



Microsatellites based Parental Polymorphism Survey in Elite Restorer Lines of Rice for Stacking Wide-compatibility Trait

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ABSTRACT

A study was undertaken during *kharif* (June, 2021–November, 2021) at ICAR–Central Rice Research Institute (CRRI), Cuttack, Odisha, India to identify polymorphic simple sequence repeat (SSR) markers between the parental lines of two rice crosses: Cross-I (CR 22-153-1×Lemont) and Cross-II (CR 22-153-1×CR 22-1-5-1). The primary goal was to screen for parental polymorphisms that could facilitate marker-assisted background selection (MABS) to expedite the recovery of the recurrent parent genome (CR 22-153-1) in a marker-assisted backcross breeding (MABB) program aimed at introgressing the wide compatibility gene ‘*S5n*’ into the elite restorer line CR 22-153-1. A total of 320 hypervariable SSR markers, uniformly distributed across all 12 rice chromosomes, were employed for the polymorphism survey. Among these, 96 markers (30%) were polymorphic between CR 22-153-1 and Lemont, while 89 markers (27.81%) showed polymorphism between CR 22-153-1 and CR 22-1-5-1. The extent of polymorphism observed between CR 22-153-1 and Lemont ranged from 20.7% to 48.2% across the twelve chromosomes, while that between CR 22-153-1 and CR 22-1-5-1 ranged from 20.7% to 41.4%. These identified polymorphic SSR markers provided a valuable resource for precise background selection in backcross and near-isogenic line (NIL) populations in both the crosses. Their use enabled the effective introgression of the ‘*S5n*’ gene while minimizing linkage drag, thereby facilitating the development of wide-compatible, high-yielding restorer lines.

KEYWORDS: Polymorphic marker survey, compatibility, *S5n*, background selection, MABB

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1. INTRODUCTION

Rice has nourished billions of people for millennia and remains one of the most important staple food crops globally (Dey et al., 2021). India, the world's second-largest producer and consumer of rice, also holds the distinction of having the largest area under rice cultivation (Ali et al., 2016 and Das et al., 2022). However, the country's rice supply faces looming challenges due to a combination of factors: a rapidly growing population, diminishing cultivable land caused by urbanization and infrastructural development, and stagnation in the yield potential of existing high-yielding varieties (HYVs) (Choudhury et al., 2013 and Pratap et al., 2018). As HYVs approach their yield ceiling, improving rice productivity through genetic enhancement becomes critical (Khush 2005 and Devi et al., 2022). One promising approach is the replacement of HYVs with hybrid rice, which offers a sustainable means of increasing yield potential (Revathi, 2015). All commercial rice hybrids in India are intra-specific hybrids, derived from crosses between *indica* cytoplasmic male sterile (CMS) lines and *indica* restorer lines and These hybrids often fail to express sufficient heterosis to offer a significant yield advantage over elite HYVs (Nanda et al., 2024). To overcome these limitations, rice breeders are turning to inter-sub-specific hybrids, which have shown much greater yield heterosis than intra-specific ones (Ikehashi and Araki, 1984). Notably, *indica*×*japonica* hybrids demonstrate the highest heterotic potential, followed by *indica*×*javanica*, *japonica*×*javanica*, *indica*×*indica*, and *japonica*×*japonica* hybrids (Dwivedi and Pandey 2012). However, earlier attempts at producing inter-sub-specific hybrids-especially *indica*×*japonica*-were largely unsuccessful due to post-zygotic hybrid sterility, primarily caused by embryo sac abortion (Zeng et al., 2007). Subsequent research has identified that this hybrid sterility is mainly regulated by a gene located at the S5 locus on chromosome 6 (Yang et al. 2012), which comprises three allelic forms: *S5i* (*indica*), *S5j* (*japonica*), and *S5n* (neutral) (Kato et al. 1928). The presence of both *S5i* and *S5j* alleles in hybrids results in partial sterility, whereas the *S5n* allele, when paired with either *S5i* or *S5j*, produces fertile embryo sacs (Sundaram et al., 2010). The *S5n* allele confers wide compatibility and is characterized by a 136 bp deletion at the S5 locus, rendering the gene non-functional (Chen et al., 2008 and Ji et al., 2010). Genotypes carrying this allele-referred to as wide compatible varieties (WCVs)-act as genetic bridges, enabling successful *indica*×*japonica* hybridization (Kallugudi et al., 2022). The development of functional molecular markers such as the S5 InDel, which can accurately distinguish the neutral '*S5n*' allele from the *indica* and *japonica* alleles, has significantly simplified marker-assisted backcross breeding (MABB) efforts (Sundaram et al., 2010). Before initiating any MABB

program to introgress a desired trait from a donor genotype into a recipient variety, it is essential to conduct a parental polymorphism analysis (Acharjee et al., 2021; Sahoo et al., 2022). Without detectable polymorphisms between the parental alleles, it becomes difficult to select plants that carry the desired features (Majhi et al., 2022). Simple Sequence Repeats (SSRs) have long been the markers of choice in rice breeding due to their co-dominant inheritance, high polymorphism, reproducibility, genome-wide distribution, cost-effectiveness, and ease of analysis (McCouch et al., 2002 and Singh et al., 2022). The present study focused on identifying polymorphic SSR markers between the parents of two crosses-Cross-I: CR 22-153-1×Lemont and Cross-II: CR 22-153-1×CR 22-1-5-1. The crosses were designed to introgress the *S5n* gene into the genetic background of an elite restorer line, CR 22-153-1. The polymorphic markers identified through this study would facilitate marker-assisted backcross breeding, enabling the efficient transfer of the wide compatibility gene '*S5n*' and thereby supporting the development of inter-sub-specific hybrids with enhanced heterosis and yield potential.

2. MATERIALS AND METHODS

The present study was carried out during *kharif* (June, 2021–November, 2021) at the ICAR–Central Rice Research Institute (CRRI), Cuttack, Odisha. The experimental materials comprised four elite restorer lines: CR 22-153-1, SLPP 1033, CR 22-1-5-1, and Lemont. A marker-assisted backcross breeding (MABB) programme was initiated during *rabi* (January, 2021–May, 2021) to introgress the wide compatibility gene '*S5n*' into the genetic background of the elite restorer line CR 22-153-1, which served as the recurrent parent (RP). The donor parents for the '*S5n*' gene were CR 22-1-5-1 and Lemont. To facilitate the recovery of the recurrent parent genome, it was essential to identify polymorphic SSR markers between the recurrent and donor parents for effective background selection. For this purpose, a parental polymorphism survey was undertaken during *kharif* (June, 2021–November, 2021). A total of 320 hypervariable simple sequence repeat (SSR) markers, uniformly distributed across all 12 rice chromosomes (approximately ≈27 markers per linkage group), were employed in this survey. These markers were randomly selected to ensure comprehensive genome coverage. Information regarding the chromosomal location, physical position, primer sequences, and repeat motifs of the SSR markers was obtained from the Gramene marker database (<https://www.gramene.org/>). Genomic DNA was extracted from young leaf tissues using the cetyl trimethyl ammonium bromide (CTAB) method. The extracted DNA was subjected to polymerase chain reaction (PCR) using an Eppendorf Thermocycler (USA). The PCR protocol

included an initial denaturation at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 45 s, annealing at approximately 55°C for 45 s (adjusted according to the T_m of individual primers), and extension at 72°C for 1 min. A final extension step was performed at 72°C for 7 min, after which the samples were held at 4°C. Electrophoresis was performed to observe the amplified PCR products, and polymorphism detection was done based on the analysis of distinct banding patterns observed between the parental lines. The molecular data were analyzed using GGT 2.0 software, which provided graphical genotyping outputs by organizing the marker scores based on their physical positions (in Mb) on the rice chromosomes (Berloo, 2007 and 2008). This visual representation allowed for effective assessment of marker distribution along each chromosome. Polymorphism % was calculated by using the following formula:

Polymorphism % = (Number of polymorphic markers identified per chromosome / Total number of markers run per chromosome) × 100

3. RESULTS AND DISCUSSION

3.1. Identifying polymorphic SSR markers between the parents for background selection

The identification of polymorphic SSR markers between the donor and recurrent parent genotypes represented the initial and most critical step in a marker-assisted backcross breeding (MABB) program aimed at recovering the recurrent parent genome. In the present study, a total of 320 SSR markers were screened to detect polymorphism between the donor genotypes (Lemont and CR 22-1-5-1) and the recurrent parent (CR 22-153-1). Among these, 96 markers (30%) were found to be polymorphic between CR 22-153-1 and Lemont, while 89 markers (27.81%) exhibited polymorphism between CR 22-153-1 and CR 22-1-5-1. These polymorphic markers were subsequently utilized for background selection in their respective backcross and near-isogenic line (NIL) derivative populations to facilitate the efficient and progressive recovery of the recurrent parent genome. Representative gel electrophoresis banding patterns of selected polymorphic SSR markers were illustrated in Figure 1, demonstrating their capacity to distinguish between donor and recurrent parent alleles. A summary of the polymorphic markers identified in Cross-I (CR 22-153-1 × Lemont) and Cross-II (CR 22-153-1 × CR 22-1-5-1) is presented in Tables 1 and 2, respectively. Comparable findings have been reported by other researchers. Singh et al. (2022) screened 510 SSR markers and identified 17.7% polymorphism between HUR-1309 (recurrent parent) and CR Dhan 801 (donor) during the introgression of drought-tolerant QTLs. Similarly, Majhi et al. (2022) observed 21.3% polymorphism between Pratikshya (recurrent parent)

