International Journal of Agriculture, Environment and Biotechnology
Citation: IJAEB: 10(4): 415-421, August 2017
DOI: 10.5958/2230-732X.2017.00051.1

BIOTECHNOLOGY

Assessment of Genetic Diversity of Ricebean [(*Vigna umbellate)* (Thunb.) Ohwi & Ohashi)] Varieties and their Narrow Leaf Cross Derivatives using RAPD Markers

Islam Shafiqul[1], M. Dutta[2], Shah Shachi*[3], Pankaj Kumar[4] and K.V. Bhatt[2]

[1]G.B. Pant University of Agriculture and Technology, College of Forestry and Hill Agriculture, Hill Campus, Ranichauri, Tehri Garhwal, Uttarakhand, India
[2]National Bureau of Plant Genetic Resources, New Delhi, India
[3]Indira Gandhi National Open University, New Delhi, India
[4]Sardar Bhallbh Bhai Patel University of Agriculture and Technology, Meerut, UP, India

*Corresponding author: sshah@ignou.ac

Paper No. 596 Received: 12-5-2017 Accepted: 3-7-2017

ABSTRACT

Genetic diversity in 13 ricebean (V*igna umbellata* (Thunb.) Ohwi & Ohashi) varieties and their 11 narrow leaf crosses was studied using in RAPD markers. A total of 147 amplicons were scored out of which 91 (61.9%) showed polymorphism indicating fair amount of variation at DNA levels. Per cent polymorphism among twenty four ricebean varieties and their crosses ranged from 38.9 per cent to 59.8 per cent, on the other hand, percent polymorphism among eleven crosses alone ranged from 40.5% to 53.0%. Cluster analysis based on UPGMA (Unweighted Pair-Group Method for Arithmetic Average) analysis with Squared Euclidean Distance revealed the patterns of relatedness among the ricebean varieties and their crosses. The polymorphism observed between the varieties and crosses were used as markers for hybrid analysis. The patterns of RAPD markers were classified into seven types according to the presence or absence of bands. The present investigation indicated that out of the seventeen RAPD primers used most of them were useful with good amount of reliability to identify ricebean crosses showing 49.5% to 68.0% shared markers with the parents (Type I, III and IV). However, Type VII markers were useful in identifying new cultivars of ricebean with 5% to 17.4% non-parental bands.

Highlights

- The present study aimed to elaborate on the application of RAPD marker technique to characterize and determine genetic relationship between the parental lines and their near homozygous progenies would be useful in marker assisted breeding programme in ricebean. The information obtained through germplasm characterization using RAPD will also be useful for screening of duplicates, assessing genetic diversity and monitoring the genetic stability of conserved germplasm.

Keywords: Polymorphism, parents and crosses, RAPD technique, hybrid analysis

Ricebean [*Vigna umbellata*, (Thunb.) Ohwi and Ohashi], one of the underutilized pulse is a multipurpose grain legume crop mainly cultivated for food, fodder and green manure (Thakur *et al*. 2017). Ricebean also known as climbing mountain bean, mambi bean, oriental bean and haricot bean, a native of South and South East Asia (Ohwi J. 1965), occurs wild in the Himalaya to Central China and Malaysia. It is a is a short-day, warm-season annual legume that is cultivated mainly in India, Nepal, Bhutan, northeast India, Myanmar, southern China, northern Thailand, Japan, Laos, Vietnam, Indonesia and East Timor (Dutta M. *et al.* 2007; Tian J. *et al.* 2013). This non-traditional and underutilized legume has gained attention as supplementary food crop (Gruere *et al.* 2006). Rice

bean seed contains 25% protein, 0.49% fat and 5% fiber. It is also rich in methionine and tryptophan as well as vitamins (thiamine, niacin and riboflavin) and restores soil fertility through biological nitrogen fixation. Despite many useful characteristics, it has not been subjected to systematic breeding including disease resistance and the highest potential grain yield among Ceratotropis species and hence little exploited. Thus, there is substantial dearth of scientific studies to assess diversity, use value and marketability (Meena *et al.* 2017)

