The Bioscan 8(2): 359-362, 2013
AN INTERNATIONAL QUARTERLY JOURNAL OF LIFE SCIENCES
www.thebioscan.in

MOLECULAR STUDIES OF AROMATIC AND NON AROMATIC RICE (*ORYZA SATIVA* L.) GENOTYPES FOR QUALITY TRAITS USING MICROSATELLITE MARKERS

ZAINAB VHORA, RUCHI TRIVEDI, SUDESHNA CHAKRABORTY*, RALLAPALLI RAVIKIRAN AND N. SASIDHARAN
Department of Agricultural Botany, Anand Agricultural University, Anand - 388 110, Gujarat, INDIA
e-mail: schakraborty.bio@gmail.com

KEY WORDS
Rice
Aromatic
Non aromatic
SSR
Polymorphism

Received on :
23.11.2012

Accepted on :
06.03.2013

***Corresponding author**

ABSTRACT
The present study was performed to analyze the genetic diversity among aromatic and non-aromatic rice genotypes using microsatellite markers (SSR). For the investigation, 20 rice cultivars of aromatic, non-aromatic, and quality traits were studied using 25 Rice Microsatellite (RM) markers, among which 15 markers were used for analyzing aromatic and nonaromatic rice genotypes. These markers generated higher level of polymorphism due to which they generated 356 polymorphic reproducible bands with 164 loci. The remaining ten markers are used for the study of quality traits which shown 222 polymorphic bands with 101 alleles. The cluster analysis using SSR markers could distinguish the different genotypes. The dendogram generated on the principle of Unweighted Pair Wise Method using Arithemetic Average (UPGMA) was constructed by Jaccard's Coefficient and the genotypes were grouped in to clusters. The dendogram developed for aroma and quality traits showed that the genotypes with common phylogeny and geographical orientation tend to cluster together.

INTRODUCTION

Rice (*Oryza sativa* L.) belonging to the family *Graminae* is the staple food for one third of the world's population. The aromatic rice is preferred over non aromatic during special occasions and for export and thus they command a higher market price (Kibiria *et al.,* 2009). Fragrance considered one of the most important grain quality traits in rice, as it is a key factor in determining market price and related to both local and national identity. Since early, the genetic basis of fragrance has been studied by various workers and reported to be controlled by one or two or three dominant or recessive genes or by QTLs (Shobha Rani *et al.,* 2008). Most of these grain quality attributes are controlled by quantitative trait loci (QTLs) as inferred from continuous phenotypic variation in the segregating progeny of intervarietal crosses. It is difficult for the breeders to select for quality using conventional methods due to lack of discrete phenotypic classes in the progeny and tedious methodologies for quality testing (He *et al*., 1999). More recently molecular markers such as SNPs and SSRs, which are genetically linked to fragrance and to identify the nature of the locus and have the advantage of being inexpensive, simple, rapid and only requiring small amount of tissue, may also be useful for the rapid incorporation of the scent character into breeding lines. The microsatellite markers were used for the identification of aromatic rice genotypes with their wild relatives and showed to have high degree of genetic similarity (Kibiria *et al.,* 2009). Compared with other markers microsatellites are abundant, codominant and interspersed throughout the genome. These markers can detect a significantly higher degree of polymorphism in rice, which becomes ideal for studies on genetic diversity and intensive genetic mapping. SSR markers can estimate genetic diversity between cultivars e.g. between parents of a genepool or between plants extracted from a population or between populations (Panaud *et al*., 1996). In the present study, 20 rice varieties released at National and State (Gujarat) level having aroma and superior quality attributes were analyzed for genetic variation using SSR markers. These markers are highly polymorphic and easy to detect. The present work aims for the assessment of aromatic and non-aromatic rice genotypes and comparison of the efficiency of SSR markers employed for the study.

MATERIALS AND METHODS

The study was conducted at Plant Biotechnology Laboratory, Department of Agricultural Botany, B.A College of Agriculture, Anand Agricultural University, Anand during the year 2012. The seeds of 20 rice genotypes comprising of aromatic non-aromatic and grain quality traits were used in the present study and were obtained from the Main Rice Research Station, Anand Agricultural University, Nawagam (Table - 1). Total DNA was extracted from three weeks old seedlings by Cetyl Trimethyl Ammonium Bromide (CTAB) method (Ahmadikhah, 2009) with minor modifications. Spectrophotometry was performed to determine absorption spectrum of isolated DNA by using Nanodrop N.D. 1000 (Software V.3.3.0). In all 25 SSR primers were used for the present study (Table 2). The PCR reactions for SSR were carried out in a reaction volume of 20μL which consisted of 2μL of PCR buffer (including 20mM

