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Evaluation of Genetic Diversity in Rice (*Oryza sativa* L.) Using Simple Sequence Repeats (SSR) Markers

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ABSTRACT

In the study, 10 rice (*Oryza sativa* L.) accessions comprising of biofortified, aerobic, japonica donors, checks represented by indica erstwhile mega-varieties and traditional accessions differing in drought tolerance were screened for genetic diversity. These biofortified lines are a product of intense breeding efforts. Out of the 45 SSR markers used, 12 SSR markers were highly informative and polymorphic. A total of 36 alleles were detected with an average of three alleles per locus. The polymorphic information content values ranged from 0.325 (RM315) to 0.791 (RM260) in all the 12 loci with an average of 0.494. RM 260 found as the best marker for identification of genotypes as revealed by PIC values. The informative and highly informative markers identified in the study could be utilized in further studies for association mapping and marker assisted selection for drought tolerance in rice genotypes. The studied genotypes revealed high level of genetic diversity indicating the availability of such materials for rice breeding program for drought tolerance.

Keywords : Drought, oryza sativa, SSR markers, genetic diversity

DROUGHT is recognized as a major abiotic stress that limits rice productivity and adversely affects grain quality in rainfed and upland ecosystems. Rice is most sensitive to drought stress during reproductive development, when moderate water shortages can result in a significant reduction in grain yield (Venuprasad *et al*., 2008). Aerobic rice cultivation is the method of cultivation, where the rice crop is established by direct seeding in non-puddled field condition (Jana *et al*., 2015).

Characterization and quantification of genetic diversity has long been a major goal in evolutionary biology. Information on the genetic diversity within and among closely related crop varieties is essential for a rational use of genetic resources. The repeated use of the indigenous germplasm in rice breeding programs has narrowed the genetic base of newly developed rice varieties. Simple sequence repeat (SSR) is an important tool for genetic variation identification of germplasm. SSR markers have some merits such as quickness, simplicity, rich polymorphism and stability. Therefore, these are being widely applied in genetic diversity analysis, molecular map construction and gene mapping, construction of fingerprints, genetic purity test and analysis of germplasm diversity (Jin *et al.*, 2010). Many SSR markers have been reported to be linked to drought tolerance traits or QTLs in rice such as yield under drought (Vikram *et al*., 2011), maximum root length and root dry weight (Kanbar and Shashidhar, 2011).

The main aim of the study is to investigate the genetic diversity among the ten rice genotypes which differ in their tolerance to drought using SSR markers. The genotypes chosen for the study are a product of intense breeding efforts over 10 years of work at the Department of Biotechnology, Bangalore (Sumantha *et al*., 2016 and Bekele *et al*., 2013).

MATERIAL AND METHODS

Plant materials and experimental conditions

A total of 10 rice genotypes were evaluated in the study including Moroberekan, ARB-6, AM-65, AM-72, AM-1, Black rice, AM-143, Jeerigesanna, IR-64 and Azucena, which were grown in the field of aerobic rice laboratory, Department of Plant Biotechnology, University of Agricultural Sciences, Bangalore, India in Randomized Complete Block Design (RCBD) with three replications during *kharif* -2016. Aerobic rice genotypes have traits with

improved lodging resistance, input responsiveness and tolerance to occasional flooding. Donor lines like Azucena are genotypes having one or few traits of economic importance like drought tolerance. While Moroberekan is a *japonica* type which possesses a deep and thick root system, IR-64 is an *indica* type with short stature and a shallow root system.

Isolation of genomic DNA

DNA was isolated adopting CTAB method from young leaves of 21 days old plants.

SSR markers

A total of forty-five SSR primer pairs, well distributed on twelve chromosomes of rice, based on the Gramene Markers Database (http://www. gramene. org/markers) were used for studying molecular diversity. These markers were chosen based on the ability to be highly polymorphic among rice genotypes in the preliminary screening performed in previous studies and most of them were reported to be related to drought tolerance traits / QTLs (Ramadan *et al*., 2015). The polymorphism information content (PIC) value is a measure of polymorphism among varieties for a marker locus used in linkage analysis. The details of SSR primers which were highly polymorphic are presented in Table I.

PCR amplification

PCR amplification of the markers was carried out in Mastercycler® Nexus Gradient, Eppendorf. PCR amplification reactions were done in 10 µl reaction mixtures, containing 1 µl of template DNA (125 ng/µl), 0.5 µl of each forward and reverse primer (5pM), 0.5 mM dNTP, 1.5 µl Taq buffer with $MgCl_2$ (10X), 0.3 µl Taq DNA polymerase (3U/µl) and 5.7 µl ddH_2O. The following PCR program was used: an initial denaturation step at 94 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 1 min, annealing at 55-65 ^{0}C for 30 seconds and primer elongation at 72 °C for 1 min and then a final extension at 72 °C for 10 min. Amplified products were stored at -20 °C until further use.

