International Journal of Biosciences (IJB)
ISSN: 2220-6655 (Print) 2222-5234 (Online)
Vol. 2, No. 10(2), p. 64-72, 2012
http://www.innspub.net

RESEARCH PAPER OPEN ACCESS

Molecular marker based (SSR) genetic diversity analysis in deep water rice germplasms of Bangladesh

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Received: 22 September 2012
Revised: 15 October 2012
Accepted: 16 October 2012

Key words: SSR markers, genetic diversity, deep water rice.

Abstract

The study was undertaken to assess the genetic diversity among deep water rice genotypes using Simple Sequence Repeat (SSR) markers through marker aided selection (MAS). Twelve deep water rice (*Oryza sativa* L.) germplasms of Bangladesh was selected for genetic diversity analysis using eighteen SSR markers. Upon PCR amplification the alleles were separated on Polyacrylamide Gel Electrophoresis (PAGE) system. Initial polymorphism detection was conducted using eighteen primer pairs distributed on twelve rice chromosomes. The chosen microsatellite marker panel consisted of RM1, RM452, RM130, RM252, RM13, RM204, RM11, RM25, RM205, RM244, RM206, and RM463 with one representative from each chromosome. A total of 79 alleles were detected with an average of 4.38 alleles per locus. The polymorphism information content (PIC) reflections of alleles diversity frequency among the varieties, which is ranged from 0.477 to 0.782, with an average of 0.634. RM 13 was found as the best marker for identification of genotypes as revealed by PIC values. The Unweighted Pair Group Method with Arithmetic Mean (UPGMA) dendrogram revealed 2 major groups with 4 clusters and the wide range of dissimilarity values (0.14-0.89) which showed a high degree of diversity among the cultivars. The results of the genetic diversity will be useful for the selection of the parents for developing submergence tolerant and flash flood tolerant rice variety through molecular breeding program.

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Introduction

Rice (*Oryza sativa* L.) belonging to the family Graminae is the staple food for one third of the world's population (Chakravarthi and Naraveni, 2006). Deepwater rice is grown in flooded conditions with water more than 50 cm (20 inch) deep. More than 100 million people in South and Southeast Asia rely on deepwater rice for their sustenance. Many districts of Bangladesh are flooded during the rice cultivation season every year and thus curtail the national rice yield by causing severe damage to the rice cultivated field. Therefore it is high time to select potential rice cultivars for breeding program to develop submergence tolerant as well as flash flood resistant rice variety.

Molecular markers have proven to be powerful tools in the assessment of genetic variation and in the elucidation of genetic relationships within and among species. The recent development of DNA markers has provided new opportunities for the genetic improvement of rice cultivars (Causse *et al.*, 1994). Satellite loci also known as simple sequence repeats (SSRs) are the most commonly used molecular markers. Microsatellites are PCR-based markers that are efficient and cost-effective to use. Compared with other markers, they are abundant, co-dominant, highly reproducible and interspersed throughout the genome (Panaud *et al.*, 1996, Temnykh *et al.*, 2000). In particular, microsatellite markers have been widely applied in rice genetic studies as they are able to detect high levels of allelic diversity (McCouch *et al.*, 1997). These markers can detect a significantly higher degree of polymorphism in rice (Ni *et al.*, 2002, Okoshi *et al.*, 2004) which becomes ideal for studies on genetic diversity and intensive genetic mapping (Cho *et al.*, 2000). They have been used for characterizing genetic diversity in several crop species including sorghum (Dean *et al.*, 1999, Smith *et al.*, 2000), maize (Senior *et al.*, 1998), cotton (Liu *et al.*, 2000) and wheat (Prasad *et al.*, 2000). In rice, SSRs have been used to assess the genetic diversity of both wild and cultivated species (Siwach *et al.*, 2004, Brondani *et al.*, 2005, Neeraja *et al.*, 2005). Rice microsatellites also have a demonstrated utility for gene-tagging and marker-assisted selection (Chen *et al.*, 1997) and are polymorphic between (Akagi *et al.*, 1996, Panaud *et al.*, 1996) and within rice varieties (Olufowote *et al.*, 1997).These studies showed that SSR markers are efficient in detecting genetic polymorphisms and discriminating among genotypes. The important advantages of microsatellites are that they are usually single locus and because of the high mutation rate, are often multi-allelic. They are very efficient tools that can be exchange between laboratories and their data are highly informative (Morgante and Olivicri, 1993).

