Available online at www.ijpab.com

DOI: http://dx.doi.org/10.18782/2320-7051.2788

ISSN: 2320 – 7051

Int. J. Pure App. Biosci. **5 (6):** 926-937 (2017)

International Journal of Pure & Applied Bioscience

Research Article

OPEN ACCESS

Identification of Efficient Primers for Developing DNA Finger Printing Pattern of Rice Cultivars of West Bengal

P. Panigrahi[1], K. K. Panigrahi[2*] and R. Sadhukhan[3]

[1]Department of Horticulture, Govt. of Odisha, India

[2]College of Agriculture, Chiplima, OUAT, Odisha, India

[3]Department of Genetics and Plant Breeding, BCKV, West Bengal, India

*Corresponding Author E-mail: kaushikpbg@gmail.com

Received: 2.04.2017 | Revised: 30.05.2017 | Accepted: 4.06.2017

ABSTRACT

In this study 18 rice cultivars of West Bengal were subjected to SSR assay to identify the efficient primers for developing DNA fingerprinting. Five SSR primers of rice (RM206, RM219, RM251, RM231: 27.77% each and RM253: 22.22%) revealed a considerable DNA polymorphism among the selected cultivars of West Bengal considered for the study. The SSR primer RM219 of rice had showed highest discriminating power (0.817) followed by RM231 (0.797) then RM251 (0.758), RM206 (0.719), RM265 (0.679), RM3 (0.542), RM253 (0.471), RM236 (0.366) respectively and were produced polymorphic banding pattern, where as RM190 and RM349 showed monomorphic banding pattern. The highest PIC value had showed by RM265 (0.427), then followed by RM231 (0.376), RM251 (0.357), RM3 (0.341), RM206 (0.339), RM219 (0.308), RM236 (0.230) and RM253 (0.222) respectively. A combination of SSR primers RM219, RM251, RM231 distinguished the cultivar Gontra bhog and Gontra Bidhan-1 from all other cultivars used in the study by generating amplicon of 200, 150, 180 bp and 200, 150, 194 bp fragments respectively. Similarly a combination of RM219, RM206, RM231 distinguished the cultivar CR-1018 and Gontra Bidhan-4 from all other cultivars used in the study by generating amplicon of 210, 150, 180 bp and 200, 150, 200 bp fragments respectively. A combination of RM206, RM231 for IR36; RM206, RM219 for Gontra Bidhan-3 and IR64; RM206, RM231 for MTU-1001 and Lalat; RM251, RM219 for Satabadi, Ratna, and Gobindabhog; were distinguished from all other cultivars used in the study.

Key words: *Variability, Primers, SSR, Rice, Combinations.*

INTRODUCTION

Variety identification and varietal purity assessment are very important for varieties, hybrids and their parents. This is essential for maintenance, multiplication and seed certification. Under the New Seed Policy Act, 2001, all the new varieties have to be registered based on the criteria of novelty, distinctness, uniformity and stability (DUS). Hence, there is a need to develop and identify the gene markers of the variety/hybrid and also to standardize the laboratory based techniques for genetic purity testing in support of the grow-out test.

Cite this article: Panigrahi, P., Panigrahi, K.K. and Sadhukhan, R., Identification of Efficient Primers for Developing DNA Finger Printing Pattern of Rice Cultivars of West Bengal, *Int. J. Pure App. Biosci.* **5(6):** 926-937 (2017). doi: http://dx.doi.org/10.18782/2320-7051.2788

The grow-out test is tedious, laborious, time consuming and expensive, requiring large areas of land and skilled personnel often making subjective decisions[8] and also the marketing of seeds is hindered due to late receipt of results. However, grow-out test requires one full season thus precluding the immediate cultivation of the variety seed produced. This limitation and the environmental dependence of the entire procedure can be managed effectively by employing the molecular markers. While the differential response of seeds or seedlings to various chemical solution and bio-chemical test (PCR based markers) can be used as a tool to identify the hybrids/varieties, which is less time consuming, simple and reproducible. The Biochemical and Molecular Techniques group of the International Union for the Protection of New Varieties of Plants (UPOV) is evaluating different DNA marker parameters prior to its routine use in establishing Distinctness, Uniformity and Stability (DUS) of plant varieties[3]. Identification and successful deployment of efficient molecular markers can minimize ambiguity in varietal identification. Since based on the importance of genetic purity of a cultivar for the good economic production, the various agencies has been interested in the identification and conformity analysis of the cultivars of different crops lines. In particular, problems revolve around the identification of young plants during the process of crop production. Classical phenotypic methods of identification are not always sufficient to solve these problems because of the instability of the morphological characters (environmental variability), as well as an inability to use such information for identification at juvenile stages or of isolated plant parts. DNA markers such as restriction fragment length polymorphisms (RFLPs), random amplified polymorphic DNAs (RAPDs), amplified fragment length polymorphisms (AFLPs), microsatellites or simple sequence repeats (SSRs) and inter-SSR (ISSR) portray genetic variation at the DNA level thereby overcoming the influence of environments, independent of tissue effects, and providing more precise characterization of genotypes and measurement of genetic relationships than other markers[1,2,17]. But such studies do not focus on the important problem that is the way to optimally apply these new techniques for elite variety identification purposes. In particular, the greatest challenges are to reduce the cost of analysis, (i.e. the number of amplifications, and thus the number of primers) as well as the risk of confusing one of these elite genotypes with a randomly chosen genotype taken from a larger sample.

