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RESEARCH ARTICLE

Efficiency of RAPD and ISSR markers system in accessing genetic variation of rice bean (*Vigna umbellata*) landraces

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Abbreviations: AFLP: amplified fragment length polymorphism
ISSRs: inter simple sequence repeats
PCR: polymerase chain reaction
PIC: polymorphism information content
RAPD: random amplified polymorphic DNA
RFLP: restriction fragment length polymorphism
UPGMA: unweighted pair group method with arithmetic averages
URP: universal rice primer
Z: Mantel test statistic

Vigna umbellata **(Thunb.) Ohwi and Ohashi commonly known as rice bean or climbing mountain bean is under-exploited tropical legume. Genetic variation between 10 landraces of rice bean was evaluated using random amplified polymorphic DNA (RAPD) and inter simple sequence repeats (ISSR) markers. Among these markers, RAPD primers generated 987 amplification products of which 719 were polymorphic and ISSR markers produced 479 amplification products, out of which 296 were polymorphic. RAPD fingerprinting detected more polymorphic loci (70.30%) than the ISSR fingerprinting (61.79%). Mean PIC (polymorphism information content) for each of these marker systems (0.243 for RAPD and 0.203 for ISSR) suggested that both the marker systems were equally effective in determining polymorphisms. The dendrograms constructed using RAPD and ISSR marker systems were highly correlated with each other as revealed by high Mantel correlation (r = 0.95). Pairwise similarity index values ranged from 0.530 to 0.782 (RAPD), 0.608 and 0.862 (ISSR) and 0.559 to 0.777 (RAPD and ISSR) and mean similarity index value of 0.677, 0.729 and**

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0.694 for RAPD, ISSR and combined data, respectively. RAPD and ISSR marker systems were found to be useful for the genetic diversity studies in *V. umbellata* and identify variation within landraces.

Rice bean (*Vigna umbellata* (Thunb.)) Ohwi and Ohashi is an important tropical leguminous plant, identified as an under exploited species, and it is used as vegetable, folk medicine and fodder in Southeast Asian countries (Wu et al. 2001). It belongs to genus *Vigna*, subgenus *Ceratotropis* (Piper) Verdc which includes azuki bean group. Among the various species of azuki bean, *V. umbellata* having different centers of diversity and cradles of the cultivated rice bean have been reported in India, China, Malaysia, Myanmar and Thailand (Tomooka et al. 2002).

Traditionally, classification of the various subgenera, species, and subspecies is based primarily on morphological attributes. However, these traits may not be significantly distinct and usually require growing plants to maturity prior to identification. Moreover, morphological characters may be unstable due to environmental influences. Over the years, the methods for detecting and assessing genetic diversity have extended from analysis of discrete morphological traits to biochemical and molecular traits. Several DNA marker systems are now common use in diversity studies of plants. The most commonly used marker systems are restriction fragment length polymorphism (RFLP) (Soller and Beckmann, 1983), random amplified polymorphic DNA (RAPD) (Williams et al. 1990), amplified fragment length polymorphism (AFLP) (Vos et al. 1995), inter simple sequence repeats (ISSRs) (Zietkiewicz et al. 1994) and microsatellites or simple sequence repeats (SSRs) (Becker and Heun, 1994). Among them to characterize DNA variation patterns within species and among closely related taxa in *Vigna* species have been RAPD (Ba et al. 2004; Dikshit et al. 2007), AFLP (Sivaprakash et al. 2004; Seehalak et al. 2006; Yoon et al. 2007; Fang et al. 2007), RFLP (Kaga et al. 2000), ISSR (Ajibade et al. 2000), SSRs (Li et al. 2001; Dikshit et al. 2007) and sequence tagged microsatellite site (STMS) (Phansak et al. 2005).

Considering the potentials of the DNA marker based genetic diversity analysis, the present study aimed to evaluate the usefulness of molecular markers *viz*. RAPD and ISSRs, in assessing and analysing the nature and the extent of genetic diversity among the genotypes of rice bean collected from a narrow geographical region of Meghalaya State of India.

