Indian J. Genet. Plant Breed., (2022); 82(4): 448-457
doi: 10.31742/ISGPB.82.4.8
ISSN: 0975-6906
www.isgpb.org

RESEARCH ARTICLE

Genetic variability and population structure analysis in ricebean (*Vigna umbellata*) genotypes from northeast India

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Abstract

Assessing the genetic variability at molecular level gives a clear idea of plant genetic resource distribution and extent of diversity among and within the available population. In the present study, 120 ricebean (*Vigna umbellate* L.) genotypes collected from different states of Northeast (NE) regions of India. The genetic variability and population structure estimated from the information generated by 23 SSR markers. A total of 59 alleles with an average of 2.57 alleles per SSR locus were detected. The polymorphic information content (PIC) value ranges from 0.129 to 0.548. The genotypes were grouped into 3 major clusters in both UPGMA and Model-based (STRUCTURE) cluster analysis. Clusters I genotypes were observed with the highest mean yield. The clustering pattern did not show a relationship between geographic distribution and the pattern of grouping of genotypes. The results suggested that the elite genotypes can be used as parent for further crop improvement strategies and creates the necessity of diverged germplasm conservation.

Keywords: Genetic diversity, PIC, SSR marker, UPGMA, ricebean

Introduction

The genus *Vigna* comprises 150 species which are distributed throughout the globe. These species can be grouped into six subgenera *Vigna, Ceratropis, Plectropis, Sigmoidotropis, Lasiospora* and *Haydonia* (Vaillancourt et al. 1993; Vijaikumar et al. 2010). Ricebean (*Vigna umbellata;* Family: Leguminocea, Thunb, Ohwi and Ohashi), earlier classified as *Phaseolus calcaratus*, is an annual self-pollinated warm legume crop having somatic chromosome number, 2n=22. It has been regarded as an under-exploited and neglected tropical legume crop, cultivated for only small areas by subsistence farmers in hilly regions of Nepal, northern India and some parts of South East Asia. Recently, this crop has been included in All India Co-ordinated Arid Legume Crops.

Mainly, ricebean is cultivated in the North Eastern Hill Region and a limited area in India's western peninsular region (Arora et al.1980). Due to different biotic and abiotic stresses, soil-borne diseases, and lack of exploitation of their available genetic resources in breeding programs, the productivity of Asian *Vigna umbellata* has been reported low comparing to their expected potential yield. Several studies have been conducted earlier to determine the genetic diversity and the stability of genotypes for a particular environment (Muthusamy et al.2008; Iangari et al. 2017; Philanim et al. 2022). It is a multi-purpose legume crop (Joshi et al. 2006; Linda 2013). It can be used in different forms, right from human dietary to fodder, and serve as the cheapest protein source. Legume-cereal cropping system increases soil nutrition and avails sustainable agriculture production.

Detailed knowledge of genetic diversity is required to add desirable characters in already popular and in-use landraces and select superior parent combinations depending on the proportion of diversity level present within the available genotypes. Since, the estimation of germplasm diversity based on only phenotypic study cannot give actual variation within species, the molecular analysis

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How to cite this article: Devi Y.S., Pandey A., Kumar A., Rai M., Tyagi W. and Aochen C. 2022. Genetic variability and population structure analysis in ricebean (*Vigna umbellata*) genotypes from Northeast India. Indian J. Genet. Plant Breed., **82**(4): 448-457.

Source of support: ICAR-RC for NEH Region, Umiam (Project code: 1XX08769)

Conflict of interest: None.

Received: Feb. 2022 **Revised:** Aug. 2022 **Accepted:** Oct. 2022

is expected to divulge the useful information. Different type of molecular markers have been used to detect the genetic diversity in various *Vigna* species, such as AFLP in ricebean (Seehalak. 2006), RAPD in cowpea (Ba et al. 2004) and mungbean (Datta et al. 2012; Lavanya et al. 2008), ISSR and SSR in ricebean (Muthusamy et al. 2008; Isemura et al. 2010; Wang et al. 2012; Chen et al. 2016). Several studies on characterization and assessment of genetic diversity in ricebean using SSR marker have been carried out earlier (BaSjracharya et al. 2008; Chaitieng et al. 2006; Iangrai et al. 2017; Misra and Swain 2010; Tian et al. 2013; Wang et al. 2015). To generate useful and additional information on the ricebean germplasm distributed across the states of Mizoram, Meghalaya, Nagaland and Manipur geographical locations a study was, therefore, carried out to analyse genetic diversity using morphological and molecular markers for utilization in the crop improvement programme.

