JEB
Website : www.jeb.co.in
E-mail : editor@jeb.co.in

Journal of Environmental Biology

ISSN: 0254-8704 (Print)
ISSN: 2394-0379 (Online)
CODEN: JEBIDP

Isolation of useful scented rice mutants and comparative assessment of genetic diversity

Swapan K. Tripathy*, Rajesh Ranjan, Sasmita Dash, Devraj Lenka Debendra N. Bastia, Bhabendra Baisakh, Pramod C. Satpathy and Simanchal Sahu

S.K. Sinha Molecular Breeding Laboratory, Department of Plant Breeding and Genetics, College of Agriculture, Ouat, Bhubaneswar-751 003, India

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

Publication Info

Paper received:
17 June 2014

Revised received:
16 January 2015

Accepted:
01 March 2015

Abstract

A set of 36 scented rice mutants were developed through recurrent mutagenesis Pusa basmati-1, Pusa Sugandha-2 and Ketakijoha local with ethyl methane sulphonate (EMS), N-methyl N-nitro N- nitrosoguanidine (NG) and a combination of 0.4% EMS and 0.015%NG over two successive generations. ORM 256-8-10 and ORM 256-8-6 (mutants of PB-1) and ORM 228-1 (mutant of Pusa Sugandha 2) had shown significantly higher grain yield than Geetanjali (standard check), as well as, their respective parent varieties. The above test genotypes were analysed by 12 RAPD and 11 ISSR primers. RAPD and ISSR primers amplified 92 and 77 bands ranging from 4-15 and 4-12 bands per respective primer exhibiting higher level of polymorphism (86.95% and 94.80%). RAPD primer OU 1 and ISSR primer OUAT 7 produced maximum number of 15 (280-1830bp) and 12 polymorphic bands (420-1260bp), respectively. ISSR revealed higher polymorphic information content (PIC) values than RAPD primers indicating better allelic diversity. Resolving power revealed higher efficiency of RAPD primers. OU-1 with high GC content (80%) and two ISSR primers OUAT-7 (GC=66.7%) and OUAT-15 (GC=88.2%) produced higher number of polymorphic amplicons. ORM 228-1, Pusa Sugandha-2, ORM 256-8-10 and Ketakijoha were identified to be highly divergent genotypes based on RAPD and ISSR analyses. RAPD analysis revealed divergence of ORM 256-2 and ORM 256-8-12 while ORM 256-8-6 isolated from rest of the genotypes in case of ISSR. This could be attributed to genotype-specific RAPD and ISSR alleles. The above diverse genotypes with high yield identified in the present pursuit would enrich the basmati gene pool for further genetic improvement for grain quality and yield per se.

Key words

Genotypes, Genetic diversity, Inter-simple sequence repeats, Random amplified polymorphic DNA, Rice mutants

Introduction

Rice is the staple food for more than half of world's population and 70% Indians. Increasing productivity is continuing to be the top most priority to meet the food demand of expanding human population which overshadowed grain quality for a pretty long time. Continued upliftment in economic condition have resulted in changing life style and awareness among consumers to improve and diversify diets, including the quality of rice consumed. In recent years, quality improvement assumed greater importance in breeding rice to track market potential and to meet consumer preference. India is the largest producer and exporter of basmati rice as it is acclaimed as the best quality rice in the world market. Traditional basmati varieties are specifically adapted to their native area of cultivation (foot hills of Himalayas in UP and Bihar of India; and Tarai region of Nepal) and are sensitive to photoperiod, prone to lodging and have poor response to fertilizer application. The crosses made so far involving basmati quality donors *i.e.,* PB-1, Taraori basmati, Basmati 370 *etc.,* have not rendered any remarkable break-through in genetic enhancement for

productivity which could be due to lack of diversity among the aromatic rices (Nagaraju *et al*., 2002). Very often delicate genetic background conforming to basmati standard is disturbed in the segregating population. Further, low genetic diversity in basmati types is a problem for development of basmati hybrids. However, induction of mutation in popular high yielding basmati rice varieties or local scented landraces fulfilling the quality features could be a feasible proposition. Mutants are traditionally identified on the basis of their morphological features, but development of DNA marker based genotyping has made this process quicker and more reliable (Abdulla and Gamal 2010, Barakat and El-Sammak 2011). Subtle difference at genotypic level that could not be differentiated by phenotyping can be confirmed using markers. RFLP (Chuan-Qing *et al.,* 2000), Microsatellite (Gao *et al.,* 2009, Mukherjee *et al.,* 2013), RAPD (Tripathy *et al.,* 2012, Singh *et al.,* 2013), AFLP (Jeung *et al.,* 2005) and ISSR (Reddy *et al.,* 2009, Tripathy *et al*., 2012) markers are commonly used for genome analysis. RAPD markers can be used, owing to their flexibility and low cost involved as compared to RFLP and AFLP, but, ISSR technique is more preferred as it combines the advantage of both RAPD and reproducible microsatellite marker system (Girma *et al.,* 2010, Li Ya-li *et al*., 2006). Keeping in view the above facts, a set of high yielding mutants, with desirable attributes from the popular scented rice varieties, were developed using EMS and NG. These mutants were characterized for varietal identification and study of *inter se* genetic diversity using RAPD and ISSR markers for their use in further breeding programme.

Materials and Methods

Plant materials and isolation of DNA : Genomic DNA was isolated from 2g tender young leaves of aseptically grown seedlings of 40 scented rice genotypes including 36 M_8-mutants derived through recurrent mutagenesis with EMS (0.2, 0.4 and 0.6%), NG (0.01, 0.015 and 0.02%) and a combination of 0.4% EMS and 0.015%NG over two successive generations, three parent varieties (Pusa basmati-1, Pusa Sugandha-2 and Ketakijoha local) and one popular check variety (Geetanjali) using standard sodium dodecyl sulphate method (Dellaporta *et al.,* 1983) with minor modification. Plant materials were homogenized in liquid nitrogen and extracted with extraction buffer (100mM Tris-HCl pH 8.0, 20mM EDTA, 0.5M NaCl, 7M Urea, 0.1% β-ME and 2% SDS) at 65ºC for one hour with occasional shaking and an equivalent volume of phenol-chloroform-isoamyl alcohol (25:24:1) mixture was added and centrifuged at 12,000 rpm for 10 min. at 4ºC. The supernatant was added with an equal amount of ice cold absolute ethanol and kept overnight to precipitate the DNA. Intact genomic DNA was hooked out and washed with 70% ethanol and finally re-dissolved in TE buffer (10mM Tris-HCl, pH-8.0 and 1mM EDTA). DNA was purified by DNase free RNase-A (GeNei, Bangalore, India) @ 20µg per ml of DNA extract to remove contaminating RNAs. Finally, the quality of DNA was checked using the ratio of absorbance at 260nm and 280nm and also rechecked by running each sample in 0.8% agarose gel. DNA was quantified through UV-VIS Nanodrop-2000 spectrophotometer (Thermo Electron Scientific Instruments LLC, USA) at 260nm and diluted to a working concentration of 10 ng μl^{-1} for PCR analysis.

