International Journal of Bio-resource and Stress Management https://ojs.pphouse.org/index.php/IJBSM

Crossref

IJBSM December 2025, 16(12): 01-16 **Article** AR6617

Research Article *Natural Resource Management*

DOI: HTTPS://DOI.ORG/10.23910/1.2025.6617

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

***Citation* (VANCOUVER):** Nanda et al., Microsatellites based Parental Polymorphism Survey in Elite Restorer Lines of Rice for Stacking Wide-compatibility Trait. *International Journal of Bio-resource and Stress Management*, 2025; 16(12), 01-16. HTTPS://DOI.ORG/10.23910/1.2025.6617.

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.

Conflict of interests: The authors have declared that no conflict of interest exists.

RECEIVED on 04th August 2025 RECEIVED in revised form on 18th November 2025 ACCEPTED in final form on 02nd December 2025 PUBLISHED on 19th December 2025

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 Tm 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)

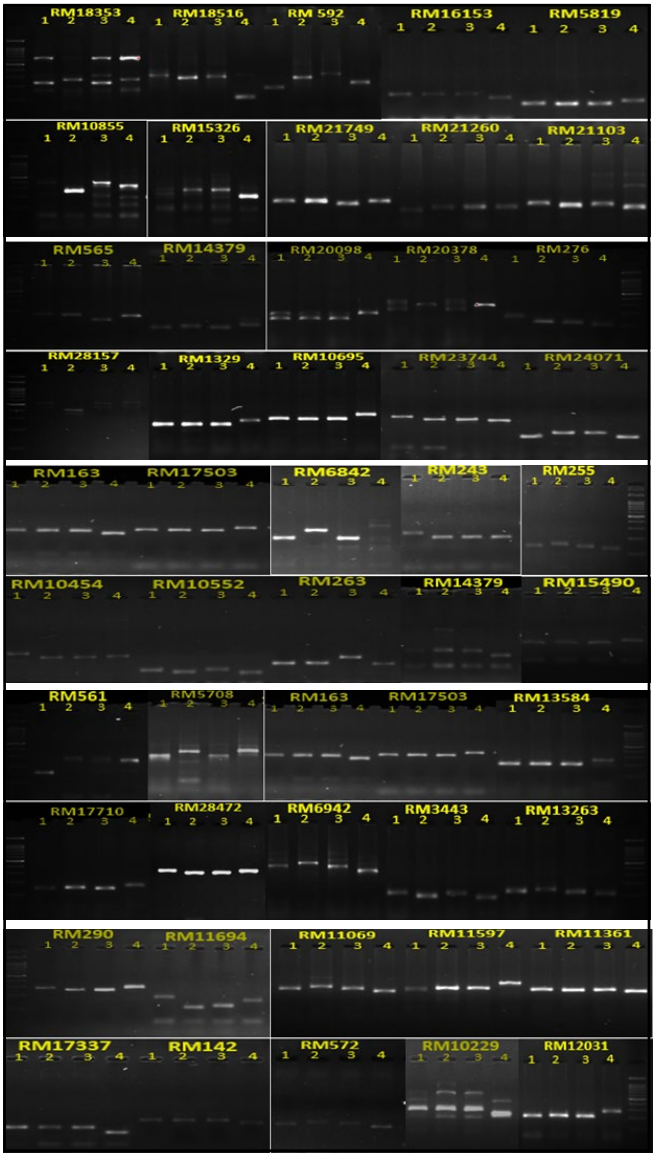

Figure 1: Gel images of the polymorphic markers identified between the parents involved in the crosses I and II; (*Parents: 1- CR 22-153-1, 2- SLPP 1033, 3- CR 22-1-5-1, 4- Lemont)

