Received: 24 Mar 2023 | Revised: 28 Jun 2023 | Accepted: 29 Jun 2023

Electronic Journal of Plant Breeding

Research Article

An insight on molecular diversity in ricebean (*Vigna umbellata*) genotypes using microsatellite markers

S. Anandhinatchiar[1], P. Jayamani[1]*, D. Kumaresan[2], K. Bhuvaneswari[3] and M. Sudha[4]

[1]Department of Pulses, Centre for Plant Breeding and Genetics, Tamil Nadu Agricultural University, Coimbatore, India
[2]Department of Rice, Centre for Plant Breeding and Genetics, Tamil Nadu Agricultural University, Coimbatore, India
[3]Department of Agricultural Entomology, Tamil Nadu Agricultural University, Coimbatore, India
[4]Department of Plant Biotechnology, Centre for Plant Molecular Biology and Biotechnology, Tamil Nadu Agricultural University, Coimbatore, India
***E-Mail**: jayamani1108@gmail.com

Abstract
A total of 25 adzukibean derived SSR markers were used for molecular diversity analysis among 45 ricebean genotypes and a greengram cultivar, CO 7. Cross species amplification was observed for all the markers in ricebean. Number of alleles, in the present study, varied from one to three. In ricebean, the allele size ranged from 110 to 250 bp, whereas in greengram cultivar, it ranged from 110 to 250 bp. PIC values ranged from 0.198 to 0.689. Out of 25 markers, 13 markers were found to be polymorphic among ricebean genotypes. Apart from this, 13 markers differentiated the ricebean genotypes from the greengram cultivar, CO 7. Dissimilarity values between the ricebean genotypes and the greengram cultivar, CO 7 were greater when compared to the dissimilarity values within the ricebean genotypes. Dendrogram and the tree generated based on Ward's and neighbor joining methods classified the genotypes into five clusters and three groups, respectively.

Keywords: SSR, rice bean, diversity, cross species amplification

INTRODUCTION

Ricebean [*Vigna umbellata* (Thunb.) Ohwi and Ohashi 2n - 2x = 22] a minor pulse crop, is also known as climbing mountain bean, oriental bean and red bean (Pattanayak *et al*., 2019). It originated in South and Southeast Asia and cultivated mainly in China, India, Nepal, Bhutan, Myanmar, Thailand, Laos, Vietnam and Indonesia (Tian *et al*., 2013). In India, it is mainly grown in Northern and North-eastern parts wherein, it is predominantly grown as rainfed crop in mixed farming system under shifting cultivation (Iangrai *et al*., 2017). The dried seeds are usually boiled and eaten as dhal and young immature pods as vegetable, whereas whole plant can be used as forage for livestock. Despite its high harvest index, disease and pest resistance, high nutritional values, it has been hardly studied, because of its use within secluded regions, which resulted in paucity of high yielding commercial varieties. Therefore, considering the present situation, any earnest effort taken to develop superior high yielding cultivars, either with specific or wider adaptation is extremely warranted.

It is regarded that effective evaluation and incisive utilization of genetic diversity forms the core component in every crop improvement programme. Hence, an empirical insight regarding the degree of genetic diversity present within the genetic material, by and large, enables the breeder to select superior genotypes. In general, morphological examination of genetic diversity is definitive, economical and simple, compared to other ways of examination, however, associated with numerous shortcomings like presence of low level of

polymorphism, more subjective due to the influence of environment, expressions (of morphological traits) are dependent on growth stage. On the other hand, molecular markers enable a better way for determining genetic diversity since the genotypic data are not affected by the environment, independent of growth stage and gives a true picture about the level of polymorphism. Especially in ricebean, morphological markers exhibit extremely low level of polymorphism except for seed related traits *viz*., seed coat colour, seed coat pattern, seed size *etc*., which prolongs the investigation period up to the maturation stage. Hence, the use of molecular markers could bypass the time required by the morphological markers, as they are independent of growth stage and also could provide authentic results, as they are unaffected by the environment.

In ricebean, DNA markers such as, random amplified polymorphic DNA [RAPD] (Muthusamy *et al*., 2008, Shafiqul *et al*., 2017 and Meena *et al*., 2017), inter-simple sequence repeat [ISSR] (Muthusamy *et al*., 2008) and simple sequence repeat [SSR] (Tian *et al*., 2013, Wang *et al*., 2015, Iangrai *et al*., 2017 and Thakur *et al*., 2017) have been used to investigate genetic diversity. Among the DNA markers, SSRs are recognized to be more reliable to assess genetic diversity due to its abundance, high reproducibility and co-dominant nature (Rathore *et al*., 2020). Given the paucity of availability of microsatellite markers for rice bean and dearth of molecular genetic diversity studies in ricebean, the present study was aimed to investigate the transferability of SSR markers derived from adzuki bean to rice bean as well as to analyze the molecular diversity among 45 ricebean genotypes and the greengram cultivar, CO 7 using adzuki bean derived SSR markers.