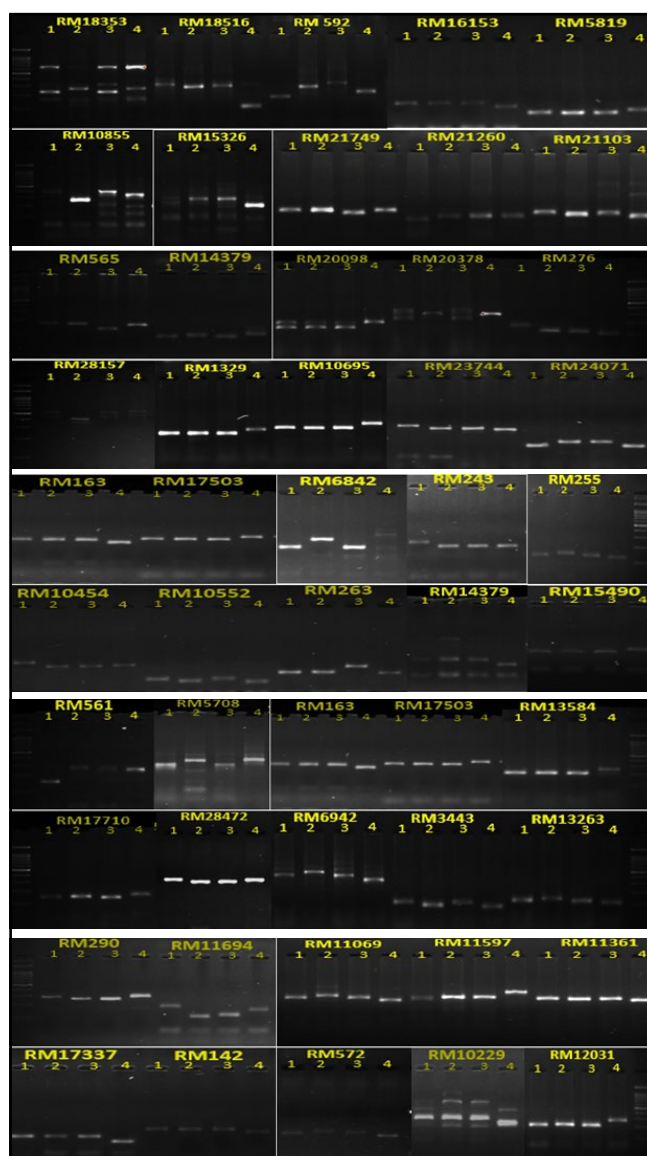


Figure 1: Gel images of the polymorphic markers identified between the parents involved in the crosses I and II; (*Parents: 1- CR 22-153-1, 2- SLPP 1033, 3- CR 22-1-5-1, 4- Lemont)

and CR Dhan 801 (donor) in a similar effort to incorporate drought-tolerant QTLs. List of polymorphic SSR markers detected between (CR 22-153-1 × Lemont) and (CR 22-153-1 × CR 22-1-5-1) are given in table 3 and 4 respectively.

3.2. Cross-I: CR 22-153-1 × Lemont

In this cross, the highest level of polymorphism between CR 22-153-1 and Lemont was observed on chromosome 1, with 48.15% of the SSR markers showing polymorphism. In contrast, chromosome 7 exhibited the lowest polymorphism at 20.69%. The remaining chromosomes—chromosomes 2, 3, 4, 5, 8, 9, 10, 11, and 12—showed polymorphism levels of 37%, 26.9%, 29.6%, 25%, 36%, 24%, 28%, 28%, and 24%, respectively (Table 1). On average, 30% polymorphism

Table 1: List of polymorphic markers between CR 22-153-1 and Lemont in cross I				
Chrom. No.	Total SSRs	Polymorphic SSRs	% polymorphism	Polymorphic SSR markers between CR 22-153-1 and Lemont
1	27	13	48.15	RM10793, RM1282, RM10596, RM11358, RM10855, RM493, RM1329, RM10695, RM11069, RM11694, RM11361, RM12031, RM572
2	27	10	37.04	RM279, RM207, RM530, RM7288, RM13584, RM5706, RM13630, RM6942, RM341, RM13679
3	26	7	26.92	RM 426, RM16153, RM5819, RM15630, RM14239, RM14379, RM15404
4	27	8	29.63	RM255, RM307, RM17600, RM16569, RM17337, RM142, RM16256, RM17503
5	28	7	25.00	RM440, RM122, RM18353, RM592, RM17710, RM163, RM18913
6	31	10	32.26	RM510, RM469, RM20377, RM20096, RM20378, RM19469, RM276, RM20150, RM162, S5 InDel
7	29	6	20.69	RM336, RM21260, RM21478, RM21521, RM320, RM20775
8	25	9	36.00	RM8271, RM152, RM22273, RM22523, RM6369, RM23345, RM22825, RM3231, RM502
9	25	6	24.00	RM219, RM23959, RM23778, RM23744, RM257, RM107
10	25	7	28.00	RM216, RM184, RM171, RM25735, RM474, RM6364, RM5708
11	25	7	28.00	RM2620, RM224, RM21, RM7283, RM26656, RM26213, RM201
12	25	6	24.00	RM28346, RM27638, RM27701, RM28464, RM19, RM28034
Total	320	96	30.00	

Table 2: List of polymorphic markers between CR 22-153-1 and CR 22-1-5-1 in cross II				
Chrom. No.	Total SSRs	Polymorphic SSRs	% polymorphism	Polymorphic SSR markers between CR 22-153-1 and CR 22-1-5-1
1	27	7	25.93	RM1282, RM11597, RM11694, RM151, RM10454, RM243, RM306
2	27	11	40.74	RM 279, RM7288, RM13630, RM13263, RM561, RM290, RM263, RM3501, RM5345, RM12349, RM341
3	26	6	23.08	RM 426, RM14270, RM14308, RM3586, RM14981, RM15806
4	27	7	25.93	RM518, RM307, RM16569, RM16913, RM17322, RM16739, RM16626
5	29	6	20.69	RM5970, RM122, RM592, RM5844, RM3068, RM163
6	30	7	23.33	RM20377, RM276, RM20150, RM20615, RM19469, RM20096, S5 InDel
7	29	12	41.38	RM21693, RM336, RM6326, RM5847, RM21749, RM21260, RM21521, RM21478, RM21810, RM21352, RM70, RM125
8	25	6	24.00	RM8271, RM6369, RM23345, RM22613, RM22571, RM22914
9	25	8	32.00	RM285, RM219, RM24664, RM23744, RM24071, RM257, RM24542, RM24717
10	25	7	28.00	RM216, RM184, RM171, RM25735, RM474, RM271, RM25817
11	25	6	24.00	RM7283, RM26213, RM21, RM26269, RM26302, RM26339
12	25	6	24.00	RM27701, RM28472, RM28067, RM19, RM28034, RM28464
Total	320	89	27.81	

