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Life Sciences Research

Research Article

Microsatellites based parental polymorphism survey for moisture stress in Rice (*Oryza sativa* L.) between the parental genotypes HUR-1309 and CR Dhan 801

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Received: 09 September 2022
Accepted: 21 December 2022
Online: 28 December 2022

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Emer Life Sci Res (2022) 8(2): 248-258

E-ISSN: 2395-6658
P-ISSN: 2395-664X

DOI: https://doi.org/10.31783/elsr.2022.82248258

Abstract

The present investigation was conducted during Rabi 2018-19. The parental polymorphism research was done at the Molecular Drought Breeding Laboratory (Department of Genetics and Plant Breeding), Institute of Agricultural Sciences, BHU, and the Crop Improvement Division, ICAR-NRRI, Cuttack. The purpose of this research was to identify the parental polymorphism markers, analyze their chromosomal distribution, and determine the repetitive motifs. The polymorphic markers will be helpful to introgress the drought tolerant yield QTLs (qDTY) from the donor CR Dhan 801 into the background of HUR-1309, a popular aromatic short duration variety through marker-assisted backcross breeding program. Polymerase chain reaction (PCR), agarose gel electrophoresis, and genomic DNA separation were carried out according to standard procedures. A total of 510 microsatellites were used for evaluation of the parental polymorphism and 90 markers showed polymorphism among the parents and the rest 420 markers are monomorphic for the studied QTLs ($qDTY_{1.1}$, $qDTY_{2.1}$, and $qDTY_{3.1}$). The parental polymorphism in the present study ranged between 11.43% to 30.61%. Considering the 12 chromosomes of rice, the average polymorphism was observed 17.65%. Out of 90 polymorphic markers, 59 were dinucleotide repeats, 24 were trinucleotide repeats and 4 were tetra nucleotide repeats. The dinucleotides were highly present on chromosomes 1 (12), 2 (9), and 3 (9) and repeated 12, 9, and 9 times respectively. The markers which are found to be polymorphic can be used in the marker-assisted backcross breeding (MABB) program for grain yield under moisture stress.

Keywords drought tolerance, marker-assisted backcross breeding, microsatellites, parental polymorphism, rice

Introduction

Rice (*Oryza sativa* L.) is the staple food for more than half of the world's population [1]. Many biotic and abiotic factors can reduce rice yields, but the drought is a prominent one. Crops are more susceptible to the negative impacts of drought stress throughout their reproductive

stages. Drought is one of the major abiotic stresses in rainfed rice habitats, causing decreased output. Many morphological and ecological factors influence drought resistance, making it a highly nuanced characteristic. Thus, rice's ability to withstand drought throughout the reproductive phase is crucial [2]. Rice cultivars that naturally have the ability to survive drought conditions might be useful for maintaining consistent rice production in ecosystems where the water supply comes mostly from rainfall. The genetic development of rice for water-limiting conditions has been sluggish due to a lack of advanced molecular techniques [3]. In recent years, research has shown that grain yield under drought is a useful selection criterion. In rice, most of the QTLs for drought tolerance in grain production has been found in low-yielding vulnerable cultivars. Marker-assisted selection is hindered by the fact that these QTLs have not been found to have the same effect in several high-yielding drought-susceptible cultivars [4]. Prior to starting marker assisted selection or marker assisted backcross breeding to introduce a desired trait into a variety from the donor genotype, parental polymorphism analysis is required. Without polymorphism in the relevant parental alleles, further selection for plants with the features of interest is impossible [5].

Plant breeders can play a crucial part in this field by screening genetic variability, locating the genomic region responsible for complicated polygenic characteristics, mapping out those features, and using markers to help with selection. Due to their wide availability, high genome coverage, low cost for analysis, co-dominant nature, strong repeatability, high polymorphism, and tremendous value in marker assisted selection, SSRs have long been the marker of choice for plant breeders [6]. High polymorphism and straightforward identification have made microsatellites the most often used form of the marker in rice breeding [7]. In the case of rice, SSRs have been exploited extensively, and these are most helpful for single-marker analysis, QTL mapping, linkage map construction, tracing marker-trait relationships, and tagging genes for future study. The survey of parental polymorphisms is a critical phase in the marker-assisted backcross breeding program. As a result, the current study was carried out to screen a large number of genome-wide dispersed SSR markers between two parental genotypes of rice, namely HUR-1309 (recurrent parent) and CR Dhan 801 (donor parent). The purpose of this research was to locate polymorphic parental marker loci for allelic variation, examine their chromosomal distribution, and look for repetitive motifs. With the aid of polymorphic markers, a marker-assisted backcross breeding procedure can be carried out to introduce the drought-tolerant yield QTLs (*qDTY*) into the genetic background of the popular high-yielding variety HUR-1309.

Methodology

Experimental site and materials

The present study was carried out at the Agricultural Research Farm, Institute of Agricultural Sciences, BHU, Varanasi, Uttar Pradesh, India, and the ICAR-National Rice Research Institute (ICAR-NRRI), Cuttack, Odisha, India. During Rabi 2018-19, field experimentation began at the ICAR-National Rice Research Institute (NRRI), Cuttack. Parental polymorphism was performed at the Molecular Drought Breeding Laboratory, BHU; and at the Crop Improvement Division, ICAR-NRRI, Cuttack. The genotypes in the study were HUR-1309 (recurrent parent) which is high yielding but susceptible to drought, whereas CR Dhan 801 (donor parent) is a drought tolerant genotype and contains three QTLs for drought tolerance ($qDTY_{1.1}$, $qDTY_{2.1}$, and $qDTY_{3.1}$). The other desirable characteristic features of both the parents are discussed in Table 1.

