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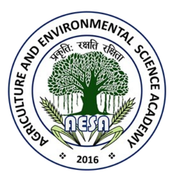

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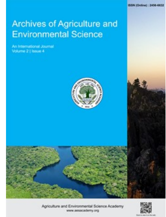

ORIGINAL RESEARCH ARTICLE

Screening microsatellite markers for establishing parental polymorphism in Indian rice (*Oryza sativa* L.)

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ABSTRACT

The experiment was conducted to investigate the parental diversity along the rice genome and to understand and screen out the SSR markers-indicated polymorphism between two indica rice (*Oryza sativa* L.) cultivars. Namely K343, the most well-liked rice variety in the hill zone of the Jammu Region, and RML22, a rice line created at IRRI, Philippines. The study is to select polymorphic markers (Simple Sequence Repeat- SSR) associated with hill ecologies rice cultivars and additional research projects like gene pyramiding and background selection to recover the recurrent parent genome (RPG) in blast gene introgression in elite lines. 450 SSR markers, evenly distributed throughout the rice genome, were used to assess the parental polymorphism between these genotypes. Of these two cultivars, 51 markers (11.33%) showed polymorphism with bands in different spectrums throughout the genome. The study has been used to Marker Assisted Backcross (MAB) breeding to integrate rice blast resistance genes in the parental genotype. The pool of polymorphic markers has the potential to use in similar studies and work, with a high probability of polymorphism for the cultivars of hill ecologies, and thus increase the chance of selection of probability in marker selection.

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INTRODUCTION

Rice (*Oryza sativa* L.) is one of the most important staple food crops for over 3.5 billion people (Gnanamanickam, 2009). Rice is a monocot plant that belongs to the genus Oryza and the family Poaceae with two domestic species and 23 wild species. Despite significant increases in rice production across the globe in recent decades, the amount produced still needs to catch up with the world's rising demand. To meet the demand of the expanding human population by 2050, rice production should increase by at least 70% (Leegood *et al.*, 2010). Conventional plant breeding methods and techniques are extensively used and have improved the quality and quantity of rice plants, which are well-known and still in practice. Conventional plant breeding approaches are time-consuming and laborious and have several other ecological, physiological, and biological constraints. Researchers are now, therefore, concentrating on new modern breeding methods such as marker-assisted breeding, recombinant DNA technology, and 'omics' sciences (genomics, proteomics, metabolomics) to improve the yield of rice plants by generating improved disease resistance (Chen *et al.*, 2018; Huang *et al.*, 2015; Liang *et al.*, 2020; Raboin *et al.*, 2016; Shen *et al.*, 2004; Wang and Zhao, 2010; Ying *et al.*, 2022) and grain quality improvement in rice plants to address the issues described above (Feng *et al.*, 2016).
However, the precision of biotechnological approaches is mainly genetic engineering and genetic mapping. These approaches contribute rapidly and significantly to crop improvement by offering a wide array of novel genes and trait identification. The various molecular markers employed in plants and their usage in the creation of linkage maps, genetic mapping, and marker-assisted selection (MAS) approaches have been the subject of

several excellent reviews (Agarwal *et al.*, 2008). The earlier efforts of the breeders to assess genetic diversity using conventional phenotypic traits were less efficient because of their interaction with the environment. Besides, assessing the transfer of genetic material, specifically disease resistance genes or with a quality attributed one, cannot be ensured and quantified. Additionally, the molecular markers have made it possible to assess the diverse cultivars more efficiently for prospective utilization as parents. Simple sequence repeat (SSR) is one of the most useful molecular markers for assessing the genetic relationships among plant cultivars (Choudhary *et al.*, 2013; Flint-Garcia *et al.*, 2005; Gaikwad *et al.*, 2014; Gawenda *et al.*, 2011; Huang *et al.*, 2015; Zhao *et al.*, 2014). They are multiallelic, highly polymorphic, co-dominant, and abundant in the genome. Hence, it is crucial to identify and select the ideal parents for hybridization and a back-cross breeding program with the assistance of marker-assisted selection using polymorphic markers among the parents.

Parental polymorphism and the polymorphic markers are the choice tool to assess the percent genome recovery in subsequent generations. Furthermore, combined with the phenotypic trait analysis, it is a choice tool for varietal development in the Indian subcontinent. The SSRs are therefore used in this study to assess the polymorphism among the parents.

MATERIALS AND METHODS

Plant material

The plant material consisted of two non-basmati *indica* rice genotypes, K343, a rice cultivar developed and released by SKUAST-K for hill and temperate ecologies of J&K in 1996. It is a bold grain, long-duration (130-140 days) rice cultivar with a yield potential of 50-60 q/ha. The nucleus seed of this cultivar was obtained from Mountain Rice Research Station, SKUAST-K, Khudwani, Kashmir. It is a predominant rice cultivar in the hill zone of Jammu and Kashmir, and RML22 is an *indica* rice line developed at IRRI, Philippines. It is the donor of the blast resistance gene (*Pi9)* against the prevalent races of *Magnaporthe oryzae*. It is a long-duration (130-140 days) genotype.