MATERIALS AND METHODS

The experimental material of the present study encompasses 45 ricebean genotypes and a greengram cultivar CO 7, the details of the above are listed in **Table1**. Genomic DNA was extracted from young leaves collected from one week old seedlings by adopting cetyltrimethyl ammonium bromide (CTAB) method as described by Murray and Thompson (1980). The DNA pellet, thus extracted was dissolved in TE buffer and later diluted for appropriate concentration. To get rid of RNA, the extracted DNA was treated with 5µl RNAase at 37°C for 20 minutes. Prior to PCR amplification, the quality of the extracted DNA was appraised by 0.8% agarose gel electrophoresis.

A set of 25 SSR markers derived from adzuki bean were used for genotyping. The details of the SSR markers along with their annealing temperature, forward and reverse primer sequences are given in **Table 2**. Polymerase chain reaction (PCR) was carried out in a total reaction volume of 11µl comprising of 2 µl of 50 ng DNA, 2 µl of 5 µM primer (including both forward and reverse primer) and 7 µl of PCR master mix. BIO-RAD Thermal cycler was used to perform amplification reactions. Amplification cycles were set with an initial denaturation at 94°C for 3 minutes

Table 1. List of genotypes investigated in the present study

S.No.	Genotypes	S.No.	Genotypes
1	IC 520980	24	IC 520966
2	IC 520905	25	IC 342375
3	IC 444185	26	IC 342238
4	IC 521013	27	IC 469191
5	IC 10283	28	IC 521174
6	IC 102832	29	IC 342376
7	IC 362094	30	IC 520929
8	IC 520961	31	IC 520943
9	IC 444189	32	IC 469171
10	IC 520960	33	IC 417830
11	IC 520958	34	IC 520942
12	IC 444164	35	IC 520900
13	IC 520977	36	IC 520956
14	IC 520996	37	IC 360608
15	IC 520932	38	IC 969187
16	IC 469202	39	IC 520916
17	IC 360617	40	IC 341969
18	IC 520945	41	IC 520964
19	IC 361364	42	IC 52068
20	IC 520996	43	IC 521034
21	IC 520971	44	IC 520972
22	IC 521171	45	IC 520925
23	IC 361365	46	Greengram, CO 7

https://doi.org/10.37992/2023.1402.082

Table 2. List of 25 SSR markers employed in the present study and their observed level of polymorphism

S.No.	Marker name	Forward primer (5'-3')/ Reverse primer (3'-5')	Annealing temperature (°C)	Rice bean				Approximate allele size (bp) in greengram
				Approximate allele size range (bp)	Number of alleles	PIC value	Remarks	
1	CEDG AG 001	F: CTCATCAGGGACATCCTCCC R:GATCGTGATCGATCCAACGGTC	60	180	1	0.000	Monomorphic	170
2	CEDG 008	F: AGGCGAGGTTTCGTTTCAAG R:GCCCATATTTTTACGCCCAC	60	110 - 130	3	0.236	Polymorphic	140
3	CEDG 10	F: TGGGCTACCAACTTTTCCTC R: TGAGCGACATCTTCAACACG	60	180 - 190	2	0.506	Polymorphic	190
4	CEDG 15	F: CCCGATGAACGCTAATGCTG R:CGCCAAAGGAAACGCAGAAC	60	170 - 190	3	0.497	Polymorphic	170
5	CEDG 24	F: CATCTTCCTCACCTGCATTC R: TTTGGTGAAGATGACAGCCC	60	150 – 160	2	0.500	Polymorphic	160
6	CEDG 26	F: TCAGCAATCACTCATGTGGG R: TGGGACAAACCTCATGGTTG	60	160	1	0.000	Monomorphic	150
7	CEDG 27	F: ACTTGGGGTTTGAGATGTGG R:TCATTTTGGCCACTCAGTGC	60	150	1	0.000	Monomorphic	150
8	CEDG 43	F: AGGATTGTGGTTGGTGCATG R: ACTATTTCCAACCTGCTGGG	60	170 – 180	2	0.391	Polymorphic	180
9	CEDG 44	F: TCAGCAACCTTGCATTGCAG R: TTTCCCGTCACTCTTCTAGG	60	160 – 180	3	0.654	Polymorphic	180
10	CEDG 50	F: GGCAGAATCGTACAAGTG R:GTCAGATTCTCGCTTGCATG	60	120 – 140	2	0.497	Polymorphic	140
11	CEDG 88	F: TCTTGTCATTTAGCACTTAGCACG R:TTGTTGTTTACTAAGAGCCCGTGT	60	120	1	0.000	Monomorphic	130
12	CEDG 97	F: GTAAGCCGCATCCATAATTCCA R: TGCGAAAGAGCCGTTAGTAGAA	60	110	1	0.000	Monomorphic	100
13	CEDG 99	F:TGGGTGAGCATGGATGTGGA R:GGTTCAAGGTGGAAGGCAGA	60	220	1	0.000	Monomorphic	220
14	CEDG 115	F: GGCTCATTGTACCACTGGATAT R: ATGCCTCCTTTCAGGTGATTGT	60	150 – 170	3	0.689	Polymorphic	100
15	CEDG 143	F: GATGAACTCGTCTCGCTCATCG R: CTGGACGCGTCTACTCAGAC	60	120 – 140	3	0.535	Polymorphic	110