Materials and methods

The study was performed at the Plant Breeding Farm of ICAR Research Complex for NEH Region, Umiam, Meghalaya. One hundred and twenty genotypes from different parts of North East India, namely, Manipur, Mizoram, Meghalaya and Nagaland (Table 1), were grown in augmented design and data was recorded on 23 agronomic traits, namely, plant height (cm) at 50% flowering, days to 50% flowering, days to 80% maturity, terminal leaf length (cm), terminal leaf width (cm), petiole length of terminal leaf (cm), leaf length (cm), leaf width(cm), petiole length of leaf (cm), primary branch length (cm), no. of clusters/plant, No. of pods/plant, No. of branches, peduncle length (cm), no. of Seeds/pod, no. of seeds/plant, 100 seed weight (g), pod length (cm), pod width (cm), seed length (cm), seed width (cm) and grain yield/plant (g).

DNA isolation, purification and gel electrophoresis

The investigation used 23 polymorphic primers and their sequence information (Chen et al. 2016; Tian et al. 2013; Isemura et al. 2010) to analyse genetic diversity.The mean and range values per cluster were calculated based on the observation of randomly selected plant. Plant DNA extraction (Sanghai-Maroof et al. 1984) was done using CTAB based method with some modification. DNA was purified by adding RNase (20 mg/mL) followed by ethanol precipitation of DNA and washing with ethanol 70% to remove the extra salt present. The DNA pellets were dried and further dissolved in 1XTE then stored in 4°C for further use.DNA quantification was estimated using a Thermo Scientific NanoDrop 2000 spectrophotometer. DNA samples were diluted in nuclease-free water accordingly obtained from the ratios. The total volume of 10 µL PCR mixture containing 1-µL diluted DNA, 1-µL $MgCl_2$, 0.1 µL *Taq* DNA polymerase, 1-µL of 1x PCR buffer without $MgCl_2$, 0.5 µL of each forward and reverse primers and 0.25 µL of dNTPs and 6.15 µL ddH2O. For the PCR amplification, the Veriti™ thermal cycler (Applied Biosystems) was set at 94°C for 5 minutes with 35 cycles followed by primer annealing at the respective annealing temperature primers for 1 min and final primer extension at 72°C for 10 minutes. One microliter (1-µL) of 6x loading dye was mixed with reaction products and spun briefly before loading to the gel. All PCR products were electrophoresed on 3% agarose gel at 90 V for 3.5 hours. The gels were stained with ethidium bromide (3 mL/100 mL of gel) and documented using a Chemidoc (BioRad).

Statistical analysis

For all 23 agronomical traits, the cluster mean and range were calculated. Each SSR marker bands were scored as '1/0' for the presence/absence of distinct bands. The allelic data were subjected to specific software to generate an informative result.Using POPGENE V1.32, the level of genetic diversity was estimated. Nie's genetic distance, genetic similarities, observed heterozygosity and expected

Table 1. Ricebean accessions with their sources used in the study

S. no.	Collection site	Genotypes
1.	Mizoram lines (35)	BKSB- 31, BKSB-10, BKSB-11, BKSB-12, BKSB-13, BKSB-14, BKSB-15, BKSB-16, BKSB-17, BKSB-19, BKSB-2, BKSB-20, BKSB-21, BKSB-22, BKSB-23, BKSB-25, BKSB-26, BKSB-27, BKSB-28, BKSB-29, BKSB-3, BKSB-30, BKSB-32, BKSB-4, BKSB-5, BKSB-6, BKSB-7, BKSB-8, BKSB-9, LRGP- 12, LRGP- 26, LRGP- 3
2.	Manipur lines (34)	CHAKHAWAI-10,CHAKHAWAI-15, CHAKHAWAI-17, CHAKHAWAI-19, CHAKHAWAI-21, CHAKHAWAI-26, CHAKHAWAI-28, CHAKHAWAI-31, CHAKHAWAI-32, CHAKHAWAI-34, CHAKHAWAI-41, CHAKHAWAI-1, CHAKHAWAI-11, CHAKHAWAI-13, CHAKHAWAI-14, CHAKHAWAI-16, CHAKHAWAI-18, CHAKHAWAI-2, CHAKHAWAI-20, CHAKHAWAI-22, CHAKHAWAI-24, CHAKHAWAI-27, CHAKHAWAI-29, CHAKHAWAI-3, CHAKHAWAI-30, CHAKHAWAI-35, CHAKHAWAI-36, CHAKHAWAI-5, CHAKHAWAI-6, CHAKHAWAI-7, CHAKHAWAI-8, LRGP- 24
3.	Meghalaya lines (32)	BKSB-33, BKSB-35, BKSB-36, BKSB-37, BKSB-40, BKSB-42, LRGP- 1, LRGP- 10, LRGP- 17, LRGP- 18, LRGP- 20, LRGP- 25, LRGP- 27, LRGP- 29, LRGP- 31, LRGP- 34, LRGP- 35, LRGP- 37, LRGP- 38, LRGP- 43, LRGP- 46, LRGP- 50, LRGP- 59, LRGP- 60, LRGP- 61, LRGP- 62, LRGP- 32, LRGP-11, LRGP-22 LRGP-39, LRGP-6, LRGP-7
4.	Nagaland lines (19)	BKSB-34, BKSB-43, BKSB-44, BKSB-45, BKSB-46, BKSB-47, LRGP- 14, LRGP- 21, LRGP- 40, LRGP- 36, LRGP- 44, LRGP- 48, LRGP- 52, LRGP- 54, LRGP- 55, LRGP- 57, LRGP-13, LRGP-15, LRGP-56