Table 1: List of genotypes used in the present study

Sr. No.	Genotype	Sr. No.	Genotype
1	GR7	11	CSR30
2	GR9	12	DUBRAJ
3	GR11	13	IR64
4	GR12	14	JIRASAR
5	GR101	15	KRISHNA KAMOD
6	GR102	16	MAHISUGANDHA
7	GR104	17	PANKHALI203
8	GAR1	18	PUSA1121
9	GAUR10	19	PUSABASMATI
10	BPT5204	20	SWARNA

$MgCl_2$), 0.5μL forward and reverse primer, 0.5μL of 25mM dNTPs mix, 0.5μL *Taq* DNA polymerase, 2μL DNA template, 0.5μL formamide and nuclease free water. The amplified product of SSR was analyzed electrophoretically using 2.3-2.5% agarose gel containing 0.5 μg/mL of Ethidium bromide prepared in 1X TBE buffer at a constant voltage of 80V for period of 2h. The separated bands were visualized under UV transilluminator and photographed using Syngene gene snap-G box. Coefficients of similarity were calculated as Jaccard's similarity coefficient by SIMQUAL subroutine in SIMILARITY routine. The matrix of similarity was clustered using UPGMA algorithm under Sequential Agglomerative Hierarchical Nesting module of the NTYSYSpc Version 2.02 (Rohlf *et al.,* 1994). Identity software was also used for data analysis (Wagner and Sefc, 1999) which revealed information about expected heterozygosity, observed heterozygosity, number of alleles and allele frequency and standard deviation

RESULTS AND DISCUSSION

In the present study, total of 25 SSR primers were used for molecular study of 20 genotypes of *Oryza sativa* among which 20 primers succeeded in producing polymorphic or monomorphic alleles when applied to rice cultivars. 15 primers of which were used for aroma analysis and remaining for analyzing qualitative traits. During analyzing for aroma, an average expected heterozygosity observed was 0.85, with the SSR markers generating in total of 356 bands with 164 alleles. The genotype GR7 showed highest allele length of 154 bp whereas genotype GAR1 showed the lowest of 124bp with primer RM 152 (Table 3). The allele frequency varied from 0.05 to 0.25. The highest allele frequency (0.25) was observed in the genotypes viz., CSR30, DURAJ, JIRASAR, Krishnakamod and Pankhali 203. Krishnakamod showed an allele length of 254bp in RM515. Pusa Basmati showed an allele length of 134bp when screened with SSR primer RM310. The data were utilized for calculating genetic similarity coefficients and constructing dendrogram (Fig1). Twenty aromatic and non aromatic rice genotypes which were subjected to molecular characterization using 15 microsatellite markers got distributed into two major clusters A and B. The cluster A was further subdivided into two minor clusters A1 and A2. A1 was further subdivided into two subclusters A11 and A12. The subclustering pattern revealed that the non aromatic rice genotypes such as GR7 and GR9 clustered together as was the case with GR11 and GR12. The aromatic genotypes such as Jirasar, Mahisugandha, Krishnakamod, CSR30, Dubraj etc. also tend to cluster together in different subclusters. Similarly cluster B contained Pankhali 203, Pusa1121, Pusa Basmati and Swarna. The clustering of aromatic genotypes with non aromatic genotypes may be due to the common phylogenetic origin and also due to the limitations of microsatellite markers used in this study. These limitations can be overcome by using more number of microsatellite markers or SNPs which can