MATERIALS AND METHODS

Plant material

Ten rice bean landraces LRB40-1, LRB85, LRB89, LRB111, LRB113, LRB173, LRB281, LRB282, LRB293 and LRB297 are used for the present study. These landraces obtained from Department of Pulses, Centre for Plant Breeding and Genetics, Tamil Nadu Agricultural University, Coimbatore, India, which were collected from, Meghalaya State, India by the National Bureau of Plant Genetic Resources (NBPGR) germplasm collection, New Delhi, India. Ten individuals of each landrace were sampled. Plants were grown in greenhouse of Department of Plant Molecular Biology and Biotechnology, Centre for Plant Molecular Biology, Tamil Nadu Agricultural University, Coimbatore, India and leaves were harvested from two-week old seedlings.

Isolation of DNA

Genomic DNA was isolated from frozen leaf tissue of ten plants (200 mg each) following the procedure described by (McCouch et al. 1988). The quality and quantity of DNA was checked by agarose gel electrophoresis. The final DNA concentration of each sample was adjusted to 25 ng/µl.

DNA amplification conditions and gel electrophoresis

A total of 114 random primers obtained from Operon Technologies, Alameda, California, USA were used for the polymorphism survey. Among these, 74 primers produced clear and unambiguous bands (Table 1). Amplification reactions were carried out in volume of 15 µl containing 25-50 ng of genomic DNA, 0.5 µM of primer, 10 mM of dNTPs (2.5 mM each), 50 mM KCl, 10 mM Tris HCl, (pH 8.3), 2.5 mM $MgCl_2$, 0.15 units (U) of *Taq* DNA polymerase (Bangalore Genei Pvt. Ltd., Bangalore, India). Amplifications were performed in PTC Thermal Cycler (MJ Research Inc., San Francisco, USA) programmed for an initial denaturation at 94ºC for 5 min, 35 cycles of 1 min denaturation at 94ºC, 1 min annealing at 36ºC and 2 min extension at 72ºC, followed by final extension for 7 min at 72ºC.

ISSR primers (University of British Columbia, Canada) synthesized by Sigma Aldrich Inc., were used for the polymorphism survey. Of which, 37 primers were subjected

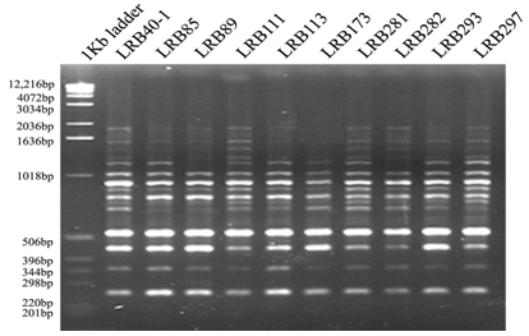

Figure 1. RAPD marker profiles of 10 landraces of *V. umbellata* generated by primer OPBB1 in 1.5 per cent agarose gel.

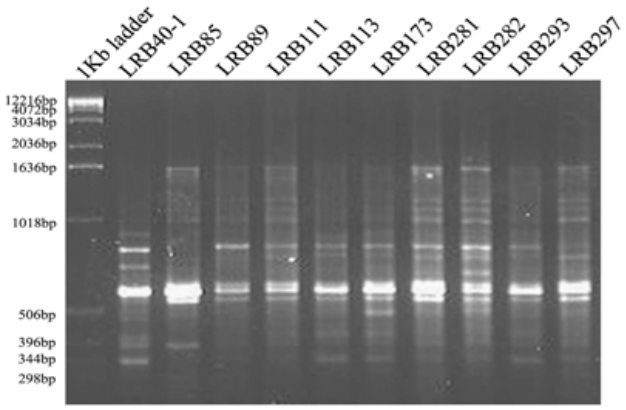

Figure 2. ISSR marker profiles of 10 landraces of *V. umbellata* generated by primer UBC864 in 1.5 per cent agarose gel.

