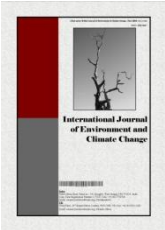

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Parental Polymorphism Survey for Evaluation and Selection of Contrasting Parents for Drought Tolerance in Rice (*Oryza sativa* L.) by Using SSR Markers

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aim: This study aims to find the informative polymorphic Microsatellite (SSR) markers for drought tolerance between the recipient parent (Pratishya) and donor parent (CR Dhan 801) of rice. The drought tolerant QTLs (qDTY1.1, qDTY2.1, and qDTY3.1) from the donor parent were subsequently introgressed into the popular variety Pratikshya via marker-assisted backcross breeding.
Place and Duration of Study: The parental polymorphism study was conducted at Molecular Drought Breeding Laboratory, Department of Genetics and Plant Breeding and Central Laboratory,

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Institute of Agricultural Sciences, Banaras Hindu University, Varanasi; and at Crop Improvement Division, ICAR-NRRI, Cuttack during Rabi 2018-19.

Methodology: The parents used for the study were Pratikshya (recurrent parent) and CR Dhan 801 (donor parent). The leaf samples were collected from 20-25 days old seedlings which were used for parental polymorphism survey after DNA extraction. The genomic DNA isolation was done by using CTAB method. A set of 510 SSR markers covering all the 12 chromosomes of rice were used for parental polymorphism survey in the present study. The standard protocol of polymerase chain reaction (PCR) and agarose gel electrophoresis was used. The DNA fragments were documented using gel documentation system. The graphical representation of marker data was obtained with GGT 2.0 software.

Results: Out of 510, total 108 SSR primer pairs exhibited polymorphism between recipient parent (Pratikshya) and donor parent (CR Dhan 801) and remaining 392 SSR primers were monomorphic. The highest percentage of parental polymorphism was observed on chromosome 2 (26.53%) and 3 (26.46%) followed by chromosome 1 (23.08%); and least on chromosome number 10 (14.71%). The average per cent of polymorphism on all the 12 chromosomes were 21.28%. Out of 108 polymorphic markers obtained from screening of 510 SSRs, 69 contained dinucleotide repeats, 32 showed trinucleotide repeats and 5 were having tetranucleotide repeats.

Conclusion: The screening of markers by parental polymorphism in rice cultivars gives the basis for tagging of the target gene, fine mapping of the gene on the rice chromosome, and subsequent application in Marker Assisted Selection (MAS) programmes. The polymorphic markers identified in this study were be used for genotyping the entire mapping population and for the introgression of the QTLs/genes linked with grain yield during the reproductive stage of drought.

Keywords: Abiotic stress; drought; molecular markers; parental polymorphism; rice cultivars.

1. INTRODUCTION

More than half of the world's population relies on rice (*Oryza sativa* L.) as their primary source of nutrition. Rice is a "Global Grain" that is farmed globally and feeds millions of people, with 90% of the rice consumed by Asians [1]. Low output in rainfed ecosystems is blamed on the prevalence of abiotic disturbances including drought and flooding. In spite of accounting for 38% of cultivated land, rain-fed highland and lowland ecosystems only account for 21% of overall output. Drought is the main cause of poor rice yield in rainfed uplands, rainfed shallow low lands, and rainfed medium low lands. The reproductive stage of a crop is particularly vulnerable to the effects of drought during the growing season, reducing grain yield [2,3]. Recent climate change projections suggest that the water deficit will continue to increase and the drought scenario will continue to worsen in the years to come [4]. Drought-resistant rice varieties have received some research and development. Most high-yielding cultivars, including IR36, IR64, Swarna, and Samba Mahsuri, were originally bred for irrigated environments and were never chosen for drought tolerance, despite the fact that they are now planted in rainfed areas. These types cause severe yield losses during drought years, which causes an abrupt drop in rice production across the country. Farmers in drought-prone areas cannot keep growing certain rice varieties since there are not any variety that produce high yields with good-quality despite the drought. Drought-prone area farmers need cultivars that can avoid yield loss when exposed to drought [1].

The analysis of parental polymorphism is necessary before beginning marker aided selection or marker assisted back cross breeding to introgress a desirable characteristic into a variety from the donor parent. Further selection of plants possessing the traits of interest is not achievable in the progenies unless the parents are polymorphic for the traits of interest [5]. Polymorphism is an index of genetic variation that shifts with different sets of parents [6]. According to the survey of parental polymorphism, there is demonstrable variation between parents. Microsatellites (SSRs) have been used more than any other type of molecular marker due to their many desirable characteristics. These include being highly polymorphic and co-dominant, being widely distributed over the genome, and being amplified by Polymerase chain reaction. These markers have also shown great promise in enhancing breeding efficiency by facilitating the accurate transfer of specific areas of the genome [7]. The majority of crop species, including rice, for which sequence information is available have made

extensive use of SSRs. These are helpful in a wide variety of genetics-related fields, including gene tagging, genetic diversity research, mapping population genotyping, linkage map construction, marker-trait association tracking, single marker analysis, and quantitative trait loci (QTL) mapping [8]. Accordingly, the purpose of the current study was to locate the donor and recipient parents informative polymorphic SSR markers. These markers were then incorporated into a marker-assisted backcross breeding programme, which was utilised to introduce drought-resistant QTLs from the donor parent CR Dhan 801 into the background of the widely-grown rice variety Pratikshya.

2. MATERIALS AND METHODS

2.1 Experimental Site and Plant Materials

The present investigation was conducted at two different locations such as Agricultural Research Farm, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, Uttar Pradesh, India and ICAR-National Rice Research Institute (ICAR-NRRI), Cuttack, Odisha, India. The crop season for field experiment was started at ICAR-National Rice Research Institute (NRRI), Cuttack, during *Rabi* 2018-19 and the parental polymorphism work was conducted at Molecular Drought Breeding Laboratory, Department of Genetics and Plant Breeding and Central Laboratory, Institute of Agricultural Sciences, BHU; and at Crop Improvement Division, ICAR-NRRI, Cuttack. The parents used for the study were Pratikshya (recurrent parent) and CR Dhan 801 (donor parent) and their characteristics were mentioned in Table 1.