Table 2: List of Ssr Primers

Sr. No.	PRIMERS	FORWARD SEQUENCE	REVERSE SEQUENCE
Primers used for analysis of Aroma			
1.	RM137	GACATCGCCACCAGCCCACCAC	CGGCTGGTCCCCGAGGATCTTG
2.	RM515	TAGGACGACCAAAGGCTGAG	TGGCCTGCTCTCTCTCTCTC
3.	RM342A	CCATCCTCCTACTTCAATGAAG	ACTATGCAGTGGTGTCACCC
4.	RM44	ACGGGCAATCCGAACAACCC	TCGGGAAAACCTACCCTACC
5.	RM342B	CCATCCTCCTACTTCAATGAAG	ACTATGCAGTGGTGTCACCC
6.	RM42	ATCCTACCGCTGACCATGAG	TTTGGTCTACGTGGCGTACA
7.	RM210	TCACATTCGGTGGCATTG	CGAGGATGGTTGTTCACTTG
8.	RM310	CCAAAACATTTAAAATATCATG	GCTTGTTGGTCATTACCATTC
9.	RM339	GTAATCGATGCTGTGGGAAG	GAGTCATGTGATAGCCGATATG
10.	RM284	ATCTCTGATACTCCATCCATCC	CCTGTACGTTGATCCGAAGC
11.	RM256	GACAGGGAGTGATTGAAGGC	GTTTTCGCCAAGGGC
12.	RM195	AGAAAGAGAGGCCGTCGGCGGC	GGGCTCACCCCCAAACCTGCAG
13.	RM152	GAAACCACCACACCTCACCG	CCGTAGACCTTCTTGAAGTAG
14.	RM223	GAGTGAGCTTGGGCTGAAAC	GAAGGCAAGTCTTGGCACTG
15.	RM331	GAACCAGAGGACAAAAATGC	CATCATACATTTGCAGCCAG
Primers used for analysis of Quality trait			
16.	RM11	TCTCCTCTTCCCCCGATC	ATAGCGGGCGAGGCTTAG
17.	RM211	CCGATCTCATCAACCAACTG	CTTCACGAGGATCTCAAAGG
18.	RM431	TCCTGCGAACTGAAGAGTTG	AGAGCAAAACCCTGGTTCAC
19.	RM435	CTCGTTTATTACCTACAGTACC	CTACCTCCTTTCTAGACCGATA
20.	RM201	ATTACGTGCATGTCTGGCTG	CGTACCTGACCATGCATCTG
21.	RM242	GGCCAACGTGTGTATGTCTC	TATATGCCAAGACGGATGGG
22.	RM282	CTGTGTCGAAAGGCTGCAC	CAGTCCTGTGTTGCAGCAAG
23.	RM257	CAGTTCCGAGCAAGAGTACTC	GGATCGGACGTGGCATATG
24.	RM336	CTTACAGAGAAACGGCATCG	GCTGGTTTGTTTCAGGTT
25.	RM212	CACCCATTTGTCTCTCATTATG	CCACTTTCAGCTACTACCAG