Molecular markers have been used in many plant breeding programmes, especially in the hybrid and cultivar identification as well as in genetic mapping of quantitative traits. Morphological traits have been traditionally used to identify cultivars. But to overcome the limitations associated with the morphological markers that are highly influenced by environment, large number of molecular markers has come up in the recent past. Molecular markers like RAPD (Random amplified polymorphic DNA) give high degree of polymorphism and thus aid in differentiating even closely associated cultivars (Williams *et al*. 1990; Meena *et al*. 2017) RAPD has been standardized and employed successfully by different workers (Xu *et al*. 2000; Choudhury *et al.* 2008; Bora *et al.* 2016) to analyze samples of various crops including Vigna species. For the simplicity and rapidity of the technique, RAPD technique has also been successfully employed for identification of genuineness of parents and their hybrids in many crop species (Santhy *et al*. 2003; Ilbi *et al.* 2004). Keeping this in view the present study was aimed to find out the genetic variability of ricebean (V*igna umbellata* (Thunb.) Ohwi & Ohashi) varieties and their narrow leaf cross derivatives by using RAPD analysis.

MATERIALS AND METHODS

Plant Material

Thirteen ricebean varieties and their eleven narrow-leaf cross derivatives developed at the Department of Plant Breeding, G B Pant University of Agriculture and Technology, Hill Campus, Ranichauri, Tehri Garhwal, Uttarakhand, India were included in the present study as detailed in Table 1. The inter-varietal cross derivatives used for the study were evaluated in their F_6 generation.

Genomic DNA Isolation

The molecular analysis was carried out at the DNA Fingerprinting Laboratory, National Bureau of Plant Genetic Resources, New Delhi. Total genomic DNA was isolated from 7-day old etiolated seedlings of different ricebean varieties and their crosses by extraction with CTAB buffer using the method developed by Saghi-Maroof *et al.* (1984). The quantification of isolated DNA was done by fluorometric determination using Calf Thymus as DNA standard with Bisbenzenide (Hoechst Dye-H 33258). The genomic DNA samples were diluted to a uniform concentration of 20 $ng^{-1}\mu l$ for PCR.

RAPD Amplification

The PCR amplification was performed according to the protocol developed by Saghi-Maroof *et al.* (1984). The PCR amplification was carried out in a 25 µl volume of reaction set consisting of 2 µl DNA template (20ng/µl) 2 µl, 0.3 µl of Taq DNA polymerase (Genei, Bangalore), 15.67 µl of sterilized distilled water, 2.0 µl of dNTP mix (0.2 MM each of d ATP, d CTP, d TTP, and d GTP) and 2.5 µl of buffer.

Thermal cycler programmed for 5 min at 94 °C for initial denaturation and 40 cycles consisting of 30 sec at 95 °C, 1 min at 35 °C and 2 min at 72 °C with final 5 min extension at 72 °C using fastest ramp time between the temperature extensions were followed. PCR products were electrophoresed on 1.8% agarose gel in TAE buffer, stained in Ethidium Bromide solution and gel images recorded. The profiles of the amplified products generated by seventeen decamer primers were evaluated to screen polymorphisms.

Data Analysis

RAPD amplification with each primer was performed twice and bands in the range of 250-3500 bp were scored. DNA banding pattern generated by RAPD for all the varieties and crosses, were scored as present (1) or absent (0) of the fragment in the data sheet. Only those fragments with medium to high intensity were taken into account. The data matrices generated were entered into STATISTICA programme. The similarity coefficient was used to construct dendrogram using UPGMA (Unweighted Pair-Group Method for Arithmetic Average) analysis

with Squared Euclidean Distance by computing polymorphic as well as monomorphic markers.