Table 3: List of polymorphic markers between CR 22-153-1 and Lemont

Sl. No.	Primer name	Chromosome number	Forward sequence	Reverse sequence	Position
1.	RM10793	1	GACTTGC-CAACTCCTTCAATTCG	TCGTCGAG-TAGCTTCCCTCTCTACC	12,569,890-12,570,013 bp
2.	RM1282	1	TCGTGCAGGAG-GTCTTCATGG	TTGAGGATGGTAAC-GAACCTTGC	548,511-548,910 bp
3.	RM10596	1	CAACAACCTGGCAGAGA-ATTTCG	CTGCACGTGATGT-CAGAGTTCG	9,489,807-9,490,070 bp
4.	RM11358	1	TTGGTGTCTTCTGACCG-GATAAGG	CTAGTGAGCGCCGGTAT-TAAAGG	24,889,769-24,890,412 bp
5.	RM10855	1	CCAAACAAGATG-TAGTGGGAACATCC	CAAGATGATCAGTGGGC-TATTCTTGG	14,067,612-14,067,967 bp
6.	RM493	1	GTACGTAAACGCG-GAAGGTGACG	CGACGTACGAGATGCC-GATCC	12,280,117-12,280,294 bp
7.	RM1329	1	CGGAGCTCAATCGAATC-TAGACC	TTCTCATATCCTC-CAGCTCTTAGC	3,946,854-3,947,045 bp
8.	RM10695	1	CCTTCGACTCCATGAAA-CAAACG	TCTCTTTGCCCTA-ACCCTATGTCC	10,981,692-10,981,986 bp
9.	RM11069	1	GGTACAATGAAGCTTG-GCAACG	CGGTGGAGTAGAAC-CACGAAGC	19,370,286-19,370,565 bp
10.	RM11694	1	GCGTCTATGCG-TATCTTCATCTTACC	CAACTCTGCTAGTGT-GCCTCTGC	31,896,904-31,897,097 bp
11.	RM11361	1	TGCTAGCTGCCTACCCTC-GAAGC	AGGCAGGCATCGGC-TATATTCTCG	24,949,694-24,949,944 bp
12.	RM12031	1	TCCCTAGCTAGCTCTC-CATCTCC	AGTACTACCGCTACAT-GTCTTCTTGG	39,187,120-39,187,415 bp
13.	RM572	1	CGCGGTTAATGTCATCT-GATTGG	CCATACTTCGAGATC-CAAGACTGACC	9,865,568-9,865,718 bp
14.	RM279	2	CCTCTCACTCACGTG-GACTCTCC	CCTCACCTAGGCTTT-GATATGC	2,882,082-2,882,252 bp
15.	RM207	2	ATCCTAGTGGATAAGGCA-CAGACTGG	CCCTTGCTCTTCCACCT-CATCC	35,369,336-35,369,671 bp
16.	RM530	2	TTCTTTATTCCCTCGCACT-GACC	CAATGATGCCACAAAC-CGTAACC	30,532,198-30,532,386 bp
17.	RM7288	2	CGCTATGTGGCAGTTTA-AGAGC	CTGTTGGAATCAAAGT-GAGTGC	9,033,547-9,033,882 bp
18.	RM13584	2	TCCATCTTCACAATCAG-CAACC	CCCTGTAACAATGTT-GAGAACACC	23,882,924-23,883,210 bp
19.	RM5706	2	GTGGCTTGATTCTG-CAACTTCC	GCTGGTACCTACCAAG-TATCATTTTCG	26,479,540-26,479,734 bp
20.	RM13630	2	GCCGCTGATCTCTTACCT-GTTACGG	GTGCTTCGGCACAGGT-CAATGC	25,119,299-25,119,394 bp

Table 3: Continue...

Sl. No.	Primer name	Chromosome number	Forward sequence	Reverse sequence	Position
21.	RM6942	2	CGAACTCCCAATTACAGAT-CACG	TCATCCAAGACTTAG-GTGGAGAAGC	17,436,262-17,436,547 bp
22.	RM341	2	CAAGAAACCTCAATCC-GAGC	CTCCTCCCGATCCCAATC	94.4-94.4 cM
23.	RM13679	2	AGATGACAAGGTGAGAG-CACTGG	TGGAGCCCAGAATTTC-TAGATCG	26,102,434-26,102,711 bp
24.	RM 426	3	CATCGCCGAAATC-CATCTTCC	AAGGCCCATTTTCATTG-TAGAGTGC	27,588,613-27,588,762 bp
25.	RM16153	3	TGGTTGTGGTATAGCACG-GTAAGC	TGACCCAAGGAGATAC-TAGGTTGC	35,013,535-35,013,799 bp
26.	RM5819	3	TACGCCTAACGGCCTAAG-TACC	AATCACTC-CAGAACCCAGTAGTAGC	4,285,381-4,285,551 bp
27.	RM15630	3	AACTCGAAGGATCTC-GCCCAACC	ACCCACCTCCTCACGCT-GTACG	26,073,971-26,074,256 bp
28.	RM14239	3	CAAGTTCACCC-GCCTTCTCG	TTTCCATCATTAGCAG-GCAGTAGC	85,314-85,337 bp
29.	RM14379	3	ATGCAACAGGGAGGT-GCTTGG	TGAATCTGAAGAT-GCCCAAGAGG	2,435,994-2,436,033 bp
30.	RM15404	3	TTTGGAAGGC-TATCTTCTCTGG	GGAGAGCCGAAAC-TATTTGATTGG	22,489,928-22,490,088 bp
31.	RM255	4	GAGGAGGAGGAGGAGA-GATCAGG	AACGAAACCGCT-CAGTTCAACC	30,772,388-30,772,538 bp
32.	RM307	4	GTACTACCGACCTACC-GTTCAC	CTGCTATGCATGAACT-GCTC	10.5-10.5 cM
33.	RM17600	4	CCTCGAAATGAATTG-CAGTCGAACG	GTCTTGTGCCTTGTGC-CGATGG	33,596,398-33,596,876 bp
34.	RM16569	4	ATAGACTTCACCCGGTA-CAACG	CTGGTATCGACATCGGC-TAGG	9,666,957-9,667,308 bp
35.	RM17337	4	CCCTCCCGTAGACCTTG-TACCC	CCACAGCTAAC-CAATCCTTCTCC	28,248,941-28,248,982 bp
36.	RM142	4	TCTTCCTCTCCACTTC-CATTTC	AAGAAGCTC-GGGATCTTCACC	20,518,899-20,519,086 bp
37.	RM16256	4	GAAGGCGTGCTCCGAGA-ACTGG	GTGATCGCCTTGTC-GTCTGC	113,340-113,363 bp
38.	RM17503	4	CCAGATCATCCAGGCATAA-CATCACC	CGGCGCTGGTAAACTC-CATTCC	31,717,625-31,717,670 bp
39.	RM440	5	GGTAGGCAC-CAAAGAGTTTGACG	GGCATCACCTTATC-CAATCACC	19,912,517-19,912,685 bp
40.	RM122	5	CTTCTTCC-GCTTCCTCCCTTCC	TGTACCAGTGCACC-GAGAGTTGG	311,055-311,218 bp
41.	RM18353	5	AGATCTCACTATTGAG-TAGCCCATGC	CACCTTGCCCTTA-AATACCAACC	13,949,549-13,949,835 bp

Table 3: Continue...

Sl. No.	Primer name	Chromosome number	Forward sequence	Reverse sequence	Position
42.	RM592	5	ACCTCACCCGAATTACTGT-GATATGC	GTTGAATTGCACGC-GACTCTGG	2, 796, 876-2, 797, 130 bp
43.	RM17710	5	GACGAGGCAGGCGAT-GATAAGG	CAGCACCTCAAGC-GTCCTAACC	46, 634-46, 802 bp
44.	RM163	5	ATCCATGTGCGCCTTTAT-GAGGA	CGCTACCTCCTTCACT-TACTAGT	19,189,417-19,189,547 bp
45.	RM18913	5	CAAGATCTTTGCAACT-GAGGAAGG	TTGCTACTTTCGTGTTGT-CACTCTACG	24,100,757-24,101,244 bp
46.	RM510	6	GTTTGACGCGATAAACC-GACAGC	ATGAGGACGACGAGCA-GATTCC	2, 831, 443-2, 831, 635 bp
47.	RM469	6	TTACGTGATCACACAG-GCTCTCC	AAGCTGAACAAGCCCT-GAAAGG	564, 135-564, 314 bp
48.	S5-InDel	6	CCTACGTTTGACTGCCT-GCCTG	CTACACGCGGCTTC-GGGAAAGC	5.8-5.8 cM
49.	RM20377	6	GTGTGTGATGTGCAT-GTTTCTGC	CATGTGATGCCCTGTAG-GAACC	24,320,992-24,321,271 bp
50.	RM20096	6	CGGTAAGCCATAAATAG-ATCCCAAGG	TTTGAACAGCGACACG-GTTTCC	17,431,904-17,432,300 bp
51.	RM20378	6	ATGTCCTTCTTCGAGATT-GCTTCG	AGAGCCCAACATATAGC-GGATGC	24,323,763-24,324,051 bp
52.	RM19469	6	TTCTTTTCTTGGGT-CATATCC	ATCGAGTCAGAGAGTG-GCTTGG	4, 049, 892-4, 050, 188 bp
53.	RM276	6	GTCCTCCATCGAGCAGTAT-CAGC	CTAGCAAGACATG-GACCTCAACG	6, 230, 045-6, 230, 185 bp
54.	RM20150	6	TGTTTGTGTGGTTAGGG-TTTGG	TTGTGGTTCTGGGTG-TAGTTATCC	19,513,580-19,513,857 bp
55.	RM162	6	TTGTTCCAGTTCAG-GTCTTGTGC	CCCTACAAACACCATA-AGAAGCAACC	24,035,491-24,035,615 bp
56.	RM336	7	GTATCTTACAGAGAAACG-GCATCG	GGTTTGTTCAGGTTC-GTCTATCC	21,871,205-21,871,356 bp
57.	RM21260	7	CTGCACAACCAGGAGA-AATTAAGC	CTGACCACTCTAGCTT-GCCTACC	7, 270, 888-7, 270, 987 bp
58.	RM21478	7	TAACACAGTTCTTCTCG-CAACG	AAGTTCCCTTGTGT-GATTGACC	14,734,925-14,735,053 bp
59.	RM21521	7	TACTCGAGCAGGCAGAG-CAACC	AGCATAGTCATCCAAC-CGAGACAGC	15,985,258-15,985,525 bp
60.	RM320	7	TACAGCAGGGTGGAATAT-CGACAAGC	TCGTTGTAACGCGTG-GATATTTGG	18,693,223-18,693,635 bp
61.	RM20775	7	GGTGTTCATCTTTGTT-GAGTCAGG	AGGATACAGCGCACCA-GATTAGC	51,912-52,093 bp
62.	RM8271	8	AGCAGCTCCGATTGTGT-TAGCC	AATGGCGTCTGTGG-TACTTTGC	7, 616, 956-7, 617, 315 bp

Table 3: Continue...