2.2 Methodology

2.2.1 Leaf sample collection and DNA isolation

DNA was extracted from leaf samples collected from 20-25 days old seedlings, and utilised to conduct a survey of parental polymorphism. As proposed by Murray and Thompson [9], the CTAB technique was used to isolate the genomic DNA. The leaf samples were cut into small pieces and were ground with 100µl 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) in a Geno-grinder set at 1000rpm for 10 minutes. Each Eppendorf tube holding a leaf homogenate received an additional 700 µl of extraction buffer. For 30 minutes, samples were heated to 65 degrees Celsius in a water bath. After removing the supernatant, the mixture was centrifuged at 12,000 rpm for 15 minutes while being combined with an equal volume of chloroform and isoamyl alcohol (24:1). After transferring the supernatant to a fresh, sterile 1.5 ml centrifuge tube, 800 µl of cold isopropanol was added, and the tubes were agitated for 10-15 minutes before being stored at -20ºC overnight. The following day, at a temperature of 24 degrees Celsius, the tubes were centrifuged for 18 minutes at a speed of 12000 rpm. Without disrupting the DNA pellet, the supernatant was drained and around 200 µl 70% ethanol was added to the particle before centrifuging again for 10 minutes at 24 ^{0}C at 10000 rpm. After being permitted to air dry at room temperature overnight, the pellet was dissolved in 50-100 µl of 1X TE buffer. The Nanodrop test was utilised to evaluate DNA purity.

2.2.2 Polymerase Chain Reaction (PCR) using SSR markers

A set of 510 SSR markers covering all the 12 chromosomes of rice were used for parental polymorphism survey in the present study. The polymerase chain reaction (PCR) was carried out in a total volume of 10 µL containing 2 µl of template DNA, random (forward and reverse) markers (0.25µl each), 4.0µl Takara PCR master mix and 3.5 µl sterile distilled water. After centrifuging the PCR mixture at 1000 rpm for a minute, it was placed in a PCR thermal cycler with 96 wells. The protocol begins with a 5-minute denaturation at 94ºC and continues with 35 cycles of 45 seconds at 94ºC, 45 seconds at 56ºC for primer annealing, 1 minute at 72ºC for extension, and 10 minutes at 72ºC for final extension.

2.2.3 Agarose gel electrophoresis and image visualization

The PCR products were analysed by electrophoresis using a 3.5% agarose gel using a gel Electrophoresis Unit (CBS Scientific, USA). About 9.0 g of agarose was weighed and transferred to a conical flax and 400 ml of 1X TAE buffer was added into it and mixed well. The ingredients were boiled slowly while being stirred intermittently in a microwave. Complete agarose melting and a transparent solution were maintained throughout the process. In order to clean the gel-casting tray, it was soaked in water and then wiped down with ethanol. After the

agarose had cooled to room temperature, two millilitres of ethidium bromide (10 mg per millilitre) were added to the molten substance, and the mixture was then poured onto a gel cast tray fitted with the necessary gel combs and left to settle for 20 to 30 minutes. The gel was transferred to the Electrophoresis Unit containing 1X TAE buffer. Before loading, PCR amplified products were mixed with 1/6th volume of gel loading dye (40% sucrose and 0.25% bromophenol blue) and loaded into the wells. 50 bp DNA ladder was added in one well to determine the size of amplified fragments. The DNA fragments were visualized under UV transilluminator and documented using gel documentation system (Gel Doc^{TM} XR**+**, BIO-RAD, USA).

2.3 Statistical Analysis

The percentage of polymorphism was calculated by using the formula :

$$= \left(\frac{\text{Number of molecular markers showing polymorphism}}{\text{Total number of molecular markers run}}\right) \times 100 \quad (1)$$

To visualise the marker data, GGT 2.0 programme was used [10]. Visualization and analyses of molecular marker scores are the focus of GGT 2.0 [10,11]. Visualization of the distribution of polymorphic markers along the length of the chromosome according to their physical positions (Mb) was obtained by GGT, as shown in Fig. 1, based on inputs of physical positions of markers given in row and column data matrix.

3. RESULTS AND DISCUSSION

The presence of two or more discontinuous genotypes or alleles on a specific locus in a given population is referred to as genetic polymorphism. The prevalence rate of gene polymorphism in the population is believed to be ≥1% [12], and it determines the diversity of the individuals. According to the survey of parental polymorphism, there is demonstrable variation between parents. In the present study, 510 SSR markers (forward and reverse) were utilised to screen for polymorphism between parents, with markers located throughout all 12 chromosomes. Only 108 SSR primer pairs (Table 2) showed polymorphism between the recipient parent (Pratikshya) and the donor parent (CR Dhan 801), whereas the remaining 392 SSR primers were monomorphic. The donor parent contains three drought tolerant yield QTLs; *qDTY*$_{1.1}$, *qDTY*$_{2.1}$ and *qDTY*$_{3.1}$ which are present on the chromosome 1, 2 and 3 respectively. Therefore, more emphasis was given on these three chromosomes and more number of molecular markers were run for the survey. There were 65, 49 and 53 numbers of random markers were run for chromosome number 1, 2 and 3 respectively for polymorphism study. The highest percentage of parental polymorphism was observed on chromosome 2 (26.53%) and 3 (26.46%) followed by chromosome 1 (23.08%); and least on chromosome number 10 (14.71 %). The average per cent of polymorphism on all the 12 chromosomes was 21.28% (Table 3). The representation of polymorphism and monomorphism of molecular markers depicted in Fig. 1 whereas, the distribution of polymorphic markers on 12 chromosomes represented in Fig. 2. Previous research by Marathi et al. [13] found that most variation occurred on chromosome 4. Among 500 SSR markers covering all 12 chromosomes, our results matched those of Yadav et al. [14], who revealed that 14% of polymorphism occurred between the ARC10531 and BPT-5204 parents. In another work, Yerva et al. [5] examined two parents namely PR122 and IR10M196 for parental polymorphism using 647 SSR markers, of which 108 markers displayed polymorphism with the level of 16.69 %. Perhaps the fact that both Pratikshya and CR Dhan 801 are indica lines accounts for the lack of visible variation between the two parents. Studies by Xu et al. [15] and Biradar et al. [16] found that there was a lack of molecular marker polymorphism between indica genotypes.