heterozygosity was calculated using POWERMAKER v3.25 software (Liu et al. 2005). Unweighted Pair Group Method of Arithmetic Average (UPGMA) clustering method was performed using NTSYS-PC ver. 2.02, Exeter Software (Rolf et al. 1993).The possible population was analysed using the model-based program Structure 2.2 (Pritchard et al. 2000).

Results and discussion

Genetic variation in the 120 ricebean accessions was detected using 23 polymorphic SSR markers, producing 59 alleles. The primer information, number of alleles per polymorphic loci, major allelic frequency for each locus, genetic diversity (%), and PIC value are summarized in Table 2. A similar frequency of alleles per locus has been reported with a mean of 2.4 per marker in mungbean (*Vigna radiata*). However, a very high number of alleles (12.9/marker) using SSR markers was reported by Tian et al. (2013), which was contrastingly higher than the present results. Usually such a high number of alleles per locus is difficult to find. Further, Muthusamy et al. (2008) evaluated ricebean landraces using random amplified polymorphic DNA (RAPD) and inter simple sequence repeats (ISSR) markers. Both RAPD and ISSR markers detected high polymorphic loci suggesting both markers are equally effective in determining the diversity. Differences in reports on variable number of alleles per locus may be overcome by a large collection of accessions and selection of suitable primers, which create a significant

Table 2. Summary of primer sequences and polymorphic SSR makers in 120 genotypes of ricebean