Leaf sample collection and isolation of genomic DNA

DNA was extracted from leaf samples obtained from seedlings aged 20-25 days, allowing for a survey of parental polymorphism. Murray and Thompson's [8], CTAB method was utilized to extract the genomic DNA. The samples of the leaf was cut into small pieces and ground in a Geno-grinder set at 1000 rpm for 10 minutes with 100lµl of CTAB extraction buffer (2% CTAB, 100 mM Tris, pH 8.0,

20 Mm), Ethylene diamine tetra acetate (EDTA) pH 8, 1.4 M NaCl). Each Eppendorf tube containing the homogenized leaf sample received another 700 µl of the extraction buffer. The samples were heated in a water bath at 65 degrees Celsius for 30 minutes. After collecting the supernatant, an equal volume of Chloroform: Isoamyl alcohol (24:1) was added, and vortex the tubes for 10 minutes. After that, tubes were kept for centrifugation at 12,000 rpm for 15 minutes. The supernatant was transferred to a fresh, sterile 1.5 ml centrifuge tube after shaking for 10-15 minutes and an equal volume (800 µl) of cool isopropanol was added; the tubes were then stored at -20°C overnight. The tubes were centrifuged at 24°C for 18 minutes at 12000 rpm the next day. Without disrupting the DNA pellet, the supernatant was carefully drained. Then, about 200 µl of 70% ethanol was added to the pellet, and it was centrifuged for 10 minutes at 24°C and 10000 rpm. Lastly, the pellet was left to dry in the air at room temperature for one night. Depending on the pellet size, 50-100 µl of 1X TE buffer was added to dissolve it. The Nanodrop method was used to determine the purity of DNA.

Table 1. The experimental materials and their characteristics

SN.	Characteristics	Donor Parent	Recurrent Parent
		CR Dhan 801 (IET 25667)	**HUR-1309 (Malaviya Sugandh Dhan 1309) (IET 23873)**
1	Yield (q/ha)	50.0	50.0-55.0
2	Duration (days)	140	115-120
3	Suitable Ecosystem	Shallow low land	Irrigated medium land
4	Released year	2018	2019
5	Releasing center	ICAR-NRRI, Cuttack	BHU, Varanasi
6	Parentage	IR81896-B-B-195 / 2* SwarnaSub1 // IR91659-54-35.	Taroari Basmati Drafy-2//Sugadh-2
7	Recommended zone	Odisha, West Bengal, Uttar Pradesh, Andhra Pradesh and Telangana	Uttar Pradesh
8	Resistance/Tolerance	Drought and Submergence	Neck Blast and glumes discoloration

Microsatellite marker based genomic DNA amplification by PCR

For this study, 510 microsatellites (SSRs) were used to find out the rice genome across all 12 chromosomes. This ensures that all chromosomes will be covered and that the distribution of markers will be consistent throughout the genome. The Gramene markers database was used to find out the marker's chromosome number, physical location, sequence of forward and reverse primer, number of repeat motifs, etc. (https://www.gramene.org/). DNA amplification has been carried out in PCR using SSR markers. In 0.2ml 96-well PCR plates, PCR reactions were done by adding 2 µl of template DNA, 0.25 µl of forward and reverse markers each, 4.0 µl of Takara PCR master mix, and 3.5 µl of sterile distilled water to make a total volume of 10 µl for each reaction. The PCR plate was shielded with a cover and kept in a thermal cycler which was set to a thermal profile as follows: (a) Initial denaturation for 5 minutes at 94°C, Denaturation for 35 seconds at 94°C, (b) Primer annealing for 45 seconds at 56°C, (c) 35 cycles of Elongation for 1 minute at 72°C and (d) Final elongation for 10 minutes at 72°C.

Agarose gel electrophoresis and gel image documentation

The DNA was tested for its purity by running it on an 8% agarose gel alongside a standard ladder and comparing the relative intensities of the bands. For this experiment, 800 mg of 0.8% Agarose was dissolved in 400 ml of 1X TAE buffer and allowed to melt until become transparent. The gel solution was cooled down to nearly 50°C, and added with 2.5 µl of ethidium bromide. Then the

solution was poured into the gel casting tray with care as it doesn't form any bubbles. After casting the gel, 2µl of DNA samples were mixed with loading dye and loaded in agarose gel at 60 Volts in TAE buffer for 1.5 hr. To measure the size of the amplified products, a DNA ladder of 50 bp was placed in one of the wells. The Gel documentation device (SYNGENE GBox, UK) was used to examine the band intensity of genomic DNA following electrophoresis. The image of the gel was saved for additional scoring of polymorphic and monomorphic markers and for permanent records, providing a visual estimate of the purity and integrity of the DNA.