According to previous studies, the largest polymorphism was found on chromosome 5 (17.02%) and the lowest was found on chromosome 10 (5.36%), in a survey of chromosome polymorphism performed by Challa and Kole [1] employing parents with different responses to drought. Out of a total of 197 markers examined, a parental polymorphism study between N22 and Uma found 20.82 percent polymorphism, with 41 polymorphic markers [17]. Another study evaluated 647 SSR markers from parental lines PR122 and IR10M196, and detected 108 polymorphic markers, for a total polymorphism level of 16.69% [5]. Repeat motifs (di, tri, tetra) are what define SSR-based polymorphism, and it was shown that dinucleotide repeats inside SSRs were more polymorphic than those within tri and tetra repeats in the current study. A distinction can be made between extremely repetitive sequences and moderately repetitive sequences

when discussing repetitions. Additional subcategorization of the moderately repetitive sequences into tandem repeats and interspersed repetitions is possible. Any type of tandem repeat can contain microsatellites [18]. Out of 108 polymorphic markers obtained from screening of 510 SSRs, 69 contained dinucleotide repeats, 32 showed trinucleotide repeats and 5 were having tetranucleotide repeats (Fig. 3). The frequency distribution of repeat motifs showed that out of the 60, dinucleotide repeats AG motif appeared more polymorphic (34 times) than others, which constitutes 31.48% of dinucleotide repeats, followed by AT repeats 20 times and constitutes 18.5 % (Fig. 4). Previous research has noted the prevalence of the dinucleotide repeats (AG)n and (AT)n in parental polymorphism survey [19,20]. Out of 43 trinucleotides AAG, CCG, AGG repeated 10, 5 and 5 times respectively. The frequency AAG trinucleotide repeat motif constitute 9.25% of the total polymorphic markers. It was also observed that maximum trinucleotides (GTC, CAT, GTT, CTT, GCT and CCT) repeated only single time. The reported results were consistent with previous conclusions on the prevalence of recurrent motifs. Temnykh et al. [21] observed the presence of an 80% trinucleotide GC rich repeat pattern in the non-coding intergenomic region, which was frequently associated with genes (or ESTs) and (AC) n/(AT)n dinucleotides and tetranucleotide repeat motif. The percentage of AGAT repeats among the total of 23 tetranucleotide repeats was 26.08 percent (6 of 23). Grover et al. [19] conducted a comparative investigation of the genomes of two *Oryza sativa* subspecies, *ssp. indica* and *ssp. japonica*, and found that (AGAT)n repeats are abundant in both the sub-species.

The efficiency of breeding programmes has been substantially improved by molecular marker technology and resistance genes are now easily introgressed. Molecular markers would allow for the straightforward tagging of polygenic traits that were previously impossible to examine using conventional breeding techniques. Marker Assisted Selection (MAS) programmes rely on the identification of parental polymorphism in rice cultivars through the screening of markers. This allows for the tagging of the target gene and the precise mapping of that gene within the rice chromosome. The polymorphic markers identified here can be utilised in a marker-assisted backcross breeding (MABB) strategy to introgress drought-tolerant QTLs from the donor parent CR Dhan 801 into the drought-susceptible recurrent parent Pratikshya, *via* foreground, recombinant and background selection.

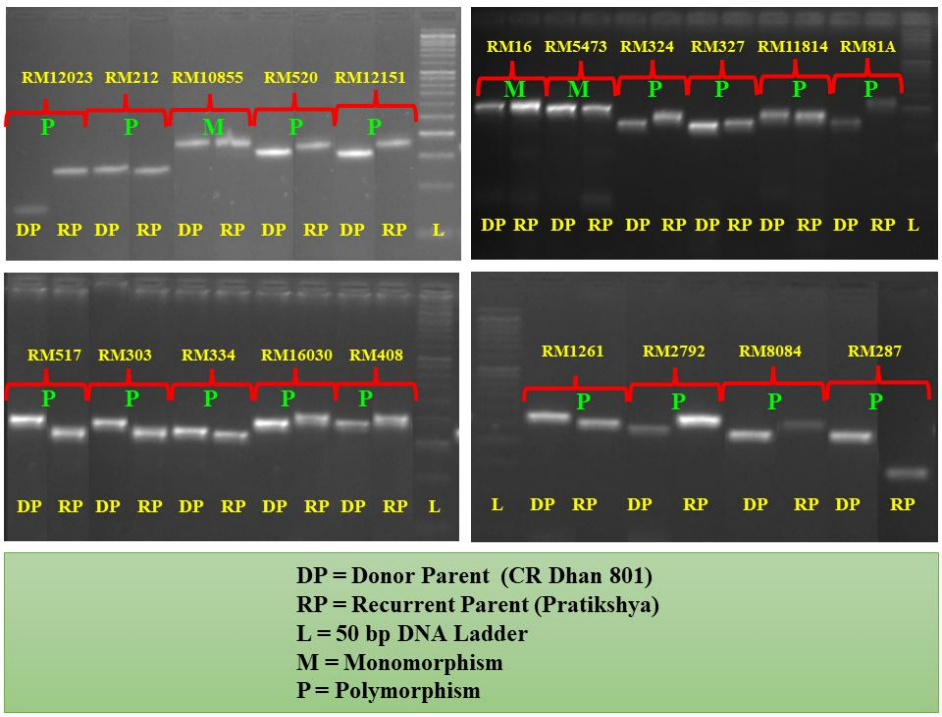

Fig. 1. Representation of the polymorphism and monomorphism of the SSR markers between the two parents pratikshya (recurrent) and CR dhan 801 (donor)