The SSR amplification products were separated on 2.5 per cent agarose gel supplemented with

TABLE 1

Details of the microsatellite markers

Name	Product size (bp)	ForwardPrimer (5'——>3')	ReversePrimer (5'——>3')	Repeat motif
RM201	158	CTCGTTTATTACCTACAGTACC	CTACCTCCTTTCTAGACCGATA	(CT)17
RM302	156	TCATGTCATCTACCATCACAC	ATGGAGAAGATGGAATACTTGC	(GT)30(AT)8
RM7	180	TTCGCCATGAAGTCTCTCG	CCTCCCATCATTTCGTTGTT	(GA)19
RM212	136	CCACTTTCAGCTACTACCAG	CACCCATTTGTCTCTCATTATG	(CT)24
RM166	321	GGTCCTGGGTCAATAATTGGGTTACC	TTGCTGCATGATCCTAAACCGG	(T)12
RM60	165	AGTCCCATGTTCCACTTCCG	ATGGCTACTGCCTGTACTAC	(AATT)5
RM231	182	CCAGATTATTTCCTGAGGTC	CACTTGCATAGTTCTGCATTG	(GA)16
RM234	156	ACAGTATCCAAGGCCCTGG	CACGTGAGACAAAGACGGAG	(CT)25
RM1153	114	ACCAACGCCAAAAGCTACTG	TACTCGCCCTGCATGAGC	(AG)13
RM315	133	GAGGTACTTCCTCCGTTTCAC	AGTCAGCTCACTGTGCAGTG	(AT)4(GT)10
RM260	120	ACTCCACTATGACCCAGAG	GAACAATCCCTTCTACGATCG	(CT)34
RM252	216	TTCGCTGACGTGATAGGTTG	ATGACTTGATCCCGAGAACG	(GA)19

ethidium bromide. The TAE 1X was used as a running buffer and 100 bp DNA ladder was used to estimate the molecular size of the amplified fragments. Electrophoresis was conducted at 60 Volts for two hours. Gels were then visualized and photographed using Alpha Innotech gel documentation instrument.

Data analysis

All the genotypes were scored for the presence and absence of the SSR bands throughout all 10 genotypes and the data were exported to binary data for the presence (1) or absence (0) or as a missing observation for further analysis with NTSYS-pc version 2.2.

Results and Discussion

The ten rice genotypes used initially in the present study were subjected to DNA polymorphism screening and assessment using SSR markers which offer great potential for generating large numbers of markers evenly distributed throughout the genome and have efficiently been used to give reliable and reproducible genetic markers. A total of 45 SSR primer pairs with known map positions covering the whole rice genome were used to screen a set of ten selected *indica* and *japonica* rice genotypes with different levels and mechanisms of drought tolerance. Polymorphism level among rice cultivars was evaluated by calculating allelic number and PIC values. A total of 36 alleles were detected at the loci of twelve microsatellite markers across ten rice genotypes.

The number of alleles per locus generated by each marker ranged from 2 to 6 alleles with an average of 3 alleles per locus. Among the polymorphic markers, 6 produced two alleles each, 3 produced three alleles each, 2 generated four alleles each and only one produced six alleles (Table II). The highest number of alleles (6.0) was detected in the locus RM260 and the lowest number of alleles (2.0) was detected on each of locus RM201, RM302, RM212, RM166, RM231 and RM315. Similar results were obtained by Fayed *et al*. (2016) where highest polymorphism was shown by RM260 loci with PIC value (0.79) and also showed the highest number of alleles (5).

Table II

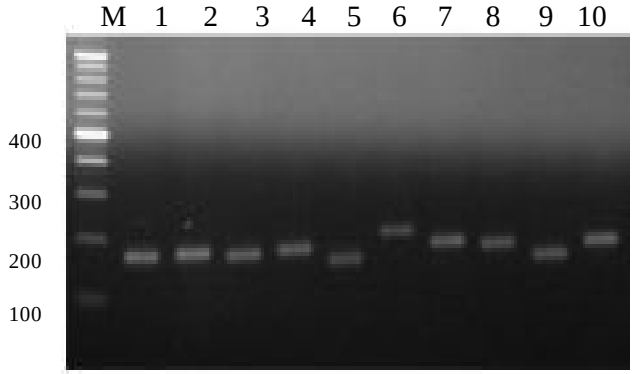
Number of alleles and polymorphism information content (PIC) values found among ten rice genotypes for 12 SSR markers