https://doi.org/10.37992/2023.1402.082

Table 2. Continued..

S.No.	Marker name	Forward primer (5'-3')/ Reverse primer (3'-5')	Annealing temperature (°C)	Ricebean				Approximate Allele size (bp) in greengram
				Approximate Allele size range (bp)	Number of alleles	PIC value	Remarks	
16	CEDG 154	F: GTCCTTGTTTTCCTCTCCATGG R: CATCAGCTGTTCAACACCCTGTG	60	240 – 250	2	0.488	Polymorphic	240
17	CEDG 156	F: CGCGTATTGGTGACTAGGTATG R:CTTAGTGTTGGGTTGGTCGTAAGG	60	190	1	0.000	Monomorphic	230
18	CEDG 171	F: CTTGAGAACCAACTCGAACTTC R:GGGAAATCGAAGAGGGACAG	60	210	1	0.000	Monomorphic	190
19	CEDG 174	F:GAGGGATCTCCAAAGTTCAACGG R:GAAGGCTCCGAAGTTGAAGGTTG	60	190	1	0.000	Monomorphic	190
20	CEDG 176	F: GGTAACACGGGTTCAGATGCC R: CAAGGTGGAGGACAAGATCGG	60	160	1	0.000	Monomorphic	150
21	CEDG 180	F: GGTATGGAGCAAAACAATC R:GTGCGTGAAGTTGTCTTATC	60	130 – 140	2	0.198	Polymorphic	130
22	CEDG 181	F: CGCGAGATCTGGATCGTTGATC R:GCAGTACGGTAACGTCCTTGAC	60	150 – 160	2	0.494	Polymorphic	130
23	CEDG 198	F: CAAGGAAGATGGAGAGAATC R:CCTTCTAAGAACAGTGACATG	60	220 – 240	3	0.663	Polymorphic	240
24	CEDG 204	F: CCTTGGTTGGAGCAGCAGC R: CACAGACACCCTCGCGATG	60	190	1	0.000	Monomorphic	180
25	CEDG 214	F: CACTCACTGCAAAGAGCAAC R: CTACCTATCTGAGGGACAC	60	180	1	0.000	Monomorphic	190
	Range			110 - 250	1 - 3	0.198 – 0.689		100 - 240
	Total				44	6.348		
	Mean				1.76	0.488		

SSR – simple sequence repeat; bp – basepair; PIC – polymorphic information content

and subsequent 35 cycles of denaturation at 94°C for 45 seconds each, trailed by annealing step at 60°C for 1 minute, followed by termination at 72°C for10 minutes. At the end, the amplified products were retained at 4°C. Then, the amplified products were electrophoresed for 2 hours at 110 V on 3% (w/v) agarose gels stained with ethidium bromide in 1X TBE buffer. Snapshots of the gels were captured by the BIO-RAD documentation unit. Sizes of the fragments were determined by a 100 bp ladder.

