Identification of Variety Diagnostic Molecular Marker of High Yielding Rice Varieties

S. S. Kshirsagar · Kailash Chandra Samal ·
Mihir Rabha · Debendra Nath Bastia ·
Gyana Ranjan Rout

Received: 5 March 2013 / Revised: 31 May 2013 / Accepted: 3 July 2013 / Published online: 11 August 2013
© The Author(s) 2013. This article is published with open access at Springerlink.com

Abstract Twenty eight inter simple sequence repeat (ISSR) markers were used to determine the genotypic identification and phylogenetic relationship within 48 varieties of Indica rice including four aromatic rice grown in eastern part of India. Out of 44 upland and low land varieties, 39 varieties released by the Orissa University of Agriculture and Technology, Orissa and five released by the other National Institutes of India. A total of 28 ISSR primers were screened representing di- and tri- nucleotide repeats, out of which 12 ISSR markers were selected for varietal analysis as diagnostic markers. The number of bands per locus ranged from 4 to 8, with an average of 6. The results revealed that all the primers showed distinct polymorphism among the varieties indicating the robust nature of ISSR markers. Most of the primers showed highest polymorphic information content and resolving power. The cluster analysis indicates that all the rice genotypes are grouped into two classes in which aromatic rice varieties clustered separately from upland and lowland varieties. Principal component analysis showed the distinct differences among themselves. Based on this study, the larger range of similarity values using ISSR markers provides greater confidence for the assessment of genetic relationships among the varieties. The information obtained from the DNA profile helps to identify the variety

diagnostic markers in 48 rice genotypes. This outcome of the investigation can be used in background selections during backcross breeding programs.

Keywords DNA profile · Genetic similarity · Polymorphism · PCR · Rice

Introduction

Rice, *Oryza sativa* ($2n = 24$) belonging to the family Poaceae and subfamily Oryzoidea is the staple food for one-third of the world's population and occupies almost one-fifth of the total land area covered under cereals. It is grown under diverse conditions and over wide geographical range. Most of the world's rice is cultivated and consumed in Asia, which constitutes more than half of the global population. Approximately 11 % of the world's arable land is planted annually to rice, and it ranks next to wheat. The world's rice production has doubled during last 25 years, largely due to the use of improved technology such as high yielding varieties and better crop management practices [1]. Further scope of crop improvement depends on the availability, genetic diversity and variability and use of new biotechnological tools. There exists a rich diversity in rice. Except for a few earlier studies on classification of germplasm using isozymes, there have been a few studies to assess the diversity of Indian rice varieties including landraces, at the molecular level. Presently some of the varieties have gone threatened. Hence, their conservation and characterization is of utmost important. Some of the traditional varieties are not sufficiently represented to study the genetic diversity. A number of molecular markers have been used for diversity study in rice [2–4]. Inter simple sequence repeat (ISSR) is a microsatellite-based multi

S. S. Kshirsagar · K. C. Samal · M. Rabha · G. R. Rout (✉)
Department of Agricultural Biotechnology, Orissa University
of Agriculture and Technology, Bhubaneswar 751003, Odisha,
India
e-mail: grrout@rediffmail.com

D. N. Bastia
Department of Plant Breeding & Genetics, Orissa University
of Agriculture and Technology, Bhubaneswar 751003, Odisha,
India

locus marker technique, which is simple and useful for estimating genetic diversity in several crop plants. The technique has the advantages of random amplified polymorphic DNA (RAPD) and in addition shows higher level of polymorphism, reproducibility and cost-effectiveness per polymorphism. ISSR-PCR has been used in genetic diversity studies in several crop plants. In rice, ISSR-PCR has been used for the analysis of micro satellite motif frequency and fingerprinting of varieties, determining phylogenetic relationships among *Oryza* species [5–9]. The usefulness of multi locus ISSR-PCR markers in determining diversity within improved varieties, landraces of rice of Orissa has not been previously investigated. The objectives of this study are: (i) to assess the extent of genetic diversity in Indian landraces of rice as compared to improved cultivars and other accessions using ISSR primers; (ii) to compare the level of polymorphism revealed by each primer, and between landraces and varieties, (iii) to develop a rational basis for the choice of informative primers using polymorphic information content, resolving power and mean genetic similarity and (iv) to identify the variety diagnostic ISSR markers for rice improvement program.