MATERIALS AND METHODS

To fulfil the precise objectives of the present study, experiments were conducted in the molecular genetics research laboratory of department of Genetics and Plant Breeding, B.C.K.V. The materials used and the protocols and the methodologies followed in the research study are summarized below (Table 1 & Fig 1). As a part of the development of a molecular tool kit for the study of diversity within the collected crop cultivars, SSR technology has been applied to the selected cultivars (Table 2).

Chemicals required for the cultivars analysis:-

1. DNA extraction buffer: 100 mM Tris-Cl (at pH 8.0), 25 mM EDTA (Ethylene Diamine Tertra Acetic Acid) (at pH 8.0), 1.25 M NaCl, 2% CTAB (CetylTrimethyl Ammonium Bromide), 3% PVP (Polyvinylpyrrolidone), double-distilled sterile water.
2. PCR reaction mixture: 2.5 mM dNTPs, 40 ng template DNA, 10X Taq polymerase assay buffer, 0.5 U Taq polymerase enzyme and 100 ng forward and 100 ng reverse primers, HPLC grade sterile water.
3. TBE buffer: 0.1 M Tris base, 0.083M Boric acid, 1mM EDTA.
4. TE buffer: 10 mM TrisCl (pH-8), 1mM EDTA (pH-8), double-distilled sterile water.
5. 10X Gel loading buffer: TE buffer, Glycerol (50 % of TE), Bromophenol blue (0.025 % of total solution).

6. Ethydium bromide solution: 10 mg ethydium bromide per 1 ml double-distilled sterile water.
7. Chloroform
8. Potassium acetate (5 M) solution: 47.575 g potassium acetate, 100 ml double-distilled sterile water.
9. RNase A
10. Isopropanol (ice cold)
11. 70%Ethanol: 70ml absolute ethanol in 100 ml double-distilled sterile water.
12. Agarose.

N.B.: The chemicals were manufactured by SISCO, Spectrochem, MERCK, Chromous Biotech, SIGMA.

DNA extraction from the seed samples:

DNA was extracted from single seed sample as described below.

DNA Extraction procedure:

DNA was extracted by the modified CTAB method[11], for isolation of high quality and quantity of DNA from single rice grain, described as follows:-

The procedure involves soaking of dehusked rice seed (or) grain and whole seed of green gram, black gram and mustard in 600 µl extraction buffer (100mM Tris-Cl, pH 8.0, 25 mM EDTA, pH 8.0, 1.25 M NaCl, 2% CTAB and 3% PVP) for 30-45 minutes at 37°C water bath (TB-85 Thermo bath) in a sterile 1.5 ml micro centrifuge tube and grinding the sample using a sterile mortar pestle till the tissue disintegrates. Then, 600 µl of chloroform is added; the contents are mixed gently for 2-3 minutes and centrifuged at 12000 x g for 10 minutes at room temperature in high speed cooling centrifuge (HERMLE). The supernatant is carefully transferred to a fresh sterile 1.5 ml micro centrifuge tube; and 2 µl RNase A was added into it and kept at 37º C water bath (TB-85 Thermo bath, Shimadzu) for 30 minutes to degrade the RNA present in the sample. The DNA is precipitated using an equal volume of ice-cold isopropanol. The DNA is pelleted by centrifugation at 12000 x g for 10 minutes at room temperature. After centrifugation, the supernatant is discarded and the DNA pellet is washed twice with 70% ethanol. The pellet is air dried for 1 hr and dissolved in 50 µl, 100 µl, 100 µl and 50 µl of sterile TE buffer (10 mM Tris HCl, pH 8.0 and 1mM EDTA, pH 8.0) for rice, green gram, black gram and mustard respectively and centrifuged at 12000 x g for 10 minutes at room temperature in high speed cooling centrifuge (HERMLE). The supernatant is carefully transferred to a fresh sterile 1.5 ml micro centrifuge tube. Then the transferred supernatant is kept for PCR analysis and the pellet precipitated is discarded.

DNA quality and quantity estimation

Extracted DNA samples were quantified through electrophoresis in 1 % agarose gel at constant voltage (65 V) for 90 minutes and further diluted accordingly to a uniform concentration of 50 ng / µl. The extracted DNA samples, along with the diluted samples were stored in -20^0C freezer (VESTFROST) with proper labeling.

Primers used for the study

We were selected 10 primers of those having high PIC value in another study for SSR marker based characterization[6] and are distributed throughout the genome of rice. Primers were synthesized from Metabion (Germany). Details of the primers are given in the following table 2.