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	GACTTGCCAACTCCTTCAATTCG	TCGTCGAGTAGCTTCCCTCTCTACC	12,569,890-12,570,013 bp
2.	RM1282	1	TCGTGCAGGAGGTCTTCATGG	TTGAGGATGGTAACGAACCTTGC	548, 511-548, 910 bp
3.	RM10596	1	CAACAACCTGGCAGAGAATTTCG	CTGCACGTGATGTCAGAGTTCG	9, 489, 807-9, 490,070 bp
4.	RM11358	1	TTGGTGTTTCTGACCGGATAAGG	CTAGTGAGCGCCGGTATTAAAGG	24,889,769-24,890,412 bp
5	RM10855	1	CCAAACAAGATGTAGTGGGAACATCC	CAAGATGATCAGTGGGCTATTCTTGG	14,067,612-14,067,967 bp
6.	RM493	1	GTACGTAAACGCGGAAGGTGACG	CGACGTACGAGATGCCGATCC	12,280,117-12,280,294 bp
7.	RM1329	1	CGGAGCTCAATCGAATCTAGACC	TTCCTCATATCCTCCAGCTCTTAGC	3, 946, 854-3, 947,045 bp
8.	RM10695	1	CCTTCGACTCCATGAAACAAACG	TCTCTTTGCCCTAACCCTATGTCC	10,981,692-10,981,986 bp
9.	RM11069	1	GGTACAATGAAGCTTGGCAACG	CGGTGGAGTAGAACCACGAAGC	19,370,286-19,370,565 bp
10.	RM11694	1	GCGTCTATGCGTATCTTCATCTTACC	CAACTCTGCTAGTGTGCCTCTGC	31,896,904-31,897,097 bp
11.	RM11361	1	TGCTAGCTGCCTACCCTCGAAGC	AGGCAGGCATCGGCTATATTCTCG	24,949,694-24,949,944 bp
12.	RM12031	1	TCCCTAGCTAGCTCTCCATCTCC	AGTACTACCGCTACATGTCTTCTTGG	39,187,120-39,187,415 bp
13.	RM572	1	CGCGGTTAATGTCATCTGATTGG	CCATACTTCGAGATCCAAGACTGACC	9, 865, 568-9, 865,718 bp
14.	RM279	2	CCTCTCACTCACGTGGACTCTCC	CCTCACCCTAGGCTTTGATATGC	2, 882, 082-2, 882,252 bp
15.	RM207	2	ATCCTAGTGGATAAGGCACAGACTGG	CCCTTGCTCTTCCACCTCATCC	35,369,336-35,369,671 bp
16.	RM530	2	TTCTTTATTCCCTCGCACTGACC	CAATGATGCCACAAACCGTAACC	30,532,198-30,532,386 bp
17.	RM7288	2	CGCTATGTGGCAGTTTAAGAGC	CTGTTGGAATCAAAGTGAGTGC	9, 033, 547-9, 033,882 bp
18.	RM13584	2	TCCATCTTCACAATCAGCAACC	CCCTGTAACAATGTTGAGAACACC	23,882,924-23,883,210 bp
19.	RM5706	2	GTGGCTTGATTTCTGCAACTTCC	GCTGGTACCTACCAAGTATCATTTCG	26,479,540-26,479,734 bp
20.	RM13630	2	GCCGCTGATCTCTTACCTGTTACGG	GTGCTTCGGCACAGGTCAATGC	25,119,299-25,119,394 bp

Table 3: Continue...

Sl. No.	Primer name	Chromosome number	Forward sequence	Reverse sequence	Position
21.	RM6942	2	CGAACTCCCAATTACAGATCACG	TCATCCAAGACTTAGGTGGAGAAGC	17,436,262-17,436,547 bp
22.	RM341	2	CAAGAAACCTCAATCCGAGC	CTCCTCCCGATCCCAATC	94.4-94.4 cM
23.	RM13679	2	AGATGACAAGGTGAGAGCACTGG	TGGAGCCCAGAATTTCTAGATCG	26,102,434-26,102,711 bp
24.	RM 426	3	CATCGCCGAAATCCATCTTCC	AAGGCCCATTTCATTGTAGAGTGC	27,588,613-27,588,762 bp
25.	RM16153	3	TGGTTGTGGTATAGCACGGTAAGC	TGACCCAAGGAGATACTAGGTTGC	35,013,535-35,013,799 bp
26.	RM5819	3	TACGCCTAACGGCCTAAGTACC	AATCACTCCAGAACCCAGTAGTAGC	4, 285, 381-4, 285, 551 bp
27.	RM15630	3	AACTCGAAGGATCTCGCCCAACC	ACCCACCTCCTCACGCTGTACG	26,073,971-26,074,256 bp
28.	RM14239	3	CAAGTTCACCCGCCTTCTCG	TTTCCATCATTAGCAGGCAGTAGC	85, 314-85, 337 bp
29.	RM14379	3	ATGCAACAGGGAGGTGCTTGG	TGAATCTGAAGATGCCCAAGAGG	2, 435, 994-2,436,033 bp
30.	RM15404	3	TTTGGCAAGGCTATCTTCTCTGG	GGAGAGCCGAAACTATTTGATTGG	22,489,928-22,490,088 bp
31.	RM255	4	GAGGAGGAGGAGGAGAGATCAGG	AACGAAACCGCTCAGTTCAACC	30,772,388-30,772,538 bp
32.	RM307	4	GTACTACCGACCTACCGTTCAC	CTGCTATGCATGAACTGCTC	10.5-10.5 cM
33.	RM17600	4	CCTCGAAATGAATTGCAGTCGAACG	GTCTTGTGCCTTGTGCCGATGG	33,596,398-33,596,876 bp
34.	RM16569	4	ATAGACTTCACCCGGTACAACG	CTGGTATCGACATCGGCTAGG	9, 666, 957-9,667,308 bp
35.	RM17337	4	CCCTCCCGTAGACCTTGTACCC	CCACAGCTAACCAATCCTTCTCC	28,248,941-28,248,982 bp
36.	RM142	4	TCTTCCTCTCCACTTCCATTTCC	AAGAAGCTCGGGATCTTCACC	20,518,899-20,519,086 bp
37.	RM16256	4	GAAGGCGTGCTCCGAGAACTGG	GTCGATCGCCTTGTCGTCTGC	113, 340-113, 363 bp
38.	RM17503	4	CCAGATCATCCAGGCATAACATCACC	CGGCGCTGGTAAACTCCATTCC	31,717,625-31,717,670 bp
39.	RM440	5	GGTAGGCACCAAAGAGTTTGACG	GGCATCACCTTATCCAATCACC	19,912,517-19,912,685 bp
40.	RM122	5	CTTCTTCCGCTTCCTCCCTTCC	TGTACCAGTGCACCGAGAGTTGG	311, 055-311, 218 bp
41.	RM18353	5	AGATCTCACTATTGAGTAGCCCATGC	CACCTTGCCCTTAAATACCAACC	13,949,549-13,949,835 bp