to diversity analysis as they produced clear and unambiguous bands (Table 2). PCR (polymerase chain reaction) amplification was carried out in 15 µl reaction containing 25 ng of genomic DNA, 1 µM of primer, 10 mM of dNTPs (2.5 mM each), 50 mM KCl, 10 mM Tris HCl (pH 8.3), 2.5 mM $MgCl_2$, 0.3 units (U) of *Taq* DNA polymerase (Bangalore Genei Pvt. Ltd., Bangalore, India). PCR conditions were programmed for initial denaturation at 94ºC for 5 min, 40 cycles of 1 min denaturation at 94ºC, 45 sec annealing at 46-48ºC, 2 min extension at 72ºC, and final extension for 5 min at 72ºC. The annealing temperature for PCR amplification was maintained based on the specificity of the primer pair used. PCR amplified products of RAPD and ISSR primers were subject to horizontal gel electrophoresis using 1.5% agarose gel in 1X TBE buffer at 80 V for 4 hrs using Broviga™ standard submarine gel electrophoresis unit. As size marker we used a 1 kb DNA ladder (GIBCO BRL life technologies, Paisley, UK) and the ethidium bromide stained gels were documented using Alpha Imager 1200™ (Alpha Innotech Corporation, USA).

Data analysis

Marker index for RAPD and ISSR markers was calculated in order to characterize the capacity of each primer to detect polymorphic loci among the genotypes. It is the sum total of the polymorphism information content (PIC) values of all the markers produced by a particular primer. PIC value was calculated using the formula PIC = $1-\sum p_i^2$, where p_i is the frequency of the i^{th} allele (Smith et al. 1997).

The data obtained by scoring the RAPD and ISSR profiles with different primers individually as well as collectively were subjected to the construction of similarity matrix using Jaccard's (Jaccard, 1908) coefficients. The similarity values were used for cluster analysis. Sequential agglomerative hierarchical non-overlapping (SAHN) clustering was done using unweighted pair group method with arithmetic averages (UPGMA) method. Data analysis was done using NTSYSpc software 2.02 (Rohlf, 1998). The product-moment correlation (r) based on Mantel Z-value (Mantel, 1967) was computed to measure the degree of relationship between similarity index matrices produced by any two-marker systems.

RESULTS

RAPD analysis

The total number of markers observed among the rice bean genotypes based on RAPD analysis with 74 primer pairs was 987. The number of scorable markers produced per primer ranged from 2 to 31 and size of the products ranged from 201 bp to 3034 bp. The total number of polymorphic markers and the percentage of polymorphism were 719 and 70.30 respectively (Table 1). RAPD marker profile produced by the primer OPBB1 is shown (Figure 1). The PIC values, a reflection of allele diversity and frequency among the varieties, were not uniformly higher for all the RAPD loci tested. The PIC value ranged from 0.053 (OPH20) to 0.404 (OPH19) with a mean of 0.243.

The similarity coefficients based on 987 RAPD markers ranged from 0.530 to 0.782. Of the 45 pair wise combinations generated by rice bean genotypes, LRB113 and LRB173 showed the highest similarity index (0.782), and LRB40-1 and LRB293 showed the lowest similarity index (0.530). The mean similarity index was 0.677.

Cluster analysis was performed based on the Jaccard's (Jaccard, 1908) similarity coefficient matrices, calculated from RAPD markers to generate a dendrogram of rice bean genotypes. The dendrogram separated rice bean genotypes into two clusters. First cluster included LRB40-1 and the second cluster was further divided into three subclusters. Among the three subclusters, first subcluster formed a separate subcluster with LRB85 and LRB89 landraces and second subcluster included LRB111, LRB113, LRB173, LRB281, LRB297 and LRB282 landraces. The rice bean landrace LRB293 was out-grouped. Hundred per cent similarity was found between LRB113 and LRB173 rice bean genotypes (Figure 3a).

ISSR analysis

Thirty seven primers produced a total of 479 scorable markers among the genotypes. The size of amplified products ranged from 201 bp to 5090 bp. The number of scorable markers produced per primer ranged from 3 to 26. The total number of polymorphic markers and the percentage of polymorphism were 296 and 61.79 respectively (Table 2). ISSR marker profile produced by the primer UBC864 in agarose gel is shown (Figure 2). In the case of ISSR analysis, the mean PIC value was 0.203

Table 1. RAPD primers used to detect polymorphism, number of bands for polymorphism between landraces per primer.

