at Institute of Biotechnology (IBT), PJTSAU for BPH resistance using three hundred and ninety-one MAGIC *indica* lines (Satturu et al., 2020) along with a set of predefined lines identified a local land race, 10-3 as BPH resistant. Marker validation studies for the presence of reported foreground markers identified that these donor is not validated with *Bph17* and *Bph32* gene specific markers which were reported to be effective against biotype 4 of BPH, which is prevalent in India and hence may harbor novel genes/QTLs associated with resistance (Lakshmi et al., 2022). To facilitate mapping of these genes/QTLs responsible for BPH resistance and enable marker-assisted backcross breeding parental polymorphism survey was conducted in the present study.

Simple Sequence Repeats (SSRs) are widely utilized among available molecular markers due to advantages such as species specificity, genomic abundance, high reproducibility and co-dominant nature (Miah et al., 2013). The screening of markers to detect parental polymorphism among rice cultivars is a crucial step in gene tagging and mapping. It is important to identify clear polymorphism between donor and recurrent parent to successfully undertake gene/quantitative trait loci (QTL) introgression programs. Thus, in the present study genome-wide SSR markers were employed to identify informative polymorphic SSR primer pairs between 10–3 and Telangana Sona.

2. MATERIALS AND METHODS

2.1. Plant material used

This study was conducted at Institute of Biotechnology (IBT), PJTSAU, Hyderabad, Telangana, India in *kharif* August to November, 2021. Two contrasting parents for BPH resistance i.e., BPH susceptible Telangana Sona (RNR 15048) and BPH resistant 10–3 used for developing mapping population were assayed for polymorphism between them Telangana Sona is a well-known high yielding, slender grain variety with short duration, developed by PJTSAU, Hyderabad. 10–3, on the other hand, is an Assam landrace, having high level of BPH resistance was obtained from the Institute of Biotechnology (IBT), PJTSAU, Hyderabad. To perform DNA isolation and conduct parental polymorphism survey, leaf samples were collected from both parents 21 days after transplanting and then stored at -20°C

2.2. Genomic DNA isolation and quality check

Genomic DNA was extracted as per the protocol described by Doyle and Doyle (1987) at Institute of Biotechnology, PJTSAU, Hyderabad. After grinding the leaf samples with 500 µl of CTAB (Cetyl Tri methyl Ammonium Bromide) buffer, they were transferred to eppendorf tubes and placed in a water bath at 65°C for 45 m. The mixture was allowed to cool at room temperature before being centrifuged for 15 m at 12000 rpm. To proceed with DNA extraction, the supernatant was collected, and mixed with an equal volume of chloroform: isoamyl alcohol mixture (24:1) by gentle inversion before centrifugation for 15 m at 12,000 rpm. The clear aqueous phase (supernatant) was transferred to new tubes and 0.5 to 0.6 volumes of chilled isopropanol was added and left overnight at 4°C. After centrifugation at 12000 rpm for 12 m, the DNA was pelleted out and washed with 200 µl of 70% ethanol. Then the pellet was air-dried and dissolved in 60 µl of TE buffer. The quality and quantity of the extracted DNA were evaluated by running samples

on a 0.8% agarose gel with a standard ladder and the DNA was compared based on the intensity and thickness of the bands. Additionally, DNA quantification and purity were assessed by measuring the O.D. values at 260 and 280 nm using a NanoDrop ND1000 spectrophotometer.

2.3. SSR markers

To identify polymorphism between 10–3 and Telangana sona, eight hundred and sixteen SSR markers which included 39 gene linked markers for BPH resistance were obtained from Institute of Biotechnology, PJTSAU and ICAR- Indian Institute of Rice Research (ICAR-IIRR). The information about these markers, such as chromosomal location, forward and reverse primer sequences and physical position (SSR start and end) was curated from Gramene Markers Database. For those markers whose information was not available in the gramene database, Phytozome (*Oryza sativa* v7.0) was used. The BLAST tool of Phytozome was used to submit query sequences for running a search against DNA databases.