Table 3: Continue...

Sl. No.	Primer name	Chromosome number	Forward sequence	Reverse sequence	Position
42.	RM592	5	ACCTCACCCGAATTACTGTGATATGC	GTTGAATTGCACGCGACTCTGG	2, 796, 876-2, 797, 130 bp
43.	RM17710	5	GACGAGGCAGGCGATGATAAGG	CAGCACCTCAAGCGTCCTAACC	46, 634-46, 802 bp
44.	RM163	5	ATCCATGTGCGCCTTTATGAGGA	CGCTACCTCCTTCACTTACTAGT	19,189,417-19, 189, 547 bp
45.	RM18913	5	CAAGATCTTTGCAACTGAGGAAGG	TTGCTACTTCGTGTTGTCACTCTACG	24,100,757-24, 101, 244 bp
46.	RM510	6	GTTTGACGCGATAAACCGACAGC	ATGAGGACGACGAGCAGATTCC	2, 831, 443-2, 831, 635 bp
47.	RM469	6	TTACGTGATCACACAGGCTCTCC	AAGCTGAACAAGCCCTGAAAGG	564, 135-564, 314 bp
48.	S5-InDel	6	CCTACGTTTGACTGCCTGCCTG	CTACACGCGGCTTCGGGAAAGC	5.8-5.8 cM
49.	RM20377	6	GTGTGTGATGTGCATGTTTCTGC	CATGTGATGCCCTGTAGGAACC	24,320,992-24, 321, 271 bp
50.	RM20096	6	CGGTAAGCCATAAATAGATCCCAAGG	TTTGAACAGCGACACGGTTTCC	17,431,904-17, 432, 300 bp
51.	RM20378	6	ATGTCCTTCTTCGAGATTGCTTCG	AGAGCCCAACATATAGCGGATGC	24,323,763-24, 324, 051 bp
52.	RM19469	6	TTCCTTTCCTTGGGTCATATCC	ATCGAGTCAGAGAGTGGCTTGG	4, 049, 892-4, 050, 188 bp
53.	RM276	6	GTCCTCCATCGAGCAGTATCAGC	CTAGCAAGACATGGACCTCAACG	6, 230, 045-6, 230, 185 bp
54.	RM20150	6	TGTTTGTGTGGTTAGGGTTTGG	TTGTGGTTCTGGGTGTAGTTATCC	19,513,580-19, 513, 857 bp
55.	RM162	6	TTGTTCCAGTTCAGGTCTTGTGC	CCCTACAAACACCATAAGAAGCAACC	24,035,491-24, 035, 615 bp
56.	RM336	7	GTATCTTACAGAGAAACGGCATCG	GGTTTGTTTCAGGTTCGTCTATCC	21,871,205-21, 871, 356 bp
57.	RM21260	7	CTGCACAACCAGGAGAAATTAAGC	CTGACCACTCTAGCTTGCCTACC	7, 270, 888-7, 270, 987 bp
58.	RM21478	7	TAACACAGTTCTTCTCGCAACG	AAGTTCCCTTGTGTGATTGACC	14,734,925-14, 735, 053 bp
59.	RM21521	7	TACTCGAGCAGGCAGAGCAACC	AGCATAGTCATCCAACCGAGACAGC	15,985,258-15, 985, 525 bp
60.	RM320	7	TACAGCAGGGTGGAATATCGACAAGC	TCGTTGTAACGCGTGGATATTTGG	18,693,223-18, 693, 635 bp
61.	RM20775	7	GGTGTTCATCTTTGTTGAGTCAGG	AGGATACAGCGCACCAGATTAGC	51, 912-52, 093 bp
62.	RM8271	8	AGCAGCTCCGATTGTGTTAGCC	AATGGCGTCTGTGGTACTTTGC	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-TACCCTACC	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-TATAGAAGAGCATTGG	1, 818, 800-1, 819, 051 bp
82.	RM6364	10	ACCTTCTCCGCTCATC-CCTACC	ATTTCGTCCTTCTCG-GATTTGC	66, 371-66, 628 bp