RAPD and ISSR analysis : Genomic DNA sample of each genotype was individually primed and amplified using 13 random decamer RAPD primers and 11 ISSR primers (Chromos Biotech. Pvt. Ltd., Bangalore, India). PCR amplification was performed in a reaction volume 25µl containing 1X reaction buffer (10mM Tris HCl, pH 9.0, 1.5mM MgCl2, 50mM KCl, 0.01% gelatin), 1 unit of Taq polymerase (GeNei, Bangalore, India), 2.5mM each of dNTPs, 10ng primer and 20ng of genomic DNA; and. amplifications were performed in a GenePro Thermal Cycler (Bioer Tech. Co., Ltd, Japan), programmed for 5min at 94°C, 40 cycles of 1min at 94°C, 1.5 min at annealing temperature as mentioned in Table 2 and 2min at 72°C and final extension for 7 min at 72°C followed by storing at 4°C till loading to agarose gel. The amplified products were loaded in agarose gel (1.5% in case of RAPD and 2.0% in case of ISSR) containing ethidium bromide @1.0µg ml^{-1} of agarose solution and electrophoresed at a constant voltage of 50V.

The gels were documented by gel doc system (Fire Reader-Uvtec, Cambridge, UK) for scoring the bands. The amplification products were checked for their reproducibility using each primer at least twice. Reproducible bands at about 0.5mm apart or more were considered for scoring. The presence and absence of bands were scored as 1 and 0 respectively; and each band corresponding to the amplified fragment was treated as a unit character (as these are dominant markers) to determine variation among genotypes. The size of amplicons was determined by comparing with lambda DNA ladder (500bp) with known size (bp) fragments. Polymorphism information content (PIC) was calculated based on the number of bands per primer, using the formula PIC = $\Sigma (1 - p_i^2)/n$ where, p_i is the frequency of i^{th} band amplified by the primer and n= total no. of bands produced by the primer. Whereas, the resolving power (R_p) of a primer was estimated as $R_p = \Sigma I_b$, where I_b (band informativeness) =1–[2× (0.5–pi)] and pi is the proportion of genotypes showing i^{th} band (Prevost and Wilkinson 1999).

Results and Discussion

Mutation breeding has been successful in obtaining new cultivars and to broaden genetic base in rice. By 2003, 440 mutant rice varieties had been developed. Of these, 264

were produced by direct application of mutagens and 176 were created by crossbreeding with induced mutants (Shehata *et al.,* 2009). In the present pursuit, mutant genotypes showed wide variation (Table 1) in important agro-economic traits e.g., maturity duration (134.6-155days), plant height (96.6-150.0cm), tiller number per plant (7.3-15.6), grain number/panicle (42.0-192.7), 1000-grain weight (14.7-31.2) and seed yield/plant (16.0-49.9g). Two mutants of PB-1 (ORM 256-8-10 and ORM 256-8-6) and one mutant of Pusa Sugandha 2 (ORM 228-1) were top yielders with significantly higher grain yield (>40 g plant^{-1}) as compared to the best standard check variety Geetanjali (17.6 g plant^{-1}), as well as, their parent varieties PB-1 (25.4 g plant^{-1}) and PS-2 (23.3 g plant^{-1}). High yield performance of these mutants could be attributed to high tiller number and/or more number of seeds/panicle.

Table 1 : Mean values of 40 test genotypes for different agro-economic traits.