Scoring was done by determining the allelic sizes of fragments in the gel and the allelic data was analyzed in Darwin 5.0 software (Perrier and Jaqeuemond-Collet, 2005). Simple matching dissimilarity index was used to compute genetic distances among the genotypes and the resulting dissimilarity matrix was utilized to generate dendrograms using Ward's clustering and neighbor joining methods. In order to assess the ability of each marker to identify polymorphic loci among the rice bean genotypes, polymorphic information content (PIC) values were computed as PIC = $1 - \Sigma p^2_i$, where'p_i' is the frequency of i^{th} allele (Anderson *et al.,* 1993).

RESULTS AND DISCUSSION

A total of 25 adzukibean derived SSR markers were used to assess the molecular genetic diversity among 45 ricebean genotypes and the greengram cultivar, CO7. All the markers exhibited amplification. Electrophoretic banding pattern of the SSR marker CEDG 180 is given in **Plate1**. Cross species amplification of adzukibean derived SSR markers in ricebean was also reported by Srimathy and Jayamani (2010), Jayamani and Sathya, (2013) and Susmitha and Jayamani (2020).This kind of transferability of SSR marker from one species to other related species is extremely useful since the development of SSR marker is expensive when species specific genome sequences are not available. The degree of sequence conservation in the primer binding sites flanking the microsatellite loci and the stability of the loci during the evolution determines the feasibility of using the same microsatellite primers in various plant species (Decroocq *et al*., 2003).

In ricebean, the allele size spanned from 110 to 250 bp, whereas in greengram, the allele size ranged from 100 to 240 bp. The aforementioned range was nearly consistent with the allele size range reported by Tian *et al*. (2013) in ricebean and Joshi *et al*. (2021) in greengram while using SSR markers. Among the ricebean genotypes, the number of alleles ranged from one to three with an average of 1.76 alleles per marker. Six markers *viz*., CEDG 008, CEDG 15, CEDG 44, CEDG 115, CEDG 143 and CEDG 198 produced three alleles each, seven markers *viz*., CEDG 10, CEDG 24, CEDG 43, CEDG 50, CEDG 154, CEDG 180 and CEDG 181 produced two alleles each and 12 markers showed monomorphic banding pattern (**Table 2**). However, Tian *et al*. (2013) and Iangrai *et al*. (2017) used SSR markers and reported an average of 12.9 and 3.68 alleles per locus in ricebean, respectively.

Polymorphism Information Content (PIC) value of a marker reflects the ability of that marker to detect the polymorphism among the individuals of a population. It indicates the discriminatory power of that marker. Higher the PIC value, higher the discriminatory power of that marker. With an average PIC value of 0.488, PIC value in the current study ranged from 0.198 to 0.689 among rice bean genotypes. In greengram, relatively similar range for PIC value of 0.204 to 0.580 and 0.122 to 0.591 was reported by Joshi *et al*. (2021) and Mwangi *et al*. (2021), respectively. Moreover, Mwangi *et al*. (2021) reported an average PIC value of 0.372 in greengram, which is quite comparable with the present study. According to Botstein *et al.* (1980), markers with PIC values greater than 0.5 are considered to be very informative, markers with PIC values between 0.25 and 0.50 are moderately informative and markers with PIC values lower than 0.25 are not very informative. In the present investigation, six markers *viz*., CEDG 10, CEDG 24, CEDG 44, CEDG 115, CEDG 143 and CEDG 198 were detected with PIC value greater than 0.50 and six markers *viz*., CEDG 008, CEDG 15, CEDG 43, CEDG 50, CEDG 154 and CEDG 181 were observed to have PIC value between 0.25 and 0.50, indicating that these markers are highly informative in discriminating and differentiating the ricebean genotypes and could be potentially used for molecular characterization of ricebean germplasm from varied sources (**Table 2**). In the present study, out of 25 markers, only 13 (AB 008, CEDG 10, CEDG 15, CEDG 24, CEDG 43, CEDG 44, CEDG 50, CEDG 115, CEDG 143, CEDG 154, CEDG 180, CEDG 181, CEDG 198) were found to be polymorphic among ricebean genotypes (52%) and could be due to low level of DNA polymorphism among the ricebean genotypes. Apart from this,13 markers *viz*., CEDG AG 001, AB 008, CEDG 26, AB 88, AB 97, CEDG 115, CEDG 143, CEDG 156, CEDG 171, CEDG 176, CEDG 181, CEDG 204 and CEDG 214 (52%) differentiated greengram and the ricebean genotypes and hence, could be used for molecular confirmation of the interspecific hybrids and subsequently could be utilized in the development of prebreeding lines (**Table 3**).