2.4. PCR analysis and agarose gel electrophoresis

The polymerase chain reaction was conducted in an applied biosystems thermal cycler using 816 SSR primers with 10 µl reaction volume. This volume included template DNA of 2 µl, 0.3 µl each forward and reverse primer, Takara PCR master mix of 4.0 µl and sterile distilled water of 3.4 µl. Prior to loading into a PCR thermal cycler with 96 wells, the PCR mix was centrifuged at 1000 rpm for 1 m. The PCR profile for amplification of genomic DNA begins with initial denaturation of 5 m at 94°C, followed by thirty-five cycles of denaturation for 0.45 m at 94°C, annealing for 0.45 m at 55°C, extension for 1 m at 72°C, and final extension for 10 m at 72°C. The amplified PCR products were resolved on a 3% agarose gel (Seakem LE), which was run for approximately 1 hour 30 m at a constant voltage of 120 V. The DNA fragments were visualized under a UV-transilluminator and recorded using a BIORAD gel documentation system.

2.5. Polymorphism percentage

The polymorphism percentage was calculated using the formula

polymorphism percentage (Markers showing the polymorphism)/(Total number of markers used)×100 ..(1)

3. RESULTS AND DISCUSSION

Parental polymorphism information is pre-requisite for QTL mapping and marker assisted breeding programme. In the current study, microsatellite markers were used to study parental polymorphism between the contrasting parents 10–3 and Telangana Sona. Many researchers viz., Deen et al. (2017), Mohanty et al. (2017), Akula et al. (2020), Ishwarya Lakshmi et al. (2021), Meshram et al. (2021) and Kiswanto et al. (2022) used SSRs to investigate parental polymorphism in rice varieties. For conducting parental polymorphism a set of 816 SSR primer pairs including 35 BPH linked markers were used across 12 chromosomes. The PCR reactions were carried out in accordance with the rice microsatellites standard protocol. The results of the survey on parental polymorphism showed there was a noticeable variation between the parents.

Out of 816 SSR primer pairs, 97 primer pairs were identified as polymorphic, which includes 15 SSR primers linked with BPH resistance from previous studies and 719 primer pairs were monomorphic (Table 1). These 15 (RM488, RM11135, RM240, RM250, RM231, RM1305, RM8213, RM273, RM261, RM435, RM19291, RM8101, RM589, RM508 and RM584) SSR primer pairs were located on chromosome number 1, 2, 3, 4, 6 and 12. Table 2 Shows the information about the number of markers tested for polymorphism, the number of polymorphic markers identified, and the percentage of polymorphism on each chromosome. The number of markers used for polymorphism survey for each chromosome varied from 97 on chromosome 7 to 49 on chromosome 3. The number of polymorphic markers identified chromosome^{-1} ranged from 3 on chromosome 5 to 15 on chromosome 6. The lower level of polymorphism observed on some chromosomes could be due to genetic similarity between the parents used in the study (Marri et al., 2005). The marker frequency distribution presented in Figure 1 revealed that chromosome 6 had the highest number of polymorphic markers (15), followed by chromosome 1 with 13, chromosomes 4 and 7 with 11, and chromosomes 2 and 9 with 10 polymorphic markers. Chromosomes 5 and 10 had the fewest markers (3), followed by chromosomes 8 and 11, which had four markers each.

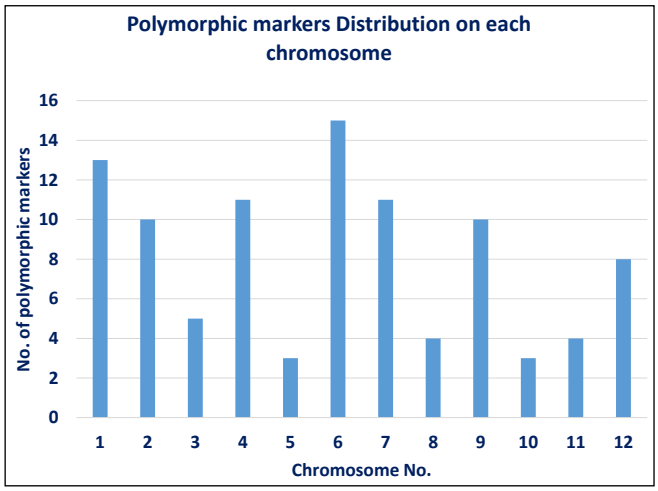

Figure 1: Histogram depicting polymorphic markers on each chromosome between 10–3 and Telangana Sona