Sl. No.	Primer name	Chromosome number	Forward sequence	Reverse sequence	Position
63.	RM152	8	AAGGAGAAGTTCTTC-GCCCAGTGC	GCCCATTAGTGACT-GCTCCTAGTCG	682, 963-683, 114 bp
64.	RM22273	8	AGTGACAGCATCAGCAT-GTTGTTTGG	TTAAGCTGGCACTG-CACTTGAAGG	849, 528-849, 810 bp
65.	RM22523	8	CGTGCTTAATTACCTG-GCTTCG	GCACGTATTCACAGC-TAGCAACC	5, 108, 770-5, 109, 207 bp
66.	RM6369	8	AGC-TAGCTTCACCTACCTACCT-CACC	ATGGTCATGTCGTTG-GTTTGC	124, 644-124, 908 bp
67.	RM23345	8	GAGATCCTGCACATCTTT-GAGACC	TGTGCCACGAAA-CAAATCTAGGC	24,080,259-24,080,487 bp
68.	RM22825	8	AGCACATCACAAACC-TACCCCTACC	CCTAATTAATCCCGCG-GAACC	11,758,311-11,758,471 bp
69.	RM3231	8	CACCGGCGTCAAGCT-CATCG	GTCAGTCCAGGTAG-GAGCATGAGAGC	3, 838, 134-3, 838, 282 bp
70.	RM502	8	CATCTCTGTTCCACTT-GCTTTGC	CTACCAACAACCCAA-CAAGAAGG	26,492,117-26,492,381 bp
71.	RM219	9	CGTCGGATGATGTA-AAGCCT	CATATCGGCATTC-GCCTG	11.7-11.7 cM
72.	RM23959	9	TGCCAAAGCTAGCTACA-CACTCC	TGCAT-CATCTTCATCTCTC-CATCC	7, 998, 131-7, 998, 274 bp
73.	RM23778	9	AACACAGCCTAAAGGT-GTTCTGAGC	GCTTCG-GCCCTATAGTCTTCTCG	3, 965, 989-3, 966, 316 bp
74.	RM23744	9	CTTAATACTCCGACGTAA-CAGTGG	CCTGACTAAATG-GAGCTTCTTCC	2, 913, 810-2, 914, 118 bp
75.	RM257	9	CCGTGCAACTTAAATC-CAAACAGG	GGAATCCTATATGAGC-CAGTGATGG	17,719,660-17,719,823 bp
76.	RM107	9	TCTTACTGCGTCCTC-TGGGTTCC	ATTCTTGCGGC-GATTCATCTTCC	20,068,688-20,068,866 bp
77.	RM216	10	GATGGTAAAGGAAGAAC-GTGTGC	CACTCATAGACGCATCA-CATAGCC	5, 352, 766-5, 352, 898 bp
78.	RM184	10	ATCCCATTCGCCAAAAC-CGGCC	TGACACTTGGAGAGCG-GTGTGG	16,358,895-16,359,111 bp
79.	RM171	10	AACGCGAGGACACGTACT-TAC	ACGAGATACGTAC-GCCTTTG	19,048,795-19,049,123 bp
80.	RM25735	10	AGGCAGGCAAGCAG-TAGTTTCG	ATCAAGATCAGGAGCCG-CAAGG	20,010,254-20,010,640 bp
81.	RM474	10	TACACGAGGGAGTACTC-GAATGG	CATGGAGG-TATAGAAGAGCATTTGG	1, 818, 800-1, 819, 051 bp
82.	RM6364	10	ACCTTCTCCGCTCATC-CCTACC	ATTTTCGTCCTTCTCG-GATTTGC	66,371-66,628 bp

Table 3: Continue...

Sl. No.	Primer name	Chromosome number	Forward sequence	Reverse sequence	Position
83.	RM5708	10	TGGTATCCATAACCTTGA-CAGC	GTCACGTATCGAACAG-TCTCC	14,456,288-14,456,536 bp
84.	RM2620	11	CTTAATCCCAGCCCTG-GAAGC	GATTGATGTCAGCCG-TAACATGG	4,459,979-4,460,147 bp
85.	RM224	11	ATCGATCGATCTTCAC-GAGG	TGCTATAAAAGGCATTC-GGG	120.1-120.1 cM
86.	RM21	11	ACAGTATTCCGTAGGCAC-GG	GCTCCATGAGGGTGG-TAGAG	85.7-85.7 cM
87.	RM7283	11	GGCATATAACGCAAACAC-CAACAAGG	CAGAAGAGCGATGGCA-GTTTGG	9,117,512-9,117,694 bp
88.	RM26656	11	GCAAAGAACATTGTG-GCAAACACC	TGGACACATTGTATCGT-GCTTCG	15,274,670-15,275,028 bp
89.	RM26213	11	GCCACAGGAGACAG-CAAGAACC	CGATCCAATTCCAGCC-TAGATAGC	4,750,713-4,751,058 bp
90.	RM201	11	CTCGTTTATTACCTACAG-TACC	CTACCTCCTTTCTAGAC-CGATA	111.1-111.1 cM
91.	RM28346	12	GCCCAAAGTTAATATCGGT-GTCTCC	AGCCTGCCTAGCACT-CATAGACC	20,919,548-20,919,838 bp
92.	RM27638	12	AGATTCCCATCCGTTAG-GAAAGC	GATGCACATGCACTTG-TAGTTCC	4,037,622-4,037,916 bp
93.	RM27701	12	CTATATGGCACCCT-GACATTTGC	TCAATAAAGCCCACC-GTTCC	5,000,889-5,001,278 bp
94.	RM28464	12	CTATATCCGCACCAATG-GAAACC	TAGGAATTGCGACGAG-TTTGTGG	22,908,956-22,909,606 bp
95.	RM19	12	CAAAAACAGAGCAGATGAC	CTCAAGATGGACGC-CAAGA	20.9-20.9 cM
96.	RM28034	12	CTCGGAAGCAGACAGC-GAAGG	GAGAGACTGGATC-GGGTTCAAGC	13,668,057-13,668,347 bp

was detected between CR 22-153-1 and Lemont across all twelve chromosomes, indicating a sufficient level of allelic diversity for effective background selection. Comparable levels of polymorphism have been reported in earlier studies. For instance, Tripathi et al. (2018) reported an average polymorphism of 68.3%, while Ishwarya et al. (2021) observed 17.1% polymorphism between donor and recurrent parents in their respective MABB programs. The chromosomal distribution of the polymorphic SSR markers detected in Cross-I is depicted in Figure 2. Notably, the S5n gene-targeted for introgression and located on chromosome 6-exhibited 32.3% polymorphism, which was considered adequate for both foreground selection and background genome recovery. Importantly, two SSR markers flanking the S5n locus-RM19469 (located at 4.05 cM) and RM276 (at 6.23 cM)-were found to be polymorphic between

the parental lines. Given that the S5n gene was located approximately at 5.8 cM, these flanking markers provide an effective means for recombinant selection, thereby enabling the precise introgression of the target gene while minimizing linkage drag from the donor genome.