In the present study, 45 ricebean genotypes and a greengram cultivar CO7 was grouped in to five clusters based on the dendrogram generated from 44 alleles by Ward's clustering method (**Table 4 and Fig. 1**.). Similarly, Susmitha and Jayamani (2020) grouped 25 greengram genotypes and five ricebean genotypes in to five clusters using unweighted pair group method with arithmetic mean (UPGMA), whereas Choudhary *et al*. (2022) classified 70 greengram cultivars in to eleven clusters based on UPGMA. Among the five clusters, cluster II was the largest and turned out to be an accommodative for 21 genotypes. It was followed by the clusters I, III and IV with twelve, seven, five genotypes, respectively. The tree derived from neighbour joining method, grouped the genotypes into three groups (**Table 5 and Fig. 2**). Group III was the largest with 19 genotypes and followed by group I with eighteen genotypes. Group II was the smallest with

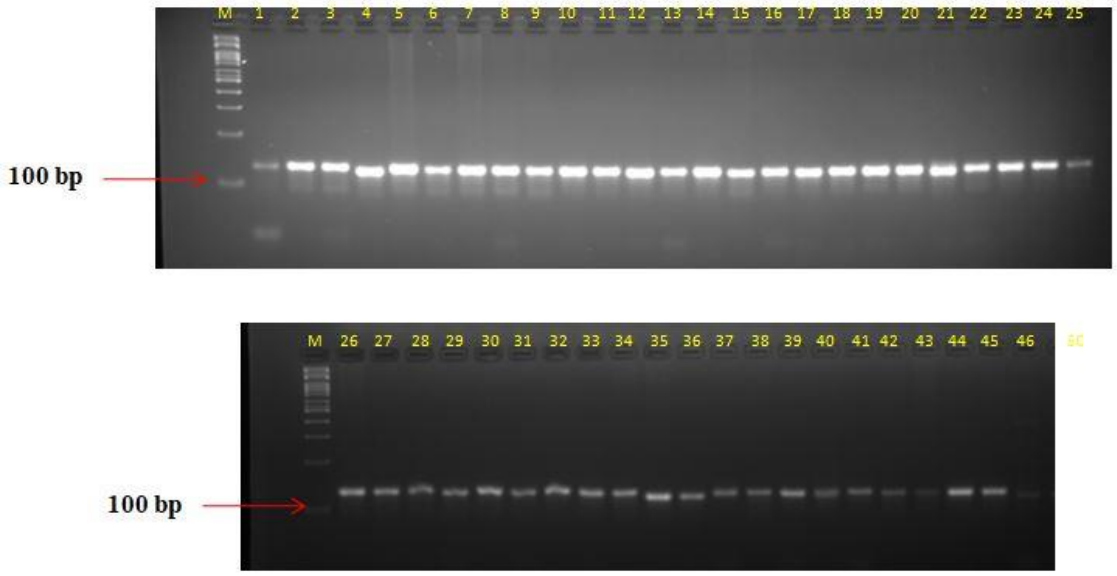

Plate1. Electrophoretic banding pattern of the SSR marker, CEDG 180 among 45 rice bean genotypes and the greengram cultivar CO7.
M: 100 bp ladder; For 1 to 46, refer Table 1 for name of genotypes

Table 3. List of polymorphic markers detected in the present study

	Number of polymorphic markers	Polymorphic markers
Among ricebean genotypes	13	AB 008,CEDG 10, CEDG 15,CEDG 24, CEDG 43,CEDG 44, CEDG 50, CEDG 115, CEDG 143, CEDG 154, CEDG 180, CEDG 181 and CEDG 198
Between greengram and ricebean genotypes	13	CEDG AG 001, AB 008, CEDG 26, AB 88, AB 97,CEDG 115, CEDG 143, CEDG 156, CEDG 171, CEDG 176, CEDG 181, CEDG 204 and CEDG 214

Table 4. Classification of 45 ricebean genotypes and a greengram cultivar, CO 7 into clusters based on a dendrogram using Ward's clustering method