Table 1. The experimental materials and their characteristics

Sl. no.	Characteristics	Recurrent parent	Donor parent
		Pratikshya (ORS-201-5) (IET 15191)	**CR dhan 801 (IET 25667)**
1.	Yield (q/ha)	48.48	63.0
2.	Duration (days)	140-142	140
3.	Ecosystem	Irrigated/ Rainfed medium land	Shallow low land
4.	Year of release	2006	2018
5.	Releasing center	OUAT, Bhubaneswar	ICAR-NRRI, Cuttack
6.	Parentage	Swarna //IR 64	IR81896-B-B-195 / 2* SwarnaSub1 // IR91659-54-35.
7.	Recommended zone	Odisha	Odisha, West Bengal, Uttar Pradesh, Andhra Pradesh and Telangana
8.	Resistance/Tolerance	Brown Spot, Leaf Folder, WBPH	Drought (*qDTY*$_{1.1}$, *qDTY*$_{2.1}$ and *qDTY*$_{3.1}$) and Submergence (*Sub-1*)

Table 2. List of polymorphic markers distributed on 12 chromosomes of rice associated with drought tolerance between the parents pratikshya (recurrent) and CR dhan 801 (donor)

Sl. No.	Marker	Chrom. No.	Expected Size	Position	Forward Primer Sequence	Reverse Primer Sequence	No. of Repeat motif
1	RM212	1	162	33.04	CCACTTTCAGCTACTACCAG	CACCCATTTGTCTCTCATTATG	(AG)14
2	RM243	1	112	7.97	GATCTGCAGACTGCAGTTGC	AGCTGCAACGATGTTGTCC	(AG)18
3	RM246	1	99	27.32	CGAGCTCCATCAGCCATTCAGC	ACTTGAGAGCGAGATTGGGAATCG	(AG)18
4	RM11814	1	247	34.88	TCCAGACTTACCGCCTATTCTAATCC	AGAGGAGGGAAGAAGAGGAATGC	(AAAG)5
5	RM8084	1	289	34.89	GCGCCCAATGCATGTAAATTCC	TGCCGATACCTGTGATCAAGTCC	(AT)18
6	RM12151	1	144	40.74	CTGCTGTCATGGTGGAACAGG	AGTGCGTGAGAGACATGAGACG	(CCG)7
7	RM11943	1	77	37.84	CTTGTTCGAGGACGAAGATAGGG	CCAGTTTACCAGGGTCGAAACC	(AG)11
8	RM12023	1	94	39.08	TGCGTACCTCTGCTCCTCTCTGC	GACGAAGCCGACCAAGTGAAGC	(ACG)8
9	RM12146	1	100	40.71	AGTATGCCCTGCCCACTACACTAGG	CAGCGAATGGCAAGAGCAACC	(AG)11
10	RM466	1	191	17.27	TCCATCACCACATTCCCC	ACCCTTCTCTCGCTCTCTCC	(AG)17
11	RM472	1	265	37.88	CCATGGCCTGAGAGAGAGAG	AGCTAAATGGCCATACGGTG	(AG)21
12	RM495	1	178	0.21	AATCCAAGGTGCAGAGATGG	CAACGATGACGAACACAACC	(AGC)7
13	RM579	1	196	8.45	TCCGAGTGGTTATGCAAATG	AATTGTGTCCAATGGGCTGT	(AG)25
14	RM572	1	168	9.86	CGGTTAATGTCATCTGATTGG	TTCGAGATCCAAGACTGACC	(AG)14
15	RM594	1	108	15.14	GCCACCAGTAAAAGCAATAC	TTGATCTGCTAGTGAGACCC	(AG)15
16	RM452	2	246	9.56	CTGATCGAGAGCGTTAAGGG	GGGATCAAACCACGTTTCTG	(GTC)9
17	RM263	2	235	25.88	CCCAGGCTAGCTCATGAACC	GCTACGTTTGAGCTACCACG	(CT)34
18	RM236	2	235	2.1	GCGCTGGTGGAAAATGAG	GGCATCCCTCTTTGATTCCTC	(AG)16
19	RM324	2	196	11.38	CTGATTCCACACACTTGTGC	GATTCCACGTCAGGATCTTC	(ATC)9
20	RM13213	2	191	16.84	GTTTCTCCACCACCGTCAGTCG	CCCTCACTTCACTAGTCCGTAGCC	(AT)21
21	RM521	2	457	10.8	ATGACCCAATTTCTGACTCTAGCC	CATGGGTGGTGCTGTAGATGG	(AG)14