Table 1: Details of rice bean varieties and their narrow leaf cross derivatives

Sl. No.	Varieties/Crosses	Place of origin
	Parents:	
1	PRR 1	Garhwal
2	PRR 2	Garhwal
3	PRR 9301	Garhwal
4	PRR 9302	Garhwal
5	PRR 9303	Garhwal
6	PRR 9401	Garhwal
7	PRR 9402	Garhwal
8	BRS 2	Kumaon
9	NAINI	Kumaon
10	KHRB 1	Karnataka
11	KHRB 3	Karnataka
12	RBL 35	Punjab
13	LRB 224	Punjab
	Crosses:	
14	PRR 1 × KHRB 1	–
15	PRR 2 × PRR 9301	–
16	PRR 2 × KHRB 3	–
17	PRR 2 × PRR 9302	–
18	PRR 9301 × KHRB 3	–
19	PRR 9303 × PRR 9401	–
20	PRR 9401 × BRS 2	–
21	LRB 224 × PRR 2	–
22	PRR 9303 × KHRB 3	–
23	PRR 2 × RBL 35	–
24	NAINI × PRR 9402	–

RESULTS AND DISCUSSION

The genomic DNA of all samples was subjected to PCR amplification using 25 random primers. Eight primers did not show polymorphic amplification and hence were not considered for further analysis. For a total level of seventeen primers, 147 markers (amplicon) were generated of which 91 (61.9%) were polymorphic and 56 (38.1%) were monomorphic bands across the genotypes. The number of amplified bands for various primers varied from 2 (D 11) to 15 (M 20) with an average of 8.6 bands per primer. However, M 8 was found to produce 85.7% polymorphic bands. The lowest polymorphism (40%) was observed in the primer D 7. The present study revealed that the RAPD analysis with a set of seventeen primers was sufficient to identify the uniqueness of twenty four ricebean varieties and their crosses.

Percent polymorphism among twenty four ricebean varieties and their crosses ranged from 38.9 per cent to 59.8 per cent as shown in Table 2. The cross, PRR 2 × PRR 9301 gave the maximum (107) scorable bands, 59.8% of which were polymorphic, while NAINI × PRR 9402 gave 64 scorable bands recording the lowest (38.9%) polymorphism. On the other hand, per cent polymorphism among eleven crosses ranged from 40.5% to 53.0%. The cross, PRR 2 × PRR 9301 though gave the maximum amplified bands (125), but only 43.2% of them were polymorphic. However, NAINI × PRR 9402 with the lowest scorable fragments (115) yielded the highest (53.0%) polymorphism. PRR 9303 × PRR 9401 though gave 116 scorable bands, but had the lowest (40.5%) polymorphism. The results illustrated that the ricebean varieties and their narrow leaf crosses manifested moderate level of polymorphism. This indicated that among the varieties and crosses moderate level of diversity was present as they originated from different places within the country.

Cluster analysis based on UPGMA (Unweighted Pair-Group Method for Arithmetic Average) analysis with Squared Euclidean Distance as shown in fig No.1 revealed the patterns of relatedness among twenty four ricebean varieties and their crosses. Fig. 2 (a and b) shows the DNA fingerprint patterns for 24 ricebean varieties and their crosses by Primer No. D17 and Primer No. D18 respectively. It was observed that when cut off value was considered at a linkage distance of 40, the ricebean varieties and their crosses were grouped into nine clusters. All the crosses except, PRR 2 × KHRB 3 were grouped into two clusters; PRR 2 × KHRB 3 was placed individually in one separate cluster. The variety, KHRB 1, NAINI and PRR 9402 were placed in three clusters individually. PRR 1 was grouped with four crosses in the same cluster. The remaining nine varieties were grouped in three clusters. Thus cluster analysis showed considerable amount of genetic diversity among the ricebean varieties and their crosses.