PCR amplification

PCR amplification through SSR markers:

The reaction volume (25 µl) contained diluted DNA sample 2 µl (40 ng) with 1 µl (100 ng) forward primer, 1 µl (100 ng) reverse primer, along with 2.5 µl 10X buffer, 1 µl 2.5 mM dNTPs, 17.5 µl HPLC grade sterile water and 5 U Taq DNA polymerase enzyme. Amplification was performed in 200 µl PCR tube (TARSONS) in a thermal cycler (Gene Amp PCR System 9700) where the reaction condition was set as follows: 94ºC for 5 minutes of initial denaturation followed by 35 cycles each of denaturation at 94ºC temperature for 45 seconds, annealing of primer pairs at appropriate temperature (55ºC) for 45 seconds, and polymerization at 72º C for 1 minute. Further extension was followed at 72º C for 7 minutes, followed by holding the samples at 4º C for 5 minutes. On completion

of PCR, the amplification products were stored in –20º C freezer.

Separation of amplification products by agarose gel electrophoresis

- The required quantity (g.) of agarose weighed by digital weighing balance and added to a conical flask containing required volume of 1X TBE buffer. (For example : a 2 % gel in 100 ml volume was prepared by dissolving 2 g of agarose in 100 ml of 1X TBE buffer).
- The agarose – TBE mixture was heated in a micro-oven (IFB) until the agarose was completely melted and was stirred interruptedly to ensure even mixing and complete dissolution of agarose.
- The solution was then cooled to about 40-450 C.
- 1 µl Ethydium bromide solution was added and mixed uniformly.
- The solution was poured into the pre levelled gel casting platform after inserting the comb in the trough whose ends were sealed. While pouring, sufficient care was taken for not allowing the air bubbles to trap in the gel.
- The gel was allowed to solidify and the comb was removed after placing the solidified gel into the electrophoretic apparatus containing sufficient buffer (1X TAE) so as to cover the well completely.
- The amplified products (25µl) to be analysed were carefully loaded along with a standard DNA marker (100 bp) into the sample wells with a micropipette, without contaminating the adjacent wells, after adding 1 µl of gel loading buffer with the help of a micropipette.
- Electrophoresis was carried out at a constant voltage (75 V) until tracking dye had migrated for about two third of the effective gel length.
- Ethidium bromide stained DNA banding patterns of PCR product were visualized and photographed for documentation on UV transilluminator (BioDoc-It™ Imaging System, UVP).

Analysis of amplified profiles

Allele scoring

The size (in nucleotide base pairs) of the most intensely amplified band for each microsatellite marker was determined based on its migration relative to a molecular-weight size marker (100 bp DNA ladder).

Primers efficiency analysis

The efficiency of primers was assessed on the basis of three criteria, viz.

i) Proportion of polymorphic bands,

ii) The polymorphism information content (PIC) value was calculated as follows:

PIC= $1/n \Sigma 2F (1-F)$,

Where, F = proportion of bands per assay unit and

n= number of loci detected and summation extends over n alleles.

iii) Marker Index (MI) = Polymorphic Information Content (PIC) x Proportion of polymorphic bands x Average number of loci per assay unit[14]

iv) The discriminating power (*D*) of each primer.

RESULT AND DISCUSSION

18 rice cultivars of West Bengal were subjected to SSR assay to identify the efficient primers for developing DNA fingerprinting. Five SSR primers of rice (RM206, RM219, RM251, RM231: 27.77% each and RM253: 22.22%) revealed a considerable DNA polymorphism among the selected cultivars of West Bengal considered for the study (Table 3). Ten SSR primers of rice cultivars used in the study produced an average of 17.17 per cent polymorphism. The number of polymorphic bandings ranged up to five (Table 3) with an average polymorphic banding patterns per SSR primer was 3.4 and average loci detected in the assay unit was 3.2 , Similar result were reported by Chakravarti *et al*[5]., Sundaram *et al*[19]., and Rahman *et al*[15].

The SSR primer RM219 of rice had showed highest discriminating power (0.817) followed by RM231 (0.797) then RM251 (0.758), RM206 (0.719), RM265 (0.679), RM3 (0.542), RM253 (0.471), RM236 (0.366) respectively and were produced polymorphic banding pattern, where as RM190 and RM349 showed monomorphic banding pattern (Table 4). The highest PIC value had showed by RM265 (0.427), then followed by RM231 (0.376), RM251 (0.357), RM3 (0.341),

RM206 (0.339), RM219 (0.308), RM236 (0.230) and RM253 (0.222) respectively.

The discriminating power (D) parameter, which evaluates the efficiency of a primer for the purpose of identification of varieties; i.e. the probability that two randomly chosen individuals have different patterns.The analysis of power discrimination revealed that the efficiency of a given primer does not depend only on the number of patterns it generates (Table 4). For example, even if two primers produce the same number of patterns (e.g. primers RM206 and RM219), they can have very different discriminating powers (0.719 and 0.817 respectively). The scale of banding pattern variation was ranging from one to five. On the contrary, two primers with quite a different number of banding patterns can have similar discrimination powers which were also judged in similar fashion while using RAPD profile in *Vitis vinifera* L. by Tessier, C. *et al*[20]., and also reported by Cao[4], Fukuoka *et al*[9]., Cho-Young *et al*[7]., in rice. This result can also be explained by the frequency differences between the banding patterns generated with these primers. From the D parameter, it could be helpful to find the optimal combination of primers among all the available primers in according with their D value one after the other, so that it could be help to minimize the number of non-differentiated pairs of cultivars for the given primer combination.