Genotypes	Pedigree	Duration (days)	Plant height (cm)	EBT/ plant	Panicle length (cm)	Grain no./ panicle	Test weight (g)	Yield/ plant (g)	Kernel dimension			Aroma
									Length (mm)	Bread th(mm)	L/B Ratio	
ORM 248-1	PB-1 mutant	140.2**	116.3	07.3	29.2	97.1	23.8**	18.7	7.6	2.0	3.6	1
ORM 249-3	-d0-	148.0	102.6**	11.6	27.6	96.8	22.2**	19.3	7.6	1.9	3.9	2
ORM 249-4	-d0-	145.0	117.3	10.7	30.4*	95.2	24.4**	30.8*	5.0	2.3	3.0	0
ORM 250-1	-d0-	140.0**	109.3*	10.0	28.5	75.8	14.7	19.1	7.0	1.6**	4.3**	0
ORM 250-2	-d0-	137.6**	113.3	10.7	29.2	102.6	19.6	20.5	8.5**	1.7**	4.9**	0
ORM 250-5	-d0-	145.0	144.3	11.9	29.1	158.3**	23.2**	32.3*	8.2*	1.8	4.2**	1
ORM 250-7	-d0-	142.0**	109.0*	15.0**	26.1	59.4	22.2**	20.7	8.2*	1.8	4.4**	0
ORM 251-4-4	-d0-	144.6	113.5	10.1	25.2	100.2	22.5**	20.4	6.9	2.0	3.4	2
ORM 254-5-3-3	-d0-	141.0**	140.0	11.0	26.3	127.9**	15.2	26.0	6.7	2.0	3.3	2
ORM 256-2	-d0-	151.0	146.5	11.4	28.7	128.3**	23.6**	37.8**	7.4	1.7**	4.3**	1
ORM 256-7-2	-d0-	146.3	137.1	12.6	26.9	97.8	19.7	22.9	7.1	2.1	3.4	3
ORM 256-8-3	-d0-	150.0	103.0**	09.7	25.5	78.1	23.5**	25.6	7.9	2.0	3.9	1
ORM 256-8-4	-d0-	155.0	105.0**	09.8	25.7	79.0	16.9	22.2	7.5	1.9	3.6	0
ORM 256-8-5	-d0-	154.6	101.0**	10.6	24.2	78.5	17.5	18.5	7.2	1.9	3.7	2
ORM 256-8-6	-d0-	155.0	138.6	12.8	26.9	153.9**	17.3	49.6**	5.5	2.2	2.5	2
ORM 256-8-7	-d0-	150.0	111.5	10.1	27.6	81.9	20.8	18.2	7.9	1.8	3.8	2
ORM 256-8-10	-d0-	141.0**	149.0	15.6**	29.3	106.7	19.2	49.9**	6.9	1.8	3.8	1
ORM 256-8-11	-d0-	147.6	107.3**	10.9	26.7	95.2	20.0	21.5	8.2*	2.0	4.1**	2
ORM 256-8-12	-d0-	146.0	130.1	10.7	30.1*	108.6	25.9**	36.8**	8.3**	2.0	4.1**	1
ORM 256-8-13	-d0-	148.0	117.0	10.3	30.0*	129.2**	17.5	22.6	7.9	2.0	3.8	1
ORM 256-8-14	-d0-	137.6**	119.3	09.5	25.3	74.6	13.6	17.3	7.0	1.8	3.8	2
ORM 256-8-15	-d0-	140.3**	103.6**	12.1	27.6	101.2	19.8	28.8	7.7	1.5**	4.0*	1
ORM 257-5-3-1	-d0-	134.6**	129.8	11.2	26.4	84.8	31.2**	32.7**	7.5	2.1	3.5	1
ORM 256-8-10	-d0-	149.0	113.5	10.8	26.5	100.2	19.7	17.6	7.7	1.8	4.2**	2
ORM 920-1	-d0-	154.6	139.1	13.1*	29.2	62.5	19.4	18.6	7.9	2.1	3.7	0
ORM 920-2	-d0-	144.6	96.6**	13.2*	26.1	62.2	25.6**	20.1	7.7	1.7**	4.3**	2
ORM 920-3	-d0-	149.0	104.3**	11.5	26.2	78.7	20.0	18.0	7.5	2.0	3.7	1
ORM 920-5	-d0-	137.6**	109.3*	08.2	25.1	92.6	28.6**	22.8	7.9	1.8	4.3**	0
ORM 920-6	-d0-	153.0	110.0*	09.8	26.5	116.6	28.3**	28.6	7.8	2.2	3.5	1
PB-1 (Parent)	Parent	137.6**	110.0*	09.6	27.0	112.0	19.3	25.4	7.8	2.2	3.5	2
ORM 223-1	PS-2 mutant	151.6	119.3	10.6	28.0	96.6	19.9	18.9	7.2	2.1	3.4	3
ORM 223-2	-d0-	141.0	96.6**	12.2	24.5	54.4	24.5**	25.2	8.3**	2.1	3.9	2
ORM 227-1	-d0-	142.3**	127.0	10.4	28.8	88.8	19.6	22.3	6.7	1.6**	4.3**	1
ORM 227-2	-d0-	147.0	117.0	10.8	24.6	42.0	27.5**	16.0	8.5**	1.8	4.6**	1
ORM 228-1	-d0-	154.6	150.0	11.5	27.2	192.7**	17.0	40.6**	7.1	2.1	3.4	2
ORM 228-3	-d0-	150.0	145.6	11.6	30.3*	115.8	22.2**	37.6**	7.2	2.0	3.5	2
PS -2	Parent	144.6	140.6	10.4	22.4	96.7	22.3**	23.3	7.4	2.0	3.6	3
ORM 240-1	Keta. mutant	144.6	141.0	09.6	26.8	100.4	27.9**	19.5	9.1**	2.0	4.5**	2
KETAKIJOHA	Parent	140.0**	116.5	07.6	29.5	97.2	23.8**	18.8	7.6	2.0	3.7	2
GEETANJALI	Check	145.3	140.6	09.5	28.3	85.9	16.5	17.6	6.0	1.9	3.1	3
GRAND MEAN		**145.6**	**121.0**	**10.9**	**27.2**	**97.7**	**21.4**	**25.1**	**7.4**	**1.9**	**3.8**	
$CD_{0.05}$		**1.29**	**10.06**	**2.16**	**2.61**	**19.2**	**0.48**	**5.65**	**0.67**	**0.11**	**0.17**	
$CD_{0.01}$		**1.69**	**13.22**	**2.84**	**3.43**	**25.2**	**0.63**	**7.42**	**0.88**	**0.15**	**0.22**	

Kernel being directly used for cooking, its dimension or shape is the first and foremost important consideration to the consumers. Long and very slender kernel types are preferred by the consumers and therefore, such kernel types fetch high premium price in the market. These types can be identified easily based on kernel length with L/B ratio. Kernel length more than 7.0 mm. and L/B ratio >3.5 fulfill the minimum standard for basmati types. A ketakijoha mutant ORM 240-1 was identified to possess kernel length more than 9.0mm with L/B ratio of 4.5 and this is comparable to the world's largest slender kernel variety Pusa sugandha-4 (Pusa 1121) (Singh and Singh 2002). Similarly, a PB-1 mutant ORM 250-2 showed significantly high kernel length (8.5 cm), as well as, kernel L/B ratio (4.91) as compared to its parent PB-1(3.5). This was due to significant iso-diametric reduction in breadth (1.7cm) as compared to length of the kernel. However, considering both productivity and quality characteristics; ORM 250-5, ORM 256-2 and ORM 256-8-12 (all mutants of PB-1) was selected as promising. Elucidation of such genetic variation at phenotypic level could be better characterized by genotyping.