Material and Methods

Plant Materials

Forty four elite upland and lowland rice genotypes and 4 aromatic genotypes ('Basumati', 'Kalajeera', 'Pumpudi-basa', 'Neelabati') were collected from the germplasm centre maintained by the Department of Plant Breeding and Genetics, Orissa University of Agriculture and Technology, Bhubaneswar for ISSR analysis. The morphological and agronomic characteristics have also been indicated in Table 1. The seeds were sown in the earthen pots and kept in the green house for germination. Leaf samples were collected and subsequently stored at -20°C until processed.

DNA Extraction and PCR reaction

DNA was extracted as described by Doyle and Doyle [10] with minor modifications. The reaction mixture composition and conditions were empirically determined. This exercise involved optimization of primer concentration and annealing temperature for each primer. Out of 28 oligonucleotide primers (Bangalore Genei, India) screened, 12 were selected for further analysis based on clarity, scorability, and reproducibility of banding patterns (Table 2). Amplification reactions contained 25 ng template DNA, 200 μM each dNTPs, 2 mM MgCl_2 , 10 pmol primer, 1 X

Taq polymerase buffer and 1 unit of *Taq* DNA polymerase (Bangalore Genei, India) in a reaction volume of 25 μl . The amplification was performed in programmable gradient Thermal Cycler (BIORAD, USA) with following programme: a pre-denaturation at 94°C for 3 min followed by 44 cycles of denaturation at 94°C for 1 min, annealing at gradient temperature for 1 min and extension at 72°C for 2 min. The final extension was made for 7 min at 72°C . Amplified products were under electrophoresis in a 1.5 % w/v agarose—1 $\times$ Tris-borate-EDTA gel at 100 volt. Sizes of the identified bands were determined by comparison with molecular weight-marker low-range DNA ruler (Bangalore Genei, India). Amplicons were scored as discrete variables, using 1 to indicate presence and 0 for absence of band. A binary matrix was obtained by visual scoring of the bands. Amplifications were repeated twice for each primer and only consistent bands were considered for further analyses. Efficiency of discrimination was assessed in terms of the number of polymorphic markers generated and the ability to generate unique band. Pair-wise-similarity matrices based on ISSR data were determined using Jaccard's similarity coefficient [11]. Principal component analysis (PCA) was made by using the binary data of the profile through NTYSIS program.

Results and Discussion

The present investigation reveals that the 48 varieties of rice belonged to two groups. First group having high yielding varieties developed by different Institutes and IR-64 (IR 5657-33-2-1/IR2061-465-1-5-5), an Indica rice variety with distinct desirable agronomic traits. It was included as a check or reference variety. Second group having aromatic rice varieties. In this group, Basmati was included which belongs to an aromatic long grain type of rice (*O. sativa*) cultivated in different parts of India and Pakistan [12]. Vaughan et al. [13] reported that there are 21 valid species of genus *Oryza*. Out of them *O. sativa* (Asian cultivar) and *O. glaberrima* (African cultivar) are the only cultivated species derived from their perennial wild progenitors *O. rufipogon* and *O. longistaminata* respectively. Further, the Asian cultivar has undergone differentiation into three distinct eco-geographical sub-species viz. *indica*, *japonica* (temperate *japonica*) and *javanica* (tropical *japonica*). The diversification of *O. sativa* is not confined with these three sub-species but is differentiated into many more varietal groups largely through human selection under different agroclimatic conditions, cultural practices and quality preferences [14]. Glaszmann [15] reported that the Asian cultivar was differentiated into six varietal groups i.e. *Indica*, *Aus*, *Ashina*, *Rayada*, *Aromatic* and *Japonica* on the basis of