It was also found that high polymorphic information content (PIC) value does not always generate a high polymorphic banding pattern. For example, the PIC value of RM236 and RM265 is 0.3456 and 0.4279 with their polymorphic banding pattern % is 11.11 and 16.66 respectively (Table 4). Whereas the D value of each primer relate proportionally with their polymorphic banding pattern %. But, higher PIC value with higher marker index (MI) for each primer relate proportionally with their polymorphic banding pattern % (Table 5) and similar results also were found by Chattopadhyay, K. *et al*[6]., while studying the "PCR-based characterization of mung bean (*Vigna radiata*) genotypes from Indian subcontinent at intra and inter specific level".

In the present study SSR primer RM206, located on chromosome 11 and RM219, located on chromosome 9 had generated maximum number of loci (5) as well as banding pattern (5), although PIC value was found maximum (0.429) in case of RM253, located on chromosome 6 (Table 3) and was the similar fashion result which was earlier found by Chattopadhyay, K. *et al*[6]., in their study on "Biochemical and SSR marker based characterization of some Bengal land races of rice suffixed with 'sail' in their name".

The marker index (MI) had showed highest value in case of RM231 (0.334) then followed by RM251 (0.318), RM206 (0.301), RM219 (0.274), RM265 (0.228), RM236 (0.204), RM253 (0.197), RM3 (0.182).

From the discussion regarding PIC and MI value (Table 4) for each primer, it was reveal that neither PIC value nor MI value alone could be used for the discriminant analysis, whereas considering with a combination to both the PIC and MI values, it could be used for the discriminant analysis. On the contrary, the D value alone could be used for the discriminant analysis and it need not require a second parameter for the same. Therefore, the D parameter could be used more efficiently for the cultivar discrimination and could also be used for diversity analysis and similar result was also found by Tessier, C. *et al*[20]., during their study on "Optimization of the choice of molecular markers for varietal identification in *Vitisvinifera* L".

RM219 had clearly distinguished Gontra Bidhan-1 (GB1), Gontra Bidhan-3 (GB3) and Gontra Bidhan-4 (GB4) by amplifying 200, 240, and 210 bp fragments respectively. These three genotypes amplified completely different bending pattern than those of MTU-7029 and Swarna. Grains/seeds of GB1, GB3, GB4, MTU7029 and Swarna were almost alike (Fig 2). So employing one single SSR primer RM219, seed purity or admixture can be ascertained. This RM219 alone also distinguished Rasi-1444 from GB4 and MTU1001 by amplifying 220 bp fragment

whose grains/seeds were appeared to be alike (Fig 2) and also distinguished Rasi-1444 from rest of all the cultivars used in the study with unique banding pattern which can be used for ascertaining genetic purity at any stage of the growth period. It was to note that RM219 was situated on chromosome 9 which had association with sub 1 locus in submergence tolerance rice FR13A[6]. So, banding pattern of RM219 also help in marker assisted introgression of sub 1 locus in to these genotypes. These results are in accordance with the findings of Nandakumar *et al*[13]., Macckill[12] and Kubo *et al*[10].

RM253 alone had clearly distinguished Swarna and MTU-7029 by amplifying 110 and 120 bp fragment respectively [Table 6 and Fig 2]. The grains/seeds of these two cultivars were almost alike (Fig 1). So, employing single SSR primer RM253, located on chromosome 6 can be used for ascertaining the seed purity or admixture and can be used for ascertaining genetic purity at any stage of the growth period.

RM206 had clearly distinguished the cultivar Sabita by amplifying 130 bp fragment (Table 6) from Lalat with 150 bp fragment (Fig 2). The grains/seeds of these two cultivars were looking alike (Fig 1). This primer alone also had distinguished from all the cultivars used in the study to the cultivar Sabita with unique banding pattern (130 bp) which can be used for ascertaining genetic purity at any stage of the growth period.

RM231 alone had clearly distinguished the cultivar Khitish by amplifying 180 and 200 bp fragment (Table 6) from IR-36 which produced only a fragment of 180 bp (Fig 2). The grains/seeds of these two cultivars were looking alike (Fig 1). This primer alone also had distinguished from all the cultivars used in the study to the cultivar Khitish with unique banding pattern (180 and 200 bp) which can be used for ascertaining genetic purity at any stage of the growth period.

RM3 alone had clearly distinguished the cultivar Pusa Basmati by amplifying 110 bp fragment (Table.6) of unique banding pattern from all the cultivars used in the study and can be used for ascertaining genetic purity at any stage of the growth period.

With a combination of SSR primer RM219, located on chromosome 9 and RM251, located on chromosome 3 had clearly distinguished Satabadi by amplifying 230 and 150 bp fragment respectively (Table 6 and Fig 2) from Gobindabhog by producing 230 and 100 bp fragment (Fig 2) respectively, whose grains/seeds of those cultivars were almost alike (Fig 1). So, employing a combination of these SSR primers, it can be used for ascertaining the seed purity or admixture and genetic purity at any stage of the growth period.