DNA isolation, PCR conditions, and Electrophoresis

DNA isolation of parental genotypes was carried out from fresh leaves of 3-week-old plants using the CTAB method (Doyle & Doyle, 1990) with certain modifications (Clarke, 2009). Quality and quantity of DNA were checked on 0.8% agarose gel, and concentration was normalized to ~50 ng μL−1. DNA amplification was carried out in Polymerase chain reaction (PCR) tubes containing a 10 μL reaction mixture. The reaction mixture contained 1 μL of template DNA (50ng/μl), 2.5 mM/ μl of each dNTP (dTTPs, dGTPs, dCTPs, dATPs), 0.5 μL each forward and reversed primer, 5 U of Taq polymerase (D1806- Sigma Aldrich, USA), 2.2 μL of 10X PCR buffer with $MgCl_2$. The PCR thermal profile in 96 well Universal Gradient Thermal Cycler (Eppendorf AG, Hamburg, Germany) was carried out with an initial denaturation step of 5 minutes programmed in the thermocycler, followed by a loop of 35 cycles, each consisting of denaturation (94°C for 30 sec), annealing (55°C - 58° C for 35 sec depending on the marker used) and extension (72°C for 30 sec). The final extension was performed at 72°C for 7 min. The PCR results were then kept at 4°C for storage. The PCR products were resolved on 3.0 percent Metaphor TM Agarose gel containing ethidium bromide in 1 x TAE buffer at 130V and visualized on ultraviolet UV light (MiniLumi by DNR Bio-Imaging System, Israel). A total of 450 SSR markers were screened to identify markers polymorphic between parental lines of K 343/RML 22. The markers that could clearly differentiate between alleles of two parents with respect to the particular locus on 3 percent agarose gel were declared polymorphic.

RESULTS AND DISCUSSION

Out of 450 SSR markers, 51 markers (11.33%) depicted variation in the location of bands/size of amplicons concerning the parents (K343/RML22) (Figure 1, Figure 2, and Figure 3). Among all the identified polymorphic SSR markers, RM6, RM11, RM1347, RM1370, RM263, RM274, RM475, RM430, RM286, RM440, RM1024, RM16, RM5720, RM242, RM240, RM480 markers were found to be highly polymorphic between the two parents who indicated close similarity between genetic backgrounds of donor and recipient parents used in the present study. The similarity in the genetic background could be attributed to the fact that donor, as well as recipient genotypes, were of *indica* type. Other studies have reported similar outcomes (Rajendrakumar *et al.*, 2007; Rani and Adilakshmi, 2011; Rathour *et al.*, 2008; Sarao *et al.*, 2010; Sharma *et al.*, 2005).

These polymorphic markers were further utilized for confirmation of hybridity of F_1 plants and background screening for analysis of percent recovery of recurrent parent genome (RPG) in target gene-positive plants using genome-wide polymorphic SSR markers. Thus, marker-assisted background selection is a potential tool to identify plants with more than average recovery of recurrent parent genome and thus accelerates the development of crop varieties compared to the conventional backcross breeding approach. Integration of foreground, background, and/ or phenotypic selection to achieve high recovery of recurrent parent genome and phenome has been practiced in various studies (Divya *et al.*, 2014; Gopalakrishnan *et al.*, 2008; Jindal *et al.*, 2012; Neeraja *et al.*, 2005; Singh *et al.*, 2012; Sundaram *et al.*, 2008). The application of MAS with the MABC breeding program thus accelerates the recovery of the recurrent parent genome, reducing the number of generations and the time for incorporating resistance against rice blasts. Molecular methods to identify novel resistance genes followed by their introgression into the elite genetic background are potential tools to improve significantly modern cultivars. Such methods would more efficiently supplement conventional breeding approaches for accelerated development of genetic stocks/lines/varieties. The list of polymorphic 51 SSR markers, their forward, reverse sequence, annealing temperature, and expected product size is given in Table 1.