Primer	Primer sequence		PS (bp)	Motif	NA	MAF	GD (%)	PIC
	Forward	Reverse						
CEDG008	AGGCGAGGTTTCGTTTCAAG	GCCCATATTTTTACGCCCAC	123	$(AG)_{26}$	2	0.529	49.82	0.374
CEDG015	CCCGATGAACGCTAATGCTG	CGCCAAAGGAAACGCAGAAC	213	$(AG)_{27}$	3	0.433	64.39	0.569
CEDG021	GCAGAATTTTAGCCACCGAG	AAAGGATGCGAGAGTGTAGC	121	$(AG)_{26}$	2	0.669	44.24	0.344
CEDG024	CATCTTCCTCACCTGCATTC	TTTGGTGAAGATGACAGCCC	146	$(AG)_{18}$	4	0.895	19.41	0.187
CEDG026	TCAGCAATCACTCATGTGGG	TGGGACAAACCTCATGGTTG	166	$(AG)_{26}$	2	0.500	50.00	0.375
CEDG029	GATTGCTTTTAGCAGAGGGC	GAAGAAACCCATCTCGATCC	189	$(AG)_{8}$	2	0.576	48.83	0.369
CEDG041	GCTGCATCTCTATTCTCTGG	GCCAACTAGCCTAATCAG	117	$(AG)_{21}$	2	0.552	49.46	0.372
CEDG043	AGGATTGTGGTTGGTGCATG	ACTATTTCCAACCTGCTGGG	158	$(AG)_{14}$	3	0.903	17.52	0.161
CEDC016	TCAGCAACCTTGCATTGCAG	TTTCCCGTCACTCTTCTAGG	135	$(GT)_{10}AT(AG)_{18}$	3	0.745	38.15	0.312
CEDG073	CCCCGAAATTCCCCTACAC	AACACCCGCCTCTTTCTCC	173	$(AG)_{24}$	5	0.728	44.53	0.419
CEDG044	ACTCTTGTCAATTGTCCAGG	TAACTTGTCACTGGAAAGGC	152	$(AT)_{5}(AC)_{9}$	3	0.525	50.27	0.380
c19149.graph_c0	AGCAATGATCTGATCCACCC	TGGGAGGTGTTTCTGTTTCC	265	$(ATA)_{7}$	3	0.730	39.61	0.321
c19719.graph_c0	CCAATCTCCAATGCCTTGAT	GACTGTTCGATGGTGGCTTT	209	$(AG)_{11}$	2	0.581	48.68	0.368
c21449.graph_c0	CAAGACCTCGGCTTTCTCAG	GCATGGAGGGTAGAAGTCCA	152	$(CT)_{9}$	2	0.729	39.44	0.316
c28613.graph_c0	GGGTGGCGTAGATCTCATGT	TTGTTCTCTTTCCGTTTGGG	255	$(TC)_{10}$	3	0.789	33.19	0.276
c28852.graph_c0	CACCCACGTCACAAATTCAG	GGGTGAAGGATAGGGAGGAG	170	$(CAA)_{7}$	3	0.855	25.70	0.241
c29169.graph_c0	CCACCTTAAGCAACCAGGAA	CTGCAGATGGCAAAGAATGA	204	$(TA)_{10}$	2	0.512	61.83	0.548
c9302.graph_c0	CCAAGAAGTTCGGGTACCAA	GCGAATAAAGGCTGAAATGG	252	$(CT)_{9}$	3	0.925	13.88	0.129
c9589.graph_c1	ATGCTTCCAAGCAGGATTGT	TGTTCTTTTCAAAGCCAGGG	205	$(TA)_{9}$	3	0.425	65.35	0.580
c9711.graph_c0	CAACAGCAAGCAAAGCAAAG	ATGAGCTGTGTGTGTGCTGA	224	$(A)_{16}$	2	0.800	32.00	0.268
c9818.graph_c0	GCGCTGAATCTTTTCTCTGC	GGAGTGGAGATGGAGATGGA	108	$(TCTA)_{5}$	2	0.571	48.98	0.369
c27353.graph_c1	CACAATTTCCCTTCCTTTGA	ACTCAACTTTGTGCAGCCCT	229	$(AT)_{9}$	2	0.775	34.88	0.287
c25883.graph_c0	TGATCATCAGGGTGGTGAAA	CAAAACGAAGAGAAATACAATGGA	263	$(T)_{17}$	2	0.917	15.15	0.140
Average value					2.57	0.681	0.406	0.335

PS = Product size, NA = Number of alleles, MAF = Major allele frequency, GD = Genetic diversity, PIC = Polymorphic Information Content.

earlier in Vigna species. Allelic frequency ranging from 2 to 5 with an average frequency of 2.4 was reported by Chen et al. (2016), while Iangari et al. (2017), reported a total of 179 alleles with an average of 6.393 alleles per locus indicating a gene diversity in the material. At the same time Kumar et al. (2002), Dikshit et al. (2012) also reported 2 to 5 alleles variation in the number of alleles per locus. According to PIC value, c9589gC1 locus detected as the most informative primer among all 23 primers.

Group-wise populations for the extent of polymorphism and expected heterozygosity within a group are presented in Table 3. The mean of observed heterozygosity was