The present investigation was made to identify the suitable SSR primers for genetic analysis of deep-water rice and to measure the genetic diversity and relatedness among twelve deep-water rice genotypes using SSR markers.

Materials and methods

The whole experiment was conducted at Biotechnology Laboratory of BRRI (Bangladesh Rice Research Institute) during the period of July/2011- December/2011.

Plant materials

Seeds of twelve deep water rice varieties were collected from BRRI (Table 1). Seeds were germinated at aseptic condition by incubating them at 30^{o} C and grown in glass house.

Isolation of genomic DNA

Genomic DNA was isolated from young leaves of 21 days old plants following the mini preparation modified CTAB method (Zheng *et al.*, 1995). DNA samples were evaluated both quantitatively and qualitatively using spectrophotometer and λ (lamda) DNA (concentration marker) respectively.

SSR analysis

PCR amplification of SSR markers was carried out using eighteen primer pairs listed in table 2. Each reaction tube contained 25 ng of template DNA, 1 x PCR buffer, 2 mM MgCl2, 0.4 mM of dNTPs, 0.4 µM each of forward and reverse primers and 1.6

units of *Taq* DNA polymerase. Amplification was performed using the following conditions: denaturation at 94°C for 5 min; 35 cycles of 1 min denaturation at 94°C, 1 min annealing at 55°C, 1 min extension at 72°C and a final extension at 72°C for 5 min. The SSR amplification products were separated in a vertical denaturing 8% polyacrylamide. DNA fragments were revealed using the ethidium bromide staining procedure. The gels were stained for 30-35 minutes and were documented using UVPRO (Uvipro Platinum EU) gel documentation unit.

Data analysis

Polymorphic information content (PIC) values were calculated for each SSR locus based on Anderson *et al.* (1993). Major allele frequency, gene diversity, polymorphism information content (PIC) values were determined using Power Marker Version 3.25 (Liu and Muse, 2005). The amplified bands were scored for each SSR primer pairs based on the presence or absence of bands, generating a binary data matrix of 1 and 0 for each marker system. Both matrices were then analyzed using the NTSYS pc statistical package version 2.2. The data matrices were used to calculate genetic similarity based on Jaccard's similarity coefficients, and two dendograms displaying relationships among 12 rice cultivars were constructed using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA). The Pearson's correlation between similarity coefficients based on SSR markers was determined from data among all twelve rice cultivars.

Results and discussion

The level of polymorphism among rice cultivars was evaluated by calculating allelic number and PIC values for each of the eighteen SSR loci evaluated. A total of 79 alleles were detected at the loci of eighteen microsatellite markers across twelve rice germplasms. The results revealed that all the primers showed distinct polymorphisms among the cultivars studied indicating the robust nature of microsatellites in revealing polymorphism. Among the polymorphic markers, 3 produced three alleles each, 9 produced four alleles each, 3 generated five alleles each, 2 produced 6 alleles each and only one produced 7 alleles (table 3). The number of alleles per locus ranged from 3 (RM1, RM 211, and RM 134) to 7 alleles (RM 13) with an average of 4.4 alleles across the 18 loci .The landraces frequency of most common allele at each locus ranged from 25% (RM25) to 50% (RM1, RM211, RM130, RM413, RM134 and RM463). On an average, 42.13% of the 12 landraces shared a common major allele at any given locus. Similar number of microsatellite markers previously used as subset for genetic diversity analysis of *Oryza sativa* (Garris *et al.*, 2005, Thomson *et al.*, 2007). The Value is comparable to 1-8 allele per SSR locus with an average number of alleles of 4.58 per locus for various classes of microsatellite (Siwach *et al.*, 2004). The amplicon size of all 12 genotypes for each marker alleles varied from 73-81 bp produced by RM130 and 267-288 bp produced by RM586. The landraces frequency of most common allele at each locus ranged from 25% (RM25) to 50% (RM1, RM211, RM130, RM413, RM134 and RM463). On an average, 42.13% of the 12 landraces shared a common major allele at any given locus. Of the 79 alleles scored all of 79 were found to be polymorphic. Maximum number of polymorphic alleles (7) was obtained with the marker RM 13, while the minimum numbers of polymorphic alleles (3) was obtained by using RM 1, RM 211, and RM 134.