A combination of SSR primers RM219, RM251, RM231 distinguished the cultivar Gontra bhog and Gontra Bidhan-1 from all other cultivars used in the study by generating amplicon of 200, 150, 180 bp and 200, 150, 194 bp fragments respectively (Table 6). Similarly a combination of RM219, RM206, RM231 distinguished the cultivar CR-1018 and Gontra Bidhan-4 from all other cultivars used in the study by generating amplicon of 210, 150, 180 bp and 200, 150, 200 bp fragments respectively (Table 6). A combination of RM206, RM231 for IR36; RM206, RM219 for Gontra Bidhan-3 and IR64; RM206, RM231 for MTU-1001 and Lalat; RM251, RM219 for Satabadi, Ratna, and Gobindabhog; were distinguished from all other cultivars used in the study (Table 6) and these discrimination can be used for ascertaining the seed purity or admixture and genetic purity at any stage of the growth period[16,18].

From the above and whole, discriminant analysis and discussion, it reveals that the SSR primers used in maximum number of cultivar cases i.e. ten (viz. Gontra bhog, Rasi-1444, Gontra Bidhan-1, Gontra Bidhan-3, Gontra Bidhan-4, CR-1018, Satabadi, IR-64, Ratna, and Gobindabhog) to discriminate among all was RM219, then followed by eight in RM231 and RM206, five in RM251, two in RM253 and one in RM3 (Table.7). This could be concluded that primer

RM219 was the most efficient followed by RM231 for the discrimination of the cultivars used in the present study and also was supported to the earlier result obtained for the discriminating power (D) value alone and also with the % of polymorphic banding pattern (Table 4) whereas the PIC and MI value alone did not supported to the results and with the % of polymorphic banding pattern. Therefore the D parameter could be used more efficiently for the cultivar discrimination and could also be used for diversity analysis.

Table 1: Name of the cultivars and source of collection of rice

Sl. No. of cultivars	Name of the cultivars	Source of collection
1	Sabita (IET-8970)	Gontra S.K.U.S. Ltd. Nadia, West Bengal and Crop Research Unit B.C.K.V.
2	MTU-7029	
3	Gontra Bhog-1 (G.Bhog)	
4	Rasi (IET-1444)	
5	Gontra Bidhan-1 (GB1) (IET-17430)	
6	Khitish (IET-4094)	
7	IR-36	
8	CR-1018	
9	Gontra Bidhan-3 (GB3)	
10	Satabadi (IET-4786)	
11	Pusa Basmati	
12	Vijetha (MTU-1001)	
13	Lalat (IET-9947)	
14	IR-64	
15	Gontra Bidhan-4 (GB4)	
16	Ratna	
17	Swarna	
18	GobindaBhog (Gobinbhog)	

Table 2: Details of SSR primers of rice

Sl. No	Primer code	Orientation	Sequence of the primer ($5^{/}$ - $3^{/}$)	Annealing temp. ^{0}C
1	RM 206	F	CCCATG CGT TTA ACT ATT CT	55
		R	CGT TCC ATC GAT CCG TAT GG	
2	RM 219	F	CGT CGG ATGATG TAA AGC CT	55
		R	CAT ATC GGC ATT CGC CTG	
3	RM 190	F	CTTTGTCTATCTCAA GAC AC	55
		R	TTGCAGATGTTCTTC CTG ATG	
4	RM 251	F	GAA TGG CAA TGG CGCTAG	55
		R	ATG CGG TTC AAG ATT CGA TC	
5	RM 253	F	TCCTTC AAG AGT GCA AAA CC	55
		R	GCA TTGTCATGTCGAAGC C	
6	RM 349	F	TTG CCA TTCGCG TGG AGG CG	55
		R	GTC CATCATCCC TAT GGT CG	
7	RM 236	F	GCG CTG GTG GAA AAT GAG	55
		R	GGC ATC CCT CTT TGA TTCCTC	
8	RM 265	F	CGA GTT CGT CCA AGT GAG C	55
		R	CAT CCA CCA TTCCACCAA TC	
9	RM 231	F	CCA GAT TAT TTC CTG AGG TC	55
		R	CACTTGCATAGTTCT GCA TTG	
10	RM 3	F	ACA CTG TAG CGG CCA CTG	55
		R	CCT CCA CTG CTCCAC ATC TT	

Table 3: Analysis of SSR banding pattern for rice cultivars

S. No.	Name of the primer code	No. of polymorphic banding patterns	Total no. of banding patterns	% of polymorphic banding patterns
1	RM206	5	18	27.77
2	RM219	5	18	27.77
3	RM190	0	18	0
4	RM251	5	18	27.77
5	RM253	4	18	22.22
6	RM349	0	18	0
7	RM236	2	18	11.11
8	RM265	3	18	16.66
9	RM231	5	18	27.77
10	RM3	3	18	16.66
	Total	**32**	**180**	**17.77**

Table 5: Comparison of PIC, MI, D and polymorphic banding pattern % value of SSR primers of rice