The polymorphism observed between the varieties and the crosses were used as markers for hybrid analysis. The patterns of RAPD markers were classified into seven types according to the presence

Table 2: Selected primers, their sequences and level of polymorphism detected

Sl. No.	Primers	Sequence (5′–3′)	Total no. of bands	No. of polymorphic bands	Per cent polymorphism
1	M 7	CCGTGACTCA	12	5	41.6
2	M 8	TCTGTTCCCC	14	12	85.7
3	M 20	AGGTCTTGGG	15	11	73.3
4	D 1	ACCGCGAAGG	8	4	50.0
5	D 2	GGACCCAACC	5	4	80.0
6	D 3	GTCGCCGTCA	4	2	50.0
7	D 4	TCTGGTGAGG	12	6	50.0
8	D 5	TGAGCGGACA	9	5	55.6
9	D 7	TTGGCACGGG	5	2	40.0
10	D 9	CTCTGGAGAC	9	7	77.7
11	D 10	GGTCTACACC	6	4	66.6
12	D 11	AGCGCCATTG	2	1	50.0
13	D 12	CACCGTATCC	9	5	55.5
14	D 14	CTTCCCCAAG	6	4	66.6
15	D 16	AGGGCGTAAG	12	6	50.0
16	D 17	TTTCCCACGG	11	8	72.7
17	D 18	GAGAGCCAAC	8	5	62.5
Total			**147**	**91**	**61.9**

Table 3 A: Seven types of RAPD markers identified from five crosses of Ricebean

Types of markers	Property of markers			RAPD markers in cross combination									
	Parent I	Parent II	Cross	PRR 2 × PRR 9301		PRR 2 × KHRB 3		PRR 2 × PRR 9302		PRR 9301 × KHRB 3		PRR 1 × KHRB 1	
				(No.)	(%)	(No.)	(%)	(No.)	(%)	(No.)	(%)	(No.)	(%)
I	1	1	1	71	56.8	68	56.2	67	56.3	62	50.8	68	56.7
II	1	1	0	5	4.0	7	5.8	12	10.1	15	12.3	11	9.1
III	1	0	1	8	6.4	3	2.5	5	4.2	10	8.2	7	5.8
IV	0	1	1	6	4.8	5	4.1	6	5.0	6	4.9	3	2.5
V	1	0	0	4	3.2	8	6.6	8	6.7	5	4.1	9	7.4
VI	0	1	0	11	8.8	9	7.4	15	12.6	7	5.7	12	9.9
VII	0	0	1	20	16.0	21	17.4	6	5.0	17	13.9	10	8.3
Total				**125**		**121**		**119**		**122**		**120**	

Table 3 B: Seven types of RAPD markers identified from six crosses of ricebean

Types of markers	Property of markers			RAPD markers in cross combination									
	Parent I	Parent II	Cross	PRR 9303 × PRR 9401		PRR 9401 × BRS 2		LRB 224 × PRR 2		PRR 9303 × KHRB 3		PRR 2 × RBL 35		NAINI × PRR 9402	
				(No.)	(%)	(No.)	(%)	(No.)	(%)	(No.)	(%)	(No.)	(%)	(No.)	(%)
I	1	1	1	66	53.2	59	50.0	69	59.5	63	51.6	60	54.5	54	46.9
II	1	1	0	7	5.6	18	15.3	10	8.6	16	13.1	15	13.6	19	16.5
III	1	0	1	10	8.1	1	0.8	4	3.4	5	4.1	1	0.9	3	2.6
IV	0	1	1	3	2.4	10	8.5	1	0.9	3	2.5	2	1.8	0	0.0
V	1	0	0	18	14.5	7	5.9	11	9.5	18	14.8	8	7.3	18	15.7
VI	0	1	0	9	7.3	8	6.8	13	11.2	12	9.8	13	11.8	11	9.6
VII	0	0	1	11	8.9	15	12.7	8	6.9	5	4.1	11	10.0	10	8.7
Total				**124**		**118**		**116**		**122**		**110**		**115**	