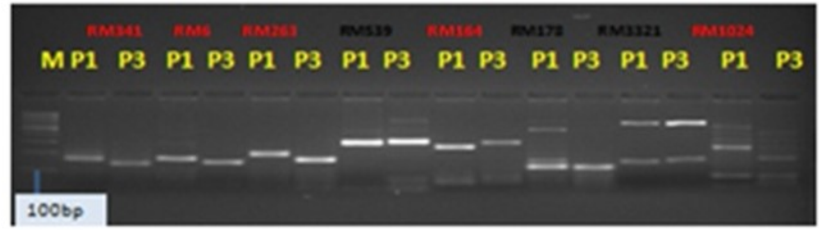

Figure 1. *The banding pattern of SSR markers with respect to parents (P1=K343, P3= RML22); Markers in red are polymorphic.*

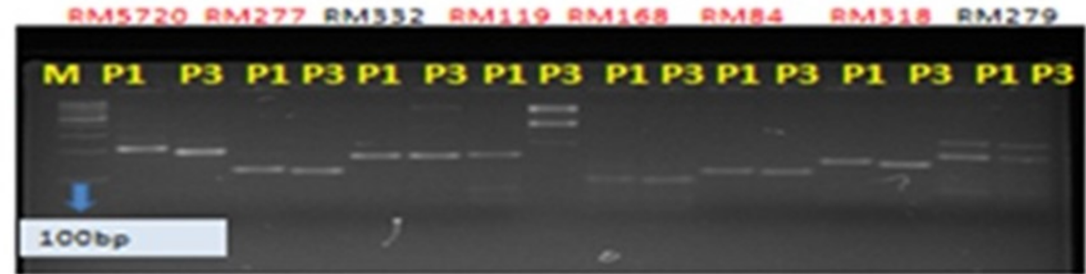

Figure 2. *Banding pattern of SSR markers with respect to parents (P1=K343, P3= RML22); Markers in red are polymorphic.*

Figure 3. *Banding pattern of SSR markers with respect to parents (P1=K343, P3= RML22); Markers in red are polymorphic.*

Table 1. List of polymorphic SSR markers for parental genotypes K343 and RML22.

S.N.	Marker	Marker Sequence	T_m °C	Expected product size (bp)
1	RM475	5' CCTCACGATTTTCCTCCAAC 3' 3' ACGGTGGGATTAGACTGTGC 5'	55	235
2	RM430	5' AAACAACGACGTCCCTGATC 3' 3' GTGCCTCCGTGGTTATGAAC 5'	55	173
3	RM440	5' CATGCAACAACGTCACCTTC 3' 3' ATGGTTGGTAGGCACCAAAG 5'	55	169
4	RM334	5' GTTCAGTGTTCAGTGCCACC3' 3'GACTTTGATCTTTGGTGGACG5'	55	182
5	RM583	5' AGATCCATCCCTGTGGAGAG3' 3'GCGAACTCGCGTTGTAATC5'	55	192
6	RM162	5' GCCAGCAAAACCAGGGATCCGG 3' 3' CAAGGTCTTGTGCGGCTTGCGG 5'	55	229
7	RM225	5' TGCCCATATGGTCTGGATG 3' 3'GAAAGTGGATCAGGAAGGC 5'	55	140
8	RM587	5' ACGCGAACAAATTAACAGCC 3' 3'CTTTGCTACCAGTAGATCCAGC 5'	55	217
9	RM11	5' TCTCCTCTTCCCCCGATC 3' 3'ATAGCGGGCGAGGCTTAG 5'	55	140
10	RM286	5' GGCTTCATCTTTGGCGAC3' 3'CCGGATTCACGAGATAAACTC5'	55	110
11	RM218	5' TGGTCAAACCAAGGTCCTTC 3' 3'GACATACATTCTACCCCCGG 5'	55	148
12	RM220	5' GGAAGGTAACTGTTTCCAAC 3' 3'GAAATGCTTCCCACATGTCT 5'	55	127
13	RM408	5' CAACGAGCTAACTTCCGTCC 3' 3' CAACGAGCTAACTTCCGTCC 5'	55	128
14	RM234	5' ACAGTATCCAAGGCCCTGG 3' 3' CACGTGAGACAAAGACGGAG 5'	55	156
15	RM263	5' CCCAGGCTAGCTCATGAACC 3' 3' GCTACGTTTGAGCTACCACG 5'	55	199

