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Research Article

Parental evaluation and polymorphism survey of drought contrasting donor and recurrent parents in rice (*Oryza sativa*. L) using microsatellite markers

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Abstract

Rice is an important food crop which is considered as lifeline for more than half of the world's population. Rice requires larger amount of water throughout its life cycle than other crops. Parental lines of Kasturi and Chaw Khao were evaluated and exhibited variation for panicle length, plant height and plot yield in stress condition. Association analysis also shown plant height and plot yield was positively associated with plot yield. In this study parental polymorphism survey was conducted between Kasturi and Chaw Khao using 721 microsatellite (SSR) markers spanning over the entire 12 chromosome and resulted in 95 polymorphic SSR markers. The overall polymorphism survey for all 12 chromosomes was found to be 13.17%. The highest and lowest polymorphism was noticed in chromosome 5 (17.02 %) and chromosome 10 (5.36 %), respectively. This investigation will be helpful to genotype mapping population, construction of linkage map and grain yield QTL identification for drought stress.

Keywords

Rice, drought, SSR markers, parental polymorphism

Introduction

Rice (*Oryza sativa* L.) is a 'Global Grain' cultivated widely across the world and feeding millions of people and 90% of the rice mostly consumed by the Asians. In 2018, the world produced 725.17 million tons of rice from 155.7 million ha of area. Of these, Asian farmers produced around 600 million tons, which represents more than 90% of global rice production. India and China together accounted for 366.65 million tons, with India producing 163.52 million tons (<http://ricestat.irri.org:8080/wrsv3/entrypoint.htm>, 2018). Rice is cultivated under diverse ecologies ranging from irrigated to rainfed upland to rainfed lowland to deep water. In India, the total area under irrigated, rain fed lowland and upland rice is 22.0, 14.4, and 6.3 million ha, respectively (Singh, 2009).

In rainfed lowland areas, one of the major abiotic constraints in rice production is water stress, including deficit (drought) or excess water (flood) (O' toole 1979). Drought stress is a serious limiting factor to rice production and yield stability in rainfed rice area. A recent estimate on climate change predicts the water deficit to deteriorate further in years to come (Wassmann *et al.*, 2009) the situation for drought is predicted to become worse. Out of the total 20.7 million ha of rainfed rice area reported in India, approximately 16.2 million ha grown in

eastern India (Singh and Singh, 2000), of which 6.3 million ha of upland area and 7.3 million ha of lowland area are highly drought-prone (Pandey and Bhandari, 2009). Despite the importance of drought as a constraint, little effort has been devoted to developing drought-tolerant rice cultivars. Most of the high-yielding varieties like IR36, IR64, Swarna, and Samba Mahsuri which are grown in rain fed areas but bred for irrigated ecosystems and they were never selected for drought tolerance. In drought years, these varieties inflict high yield losses, leading to a sudden decline in the country's rice production. Because of the absence of high-yielding, good-quality drought-tolerant varieties, farmers are not able to continue to grow rice varieties in drought affected areas. Farmers of drought-prone areas require varieties that are able to overcome yield penalty under drought stress condition. Keeping the above situation in view, the present investigation was taken up to meet the upcoming challenges.

Materials and Methods

The experiment was conducted in IRRI South Asia Hub, Hyderabad during wet season 2015. Twenty five days old seedlings of Kasturi and donor Chaw Khao were transplanted in the main field for evaluation. Leaf samples were collected 15-20 days after transplanting which were used for parental

polymorphism after DNA extraction. Genomic DNA was isolated from parents by TPS method for genotyping (Lenie Quiatchon unpublished, IRRRI - Japan Collaboration project). The step wise protocol is presented below for the TPS method, ~2-cm lengths of rice leaf tips were collected and ground by using a Geno Grinder after placing two magnetic beads in each well, after which TPS buffer of nearly 600 μ l [100 mM Tris HCl (pH 8.0), 1 M KCl, 10 mM EDTA] was added to each tube. Samples were incubated in water bath at 55^o C for 30 minutes which were then centrifuged at 3000 rpm for 30 minutes. After centrifugation, supernatant were transferred into new wells, and isopropyl alcohol was added. Samples were then incubated at -20^o C refrigerator for 24 hours. After 24 hours, pellets were washed using 100 μ l 70% ethanol by short centrifugation. Ethanol wash step removes salt and other impurities which are then allowed to dry to remove ethanol smell. Dried samples were dissolved using 200 μ l TE buffer TE [10 mM Tris HCl (pH 8.0), 1 mM EDTA]. The purified DNA samples were then genotyped by using SSR markers. Quantification of DNA was done by analyzing the purified DNA on 0.8 percent agarose gel. Based on the intensity and thickness of genomic DNA bands when compared to DNA, the concentration and quality of DNA in individual samples were determined.