Table 3. Group wise summary of heterozygosity statistics for all loci

Locus	Mizoram lines				Nagaland lines				Manipur lines				Meghalaya lines			
	Obs. Homo	Obs. Hete.	Exp. Hete*	Nei**	Obs. Homo.	Obs. Hete.	Exp. Hete*	Nei**	Obs. Homo.	Obs. Hete	Exp. Het*	Nei**	Obs. Homo.	Obs. Hete.	Exp. Het*	Nei**
CEDG008	1	0	0.50	0.49	1	0	0.51	0.49	1	0	0.47	0.46	1	0	0.50	0.49
CEDG015	1	0	0.64	0.63	0.88	0.11	0.68	0.66	1	0	0.61	0.60	1	0	0.58	0.57
CEDG021	1	0	0.50	0.49	1	0	0.38	0.37	1	0	0.16	0.16	1	0	0.48	0.47
CEDG024	0.94	0.05	0.21	0.20	0.93	0.06	0.17	0.17	0.93	0.06	0.06	0.06	1	0	0.26	0.26
CEDG026	1	0	0.49	0.48	1	0	0.50	0.49	1	0	0.50	0.49	1	0	0.47	0.46
CEDG029	1	0	0.50	0.49	1	0	0.21	0.20	1	0	0.48	0.47	1	0	0.49	0.49
CEDG041	1	0	0.38	0.37	1	0	0.38	0.37	1	0	0.47	0.46	1	0	0.48	0.48
CEDG043	0.94	0.05	0.33	0.32	1	0	0.10	0.10	1	0	0.05	0.05	1	0	0.13	0.13
CEDC016	1	0	0.46	0.45	1	0	0.51	0.49	1	0	0	0	0.96	0.03	0.41	0.40
CEDG073	0.47	0.52	0.55	0.54	0.84	0.15	0.29	0.28	0.73	0.26	0.44	0.43	0.64	0.35	0.40	0.39
CEDG044	0.31	0.68	0.51	0.51	0.38	0.61	0.5	0.48	0.60	0.39	0.50	0.49	0.32	0.67	0.47	0.46
c19149gC0	0.76	0.23	0.46	0.45	0.89	0.10	0.51	0.49	0.96	0.03	0.28	0.27	0.92	0.07	0.24	0.24
c19719gC0	1	0	0.39	0.38	1	0	0.42	0.41	1	0	0.49	0.48	1	0	0.50	0.49
c21449gC0	1	0	0.41	0.40	1	0	0.34	0.33	1	0	0.44	0.43	1	0	0.35	0.34
c28613gC0	1	0	0.15	0.15	1	0	0.50	0.48	1	0	0.46	0.46	1	0	0.12	0.12
c28852gC0	0.8	0.2	0.23	0.22	0.83	0.16	0.34	0.33	1	0	0.11	0.11	0.8	0.2	0.39	0.38
c29169gC	0.44	0.55	0.61	0.60	0.55	0.44	0.53	0.52	0.70	0.29	0.61	0.61	0.38	0.61	0.59	0.58
c9302gC	1	0	0.05	0.05	1	0	0	0	1	0	0.29	0.29	1	0	0.12	0.12
c9589gC1	1	0	0.29	0.29	0.77	0.22	0.35	0.34	1	0	0.49	0.48	0.73	0.26	0.39	0.38
c9711gC0	1	0	0.40	0.39	1	0	0.10	0.09	1	0	0.30	0.30	1	0	0.36	0.35
c9818gC0	1	0	0.32	0.32	1	0	0.39	0.38	1	0	0.40	0.39	1	0	0.50	0.49
c27353gC1	0.87	0.12	0.17	0.17	0.41	0.58	0.49	0.48	1	0	0.14	0.14	0.5	0.5	0.491	0.48
c25883gC0	1	0	0.06	0.06	1	0	0.21	0.20	1	0	0.16	0.16	1	0	0.20	0.19
Mean	0.89	0.10	0.37	0.37	0.89	0.10	0.37	0.35	0.95	0.04	0.34	0.34	0.88	0.11	0.39	0.38
SD	0.20	0.20	0.16	0.16	0.18	0.18	0.16	0.16	0.11	0.11	0.18	0.18	0.21	0.21	0.14	0.14

Obs. Homo. = Observed homozygosity; Obs. Hete.= Observed heterozygosity; Exp. Hete. = Expected heterozygosity; Nei**= Nei's expected heterozygosity and SD = Standard Deviation

Table 4. Estimates of genetic diversity within population of ricebean from different states