12 RAPD and 11 ISSR primers (Table 2) were used for genomic DNA amplification of 40 test genotypes which indicated wide range of amplicon sizes (bp). Comparatively, RAPD produced amplicons of longer size than ISSR. It ranged from 280 to 2500 bp in case of RAPD, while ISSR revealed amplicon sizes within the range of 358 to 1650 bp only. Total number of bands amplified by individual RAPD and ISSR primers ranged from 4-15 and 4-12 bands with an average of 7.66 and 7.0 bands per respective primer. The range and average number of loci reproduced were comparable to earlier studies of Saini *et al.* (2004), Rabbani *et al.* (2008) and Kiani *et al.* (2011). Five out of 12 RAPD primers produced 100% polymorphism among which OU-1 revealed highest number of polymorphic bands (15bands) of fragment size 280-1830bp. Besides, primer OU- 2 and OU- 3 (Fig 1a and b) amplified ten and nine distinct polymorphic bands respectively. Whereas, all ISSR primers except OUAT-5, OUAT-8, OUAT-11 and OUAT-13 produced 100% polymorphic bands (Table 2). Primer OUAT-7 (Fig 2a) produced 12 polymorphic bands (420-1260bp) followed by OUAT 10 (Fig 2b) and OUAT 15 that showed eight

Table 2 : Amplified products with different primers in 40 scented rice mutants

Primer code	Sequences(5'-3')	GC content (%)	*Tm (°C)	*Tan (°C)	Polymorphicbands	No. of monomorphic bands	Total bands	% polymorphism	PIC value	Resolving power (Rp)	Range of fragmentsize (bp)
					RAPD Primers						
OU-1	CAGGCGGCGT	80.0	36.0	38.0	15	0	15	100.0	0.686	14.65	**280**-1830
OU-2	ACTGAACGCC	60.0	32.0	34.0	10	0	10	100.0	0.516	12.35	400-1700
OU-3	GGTGAACGCT	60.0	32.0	34.0	9	0	9	100.0	0.783	6.20	350-1450
OU-5	AGGACGTGCC	70.0	34.0	36.0	3	2	5	60.0	0.396	7.40	550-2020
OU-10	AATCGGGCTG	60.0	32.0	34.0	7	0	7	100.0	0.707	5.80	455-1800
OU-16	CTGAGACGGA	60.0	32.0	34.0	6	1	7	85.7	0.625	7.70	420-2000
OU-20	AACGTCGAGG	60.0	32.0	34.0	3	1	4	75.0	0.615	4.50	390-2220
OU-24	TCCGCTGAGA	60.0	32.0	34.0	4	2	6	66.0	0.466	8.30	460-**2500**
OU-28	TGCTGCAGGT	60.0	32.0	34.0	4	0	4	100.0	0.636	4.75	385-1930
OU 30	CTGCTGGGAC	70.0	34.0	35.0	6	2	8	75.0	0.508	10.25	300-1820
OU 32	GAGAGCCAAC	60.0	32.0	34.0	7	2	9	77.9	0.569	11.05	290-1550
OU 35	AGGTTGCAGG	60.0	32.0	34.0	6	2	8	75.0	0.551	9.30	360-1280
Total	**80**	**12**	**92**								
					ISSR Primers						
OUAT-2	(GGTT)6C	52.0	64.6	61.0	6	0	6	100.0	0.632	5.95	380-750
OUAT-5	(AACC)6T	48.0	63.0	61.0	4	1	5	80.0	0.639	5.35	430-1250
OUAT-3	(TGGT)6C	52.0	64.6	56.0	7	0	7	100.0	0.819	5.30	450-1300
OUAT-7	CCA(GTG)4	66.7	53.3	54.0	12	0	12	100.0	0.786	9.75	420-1260
OUAT-8	CGA(CTC)4	66.7	53.3	66.7	6	1	7	85.7	0.686	6.80	480-1420
OUAT 10	(TC)8T	47.1	50.4	47.1	8	0	8	100.0	0.802	6.45	410-1540
OUAT-11	(TG)8C	52.9	52.8	56.6	3	1	4	75.0	0.632	4.30	**358**-1500
OUAT-12	(AG)8C	52.9	52.8	46.6	6	0	6	100.0	0.810	3.75	700-**1650**
OUAT 13	(GA)8C	52.9	52.8	48.5	6	1	7	85.7	0.607	8.35	650-1510
OUAT-15	(GGC)5AT	88.2	67.2	58.0	8	0	8	100.0	0.854	5.40	650-1230
OUAT-18	(AGC)5CG	70.6	60.0	61.0	7	0	7	100.0	0.774	6.30	530-1050
Total	**73**	**4**	**77**								

Note: Range of amplicon size as a whole for RAPD and ISSR marked bold. * "Tm" and "Tan" denote melting and annealing temperature of primers

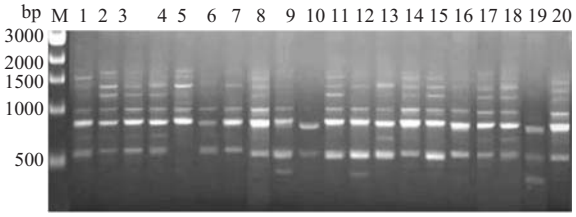

Fig. 1(a) : RAPD profile of different scented rice mutants amplified with primer OU-2. M=DNA molecular marker; Lane 1-20: ORM 248-1, ORM 249-3, ORM 249-4, ORM 250-1, ORM 250-2, ORM 250-5, ORM 250-7, ORM 251-4-4, ORM 254-5-3-3, ORM 256-2, ORM 256-7-2, ORM 256-8-3, ORM 256-8-4, ORM 256-8-5, ORM 256-8-6, ORM 256-8-7, ORM 256-8-10, ORM 256-8-11, ORM 256-8-12 and ORM 256-8-13

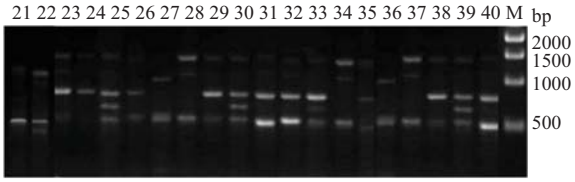

Fig. 1(b) : RAPD profile of different scented rice mutants amplified with primer OU-3. Lane 21-40: ORM 256-8-14, ORM 256-8-15, ORM 257-5-3-1, ORM 257-5-3-2, ORM 920-1, ORM 920-2, ORM 920-3, ORM 920-5, ORM 920-6, PB-1, ORM 223-1, ORM 223-2, ORM 227-1, ORM 227-2, ORM 228-1, ORM 228-3, PS-2, ORM 240-1, Ketakijoha and Geetanjali, M=DNA molecular marker