A set of 721 SSR markers covering all the 12 chromosomes of rice were used for parental polymorphism survey. About, 2 μ l of diluted template DNA (40 ng / μ l) of each line was dispensed in the bottom of 96 well PCR plates (AXYGEN-MAKE). Primers were added separately and PCR master mix (dNTPs, taq buffer, taq enzyme and sterile water) was prepared in an Eppendorf tube and added. About 8 μ l of the PCR master mix was added to each tube to make final volume to 10 μ l. The polymerase chain reaction comprised of one cycle of denaturation at 95^oC for 5 min, followed by 35 cycles at 95^oC for 30 s, 55^oC for 30 s and 72^oC for 1 min, with a final extension of 72^oC for 7 min. The amplified products were resolved on 3% SeaKem LE Agarose (Lonza USA) gel containing 0.1 mg/ml of ethidium bromide along with 100 bp DNA ladder. Amplified products were electrophoresed in 3% agarose gel matrix and documented with the help Syngene (Model: GBox F3, United Kingdom).

Results and Discussion

Field evaluation under stress condition reported variation between the two parents and checks *viz.*, IR64 and Sahbhagi Dhan in most of the traits

especially plant height, panicle length and plot yield but much variation was not observed in non-stress condition. Correlation among the traits in stress condition recorded significant positive association of plant height and panicle length with plot yield under stress (Fig. 1). In this study microsatellite markers were used to study polymorphism between parents and moreover, the advantage of SSRs is co-dominant and variable in nature (Weber and May 1989). In general, various DNA markers can be used to study the polymorphism survey and molecular mapping of target QTLs/genes (Weising *et al.*, 1995) but SSRs are having some selective advantages over other marker types. McCouch *et al.* (1997) reported that SSR markers are highly used in DNA fingerprinting, genetic diversity assessment, introgression, molecular mapping QTLs/genes and marker assisted selection. Marker assisted breeding programme or molecular breeding programme is primarily based on polymorphic molecular markers between the parental lines which are involved in the development of mapping population. The parental polymorphism survey indicated that a clear polymorphism was observed between the parents. A total of 721 SSR primers over 12 chromosomes were used for testing polymorphism between two parents. Out of 712 SSRs, 95 SSR primer pairs exhibited polymorphism (Table 1) between recipient parent Kasturi and donor parent Chaw Khao with the polymorphism percentage of 13.17 (Fig 2). Among 12 chromosomes, chromosome 5 recorded the highest polymorphism percentage of 17.02 per cent and chromosome 10 recorded the lowest polymorphism percentage of 5.36 per cent (Table 2). In earlier reports of Marathi *et al.* (2011) reported maximum polymorphism was observed in chromosome 4 with overall polymorphic percentage of 32.93. Polymorphism survey between popular rice varieties of Andaman and Nicobar Islands *viz.* C14-8, CARI Dhan 5 and donor IRBB 60 was studied using 200 highly variable SSR markers which reported that, 36 and 48 SSR markers displayed polymorphism for C14-8 and CARI Dhan 5, respectively and also chromosome 4 showed the highest polymorphic percentages in the C14-8 (53%) as well as CARI Dhan 5 (41 %) as compared to IRBB60 (Gautam *et al.*, 2015). Parental polymorphism involved 500 SSR markers spanning the entire 12 chromosomes, among which 70 markers were found polymorphic with 14% of polymorphism between two parents ARC10531 and BPT-5204 (Yadav *et al.*, 2015). Channamallikarjuna *et al.* (2010) in their investigation, 637 SSR markers were used to determine DNA polymorphism between the parents