Table 1: Polymorphic SSR markers between 10-3 and Telangana Sona

Sl. No.	Chro. No.	Marker	Forward primer sequence	Reverse primer sequence	Physical position (Mb)
1.	1	RM10009	GATGCTCCGGAATAACTAG-ATTGG	GGAATTACAGCTGTCTTGGAAGG	0.18
2.	1	RM3148	GCTTTGGTATTTGCAG-GTTCACG	CTATTGCTCGAACACTTT-GCTTCTCC	0.74
3.	1	RM8069	CGTTCAAAGCGAGCTTAATTGC	CTACGGCGGCTAAACATAACTCC	1.56
4.	1	RM10167	GTGCCTAAGATTCAGCGCT-GTGG	GCGGGACATTCATGTACACATT-GC	3.39
5.	1	RM10255	GATTCACACATAGAGTGACT-GTCG	CTTTGCCAGGAGAGTTCTAACG	4.35
6.	1	RM3604	CAGGAACCAACCTTCTTCTT-GACC	GTCAGACTCCGATCTGGGATGG	5.13
7.	1	RM151	TGCTGATCAGTTACACGAAT-CAGAGC	GCGTACGTGCACAAATTAAA-CAGACC	5.55
8.	1	RM490	ATCTGCACACTGCAAACACC	AGCAAGCAGTGCTTTCAGAG	10.18
9.	1	RM582	TCTGTTGCCGATTTGTTCG	AAATGGCTTACCTGCTGTCTC	9.19
10.	1	JGT1-15.9	GGGTGCCACTTGACGGTTTG	GGCGCTCCAACAAATGCTG	15.76
11.	1	RM312	GTATGCATATTTGATAAGAG	AAGTCACCGAGTTTACCTTC	17.9
12.	1	RM11135	CAGATGGCCACCACGTATAG-TACC	GATGTCGCAATCTTTGGTTTCC	20.64
13.	1	RM488	AACAACCAGCGTATGCGTTCTCG	CCCACGGCTTTGTAGGAAGAAGC	24.8
14.	2	2-0.12	CCGTGCATCTCGCTACCTAACC	TGCACACACAACTTAGAG-GAAGAAGG	0.12
15.	2	JGT2-9.17	CCATGTGGCGGTCTAGGAGTA	TGCCCTTGCTAAATAAATGCT	9.03
16.	2	RM13131	TGGAGTGAGGGTAG-GTGGGTTGG	CTCGAGAGTTGTGCCCATCATCC	14.6
17.	2	RM13263	AAGATTGCACACTGGTGTTCTCC	AGAAGAGCCGGTCTTTGTCTCC	18.18
18.	2	RM6318	AAGTGCCTCGAATTACA-CATCTCC	GCTGCTTCTGTCCAGTGAGACC	24.44
19.	2	RM13659	GAACAGATTCTTGCCAATGTGC	AGCGAGAAAGAACAGGAAGTGC	25.75
20.	2	2-27.3	ATCTGATCTCACGAGTCACATGG	TGCTCGAGTTGTGAAAGATAGGG	27.32
21.	2	RM240	CCTTAATGGGTAGTGTGCAC	TGTAACCATTCCTTCCATCC	31.5
22.	2	RM2578	TCCAGTTGGGCCAGGTGACG	ACTTGGTGGCTGTCATTGGTTGG	32.27
23.	2	RM250	GTTCAAACCAAGCTGATCA-CAAGC	GGCGTCAGAGTCAGAGATGAAGG	32.8
24.	3	RM231	CCAGATTATTTCCTGAGGTC	CACTTGCATAGTTCTGCATTG	0.98
25.	3	RM15188	AGCCTCCTAGAACGGCAAAGG	CTATGTTTCGTGCAACCAAGTCC	16.49
26.	3	RM273	GAAGCCGTCGTGAAGTTACC	GTTTCCTACCTGATCGCGAC	23.6
27.	3	RM15580	TTTGGTGCATGAACTTGTCTGG	TTGAATCTTGGAGCCCATACTCC	24.98
28.	3	RM347	CACCTCAAACTTTTAACCGCAC	TCCGGCAAGGGATACGGCGG	28.07
29.	4	RM335	GTACACACCCACATCGAGAAG	GCTCTATGCGAGTATCCATGG	0.69

Table 1: Continue...