Table 1 Morphological characteristics of rice varieties used for ISSR analysis

Sl. No.	Variety name	Parentage	Maturity duration (days)	1,000 grain weight (g)	Yield (q/ha)	Important features
1	Keshari	Kumar/Jagannath	90–95	20.0	32	Semi-dwarf, photo-insensitive, deep green short narrow erect leaves, profuse tillering ability
2	Meher	OBS677/IR2071// Vikram/w1263	140	28.0	45	Medium bold, multiple resistance
3	Birupa	ADT 27/IRB// Annapurna	135	24.4	42	Medium bold, multiple resistance
4	Mahalaxmi	Pankaj/Mahsuri	150	21.8	45	High grain number, suitable for shallow low land
5	Manika	CR 210-1010/Obs 677	155–160	18.7	45	Resistant to sheath blight, moderately resistant to blast, sheath rot, BLB and BPH
6	SR-26-B	Selection from kalambanka	150	25.4	20	Long bold, saline tolerant
7	Jajati	Rajeswari/T141	135	14.3	40	Medium slender, intermediate height, good grain quality matures 10 days earlier in summer, adopts well under low and high fertility condition
8	Badami	Suphala/Annapurna	100	23.7	35	Medium bold, resistant to BLB and blast, red kernel
9	Samanta	T90/IR8/Vikram/// Sanja29/Mahsuri	140	21.5	44	Medium bold, multiple resistance
10	Santep Heap	Pankaj/Sigadis	150–155	21.8	45	Resistant to BLB, sheath rot, sheath blight and gall midge
11	IR-64	Introduction	120	21.2	40	Medium slender, multiple resistance
12	CR-1017	Cross T-90/Urang	155	21.5	40	Medium bold, suitable for shallow low land
13	Pratikshya	Swarna/IR 64	142	19.6	48	Plant height semi-dwarf, long panicles, low tillering, stout stem, high grain number, MS grains, golden coloured hull with white kernel
14	Prachi	IR-9764-45-2-2/CR149-3244-198	153	21.5	42.7	Medium bold grains, brown spot-R, BPH, WBPH-MR
15	Khandgiri	Parijata/IR13429-94	95	20.8	35	Medium slender, suitable for gall midge and BPH endemic areas
16	Rajeswari	T 90/IRS	130–135	21.0	40	Semi-dwarf, photo-insensitive, profuse tillering
17	Lalitgiri	Badami/IR-19661-364	95	21.7	32	Resistance of BLB, BLS, GM, BPH
18	Rambha	Pankaj/W 1263	150	25.1	35	Medium bold, suitable for shallow and semi deep low lands
19	Lalat	OBSM 677/IR2071/ Vikram/W1263 ^a	125	23.3	40	Medium slender, multiple resistance
20	Kanchan	Jajati/Mahsuri	155	19.2	40	Medium slender, tall, suitable for shallow and semi deep low lands
21	Nilgiri	Suphala/DZ 192	95	22.4	35	Medium bold, moderately drought tolerant
22	Urbashi	Rajeshwari/Jajati	145	18.4	40	Medium bold, tall, suitable for shallow low lands
23	Surendra	OR 158-5/Rasi	130–137	11.4	47.1	Medium bold grains, suitable for rainfed a irrigated medium lands, resistant to blast, sh. rot, GM, BPH WBPH
24	Kalinga-3	AC540/Ratna	75	24.5	30	Long slender, tall suitable for drought prone area
25	Mandakini	Ghanteswari/IR 27069	100–105	23.6	45.81	Adaptable to upland, blast, sheath blight
26	Gouri	Rajeshwari/Vikram	135	19.2	43	Medium slender, multiple resistance and good grain.
27	Annada	MTU 15/Yai kyaku kantoku	110	21.2	35	Short bold, moderately drought tolerant, suitable for rainfed upland and medium land
28	Uphar	Mahalaxmi/IR 62	135	20.5	45	Medium bold, multiple resistance
29	Indrabati	IR-56/OR-142-99	152	23.3	40.7	Medium slender grains, leaf blast-R, brown spot-R, sh. rot-R, BPH, WBPH-MR
30	Ghanteswari	IR 2061-628/M22	95	29.5	35	Medium bold, Resistant to BLB, blast, gall midge and moderately tolerant for drought, Red kernel