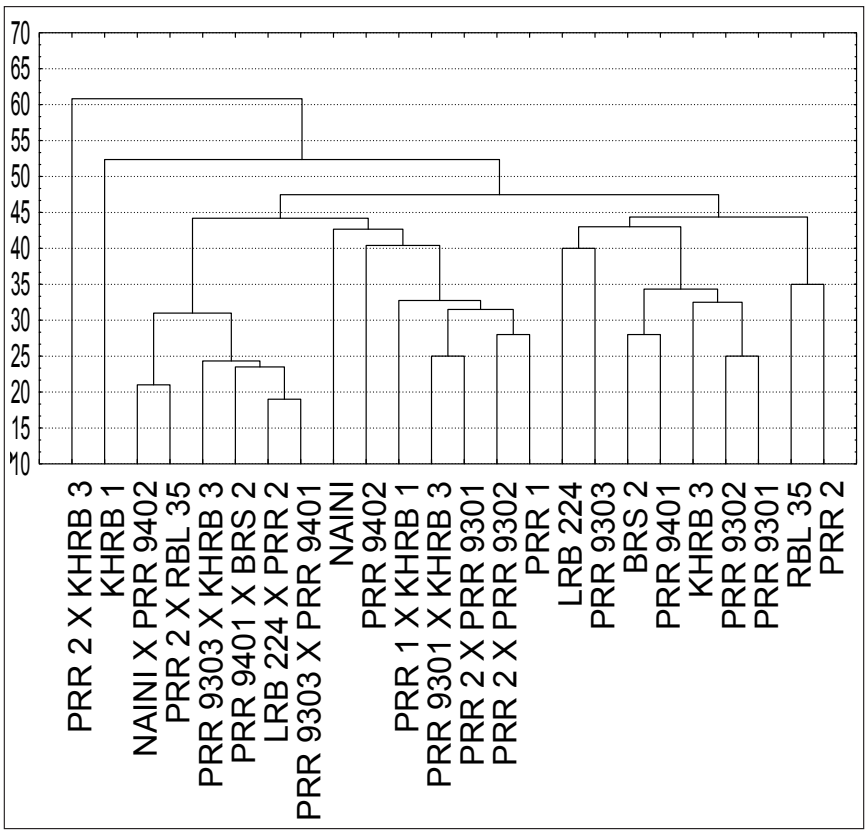

Fig. 1. Cluster analysis with UPGMA utilizing SED of the RAPD fragments generated out of twenty four rice bean varieties and their crosses