Statistical analysis

The parental polymorphism between the two parents; HUR-1309 and CR Dhan 801 was recorded based on the difference between the base pair size of both parents. Polymorphic % was calculated by using the following formula:

$$\text{Polymorphism \%} = \frac{\text{Number of polymorphism markers identified per chromosome}}{\text{Total Number of markers run per chromosome}} \times 100$$

GGT 2.0 software was used to visualize and interpret the molecular scoring data [9-10] in the form of graphical representations of the marker data. By giving the physical positions (Mb) of markers in a row-by-column data matrix, it was possible to see how they were spread out along the length of chromosomes.

Results and Discussion

The prevalence rate of gene polymorphism in the population is estimated to be less than or equal to 1%, and it is characterized as the occurrence of two or more discontinuous genotypes or alleles on a certain locus [11]. This is what establishes the range of possible ancestry within a population. Before starting a marker-aided selection or marker-aided backcross breeding program, it is necessary to conduct research on parental polymorphism. When neither parent is polymorphic, a further selection of parents with the trait of interest cannot be done [12]. The present study comprised with 510 microsatellites for evaluation of the parental polymorphism among the parents HUR-1309 and CR Dhan 801. Out of 510 microsatellites, only 90 markers showed polymorphism among the parents and the rest 420 markers are monomorphic for the studied QTLs (*qDTY*$_{1.1}$, *qDTY*$_{2.1}$, and *qDTY*$_{3.1}$) (Table 2). The gel banding pattern and visualization of polymorphic and monomorphic markers were shown in Figure 1 and the distribution of polymorphic markers on 12 chromosomes were represented in Figure 2. In this analysis, parental polymorphism was found to be anywhere from 11.43 % to 30.61 %. When looking over all 12 rice chromosomes, we found an average polymorphism of 17.65 % (Table 3). The highest and lowest polymorphism was obtained on chromosome number 2 and 8 respectively. The chromosome 1 and 3 showed polymorphism of 23.08% and 22.64% respectively. Since RM520 and RM16030 were used in so many previous rice drought investigations, they were identified as reliable and robust markers. Both HUR-1309 and CR Dhan 801 are indica lines, which may account for the lack of observable variation between the parents, or there may have been too few manufacturers employed for that particular chromosome. Biradar et al., and Xu et al., [13-14] were also identified reduced molecular marker polymorphism between indica genotypes in their prior studies. out of 1013 whole-genome SSR markers tested, Habde et al. [6] found 294 to be polymorphic, leading to a level of polymorphism between the parents of 29.02%. We found that chromosome 4 had the highest polymorphism (40.96%) whereas chromosome 9 had the lowest (16%) of all chromosomes. Rathi et al., [15] employed 576 randomly selected SSR markers to conduct a parental polymorphism study, and they discovered that 16.67% of those markers (96 in total, including 4 gene-specific markers) were polymorphic between the two

Table 2. List of Polymorphic markers distributed on 12 chromosomes of Rice associated with drought tolerance between the parents HUR-1309 (recurrent) and CR Dhan 801 (donor)