Table 3: Continue...

Sl. No.	Primer name	Chromosome number	Forward sequence	Reverse sequence	Position
83.	RM5708	10	TGGTATCCATAACCTTGACAGC	GTCACGTATCGAACAGTCTCC	14,456,288-14,456,536 bp
84.	RM2620	11	CTTAATCCCAGCCCTGGAAGC	GATTGATGTCAGCCGTAACATGG	4, 459, 979-4,460,147 bp
85.	RM224	11	ATCGATCGATCTTCACGAGG	TGCTATAAAAGGCATTCGGG	120.1-120.1 cM
86.	RM21	11	ACAGTATTCCGTAGGCACGG	GCTCCATGAGGGTGGTAGAG	85.7-85.7 cM
87.	RM7283	11	GGCATATAACGCAAACACCAACAAGG	CAGAAGAGCGATGGCAGTTTGG	9, 117, 512-9,117,694 bp
88.	RM26656	11	GCAAAGAACATTGTGGCAAACACC	TGGACACATTGTATCGTGCTTCG	15,274,670-15,275,028 bp
89.	RM26213	11	GCCACAGGAGACAGCAAGAACC	CGATCCAATTCCAGCCTAGATAGC	4, 750, 713-4,751,058 bp
90.	RM201	11	CTCGTTTATTACCTACAGTACC	CTACCTCCTTTCTAGACCGATA	111.1-111.1 cM
91.	RM28346	12	GCCCAAAGTTAATATCGGTGTCTCC	AGCCTGCCTAGCACTCATAGACC	20,919,548-20,919,838 bp
92.	RM27638	12	AGATTCCCATCCGTTAGGAAAGC	GATGCACATGCACTTGTAGTTCC	4, 037, 622-4,037,916 bp
93.	RM27701	12	CTATATGGCACCACTGACATTTGC	TCAATAAAGCCCACCGTTCC	5, 000, 889-5,001,278 bp
94.	RM28464	12	CTATATCCGCACCAATGGAAACC	TAGGAATTGCGACGAGTTTGTGG	22,908,956-22,909,606 bp
95.	RM19	12	CAAAAACAGAGCAGATGAC	CTCAAGATGGACGCCAAGA	20.9-20.9 cM
96.	RM28034	12	CTCGGAAGCAGACAGCGAAGG	GAGAGACTGGATCGGGTTCAAGC	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	TCGTGCAGGAGGTCTTCATGG	TTGAGGATGGTAACGAACCTTGC	548, 511-548, 910 bp
2.	RM11597	1	GTATTTATTGTGGGCGGCAAACC	CGTTTGCTAGTTCAGTGTTGTGTCG	29, 634, 209-29, 634, 612 bp
3.	RM11694	1	GCGTCTATGCGTATCTTCATCTTACC	CAACTCTGCTAGTGTGCCTCTGC	31, 896, 904-31, 897, 097 bp
4.	RM151	1	TGCTGATCAGTTACACGAATCAGAGC	GCGTACGTGCACAAATTAAACAGACC	5, 558, 576-5, 558, 814 bp
5	RM10454	1	AGTGATGAGAAGCGAGAACTGAAGG	GAGGGAGTACATGTTGATGTCATGG	7, 283, 578-7, 283, 788 bp
6.	RM243	1	CAGACTGCAGTTGCACGATACTACG	GAAAGCTGCAACGATGTTGTCC	7, 970, 722-7, 970, 836 bp
7.	RM306	1	CAAGGTCAAGAATGCAATGG	GCCACTTTAATCATTGCATC	98.1-98.1 cM
8.	RM 279	2	CCTCTCACTCACGTGGACTCTCC	CCTCACCCTAGGCTTTGATATGC	2, 882, 082-2, 882, 252 bp
9.	RM7288	2	CGCTATGTGGCAGTTTAAGAGC	CTGTTGGAATCAAAGTGAGTGC	9, 033, 547-9, 033, 882 bp
10.	RM13630	2	GCCGCTGATCTCTTACCTGTTACGG	GTGCTTCGGCACAGGTCAATGC	25, 119, 299-25, 119, 394 bp
11.	RM13263	2	AAGATTGCACACTGGTGTTCTCC	AGAAGAGCCGGTCTTTGTCTCC	18, 081, 502-18, 081, 656 bp
12.	RM561	2	GAGCTGTTTTGGACTACGGC	GAGTAGCTTTCTCCCACCCC	74.1-74.1 cM
13.	RM290	2	ATGACCCAATTTCTGACTCTAGCC	CATGGGTGGTGCTGTAGATGG	10, 806, 902-10, 807, 358 bp
14.	RM263	2	AATCTATGGACCTGGGAGGAACC	TGACGAGAGTGCTACGTTTGAGC	25, 865, 334-25, 865, 568 bp
15.	RM3501	2	CTACAATGATTCCATGCCTGTCC	TCCGGCTCAAGCTACAGTTAAGG	10, 187, 508-10, 187, 682 bp
16.	RM5345	2	CTCTGTTGCAATGCCCAATACC	AAGGGAGTCCATGGAGATGAGG	4, 206, 222-4, 206, 587 bp
17.	RM12349	2	CCGATTAGCGATTGATATGGAGTAGG	AGTGCACAGCCATGGAATTATGC	977, 799-977, 912 bp
18.	RM341	2	CAAGAAACCTCAATCCGAGC	CTCCTCCCGATCCCAATC	94.4-94.4 cM
19.	RM426	3	CATCGCCGAAATCCATCTTCC	AAGGCCCATTTCATTGTAGAGTGC	27, 588, 613-27, 588, 762 bp
20.	RM14270	3	ATTATCGCTCGGAGATGCTTACC	GCCAATATTGTCCACACAATCACC	434, 406-434, 793 bp
21.	RM14308	3	GCATTCTAGTGTTATGGCTGTTGC	GCTACTAAGCTGGTCACCTGTGG	1, 068, 020- 1, 068, 051 bp
22.	RM3586	3	TCTTGATTGCTGGACCACATGC	TCGAGCTAGAAGACGACACACAGC	36, 141, 002-36, 141, 177 bp