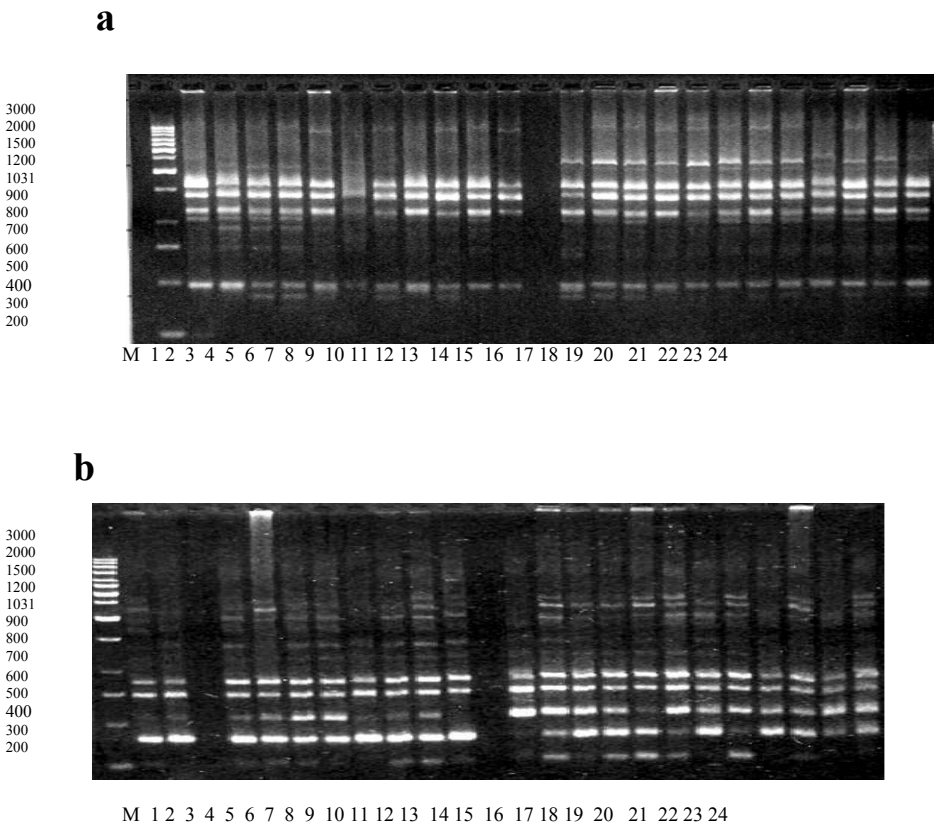

Fig. 2. DNA fingerprint patterns revealed for 24 ricebean varieties and their crosses by: a. Primer No. D17 and b. Primer No. D18

or absence of bands. Among these markers Type I, III and IV markers revealed bands common with the parents. Type II, V and VI are the bands found absent in their offspring, while, Type VII was the non-parental band as ahown in table 3A and 3B.

In the present investigation it was observed that 49.5% to 68.0% of the RAPD markers were found to reveal diversity among parents and crosses in eleven cross combination of ricebean. Arnold *et al.* (1981), identified the natural hybrids of Louisiana Irises by bands shared with both species and concluded that Type I, III and IV markers are good markers to identify the new hybrid from parents to ensure effective selection by plant breeders. The same holds true in the present investigation as well with hybrids showing 49.5% to 68.0% shared markers with the parents.

Characterization of diversity present among the genotypes is of immense importance in any crop improvement program for judicious choice of parents and efficient handling of segregating populations (Thakur *et al.* 2017). The polymorphisms of RAPD markers were observed as different-sized DNA fragments from amplification. Therefore, differences in markers from parents to crosses may be the result of DNA recombination, mutation, or random segregation of chromosomes in meiotic processing during hybridization. In the present study, 19.8% to 41.8% of markers (Type II, V and VI) were absent in eleven cross combinations of ricebean. These bands that are not shared with the parents in ricebean crosses are probably due to the segregation of heterozygous chromosomes during meiosis. Chromosomal crossing-over during meiosis may result in the loss of priming sites and thus markers are present in the parents but not in the crosses. Besides, 5.0% to 17.4% of unique markers were present in eleven cross combinations of ricebean. These non-parental bands may have been generated from the recombination and mutation in meiosis processing during hybridization (Huchett and Botha 1995) and may have also been created by heteroduplex formation as observed by Hunt and Page (1992); Ayliffe *et al*. (1994) and Novy and Vorsa (1996). The above results illustrated that of the seventeen RAPD primers used most were useful with good amount of reliability to identify ricebean crosses showing 49.5% to 68.0% shared markers with the parents (Type I, III and IV). However, Type VII markers were useful in identifying new crosses of ricebean with 5.0% to 17.4% non-parental bands. Given the paucity of research on molecular aspects of ricebean, characterization and assessment of its diversity could have great significance in designing breeding and germplasm conservation strategies**.**

CONCLUSION

In the present study RAPD marker technique was used to characterize and determine genetic relationship between the parental lines and their near homozygous progenies which in turn would be useful in marker assisted breeding programme in ricebean. The investigation indicated that out of the seventeen RAPD primers used most of them were useful with good amount of reliability to identify ricebean crosses showing 49.5% to 68.0% shared markers with the parents (Type I, III and IV). However, Type VII markers were useful in identifying new cultivars of ricebean with 5% to 17.4% non-parental bands.

REFERENCES

Arnold, M.L., Buckner, C.M. and Robison, J.J. 1991. Pollen-mediated introgression and hybrid speciation in Louisiana Irises. *Proc. Natl. Acad Sci.* **88**: 1398-1402.

Ayliffe, M.A., Lawrence, G.J., Ellis, J.G. and Pryor, A.J. 1994. Heteroduplex molecules formed between alletic sequences cause nonparental RAPD bands. *Nucleic Acids Res.* **22**: 1632-1636.