Primer name	Chromosome number	Amplicon size range (bp)	Allele number	Annealing temperature (^{0}C)	PIC value
RM201	9	140-158	2	55.0	0.480
RM302	1	120-190	2	55.0	0.440
RM7	3	159-172	4	58.0	0.570
RM212	1	122-125	2	59.0	0.495
RM166	2	305-441	2	60.3	0.495
RM60	3	164-188	3	60.3	0.425
RM231	3	175-205	2	58.4	0.495
RM234	7	133-163	4	60.3	0.570
RM1153	4	119-140	3	60.3	0.407
RM315	1	140-144	2	56.6	0.325
RM260	12	103-130	6	60.3	0.791
RM252	4	214-254	3	60.3	0.444

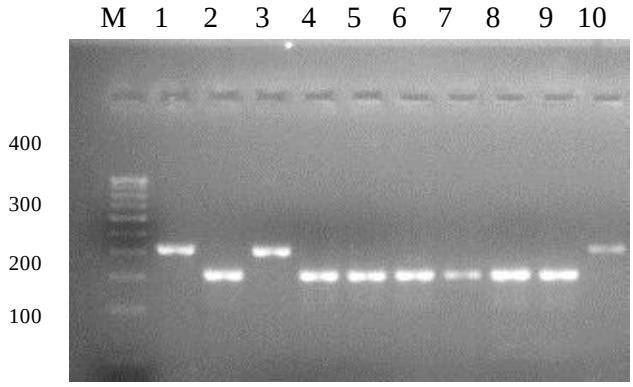
This suggests that these markers could be potentially used for molecular characterization of rice germplasm from various sources. However, there were a number of markers which produced only few alleles. Despite their ability to produce only few alleles, they were robust enough to distinguish specifically diverse genotypes or different accessions of the same genotype. Fig.1 shows a gel image of amplified fragments produced by primer RM260 and RM166.

PIC value

SSR markers are highly informative and polymorphic as evident from its PIC value. The PIC value of each marker, which can be evaluated on the basis of its alleles, varied greatly for all tested SSR loci - from 0.325 to 0.791 with an average of 0.494 (Table II). The highest PIC value of 0.791 was obtained for RM260 followed respectively by RM7 (0.570), RM234 (0.570), RM166 (0.495), RM212 (0.495) and RM231 (0.495). Table III shows the size of amplicons in different genotypes. RM201 amplified 2 alleles which were also reported by Qu *et al*. (2008). RM212 amplified 2 alleles and this result is consistent with Swamy *et al*. (2011). The different size of the amplicons shows the allelic diversity among the various genotypes which were used for the present study.



RM 260



RM166

Fig.1: Agarose gel electrophoresis of PCR amplified fragments for the polymorphic markers RM260 and RM166, M is 100bp DNA ladder; 1-Moroberekan, 2-ARB-6, 3-AM-65, 4-AM-72, 5-AM-1, 6-Black rice, 7-AM-143, 8-Jeerigesanna, 9-IR-64, 10-Azucena.

TABLE III

List of genotypes with SSR markers along with their band size

Marker name	Moroberekan	ARB-6	AM-65	AM-72	AM-1	Black rice	AM-143	Jeerigesanna	IR-64	Azucena
RM201	140	150	150	150	150	0	140	0	150	140
RM302	120	180	120	180	180	120	180	120	180	120
RM7	160	160	170	160	160	150	160	170	170	160
RM212	120	130	120	130	130	120	130	120	120	130
RM166	400	300	400	300	300	300	300	300	300	400
RM60	160	160	180	180	180	0	180	170	160	160
RM231	200	180	180	180	0	200	180	200	180	200
RM234	140	160	140	160	140	140	0	140	0	140
RM1153	140	120	140	120	120	120	120	120	120	120
RM315	140	140	140	140	140	145	140	140	140	140
RM260	170	175	175	175	160	220	200	200	160	210
RM252	200	220	200	200	200	200	200	250	220	200

The present study revealed a wide variation among the germplasms. The highly informative markers identified in the study could be utilized in further studies for association mapping and marker assisted selection for drought tolerance. The primer RM302 recognized two DNA regions (97 bp and 159 bp), the smaller one (97 bp) was found in 5 genotypes; according to the phenotypic data this type of band may be associated with drought tolerance.

The highest polymorphism was shown by RM260, RM7 and RM234 markers. These markers revealed the highest PIC values ranging from 0.570 to 0.791 and the highest number of alleles ranging from 4 to 6 alleles per locus suggesting that these markers could be used for molecular characterisation of large number of rice genotypes. The most significant application of these identified major QTLs for drought tolerance is to collect those favorable alleles into elite local line through marker assisted breeding.

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