Polymorphism information content (PIC) value is a reflection of allele diversity and frequency among varieties. PIC values ranged from 0.477 to 0.782 with an average of 0.634 (table 3). The highest PIC value 0.7818 was obtained for RM13 followed by respectively RM25 (0.760), RM85 (0.73) and RM252 (0.72). PIC value revealed that RM13 was considered as best marker for 12 test genotypes. The PIC value observed, are comparable to three previous estimates of microsatellite analysis in rice via 0.34-0.88 (Thomson *et al.*, 2009), 0.20-0.90 with an average of 0.56 (Jain *et al.*, 2003). The PIC

value was higher than the earlier observations (Joshi and Behera, 2006) also. Figure 1 showed gel pictures of amplified fragment using primer designed for the SSR marker RM 252 and RM 13.

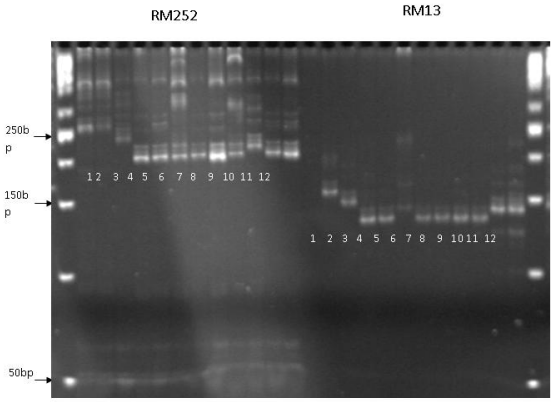

Fig. 1. DNA profile of the twelve deepwater rice land races with SSR marker RM 252 and RM 13. Legend: 1= Kata Mukul; 2= Dula Bexh; 3= Mota Kartik Sail; 4= Laxmi Digha; 5= Dulai Aman; 6= Bichi Bazal; 7= Manik Gira; 8= Aguli Aman; 9= Dudhsor; 10= Kartik Jhul; 11= Kartik Sail and 12= Kartik Gurol. L= Ladder marker.

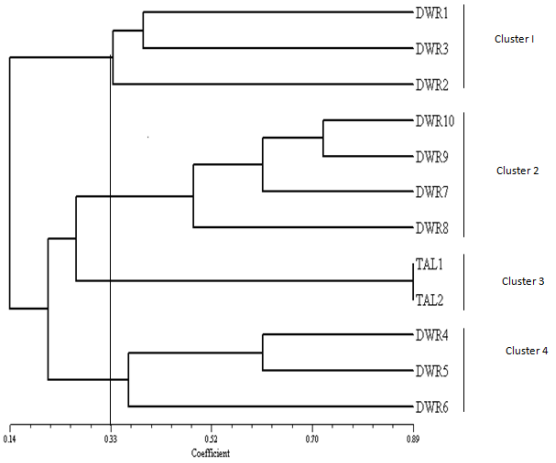

Fig. 2. A UPGMA clustering dendogram showing the genetic relationships among 12 landraces on the alleles detected by 18 microsatellite markers. Legend: DWR1= Kata Mukul; DWR2= Dula Bech; DWR3= Mota Kartik Sail; DWR4= Laxmi Digha; DWR5= Dulai Aman; DWR6= Bichi Bazal; DWR7= Manik Gira; DWR8= Aguli Aman; DWR9= Dudhsor; DWR10= Kartik Jhul; TAL1= Kartik Sail and TAL2= Kartik Gurol.