Sl. No.	Marker	Chrom. No.	Expected Size	Position	Forward Primer Sequence	Reverse Primer Sequence	No. of Repeat motif
22	RM555	2	159	4.3	TTGACATGCGAAATGGAGATGG	TTGGATCAGCCAAAGGAGACC	(AG)11
23	RM3549	2	153	11	AAAGGCGGAGGAGAAGGTATGG	CTTGGAACGAACGACCAACTCC	(AG)12
24	RM6374	2	261	15.28	TCACCAGACTCAACAAAGGATCG	TTCACCTTTCTTCTCCCTCATTCC	(AAG)16
25	RM2792	2	485	10.75	CACACGATCAACTGAATATGCACACG	GTCGTTCGACTTGAGACCGATGG	(AT)35
26	RM327	2	213	12.46	CTACTCCTCTGTCCCTCCTCTC	CCAGCTAGACACAATCGAGC	(CAT)11(CTT)5
27	RM16033	2	155	32.57	TACCTTATCCCGATTGCTCAAGG	TAAGAGAAAGCCTTCCCGACTCC	(ACG)7
28	RM5791	2	100	10.74	ACGACGTCACAAAGGGTTCTTGC	GAATACGCTTTCGCCTGCTACG	(AGC)8
29	RM15924	3	366	30.63	GGCTCAATCCCTATGATCACAATCC	TTCCACAACAGCCCTTCAAATCC	(AT)17
30	RM15791	3	87	28.56	AGTAAGTTTGCCGCGGAGGAAGC	CTCCTTGTCGATCACCACCATCG	(AGG)8
31	RM22	3	171	1.5	GGTTTGGGAGCCCATAATCT	CTGGGCTTCTTTCACTCGTC	(GA)22
32	RM60	3	168	0.08	AGTCCCATGTTCCACTTCCG	ATGGCTACTGCCTGTACTAC	(AATT)5AATCT (AATT)
33	RM411	3	168	21.23	ACACCAACTCTTGCCTGCAT	TGAAGCAAAAACATGGCTAGG	(GTT)7
34	RM517	3	190	6.13	GGCTTACTGGCTTCGATTTG	CGTCTCCTTTGGTTAGTGCC	(CT)15
35	RM523	3	252	1.3	AAGGCATTGCAGCTAGAAGC	GCACTTGGGAGGTTTGCTAG	(TC)14
36	RM7332	3	239	0.39	ACACTGTACACCACACTTCAGC	CAGGGAAATGACACTGTCCC	(ACAT)11
37	RM81A	3	180	1.92	GAGTGCTTGTGCAAGATCCA	CTTCTTCACTCATGCAGTTC	(AAG)9
38	RM135	3	111	27.21	CTCTGTCTCCTCCCCCGCGTCG	TCAGCTTCTGGCCGGCCTCCTC	(CCG)11
39	RM520	3	114	30.71	AGGAGCAAGAAAAGTTCCCC	GCCAATGTGTGACGCAATAG	(AG)10
40	RM545	3	166	4.91	CAATGGCAGAGACCCAAAAG	CTGGCATGTAACGACAGTGG	(AG)30
41	RM16030	3	100	32.5	GCGAACTATGAGCATGCCAACC	GGATTACCTGGTGTGTGCAGTGTCC	(AG)11
42	RM426	3	124	27.39	CATCGCCGAAATCCATCTTCC	AAGGCCCATTTCATTGTAGAGTGC	(AT)11
43	RM518	4	193	2.02	CTCTTCACTCACTCACCATGG	ATCCATCTGGAGCAAGCAAC	(TC)15
44	RM280	4	175	35.21	GTGCTCTCCATGTCGGATTATGC	CAAGGCAACAAGATTGGTTAGTGG	(AG)11
45	RM16577	4	491	10.61	GGTGAATTCTACTAAGACGGATCG	AGCCTTATTAGTCTCACCTCGTAACC	(AT)29
46	RM17008	4	174	21.48	TTACCTTCGATTAGCTGCTGTTGC	ATTCCTTGCATTACAGACGGTAGC	(AG)26
47	RM252	4	491	9.95	TTCGCTGACGTGATAGGTTG	ATGACTTGATCCCGAGAACG	(AT)29
48	RM303	4	218	28.73	GCATGGCCAAATATTAAAGG	GGTTGGAAATAGAAGTTCGGT	(AT)12
49	RM537	4	NA	0.17	CCGTCCCTCTCTCTCCTTTC	ACAGGGAAACCATCCTCCTC	(CCG)9
50	RM185	4	79	18.76	AGTTGTTGGGAGGGAGAAAGGCC	AGGAGGCGACGGCGATGTCCTC	(AGG)9
51	RM2441	4	390	28.02	GATTCACCACGTTGAGCAAAGG	ACGTTTACCAACCACGGATTACG	(AT)27
52	RM4743	5	477	18.29	CTGTTAGGCAGGCTAGTCTGAGAGC	GATGCTCGTGGTCTGAATTGG	(AT)25
53	RM267	5	NA	2.82	TGCAGACATAGAGAAGGAAGTG	AGCAACAGCACAACTTGATG	(AG)12
54	RM18821	5	186	22.52	CGGCTAAATCGTCATGTGTATGG	TCTCCCATCTTACATGTCCTCACC	(AAG)21
55	RM5844	5	191	9.04	AACGTGGCATCCATGTTAGTACC	AGCTAGGAGCCATTGTCGAAGG	(AAT)22
56	RM334	5	177	14.81	GTTCAGTGTTCAGTGCCACC	GACTTTGATCTTTGGTGGACG	(CTT)20
57	RM17731	5	259	0.16	GGATCAACGCGATCAGTATTCG	CAACGTAAAGTTGATGGCACTGG	(ACAT)15