2.3. Cross-II: CR 22-153-1×CR 22-1-5-1

In Cross-II, the highest level of polymorphism between CR 22-153-1 and CR 22-1-5-1 was recorded on chromosome 7, where 41.4% of the SSR markers were polymorphic. Conversely, chromosome 5 exhibited the lowest polymorphism at 20.7%. Across all twelve chromosomes, the average polymorphism observed between the recurrent and donor parents was 27.8%, indicating a moderate level of allelic differentiation suitable for marker-assisted background selection. Polymorphism levels for

Table 4: List of polymorphic markers between CR 22-153-1 and CR 22-1-5-1					
Sl. No.	Primer name	Chromosome no.	Forward sequence	Reverse sequence	Position
1.	RM1282	1	TCGTGCAGGAGGTCTT-CATGG	TTGAGGATGGTAAC-GAACCTTGC	548, 511-548, 910 bp
2.	RM11597	1	GTATTTATTGTGGGCG-GCAAACC	CGTTTGCTAGTTCAGT-GTTGTGTCG	29, 634, 209-29, 634, 612 bp
3.	RM11694	1	GCGTCTATGCG-TATCTTCATCTTACC	CAACTCTGCTAGTGT-GCCTCTGC	31, 896, 904-31, 897, 097 bp
4.	RM151	1	TGCTGATCAGTTACAC-GAATCAGAGC	GCGTACGTGCACAAAT-TAAACAGACC	5, 558, 576-5, 558, 814 bp
5.	RM10454	1	AGTGATGAGAAGCGAGA-ACTGAAGG	GAGGGAGTACATGTT-GATGTCATGG	7, 283, 578-7, 283, 788 bp
6.	RM243	1	CAGACTGCAGTTGCAC-GATACTACG	GAAAGCTGCAACGAT-GTTGTCC	7, 970, 722-7, 970, 836 bp
7.	RM306	1	CAAGGTCAAGAATG-CAATGG	GCCACTTTAATCATTG-CATC	98.1-98.1 cM
8.	RM 279	2	CCTCTCACTCACGTG-GACTCTCC	CCTCACCCCTAGGCTTT-GATATGC	2, 882, 082-2, 882, 252 bp
9.	RM7288	2	CGCTATGTGGCAGTTTA-AGAGC	CTGTTGGAATCAAAGT-GAGTGC	9, 033, 547-9, 033, 882 bp
10.	RM13630	2	GCCGCTGATCTCTTACCT-GTTACGG	GTGCTTCGGCACAGGT-CAATGC	25, 119, 299-25, 119, 394 bp
11.	RM13263	2	AAGATTGCACACTGGT-GTTCTCC	AGAAGAGCCGGTCTTT-GTCTCC	18, 081, 502-18, 081, 656 bp
12.	RM561	2	GAGCTGTTTTGGACTAC-GGC	GAG-TAGCTTTCTCCCACCCC	74.1-74.1 cM
13.	RM290	2	ATGACCCAATTTCT-GACTCTAGCC	CATGGGTGGTGCTGTA-GATGG	10, 806, 902-10, 807, 358 bp
14.	RM263	2	AATCTATGGACCTGGGAG-GAACC	TGACGAGAGTGCTAC-GTTTGAGC	25, 865, 334-25, 865, 568 bp
15.	RM3501	2	CTACAATGATTCCAT-GCCTGTCC	TCCGGCTCAAGCTA-CAGTTAAGG	10, 187, 508-10, 187, 682 bp
16.	RM5345	2	CTCTGTTGCAAT-GCCCAATACC	AAGGGAGTCCATGGA-GATGAGG	4, 206, 222-4, 206, 587 bp
17.	RM12349	2	CCGATTAGCGATT-GATATGGAGTAGG	AGTGCACAGCCATG-GAATTATGC	977, 799-977, 912 bp
18.	RM341	2	CAAGAAACCTCAATCC-GAGC	CTCCTCCC-GATCCCAATC	94.4-94.4 cM
19.	RM426	3	CATCGCCGAAATC-CATCTTCC	AAGGCCCATTTTCATTG-TAGAGTGC	27, 588, 613-27, 588, 762 bp
20.	RM14270	3	ATTATCGCTCGGAGAT-GCTTACC	GCCAATATTGTCCACA-CAATCACC	434, 406-434, 793 bp
21.	RM14308	3	GCATTCTAGTGTTATG-GCTGTTGC	GCTACTAAGCTGGT-CACCTGTGG	1, 068, 020-1, 068, 051 bp
22.	RM3586	3	TCTTGATTGCTGGACCA-CATGC	TCGAGCTAGAAGACGA-CACACAGC	36, 141, 002-36, 141, 177 bp

Table 4: Continue...

Sl. No.	Primer name	Chromosome no.	Forward sequence	Reverse sequence	Position
23.	RM15806	3	ACCATGAGGTTTCAGTTAG-GAGTGC	AATTGACGGTGGAGT-GAAGTACC	28, 975, 284-28, 975, 677 bp
24.	RM14981	3	GGCGAGCAGAAGTATA-ATCCAGAAGG	CGCTTGTGGCTTACTG-GCTTGG	13, 886, 241-13, 886, 523 bp
25.	RM518	4	AAGACACAAGCAAA-CAGCTCAACC	AAGCTTGCTTG-GTTCAAGAGAGG	2,030, 135-2,030, 305 bp
26.	RM307	4	GTACTACCGACCTACC-GTTCAC	CTGCTATGCATGAACT-GCTC	10.5-10.5 cM
27.	RM16569	4	ATAGACTTCACCCGGTACAACG	CTGGTATCGACATCG-GCTAGG	9,666,957-9,667, 308 bp
28.	RM16913	4	GTGTACGTGTTG-GCTCTCTGTACG	GATGTTGCTTGTGCTG-CAACC	19, 646, 468-19, 646, 859 bp
29.	RM17322	4	TCTGCTAGCCTGCACA-CAGAAGG	GCACAGGAGAC-CAAAGAATCAGG	27, 782, 214-27, 782, 253 bp
30.	RM16739	4	TGCATAGTCCACCTA-AGAGTTCAAGG	TTTCTGCAGGCT-GATTAGTGTAGG	16, 460, 287-16, 460, 641 bp
31.	RM16626	4	ACATGATTGCTGGCTT-GCTTACC	GCCACGCAGTGTT-GTTTCAGC	12, 717, 126-12, 717, 307 bp
32.	RM5970	5	CCATCTG-GTTCACCTTCACG	ACGTCTTGAAGAAGCT-GAATGC	23, 946, 271-23, 946, 390 bp
33.	RM122	5	CTTCTTCC-GCTTCCCTCCCTTCC	TGTACCAGTGCACC-GAGAGTTGG	311,055-311,218 bp
34.	RM592	5	ACCTCACCCGAATTACT-GTGATATGC	GTTGAATTGCACGC-GACTCTGG	2,796,876-2,797, 130 bp
35.	RM5844	5	AACGTGGCATCCATGT-TAGTACC	AGCTAGGAGCCATT-GTCGAAGG	9,149,429-9,149, 623 bp
36.	RM3068	5	CAACCCGAACGATAT-CAAGTTACC	CAGGCAAGATGGTGAT-GAACC	29, 251, 643-29, 251, 896 bp
37.	RM163	5	ATCCATGTGCGCCTTTAT-GAGGA	CGCTACCTCCTTCACT-TACTAGT	19, 189, 417-19, 189, 547 bp
38.	RM20377	6	GTGTGTGATGTGCAT-GTTTCTGC	CATGTGATGCCCTG-TAGGAACC	24, 320, 992-24, 321, 271 bp
39.	S5-InDel	6	CCTACGTTTGACTGCCT-GCCTG	CTACACGCGGCTTC-GGGAAAGC	5.8-5.8 cM
40.	RM276	6	GTCCTCCATCGAGCAG-TATCAGC	CTAGCAAGACATG-GACCTCAACG	6,230,045-6,230, 185 bp
41.	RM20150	6	TGTTTGTGTGGT-TAGGGTTTGG	TTGTGGTTCTGGGTG-TAGTTATCC	19, 513, 580-19, 513, 857 bp
42.	RM20615	6	TTACACCAGGC-TACCCAAACTCG	TTGCTGAGTTCCCTC-GTCTATCC	28, 049, 016-28, 049, 204 bp
43.	RM20096	6	CGGTAAGCCATAAATAG-ATCCCAAGG	TTTGAACAGCGACACG-GTTTCC	17, 431, 904-17, 432, 300 bp
44.	RM19469	6	TTCCTTTCCTTGGGT-CATATCC	ATCGAGTCAGAGAGTG-GCTTGG	4,049,892-4,050, 188 bp

Table 4: Continue...