HP2216 and Tetep and found only 74 markers were polymorphic in order to map the QTLs for sheath blight resistance in rice. In many cases, parents that provide adequate polymorphism were selected on the basis of level of genetic diversity between them (Anderson *et al.*, 1993). In an another study, Yerva *et al.* (2018) screened two parents namely PR122 and IR10M196 for parental polymorphism using 647 SSR markers, of which 108 markers exhibited polymorphism with the level of 16.69%. Molecular marker technology has greatly increased the efficiency and made introgression of genes from wild source easy. Polygenic markers which were previously difficult to analyze using traditional breeding methods, would now be easily tagged using molecular markers. The screening of markers for parental polymorphism among the rice cultivars forms the basis for tagging of the desired gene, fine mapping of gene in the rice chromosome and in the subsequent Marker Assisted Selection (MAS) programmes. The markers which are found to be polymorphic can be used in molecular breeding study for grain yield under drought.

The identified polymorphic markers from the present study will be utilized for genotyping the whole mapping population and mapping of QTLs/genes associated with grain yield in reproductive stage drought. Furthermore, the identified QTLs can be validated and utilized in markers assisted introgression programmes to improve economic yield under drought prone environment.

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Table 1. Identified polymorphic markers between the parents Kasturi and Chaw Khao

S. no	Primer name	Chr. no	Forward sequence (5'-3')	Reverse sequence (3'-5')
1	RM9	1	GGTGCCATTGTCGTCCTC	ACGGCCCTCATCACCTTC
2	RM212	1	CCACTTTCAGCTACTACCAG	CACCCATTTGTCTCTCATTATG
3	RM243	1	GATCTGCAGACTGCAGTTGC	AGCTGCAACGATGTTGTCC
4	RM466	1	TCCATCACCACATTCCCC	ACCCTTCTCTCGCTCTCTCC
5	RM472	1	CCATGGCCTGAGAGAGAGAG	AGCTAAATGGCCATACGGTG
6	RM488	1	CAGCTAGGGTTTTGAGGCTG	TAGCAACAACCAGCGTATGC
7	RM490	1	ATCTGCACACTGCAAACACC	AGCAAGCAGTGCTTTCAGAG
8	RM493	1	TAGCTCCAACAGGATCGACC	GTACGTAAACGCGGAAGGTG
9	RM495	1	AATCCAAGGTGCAGAGATGG	CAACGATGACGAACAACAACC
10	RM579	1	TCCGAGTGGTTATGCAAATG	AATTGTGTCCAATGGGCTGT
12	RM572	1	CGGTAAATGTCATCTGATTGG	TTCGAGATCCAAGACTGACC
13	RM1	1	GCGAAAACACAATGCAAAAA	GCGTTGGTTGGACCTGAC