Locus	Mizoram			Nagaland			Manipur			Meghalaya		
	Na	Ne	I	Na	Ne	I	Na	Ne	I	Na	Ne	I
CEDG008	2	1.99	0.69	2	1.99	0.69	2	1.88	0.66	2	2.00	0.69
CEDG015	3	2.73	1.05	3	2.98	1.10	3	2.52	1.01	3	2.35	0.94
CEDG021	2	1.97	0.69	2	1.60	0.56	2	1.19	0.30	2	1.91	0.67
CEDG024	3	1.26	0.42	3	1.21	0.37	2	1.07	0.14	3	1.35	0.50
CEDG026	2	1.96	0.68	2	1.97	0.69	2	1.98	0.69	2	1.87	0.66
CEDG029	2	2.00	0.69	2	1.26	0.36	2	1.90	0.67	2	1.97	0.68
CEDG041	2	1.60	0.56	2	1.60	0.56	2	1.87	0.66	2	1.92	0.67
CEDG043	3	1.48	0.55	2	1.12	0.21	2	1.06	0.14	2	1.16	0.26
CEDC016	2	1.84	0.65	2	1.99	0.69	1	1.00	0.00	3	1.68	0.65
CEDG073	5	2.22	1.07	5	1.40	0.65	4	1.77	0.86	4	1.67	0.77
CEDG044	3	2.05	0.76	2	1.95	0.68	2	1.97	0.69	2	1.88	0.66
c19149gC0	2	1.84	0.65	2	1.99	0.69	2	1.38	0.45	2	1.32	0.41
c19719gC0	2	1.64	0.58	2	1.71	0.61	2	1.94	0.68	2	1.98	0.69
c21449gC0	2	1.69	0.60	2	1.51	0.52	2	1.78	0.63	2	1.53	0.53
c28613gC0	2	1.19	0.29	2	1.95	0.68	2	1.86	0.66	2	1.14	0.24
c28852gC0	3	1.30	0.45	3	1.50	0.63	2	1.12	0.22	3	1.62	0.70
c29169gC	3	2.53	1.01	3	2.10	0.89	3	2.57	1.00	3	2.41	0.97
c9302gC	2	1.06	0.13	1	1.00	0.00	2	1.41	0.47	2	1.14	0.24
c9589gC1	2	1.41	0.47	2	1.53	0.53	2	1.93	0.68	2	1.63	0.57
c9711gC0	2	1.66	0.59	2	1.11	0.21	2	1.44	0.48	2	1.56	0.54
c9818gC0	2	1.47	0.50	2	1.63	0.58	2	1.66	0.59	2	1.98	0.69
c27353gC1	2	1.21	0.32	2	1.94	0.68	2	1.17	0.27	2	1.93	0.67
c25883gC0	2	1.06	0.14	2	1.26	0.36	2	1.20	0.30	2	1.25	0.35
Mean	2.3913	1.70	0.59	2.2609	1.67	0.56	2.1304	1.64	0.53	2.3043	1.71	0.60
SD	0.7223	0.45	0.25	0.7518	0.44	0.23	0.5481	0.45	0.27	0.5588	0.36	0.20

Na = Number of observed alleles, Ne = Number of effective alleles and I = Shannon's index and SD = Standard Deviation

found to be lower than expected heterozygosity for all Mizoram, Nagaland, Manipur and Meghalaya germplasms. This level of heterozygosity in ricebean may be because it is predominantly a self-pollinated crop in nature like other legumes. The results on the level of heterozygosity in ricebean are supported by Wang et al. (2004) who worked on Adzuki bean. Although, at the same locus (CEDG044), accessions from respective sources showed greater observed heterozygosity than expected heterozygosity due to segregation occurs in this specific locus. The proportion of polymorphic products (a product considered polymorphic when it is absent in at least 5% of the accessions) varied from 100% in Mizoram and Meghalaya germplasm 95.65% in Nagaland and Manipur germplasm (Table 4). The locus c9302gC was found to be monomorphic for Nagaland germplasm, while locus CEDC016 was monomorphic for Manipur germplasm. Out of 59 alleles, 52 alleles were found common to all regions, whereas seven alleles were unique to the various areas where the germplasm was procured.

Cluster analysis and population structure

The mean value of 23 quantitative characters was shown with the cluster in Table 5. Among the clusters, cluster I genotypes were the highest yield but took the longest day of maturity and followed by cluster III. Genotypes were characterized by relatively early maturing and the highest number of seed per plant compared to clusters I and II genotypes. Larger seed size and larger pod size were found in cluster I genotypes which can be selected for fresh vegetable purpose. The three distinct clusters, Cluster I, II and III comprised 8 (6.67%), 47 (39.16%) and 65 (54.16%) lines, respectively. Most of the similar-source genotypes tend to form a single cluster, i.e., most Manipur lines are distributed in cluster I, and Mizoram lines are in cluster II. Also, a Dendrogram was created using (UPGMA) Unweighted Pair Group Method with Arithmetic Mean Algorithm. Genotypes were classified into three major clusters (Fig. 1) from SSR data. Cluster I comprised 37 (30.83%) accessions, while cluster III has 71 (59.16%) accession, and the rest of the 12 (10%) accessions were present in cluster II.