Sl. No.	Primer name	Chromosome no.	Forward sequence	Reverse sequence	Position
45.	RM21693	7	GCACAGACCAGAACT-TTCTTCG	TGGCGAGTGTAGATG-TAATTGG	19, 510, 426-19, 510, 888 bp
46.	RM336	7	GTATCTTACAGAGAAACG-GCATCG	GGTTTGTTCAGGTTC-GTCTATCC	21, 871, 205-21, 871, 356 bp
47.	RM6326	7	CGATCTCTC-TATCCTTCCTCTTCTCC	CAGATTGTTAGCAACCC-GAATCC	24, 195, 104-24, 195, 325 bp
48.	RM5847	7	CTTTAGGTAGCGT-CATCTTCC	TGGAAATA-CAGAAGGAGTCG	23, 648, 589-23, 648, 782 bp
49.	RM21749	7	TAGCTTCCTACTTCC-GCCTTCTATCG	ACAGTGGGAGGGAGTA-CATACACTGG	20, 547, 379-20, 547, 591 bp
50.	RM21260	7	CTGCACAACCAGGAGA-AATTAAGC	CTGACCACTCTAGCTT-GCCTACC	7, 270, 888-7, 270, 987 bp
51.	RM21521	7	TACTCGAGCAGGCAGAG-CAACC	AGCATAGTCATCCAAC-CGAGACAGC	15, 985, 258-15, 985, 525 bp
52.	RM21478	7	TAACACAGTTCTTCTCG-CAACG	AAGTTCCTTGTGT-GATTGACC	14, 734, 925-14, 735, 053 bp
53.	RM21810	7	CTAGTTGCCAAGAGGAG-GCATCG	CTGTAGCTGAGGCAC-CAGAATGG	22, 188, 009-22, 188, 196 bp
54.	RM125	7	ATCAGCAGCCATGGCAGC-GACC	AGGGGATCATGTGCC-GAAGGCC	24.8-24.8 cM
55.	RM70	7	GTG-GACTTCATTTCAACTCG	GATGTATAAGATAGTCCC	64.6-64.6 cM
56.	RM21352	7	GGACGAAAGGGTATTT-GATTGG	CCTCAAGGTG-GTCTCCTTCTCC	10, 020, 417- 10, 020, 466 bp
57.	RM8271	8	AGCAGCTCCGATTGTGT-TAGCC	AATGGCGTCTGTGG-TACTTTGC	7, 616, 956-7, 617, 315 bp
58.	RM6369	8	AGCTAGCTT-CACCTAC-CTACCTCACC	ATGGTCATGTCGTTG-GTTTGC	124, 644-124, 908 bp
59.	RM23345	8	GAGATCCTGCACATCTTT-GAGACC	TGTGCCACGAAA-CAAATCTAGGC	24, 080, 259-24, 080, 487 bp
60.	RM22613	8	TTTCTGGCCCAGTTCAG-TACAGC	TGGTGCGTA-CATATCCCTTTAACC	6, 731, 353-6, 731, 546 bp
61.	RM22571	8	TCCTCCTCCACCTCAAT-CACACC	CCGAGCTCGC-CATTAGCTTGC	5, 844, 242- 5, 844, 283 bp
62.	RM22914	8	GGAGCAGCTAAGGCA-GATAAGAGG	GCCTTCAT-GCTTCAGAAGACAGC	15, 433, 651- 15, 433, 708 bp
63.	RM285	9	CTGTGGGCCCAATATGT-CAC	GGCGGTGACATGGAGA-AAG	1.8-1.8 cM
64.	RM219	9	CGTCGGATGATGTA-AAGCCT	CATATCGGCATTC-GCCTG	11.7-11.7 cM
65.	RM24664	9	ATCTAAACACAGCCC-TAGTTGC	GAGCCATAACTTTAC-CATGTGC	20, 360, 221-20, 360, 583 bp
66.	RM23744	9	CTTAATACTCCGACGTAA-CAGTGG	CCTGACTAAATG-GAGCTTCTTCC	2, 913, 810-2, 914, 118 bp

Table 4: Continue...

Sl. No.	Primer name	Chromosome no.	Forward sequence	Reverse sequence	Position
67.	RM24071	9	TACTGAAGGCCAAGGA-AGAGGTAGC	GAGACTATGGTGTGGC-GTCAATGG	10, 492, 629-10, 492, 782 bp
68.	RM257	9	CCGTGCAACTTAAATC-CAAACAGG	GGAATCCTATATGAGC-CAGTGATGG	17, 719, 660-17, 719, 823 bp
69.	RM24542	9	ATCCACAAGAGCACCGAT-GAGG	TGACCTGGTAGTGGT-GAGTGTGC	18, 119, 774- 18, 119, 875 bp
70.	RM24717	9	CCTCACTCCCGTACAGTT-GAACC	TAAGGCCATTCCGTT-GATGTGG	20, 895, 370- 20, 895, 453 bp
71.	RM216	10	GATGGTAAAGGAAGAAC-GTGTGC	CACTCATAGACGCATCA-CATAGCC	5, 352, 766-5, 352, 898 bp
72.	RM184	10	ATCCCATTCGCCAAAAC-CGGCC	TGACACTTGAGAGAGCG-GTGTGG	16, 358, 895-16, 359, 111 bp
73.	RM171	10	AACGCGAGGACACG-TACTTAC	ACGAGATACGTAC-GCCTTTG	19, 048, 795-19, 049, 123 bp
74.	RM25735	10	AGGCAGGCAAGCAG-TAGTTTCG	ATCAAGATCAGGAGCC-GCAAGG	20, 010, 254-20, 010, 640 bp
75.	RM474	10	TACACGAGGGAGTACTC-GAATGG	CATGGAGG-TATAGAAGAGCATTGG	1, 818, 800-1, 819, 051 bp
76.	RM25817	10	GCCTCGAATCAAC-CAAATAGTGG	GGGCTGAGACCTTGTG-GTATGG	21, 125, 455- 21, 125, 514 bp
77.	RM271	10	TCAGATCTACAATTC-CATCC	TCGGTGAGA-CCTAGAGAGCC	59.4-59.4 cM
78.	RM7283	11	GGCATATAACGCAAACAC-CAACAAGG	CAGAAGAGCGATG-GCAGTTTGG	9, 117, 512-9, 117, 694 bp
79.	RM26213	11	GCCACAGGAGACAG-CAAGAACC	CGATCCAATTC-CAGCCTAGATAGC	4, 750, 713-4, 751, 058 bp
80.	RM21	11	ACAGTATTCGGTAGGCAC-GG	GCTCCATGAGGGTGG-TAGAG	85.7-85.7 cM
81.	RM26269	11	GGAGGTAGGGAAATCAG-GTGAGG	GTGCACGTGACCATA-AACACTCC	6, 114, 008-6, 114, 029 bp
82.	RM26302	11	CTTTCTTCGAGGGTCAG-GTTGC	CAAGGAAGTTGAGTTG-GAAACTGC	6, 992, 366- 6, 992, 431 bp
83.	RM26339	11	CTGACACATTTGACTG-TATGG	GTGTTTAGGAGGTG-GAGTTTGG	7, 554, 952- 7, 555, 003 bp
84.	RM27701	12	CTATATGGCACCCT-GACATTTGC	TCAATAAAGCCCACC-GTTCC	5, 000, 889-5, 001, 278 bp
85.	RM28472	12	GCTATGAACCTGTACA-CATGTAGG	GAGCACCATAACAAT-CAGTTGC	22, 997, 129-22, 997, 459 bp
86.	RM28067	12	GAGGGAGTACTA-AACTTCCTAACG	ATGTGGATGTGATCTC-CATAGG	14, 694, 190-14, 694, 576 bp
87.	RM19	12	CAAAAACAGAGCAGAT-GAC	CTCAAGATGGACGC-CAAGA	20.9-20.9 cM
88.	RM28034	12	CTCGGAAGCAGACAGC-GAAGG	GAGAGACTGGATC-GGGTTCAAGC	13, 715, 095- 13, 715, 130 bp
89.	RM28464	12	CTATATCCGCACCAATG-GAAACC	TAGGAATTGCGAC-GAGTTTGTGG	22, 908, 956-22, 909, 606 bp