Table 4: Continue...

Sl. No.	Primer name	Chromosome no.	Forward sequence	Reverse sequence	Position
23.	RM15806	3	ACCATGAGGTTCAGTTAGGAGTGC	AATTGACGGTGGAGTGAAGTACC	28, 975, 284-28, 975, 677 bp
24.	RM14981	3	GGCGAGCAGAAGTATAATCCAGAAGG	CGCTTGTGGCTTACTGGCTTGG	13, 886, 241-13, 886, 523 bp
25.	RM518	4	AAGACACAAGCAAACAGCTCAACC	AAGCTTGCTTGGTTCAAGAGAGG	2,030,135-2,030, 305 bp
26.	RM307	4	GTACTACCGACCTACCGTTCAC	CTGCTATGCATGAACTGCTC	10.5-10.5 cM
27.	RM16569	4	ATAGACTTCACCCGGTACAACG	CTGGTATCGACATCGGCTAGG	9,666,957-9,667, 308 bp
28.	RM16913	4	GTGTACGTGTTGGCTCTCTGTACG	GATGTTGCTTGTGCTGCAACC	19, 646, 468-19, 646, 859 bp
29.	RM17322	4	TCTGCTAGCCTGCACACAGAAGG	GCACAGGAGACCAAAGAATCAGG	27, 782, 214-27, 782, 253 bp
30.	RM16739	4	TGCATAGTCCACCTAAGAGTTCAAGG	TTTCTGCAGGCTGATTAGTGTAGG	16, 460, 287-16, 460, 641 bp
31.	RM16626	4	ACATGATTGCTGGCTTGCTTACC	GCCACGCAGTGTTGTTTCAGC	12, 717, 126-12, 717, 307 bp
32.	RM5970	5	CCATCTGGTTCACCTTCACG	ACGTCTTGAAGAAGCTGAATGC	23, 946, 271-23, 946, 390 bp
33.	RM122	5	CTTCTTCCGCTTCCTCCCTTCC	TGTACCAGTGCACCGAGAGTTGG	311,055-311,218 bp
34.	RM592	5	ACCTCACCCGAATTACTGTGATATGC	GTTGAATTGCACGCGACTCTGG	2,796,876-2,797, 130 bp
35.	RM5844	5	AACGTGGCATCCATGTTAGTACC	AGCTAGGAGCCATTGTCGAAGG	9,149,429-9,149, 623 bp
36.	RM3068	5	CAACCCGAACGATATCAAGTTACC	CAGGCAAGATGGTGATGAACC	29, 251, 643-29, 251, 896 bp
37.	RM163	5	ATCCATGTGCGCCTTTATGAGGA	CGCTACCTCCTTCACTTACTAGT	19, 189, 417-19, 189, 547 bp
38.	RM20377	6	GTGTGTGATGTGCATGTTTCTGC	CATGTGATGCCCTGTAGGAACC	24, 320, 992-24, 321, 271 bp
39.	S5-InDel	6	CCTACGTTTGACTGCCTGCCTG	CTACACGCGGCTTCGGGAAAGC	5.8-5.8 cM
40.	RM276	6	GTCCTCCATCGAGCAGTATCAGC	CTAGCAAGACATGGACCTCAACG	6,230,045-6,230, 185 bp
41.	RM20150	6	TGTTTGTGTGGTTAGGGTTTGG	TTGTGGTTCTGGGTGTAGTTATCC	19, 513, 580-19, 513, 857 bp
42.	RM20615	6	TTACACCAGGCTACCCAAACTCG	TTGCTGAGTTCCCTCGTCTATCC	28, 049, 016-28, 049, 204 bp
43.	RM20096	6	CGGTAAGCCATAAATAGATCCCAAGG	TTTGAACAGCGACACGGTTTCC	17, 431, 904-17, 432, 300 bp
44.	RM19469	6	TTCCTTTCCTTGGGTCATATCC	ATCGAGTCAGAGAGTGGCTTGG	4,049,892-4,050, 188 bp