RAPD Primer	Sequence (5'-3')	Number of bands	Number of polymorphic markers	Percentage polymorphism	PIC value
OPC9	CTCACCGTCC	2	2	100.00	0.353
OPC10	TGTCTGGGTG	8	4	50.00	0.283
OPC11	AAAGCTGCGG	8	3	37.50	0.122
OPC12	TGTCATCCCC	20	10	50.00	0.184
OPC13	AAGCCTCGTC	8	5	62.50	0.253
OPC14	TGCGTGCTTG	11	2	18.18	0.045
OPC15	GACGGATCAG	8	6	75.00	0.313
OPC16	CACACTCCAG	9	6	66.67	0.255
OPC17	TTCCCCCCAG	14	8	57.14	0.162
OPC18	TGAGTGGGTG	8	5	62.50	0.240
OPC19	GTTGCCAGCC	14	7	50.00	0.146
OPC20	ACTTCGCCAC	16	7	43.75	0.137
OPH12	ACGCGCATGT	10	7	70.00	0.242
OPH14	ACCAGGTTGG	17	16	94.12	0.287
OPH17	CACTCTCCTC	2	0	0.00	0.180
OPH19	CTGACCAG.CC	10	9	90.00	0.404
OPH20	GG.GAGACATC	16	3	18.75	0.053
OPM1	GTTGGTGGCT	10	8	80.00	0.228
OPM2	ACAACGCCTC	14	10	71.43	0.242
OPM4	GGCGGTTGTC	8	6	75.00	0.323
OPM5	GGGAACGTGT	5	4	80.00	0.396
OPM6	CTGGGCAACT	17	16	94.12	0.300
OPM7	CCGTGACTCA	7	7	100.00	0.254
OPM8	TCTGTTCCCC	16	16	100.00	0.436
OPM9	GTCTTGCGGA	24	24	100.00	0.283
OPM10	TCTGGCGCAC	15	13	86.67	0.251
OPO2	ACGTAGCGTC	22	19	86.36	0.306
OPO3	CTGTTGCTAC	15	15	100.00	0.369
OPO4	AAGTCCGCTC	18	16	88.89	0.354
OPO5	CCCAGTCACT	11	7	63.64	0.167
OPO8	CCTCCAGTGT	7	5	71.43	0.240
OPO10	TCCCACGCAA	31	31	100.00	0.269
OPO11	CCTCCAGTGT	7	6	85.71	0.333
OPO13	GACAGGAGGT	12	12	100.00	0.305
OPQ1	GGGACGATGG	17	4	23.53	0.082
OPQ2	TCTGTCGGTC	14	13	92.86	0.354
OPQ3	GGTCACCTCA	14	10	71.43	0.169
OPQ4	AGTGCGCTGA	17	13	76.47	0.193
OPQ5	CCGCGTCTTG	14	11	78.57	0.227
OPQ6	GAGCGCCTTG	15	12	80.00	0.189
OPQ7	CCCCGATGGT	9	8	88.89	0.263
OPQ9	GGCTAACCGA	22	19	86.36	0.223
OPQ10	TGTGCCCGAA	12	7	58.33	0.193
OPY9	GCAGCGCAC	14	10	71.43	0.239
OPZ1	TCTGTGCCAC	13	8	61.54	0.118
OPZ05	TCCCATGCTG	12	10	83.33	0.246
OPZ08	TCCCATGCTG	14	9	64.29	0.203
OPZ11	CTCAGTCGCA	16	15	93.75	0.280
OPZ12	TCAACGGGAC	12	3	25.00	0.065
OPZ13	GACTAAGCCC	1	0	0.00	0.260
OPZ16	TCCCCATCAC	12	6	50.00	0.142
OPBA13	AGGGCGAATG	17	8	47.06	0.266
OPBA15	GAAGACCTGG	13	11	84.62	0.212
OPBA16	CCACGCATCA	17	8	47.06	0.121
OPBB1	ACACTGGCTG	18	7	38.89	0.120
OPBB5	GGGCCGAACA	7	5	71.43	0.317
OPBB6	CTGAAGCTGG	18	15	83.33	0.277
OPBB7	GAAGGCTGGG	18	11	61.11	0.350
OPBB9	AGGCCGGTCA	9	4	44.44	0.122
OPBB11	TGCGGGTTCC	16	11	68.75	0.259
OPBB13	CTTCGGTGTG	14	12	85.71	0.233
OPBB14	GTGGGACCTG	7	5	71.43	0.300
OPBC2	ACAGTAGCGG	15	15	100.00	0.448
OPBC3	GGCTTGACCT	10	8	80.00	0.422
OPBC4	CCACGTGCCA	15	6	40.00	0.149
OPBC10	AACGTCGAGG	16	14	87.50	0.304
OPBC20	AGCACTGGGG	15	15	100.00	0.219
OPBD9	CCACGGTCAG	19	11	57.89	0.180
OPBD10	CTGCTGGGAC	18	17	94.44	0.257
OPBD16	GAACTCCCAG	11	8	72.73	0.273
OPBD17	GTTCGCTCCC	16	12	75.00	0.245
OPBD18	ACGCACACTC	18	18	100.00	0.298
OPBD19	GGTTCCTCTC	14	11	78.57	0.271
OPBD20	AGGCGGCACA	18	14	77.78	0.194
	Total	987	719		
	Average per primer	13.35		70.30	0.243