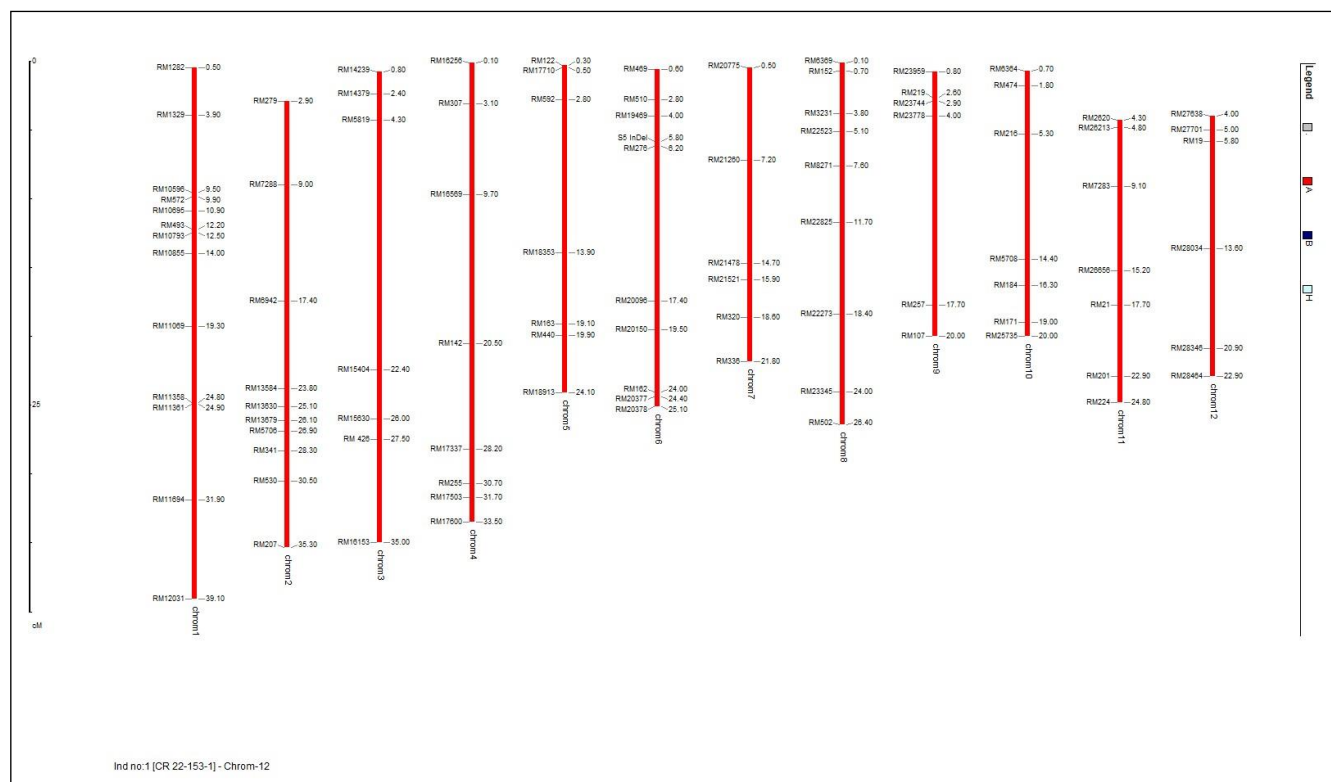


Figure 2: The distribution of the polymorphic SSR markers detected between CR 22-153-1 and Lemont on the twelve chromosomes

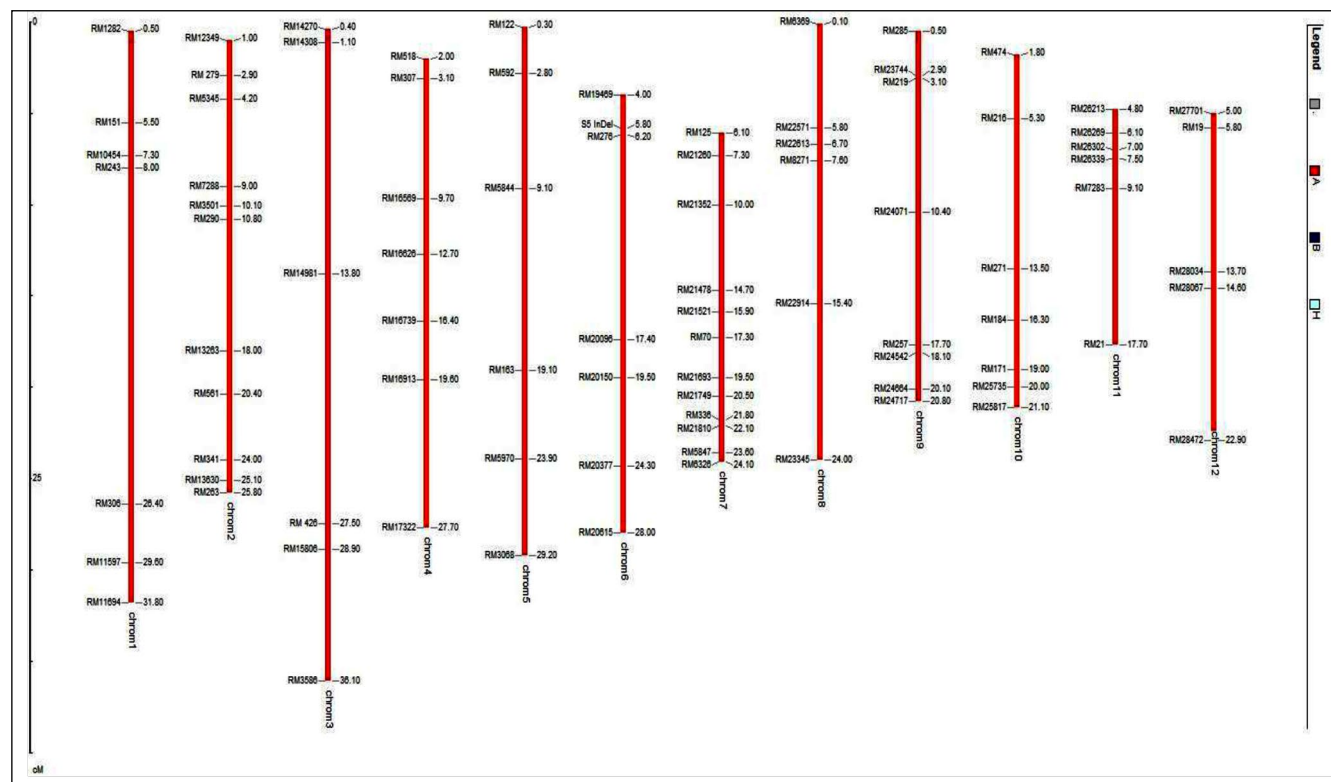


Figure 3: The distribution of the polymorphic SSR markers detected between CR 22-153-1 and CR 22-1-5-1 on the twelve chromosomes

individual chromosomes were as follows: chromosome 1 (25.9%), chromosome 2 (40.7%), chromosome 3 (23.1%), chromosome 4 (25.9%), chromosome 6 (23.3%), chromosome 8 (24%), chromosome 9 (32%), chromosome 10 (28%), chromosome 11 (24%), and chromosome 12 (24%) (Table 2). These results aligned with earlier reports. For example, Kumar et al. (2011) documented a range of 43.4% to 48.1% polymorphism among twelve chromosomes, while Challa and Kole (2019) observed lower polymorphism levels ranging from 5% to 17% between donor and recurrent parents. The distribution of the polymorphic SSR markers identified in Cross-II across the twelve chromosomes was illustrated in Figure 3. Importantly, chromosome 6, which harbors the *S5n* gene targeted for introgression, exhibited 23.3% polymorphism. This level of marker informativeness was adequate to monitor the introgression of the target locus during backcrossing. The same flanking SSR markers-RM19469 and RM276-previously validated in Cross-I were also polymorphic in this cross. These markers could therefore be effectively employed for recombinant selection, ensuring precise transfer of the *S5n* gene with minimal retention of the linked donor genomic segments.

4. CONCLUSION

The identified polymorphic markers served as a robust tool for marker-assisted background selection, facilitating efficient and targeted introgression of the *S5n* gene into CR 22-153-1 while accelerating the recovery of the recurrent parent genome. The polymorphic markers around the *S5n* locus, ensured the feasibility of precise recombinant selection in both crosses.

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6. REFERENCES

Acharjee, S., Chakraborty, N.R., Das, S.P., 2021. Marker based genetic variability analysis of rice (*Oryza sativa* L.) landraces for drought tolerance. African Journal of Biological Sciences 17(1), 117–136. <https://doi.org/10.21608/ajbs.2021.158916>.

Ali, M.N., Alam, M.P., Ahmad, E., Sah, A., 2016. Effect of different residue management practices of rice on growth and yield of wheat and soil health in rice-wheat system. International Journal of Bio-resource and Stress Management 7(4), 567–574. <https://ojs.pphouse.org/index.php/IJBSM/article/view/934>.

Berloo, R.V., 2008. GGT 2.0: Versatile software for visualization and analysis of genetic data. Journal of Heredity 99, 232–236. DOI: 10.1093/jhered/esm109.

Berloo, R.V., 2007. GGT: user manual Version 2.0. Wageningen (The Netherlands): Wageningen University. Available from: http://www.plantbreeding.wur.nl/Software/ggt/ggt2_manual.

Challa, V., Kole, P.C., 2019. Parental evaluation and polymorphism survey of drought contrasting donor and recurrent parents in rice (*Oryza sativa* L.) using microsatellite markers. Electronic Journal of Plant Breeding 10(2), 406–412. <https://www.ejplantbreeding.org/index.php/EJPB/article/view/3148>.