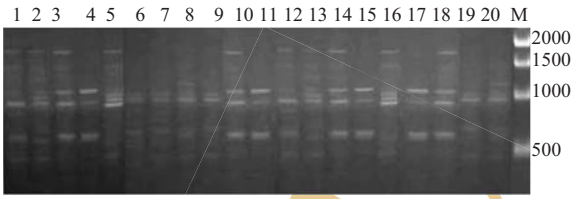

Fig. 2(a) : ISSR profile of different scented rice mutants amplified with primer OUAT-7, Lane 1-20: ORM 248-1, ORM 249-3, ORM 249-4, ORM 250-1, ORM 250-2, ORM 250-5, ORM 250-7, ORM 251-4-4, ORM 254-5-3-3, ORM 256-2, ORM 256-7-2, ORM 256-8-3, ORM 256-8-4, ORM 256-8-5, ORM 256-8-6, ORM 256-8-7, ORM 256-8-10, ORM 256-8-11, ORM 256-8-12 and ORM 256-8-13, M=DNA molecular marker

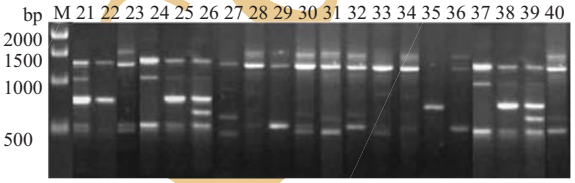

Fig. 2(b) : ISSR profile of different scented rice mutants amplified with primer OUAT-10. M=DNA molecular marker, Lane 21-40: ORM 256-8-14, ORM 256-8-15, ORM 257-5-3-1, ORM 257-5-3-2, ORM 920-1, ORM 920-2, ORM 920-3, ORM 920-5, ORM 920-6, PB-1, ORM 223-1, ORM 223-2, ORM 227-1, ORM 227-2, ORM 228-1, ORM 228-3, PS-2, ORM 240-1, Ketakijoha and Geetanjali

distinct bands each. OU 1, OU 2 and OUAT-7 could be considered highly useful primers for genetic diversity analysis and for detecting genotype variation in mutants. RAPD and ISSR analysis revealed 92 and 77 scorable bands respectively, out of which 80 and 73 amplicons were polymorphic which resulted in high level of polymorphism (86.95% and 94.80%). Singh *et al.,* (2013) and Saini *et al*.(2004) reported 92% and 78.33% polymorphism using RAPD and ISSR markers.

Total number of bands scored in genotype was invariably higher in RAPD than ISSR and it varied widely among the genotypes. RAPD revealed 53, 52, and 46 bands in PB-1, PS-2 and Ketakijoha, but it varied from 39 (ORM 256-8-12) to 61 bands (ORM 248-1) among mutants. In contrast, ISSR amplified 32, 28, and 24 bands in PB-1, PS-2 and Ketakijoha respectively, while it ranged from 22 (ORM 256-8-6) to 44 bands (ORM 257-5-3-1) among the mutants. The resulting data matrix resolved a total of 1,601 polymorphic amplicons out of 2,081 PCR products across the test genotypes using 12 RAPD primers which revealed 76.93% polymorphism. Whereas, 11 ISSR primers visualized 1,194 polymorphic bands out of 1,354 amplified products in the present set of materials and revealed 88.18% polymorphism. High level of polymorphism could be ascribed to higher genetic variation induced by recurrent mutagenesis in aromatic rice mutants. Kaushik *et al.* (2003) reported 149 bands (200-3530bp) in two rice varieties and their selected segregating F_3 plants using 26 ISSR primers.

In the present investigation, a poly-AG primer OUAT-12 with anchored Cytosine (C) at 3' end produced 100% polymorphism than Poly-GA primer OUAT-13. Joshi *et al*. (2000) obtained highly polymorphic pictures of DNA fingerprints using poly-GA or Poly -AG, irrespective of any anchored nucleotide at either 3' or 5' end. A Poly –TC ISSR primer with anchored T at 3' end (OUAT-10) was found to be more informative(100% polymorphism) than Poly-TG primer with anchored C at 3' (OUAT-11), which may be ascribed to distribution of repeats in rice genome being within the range of amplification by Taq DNA polymerase.

Polymorphism information content (PIC) -a measure of allelic diversity produced by a primer ranged from 0.396 (OU-5) to 0.783 (OU-3) for RAPD, while it was 0.607 (OUAT-13) to 0.854 (OUAT-15) for ISSR primers (Table 2). High PIC values of ISSR suggested its superiority over RAPD primers. Three RAPD primers (OU-1, OU-3 and OU-10) and six ISSR primers (OUAT-3, OUAT-7, OUAT-10, OUAT-12, OUAT-15 and OUAT 18) with high PIC values

than the mean were considered highly polymorphic. Reddy *et al.* (2009) reported (GA) 8G as the most informative primer that showed highest polymorphic information content (PIC=0.75) and resolving power (Rp=14.8).

Resolving power (Rp) of different primers as indicative of genotype discrimination ranged from 4.50 (OU-20) to as high as 14. 65(OU-1) with a mean value of 8.52 for RAPD primers. Narrow range of resolving power was revealed for ISSR primers (3.75 in OUAT-12 to 9.75 in OUAT-7). ISSR primer OUAT-7 revealed highest number of polymorphic bands (12 bands) with 100% polymorphism, as well, as high PIC and Rp values. Thus, such an informative and discriminative ISSR primer is of immense value for the study of genetic diversity in rice. On similar considerations, RAPD primers OU-1 and OU-2 could be considered for genetic analysis and varietal discrimination. For instance, OU-1 alone could discriminate all 40 scented rice genotypes, while highly informative ISSR primer OUAT -7 produced distinct fingerprint in all the genotypes except ORM 254-5-3-3 and ORM 256-8-12 at 100% phenon level. Jeung *et al.* (2005) compared relative efficiency of RAPD, ISSR and AFLP. AFLP assay discriminated the genotypes effectively with a good discriminating power (0.99), followed by ISSR (0.76) and RAPD (0.61). Ye Chunjiang *et al.* (2005) proposed that RAPD primers could be successfully used with ISSR primers for genome analysis.