SN.	Marker	Chr. N.*	Expected Size	Position	Forward Primer Sequence	Reverse Primer Sequence	No. of Repeat motif
1	RM12091	1	142	40.25	CTGCAAATGCACAGGAATCAGG	TCCTCTCGCCTTTCTTTCTCTCC	(AG)31
2	RM243	1	112	7.97	GATCTGCAGACTGCAGTTGC	AGCTGCAACGATGTTGTCC	(AG)18
3	RM246	1	99	27.32	CGAGCTCCATCAGCCATTCAGC	ACTTGAGAGCGAGATTGGGAATCG	(AG)18
4	RM11943	1	77	37.84	CTTGTTCGAGGACGAAGATAGGG	CCAGTTTACCAGGGTCGAAACC	(AG)11
5	RM472	1	265	37.88	CCATGGCCTGAGAGAGAGAG	AGCTAAATGGCCATACGGTG	(AG)21
6	RM488	1	110	24.8	CAGCTAGGGTTTTGAGGCTG	TAGCAACAACCAGCGTATGC	(AG)17
7	RM493	1	178	12.26	TAGCTCCAACAGGATCGACC	GTACGTAAACGCGGAAGGTG	(AAG)9
8	RM495	1	178	0.21	AATCCAAGGTGCAGAGATGG	CAACGATGACGAACACAACC	(AGC)7
9	RM579	1	196	8.45	TCCGAGTGGTTATGCAAATG	AATTGTGTCCAATGGGCTGT	(AG)25
10	RM572	1	168	9.86	CGGTTAATGTCATCTGATTGG	TTCGAGATCCAAGACTGACC	(AG)14
11	RM594	1	108	15.14	GCCACCAGTAAAAGCAATAC	TTGATCTGCTAGTGAGACCC	(AG)15
12	RM449	1	581	15.1	AGACTACAGGCTTGTTCAGATTGG	TTGAGCTGTGCATAGGTGAGG	(AG)12
13	RM431	1	499	38.88	GCTTGCTTGTATCTGCATTGGTAGG	GGGATGATCCACTCTCTGTTTGG	(AG)16
14	RM8100	1	384	41.01	GCGTGGAGATTCAGTTAAGTTTCACC	AATTCCGTGTCCGATTTCCTACC	(AT)21
15	RM11820	1	269	35.02	CACCGAGACAGTGAGACGTACAGC	TATCGGTAGGCCGTTGGATTCG	(GTA)8
16	RM452	2	246	9.56	CTGATCGAGAGCGTTAAGGG	GGGATCAAACCACGTTTCTG	(GTC)9
17	RM263	2	235	25.88	CCCAGGCTAGCTCATGAACC	GCTACGTTTGAGCTACCACG	(CT)34
18	RM13151	2	210	15.04	CGAATGTCGCGAAAGAGAAGAAACG	TATTTGCCCACGAACCTCCATCC	(ACCC)5
19	RM208	2	286	35.16	AGTACCACCACCATTCTCTGCAAGC	TCGATTGGCCATGAGTTCTCG	(AG)12
20	RM236	2	299	2.1	GCGCTGGTGGAAAATGAG	GGCATCCCTCTTTGATTCCTC	(AG)16
21	RM324	2	196	11.38	CTGATTCCACACACTTGTGC	GATTCCACGTCAGGATCTTC	(ATC)9
22	RM492	2	283	7.28	CCAAAAATAGCGCGAGAGAG	AAGACGTACATGGGTCAGGC	(AG)11
23	RM13213	2	191	16.84	GTTTCTCCACCACCGTCAGTCG	CCCTCACTTCACTAGTCCGTAGCC	(AT)21
24	RM521	2	457	10.8	ATGACCCAATTTCTGACTCTAGCC	CATGGGTGGTGCTGTAGATGG	(AG)14
25	RM530	2	189	30.55	TTCTTTATTCCCTCGCACTGACC	CAATGATGCCACAAACCGTAACC	(AG)23
26	RM3549	2	153	11	AAAGGCGGAGGAGAAGGTATGG	CTTGGAACGAACGACCAACTCC	(AG)12
27	RM6374	2	261	15.28	TCACCAGACTCAACAAAGGATCG	TTCACCTTTCTTCTCCCTCATTCC	(AAG)16
28	RM2792	2	485	10.75	CACACGATCAACTGAATATGCACACG	GTCGTTCGACTTGAGACCGATGG	(AT)35
29	RM6378	2	167	5.47	CTGATCATCTCATGCCTCCTACG	TCCATCTCCCAATATGACCAACC	(AAG)19
30	RM327	2	213	12.46	CTACTCCTCTGTCCCTCCTCTC	CCAGCTAGACACAATCGAGC	(CAT)11 (CTT)5
31	RM411	3	168	21.23	ACACCAACTCTTGCCTGCAT	TGAAGCAAAAACATGGCTAGG	(GTT)7
32	RM517	3	190	6.13	GGCTTACTGGCTTCGATTTG	CGTCTCCTTTGGTTAGTGCC	(CT)15
33	RM514	3	491	35.07	CTTCTCAGATTGATCTCCCATTCC	GGGAGAGAGGAAGAAGACAAGG	(AC)12
34	RM81A	3	180	1.92	GAGTGCTTGTGCAAGATCCA	CTTCTTCACTCATGCAGTTC	(AAG)9
35	RM520	3	114	30.71	AGGAGCAAGAAAAGTTCCCC	GCCAATGTGTGACGCAATAG	(AG)10
36	RM15925	3	200	30.65	CCCACTGGACAGTGAGAGTTGG	AACGCGAGGCTTTATTGACAACC	(AG)10
37	RM545	3	166	4.91	CAATGGCAGAGACCCAAAAG	CTGGCATGTAACGACAGTGG	(AG)30
38	RM16030	3	100	32.5	GCGAACTATGAGCATGCCAACC	GGATTACCTGGTGTGTGCAGTGTCC	(AG)11
39	RM7332	3	238	0.39	ACACTGTACACCACACTTCAGC	CACACCAAAGGGAAATTAGG	(ACAT)12
40	RM426	3	124	27.39	CATCGCCGAAATCCATCTTCC	AAGGCCCATTTCATTGTAGAGTGC	(AT)11
41	RM3829	3	187	30.62	ATTCTCAGCCTCTCAGGAATCTGC	AGGCGAGGGAAATGGATTTGG	(AG)30
42	RM168	3	194	27.89	TGTCGTCGAGGATTTGGAGATCG	GAATCAATCCACGGCACAGTCC	(AC)10
43	RM518	4	193	2.02	CTCTTCACTCACTCACCATGG	ATCCATCTGGAGCAAGCAAC	(TC)15
44	RM252	4	491	9.95	TTCGCTGACGTGATAGGTTG	ATGACTTGATCCCGAGAACG	(AT)29
45	RM303	4	218	28.73	GCATGGCCAAATATTAAAGG	GGTTGGAAATAGAAGTTCGGT	(AT)12
46	RM537	4	NA	0.17	CCGTCCCTCTCTCTCCTTTC	ACAGGGAAACCATCCTCCTC	(CCG)9
47	RM185	4	79	18.76	AGTTGTTGGGAGGGAGAAAGGCC	AGGAGGCGACGGCGATGTCCTC	(AGG)9
48	RM2441	4	390	28.02	GATTCACCACGTTGAGCAAAGG	ACGTTTACCAACCACGGATTACG	(AT)27
49	RM164	5	NA	19.11	TCTTGCCCGTCACTGCAGATATCC	GCAGCCCTAATGCTACAATTCTTC	(AC)16
50	RM169	5	144	7.39	TGGCTGGCTCCGTGGGTAGCTG	TCCCGTTGCCGTTCATCCCTCC	(AG)12
51	RM267	5	NA	2.82	TGCAGACATAGAGAAGGAAGTG	AGCAACAGCACAACTTGATG	(AG)12
52	RM334	5	177	14.81	GTTCAGTGTTCAGTGCCACC	GACTTTGATCTTTGGTGGACG	(CTT)20
53	RM440	5	200	19.83	CATGCAACAACGTCACCTTC	ATGGTTGGTAGGCACCAAAG	(AAG)22
54	RM136	6	299	8.76	GAGAGCTCAGCTGCTGCCTCTAGC	GAGGAGCGCCACGGTGTACGCC	(AGG)7
55	RM276	6	141	6.24	CTCAACGTTGACACCTCGTG	TCCTCCATCGAGCAGTATCA	(AG)8A3 (GA)33
56	RM20416	6	259	24.78	GAGACATCATAGCCGGATCTTCC	TCGGTAGAACATCACCTCCAAGG	(AG)33
57	RM176	6	299	8.76	CGGCTCCCGCTACGACGTCTCC	AGCGATGCGCTGGAAGAGGTGC	(CCG)7
58	RM494	6	387	30.57	GGGAGGGGATCGAGATAGAC	TTTAACCTTCCTTCCGCTCC	(AAG)16
59	RM508	6	159	0.44	GGATAGATCATGTGTGGGGG	ACCCGTGAACCACAAAGAAC	(AG)18
60	RM597	6	466	1.36	CCTGATGCACAACTGCGTAC	TCAGAGAGAGAGAGAGAGAGAGAG	(AG)11