14	RM594	1	GCCACCAGTAAAAGCAATAC	TTGATCTGCTAGTGAGACCC
15	RM154	2	GACGGTGACGCACTTTATGAACC	CGATCTGCGAGAAACCCTCTCC
16	RM174	2	AGCGACGCCAAGACAAGTCGGG	TCCACGTGATCGACACGACGG
17	RM211	2	CCGATCTCATCAACCTTCTG	CTTCACGAGGATCTCAAAGC
18	RM236	2	GCGCTGGTGAAAAATGAG	GGCATCCCTCTTTGATTCTCTC
19	RM300	2	GCTTAAGGACTTCTGCGAACC	CAACAGCGATCCACATCATC
20	RM324	2	CTGATTCCACACACTTGTGC	GATTCCACGTCAGGATCTTC
21	RM341	2	CAAGAAAACCTCAATCCGAGC	CTCCTCCCGATCCCAATC
22	RM492	2	CCAAAAATAGCGCGAGAGAG	AAGACGTACATGGGTACGGC
23	RM13213	2	GTTTCTCCACCACCGTCAGTCG	CCCTCACTTCACTAGTCCGTAGCC
24	RM71	2	CTAGAGGCGAAAACGAGATG	GGGTGGGCGAGGTAATAATG
25	RM7	3	TTCGCCATGAAGTCTCTCG	CCTCCCATCATTTCTGTTGTT
26	RM81a	3	GAGTGCTTGTGCAAGATCCA	CTTCTTCACTCATGCAGTTC
27	RM135	3	CTCTGTCTCCTCCCCGCGTCG	TCAGCTTCTGGCCGGCTCCTC
28	RM143	3	GTCCCGAACCCTAGCCCCGAGGG	AGAGGCCCTCCACATGGCGACC
29	RM251	3	GAATGGCAATGGCGCTAG	ATGCGGTTCAAGATTCTGATC
30	RM520	3	AGGAGCAAGAAAAGTTCCCC	GCCAATGTGTGACGCAATAG
31	RM545	3	CAATGGCAGAGACCCAAAAG	CTGGCATGTAACGACAGTGG
32	RM565	3	AGTAACGAGCATAGCAGGCG	GCAAAGCCTTCAGGAATCAG
33	RM16030	3	GCGAACTATGAGCATGCCAACC	GGATTACCTGGTGTGTGCAGTGCC
34	RM227	3	ACCTTTCGTCATAAAGACGAG	GATTGGAGAGAAAAGAAGCC
35	RM347	3	CACCTCAAACCTTTTAACCGCAC	TCCGGCAAGGGATACGGCGG
36	RM119	4	CATCCCCCTGCTGCTGCTGCTG	CGCCGGATGTGTGGGACTAGCG
37	RM252	4	TTCGCTGACGTGATAGGTTG	ATGACTTGATCCCAGAAACG
38	RM303	4	GCATGGCCAAATATTAAGG	GGTTGGAAATAGAAGTTCGGT
39	RM537	4	CCGTCCCTCTCTCCTTTC	ACAGGGAAACCATCCTCTCTC
40	RM185	4	AGTTGTTGGGAGGGAGAAAGGCC	AGGAGGCGACGGCGATGTCCTC
41	RM2441	4	GATTACCACGTTGAGCAAAGG	ACGTTTACCAACCACGGATTACG
42	RM39	5	GCCTCTCTCGTCTCCTTCTC	AATTCAAACCTGCGGTGGC
43	RM87	5	CCTCTCCGATACACCGTATG	GCGAAGGTACGAAAGGAAAG
44	RM164	5	TCTTGCCCGTCACTGCAGATATCC	GCAGCCCTAATGCTACAATTCTTC
45	RM169	5	TGGCTGGCTCCGTGGGTAGCTG	TCCCGTTGCCGTTTCTCCCTCC
46	RM267	5	TGCAGACATAGAGAAGGAAGTG	AGCAACAGCACAACTTGATG
47	RM289	5	TTCCATGGCACACAAGCC	CTGTGCACGAACTTCCAAG
48	RM334	5	GTTTCAGTGTTTCAGTGCCACC	GACTTTGATCTTTGGTGGACG
49	RM440	5	CATGCAACAACGTCACCTTC	ATGGTTGGTAGGCACCAAAG
50	RM170	6	TCGCGCTTCTCCTCGTCGACG	CCCCTTGCAGAGGAAGCAGCC
51	RM176	6	CGGCTCCCGCTACGACGCTCCTC	AGCGATGCGCTGGAAGAGGTGC
52	RM276	6	CTCAACGTTGACACCTCGT	TCCTCCATCGAGCAGTCA
53	RM494	6	GGGAGGGGATCGAGATAGAC	TTTAACTTCTTCCGATCC
54	RM508	6	GGATAGATCATGTGTGGGGG	ACCCGTGAACCACAAAGAAC
55	RM588A	6	GTTGCTCTGCCTCACTCTTG	AACGAGCCAACGAAGCAG
56	RM597	6	CCTGATGCACAACCTGCGTAC	TCAGAGAGAGAGAGAGAGAGAG