Twelve monomorphic RAPD bands (Table 1**)** were observed by different primers. OU-5, OU-24, OU-30, OU-32 and OU-35 produced two monomorphic bands each; and OU-16 and OU-20 revealed single monomorphic band each. The presence or absence of few bands was found to be specific to certain test genotypes. 420bp and 350bp bands produced by OU-3 were unique to mutant ORM 228-1 and ORM 256-8-15 respectively (Fig. 1b). OU-2 produced a specific 850bp band in ORM 256-2 (Fig 1a). Similarly, 1000bp and 480bp bands amplified by OU-3 were specific to ORM 920-3 and ORM 228-3; and ORM 223-1 and Geetanjali respectively. The RAPD primer OU-10 could reveal 1430bp band in both the mutants ORM 249-4 and ORM 256-8-6. In contrast, 1450bp, 1000bp, and 900bp bands produced by OU-2 were specifically absent in PB-1 mutant ORM 256-2 (Fig. 1a). Such mutant-specific fingerprinting is indicative of clear genetic variation possibly due to deletion or point mutation. These bands could serve as valuable markers for varietal identification and elimination of duplicates. Mathure *et al.* (2010) identified specific bands present among three accessions of aromatic rice using RAPD and ISSR markers. Similarly, Tripathy *et al*. (2012) reported genotype-specific

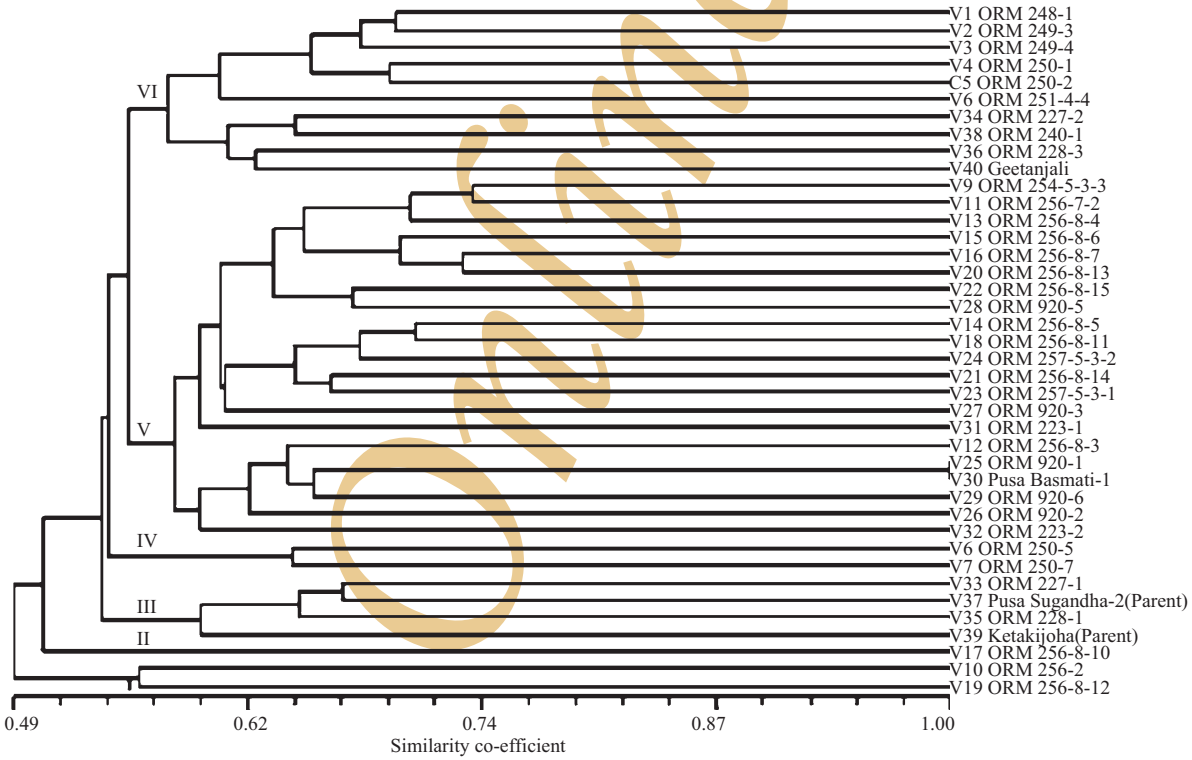

Fig. 3 : Dendrogram showing genetic diversity in a set of 40 scented rice genotypes based on RAPD markers

RAPD and ISSR profiles to certify the genetic makeup in short duration rice mutants.

ISSR primers OUAT-5, OUAT-8, OUAT-11 and OUAT-13 produced one monomorphic band each (Table 1) having fragment size 430bp, 1230bp, 840bp and 1020bp respectively. OUAT-7 could generate unique bands of size 1400bp in ORM 249-3 and ORM 256-8-4; and 900bp band in ORM 248-1 and ORM 256-8-3 (Fig. 2a). OUAT-10 produced a unique 1380bp amplicon specifically in ORM 257-5-3-2, ORM 920-1 and ORM 920-2 (Fig 2b); while OUAT-12 amplified 1200bp and 700bp bands specifically in ORM 256-8-3; ORM 256-8-5 and ORM 256-8-13 respectively. Similar to RAPD, ISSR markers are dominant in nature, and therefore absence of specific band could serve as valuable marker for varietal discrimination. OUAT-2 revealed 480bp fragment in all the scented rice genotypes except ORM 250-2. A 950bp amplicon was specifically absent in ORM 256-8-12, ORM 920-5 and PS-2. Absence of bands in few test genotypes might be due to induced deletion or point mutation and such markers could be associated with negative traits. Gao *et al*. (2009) used transposon markers to identify somaclonal mutants and SSR markers to differentiate bacterial blight resistant mutant and purple sheath mutant from their respective wild types (Minghui 63 and C 418). Besides, four SSR markers i.e., RM223, RM342A, RM515 and SCU-SSR1 (Kibria *et al.* 2008 and Garland *et al.,* 2000) and RFLP marker RG 28 (Jain *et al.,* 2006) were detected for fragrance gene (fgr) on chromosome 8 of rice which could discriminate Basmati from non-Basmati rice varieties.

Similarity coefficient (S_G) value between each pair of genotypes is likely to give a clear picture of the extent of genomic homology in terms of gene content and nucleotide sequence. The overall average S_G was estimated to be 0.50 and 0.35, respectively, using RAPD and ISSR markers suggesting more efficacy of ISSR technique in genetic diversity analysis.