and the highest and lowest PIC values were 0.377 (UBC843) and 0.000 (UBC873) respectively. Similarity coefficients for the rice bean genotypes based on 479 ISSR markers ranged between 0.608 and 0.862. Of the 45 combinations among the genotypes, similarity index was highest between LRB111 and LRB113 (0.862) and it was lowest between LRB41 and LRB89 (0.608). The mean similarity index was 0.729.

The cluster analysis of ISSR markers separated the rice bean genotypes into two distinct clusters. The first cluster included only LRB40-1 while the second cluster included the remaining nine rice bean genotypes and was further divided into two subclusters. The first subcluster had LRB85 genotype and second subcluster contained LRB89, LRB173, LRB111, LRB113, LRB281, LRB293, LRB297 and LRB282 genotypes. In this subcluster, LRB111 and LRB113 had 100 per cent similarity (Figure 3b).

Combined RAPD and ISSR analysis

The similarity coefficients of the rice bean landraces based on 987 RAPD markers and 479 ISSR markers ranged from 0.559 to 0.777. Of the 45 pair wise combinations among the 10 genotypes, LRB113 and LRB173 showed the highest similarity index (0.782), while the genotypes LRB40-1 and LRB293 showed the lowest similarity index (0.530). The mean similarity index was 0.677.

Cluster analysis performed from combining data of both markers generated a dendrogram that separated the genotypes into two distinct clusters. The first cluster included only genotype LRB40-1. The second cluster was further divided into 3 subclusters. Among them, the first subcluster included LRB85 genotype, the second subcluster contained LRB89, LRB111, LRB130, LRB173, LRB281, LRB293, LRB297 and LRB282 and the third were represented by LRB113 and LRB173 that showed hundred per cent similarity between each other (Figure 3c).

The product moment correlation (r) and the Mantel test statistic (Z) were calculated to measure the degree of relationship between the similarity matrices obtained and combined (RAPD and ISSR) data and the correlation was significant (0.673). The product moment correlation (r) and the Mantel test statistic (Z) were calculated to measure the degree of relationship between the similarity matrices obtained by RAPD and integrated RAPD and ISSR data and the correlation value (r) was significant (0.955). The product moment correlation (r) and the Mantel test statistic (Z) obtained by ISSR and integrated RAPD and ISSR data also showed significant correlation value (0.861).

DISCUSSION

The rice bean landraces constitutes a rich source of biodiversity and their conservation and utilization requires that their genetic structure is well characterized and understood. DNA fingerprinting is a routine method employed to study the extent of genetic diversity across a set of germplasm or cultivars and group them into specific categories. Comparative studies in *Vigna* species involving RAPD, AFLP, ISSR and SSR marker systems were successfully used by very limited researchers (Souframanien and Gopalakrisna, 2004; Gillaspie et al. 2005; Dikshit et al. 2007). But, it has been carried out in many crops like rice (Ravi et al. 2003), capsicum (Ruanet et al. 2005) and common bean (Marotti et al. 2006). The discriminative power of DNA markers used as tool to characterize the rice bean landraces is very important because they can be used to assess the genetic diversity among the landraces of rice bean.