Table 1. *Contd…..*

16	RM333	5' GTACGACTACGAGTGTCACCAA 3' 3' GTCTTCGCGATCACTCGC 5'	55	191
17	RM304	5' TCAAACCGGCACATATAAGAC 3' 3' GATAGGGAGCTGAAGGAGATG 5'	55	160
18	RM231	5' CCAGATTATTTCCTGAGGTC 3' 3'CACTTGCATAGTTCTGCATTG 5'	55	182
19	RM240	5' CCTTAATGGGTAGTGTGCAC 3' 3' TGTAACCATTCCTTCCATCC 5'	55	132
20	RM167	5' GATCCAGCGTGAGGAACACGT 3' 3' AGTCCGACCACAAGGTGCGTTGTC 5'	55	128
21	RM82	5' TGCTTCTTGTCAATTCGCC 3' 3' CGACTCGTGGAGGTACGG 5'	55	186
22	RM274	5' CCTCGCTTATGAGAGCTTCG 3' 3'CTTCTCCATCACTCCCATGG 5'	55	160
23	RM242	5' GGCCAACGTGTGTATGTCTC 3' 3' TATATGCCAAGACGGATGGG 5'	55	225
24	RM324	5' CTGATTCCACACACTTGTGC 3' 3' GATTCCACGTCAGGATCTTC 5'	55	175
25	RM5488	5' CTCCCTCTTCCTCTGTGTGC 3' 3' CTCAGAGGAACAGCTGGGTC 5'	55	136
26	RM1112	5' TCAGGACACATGGCCCTTAC 3' 3' CAGCTCCTGACAGAGCACAC 5'	55	107
27	RM556	5' ACTCCAAACCTCACTGCACC 3' 3' TAGCACACTGAACAGCTGGC 5'	55	93
28	RM160	5' AGCTAGCAGCTATAGCTTAGCTGGAGATCG 3' 3' TCTCATCGCCATGCGAGGCCTC 5'	55	131
29	RM4601	5' CATACATGTGAACCTGACTG 3' 3' CTAGCTTAGCATCTCCTCAA 5'	55	118
30	RM262	5' CATTCCGTCTCGGCTCAACT 3' 3' CAGAGCAAGGTGGCTTGC 5'	55	154
31	RM6	5' GTCCCCTCCACCCAATTC 3' 3' TCGTCTACTGTTGGCTGCAC 5'	55	163
32	RM3732	5' ATCCACAAACTCAGATGGGC 3' 3' TGCCACGCGATTGAAGAC 5'	55	106
33	RM245	5' ATGCCGCCAGTGAATAGC 3' 3' CTGAGAATCCAATTATCTGGGG 5'	55	150
34	RM1370	5' AAACGAGAACCAACCGACAC 3' 3' GGAGGGAGGAATGGGTACAC 5'	55	173
35	RM232	5' CCGGTATCCTTCGATATTGC 3' 3' CCGACTTTTCCTCCTGACG 5'	55	158
36	RM3	5' ACACTGTAGCGGCCACTG 3' 3' CCTCCACTGCTCCACATCTT 5'	55	145
37	RM547	5' TAGGTTGGCAGACCTTTTCG 3' 3' GTCAAGATCATCCTCGTAGCG 5'	55	235
38	RM3874	5' TGGGTGATCTTAGTTTGGCC 3' 3' AATGTGCCTGCACATGTCAC 5'	55	206
39	RM70	5' GTGGACTTCATTTCAACTCG 3' 3' GATGTATAAGATAGTCCC 5'	55	170
40	RM16	5' CGCTAGGGCAGCATCTAAA3' 3' AACACAGCAGGTACGCGC 5'	55	181
41	RM471	5' ACGCACAAGCAGATGATGAG 3' 3' GGGAGAAGACGAATGTTTGC 5'	58	106
42	RM480	5' GCTCAAGCATTCTGCAGTTG 3' 3' GCGCTTCTGCTTATTGGAAG 5'	58	225
43	RM310	5' CCAAAACATTTAAAATATCATG 3' 3' GCTTGTTGGTCATTACCATTC 5'	55	105
44	RM25003	5' GATTGATCCGAGAGACAAATCC 3' 3' TCGATCAATAGTAGCAGCAGTAGG 5'	55	115
45	RM247	5' TAGTGCCGATCGATGTAACG 3' 3' CATATGGTTTTGACAAAGCG 5'	55	160
46	RM171	5' AACGCGAGGACACGTACTTAC 3' 3' ACGAGATACGTACGCCTTTG 5'	55	328
47	RM5095	5' CTATATGACTATGCGAATGG 3' 3' ACAAATGCAACTAAGGTAGA 5'	55	182
48	RM201	5' CTCGTTTATTACCTACAGTACC 3' 3' CTACCTCCTTTCTAGACCGATA 5'	55	158
49	RM259	5' TGGAGTTTGAGAGGAGGG 3' 3' CTTGTTGCATGGTGCCATGT 5'	55	162
50	RM562	5' CACAACCCACAAACAGCAAG 3' 3' CTTCCCCCAAAGTTTTAGCC 5'	55	243
51	RM1347	5' AACAAATTAAACTGCCAAG 3' 3' GTCTTATCATCAGAACTGGA 5'	55	119

Conclusion

The result of this study clearly indicates the close association between the *indica* cultivars of rice. It is also understandable that the genome-wide association is considerable between the cultivars. Further screening of markers can indicate more polymorphic associations of the markers and thus increase the probability of selection of the same.

Conflict of interest

The authors declare no conflict of interest.

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