Sl. No.	Chro. No.	Marker	Forward primer sequence	Reverse primer sequence	Physical position (Mb)
30.	4	RM261	CTACTTCTCCCCTTGTGTCG	TGTACCATCGCCAAATCTCC	6.57
31.	4	RM8213	TGTTGGGTGGGTAAAGTAG-ATGC	CCCAGTGATACAAAGATGAGTTGG	4.41
32.	4	RM1305	GGTACTACAAAGAAACCTG-CATCG	TCCTAGCTCAAATGTGCTATCTGG	5.62
33.	4	RM16649	CTCCCTTCATGCGTAAGCTCTCC	GCAAACAGGATCCTCCACAAAGG	13.62
34.	4	RM16738	AAACGGAGTGGTCCATTAGC	GAAAGGTAGATGGGTTTGTGG	16.61
35.	4	HRM17127	CTCAATGTTTCCCACAGTTACCG	TGTGTTATGTGTGCGTGATGAGC	23.55
36.	4	RM17345	TTTAGCTGTTTCGCACACACG	AAGGACATCCTTCATCCTCTTGC	28.37
37.	4	RM17377	ATATTACTTCGACGCTGGAT-CAGG	GTCAGTTCGTCAGGCACAACG	29.12
38.	4	RM6748	AGAAGAAGCAGCTGGT-GATTAGCC	CAACGATGTACCAGTTGAATACCC	30.9
39.	4	RM17686	GAACGAAGTGAACAAGC-CAATCC	CCCATTACGGCTTAGGCTCAGG	35.31
40.	5	RM334	GTTCAGTGTTCAGTGCCACC	GACTTTGATCTTTGGTGGACG	39.65
41.	5	RM592	ACCTCACCCGAATTACTGT-GATATGC	GTTGAATTGCACGCGACTCTGG	2.73
42.	5	RM18081	TGAAGTGCGTTGAAACATCTCC	ATTGCGCATCCAATCTATGG	6.32
43	6	RM508	AGAAGCCGGTTCATAGTTCATGC	ACCCGTGAACCACAAAGAACG	0.44
44	6	RM435	ATTACGTGCATGTCTGGCTG	CGTACCTGACCATGCATCTG	0.54
45.	6	RM469	TTACGTGATCACACAGGCTCTCC	AAGCTGAACAAGCCCTGAAAGG	0.56
46.	6	RM19291	CACTTGCACGTGTCCTCTGTACG	GTGTTTCAGTTCACCTTGCATCG	1.22
47.	6	RM589	ATCATGGTCGGTGGCTTAAC	CAGGTTCCAACCAGACACTG	1.38
48.	6	RM19410	TGCTGATTGCTCAC-TACTTCATCC	GCGGGATACCATGGTCTAAAGG	2.91
49	6	RM19426	CGGTGTCTTCTTTAAACAGC	ATGGATAAGCGGTATGTTCC	3.35
50.	6	RM584	TATGTGGTTGGCTT-GCCTAGTGG	TGCCCATATGGTCTGGATGTGC	3.42
51.	6	RM253	CCATCTCTGCCTCTGACTCACC	TCCTTCAATGGTCGTATCTTCTCC	5.44
52.	6	RM19660	TTTGTCCCTGCCGTACTTGC	AGCCACGTTGGGTGAAATTAGC	6.75
53.	6	JGT6-6.8	GAGCGTTTGTAG-GAAGTTTCATGGAT	GGACAACCACAAGCACACCACTCT	6.86
54.	6	RM314	CTAGCAGGAACTCCTTTCAGG	AACATTCCACACACACACGC	8.03
55.	6	RM527	CGGTTTGTACGTAAGTAGCAT-CAGG	TCCAATGCCAACAGCTATACTCG	9.87
56.	6	RM7213	ATCCATCCGCACTGCCATCG	CAGCTCGGTGCACAAGAAGTTGC	10.45
57.	6	RM400	TTACACCAGGCTACCCAAACTCG	TTGCTGAGTTCCCTCGTCTATCC	28.05
58.	7	RM8101	GTGTAGTTACGACCAATGATAC-GC	TATAATGAGTTCGAGCCGATCC	1.7
59.	7	RM6728	TCACCCTTCTTGTCAAGCAAACC	GAGGATCAGGATAGAACATGAGA-TGC	5.76

Table 1: Continue...