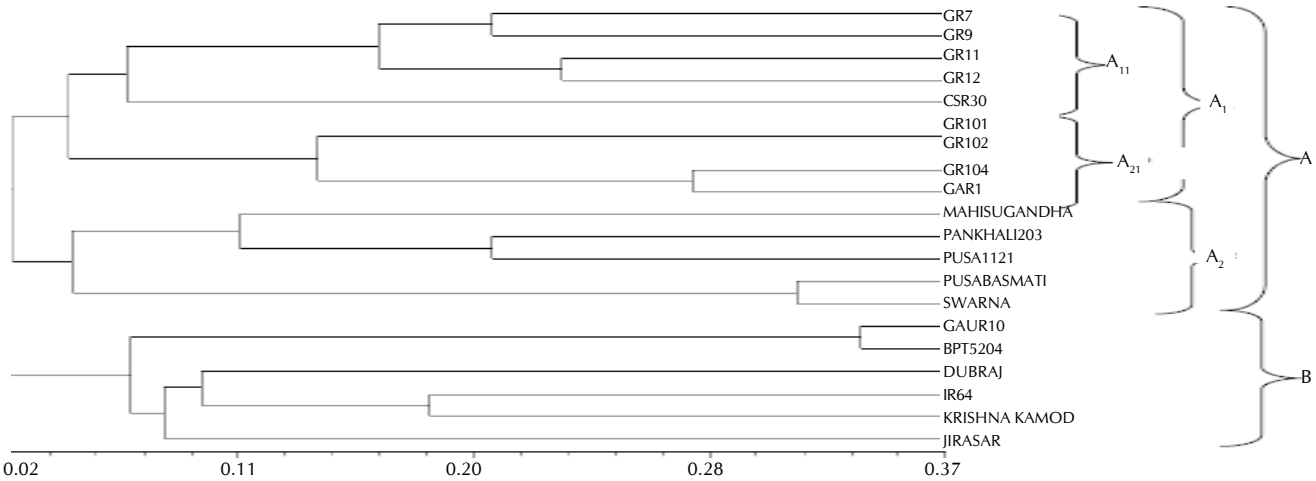

Figure 1: Dendogram of 15 SSR primers for Aroma using UPGMA algorithm

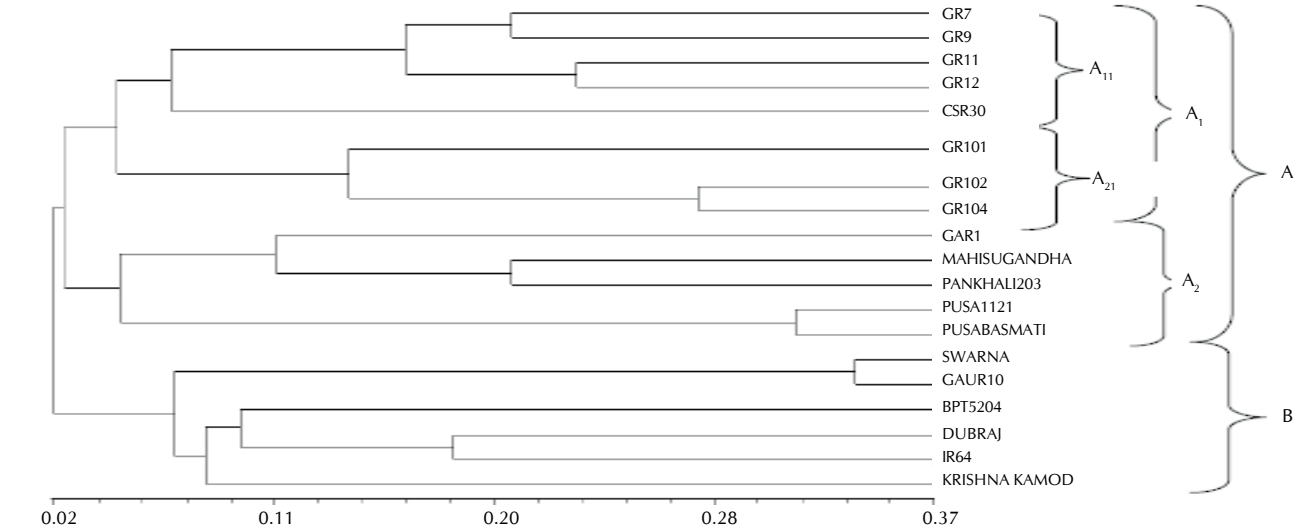

Figure 2: Dendogram of 15 SSR primers for Aroma using UPGMA algorithm

Table 3: Details of SSR markers used for analysis of aroma

Sr.No	Primer	No. of Alleles	Range of alleles length(bp)	Genotypes with highest allele length
1	RM137	7	211-232	Swarna
2	RM515	15	184-254	Krishnakamod
3	RM342A	12	118-150	Swarna, Pankhali203
4	RM44	8	109-135	Mahisugandha, Pankhali 203, Pusa1121, Pusa BasmatiSwarna
5	RM342B	13	116-159	Swarna
6	RM42	15	124-175	PusaBasmati, Swarna
7	RM210	10	118-160	Jirasar, Pusa1121
8	RM310	10	100-134	PusaBasmati
9	RM339	13	120-197	Pankhali203, Pusa1121
10	RM284	8	123-154	GR104
11	RM256	13	120-197	GR104
12	RM195	11	252-310	Mahisugandha
13	RM152	9	124-154	GR7
14	RM223	6	128-150	GR7, IR64, Swarna, Pankhali 203, Jirasar
15	RM331	12	157-214	Pusa1121

show more specificity.

For microsatellite analysis for quality traits, 10 primers are used (Table 4). SSR primer RM 211 showed highest allele length of 181bp with Pusa Basmati. Another primer RM 435 showed highest allele length of 189bp with Mahisugandha and Pankhali 203 whereas genotypes GR7, GR9, GR11 and GR12 showed lowest of 162bp. Genotype GR104 contained a null allele. All the SSR markers were found polymorphic and generated 222 bands with 101 alleles. The maximum similarity index of 0.4 was obtained between BPT5204 and GR102. The present work corroborates with the work of Matin *et al.* (2012) where RM 211 primer is been used for studying the genetic diversity of rice genotypes. Ten SSR primers were utilized in these studies which are specific for various physical and chemical quality parameters of rice such as kernel length, kernel breadth, scent, amylose content and high water uptake. The 20 genotypes considered for this study formed two major clusters A and B which were then further subdivided into many major and minor subclusters. On the whole it was observed that genotypes with common geographical and phylogenetic origin such as GR101, GR102 etc were found to cluster together for different