The whole range of RAPD markers were grouped into 40 test genotypes into six genetic clusters at 55% phenon level (Fig. 3). But, Singh *et al.* (2013) revealed only two major genetic groups having two and 43 aromatic rice genotypes at the said similarity coefficient level using 45 RAPD primers. Among the test genotypes, two PB-1 mutants ORM 256 -8-12 and ORM 256-2 constituting Cluster- I were separated from rest of the genotypes. Cluster II included a single PB-1 mutant ORM 256-8-10, while Cluster III comprised of four diverse genotypes e.g., Ketakijoha, Pusa Sugandha-2 and its two mutants ORM 228-1 and ORM 227-1. Cluster IV included two PB-1 sister mutant lines (ORM

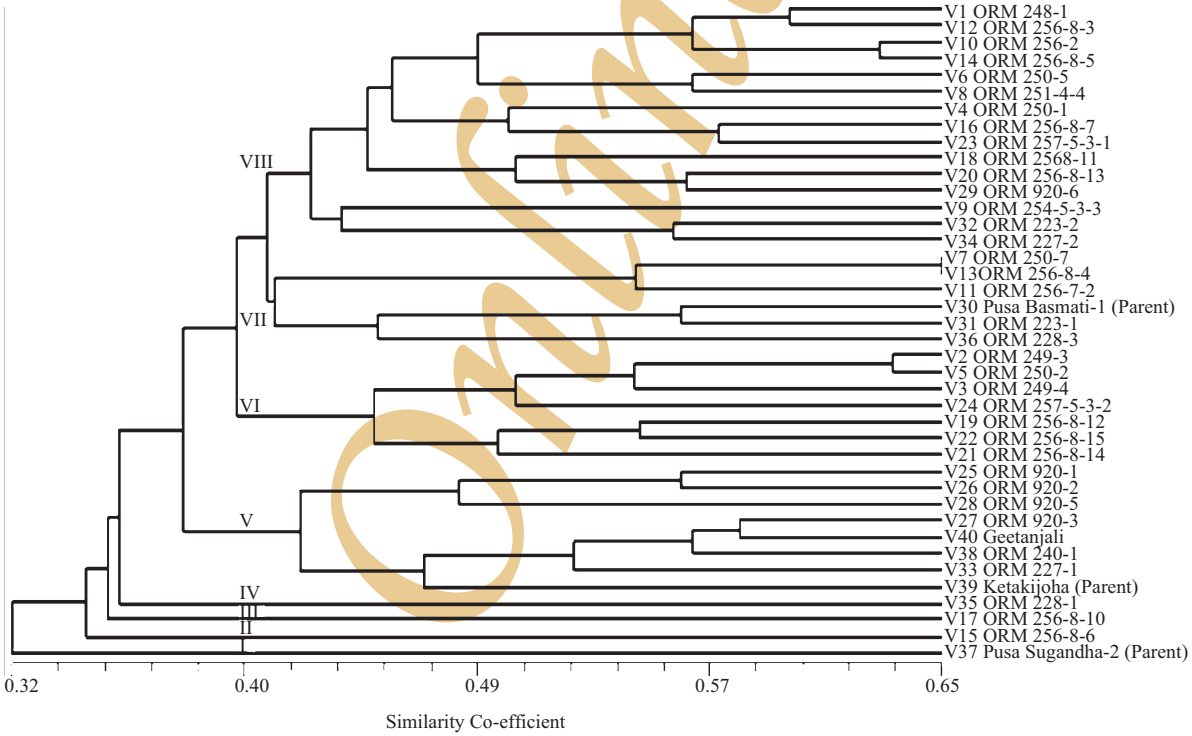

Fig. 4 : Dendrogram showing genetic diversity in a set of 40 scented rice genotypes based on ISSR markers

250-7 and ORM 250-5). The rest of the genotypes were distributed into two multi-variety clusters (Cluster-V and Cluster VI) which grouped in 21 and 10 scented rice genotypes. RAPD markers were potent enough to discriminate all test the genotypes except PB-1 and ORM 920-1, even at 100% phenon level. The mutants included in Cluster-I, II and III maintained wide genetic divergence with rest of the genotypes and hence, these could be sorted out as valuable materials for recombination breeding..

In contrast to RAPD, ISSR technique discriminated the present set of genotypes into eight distinct genetic clusters at even 40% phenon level (Fig. 4). However, Nagaraju *et al.* (2002) observed lowest genetic diversity among the traditional basmati varieties. In the present pursuit, PS-2, two mutants of PB-1(ORM 256-8-6, ORM 256-8-10) and a mutant of PS-2 (ORM 228-1) showed distinct genetic dissimilarity from rest of the genotypes and these individually constituted single clusters *i.e.* Cluster-I, II, III and IV respectively. Eight genotypes were grouped together to form Cluster V which included of Ketakijoha, ORM 227-1(a mutant of PS-2), ORM 240-1(a mutant of Ketakijoha), Geetanjali, and four sister mutant lines of PB-1 *i.e.* ORM 920-3, ORM 920-5, ORM 920-2 and ORM 920-1. Remaining genotypes were distributed into three clusters i.e., Cluster-VI, VII and VIII which included of 7, 6 and 15 genotypes, respectively. ORM 256-8-4 and ORM 250-7, both derived from PB-1 following mutagenesis, were clubbed together within Cluster-VII which could be well discriminated beyond 65% phenon level. Thus, ISSR could discriminate all genotypes and/or mutants beyond 65% phenon level, while RAPD failed to do so. Superiority of ISSR over RAPD could be due to the fact that ISSR markers can map even near to centromeres.

Considering both RAPD and ISSR; ORM 228-1 and its parent Pusa Sugandha-2; one PB-1 mutant ORM 256-8-10 and the aromatic land race Ketakijoha were identified to be highly divergent genotypes. Besides, a few mutants of PB-1 *i.e.,* ORM 256-2 and ORM 256-8-12 were seen to be highly divergent using RAPD markers, while ORM 256-8-6 revealed clear isolation from remaining genotypes as assessed by ISSR analysis. This could be attributed to genotype-specific RAPD and ISSR alleles (Tripathy *et al.,* 2012).

Thus, the present pursuit revealed ISSR marker system to be more informative and highly potent to generate allelic diversity than RAPD to justify hereditary mutational change in mutants. In fact, most of the above divergent mutants had shown genetic improvement in overall productivity and these may enrich the basmati gene pool for further genetic improvement of grain quality and seed yield *per se.*

References

Abdulla, M. and O. Gamal: Investigation on molecular phylogeny of some date palm cultivars by protein, RAPD and ISSR markers in Saudi Arabia. *Aust J Crop Sci.,* **4**, 23-28(2010).