Table 5. Mean and range analysis in the 3 major clusters for 23 morphological traits of ricebean

S. no.	Characters	Statistical parameters	Cluster I	Cluster II	Cluster III
1.	Plant height (cm) at 50% flowering	Mean	75.0	94.4	99.3
		Range	33.0–151.7	47.7–132.7	35.7–140.6
2.	Plant height (cm) at maturity	Mean	86.7	112.2	114.1
		Range	36.7–169.7	75.3–150.0	41.0–164.7
3.	Days to 50% flowering	Mean	52.3	56.5	56.6
		Range	52.3–72.7	53.3–63.3	44.0–67.7
4.	Days to 80% maturity	Mean	123.1	116.4	116.0
		Range	106.7-137.7	106.3–141.0	103.0–139.0
5.	Terminal leaf length (cm)	Mean	4.9	4.7	4.9
		Range	2.0–6.7	3.2–6.4	3.0–17.2
6.	Terminal leaf width(cm)	Mean	3.0	2.5	2.3
		Range	1.6–5.3	1.5–4.3	1.1–4.3
7.	Petiole length of terminal leaf (cm)	Mean	3.8	2.9	2.7
		Range	1.5–10.2	1.5–4.8	1.2-5.0
8.	Leaf length (cm)	Mean	9.7	10.5	10.3
		Range	3.8–12.6	8.7–14.9	8.0–13.4
9.	Leaf width(cm)	Mean	7.1	7.1	6.9
		Range	2.3–8.9	3.8–8.4	5.2-8.8
10.	Petiole length of leaf (cm)	Mean	17.2	16.8	17.3
		Range	5.3–25.2	10.8–21.4	8.6–24.2
11.	Primary branch length (cm)	Mean	48.3	56.8	63.8
		Range	10.3–110.7	25.7–75.3	25.3–107.3
12.	No. of clusters/plant	Mean	14.2	13.6	13.6
		Range	4.0–25.0	7.0–21.7	5.3–21.7
13.	No. of pods/plant	Mean	40.1	39.6	39.5
		Range	10.7-73.7	16.7–75.0	11.7–75.0
14.	No. of branches	Mean	2.9	2.9	2.9
		Range	1.0-4.0	2.0–3.7	1.0–4.7
15.	Peduncle length (cm)	Mean	9.7	9.9	10.3
		Range	4.8-14.7	7.7–12.2	6.3–16.6
16.	No. of seeds/pod	Mean	6.4	7.6	7.7
		Range	3.0-9.7	4.0–8.7	3.7–9.7
17.	No. of seed/plant	Mean	217.5	263.8	295.1
		Range	18.3–545.0	58.0–527.0	38.0–816.7
18.	100 Seed weight (g)	Mean	18.7	12.1	10.9
		Range	6.7–52.4	7.4–24.3	6.5–42.0
19.	Pod length (cm)	Mean	10.5	10.0	9.8
		Range	8.7–13.0	8.3–11.2	7.0–12.5
20.	Pod width (cm)	Mean	0.8	0.7	0.6
		Range	0.5–1.3	0.5–1.0	0.5–1.2
21.	Seed length (cm)	Mean	0.9	0.8	0.8
		Range	0.7–1.3	0.7–1.1	0.5–1.2
22.	Seed width (cm)	Mean	0.5	0.5	0.5
		Range	0.3–0.8	0.4–0.7	0.3–0.8
23.	Yield/ plant (g)	Mean	33.0	27.2	27.8
		Range	3.7–80.5	6.1–49.3	2.1–67.9

Here, the clustering pattern did not show any relationship between geographic distribution and genotypic diversity as some genotypes of different geographic origin were also grouped in the same cluster. The present findings are also supported by Bisht et al. (2005) and Wang et al. (2015), where genotypes' clustering did not show distinct cluster according to their geographic distribution. These results indicated that genetic drift and selection, in a different environment, could cause greater diversity than geographic distances. Chen et al. (2016) reported the opposite result was explaining the ricebean germplasm of different geographical origins clustered together. Contrasting results were also reported in chickpea (Jha et al. 2021) who evidenced close evolutionary relationship among the genotypes from different geographical regions based on molecular analysis. The geographical distribution of accessions per cluster was shown in Table 6.