The analyses were conducted using one bulk of each landrace (Michelmore et al. 1991). Bulk analyses are useful to obtain information on genetic variability between different populations (Loarce et al. 1996). During the

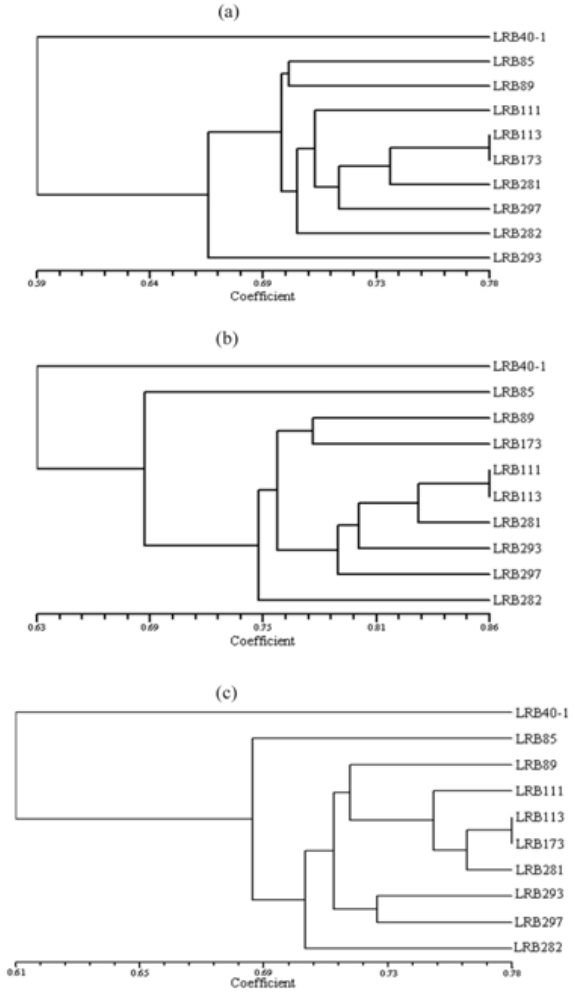

Figure 3. Dendrogram generated using UPGMA analysis, showing relationships between rice bean landraces, using RAPD, ISSR and combining RAPD and ISSR data.
(a) RAPD.
(b) ISSR.
(c) RAPD and ISSR.

Table 2. ISSR primers used to detect polymorphism, number of bands for polymorphism between landraces per primer.