Table 1 continued

Sl. No.	Variety name	Parentage	Maturity duration (days)	1,000 grain weight (g)	Yield (q/ha)	Important features
31	Jogesh	CR 544-1-3-4/NDR 1008	89–90	26.9	40	Rainfed, irrigated uplands, resistant to BS, neck blast, mod. rest. to blast and Sh. B
32	Manaswini	Swarna/Lalat	140	25.2	49–75	Resistant to brown spot, blast, sheath blight, WBPH and BPH
33	Parijata	TN 1/TKM6	90–95	18.0	35	Medium slender grains, semi-dwarf, photo-insensitive, deep green leaves
34	Basumati-7	Land race	155	16.9	23.95	Grain wt. 10.3 %, white grain with white kernel, let aromatic variety
35	Pratap	Kumar/CR57-49	135–140	17.1	40	Semi-dwarf leaves, medium bold grains with translucent white kernel
36	Kalajeera	Land race	150–160	13.9	18.47	Well adopted rice variety in Orissa, let variety, black colour grain with husk, fragrance like jeera
37	Pathara	Co 18/Hems.	95	23.3	35	Medium bold, Moderately drought tolerant, suitable for upland and low land
38	Sidhanta	Jajati/Arnapurna	95	15.6	35–45	Intermediate height, golden hull, tolerant of RTV and leaf folder. Adopted to rainfed and irrigated uplands
39	MTU 1010	Krishnaveni/IR 64	120	21.4	35–45	Early variety, grains medium slender, husk straw coloured, moderately resistant to stem borer, BPH
40	Mahanandi	IR-19661-131-1-3-1-3/Savitri	150	20.8	44.21	Medium bold grains, resistant to blast, brown spot, sh.rot
41	Bhubana	OR 158-5/Rasi	135	22.4	42	Medium bold, multiple resistance
42	Uadaygiri	IRAT138/IR 13543-66	90–95	21.0	35	suitable for rainfed and irrigated uplands of moderate tolerant to drought resistant to leaf blast brown spot, stem borer
43	Ranidhan	CR 151-179/CR 1014	180	17.5	40	Medium slender, tall, suitable for semi deep low land, tolerant flood
44	Bhanja	IR 36//Hema/Vikram	140	22.0	40	Medium bold, multiple resistance
45	Gajapati	IR-13429-196-1-120	130	25.5	44	Resistance of BLB < GM, BPH, LF
46	Pumpudibasa-55	Land race	150–160	13.3	20–25	Very good aromatic rice variety, well adopted to Orissa
47	Neelabati-51	Land race	155–165	14.0	20–28	Medium to let duration variety of aromatic rice variety in Orissa
48	Suphala	T 141/T (N)	90–95	15.7	35	Resistant to bacterial leaf blight and bacterial leaf streak.

isozyme analysis. Basumati rice is a distinct group, well separated from *indica* and *japonica* varieties on the basis of morpho-physiological traits as reported earlier [16]. The assessment of genetic diversity and variety diagnostic molecular markers are an essential component in germ-plasm characterization and conservation. Out of 28 ISSR markers screened, 12 markers were selected to characterize and assess the genetic variability among 48 rice genotypes including four aromatic rice genotypes belonging to *indica* types because of distinct amplification and highly reproducible bands. The selected ISSR primers yielded 71 bands with an average of 5.92 bands per primer of sizes ranging from 250 bp to 2,000 bp (Fig. 1; Table 2). No unique band was observed, but there were 12 low frequency bands. All other bands (59) were high frequency bands. The

ISSR primer (UBC-825) produced the maximum eight bands followed by UBC-872 with seven bands. The lowest number of bands was observed in UBC-864 with four bands. The maximum resolving power (11.417) was observed in primer UBC 825 and the maximum PIC value was obtained by using the ISSR primer (UBC-872). ISSR markers are frequently used for varietal diagnostic purposes in many crop species [17, 18].