Clusters	Number of genotypes	Genotypes
I	12	IC 469202, IC 520996, IC 520958, IC 521013, IC 444189, IC 362094, IC 520977, IC 102832, IC 444185, IC 520960, IC 10283, IC 520905
II	21	IC 361364, IC 520961, IC 520943, IC 521034, IC 52068, IC 520916, IC 969187, IC 360608, IC 520956, IC 520900, IC 520942, IC 520929, IC 342376, IC 342238, IC 520966, IC 360617, IC 520932, IC 520925, IC 520972, IC 520945, IC 444164
III	7	IC 520964, IC 341969, IC 521171, IC 361365, IC 469191, IC 520971, IC 342375
IV	5	IC 469171, IC 521174, IC 520980, IC 417830, IC 520901
V	1	CO 7

nine genotypes. Both Ward's and the neighbour joining methods classified majority of the genotypes in to two major clusters and groups, respectively. The genotypes in the sub group Ia of the group I as demarcated by the neighbor joining method, predominantly coincided with genotypes in cluster III, as classified by Ward's method. Moreover, most of the genotypes in subgroup Ib of the group I perfectly matched with the genotypes of cluster IV, classified by Ward's method. In the group II, a number of genotypes from sub group IIa and few genotypes from sub group IIb classified by the neighbor joining method partially coincided with the genotypes of cluster II and I generated by the Ward's method, respectively. The genotypes in the sub group IIIa of the group III established by the neighbour joining method, were partly similar to those in cluster I generated by Ward's clustering method.

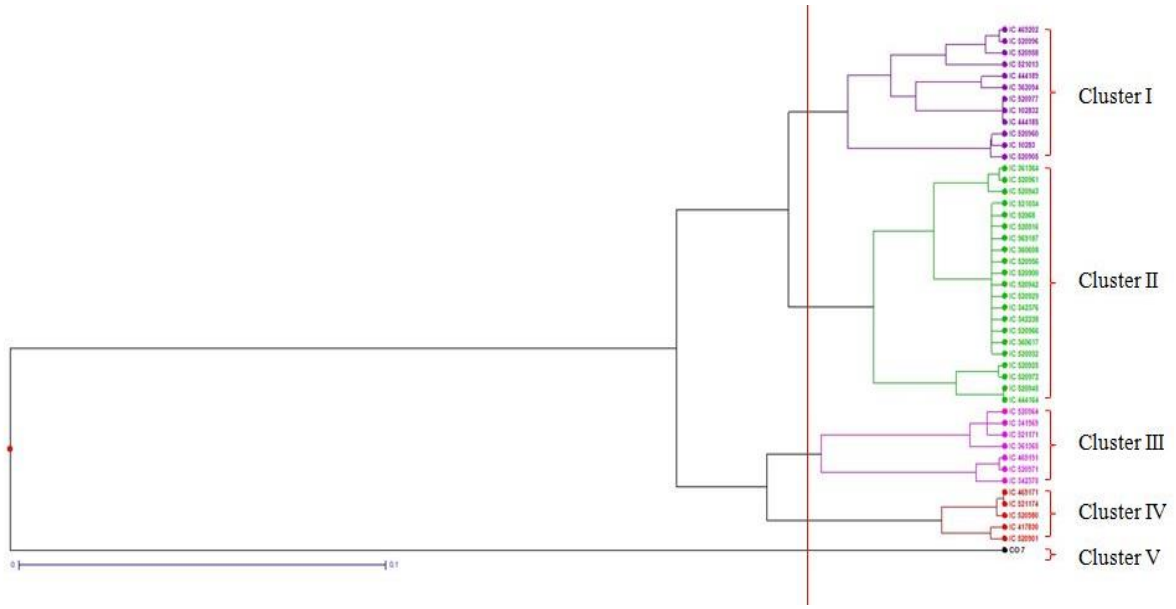

Fig. 1. Dendrogram of 45 ricebean genotypes and a greengram cultivar CO 7 based on Ward's method

Table 5. Classification of 45 ricebean genotypes and a greengram cultivar, CO 7 into groups based on neighbor joining method

Groups	Subgroups	Number of genotypes	Genotypes
I	Ia	10	IC 520964, IC 521171, IC 341969, IC 361365, IC 469191, IC 520971, IC 342375, IC 520916, IC 969187, IC 342238
	Ib	8	IC 469171, IC 521174, IC 520980, CO 7, IC 417830, IC 520901, IC 52068, IC 360608
II	IIa	6	IC 520900, IC 520942, IC 362094, IC 444189, IC 360617, IC 520932
	IIb	3	IC 520977, IC 102832, IC 444185
III	IIIa	5	IC 520960, IC 10283, IC 520905, IC 520943, IC 521013
	IIIb	14	IC 520929, IC 520966, IC 521034, IC 520945, IC 444164, IC 361364, IC 520961, IC 520925, IC 520972, IC 520956, IC 342376, IC 469202, IC 520996, IC 520958

In addition, the genotypes in the sub group IIIb of the above group III were largely the same as those in cluster II, established through the Ward's method. Furthermore, the greengram cultivar CO 7, even though present in subgroup Ib of group I along with the ricebean genotypes, was positioned at a greater distance, on comparison with its other group members. This was also precisely in tune with the Ward's method, in which the greengram cultivar was classified as solitary cluster. In the same way, distinct demarcation of ricebean and greengram genotypes was reported by Jayamani and Sathya (2012) and Susmitha and Jayamani (2020). The results from both the methods were relatively comparable with each other and stand evident to the presence of genetic diversity among the germplasm studied. Similarly, Susmitha and Jayamani (2020) in greengram reported that distinctness and stability of the clusters stay unaffected by the method of analysis.