A cluster analysis using UPGMA based on similarity coefficients was done to resolve the phylogenetic relationships among the different deepwater rice genotypes considered for the present study. The UPGMA clustering system generated four genetic clusters with similarity coefficient 33% (fig. 2). Cluster 2 was the biggest group which contained four landraces viz. Kartik Jhul, Dudhsor, Manik Gira and Aguli Aman. The cluster analysis revealed that Kartik Jhul, Dudhsor and Manik Gira are closer than Anguli Aman while Kartik Jhul and Dudhsor are closer than Manik Gira. Cluster 1 and 4 contained three landraces in each cluster. The three genotypes, Kata Mukul, Mota Kartik sail and Dula Bech were clustered distinctly in the same group (cluster 1) but Kata Mukul and Mota Kartik are closer than the Dula Bech. Again, Laxmi Digha, Dulai Aman and Bhchi Bazal were clustered in same group (cluster 4) but Laxmi Digha and Dulai Aman are closer than the Bhchi Bazal. Cluster 3 was the smallest group which contains two T. Aman landraces viz. Kartik Sail and Kartik Gurol.

Table 1. List of twelve test genotypes for diversity analysis.

SI No.	BRRI accession No.	Germplasms
G39	2047	Kata Mukul
G40	2050	Dula Bech
G41	6351	Mota kartik sail
G42	6352	Laxmi Digha
G43	6353	Dulai Aman
G44	6355	Bichi Bazal
G45	6356	Manik Gira
G46	6357	Aguli Aman
G47	6358	Dudhsor
G48	6359	Kartik Jhul
G49	6360	Katik Sail
G50	6362	Kartik Gurol

This cluster tree analysis agreed with the allelic diversity observed among Basmati and Non-basmati long grain indica rice varieties using microsatellite markers (Siwach *et al*, 2004). DNA fingerprinting and phylogenic analysis of Indian aromatic high quality rice germplasms also showed similar trend (Jain *et al*, 2003).

The pair wise genetic dissimilarity coefficient indicated that the highest (100%) genetic

dissimilarity was found between Kata Mukul with Kartik Sail and Kartik Gurol; Dula Bech with Aguli Aman, Kartik Sail & Kartik Gurol and Manik Gira with Kartik Gurol (table 4). Besides, 94% dissimilarity was found between Dula Bech with Bichi Bazal as well as Mota Kartik Sail with Bichi Bazal and Kartik Gurol. However, potential hybrid line can be produced by inter-varietal crossing based on the genetic dissimilarity value since the more the genetic dissimilarity value the more chance of getting vigorous heterosis in the progeny. Hence microsatellite marker based molecular fingerprinting could serve as a potential basis in the identification of genetically distance genotypes as well as in sorting of duplication for morphologically close accession.

Table 2. List of eighteen SSR primers with position, and expected PCR product sizefor the study.

Name	Position	Product size (bp)	Forward primer (5/ - 3/)	Reverse primer (3/- 5/)
RM 1	4.63	113	gcgaaaacacaatgcaaaaa	gcgttggttggacctgac
RM452	9.50	105	Ctgatcgagagcgttaaggg	gggatcaaaccacgtttctg
RM 211	4.16	161	Ccgatctcatcaaccaactg	cttcacgaggatctcaaagg
RM 85	66.76	107	ccaaagatgaaacctggattg	gcacaaggtgagcagtcc
RM 130	33.33	85	tgttgcttgccctcacgcgaag	ggtcgcgtgcttggtttggttc
RM127	34.19	223	gtgggatagctgcgtcgcgtcg	aggccagggtgttggcatgctg
RM252	45.21	216	Ttcgctgacgtgataggttg	atgacttgatcccgagaacg
RM13	8.27	141	tccaacatggcaagagagag	ggtggcattcgattccag
RM413	2.19	79	Ggcgattcttggatgaagag	tccccaccaatcttgtcttc
RM204	3.17	169	Gtgactgacttggtcataggg	gctagccatgctctcgtacc
RM586	1.47	271	Acctcgcgttattaggtaccc	gagatacgccaacgagatacc
RM11	19.25	140	Tctcctcttcccccgatc	atagcgggcgaggcttag
RM134	26.63	93	acaaggccgcgagaggattccg	gctctccggtggctccgattgg
RM 25	2.59	146	ggaaagaatgatcttttcatgg	ctaccatcaaaaccaatgttc
RM205	22.72	122	Ctggttctgtatgggagcag	ctggcccttcacgtttcagtg
RM 244	4.34	163	Ccgactgttcgtccttatca	ctgctctcgggtgaacgt
RM 206	21.97	147	Cccatgcgtttaactattct	cgttccatcgatccgtatgg
RM 463	22.09	192	Ttcccctccttttatggtgc	tgttctcctcagtcactgcg