ISSR Primer	Sequence (5'-3')	Number of	Number of	Percentage	PIC value
UBC807	AGAGAGAGAGAGAGAGT	16	2	12.50	0.041
UBC808	AGAGAGAGAGAGAGAGC	10	7	70.00	0.260
UBC810	GAGAGAGAGAGAGAGAT	3	2	66.67	0.200
UBC811	GAGAGAGAGAGAGAGAC	6	1	16.67	0.080
UBC814	CTCTCTCTCTCTCTCTA	6	2	33.33	0.160
UBC816	CACACACACACACACAT	11	6	54.55	0.247
UBC820	GTGTGTGTGTGTGTGTC	10	10	100.00	0.296
UBC821	GTGTGTGTGTGTGTGTC	19	12	63.16	0.404
UBC824	TCTCTCTCTCTCTCTCG	8	8	100.00	0.308
UBC826	ACACACACACACACACC	12	3	25.00	0.097
UBC827	ACACACACACACACACG	12	3	25.00	0.045
UBC828	TGTGTGTGTGTGTGTGA	5	0	0.00	0.000
UBC829	TGTGTGTGTGTGTGTGC	13	5	38.46	0.131
UBC834	AGAGAGAGAGAGAGAGYT	26	6	23.08	0.058
UBC836	AGAGAGAGAGAGAGAGYA	8	6	75.00	0.135
UBC840	GAGAGAGAGAGAGAGAYT	16	2	12.50	0.043
UBC841	GAGAGAGAGAGAGAGAYC	16	11	68.75	0.233
UBC843	CTCTCTCTCTCTCTCTRA	18	17	94.44	0.377
UBC844	CTCTCTCTCTCTCTCTRC	17	16	94.12	0.374
UBC846	CACACACACACACACART	12	5	41.67	0.127
UBC849	GTGTGTGTGTGTGTGTYA	10	10	100.00	0.296
UBC851	GTGTGTGTGTGTGTGTYG	5	3	60.00	0.332
UBC852	TCTCTCTCTCTCTCTCRA	4	2	50.00	0.125
UBC853	TCTCTCTCTCTCTCTCRT	9	4	44.44	0.149
UBC854	TCTCTCTCTCTCTCTCRG	20	19	95.00	0.259
UBC855	ACACACACACACACACYT	24	23	95.83	0.253
UBC857	ACACACACACACACACYG	18	13	72.22	0.250
UBC859	TGTGTGTGTGTGTGTGRC	15	13	86.67	0.325
UBC862	AGCAGCAGCAGCAGCAGC	13	12	92.31	0.311
UBC864	ATGATGATGATGATGATG	20	19	95.00	0.287
UBC867	GGCGGCGGCGGCGGCGGC	5	5	100.00	0.264
UBC873	GACAGACAGACAGAC A	9	0	0.00	0.000
UBC880	GGAGAGGAGAGGAGA	23	19	82.61	0.298
UBC884	HBHAGAGAGAGAGAGAG	14	3	21.43	0.059
UBC885	HBHAGAGAGAGAGAGAG	13	7	53.85	0.168
UBC886	VDVCTCTCTCTCTCTCT	18	9	50.00	0.267
UBC895	AGGTCGCGGCCGCNNNNNNAT	15	11	73.33	0.240
	Total	479	296		
	Average per primer	12.94		61.79	0.203

Single letter abbreviations for mixed base positions
N = (A, G, C, T); R = (A, G); Y = (C, T); B = (C, G, T); (*i.e.* not A); D = (A, G, T) (*i.e.* not C); H = (A, C, T) *i.e.* not G); V = (A, C, G) (*i.e.* not T).

characterization of rice bean landraces, from Meghalaya State, we evaluated the discriminative power of primers for RAPD and ISSR marker systems. Among the two marker systems employed, 74 RAPD primer pairs produced a total of 987 markers whereas thirty seven ISSR primers produced only 479 markers. The level of polymorphism revealed by RAPD (70.30%) is higher than ISSR (60.79%). Yu and Nguyen (1994) detected 80 % of polymorphism in 13 *Oryza sativa* cultivars with RAPDs. Godwin et al. (1997) also reported similar results for sorghum (*Sorghum bicolor*) and banana (*Musa acuminata*) and Patzak (2001) in hop. It has been reported that the ability to resolve genetic variation may be more directly related to the number of polymorphisms detected by the marker system (Sivaprakash et al. 2004).

In the present investigation, the average number of fragments amplified by RAPD primers among the genotypes was 13.35 with a range of 2 to 31. Ratnaparkhe et al. (1995) reported an average of 8 markers per primer in *Cajanus cajan*. However, Maciel et al. (2001) reported the generation of RAPD fragments ranging from 7 to 31 in common beans. Such a high variation in the number of fragments produced by these arbitrary primers may be attributed to the differences in the binding sites throughout genome of the genotypes included. ISSR primers generated 3 to 26 markers with average of 12.94 per genotype. Earlier, generation of 4 to 12 markers in *Vigna* (Ajibade et al. 2000) and 8 markers in *Phaseolus vulgaris* (Galvan et al. 2003) by ISSR primers were reported. The distribution of different microsatellite sequences in different plant genomes determines the possibility of using this method for DNA fingerprinting.

Comparison of PIC values for two marker systems (a parameter associated with the discriminating power of markers) indicated that the range of PIC values for RAPD primers was from 0.045 (OPC14) to 0.0448 (OPBC2). Among the 74 ISSR markers surveyed across the rice bean genotypes, only five markers possessed less than four alleles indicating better resolving power of the RAPD markers. This is because of polyallelic nature of RAPD markers. The comparison of the average PIC values of ISSR primers revealed that the lowest was with UBC828 and UBC873 (0.000) and the highest was with UBC821 (0.404).