The results indicate that microsatellite markers derived from adzukibean could be effectively used for molecular studies in ricebean, in which the availability of SSR markers is limited. Six microsatellite markers with PIC value greater than 0.5 identified in the present study could be used in future genetic diversity analysis of ricebean germplasm. The polymorphic markers for the ricebean genotypes, determined from the present study, could be used for molecular fingerprinting of the genotypes and could also aid in mapping of key traits in ricebean. Thirteen markers that distinguished the ricebean genotypes and the greengram cultivar could be utilized in molecular confirmation of interspecific hybrids, thereby in the

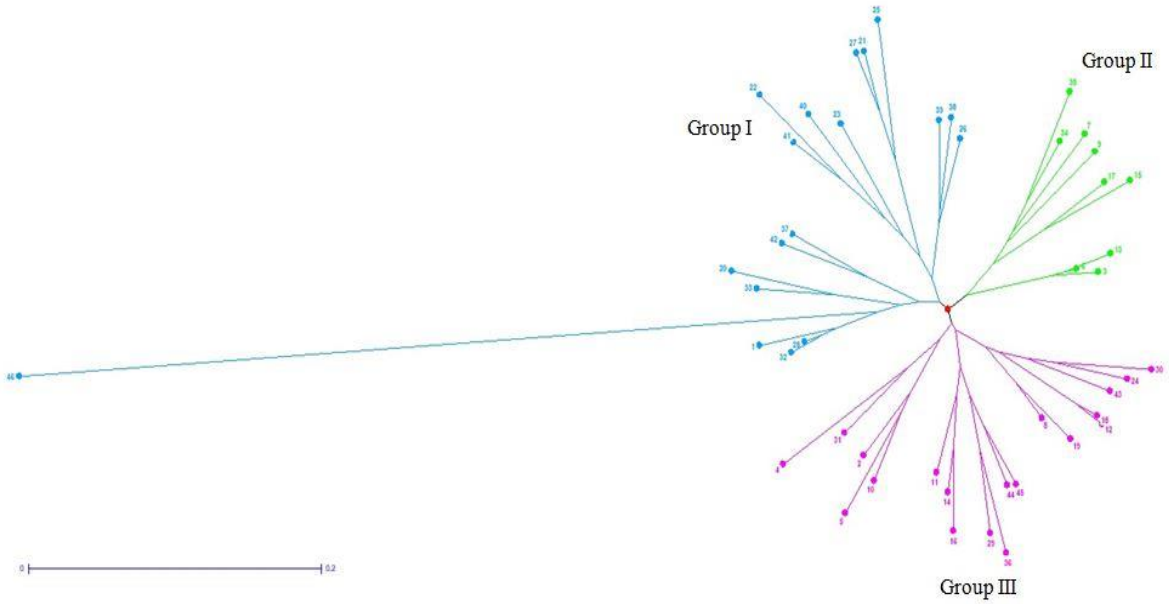

Fig. 2. Neighbour joining tree of 45 ricebean genotypes and a greengram cultivar CO 7

development of prebreeding lines. The distinct genotypes identified in dendrogram and neighbour joining tree could be used as parents in the hybridization programmes for the improvement of ricebean.

REFERENCES

Anderson, J.A., Churchill, G.A., Autroque, J.E., Tanksley, S.D. and Swells, M.E. 1993. Optimising selection for plant linkage map. *Genome,* **36**:181-186. [Cross Ref]

Botstein, D., White, R.L., Skolnick, M. and Davis, R.W. 1980. Construction of a genetic linkage map in man using restriction fragment length polymorphisms. *American journal of human genetics*, **32**(3): 314 – 331.