There have been a number of studies that have reported on the assessment of genetic diversity in a relatively large set of cultivated germplasm. This include diversity analysis of high yielding cultivars (Ni *et al.*, 2002), aromatic rice (Nagaraju *et al.*, 2002), indigenous aromatic rice (Joshi and Behera, 2006) and even lowland rice (Bhuyan *et al.*, 2007, Yu and Nguyen, 1994) using various molecular fingerprinting techniques like RFLP, RAPD, SSR, AFLP etc. Since the long term objective is to utilize rice germplasm for broadening the genetic base of the cultivated rice, the present study is an attempt at characterizing diversity at the molecular level in this set of lowland rice genotypes to broaden the genetic base of cultivated rice in the rain fed and lowland areas of Bangladesh such as Sunamganj, Hobigonj, Bogra and the Sirajgonj. Moreover, the cultivars with wide genetic distance can be crossed to widen the genetic base and exploit heterosis. The informative primers would prove useful in marker-assisted selection, linkage mapping and gene tagging for specialty traits.

Table 3. Number of alleles, highest frequency allele and Polymorphism Information Content (PIC) Values found among twelve rice germplasms for eighteen SSR markers.

Primer names	Chromosome no.	Allele no.	Amplicon size range	Major Allele	Major Allele frequency	PIC value	Gene Diversity
RM 1	1	3	76-111	82	0.5000	0.4768	0.5694
RM452	2	5	199-211	211	0.4167	0.6990	0.7361
RM 211	2	3	144-150	150	0.5000	0.5355	0.6111
RM 85	3	5	91-113	113	0.3333	0.7260	0.7639
RM 130	3	4	73-81	75	0.5000	0.5593	0.6250
RM127	4	4	218-226	224	0.4167	0.6218	0.6806
RM252	4	6	195-265	200	0.4167	0.7193	0.7500
RM13	5	7	136-152	138	0.3333	0.7818	0.8056
RM413	5	4	74-82	80	0.5000	0.6204	0.6667
RM204	6	4	117-124	117	0.4167	0.6218	0.6806
RM586	6	4	267-288	267	0.3333	0.6874	0.7361
RM11	7	5	127-149	127	0.4167	0.6437	0.6944
RM134	7	3	92-94	94	0.5000	0.5355	0.6111
RM 25	8	6	138-155	145	0.2500	0.7601	0.7917
RM205	9	4	123-134	134	0.4167	0.6218	0.6806
RM 244	10	4	162-168	165	0.4167	0.6218	0.6806
RM 206	11	4	137-166	137	0.4167	0.6218	0.6806
RM 463	12	4	195-205	205	0.5000	0.5593	0.6250
Mean		**4.3889**			**0.4213**	**0.6341**	**0.6883**

Table 4. Genetic dissimilarity pair (below diagonal)) values among studied twelve deep water rice genotypes.

Germplasm	Kata Mukul	Kartik Jhul	Dula Bech	Mota Kartik sail	Laxmi Digha	Dulai Aman	Bichi Bazal	Manik Gira	Aguli Aman	Dudhsor	Kartik Sail	Kartik Gurol
Kata Mukul	0.0000											
Kartik Jhul	0.8333	0.0000										
Dula Bech	0.6667	0.7778	0.0000									
Mota Kartik sail	0.6111	0.7222	0.6667	0.0000								
Laxmi Digha	0.8889	0.8333	0.7222	0.7222	0.0000							
Dulai Aman	0.8333	0.8889	0.8889	0.8333	0.3889	0.0000						
Bichi Bazal	0.8889	0.8333	0.9444	0.9444	0.6667	0.611	0.0000					
Manik Gira	0.7778	0.3333	0.6111	0.5556	0.7222	0.8333	0.6667	0.0000				
Aguli Aman	0.8889	0.5000	1.0000	0.8889	0.6667	0.7222	0.6667	0.6111	0.0000			
Dudhsor	0.8889	0.2778	0.8333	0.8889	0.7778	0.8333	0.7222	0.4444	0.4444	0.0000		
Kartik Sail	1.0000	0.7778	1.0000	0.8889	0.8333	0.8889	0.7778	0.8889	0.6667	0.5556	0.0000	
Kartik Gurol	1.0000	0.7778	1.0000	0.9444	0.7778	0.8333	0.8889	1.0000	0.6667	0.5556	0.1111	0.0000