Continued

Table 2. List of Polymorphic markers distributed on 12 chromosomes of Rice associated with drought tolerance between the parents HUR-1309 (recurrent) and CR Dhan 801 (donor) (Continued)

61	RM125	7	127	24.81	ATCAGCAGCCATGGCAGCGACC	AGGGGATCATGTGCCGAAGGCC	(GCT)8
62	RM320	7	413	18.64	CAACGTGATCGAGGATAGATC	GGATTTGCTTACCACAGCTC	(AT)18
63	RM432	7	186	18.9	TTCTGTCTCACGCTGGATTG	AGCTGCGTACGTGATGAATG	(ATCC)9
64	RM248	7	271	29.28	TCCTTGTGAAATCTGGTCCC	GTAGCCTAGCATGGTGCATG	(AG)15
65	RM445	7	277	27.4	CGTAACATGCATATCACGCC	ATATGCCGATATGCGTAGCC	(AG)12
66	RM473	7	191	28.12	TATCCTCGTCTCCATCGCTC	AAGGATGTGGCGGTAGAATG	(AGAT)14
67	RM149	8	231	24.71	GCTGACCAACGAACCTAGGCCG	GTTGGAAGCCTTTCCTCGTAACACG	NA
68	RM408	8	213	0.11	CAACGAGCTAACTTCCGTCC	ACTGCTACTTGGGTAGCTGACC	(AG)13
69	RM515	8	380	20.27	TAGGACGACCAAAGGGTGAG	TGGCCTGCTCTCTCTCTCTC	(AG)11
70	RM210	8	194	22.46	TCACATTCGGTGGCATTG	CGAGGATGGTTGTTCACTTG	NA
71	RM215	9	174	20.88	CAAAATGGAGCAGCAAGAGC	TGAGCACCTCCTTCTCTGTAG	(CT)16
72	RM257	9	172	17.66	CAGTTCCGAGCAAGAGTACTC	GGATCGGACGTGGCATATG	(AG)30
73	RM464	9	75	21.14	GAAGCAGGAAACAAGAAGAGAAGG	GTCTTCACCACAGTAAATGCTTGC	(AT)21
74	RM23668	9	160	0.6	TGCATAGCATATCAACTAGCCCTACC	GCTGAAACAGAATGAAAGCACAGC	(ACG)10
75	RM23911	9	270	17.14	TGCCTGCACTTATCTCTTGATGC	GATGAACCTAAAGGGCAGTTTCC	(AC)13
76	RM566	9	143	14.65	AATATGGTGGCGCGTACATCC	TGATCGAGCCAACAACAACTGG	(AG)15
77	RM216	10	91	4.98	GCATGGCCGATGGTAAAG	TGTATAAAACCACACGGCCA	(CT)18
78	RM496	10	186	21.98	GACATGCGAACAACGACATC	GCTGCGGCGCTGTTATAC	(AG)24
79	RM6100	10	173	18.37	TCCTCTACCAGTACCGCACC	GCTGGATCACAGATCATTGC	(ACG)8
80	RM304	10	182	18.21	TCAAACCGGCACATATAAGACC	CGTTGTAGTGTCAGCAAGATAGGG	(AT)30
81	RM144	11	245	28.17	TGCCCTGGCGCAAATTTGATCC	GCTAGAGGAGATCAGATGGTAGTGCATG	(AAT)7
82	RM224	11	NA	26.79	ATCGATCGATCTTCACGAGG	TGCTATAAAAGGCATTCGGG	NA
83	RM287	11	299	16.61	TTCCCTGTTAAGAGAGAAATC	GTGTATTTGGTGAAAGCAAC	(AG)15
84	RM286	11	235	0.38	CTGGCCTCTAGCTACAACCTTGC	AAACTCTCGCTGGATTCGATAGG	(AG)21
85	RM463	12	159	32.46	TTCCCCTCCTTTTATGGTGC	TGTTCTCCTCAGTCACTGCG	(AAT)5
86	RM28052	12	390	14.38	TTCAGCCGATCCATTCAATTCC	GCTATTGGCCGGAAAGTAGTTAGC	(CGC)8
87	RM1261	12	218	17.57	GTCCATGCCCAAGACACAAC	GTTACATCATGGGTGACCCC	(AG)16
88	RM19	12	226	2.1	CCCATCCTCACCGATCTCTCTAAAC	GTGCGCACGGAGGAGGAAAGGG	(ATC)10
89	RM28099	12	121	15.89	TGTGCGGATGCGGGTAAGTCC	CCACCTGTCAACCACCGAAACC	(CCG)7
90	RM28311	12	373	20.03	TGATGTTGTCATCAGGCATGTAGC	AGATTTGGGCTGGTTGCATTAGG	(AT)13