Choudhary, K.B., Pratap, A. and Tomar, R. 2022. Cross genera marker transferability and genetic diversity analysis in elite cultivars of mungbean [*Vigna radiata* (L.) wilczek]. *Legume Research-An International Journal*, **45**(9):1065-1073. [Cross Ref]

Decroocq, V., Fave, M.G., Hagen, L., Bordenave, L. and Decroocq, S. 2003. Development and transferability of apricot and grape EST microsatellite markers across taxa. *Theoretical and Applied Genetics*,**106**(5):912-922. [Cross Ref]

Rathore, D., Monika, M.B. and Nidhi, V. 2020. Role of microsatellite markers in molecular diversity analysis of rice bean (*Vigna umbellta*). *Asian Journal of Bio Science*, **15**(1):15-20.

Iangrai, B., Pattanayak, A., Khongwir, D.E., Pale, G., Gatphoh, E.M., Das, A. and Chrungoo, N.K. 2017. Development and characterization of a new set of genomic microsatellite markers in rice bean (*Vigna umbellata* (Thunb.) Ohwi and Ohashi) and their utilization in genetic diversity analysis of collections from North East India. *PloS one*, **12**(7): e0179801. [Cross Ref]

Joshi, D.P., Parmar, L.D., Solanki, R.S. and Patel, M.P. 2021.Assessment of molecular diversity among mungbean [*Vigna radiata* (L.) Wilczek] genotypes using EST-SSR marker. *The Pharma Innovation,* **10**(12): 757-764. [Cross Ref]

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 Ricebean [*Vigna umbellata* (Thunb.) Ohwi and Ohashi]. *International Journal of Current Microbiology and Applied Sci*ences, **6**(5):2882-2892. [Cross Ref]

Mwangi, J.W., Okoth, O.R., Kariuki, M.P. and Piero, N.M. 2021. Genetic and phenotypic diversity of selected Kenyan mung bean (*Vigna radiata* L. Wilckzek) genotypes. *Journal of Genetic Engineering and Biotechnology*, **19**(1): 1-4. [Cross Ref]

Murray, M.G. and Thompson, W. 1980.Rapid isolation of high molecular weight plant DNA. *Nucleic acids research*, **8**(19):4321-4326. [Cross Ref]

Muthusamy, S., Kanagarajan, S. and Ponnusamy, S. 2008. Efficiency of RAPD and ISSR markers system in

accessing genetic variation of ricebean (*Vigna umbellata*) landraces. *Electronic Journal of Biotechnology*, **11**(3):32-41. [Cross Ref]

Jayamani, P. and Sathya, M. 2012.Genetic diversity as assessed by morphological and microsatellite markers in greengram (*Vigna radiata* L.). *African Journal of Biotechnology,***11**(84): 15091-15097.

Pattanayak, A., Roy, S., Sood, S., Iangrai, B., Banerjee, A., Gupta, S. and Joshi, D.C. 2019. Ricebean: a lesser known pulse with well-recognized potential. *Planta*, **250**(3): 873-890. [Cross Ref]

Perrier, X. and Jaqeuemond-Collet, J.P.2005. Darwin-5.0 software http://darwin.cirad.fr/Dissimilarity analysis and representation for windows *Equipe Mathematiqueet Informatique*.

Shafiqul, I., Dutta, M., Shachi, S., Kumar, P. and Bhatt, K.V. 2017. Assessment of genetic diversity of ricebean [*Vigna umbellata* (Thunb.) Ohwi&Ohashi)] varieties and their narrow leaf cross derivatives using RAPD Markers. *International Journal of Agriculture, Environment and Biotechnology*, **10**(4):415-421. [Cross Ref]

Srimathy, M. and Jayamani, P. 2010. Cross species amplification of Adzuki bean derived microsatellite markers in Asian *Vigna* species. *Electronic Journal of Plant Breeding.***1**: 1171-1179.

Susmitha, D. and Jayamani, P. 2020. Cross-species Amplification and molecular diversity analysis in greengram [*Vigna radiata* (L.) Wilczek] and rice bean [*Vigna umbellata* Thunb.]. *Legume Research – An International journal*, DOI: http://dx.doi.org/10.18805/LR-4230. [Cross Ref]

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. [Cross Ref]

Tian, J., Isemura, T., Kaga, A., Vaughan, D.A. and Tomooka, N. 2013. Genetic diversity of the ricebean (*Vigna umbellata*) genepool as assessed by SSR markers. *Genome,* **56**(12):717-727. [Cross Ref]

Wang, L.X., Chen, H.L., Bai, P., Wu, J.X., Wang, S.H., Blair, M.W. and Cheng, X.Z. 2015. The transferability and polymorphism of mungbean SSR markers in rice bean germplasm. *Molecular breeding*, **35**: 77. [Cross Ref]