57	RM11	7	TCTCCTCTTCCCCGATC	ATAGCGGGCGAGGCTTAG
58	RM125	7	ATCAGCAGCCATGGCAGCGACC	AGGGGATCATGTGCCGAAGGCC
59	RM320	7	CAACGTGATCGAGGATAGATC	GGATTTGCTTACCACAGCTC
60	RM432	7	TTCTGTCTCACGCTGGATTG	AGCTGCGTACGTGATGAATG
61	RM51	7	TCTCGATTCAATGTCCTCGG	CTACGTCATCATCGTCTTCCC
62	RM248	7	TCCTTGTGAAATCTGGTCCC	GTAGCCTAGCATGGTGCATG
63	RM445	7	CGTAACATGCATATCACGCC	ATATGCCGATATGCGTAGCC
64	RM473	7	TATCCTCGTCTCCATCGCTC	AAGGATGTGGCGGTAGAATG
65	RM25	8	GGAAAGAATGATCTTTTCATGG	CTACCATCAAACCAATGTTC
66	RM149	8	GCTGACCAACGAACCTAGGCCG	GTTGGAAGCCTTTCCTCGTAACACG
67	RM223	8	GAGTGAGCTTGGGCTGAAAC	GAAGGCAAGTCTTGGCACTG
68	RM310	8	CCAAAACATTTAAAATATCATG	GCTTGTGGTTCATTACCATTTC
69	RM408	8	CAACGAGCTAACTTCCGTCC	ACTGCTACTTGGGTAGTGACC
70	RM515	8	TAGGACGACCAAAGGGTGAG	TGGCCTGCTCTCTCTCTCTC
71	RM72	8	CCGGCGATAAAACAATGAG	GCATCGGTCCTAACTAAGG
72	RM210	8	TCACATTCGGTGGCATTG	CGAGGATGGTTGTTCACTTG
73	RM44	9	ACGGGCAATCCGAACAACC	TCGGGAAAACCTACCCTACC
74	RM242	9	GGCCAACGTGTGTATGTCTC	TATATGCCAAGACGGATGGG
75	RM257	9	CAGTCCGAGCAAGAGTACTC	GGATCGGACGTGGCATATG
76	RM410	9	GCTCAACGTTTCGTTCCTG	GAAGATGCGTAAAGTGAACGG
77	RM464	9	GAAGCAGGAAACAAGAAGAGAAGG	GTCTTACCACAGTAAATGCTTGC
78	RM23668	9	TGCATAGCATATCAACTAGCCCTACC	GCTGAAACAGAATGAAAGCACAGC
79	RM23911	9	TGCCTGCACTTATCTCTTGATGC	GATGAACCTAAAGGGCAGTTTCC
80	RM496	10	GACATGCGAACAACGACATC	GCTGCGGCGCTGTTATAC
81	RM6100	10	TCCTTACCAGTACCGCACC	GCTGGATCACAGATCATTGC
82	RM216	10	GCATGGCCGATGGTAAAG	TGTATAAAACCACACGGCCA
83	RM21	11	ACAGTATTCGATAGGCACGG	GCTCCATGAGGGTGGTAGAG
84	RM144	11	TGCCCTGGCGCAAATTTGATCC	GCTAGAGGAGATCAGATGGTAGTGCATG
85	RM206	11	CCCATGCGTTTAACTATTCT	CGTTCCATCGATCCGATGG
86	RM224	11	ATCGATCGATCTTCACGAGG	TGCTATAAAAGGCATTCCGGG
87	RM287	11	TTCCCTGTAAAGAGAGAAATC	GTGTATTTGGTGAAGCAAC
88	RM187	11	CCAAGGGAAAGATGCGACAATTG	GTGGACGCTTTATATTATGGG
89	RM229	11	CACTCACACGAACGACTGAC	CGCAGGTTCTTGTGAAATGT
90	INDEL8	12	CCATTCTTGAGGGAGCAGTC	CACAGTGGCCAAAAATGCTA
91	RM19	12	CCCATCCTCACCGATCTCTTAAAC	GTGCGCACGGAGGAGGAAAGGG
92	RM260	12	ACTCCACTATGACCCAGAG	GAACAATCCCTTCTACGATCG
93	RM28099	12	TGTGCGGATGCGGGTAAAGTCC	CCACCTGTCAACCACCGAAACC
94	RM28130	12	CAGCAGACGTTCCGGTTCTACTCG	AGGACGGTGGTGGTGTACTGG
95	RM28311	12	TGATGTTGTCATCAGGCATGTAGC	AGATTTGGGCTGGTTGCATTAGG

Table 2. Chromosomal wise polymorphism percentage of SSR markers between Kasturi and Chaw Khao