The ISSR amplification pattern was used to assess genetic variation among the genotypes by cluster analysis and to detect the varietal diagnostic markers. A dendrogram was plotted using the similarity coefficient derived from ISSR profile data as presented in Fig. 2. The dendrogram was divided into two clusters at 40 % similarity coefficient. The maximum number of genotypes (47) were

Table 2 ISSR primers used for DNA profiling of 48 rice genotypes

Primer code	Sequence (5'-3')	Total no. of bands	High frequency band	% age poly-morphism	Size range (bp)	Resolving power (Rp)	Average PIC value
AM-1	GGCGGCGGCGGCGGCAT	5	3	100	500–2,000	5.417	0.596
AM-2	AAGAAGAAGAAGAAGGC	6	6	100	580–1,900	8.333	0.485
AM-4	AAGAAGAAGAAGAAGCC	6	2	100	500–1,700	4.833	0.758
AM-6	AGCAGCAGCAGCAGCGG	6	5	100	300–1,080	9.125	0.348
UBC-807	AGAGAGAGAGAGAGAGT	6	6	100	350–1,100	8.375	0.487
UBC-811	GAGAGAGAGAGAGAGAC	6	6	100	500–1,815	8.417	0.476
UBC-813	CTCTCTCTCTCTCTTT	5	5	100	400–1,000	5.958	0.637
UBC-818	CACACACACACACACAG	6	5	100	350–1,000	8.333	0.451
UBC-825	ACACACACACACACACT	8	7	100	250–1,050	11.417	0.409
UBC-827	ACACACACACACACACG	6	6	100	350–900	8.500	0.440
UBC-864	ATGATGATGATGATGATG	4	4	100	600–1,500	6.125	0.367
UBC-872	GATAGATAGATAGATA	7	4	100	300–1,700	5.667	0.779
Total		71	59			90.5	6.233
Mean		5.92	4.92			7.54	0.52

represented in Cluster I whereas, one genotype ‘CR 1017’, a high yielding variety developed from Central Rice Research Institute formed the cluster II, which was an out-group from the rest of the clusters. Cluster I was again divided into two sub-clusters IA and IB. In sub-cluster IA, there are two rice genotypes whereas 45 genotypes were included in Cluster IB. In sub-cluster IA, these two varieties, “Badami” and “Samant”, with similar pedigree relationship have been grouped together. Both the varieties have medium bold grain with multiple resistance. The sub-cluster IB is further divided into two minor sub-clusters i.e. BI and BII. The minor sub-cluster BI having four varieties of aromatic rice making one group with 60 % similarity coefficient. These four varieties (“Basumati”, “Pumpudibasa”, “Neelabati” and “Kalajerra”) represent aromatic indica rice. The variety “Basumati” and “Pumpudibasa” show 80 % similarity among each other. Both the varieties have 75 % similarity with “Neelabati” and 68 % with “Kalajeera”. They have been developed through natural selection. For these reasons they have been grouped together and placed separately from the other high yielding improved varieties. Further, minor sub-cluster BII is further divided into two sub minor clusters i.e. BIII and BIV. The cluster BIII has 20 varieties and cluster BIV has 21 varieties. In cluster BIII, the variety “Uphar” and “Mandakini” make two individual groups with yield potential 45 q/ha and resistance to blast disease and sheath blight. The variety “Ghanteswari” and “Pathara” make one group with 86 % similarity among themselves. Both the genotypes have same maturity duration of 95 days and equal yield potential of 35 q/ha. The variety “Jogesh” and “Bhanja” have 90 % similarity among themselves with