Sl. No.	Name of the primer code	PIC value	Marker Index (MI)	Discriminating power (D)	polymorphic (%) banding patterns
1	RM206	0.271	0.301	0.719	27.77
2	RM219	0.308	0.274	0.817	27.77
3	RM190	0.0	0.0	0	0
4	RM251	0.286	0.318	0.758	27.77
5	RM253	0.222	0.197	0.470	22.22
6	RM349	0.0	0.0	0	0
7	RM236	0.345	0.204	0.366	11.11
8	RM265	0.427	0.228	0.679	16.66
9	RM231	0.301	0.334	0.797	27.77
10	RM3	0.341	0.182	0.542	16.66

Table 4: D, PIC and Polymorphism % calculation from resulted banding pattern of SSR marker of rice cultivars:

Primer code Name	Banding pattern in bp size	No. of i^{th} banding pattern	Total no. of cultivars	Proportion of i^{th} banding pattern (F)	Confusion probability of i^{th} banding pattern	Confusion probability of j^{th} primer	Discriminating power of j^{th} primer	2F(1-F)	No. of loci detected	PIC of j^{th} primer	polymorphic (%) banding patterns
RM-206	120	3	18	3/18	0.0196	0.281	**0.719**	0.2777	4	**0.339**	**27.77**
	130	1	18	1/18	0.0			0.1049			
	140	3	18	3/18	0.0196			0.2777			
	150	9	18	9/18	0.2353			0.50			
	140,150	2	18	2/18	0.0065			0.1975			
RM-219	200	4	18	4/18	0.0392	0.1829	**0.817**	0.3456	5	**0.308**	**27.77**
	210	4	18	4/18	0.0392			0.3456			
	220	1	18	1/18	0.0			0.1049			
	230	4	18	4/18	0.0392			0.3456			
	240	5	18	5/18	0.0653			0.4012			
RM-190	100	18	18	18/18	1	1	**0**	0.0	1	**0.0**	**0**
RM-251	100	2	18	2/18	0.0065	0.2417	**0.758**	0.1975	4	**0.357**	**27.77**
	120	2	18	2/18	0.0065			0.1975			
	130	4	18	4/18	0.0392			0.3456			
	150	8	18	8/18	0.1830			0.4938			
	120,150	2	18	2/18	0.0065			0.1975			
RM-253	110	1	18	1/18	0.0	0.5294	**0.471**	0.1049	4	**0.222**	**22.22**
	120	1	18	1/18	0.0			0.1049			
	140	13	18	13/18	0.5098			0.4012			
	150	3	18	3/18	0.0196			0.2777			
RM-349	140	18	18	18/18	1	1	**0**	0.0	1	**0.0**	**0**
RM-236	140,180	4	18	4/18	0.0392	0.6339	**0.366**	0.3456	3	**0.230**	**11.11**
	140,190	14	18	14/18	0.5947			.03456			
RM-265	100	4	18	4/18	0.0392	0.3202	**0.679**	0.3456	3	**0.427**	**16.66**
	110	6	18	6/18	0.0980			0.4444			
	120	8	18	8/18	0.1830			0.4938			
RM-231	180	5	18	5/18	0.0653	0.2025	**0.797**	0.4012	4	**0.376**	**27.77**
	190	3	18	3/18	0.0196			0.2777			
	194	3	18	3/18	0.0196			0.2777			
	200	6	18	6/18	0.0980			.04444			
	180,200	1	18	1/18	0.0			0.1049			
RM-3	110	1	18	1/18	0.0	0.4574	**0.542**	0.1049	3	**0.341**	**16.66**
	120	6	18	6/18	0.0980			0.4444			
	140	11	18	11/18	0.3594			0.4753			

Table 6: Distinctiveness of Rice cultivars through SSR marker

Sl.No.	Name of the distinctive variety from all others	Name of the distinctive primer code	Particulars of the use of primers (single or in combination with)	Unique distinctive feature (amplicon in bp size)
1	Sabita	RM206	single	130
2	MTU-7029	RM253	single	120
3	GontraBhog	RM219+RM251+ RM231	combination	200+150+180
4	Rasi-1444	RM219	single	220
5	Gontra Bidhan-1	RM219+RM251+ RM231	combination	200+150+194
6	Khitish	RM231	single	180,200
7	IR-36	RM206+RM231	combination	140+180
8	CR-1018	RM219+RM206+ RM231	combination	210+150+180
9	Gontra Bidhan-3	RM206+RM219	combination	120+240
10	Satabadi	RM219+RM251	combination	230+150
11	Pusa Basmati	RM3	single	110
12	MTU-1001	RM206+RM231	combination	140+200
13	Lalat	RM231+RM206	combination	190+150
14	IR-64	RM206 + RM219	combination	140,150 + 240
15	Gontra Bidhan-4	RM219+RM206+ RM231	combination	210+150+200
16	Ratana	RM251+RM219	combination	100+200
17	Swarna	RM253	single	110
18	GobindaBhog	RM251+RM219	combination	100+230

Table 7: Efficiency features of SSR primers of Rice cultivars

Primer's code name	Polymorphic band (in bp size)													
	100	110	120	130	140	150	180	190	194	200	210	220	230	240
RM206			GB3	Sabita	IR-36, MTU-1001, IR-64	IR-64								
RM219										Gontra bhog, GB1 Ratna	CR-1018, GB4	Rasi-1444	Satabadi, Gobindabhog	GB3, IR-64
RM251	Ratna, Gobindabhog					Gontra bhog, GB1, Satabadi								
RM253		Swarna	MTU-7029											
RM231							Gontrabhog, Khitish, IR-36, CR-1018	Lalat	GB1	Khitish, MTU-1001, GB4				
RM3		Pusa Basmati												