Chromosome No.	No. of SSR markers used	No. of polymorphic markers	Polymorphism %
Chromosome 1	85	14	16.47
Chromosome 2	86	10	11.63
Chromosome 3	71	11	15.49
Chromosome 4	54	6	11.11
Chromosome 5	47	8	17.02
Chromosome 6	51	7	13.73
Chromosome 7	57	8	14.04
Chromosome 8	55	8	14.55
Chromosome 9	50	7	14.00
Chromosome 10	56	3	5.36
Chromosome 11	61	7	11.48
Chromosome 12	48	6	12.50
Total	721	95	13.17

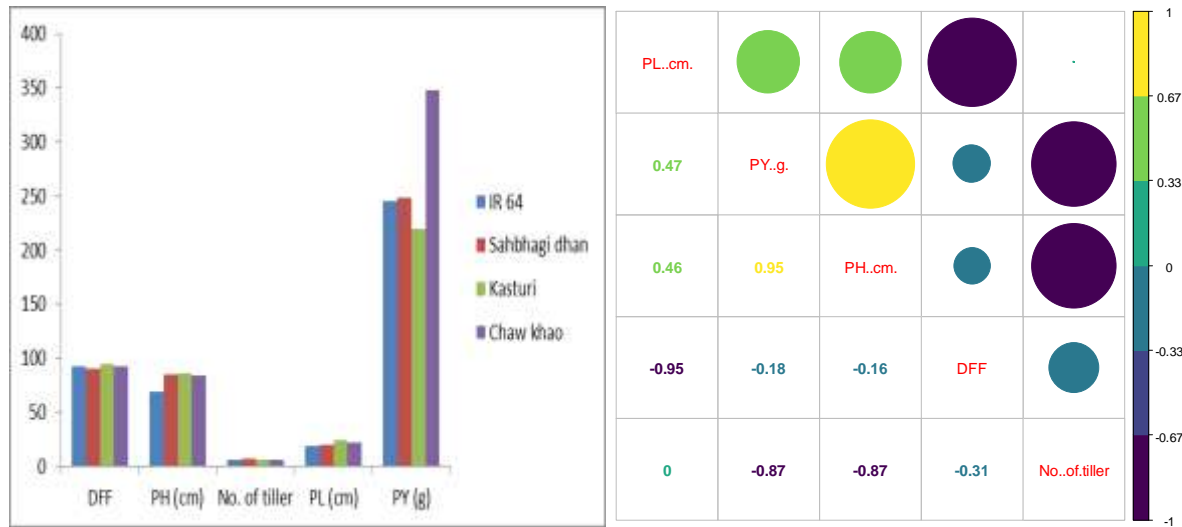
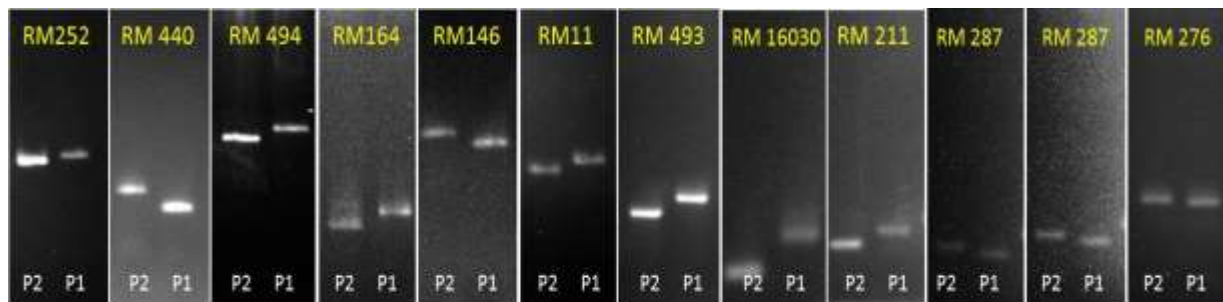


Fig. 1. Trait variation and correlation analysis of important traits among parents and checks under drought stress condition



P1- Kasturi (recipient parent), P2- Chawkhao (donor parent)

Fig. 2. Polymorphic profile of parental lines of Kasturi and Chaw Khao