The results revealed that highest genetic distance for one pair of landraces can be utilized as potential parents for improvement of varieties. The SSR markers based molecular fingerprinting could serve as a sound basis in the identification of genetically distant accessions as well as sorting of duplicate germplasm of morphologically close accessions.

Acknowledgements

The authors gratefully acknowledged to the authority of Biotechnology Division, Bangladesh Rice Research Institute (BRRI), Gazipur, Bangladesh for providing the facilities to carry out this research work.

References

Akagi H, Yokozek Y, Fujimura T. 1996. Microsatellite DNA markers for rice chromosomes.Theoretical and Applied Genetics **93,** 1071-1077.

Bhuyan N, Borah BK, Sarma RN. 2007. Genetic diversity analysis in traditional lowland rice (*Oryzae sativa* L.) of Assam using RAPD and SSR markers. Current Science **93**, 967-972.

Brondani RPV, Zucchi MI, Brondani C, Rangel PHN, Borba TCDO, Rangel PN, Magalhaes MR, Vencovsky R. 2005. Genetic structure of wild rice *Oryza glumaepatula* populations in three Brazilian biomes using microsatellite markers. Genetica, **125(2-3),** 115-123.

Causse MA, Fulton TM, Cho YG, Ahn SN, Chunwongse J. 1994. Saturated molecular map of the rice genome based on an interspecific backcross population. Genetics **138**, 1251–1274.

Chakravarthi BK, Naravaneni R. 2006. SSR marker based DNA finger printing and diversity study in rice (*Oryza sativa* L). African Journal of Biotechnology **5 (9)**, 684-688.

Chen X, Temnykh S, Xu Y, Cho YG, McCouch SR. 1997. Development of a microsatellite frame work map providing genome wide coverage in rice (*Oryza sativa* L.).Theoretical and Applied Genetics **95**, 553-567.

Cho YG, Ishii T, Temnykh S, Chen X, Lipovich L, Park WD, Ayres N, Cartinhour S, McCouch SR. 2000. Diversity of microsatellites derived from genomic libraries and GenBank sequences in rice (*Oryza sativa* L.). Theoretical and Applied Genetics **100**, 713–722.

Dean RE, Dahlberg JA, Hopkins MS, Mitchell SE, Kresovich S. 1999. Genetic redundancy and diversity among 'Orange' accessions in the U.S. national Sorghum collection as assessed with simple sequence repeat (SSR) markers. Crop Science **39(4)**, 1215-1221.

Garris AJ, Thomas HT, Coburn J, Krcsovich S, McCouch S. 2005. Genetic structure and diversity in (*Oryza* sativa L.). Genetics **169**, 1631-1638.

Jain S, Mitchell SE, Jain RK, Kresovich S, McCouch SR. 2003. DNA fingerprinting and phylogenetic analysis of Indian aromatic high quality rice germplasm using panels of fluorescent-labeled microsatellite markers. *In:* Advance in Rice Genetics, Khush GS, Brar DS and Hardy B (Eds), IRRI, Philippine, 162-165.

Joshi RK, Behera L. 2006. Identification and differentiation of indigenous non-Basmati aromatic rice genotypes of India using microsatellite markers. African Journal of Biotechnology **6(4)**, 348-354.

Liu S, Cantrell RG, McCarty JC, Stewart JMD. 2000. Simple sequence repeat-based assessment of genetic diversity in cotton race stock accessions. Crop Science **40(5)**,1459-1469.

Liu K, Muse SV. 2005. PowerMarker: an integrated analysis environment for genetic marker analysis. Bioinformatics **21**, 2128-2129.