equal yield potential (40 q/ha). These varieties are grown in rain fed and irrigated uplands area with resistant to blast, sheath blast and neck blast disease. These varieties have 86 % similarity with “Ranidhan” and “Bhubana”. The varieties “Parijata” and “MTV 1010” also have 80 % similarity among themselves with some agronomic characteristics like slender grains, photo insensitive, moderately resistant to stem borer and yield potential up to 35 q/ha. The cluster BIV is further divided into two minor clusters, one having 7 varieties and other having 14 varieties. The variety “Lalat” and “IR-64” have 78 % similarity with same yield potential (40 q/ha). Both the varieties have medium slender seeds and multiple resistance to disease and drought. Most of the varieties in this group show 70 % similarity among themselves. The high yielding varieties “Mahalaxmi” (Pankaj/Mahsuri), “Ram-bha” (Pankaj/W 1263), “Santep Heap” (Pankaj/Sigadis) were placed together in this sub-cluster. All these cultivars have the common parent “Pankaj” and they are the long duration varieties suitable for low land ecosystem. The variety “Jajati” (Rajeshwari/T141) and “Urbashi” (Rajeshwari/Jajati) have been grouped together and have originated from the same parent “Rajeshwari”. All these varieties are suitable for cultivation in the eastern part of India. The patterns of diversity revealed by the ISSR primers showed similar correlations between similarity matrices. Most of the ISSR primers especially members of the 5'-anchored dinucleotides could be freely interchanged with no loss in the power to distinguish varietal diversity. These results suggest that the use of different ISSR primers would be able to assess the genetic diversity of rice as reported previously [6, 19].

Fig. 1 ISSR profile of 48 rice genotypes by using primer UBC-827

Principal component analysis indicates that the four aromatic rice varieties (sl. no. 34, 36, 46 and 47 as per Table 1) are making one separate group (Fig. 3). The variety “Badami” and “Samant” are making one out group having the medium bold grain quality with multiple resistant to disease. The differences in ISSR profiles were perhaps due to their adaptability and other significant agronomic characteristics like yield potential, panicle type, seed morphology, photosynthesis efficiency and grain characteristics. The present study showed higher percentage of polymorphism as compared to other molecular markers as reported earlier [20, 21]. The genetic diversity in traditional lowland rice grown in north-east regions using both RAPD and ISSR markers has been reported by

Bhuyan et al. [7]. Further, Youssef et al. [9] used both RAPD and ISSR markers to identify the new promising drought tolerant lines of rice. These traits with molecular differences suggest that these rice varieties belong to *Indica* rice with introgressions from wild rice land races.

In conclusion, ISSR has generated high number of polymorphic markers which can be used in diagnostic fingerprinting studies of rice. Based on the average percentage polymorphism, PIC, Rp, diversity index and marker index, the efficiency of ISSR markers will be helpful for varietal diagnostics and for rice breeding program. In addition, this marker has an important role in securing plant variety rights as the need to protect proprietary germplasm which is likely to increase in the future.

Fig. 2 Dendrogram depicting genetic relationship among 48 rice varieties based on ISSR profile

Fig. 3 Principal component analysis (2-D) of 48 rice varieties using similarity coefficient of ISSR profile data. (Numbers denoting the plotted data points represent the respective rice varieties as listed in Table 1)

Acknowledgments The authors wish to acknowledge to Department of Biotechnology, Govt. of India for providing fund for student research under PG HRD program.

Open Access This article is distributed under the terms of the Creative Commons Attribution License which permits any use, distribution, and reproduction in any medium, provided the original author(s) and the source are credited.