Table 4: Continue...

Sl. No.	Primer name	Chromosome no.	Forward sequence	Reverse sequence	Position
45.	RM21693	7	GCACAGACCAGAACTTTCTTCG	TGGCGAGTGTAGATGTAATTGG	19, 510, 426-19, 510, 888 bp
46.	RM336	7	GTATCTTACAGAGAAACGGCATCG	GGTTTGTTTCAGGTTCGTCTATCC	21, 871, 205-21, 871, 356 bp
47.	RM6326	7	CGATCTCTCTATCCTTCCTCTTCTCC	CAGATTGTTAGCAACCCGAATCC	24, 195, 104-24, 195, 325 bp
48.	RM5847	7	CTTTAGGTAGCGTCATCTTCC	TGGAAATACAGAAGGAGTCG	23, 648, 589-23, 648, 782 bp
49.	RM21749	7	TAGCTTCCTACTTCCGCCTTCTATCG	ACAGTGGGAGGGAGTACATACACTGG	20, 547, 379-20, 547, 591 bp
50.	RM21260	7	CTGCACAACCAGGAGAAATTAAGC	CTGACCACTCTAGCTTGCCTACC	7,270,888-7,270, 987 bp
51.	RM21521	7	TACTCGAGCAGGCAGAGCAACC	AGCATAGTCATCCAACCGAGACAGC	15, 985, 258-15, 985, 525 bp
52.	RM21478	7	TAACACAGTTCTTCTCGCAACG	AAGTTCCCTTGTGTGATTGACC	14, 734, 925-14, 735, 053 bp
53.	RM21810	7	CTAGTTGCCAAGAGGAGGCATCG	CTGTAGCTGAGGCACCAGAATGG	22, 188, 009-22, 188, 196 bp
54.	RM125	7	ATCAGCAGCCATGGCAGCGACC	AGGGGATCATGTGCCGAAGGCC	24.8-24.8 cM
55.	RM70	7	GTGGACTTCATTTCAACTCG	GATGTATAAGATAGTCCC	64.6-64.6 cM
56.	RM21352	7	GGACGAAAGGGTATTTGATTGG	CCTCAAGGTGGTCTCCTTCTCC	10, 020, 417- 10, 020, 466 bp
57.	RM8271	8	AGCAGCTCCGATTGTGTTAGCC	AATGGCGTCTGTGGTACTTTGC	7,616,956-7,617, 315 bp
58.	RM6369	8	AGCTAGCTT-CACCTACCTACCTCACC	ATGGTCATGTCGTTGGTTTGC	124, 644-124, 908 bp
59.	RM23345	8	GAGATCCTGCACATCTTTGAGACC	TGTGCCACGAAACAAATCTAGGC	24, 080, 259-24, 080, 487 bp
60.	RM22613	8	TTTCTGGCCCAGTTCAGTACAGC	TGGTGCGTACATATCCCTTTAACC	6,731,353-6,731, 546 bp
61.	RM22571	8	TCCTCCTCCACCTCAATCACACC	CCGAGCTCGCCATTAGCTTGC	5,844,242- 5,844, 283 bp
62.	RM22914	8	GGAGCAGCTAAGGCAGATAAGAGG	GCCTTCATGCTTCAGAAGACAGC	15, 433, 651- 15, 433, 708 bp
63.	RM285	9	CTGTGGGCCCAATATGTCAC	GGCGGTGACATGGAGAAAG	1.8-1.8 cM
64.	RM219	9	CGTCGGATGATGTAAAGCCT	CATATCGGCATTCGCCTG	11.7-11.7 cM
65.	RM24664	9	ATCTAAACACAGCCCTAGTTGC	GAGCCATAACTTTACCATGTGC	20, 360, 221-20, 360, 583 bp
66.	RM23744	9	CTTAATACTCCGACGTAACAGTGG	CCTGACTAAATGGAGCTTCTTCC	2,913,810-2,914, 118 bp