Alvarez, A, J.L. Fuentes, J.E. Deus, M.C. Duque and M.T. Cornide: Genetic diversity analysis in rice mutants using isozyme and morphological markers. *Cultivos Tropicales*, **21**, 39-44 (2000).

Barakat, M.N. and H. El-Sammak: In vitro mutagenesis, plant regeneration and characterization of mutants via RAPD analysis in Baby's breath *Gypsophila paniculata* L. *Aust J Crop Sci*., **5**, 214-222(2011).

Chuan-Qing, S., W. Xiang-Kun, A. Yoshimura and N. Iwata: A Study of the Genetic diversity of common wild rice (*O. rufipogon* Griff) and cultivated rice (*O. sativa* L.) by RFLP analysis. *Journal of Genetics and Genomics*, **27**, 227-234(2000).

Dellaporta, S.L., J. Wood and J.B. Hick: A plant DNA minipreparation: Version II. *Plant Mol. Biol Rep.*, **1**, 19–21(1983).

Gao, D.Y., A. Vallejo Veronica, B. He, Yun-chao Gai and Li-Hua Sun: Detection of DNA changes in somaclonal mutants of rice using SSR markers and transposon display. *Plant Cell Tissue Organ Culture,* **98**, 187-196(2009).

Garland, S., L. Lewin, A. Blakeney, R. Reinke and R. Henry: PCR-based molecular markers for the fragrance gene in rice (*Oryza sativa* L.). *Theor. Appl. Genet*., **101**, 364–371(2000).

Girma, G., K. Tesfaye and E. Bekele: Inter simple sequence repeat (ISSR) analysis of wild and cultivated rice species from Ethiopia. *Afr. J. Biotechnol*., **9**, 5048-5059(2010).

Jain, N., S. Jain, N. Saini and R.K. Jain: SSR analysis of chromosome 8 regions associated with aroma and cooked kernel elongation in basmati rice. *Euphytica*, **152**, 259–273(2006).

Jeung, J.U., H.G. Hwang, H.P. Moon and K.K. Jena: Fingerprinting temperate japonica and tropical indica rice genotypes by comparative analysis of DNA markers. *Euphytica,* **146**, 239–251 (2005).

Joshi, S.P., V.S. Gupta, R.K. Agarwal, P.K. Ranjekar and D.S. Brar: Genetic diversity and phylogenetic relationship as revealed by inter-simple sequence repeat (ISSR) polymorphism in the genus *Oryza*. *Theor. Appl. Genet*., **100**, 1311–1320(2000).

Kaushik, A., N. Saini, S. Jain, P. Rana, R.K. Singh and R.K. Jain: Genetic analysis of CSR 10 (indica) x Taraori Basmati F_3 population segregating for salt tolerance using ISSR markers. Kluwer Academic Publishers, *Euphytica,* **134**, 231-238(2003).

Kiani, G.: Genetic diversity analysis of Iranian improved rice cultivars through RAPD markers. *Not. Sci. Biol*., **3**, **135** – 139(2011).

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

Li, Ya-li, Xiao-Xi Yang, Feng-Ping Zhao and Ming-Hui Xu: SSR marker analysis on indica –japonica differentiation of natural population of *Oryza* rufipogon in Yuanjiang. Yunnan province, *Rice Science*, **13**, 71-74(2006).

Mathure, S., N. Jawali and A. Nadaf : Diversity analysis in selected non-basmati scented rice collection. *Rice Sci.*, **17**, 35-42(2010).

Mukherjee, B., P. Das, Q. Alam, D. Nath and T. Dasgupta: Molecular characterization of rice genotypes using microsatellite markers. *Oryza*, **50**, 35-40(2013).

Nagaraju, J., M. Kathirvel, R. Ramesh Kumar, E.A. Siddiqi and S.E. Hasnain: Genetic analysis of traditional and evolved basmati and

non-basmati rice varieties by using fluorescence –based ISSR-PCR and SSR markers. *PNAS,* **99**, 5836-5841(2002).

Prevost, A. and M.J. Wilkinson: A new system of comparing PCR primers applied to ISSR fingerprinting of potato cultivars. *Theor. Appl. Genet*., **98**, 107–112(1999).

Rabbani, M.A., Z.H. Pervaiz and M.S. Masood: Genetic diversity analysis of traditional and improved cultivars of Pakistani rice (*Oryza sativa* L.) using RAPD markers. *Electronic J. of Biotech,* **11**, 2551-2564, (2008).

Reddy, C.S., A. Prasad Babu, B.P. Mallikarjuna Swamy, K. Kaladhar and N. Sarla: ISSR markers based on GA and AG repeats reveal genetic relationship among rice varieties tolerant to drought, flood or salinity. *J. Zhejiang Univ. , Science B*., **10**, 133-141(2009).

Saini, N., N. Jain, S. Jain and R.K. Jain: Assessment of genetic diversity within and among basmati and non-basmati rice varieties using AFLP, ISSR and SSR markers. *Euphytica,* **140** , 133 – 146, (2004).

Shehata, S.M., M.H. Amma, A.F. Abdelkalik and B.A. Zayed: Morphological, molecular and biochemical evaluation of Egyptian jasmine rice variety and its M_3 derived mutants. *Afr. J. Biotechnol.,* **8**, 6110-6116(2009).

Singh, V.P. and A.K. Singh: Pusa 1121: A rice line with exceptionally high cooked kernel elongation and basmati quality. *Int. Rice Res. Newsletter* (*IRRN)*, **27**, 25-26(2002).

Singh, Y., D.R. Pani, D. Khokhar and U.S. Singh: Agro-morphological characterization and molecular diversity analysis on aromatic rice germplasm using RAPD markers. *Oryza*, **50**, 26-34(2013).

Tripathy, S.K., R. Ekka, D. Lenka, R. Ranjan, S. Pal and B.R. Mohapatra: Performance of short duration rice mutants and assessment of genetic diversity using RAPD and ISSR markers. *Int. J. Agro. Agril. Res.*, **2**, 16-24(2012).

Ye Chunjiang, Yu Z., F. Kong, S. Wu and B. Wang: R-ISSR as a new tool for genomic finger printing, mapping and gene tagging. *Plant Mol. Bio. Reporter*, **23,** 167-177(2005).