Cluster analysis was carried out on three sets of marker profiling data based on 1) RAPD 2) ISSR 3) combination of RAPD and ISSR. The results based on all the three DNA marker profiles broadly grouped the 10 landraces into two clusters. In contrast, Yoon et al. (2007) observed no polymorphism when assessing the genetic relationship of *V. umbellata* accessions using AFLP markers. First cluster had the landrace of LRB40-1 in all the three marker systems studied. However the formation of subclusters within the cluster varied between RAPD and ISSR. The high similarity between LRB113 and LRB173 was noticed in both RAPD and combined analysis indicating that these genotypes are closely related. But high similarity of LRB111 and LRB113 was observed in ISSR marker system.

The pattern of subclustering of the cluster, which included nine genotypes varied over different marker systems. In RAPD analysis, the rice bean genotypes were grouped into three subclusters revealing sufficient amount of diversity within the cluster. Similar results have been found by Dikshit et al. (2007) for *V. umbellata* accessions and estimated 53 per cent of genetic similarity based on RAPD, URP (universal rice primer) and SSR markers data. In our study, ISSR markers grouped nine rice bean genotypes in two subclusters. Recently, ISSR markers also have been employed for genetic diversity analysis in *Vigna* species (Ajibade et al. 2000; Souframanien and Gopalakrisna, 2004). The cluster formation was observed in the landraces of rice bean at the minimum of 59 per cent similarity value, indicating presence of considerable divergence between the species of genus *Vigna.* These data indicated that RAPD and ISSR markers were equally effective for diversity studies in rice bean landraces.

Both RAPD and combined analysis grouped five landraces in the same clusters and few groups maintained the same associations in the ISSR and RAPD and ISSR combined dendrogram. There was close relationship between some of the landraces used in this study, presumably they might have been collected from similar locations or these landraces may have been derived from the same pedigree.

Close correspondence between the similarity matrices of RAPD and ISSR, RAPD and combined RAPD and ISSR and ISSR and combined RAPD and ISSR was established by means of high value of matrix correlation value of 0.673, 0.955 and 0.861 respectively. Hence, both the marker systems RAPD and ISSR either individually or combined can be effectively used in determination of genetic relationships among rice bean landraces. However, Ajibade et al. (2000) and Galvan et al. (2003) concluded that ISSR would be a better tool than RAPD for phylogenetic studies. Nagaoka and Ogihara (1997) have also reported that the ISSR primers produced several times more information than RAPD markers in wheat. The marker index values of ISSR primers also added strength to the above results.

Within the cluster combined marker system grouped the genotypes differently. RAPD showed the landraces LRB40-1 and LRB293 as most divergent ones while the landraces LRB40-1 and LRB282 were most diverse as per the ISSR results. This revealed the existence of sufficient amount of genetic variability among the landraces of same species, which could be exploited further. A close genetic similarity was found in some of the cultivars analyzed as shown by high values of similarity index. Also, the similarities detected with ISSRs are greater than the similarities observed with RAPDs. Fernández et al. (2002) have studied 16 barley cultivars form different countries and they have

found high similarity index by ISSRs than by RAPDs. It may be due to highly polymorphic, abundant nature of the microsattelites due to slippage in DNA replication. Genetic variations observed in some of the landraces are very narrow because it might have resulted during the long cultivation history of the species, as an adaptation to the local agroclimatic conditions and may be result of narrow genetic base (Seehalak et al. 2006). Li et al. (2001) also reported low genetic diversity in cowpea using SSR markers. In the long run, these could have resulted in locally adapted genotypes. This agricultural practice is maintaining and also probably contributing to the genetic uniqueness by strengthening the specific adaptations obtained by the landraces (Sivaprakash et al. 2004). Inclusion of genotypes bred for specific objectives like yield and quality parameters over different geographical location resulting in narrowing of genetic base and the marker system used could be the reason for clustering most of the cultivars in one cluster. The relationships between the landraces are not necessarily reflecting the agronomic traits. Molecular markers are scattered throughout the genome and their association with various agronomic traits is influenced by the cultivator under selection pressure induced by domestication. Exploration and evaluation of diversity among these landraces would be of great significance for *in situ* conservation and rice bean breeding programmes.

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