Fig. 1: Seed morphology of rice cultivars

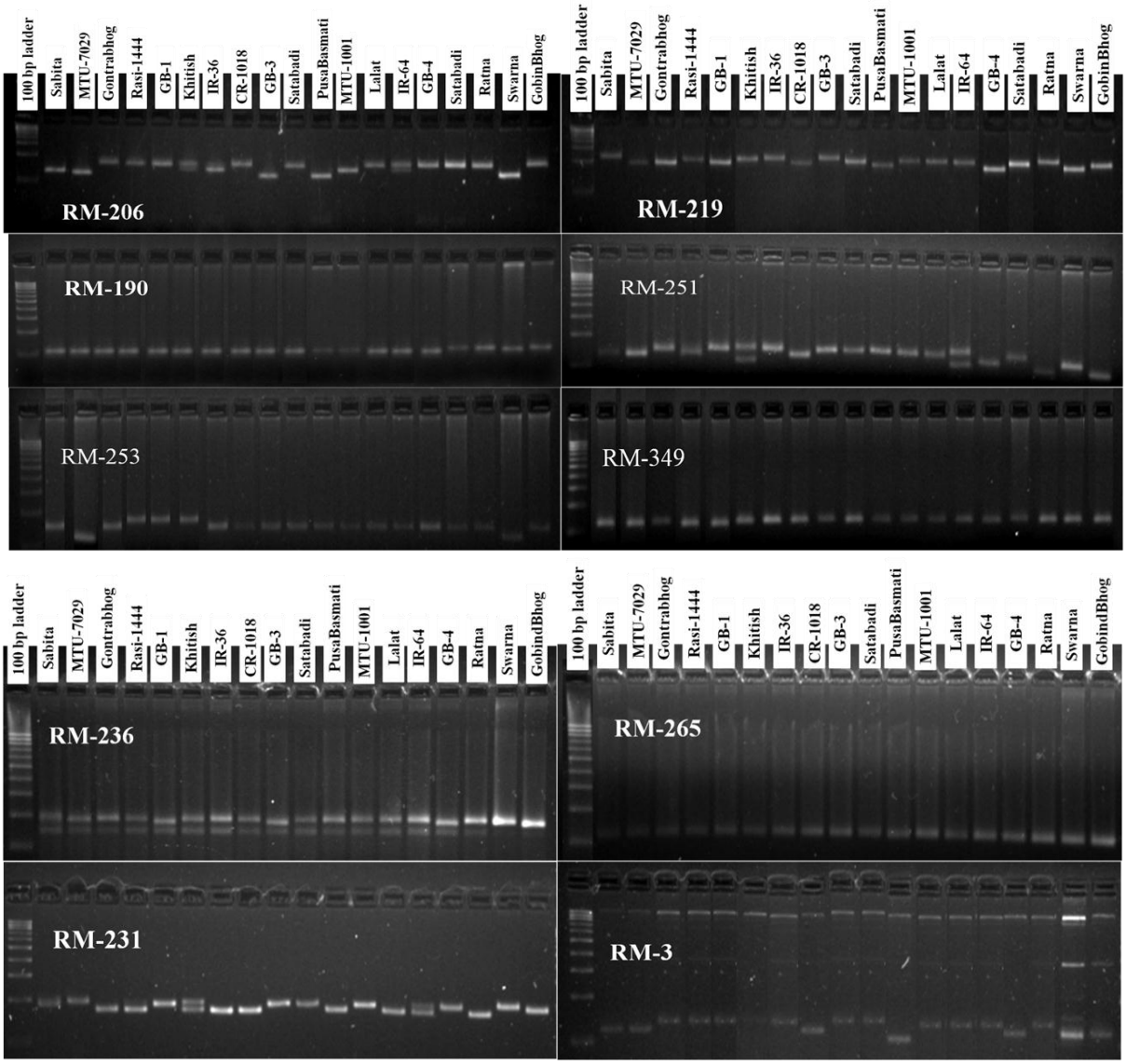

Fig. 2: Banding patterns of SSR markers of rice cultivars

CONCLUSION

The SSR primers used in the study had successfully discriminated all the rice cultivars. The rice SSR primers RM219, RM231, RM251, RM206, RM265, RM3, RM253 followed a descending order of their efficiency to discriminate the rice cultivars. This study also showed that confusion risks must not be neglected in varietal identification. It also emphasizes the necessity of a good basic knowledge of the varietal diversity of a species before choosing the best primer combination for a small set of varieties. The discriminating power *D* can be considered to be a good estimator of the efficiency of a primer or a locus. It allows one to compare different types of molecular markers. It also can be used to predict the efficiency of primers taken in combination, and the risks of confusion due to the use of this combination. This parameter can be of great interest for varietal identification by molecular techniques especially to evaluate the cost in terms of amplifications.