Bora, A., Choudhury, P.R., Pande, V. and Mandal, A.B. 2016. RAPD- Holds Promise to identify Different Genotypes of Rice for use in Breeding Programs of Diverse Genetic Stocks of Rice (*Oryza sativa* L.) Based on Genetic Diversity. *Vegetos*., **29**(Special): 69-77.

Choudhury, P.R., Singh, I.P., George, B., Verma, A.K. and Singh, N.P. 2008. Assessment of genetic diversity of pigeonpea cultivars using RAPD analysis. *Biologia Plantarum*., **52**(4): 648-653.

Huchett, B.I. and Botha, F.C. 1995. Stability and potential use of RAPD markers in sugarcane genealogy. *Euphytica.*, **86**: 117-125.

Hunt, G.J. and Page, Jr. R.E. 1992. Patterns of inheritance with RAPD molecular markers reveal novel types of polymorphism in the honey bee. *Theor. Appl. Genet*., **85**: 15-20.

Ilbi, H., Waters, C.M. and Bolkan, H.A. 2004. Sequence extension of RAPD markers to increase their utility for hybrid purity testing. *Seed Sci. & Technol.*, **32**: 193-203.

Meena V.S., Mittal, R.K., Choudhury, P.R., Rathod, S., Mahadevaswamy, H.K. and Choudhary, R. 2017. Utilization of molecular and morphometric tools for assessment of genetic diversity of Rice bean [*Vigna*

umbellata (Thunb.) Ohwi and Ohashi]. *Int.J.Curr.Microbiol. App.Sci.,* **6**(5): 2882-2892.

Novy, R.G. and Vorsa, N. 1996. Evidence for RAPD heteroduplex formation in cranberry: Implications for pedigree and genetic-relatedness studies and a source of co-dominant RAPD markers. *Theor. Appl. Genet.,* **92**: 840-849.

Santhy, V., Dadlani, M., Mohapatra, T. and Sharma, S.P. 2003. Identification of the parental lines and hybrids of rice (*Oryza sativa* L.) using RAPD markers. *Seed Sci & Technol.,* **31**(1): 187-192.

Thakur, S., Bhardwaj, N. and Chahota, R.K. 2017. Evaluation of genetic diversity in ricebean [*Vigna umbellata* (Thunb.) Ohwi and Ohashi] germplasm using SSR markers. *Electronic Journal of Plant Breeding*, **8**(2): 674-679.

Tian, J., Isemura, T., Kaga, A., Vaughan, D.A. and Tomooka, N. 2013. Genetic diversity of the rice bean (*Vigna umbellata*) genepool as assessed by SSR markers. *Genome*, **56**(12): 717–727.

Williams, J.G.K., Kubelik, A.E., Levak, R.J., Rafalski, J.A. and Tingey, S.C. 1990. DNA polymorphisms amplified by arbitrary primers are useful genetic markers. *Nucleic Acids Res.,* **18**: 6531-6535.

Xu, R.Q., Tomooka, N., Vaughan, A.D. and Doi, K. 2000. The *Vigna angularis* complex: Genetic variation and relationships revealed by RAPD analysis and their implications for in-situ conservation and domestication. *Genetic Resources and Crop Evolution,* **47**: 123-134.

Book Chapter

Ohwi, J. 1965. Flora of Japan. Smithsonian Institute, Washington DC. 567 pp.

Dutta, M., Phogat, B.S. and Dhillon, B.S. 2007. Genetic improvement and utilization of major under utilized crops in India. In Breeding of Neglected and Under-Utilized Crops, Spices and Herbs. Ochatt S and Jain M.S.(eds). Science Publishers. Enfield, NH, USA, pp. 251-298.

Gruere, G., Giuliani, A. and Smale, M. 2006. Marketing underutilized Plant Species for the Benefit of the Poor: A conceptual Framework. International Policy Research Institute. K Street, NW, Washington, DC, USA.