Sl. No.	Chro. No.	Marker	Forward primer sequence	Reverse primer sequence	Physical position (Mb)
60.	7	RM21625	AGCATGAGGTAATGACTCTTCG	AACGACTAAGGCTGAGTTTGC	18.46
61.	7	RM336	GTATCTTACAGAGAAACG-GCATCG	GGTTTGTTTCAGGTTCGTCTATCC	21.82
62.	7	RM21842	GAACGGGAGGAGGAGTTG-TAGG	GACTTCATTTCAACTCGAC-GATGG	22.72
63.	7	JGT7-22.76	AAGGGGTTATTGATTTAGTCC	AGGGGTTGAACTTTGATGCTA	22.11
64.	7	RM5847	CTTTAGGTAGCGTCATCTTCC	TGGAAATACAGAAGGAGTCG	23.6
65.	7	RM21922	NA	NA	24.39
66.	7	RM21992	ATGGGTGAGGCTTTAAACA-CAGG	TAAGCCAGTCCATTGGCAAACC	25.8
67.	7	RM22171	TAGTACCGCCATTAC-CATTCATCC	GACGGTGGGACTCCTAATTA-CAGC	29.43
68.	7	RM21596	GGTGGTAACCTTGAGAGCTACG	CGAAGAGGATAGATA-ACCCATAGTCC	17.63
69.	8	RM22266	GGGAGAAGCTGTAGCAGT-TAGAAGC	CAAATGCAGAAGCCATTGTCC	0.76
70.	8	RM23518	TTTCGCTTCTGAGTTG-GCAGAGG	GACCTGAACAACGTGCCAAACG	26.81
71.	8	RM5933	AGCGATTCAGAACGAATCAACG	TGCCAAAGCTACACAAATCTGACC	27.46
72.	8	RM264	GTTGCGTCCTACTGCTACTTC	GATCCGTGTCGATGATTAGC	29.03
73.	9	RM474	TACACGAGGGAGTACTC-GAATGG	CATGGAGGTATAGAAGAG-CATTGG	1.8
74.	9	RM23791	TCACCAACAAGTGGAGTACT-TAGGC	CCTTACCTCAGGAGTGTTCGATCC	4.26
75.	9	RM23908	GGTCACCCTTCAAAGATGT-CATGG	ATCCCGCTATCGAAGGTGAAACG	7.04
76.	9	RM24199	CTCGTAAGCCTAGGCCAT-CAAGC	AAACTTGAGCTTGGCTCGTTTCC	12.76
77.	9	SSR9-18.1	ATCCACAAGAGCACCGATGAGG	TGACCTGGTAGTGGTGAGTGTGC	18.17
78.	9	RM24542	ATCCACAAGAGCACCGATGAGG	TGACCTGGTAGTGGTGAGTGTGC	18.11
79.	9	RM242	AAACACATGCTGCTGACACTT-GC	TTACTAGATTTACCACGGCCAACG	18.64
80.	9	RM24616	CACCTTGGCCAACTAACTA-ATCG	GGGCAAGAGGAATTCACAACC	19.28
81.	9	RM3808	CAGTGGCGTGGAGAGA-AATTTGG	CTCACCTGCGACAGCAAGATCG	20.25
82.	9	RM591	CTCATAGGTGGGGT-TAGTTTCTTGG	GCTGGTTTACAACTTGCTACTC-TACC	22.45
83.	10	RM5271	CGAATCTTGGAACACATCAACG	GGGAGGAGTGCTGTGAGAGG	1.92
84.	10	RM25404	GCAACGGTTCTCCTTCCAC-TACC	CCATGATAGCGTTAGCCATAAACG	13.9
85.	10	RM258	CTCCCTGGCCTTTAAAGCT-GTCG	GACGAACAGCAGCAGAAGAGA-AGC	17.57

Table 1: Continue...