Sl. No.	Marker	Chrom. No.	Expected Size	Position	Forward Primer Sequence	Reverse Primer Sequence	No. of Repeat motif
58	RM413	5	84	2.15	CCAATCTTGTCTTCCGGATCTTGC	AGATAGCCATGGGCGATTCTTGG	(AG)11
59	RM440	5	200	19.83	GGTAGGCACCAAAGAGTTTGACG	GGCATCACCTTATCCAATCACC	(AAG)22
60	RM17954	5	198	3.59	ATTTCAGTACAAGGCACCCATGC	GTAGACGAGGGAGTACCAACTTGC	(AAT)26
61	RM136	6	299	8.76	GAGAGCTCAGCTGCTGCCTCTAGC	GAGGAGCGCCACGGTGTACGCC	(AGG)7
62	RM276	6	144	6.24	CTCAACGTTGACACCTCGTG	TCCTCCATCGAGCAGTATCA	(AG)8A3 (GA)33
63	RM510	6	193	2.83	GTTTGACGCGATAAACCGACAGC	ATGAGGACGACGAGCAGATTCC	(AG)15
64	RM133	6	230	0.02	TTGGATTGTTTTGCTGGCTCGC	GGAACACGGGGTCGGAAGCGAC	(CT)8
65	RM176	6	142	29.75	CGGCTCCCGCTACGACGTCTCC	AGCGATGCGCTGGAAGAGGTGC	(CCG)7
66	RM276	6	141	6.24	CTCAACGTTGACACCTCGTG	TCCTCCATCGAGCAGTATCA	(AG)10
67	RM20416	6	259	24.77	GAGACATCATAGCCGGATCTTCC	TCGGTAGAACATCACCTCCAAGG	(AG)33
68	RM508	6	159	0.44	GGATAGATCATGTGTGGGGG	ACCCGTGAACCACAAAGAAC	(AG)18
69	RM19697	6	329	7.55	AACAACCTGAGAACACCTCTTGG	GGACAAACACATGGTGATCTGC	(AT)52
70	RM597	6	466	1.36	CCTGATGCACAACTGCGTAC	TCAGAGAGAGAGAGAGAGAGAGAG	(AG)11
71	RM20096	6	397	17.21	CGGTAAGCCATAAATAGATCCCAAGG	TTTGAACAGCGACACGGTTTCC	(AT)52
72	RM11	7	115	19.2	TCTCCTCTTCCCCCGATC	ATAGCGGGCGAGGCTTAG	(AG)15
73	RM248	7	271	29.28	TCCTTGTGAAATCTGGTCCC	GTAGCCTAGCATGGTGCATG	(AG)15
74	RM125	7	127	24.81	ATCAGCAGCCATGGCAGCGACC	AGGGGATCATGTGCCGAAGGCC	(GCT)8
75	RM320	7	413	18.64	CAACGTGATCGAGGATAGATC	GGATTTGCTTACCACAGCTC	(AT)18
76	RM22068	7	368	27.44	ATCACGGGTGTATGTAGAACTGACC	TATTGGCTTTGACGGAGTGAACC	(AT)26
77	RM445	7	277	27.4	CGTAACATGCATATCACGCC	ATATGCCGATATGCGTAGCC	(AG)12
78	RM473	7	100	25.4	TCCCTATCCTCGTCTCCATCG	AGGATGTGGCGGTAGAATGC	(AGAT)14
79	RM22837	8	277	12.37	ACCTGGGTCAGATGTCTGTTTGG	GGTAGAGCTCCATCCATCTTAGTGC	(AT)24
80	RM25	8	146	5.2	GGAAAGAATGATCTTTTCATGG	CTACCATCAAAACCAATGTTC	(GA)18
81	RM149	8	231	24.71	GCTGACCAACGAACCTAGGCCG	GTTGGAAGCCTTTCCTCGTAACACG	NA
82	RM23553	8	314	27.24	CACTGTACGTGCACTATCCTAACC	GGAGACAAATGTATTGGGAGTAGG	(AT)43
83	RM310	8	176	5.11	CCAAAACATTTAAAATATCATG	GCTTGTTGGTCATTACCATTC	(AC)22
84	RM408	8	213	0.11	CAACGAGCTAACTTCCGTCC	ACTGCTACTTGGGTAGCTGACC	(AG)13
85	RM8019	8	663	7.48	CCATTCTATCCAATGGCTTCTCC	CCTTTCTCAGAGGATGCTATCAAGC	(AT)47
86	RM257	9	172	17.66	CAGTTCCGAGCAAGAGTACTC	GGATCGGACGTGGCATATG	(AG)30
87	RM105	9	134	3.2	GTCGTCGACCCATCGGAGCCAC	TGGTCGAGGTGGGGATCGGGTC	(CCT)6
88	RM410	9	597	17.58	GCTAGATTCACGGGCCTTGC	GTGCGTTCGGATGGAGGTAGG	(AT)13
89	RM23668	9	160	0.6	TGCATAGCATATCAACTAGCCCTACC	GCTGAAACAGAATGAAAGCACAGC	(ACG)10
90	RM5515	9	480	7.09	AGACCCTTGGCTACTGATTCC	AAGGTTCCACGAAAGATCTAGG	(AC)12
91	RM23911	9	270	7.14	TGCCTGCACTTATCTCTTGATGC	GATGAACCTAAAGGGCAGTTTCC	(AC)13
92	RM566	9	143	14.65	AATATGGTGGCGCGTACATCC	TGATCGAGCCAACAACAACTGG	(AG)15
93	RM25610	10	350	17.74	TCCTCCTCCAGTTCATCTTCTGC	GATCCGAGCATACAAGGAGTGC	(AG)35

Sl. No.	Marker	Chrom. No.	Expected Size	Position	Forward Primer Sequence	Reverse Primer Sequence	No. of Repeat motif
94	RM496	10	186	21.98	GACATGCGAACAACGACATC	GCTGCGGCGCTGTTATAC	(AG)24
95	RM6100	10	173	18.37	TCCTCTACCAGTACCGCACC	GCTGGATCACAGATCATTGC	(ACG)8
96	RM24889	10	384	0.46	CCAGAACCACTTGGACGTATTGG	TCATATAGCCACGACTCCATCACC	(AT)38
97	RM304	10	182	18.21	TCAAACCGGCACATATAAGACC	CGTTGTAGTGTCAGCAAGATAGGG	(AT)30
98	RM144	11	245	28.17	TGCCCTGGCGCAAATTTGATCC	GCTAGAGGAGATCAGATGGTAGTGCATG	(AAT)7
99	RM224	11	NA	26.79	ATCGATCGATCTTCACGAGG	TGCTATAAAAGGCATTCGGG	NA
100	RM287	11	299	16.61	TTCCCTGTTAAGAGAGAAATC	GTGTATTTGGTGAAAGCAAC	(AG)15
101	RM26868	11	169	19.15	CAACTGTACTGTGCTGACCATCG	AGTAGGGACGAGGATTTCATGG	(AAG)30
102	RM286	11	235	0.38	CTGGCCTCTAGCTACAACCTTGC	AAACTCTCGCTGGATTCGATAGG	(AG)21
103	RM463	12	159	32.46	TTCCCCTCCTTTTATGGTGC	TGTTCTCCTCAGTCACTGCG	(AAT)5
104	RM1261	12	218	17.57	ATGGTAGAGACACAAGTCCATGC	GACAAATTGGTGTAGGTGAAGG	(AG)16
105	RM19	12	226	2.1	CCCATCCTCACCGATCTCTCTAAAC	GTGCGCACGGAGGAGGAAAGGG	(ATC)10
106	RM28099	12	121	15.89	TGTGCGGATGCGGGTAAGTCC	CCACCTGTCAACCACCGAAACC	(CCG)7
107	RM28311	12	373	20.03	TGATGTTGTCATCAGGCATGTAGC	AGATTTGGGCTGGTTGCATTAGG	(AT)13
108	RM28089	12	261	15.45	GGGAGGACACCTGTGTAAGTAGG	GGTTCAAATGAGACCCAATTCC	(ATC)12