References

1. Byerlee D (1996). Knowledge-Intensive Crop Management Technologies: Concepts, Impacts, and Prospects in Asian Agriculture. International Rice Research Conference, Bangkok, 3–5 June 1996
2. Mackill DJ, Zhang Z, Redona ED, Colowit PM (1996) Level of polymorphism and genetic mapping of AFLP markers in rice. *Genome* 39:969–977
3. Zhu J, Gale MD, Quarrie S, Jackson MT, Bryan GJ (1998) AFLP markers for the study of rice biodiversity. *Theor Appl Genet* 96:602–611
4. McCouch SR, Temnykh S, Lukashova A, Coburn J, Declerck G, Cartinhour S, Harrington S, Thomson M, Septiningsi E, Semon M, Moncada P, Jiming L (2001) Microsatellite markers in rice: abundance, diversity and applications. *Rice genetics IV*. IRRI, Manila, pp 117–135
5. Akagi H, Yokozeki Y, Inagaki A, Fujimura T (1997) Highly polymorphic microsatellites of rice consist of AT repeats, and a classification of closely related cultivars with these microsatellite loci. *Theor Appl Genet* 94:61–67
6. Joshi SP, Gupta VS, Aggarwal RK, Ranjekar PK, Brar DS (2000) Genetic diversity and phylogenetic relationship as revealed by Inter simple sequence repeat polymorphism in the genus *Oryza*. *Theor Appl Genet* 100:1311–1320
7. Bhuyan N, Borah BK, Sarma RN (2007) Genetic diversity analysis in traditional lowland rice (*Oryza sativa* L.) of Assam using RAPD and ISSR markers. *Curr Sci* 93:967–972

8. Reddy CS, Babu AP, Mallikarjuna BP, Kaladhar K, Sarl N (2009) ISSR markers based on GA and AG repeats reveal genetic relationship among rice varieties tolerant to drought, flood or salinity. *J Zhejiang Univ (Sci B)* 10:133–141
9. Youssef MA, Mansour, Solliman SS (2010) Molecular markers for new promising drought tolerant lines of rice under drought stress via. RAPD-PCR and ISSR markers. *J Am Sci* 6:355–363
10. Doyle JJ, Doyle JL (1990) Isolation of plant DNA from fresh tissue. *Focus* 12:13–15
11. Sneath PHA, Sokal RR (1973) Numerical taxonomy: the principles and practice of numerical classification. W. H. Freeman, San Francisco
12. Nene YL (1998) Basmati rice: a distinct variety (cultivar) of the Indian subcontinent. *Asian Agri-history* 2:321–330
13. Vaughan DA, Morishima H, Kadowaki K (2003) Diversity in the *Oryza* genus. *Curr Opin Plant Mol Biol* 6:139–146
14. Siddiq EA, Vemiredd LR, Nagaraju J (2012) Basmati rice: genetics, breeding and trade. *Agric Res* 1(1):25–36
15. Glaszmann JC (1987) Isozymes and classification of Asian rice varieties. *Theor Appl Genet* 74:21–30
16. Vairavan S, Siddiq EA, Arunachalam V, Swaminathan MS (1973) A study on the nature of genetic divergence in rice from Assam and North East Himalayas. *Theor Appl Genet* 43(5):213–221
17. Raina SN, Rani V, Kojima T, Ogihara Y, Singh KP, Devarumath RM (2001) RAPD and ISSR fingerprints as useful markers for analysis of genetic diversity, varietal identification and phylogenetic relationships in peanut (*Arachis hypogaea*) cultivars and wild species. *Genome* 44:763–772
18. Gorji AM, Poccai P, Polgar Z, Taller J (2011) Efficiency of arbitrarily amplified dominant markers (SCOT, ISSR and RAPD) for diagnostic fingerprinting in tetraploid potato. *Am J Potato Res* 88:226–237
19. Blair MW, Panaud O, McCouch SR (1999) Inter-simple sequence repeat (ISSR) amplification for analysis of microsatellite motif frequency and fingerprinting in rice (*Oryza sativa* L.). *Theor Appl Genet* 98:780–792
20. Galvan MZ, Bornet B, Balatti P, Branchard M (2003) Inter simple sequence repeat (ISSR) markers as a tool for the assessment of both genetic diversity and gene pool origin in common bean (*Phaseolus vulgaris* L.). *Euphytica* 132:297–301
21. Kumar J, Verma V, Qazi GN, Gupta PK (2007) Genetic diversity in *Cajanus–Rhynchosia–Flemingia* group based on functional markers. *Proc Natl Acad Sci India* 77(B):369–374