Sl. No.	Chro. No.	Marker	Forward primer sequence	Reverse primer sequence	Physical position (Mb)
86.	11	RM26048	TCTTGGGTTCGCCATATCTAGG	CCCATCTCCCTTCTGTCTCTCC	1.94
87.	11	RM26550	GGGAAACATGGAAGAACACTC-TAGC	CAAGCATTTGCAGCATTCAAGG	12.07
88.	11	RM206	ATCGATCCGTATGGGTTCTAGC	GTCCATGTAGCCAATCTTATGTGG	21.63
89.	11	RM27096	AGTTAGGATCGCTTCCAG-GTTCC	TCCAACTGGAATATCGTCTTG-TAGGC	23.47
90.	12	RM27876	TGCAAATAGTGCTTCACTGACC	CAGCATGCATAGAGAAGAGTTGG	9.11
91	12	RM28085	CCCGCTGCAGCAGTTTATT-GAGG	GATCTGGTACCTGCATGGGTTGC	15.38
92.	12	SSR12-17.4	GCTTAATTTCTGACAGAC-CAGTGC	GATCTAAACACAGCCTTCCTTGC	17.4
93.	12	RM28157	GCTTAATTTCTGACAGAC-CAGTGC	GATCTAAACACAGCCTTCCTTGG	17.43
94.	12	ESSR12-20.2	GGTGTTGCAGGCGTCCTACT	TCATGGAATGGAAACAACCA	20.06
95.	12	RM28424	TCCACACACTTCGCCAATA-AACC	CCGCCACCACTCCTCTATCC	22.4
96.	12	RM28491	TACACCCACCCACATACGT-CAGC	GTCGTACTCCCGGATCTTCTTCC	23.3
97.	12	RM28739	CTCCCACAGAGAATG-CAAATAGC	TGAATAATGGAGCTAGAG-GCTTCG	26.31

Table 2: Percentage of SSR markers polymorphic between 10–3 and Telangana Sona on each chromosome

Sl. No.	Chromosome No.	Total No. of markers screened chromosome^{-1}	No. of Polymorphic markers chromosome^{-1}	No. of monomorphic markers chromosome^{-1}	Percentage of polymorphism chromosome^{-1}
1.	1	84	13	71	15.48
2.	2	56	10	46	17.86
3.	3	49	5	44	10.2
4.	4	72	11	61	15.28
5.	5	86	3	83	3.49
6.	6	50	15	35	30
7.	7	97	11	86	11.34
8.	8	55	4	51	7.27
9.	9	57	10	47	17.54
10.	10	49	3	46	6.12
11.	11	87	4	83	4.6
12.	12	74	8	66	10.81
Total		816	97	719	

The range of polymorphism varied across different chromosomes, with chromosome 6 having the highest percentage of 30 and chromosome 5 having the lowest percentage of 3.49. On average, the percentage of polymorphism was 12.50%, which suggests that 10–3 and Telangana Sona exhibited greater genetic variability

on the 6th chromosome and minimal variability on the 5th chromosome. These findings are consistent with other studies of a similar nature. Ishwarya Lakshmi et al. (2021) used 494 SSR markers to detect polymorphism between the BPH resistant line (M-229) and the susceptible line (RNR 15048), with 87 (17.1%) markers demonstrating polymorphism.

Genetically diverse parents are prerequisite for mapping of desirable traits (Collard et al., 2005). The observed markers that exhibit polymorphism, as identified in this study, can be effectively employed in QTL mapping experiments to create a linkage map and identify QTLs that are linked with BPH resistance in the mapping population generated from both parents, RNR 15048 and 10–3. They will also be used for marker assisted backcrossing of identified QTLs into elite cultivar RNR 15048 along with recovery of the maximum RNR15048 background.