*Chr. N.- Chromosome number

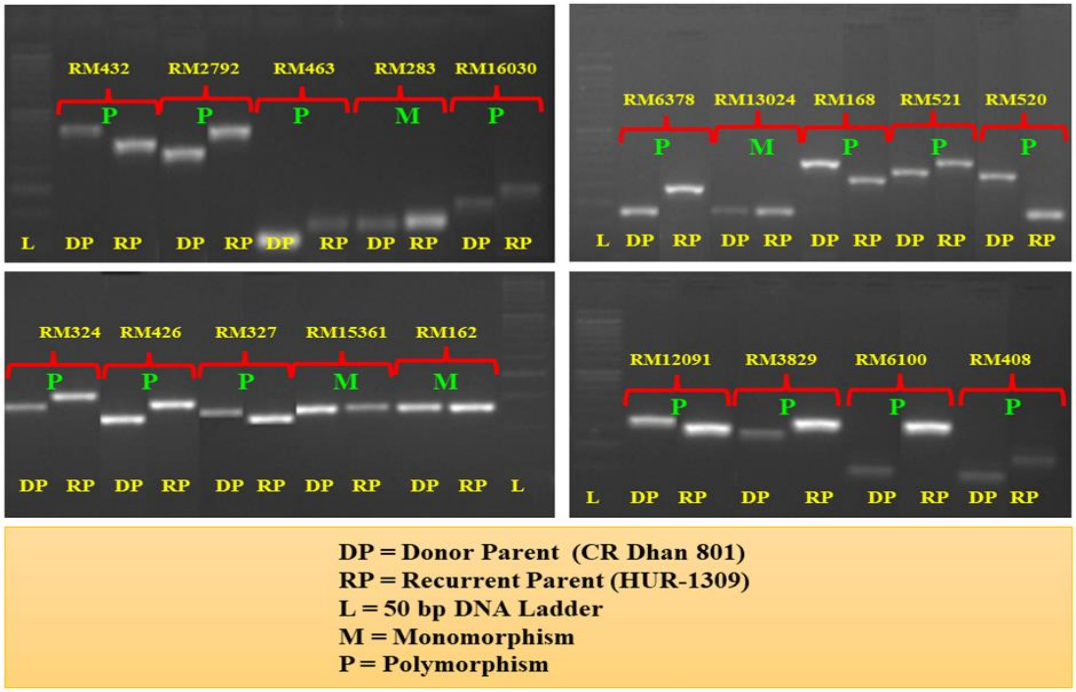

Figure 1. Banding pattern of polymorphic and monomorphic markers for drought tolerance among the two parents; HUR-1309 (recurrent) and CR Dhan 801 (donor)

genotypes (Improved Samba Mahsuri and Badshabhog). Chromosome 6 has the highest polymorphism rate (26.67%), followed by chromosome 4 (21.43%), while chromosome 10 (8.93%) had the lowest. The current results were consistent with those of numerous prior research. There were 108 polymorphic markers detected in a survey of polymorphism utilizing 647 SSR markers, representing a polymorphism level of 16.69% between the parental strains PR122 and IR10M196 [16]. In Kumar et al., [12] study the donor parents (GPP 2 and NLR 145) and the recurrent parent (JGL 1798) showed 31% polymorphism through 128 HRM primers. The polymorphism of the three most widely grown rice varieties on the Andaman and Nicobar Islands (C14-8, CARI Dhan 5, and donor IRBB 60) was surveyed using a panel of 200 highly variable SSR markers.