Chen, J., Ding, J., Ouyang, Y., Du, H., Yang, J., Cheng, K., Zhao, J., Qiu, S., Zhang, X., Yao, J., Liu, K., Wang, L., Xu, C., Li, X., Xue, Y., Xia, M., Ji, Q., Lu, J., Xu, M., Zhang, Q., 2008. A triallelic system of *S5* is a major regulator of the reproductive barrier and compatibility of indica-japonica hybrids in rice. In: Proceedings of the National Academy of Sciences 105(32), 11436–11441. <https://doi.org/10.1073/pnas.0804761105>.

Choudhury, B., Khan, M.L., Dayanandan, S., 2013. Genetic structure and diversity of indigenous rice varieties (*Oryza sativa* L.) in eastern Himalayan region of northeast India. Springer Plus 2, 228–237. <https://springerplus.springeropen.com/articles/10.1186/2193-1801-2-228>.

Das, G., Pradhan, B., Bastia, D., Samantaray, S., Jena, D., 2022. Pyramiding submergence tolerance and three bacterial blight resistance genes in popular rice variety hasanta through marker-assisted backcross breeding. Agriculture 12, 1815. <https://doi.org/10.3390/agriculture12111815>.

Devi, K.R., Hari, Y., Chandra, B.S., Prasad, K.R., 2022. Genetic association, variability and path studies for yield components and quality traits of high yielding rice (*Oryza sativa* L.) genotypes. International Journal of Bio-resource and Stress Management 13(1), 81–92. <https://www.ojs.pphouse.org/index.php/IJBSM/article/view/4182>.

Dey, S., Kumar, R., Battan, K.R., Chhabra, A.K., Reddy, A.L., 2021. Study of coefficient of variation, heritability and genetic advance for different traits of rice genotypes grown under aerobic condition. International Journal of Bio-resource and Stress Management 12(5), 426–430. <https://ojs.pphouse.org/index.php/IJBSM/article/view/4125>.

Dwivedi, D.K., Pandey, M.P., 2012. Gene action and heterosis for yield and associated traits in indica and tropical japonica crosses of rice (*Oryza sativa* L.) involving wide compatibility gene(s). International Journal of Plant Breeding and Genetics 6(3), 140–150. DOI: 10.3923/ijpb.2012.140.150.

Ikehashi, H., Araki, H., 1984. Varietal screening of compatibility types revealed in *f1* fertility of distant crosses in rice. Japanese Journal of Breeding 34(3),

- 304–313. <https://doi.org/10.1270/jsbbs1951.34.304>.
- Ishwarya Lakshmi, V.G., Sreedhar, M., Lakshmi, V.J., Gireesh, C., Vanisri, S.R.S., 2021. Parental polymorphism survey using genome-wide SSR markers for brown planthopper resistance in rice. *The Pharma Innovation* 10(6), 622–629. <https://www.thepharmajournal.com/archives/?year=2021&vol=10&issue=6&ArticleId=6592>.
- Ji, Q., Lu, J.F., Chao, Q., Zhang, Y., Zhang, M.J., Gu, M.H., Xu, M.L., 2010. Two sequence alterations, a 136bp InDel and an A/C polymorphic site, in the S5 locus are associated with spikelet fertility of indica-japonica hybrid in rice. *Journal of Genetics and Genomics* 37, 57–68. DOI: 10.1016/S1673-8527(09)60025-4.
- Kallugudi, J., Singh, V.J., Vinod, K.K., Krishnan, S.G., Nandakumar, S., Dixit, B.K., Ellur, R.K., Bollinedi, H., Nagarajan, M., Kumar, A., Chakraborti, M., Seth, R.K., Mondal, T.K., Bhowmick, P.K., Singh, A.K., 2022. Population dynamics of wide compatibility system and evaluation of intersubspecific hybrids by indica-japonica hybridization in rice. *Plants* 11(15), 1930. <https://doi.org/10.3390/plants11151930>.
- Kato, S., Kosaka, H., Hara, T., 1928. On the affinity of rice varieties as shown by fertility of hybrid plants. *Science Bulletin of the Faculty of Agriculture, Kyushu University* 3, 132–147.
- Khush, G.S., 2005. What it will take to feed 5.0 billion rice consumers in 2030. *Plant Molecular Biology* 59, 1–6. <https://link.springer.com/article/10.1007/s11103-005-2159-5>.
- Kumar, V., Rani, A., Mourya, V., Rawal, R., Verma, K., Shivakumar, M., Talukdar, A., 2011. Marker-assisted selection for development of kunitz trypsin inhibitorfree soybean varieties: I. Parental polymorphism survey using SSR markers. *Indian Journal of Genetics and Plant Breeding* 71(04), 372–376. <https://www.isgpb.org/journal/index.php/IJGPB/article/view/850>.
- Majhi, P.K., Singh, S.K., Anandan, A., Khaire, A.R., Korada, M., Habde, S.V., Singh, A., 2022. Parental polymorphism survey for evaluation and selection of contrasting parents for drought tolerance in rice (*Oryza sativa* L.) by using SSR markers. *International Journal of Environment and Climate Change* 12(11), 2507–2519. DOI: 10.9734/ijecc/2022/v12i1131244.
- McCouch, S.R., Teytelman, L., Xu, Y., Lobos, K.B., Clare, K., Walton, M., Fu, B., 2002. Development and mapping of 2240 new SSR markers for rice (*Oryza sativa* L.). *DNA Research* 9, 199–207. DOI: 10.1093/dnares/9.6.199.
- Nanda, K., Chakraborty, N.R., Jena, D., Rout, D., Verma, R., 2025. Gene action of yield and its contributing traits in wide-compatible elite rice (*Oryza sativa* L.) restorer lines. *Journal of Experimental Biology and Agricultural Sciences* 12(6), 850–859. [https://doi.org/10.18006/2024.12\(6\).850.859](https://doi.org/10.18006/2024.12(6).850.859).
- Pratap, A., Bisen, P., Loitongbam, P., Sandhya, Singh, P.K., 2018. Assessment of genetic variability for yield and yield components in rice (*Oryza sativa* L.) germplasms. *International Journal of Bio-resource and Stress Management* 9(1), 087–092. <https://www.ojs.pphouse.org/index.php/IJBBSM/article/view/3654>.
- Revathi, P., Chandra, D., Deen, R., Singh, A.K., Lal, M., Tilathoo, R., 2015. Marker assisted selection for S5 neutral allele in inter-subspecific hybridization of rice. *Molecular Plant Breeding* 6(1), 1–7. doi: 10.5376/mpb.2015.06.0001.
- Sahoo, B., Nair, S.K., Jena, D., Rout, D., Singh, V., Arsode, P., Verma, R., 2022. Genetic diversity analysis among parents and derivative NILs of rice hybrid Rajalaxmi improved for BPH tolerance. *The Pharma Innovation Journal* 11(3), 1519–1522. <https://www.thepharmajournal.com/archives/?year=2022&vol=11&issue=3&ArticleId=11511>.
- Singh, S.K., Majhi, P.K., Anandan, A., Korada, M., Habde, S.V., Khaire, A.R., Bhagvan, A.P., 2022. Microsatellites based parental polymorphism survey for moisture stress in rice (*Oryza sativa* L.) between the parental genotypes HUR-1309 and CR Dhan 801. *Emergent Life Sciences Research* 8, 248–258. https://www.emergentresearch.org/print_article.php?did=14265.
- Sundaram, R.M., Sakthivel, K., Hariprasad, A.S., Ramesha, M.S., Viraktamath, B.C., Neeraja, C.N., Balachandran, S.M., Shobha Rani, N., Revathi, P., Sandhya, P., Hari, Y., 2010. Development and validation of a PCR-based functional marker system for the major wide-compatible gene locus S5 in rice. *Molecular Breeding* 26(4), 719–727. <https://doi.org/10.1007/s11032-010-9482-5>.
- Tripathi, J., Thagele, S.N., Sharma, A.K., Tripathi, N., Tripathi, P., Singh, A.K., 2018. Parental polymorphism survey for drought tolerance in rice by using SSR marker. *Journal of Biotechnology and Biochemistry* 4(5), 82–86. <https://www.iosrjournals.org/iosr-jbb/papers/Volume%204,%20Issue%205/Version-1/K0405018286.pdf>.
- Yang, Y.X., Li, Y.H., Tong, J.F., Qasim, S.M., Chen, Z.X., Lan, W., 2012. Wide-compatibility gene S5n exploited by functional molecular markers and its effect on fertility of intersubspecific rice hybrids. *Crop Science* 52(2), 669–675. DOI:10.2135/cropsci2011.04.0232.
- Zeng, Y.X., Hu, C.Y., Lu, Y.G., Li, J.Q., Liu, X.D., 2007. Diversity of abnormal embryosacs in indica/japonica hybrids in rice demonstrated by confocal microscopy of ovaries. *Plant Breeding* 126(6), 574–580. <https://doi.org/10.1111/j.1439-0523.2007.01380.x>.