Earlier researchers, Xiao et al. (2016) improved 9311 cultivars, Mohanapriya et al. (2019) enhanced CO43Sub1, Wang et al. (2019) improved superior rice cultivar Wushansimiao, Sahoo et al. (2020) enhanced popular rice hybrid Rajalaxmi by using marker assisted backcrossing. Figure 2. shows the polymorphic SSR markers distribution on all 12 chromosomes, which was created using the map chart 2.32 software. Figure 3 Depicts a representative gel image of a polymorphism survey between 10–3 and Telangana Sona.

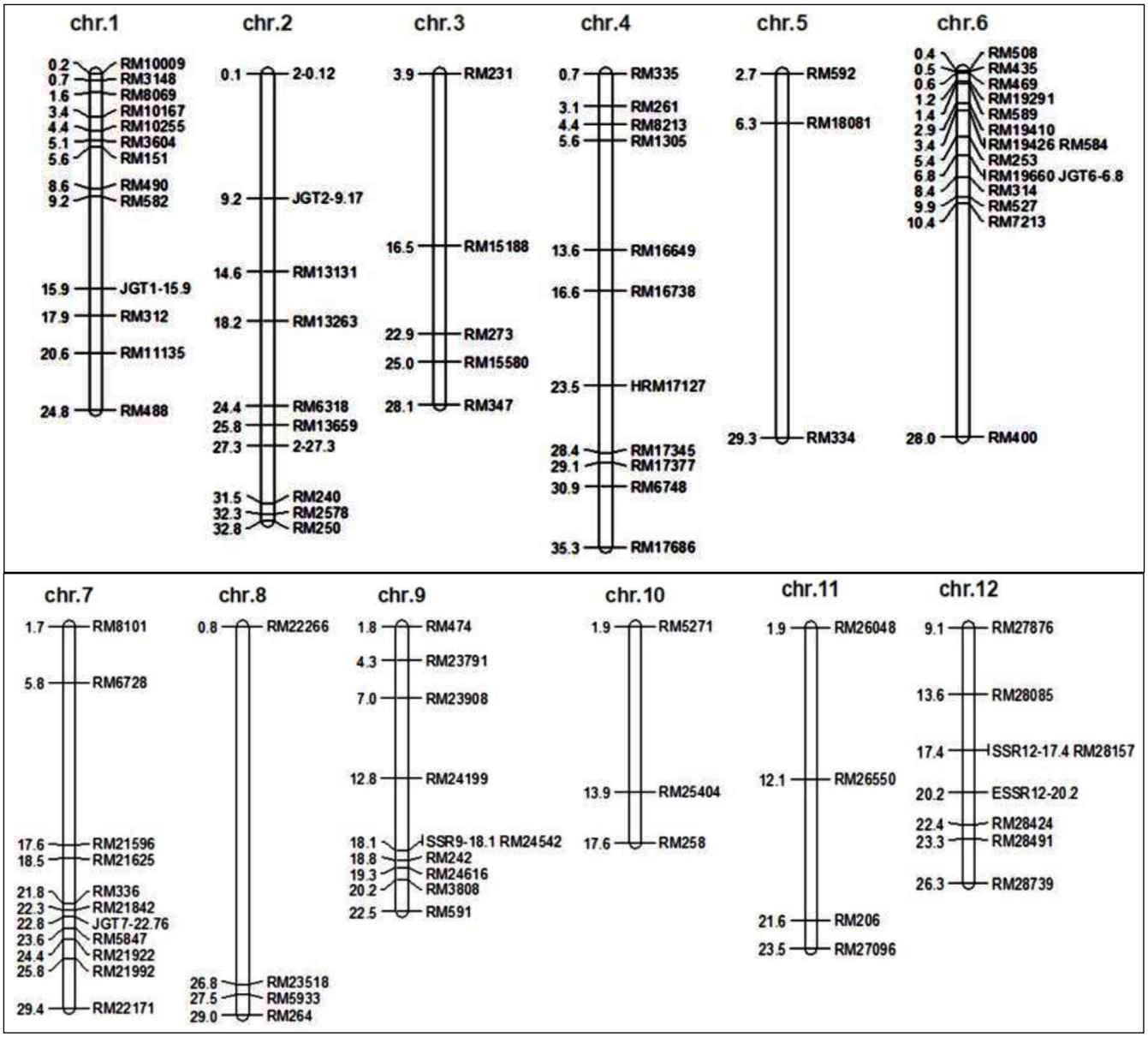

Figure 2: Graphical representation of polymorphic SSR markers across the 12 chromosomes