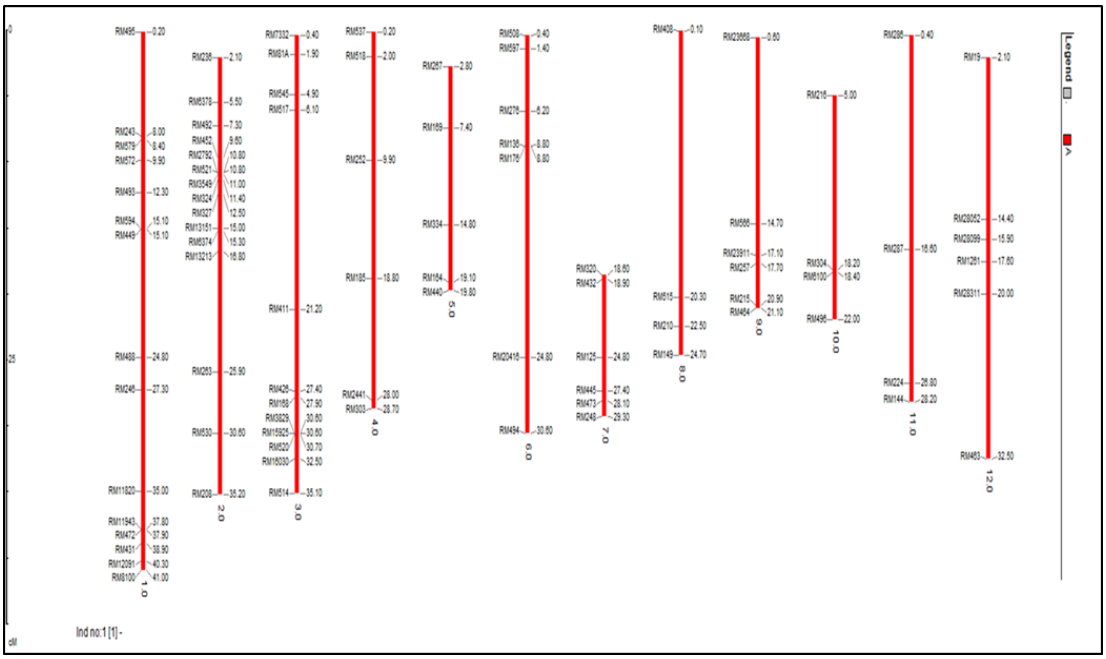

Figure 2. Distribution of Polymorphic markers for drought tolerance between the parents HUR-1309 (Recipient) and CR Dhan 801 (donor)

Table 3. Parental polymorphism percentage between the parents HUR-1309 (recipient) and CR Dhan 801 (donor)

SN.	Chr. N. *	Total no. of Markers run	No. of Polymorphic markers identified	Polymorphism (%)
1	1	65	15	23.08
2	2	49	15	30.61
3	3	53	12	22.64
4	4	44	6	13.64
5	5	43	5	11.63
6	6	49	7	14.29
7	7	35	6	17.14
8	8	35	4	11.43
9	9	35	6	17.14
10	10	34	4	11.76
11	11	32	4	12.50
12	12	36	6	16.67
Total		**510**	**90**	**17.65 (Average)**

*Chr. N.- Chromosome number

The data showed that polymorphism occurred in 36% and 48% of the SSR markers in the C14-8 and CARI Dhan 5 samples, respectively [17]. From a parental survey undertaken by Waghmare et al., [18], observed that there is 20.82 percent polymorphism between the two parents (N22 and Uma), as evidenced by 41 polymorphic markers out of 197 tested. Marathi et al., [19] discovered that chromosome 4 had the highest polymorphism rate, at 32.93 percent. The parental polymorphism was analyzed using 500 SSR markers encompassing all 12 chromosomes, and 70 of them were found to be polymorphic, which is equivalent to 14% polymorphism between ARC10531 and BPT-5204 [20]. To map the QTLs for sheath blight resistance in rice, Channamallikarjuna et al., [21] used 637 SSR markers to determine DNA polymorphism between the parents HP2216 and Tetep and found that only 74 markers were polymorphic. Based on the number of nucleotides they contain, we observed that SSR repeat motifs with dinucleotide repetitions were more polymorphic than other repeat motif classes (di, tri, or tetra).