Morgante M, Olivieri AM. 1993. PCR-amplified microsatellites as marker in plant genetics. The Plant Journal **3**, 175-182.

McCouch SR, Chen X, Panaud O, Temnykh S, Xu Y, Cho YG, Huang N, Ishii T, Blair M. 1997. Microsatellite marker development, mapping and applications in rice genetics and breeding. Plant Molecular Biology **35**, 89–99.

Nagaraju J, Kathirvel M, Ramesh KR, Siddiq EA and Hasnain SE. 2002. Genetic analysis of traditional and evolved *Basmati* and non-*Basmati* rice varieties by using fluorescence-based ISSR-PCR and SSR markers. Proceedings of the National Academy of Sciences **99**, 5836-5841

Neeraja CN, Hariprasad AS, Malathi S, Siddiq EA. 2005. Characterization of tall landraces of rice (*Oryza sativa* L.) using gene derived simple sequence repeats. Current Science **88(1)**, 149-152.

Ni J, Colowitand PM, Mackill DJ. 2002. Evaluation of genetic diversity in rice subspecies using microsatellite markers. Crop Science **42**, 601-607.

Olufowote JO, Xu Y, Chen X, Goto M, McCouch SR, Park WD, Beachell HM, Dilday RH. 1997. Comparative evaluation of within-cultivar variation of rice (*Oryza sativa* L.) using microsatellite and RFLP markers. Genome **40(3)**, 370-378.

Okoshi M, Hu J, Ishikawa R, Fujimura T. 2004. Polymorphoic analysis of landraces of Japanese rice using microsatellite markers. Breeding Research **6**, 125-133.

Panaud O, Chenayd X, McCouch SR. 1996. Development of microsatellite markers and characterization of simple sequence length polymorphism (SSLP) in rice (*Oryza sativa* L.). Molecular and General Genetics **252**, 597-607.

Prasad M, Varshney RK, Roy JK, Balyan HS, Gupta PK. 2000. The use of microsatellites for detecting DNA polymorphism, genotype identification and genetic diversity in wheat. Theoretical and Applied Genetics **100(3-4)**, 584-592.

Senior ML, Murphy JP, Goodman MM, Stuber CW. 1998. Utility of SSRs for determining genetic similarities and relationships in maize using an agarose gel system. Crop Science **38(4)**, 1088-1098.

Siwach P, Jain S, Saini N, Chowdhury VK, Jain RK. 2004. Allelic diversity among Basmati and non-Basmati long-grain indica rice varieties using microsatellite markers. Journal of Plant Biochemistry and Biotechnology **13**, 25-32.

Smith JSC, Kresovich S, Hopkins MS, Mitchell S, Dean RE, Woodman WL, Lee M, Porter K. 2000. Genetic diversity among elite sorghum inbred lines assessed with simple sequence repeats. Crop Science **40(1)**, 226-232.

Temnykh S, Park WD, Ayers N, Cartinhour S, Hauck N, Lipovich L, Cho YG, Ishii T, McCouch SR. 2000. Mapping and genome organization of microsatellite sequences in rice *(Oryza sativa L.)*. Theoretical and Applied Genetics **100**, 697–712.

Thomson MJ, Septiningsihn, Endang M, Suwardjo, Patimah, Santoso, Tiur S, McCouch SR. 2007. Genetic diversity analysis of traditional improved Indonesian rice (*Oryza sativa* L.) germplasm using microsatellite markers. Theoretical and Applied Genetics **114**, 559-568.

Thomson MJ, Polato NR, Prasetiyono J, Trijatmiko KR, Silitonga TS, McCouch SR. 2009. Genetic diversity of isolated populations of Indonesian landraces of rice (*Oryza sativa L.*) collected in East Kalimantan on the island of Borneo. Rice **2**, 80-92.

Yu LX, Nguyen HT. 1994. Genetic variation detected with RAPD markers among upland and lowland rice cultivars (*Oryza sativa* L.). Theoretical and Applied Genetics **87**, 668–672.

Zheng K, Huang N, Bennett J, Khush GS. 1995. PCR assisted marker based selection in rice breeding. IRRI Discussion Paper Series No. 12. IRRI. Manila. Philipines, 5-6.