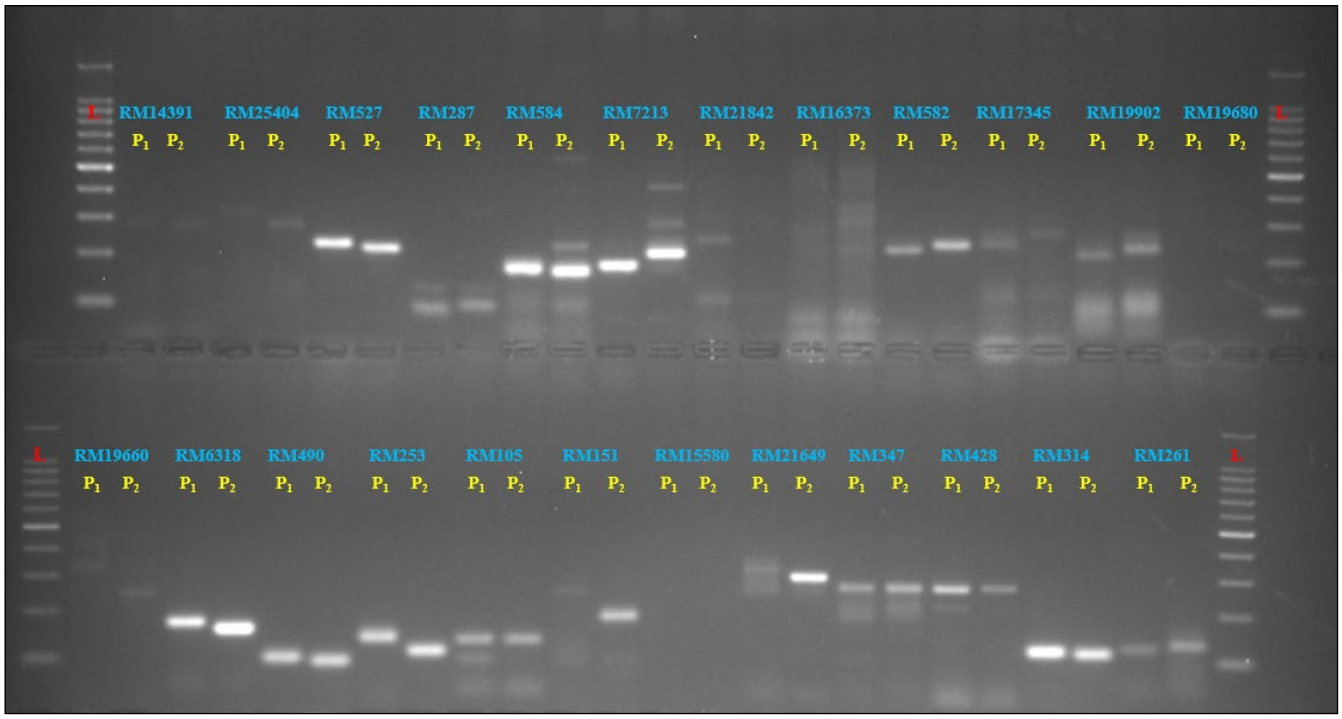

Figure 3: Survey of parental polymorphism between Telangana Sona and 10–3 with SSR markers; L: 100bp ladder; P_1: RNR15048; P_2: 10–3

4. CONCLUSION

In the current study, eight hundred and sixteen SSR primer pairs covering all the 12 chromosomes were used, with a particular focus on the target chromosomes associated with BPH resistance. 97 of them were found to be polymorphic, with 11.89% polymorphism. Chromosome 6 revealed the most polymorphic markers between parents. Polymorphic markers between 10–3 and RNR15048 will help in identifying QTLs associated with BPH resistance, marker assisted transfer of the identified QTLs into elite cultivar, RNR15048 and in recovering the maximum RNR15048 background.

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