Table 4: Details of SSR markers used for analysis of quality trait

Sr.No	Primer	No. of Alleles	Range of alleles length(bp)	Genotypes with highest allele length
1	RM11	12	119-158	Pankhali203
2	RM211	12	140-181	PusaBasmati
3	RM431	12	216-259	Mahisugandha, Pusa Basmati, Swarna
4	RM435	9	162-189	Mahisugandha,Pusa Basmati
5	RM201	8	139-168	PusaBasmati,Swarna
6	RM242	14	186-243	Pusa1121
7	RM282	5	133-161	Swarna
8	RM257	9	139-151	Swarna
9	RM336	14	122-191	GR-7
10	RM212	3	119-126	GR-11

characters. Similar work was carried out by Lapitan *et al*. (2007) where SSR markers were used for the studying the quality traits of several rice varieties. The result revealed that the rice quality exhibit a higher genetic diversity and therefore very useful for rice breeding programs, especially for genetic mapping studies and eventually for application of marker assisted selection (MAS) in the programs. Similar types of work has been carried out by Kibria *et al*. (2008) where fourteen different rice varieties were screened with the help of SSR markers and showed that these SSR markers are helpful in indentifying *fgr* locus in rice genotypes. Lestari *et al*. (2009) observed that, as nucleotide differences among genotypes are a major source heritable variation, molecular markers derived from them should provide an effective measure of genotypic variation and hence phenotypic difference among varieties. However, some genotypes such as IR64 did not cluster as expected which may be due to the limitations of SSR primers used in this study. These limitations can be overcome by using more number of SSR markers or third generation markers such as single nucleotide polymorphic (SNPs) markers, which are more specific. These markers can be profitability employed for unraveling the latent variability present in the rice germplasm for various characters which in turn can be a boon to the plant breeders for formulating their breeding programs.

REFERENCES

Ahmadikhah, A. 2009. A rapid mini-prep DNA extraction method in rice (*Oryza sativa*). *African J. Biotechnology*. **8(2):** 323-327.

He, P., Li, S.G., Quian, Q., Ma, T.Q., Li, J. Z., Wang, W.M., Chen, Y. and Zhu, L.H.1999. Genetic analysis of rice grain quality. *Theoretical and Applied Genetics*. **98**: 502-508.

Kibria, K., Islam, M. M. and Begum, S. N. 2008. Screening of aromatic rice lines by phenotypic and molecular markers. *Bangladesh J. Bot*. **37(2)**: 141-147.

Kibria, K., Nur, F., Begum, S. N., Islam, M. M., Paul, S. K., Rahman, K. S. and Azam, M. M. 2009. Molecular marker based genetic diversity analysis in aromatic rice genotypes using SSR and RAPD markers. *Int. J. Sustain. Crop. Prod.* **4(1):** 23-34.

Lapitan, V. C., Brar, D. S., Abe, T. and Redona, E. D. 2007. Assessment of genetic diversity of Philippine rice cultivars carrying good quality traits using SSR markers. *Breeding Sci.* **57:** 263-270.

Lestari, P., Ham, T. H., Woo, M. O., Jiang, W., Chu, S. H., Kwon S. W., Ma, K., Lee, J. H., Cho, Y. C. and Koh, H. J. 2009. PCR marker-based evaluation of the eating quality of *Japonica* rice (*Oryza sativa* L.). *J Agri. Food Chem*. **57:** 2754-2762.

Matin, S., Ashrafuzzaman, M., Islam, M. M., Sikdar, S. U., Zobayer, N. 2012. Molecular marker based (SSR) genetic diversity analysis in deep water rice germplasms of Bangladesh. *International J.Biosciences*. **2(10):** 2222-5234.

Panaud, O., Xhen, X. and Mc Couch, S. R. 1996. Development of microsatellite markers and characterization of simple sequence length polymorphism (SSLP) in rice (*Oryza sativa L.*). *Molecular Genetics*. **252**: 597-607.

Rohlf, F. J. 1994. NTSYS-PC Numerical Taxonomy and Multivariate analysis system, ver. 2.02. State University of New York. *Stonybrook*. New York. pp. 103-120.

Shobha Rani, N., Sheshu, M. M., Manish, K., Pandey, R. M., Sundaram, G. S. V., Prashad, I., Sudarshan, L. V., Subba, R. and Ravindrababu, V. 2008. Genetic and molecular approaches for improvement of grain quality in rice. *Indian J. Crop Science*. **3(1)**: 1-14.

Wagner, H. W. and Sefc, K. M. 1999. Identity 1.0. Centre for Applied Genetics. *University of Agricultural Science, Vienna*. pp. 113.