Table 4: Continue...

Sl. No.	Primer name	Chromo-some 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	TGACACTTGGAGAGCG-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	ACAGTATTCCGTAGGCAC-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	CTGACACATTTCGACTG-TATGG	GTGTTTAGGAGGTG-GAGTTTGG	7,554,952- 7,555, 003 bp
84.	RM27701	12	CTATATGGCACCACT-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

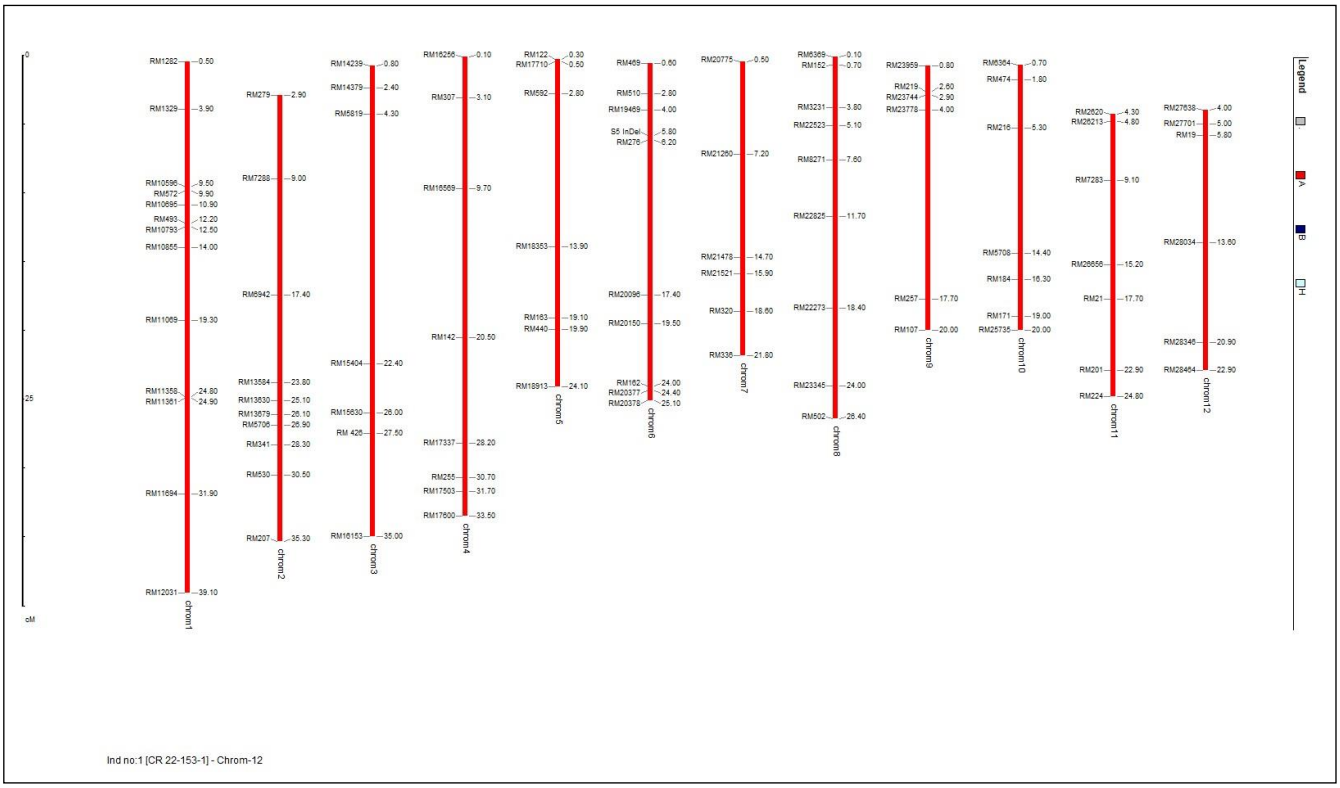

Figure 2: The distribution of the polymorphic SSR markers detected between CR 22-153-1 and Lemont on the twelve chromosomes