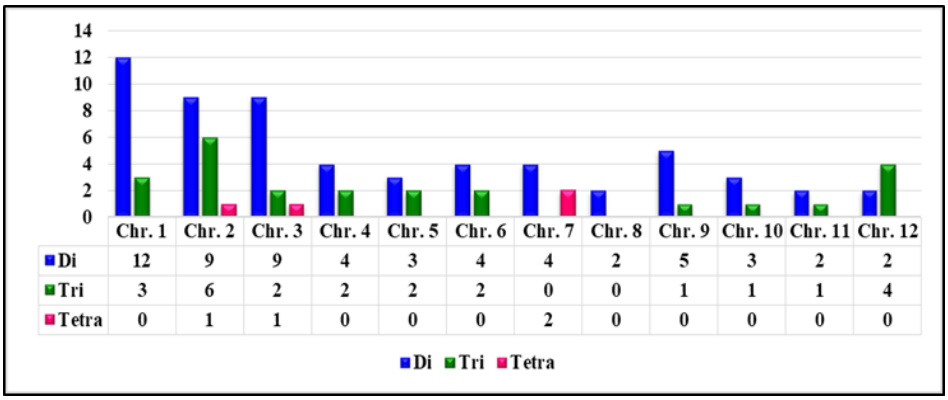

Figure 3. Presence of different types of repeat motifs on 12 chromosomes of Rice

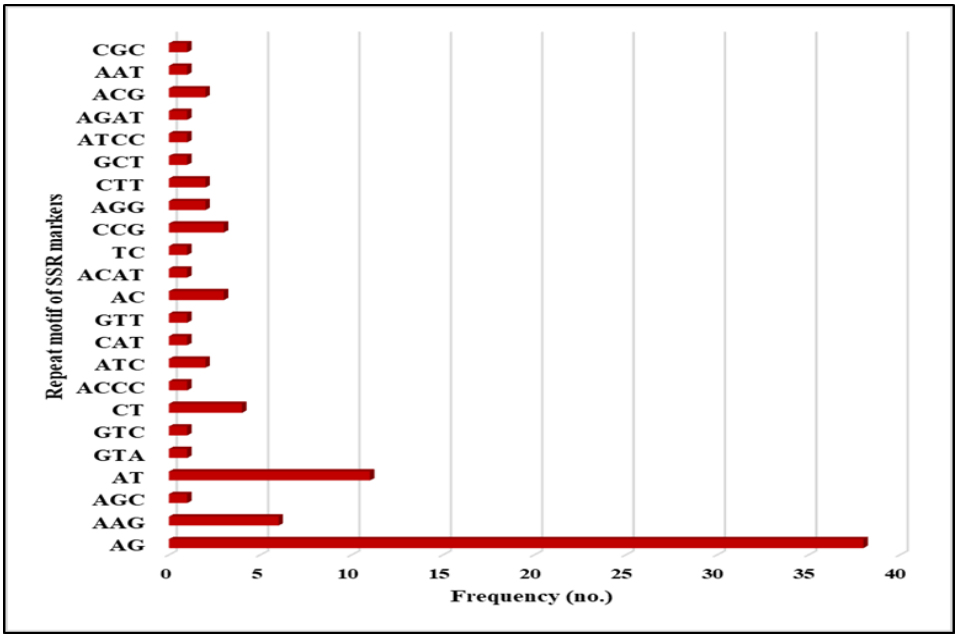

Figure 4. Frequency distribution of different repeat motifs of polymorphic SSR markers

There was a total of 90 polymorphic markers found; 59 were dinucleotide repeats, 24 were trinucleotide repeats, and 4 were tetra nucleotide repeats. Dinucleotides repeated 12 times on chromosome 1, 9 times on chromosome 2, and 9 times on chromosome 3 (Figure 3). According to the motif frequency distribution, the AG repeat motif was the most abundant among dinucleotide repeats, making up 42.2% of all repeats (Figure 4). AAG is the most commonly repeated trinucleotide, accounting for 6.7% (6 times), while the tetra nucleotides (ACCC, ATCC, and AGAT) occur only once each. McCouch et al., and Grover et al., [7, 22] have both found numerous examples of the $(AG)_n$ and (AT)n dinucleotide repeats in their research. From a total of 840 hypervariable rice microsatellite (hvRM) markers, Narshimulu et al., [23] discovered that dinucleotides markers were the most common (ranging from 5 to 63), followed by tri and tetranucleotides. Repeats of the dinucleotide sequence $(GA)_n$ were found to be the most common (39.34%), followed by (CT)n at 27.87% and (TA)n at a paltry 1.64%. The number of $(CCT)_n$ repeats was found to be the highest among the trinucleotide motifs, as stated by Rathi et al. [15]. The reported polymorphic markers between the two parents, depending on repeat motif and frequency distribution, contribute in the selection of polymorphic microsatellites for genotyping the backcrossed progeny. More SSR markers can be run for this region using the available resources and published research on the rice map, or if this area is found to be connected with a QTL interval, gene-specific markers that have been reported in the past can be used to retrieve this low polymorphism region. Once a QTL region defined by a marker interval has been established, it is possible to further refine it using fine mapping techniques.

Conclusion

Increasing climatic variability due to global warming will result in significant seasonal and annual swings in food output. The implications of climate change on rice production include that the country experiences diverse natural disasters in a given year. While floods and cyclones are instantaneous phenomena that occur within hours owing to the wrath of nature, the drought is a progressive phenomenon produced by soil conditions and atmospheric changes over time. The technology of molecular markers has dramatically boosted efficiency and simplified the introduction of genes from wild and domesticated sources. In contrast to traditional breeding methods, molecular markers would simplify the analysis of polymorphic markers. Marker Assisted Selection (MAS) programmes rely on the identification of parental polymorphism in rice cultivars through the screening of markers. This paves the way for tagging the target gene and fine mapping its location on the rice chromosome. The polymorphic markers can be deployed in the marker-assisted backcross breeding (MABB) strategy to improve drought tolerance in grain yield. Since the QTL introgression using the MABB technique followed three levels of selection (foreground, recombinant, and background selection), the polymorphic markers obtained in the current study will be used for genotyping the complete backcrossed population.

Acknowledgments

The authors would like to express their deep appreciation to the Institute of Agricultural Sciences at Banaras Hindu University (B.H.U.), Varanasi, Uttar Pradesh, India; and the Crop Improvement Division at the Indian Council of Agricultural Research (ICAR)-National Rice Research Institute (NRRI), Cuttack, Odisha, India. The authors would also like to extend their gratitude to the Indian Council of Agricultural Research (ICAR), New Delhi, for providing the lead author with an ICAR-SRF fellowship throughout the research period.

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