Table 3. Parental polymorphism percentage observed between the two contrasting parents pratikshya (recurrent) and CR dhan 801 (donor) for drought tolerances

Sl. no.	Chromosome no.	Total no. of markers run	No. of polymorphic markers identified	Polymorphism (%)
1.	1	65	15	23.08
2.	2	49	13	26.53
3.	3	53	14	26.42
4.	4	44	9	20.45
5.	5	43	9	20.93
6.	6	49	11	22.45
7.	7	35	7	20.00
8.	8	35	7	20.00
9.	9	35	7	20.00
10.	10	34	5	14.71
11.	11	32	5	15.63
12.	12	36	6	16.67
Total		**510**	**108**	**21.18 (Average)**

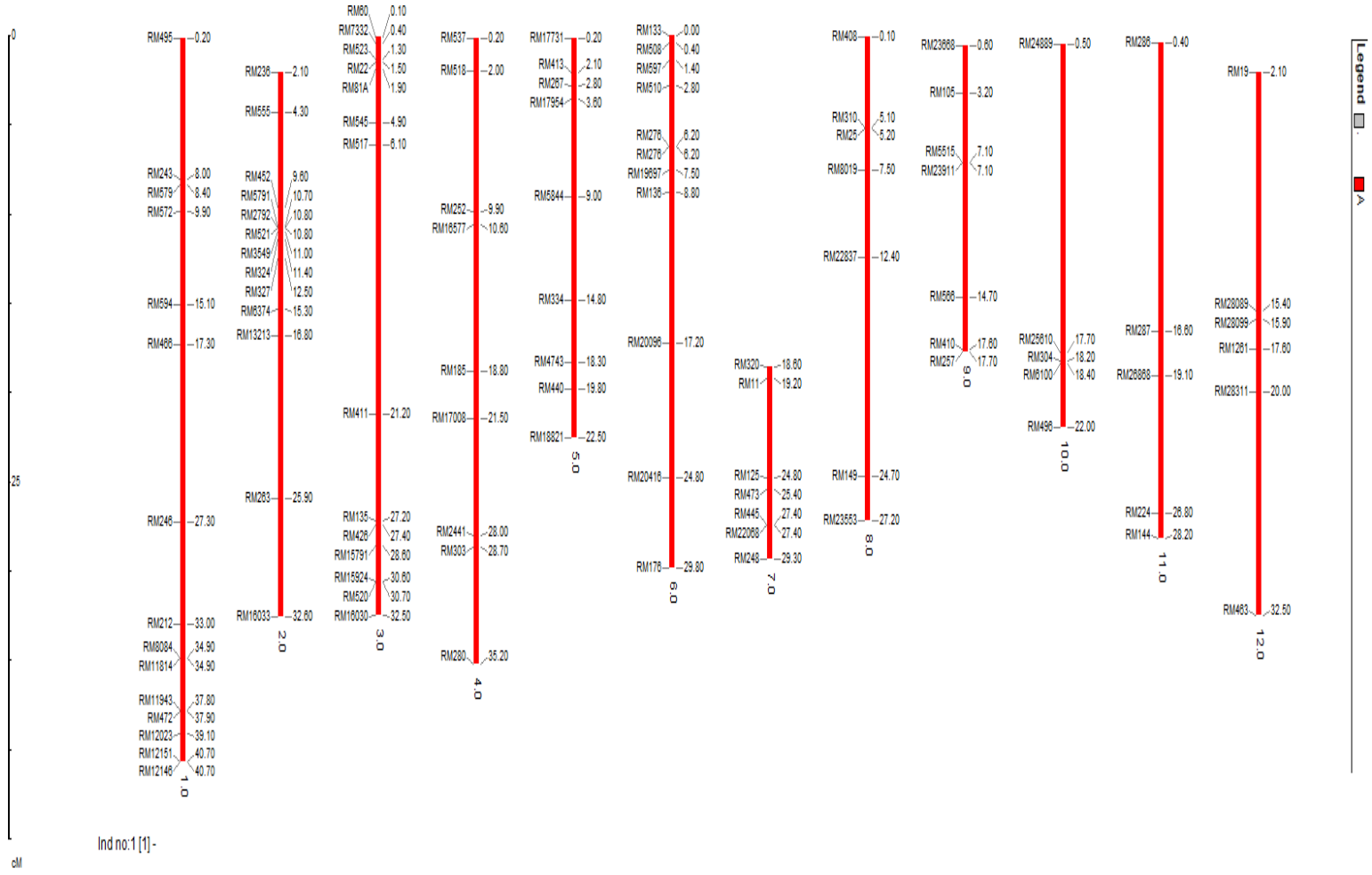

Fig. 2. Distribution of 108 polymorphic SSR markers on 12 chromosomes of rice (image by GGT 2.0)