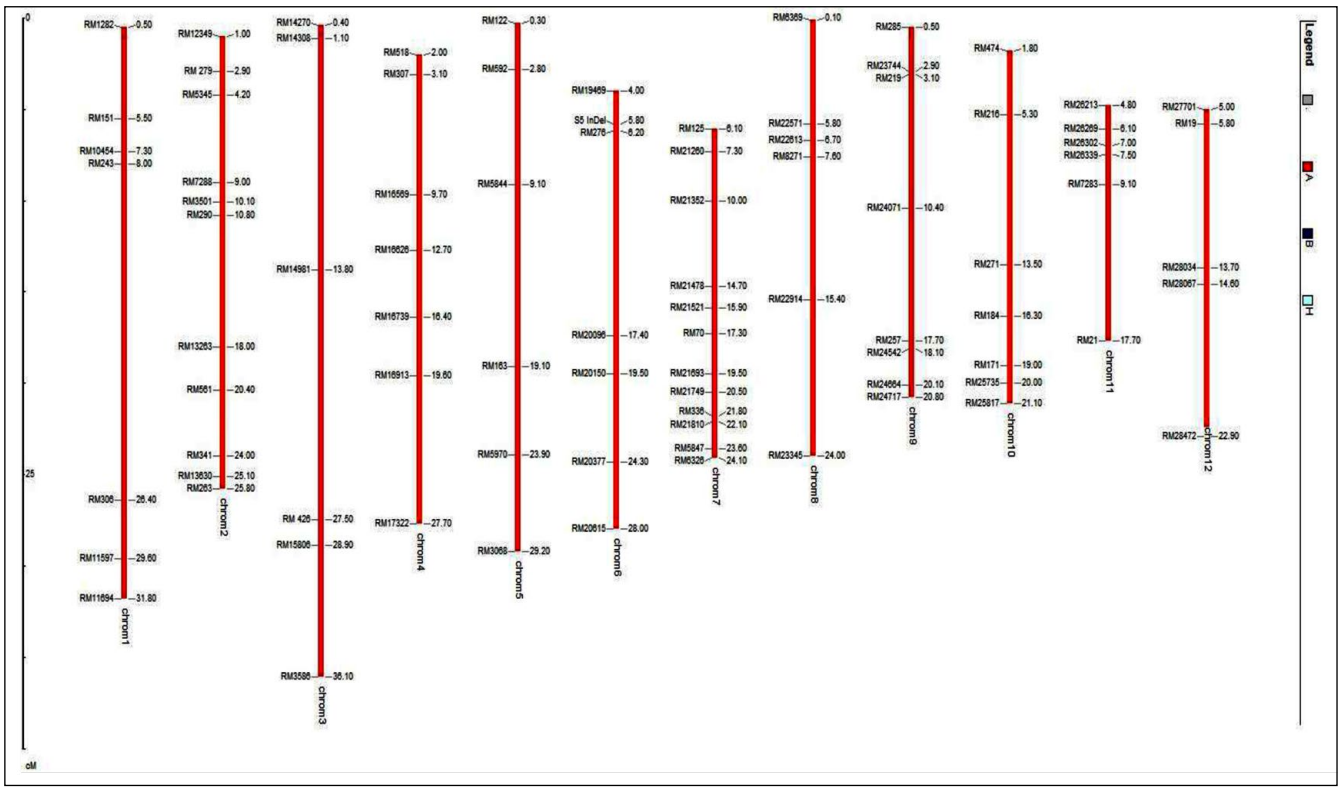

Figure 3: The distribution of the polymorphic SSR markers detected between CR 22-153-1 and CR 22-1-5-1 on the twelve chromosomes

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.

5. ACKNOWLEDGMENT

The authors are sincerely thankful to Visva-Bharati, Santiniketan; and ICAR-Central Rice Research Institute, Cuttack for providing the opportunity to carry out this research.

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