REFERENCES

1. Ajibade S.R., Weeden, N.F. and Chite, S.M., Inter simple sequence repeat analysis of genetic relationships in the genus *Vigna*. *Euphytica*., **111:** 47-55 (2000).
2. Autrique E, Nachit M.M., Monneveux, P., Tanksley, S.D. and Sorrells, M.E., Genetic diversity in durum wheat based on RFLPs, morphological traits and coefficient of parentage. *Crop Sci*., **36:** 735- 742 (1996).

3. Bredemeijer G.M.M., Cook, R.J., Ganal, M.W., Peeters, R. and Issac, P., Construction and testing of microsatellite database containing more than 500 tomato varieties. *Theor. Applied Genet*., **105:** 1019-1026 (2002).
4. Cao, D. and Oard, J.H., Pedigree and RAPD-based DNA analysis of commercial U.S. rice cultivars. *Crop Sci*., **37:** 1630–1635 (1997).
5. Chakravarthi, B., Kalyan, and Naravaneni Rambabu, SSR marker based DNA fingerprinting and diversity study in rice (*Oryza sativa*. L). *African Journal of Biotechnology*, **5(9):** 684-688 (2006).
6. Chattopadhyay, K., Bhattacharya, S., Mandal, N., Sarkar, H.K., PCR-based characterization of mung bean (*Vigna radiata*) genotypes from Indian subcontinent at intra- and inter- specific level. *Journal-of-Plant-Biochemistry-and-Biotechnology*, **17(2):** 141-148 (2008).
7. Cho-Youngchan, Shin-Youngseop, Ahn-Sangnag, Gregorio G.B., Kang-Kyongho, Brar, D., Moon-Huhnpal, DNA fingerprinting of rice cultivars using AFLP and RAPD markers. *Korean-Journal-of-Crop-Science*. **44(1):** 26-31 (1999).
8. Cooke, R.J., Modern methods for cultivar verification and the transgenic plant challenge. *Seed Sci. Technol*., **27:** 669-680 (1999).
9. Fukuoka, S., Hosaka, K. and Kamijima, O., Use of random amplified polymorphic DNAs (RAPDs) for identification of rice accessions. *Jpn J Genet*., **67:** 243–252 (1992).
10. Kubo, T., Ogata, T., Yoshimura, A., Matsue, Y. and Iwata, N., DNA fingerprinting of *japonica* rice varieties using RAPD analysis. *Science-Bulletin-of-the-Faculty-of-Agriculture, Kyushu University*, **55(1):** 5-11 (2000).
11. Kumar, P., Rajendra, Sujatha, K., Rao, K.S., Kumar, P., Nataraj, Viraktamath, B.C., Balachandran, S.M., Biswal, A.K. and Sundaram, R.M., A protocol for isolation of DNA suitable for rapid seed and grain purity assessments in rice; vol. **23** > b. Research notes > vi. *Gene, genome structure/function and methods.*
12. Mackill, D.J., Classifying japonica rice cultivars with RAPD markers. *Crop Sci*., **35:** 889–894 (1995).
13. Nandakumar, N., Singh, A.K., Sharma, R.K., Mohapatra, T., Prabhu, K.V. and Zaman, F.U., Molecular fingerprinting of hybrids and assessment of genetic purity of hybrid seeds in rice using microsatellite markers. *Euphytica*., **136:** 257-264 (2004).
14. Powell, W., Morgante, M., Andre, C., Hanafey, M., Vogel, J., Tingey, S. and Rafalski, A., The comparison of RFLP, RAPD, AFLP and SSR (microsatellite) markers for germplasm analysis. *Mol. Breed*., **2:** 225-238 (1996).
15. Rahman, M.S., Molla, M.R., Alam, M.S., Lutfur Rahman, DNA fingerprinting of rice *(Oryza sativa* L.) Cultivars using microsatellite markers. *Australian-Journal-of-Crop-Science*., **3(3):** 122-128 (2009).
16. Ray Choudhury, P., Kohli, S., Srinivasan, K., Mohapatra, T. and Sharma, R.P.,. Identification and classification of aromatic rices based on DNA Fingerprinting. *Euphytica.*, **118:** 243–251 (2001).
17. Souframanien, J. and Gopalakrishna, T., A comparative analysis of genetic diversity in black gram genotypes using RAPD and ISSR markers. *Theor. Appl. Genet*. **109:** 1687-1693 (2004).
18. Stobdan, T., Khanna, V.K., Singh, N.K. and Mani, S.C., DNA fingerprinting of Indian aromatic rice genotypes by RAPD. *Rice- Biotechnology-Quarterly*, **40:** 18-19 (1999).
19. Sundaram, R.M., Naveenkumar, B., Biradar, S.K., Balachandran, S.M. and Mishra, B., Identification of informative SSR markers capable of distinguishing hybrid rice parental lines and their utilization in seed purity assessment. *Euphytica*., **163:** 215-224 (2007).
20. Tessier, C., David, A.J., This, A.P., Boursiquot, J.M. and Charrier, A.A., Optimization of the choice of molecular markers for varietal identification in *Vitis vinifera* L. *Theor Appl Genet*., **98:** 171-177 (1999).