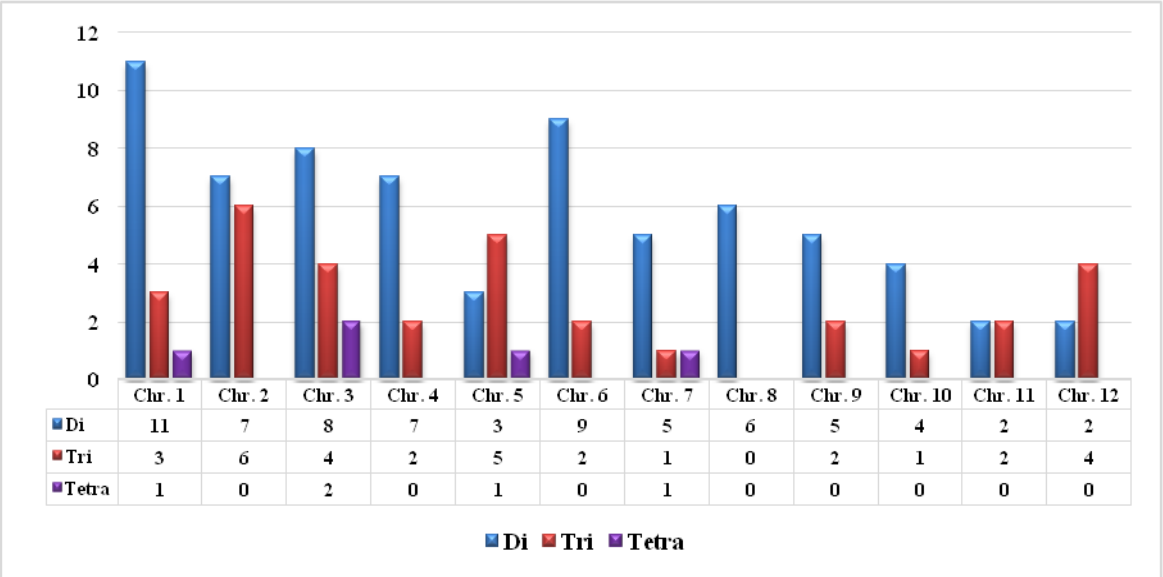

Fig. 3. Different types of repeat motifs of the polymorphic markers present on 12 chromosomes of rice

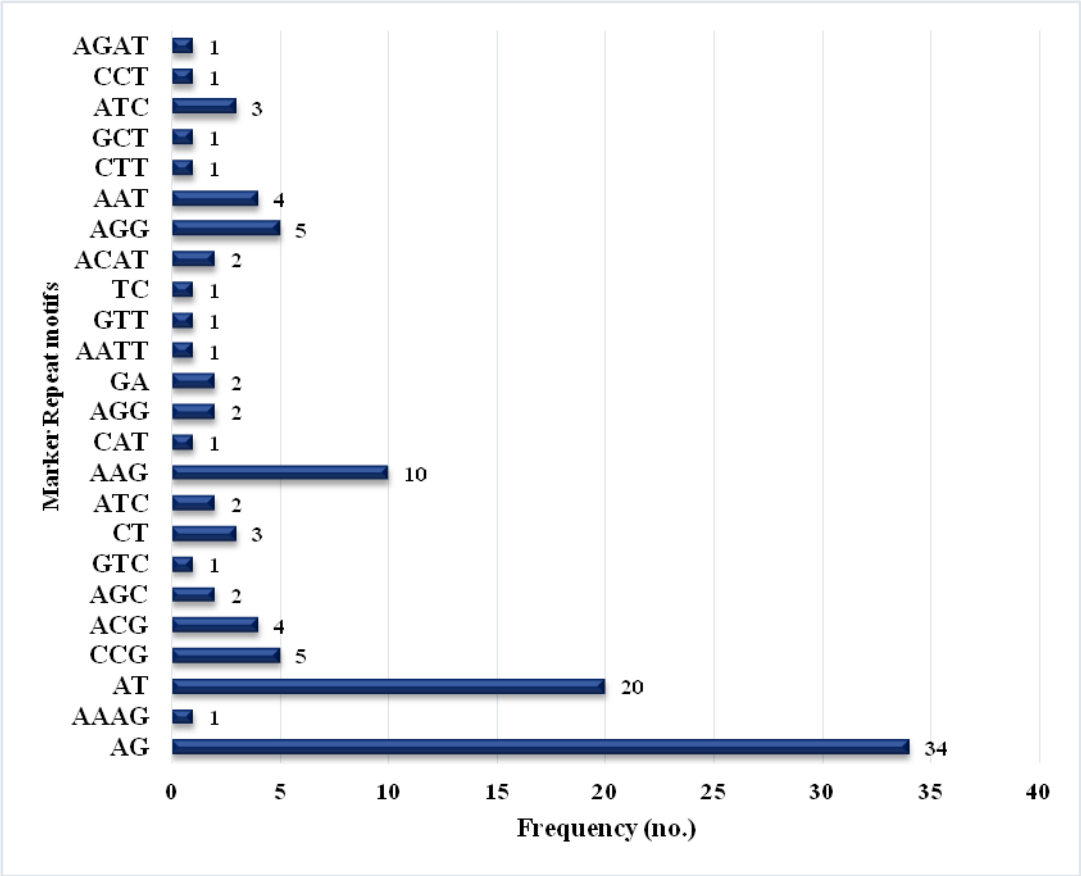

Fig. 4. Frequency distribution of different types of repeat motifs (di, tri and tetra) of polymorphic SSR markers present on 12 chromosomes of rice

5. CONCLUSION

Many morphological and environmental factors influence drought resistance, making it a highly nuanced characteristic in rice breeding. The ability of rice cultivars grown in a rainfed ecosystem to endure drought would be useful in maintaining a steady supply of rice. Genetic development of rice for water-limiting situations has slowed due to a lack of contemporary molecular techniques for screening of breeding lines for drought tolerance and a lack of understanding of the specific mechanisms of drought tolerance and the inheritance of drought tolerance traits. Breeding programmes that make use of molecular marker technologies are more productive, can move desired genes more quickly between varieties, and can introduce novel genes from related or cultivated species. Marker Assisted Selection (MAS) programmes rely on the identification of parental polymorphism in rice cultivars through the screening of markers. This allows for the subsequent tagging and fine mapping of the target gene within the rice chromosome. Polymorphic markers discovered in this work will be used to genotype the entire mapping population and facilitate the introgression of drought-responsive QTLs/genes involved in grain production throughout the reproductive stage. As a result, the findings of this study will aid in the creation of a new, better drought-resistant variety of rice.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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