Cluster III has wider geographic distribution than others, occurring genotypes from different NE areas (Manipur, Meghalaya, Mizoram and Nagaland). A Mantel's correlation value between cophenetic matrix value and genetic distances was observed as 0.82. Clustering of genotypes into sub-populations is becoming more common for the identification of genetically similar groups of accessions in many fields of genetic studies (Greenbaum et al 2016). STRUCTURE software was also used to construct Model-

Table 6. Geographical distribution of accessions per cluster

S. no.	Source of accession	No. of accession	Cluster I	Cluster II	Cluster III
1.	Manipur	34	23 (62.16%)	2 (16.67%)	9 (12.68%)
2.	Meghalaya	32	8 (10.81%)	5 (41.67%)	19 (26.76%)
3.	Mizoram	35	4 (10.81%)	3 (25.0%)	28 (39.44%)
4.	Nagaland	19	2 (5.40%)	2 (16.67%)	15 (21.13%)

Table 7. Ewens-Watterson Test for Neutrality for overall allele frequencies. Observed homozygosity (F) is calculated using 1000 simulated sample

Locus	Number of alleles (k)	Obs. F	L95	U95
CEDG008	2	0.501	0.504	0.991
CEDG015	3	0.355	0.395	0.982
CEDG021	2	0.558	0.501	0.991
CEDG024	3	0.821	0.382	0.982
CEDG026	2	0.500	0.503	0.991
CEDG029	2	0.512	0.503	0.991
CEDG041	2	0.505	0.501	0.990
CEDG043	3	0.823	0.376	0.982
CEDC016	3	0.616	0.383	0.982
CEDG073	5	0.555	0.282	0.910
CEDG044	3	0.497	0.380	0.983
c19149gC0	2	0.608	0.503	0.991
c19719gC0	2	0.512	0.505	0.991
c21449gC0	2	0.603	0.503	0.991
c28613gC0	2	0.666	0.502	0.992
c28852gC0	3	0.741	0.389	0.983
c29169gC	3	0.380	0.394	0.975
c9302gC	2	0.860	0.504	0.992
c9589gC1	2	0.605	0.503	0.991
c9711gC0	2	0.678	0.503	0.991
c9818gC0	2	0.512	0.503	0.992
c27353gC1	2	0.651	0.502	0.990
c25883gC0	2	0.849	0.503	0.991

Obs. F = Observed sum of squared of allelic frequency and L95 and U95 = Lower and Upper limit of 95% confidence region of expected F value of 23 SSR loci

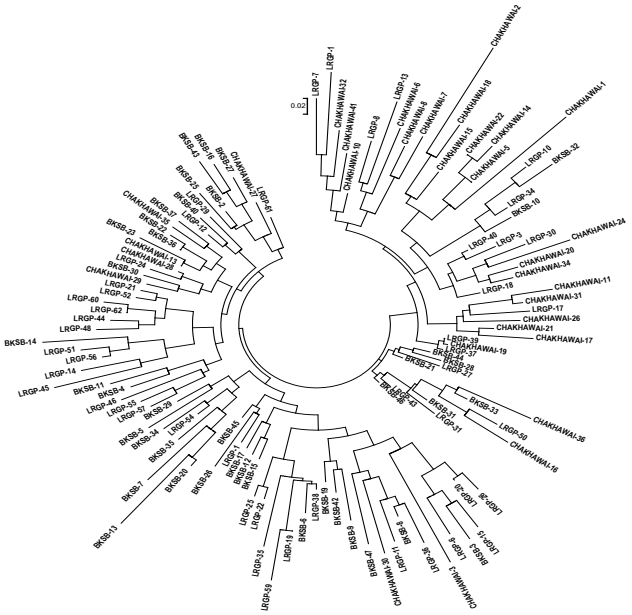

Fig. 1. UPGMA cluster dendrogram based on SSR profiles showing genetic relatedness among 120 ricebean genotypes

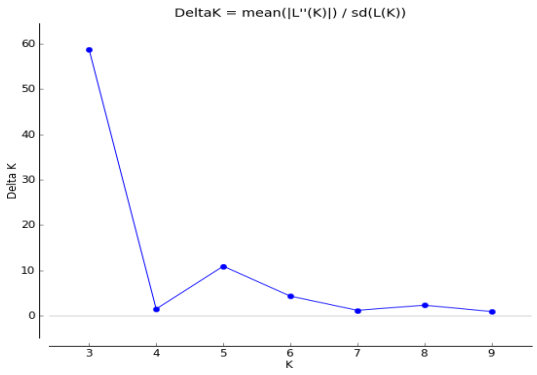

Fig. 2. Estimation of population in ricebean accessions showing K value

Based Cluster, and number of pure and admixture individuals were identified. In 10 replicated runs for different K value ranging from 2 to 9 (presumed number of populations) based on the distribution of 59 different alleles at 23 SSR loci among 120 individuals were used for analysis. The most appropriate K value was identified at K = 3. It corresponds

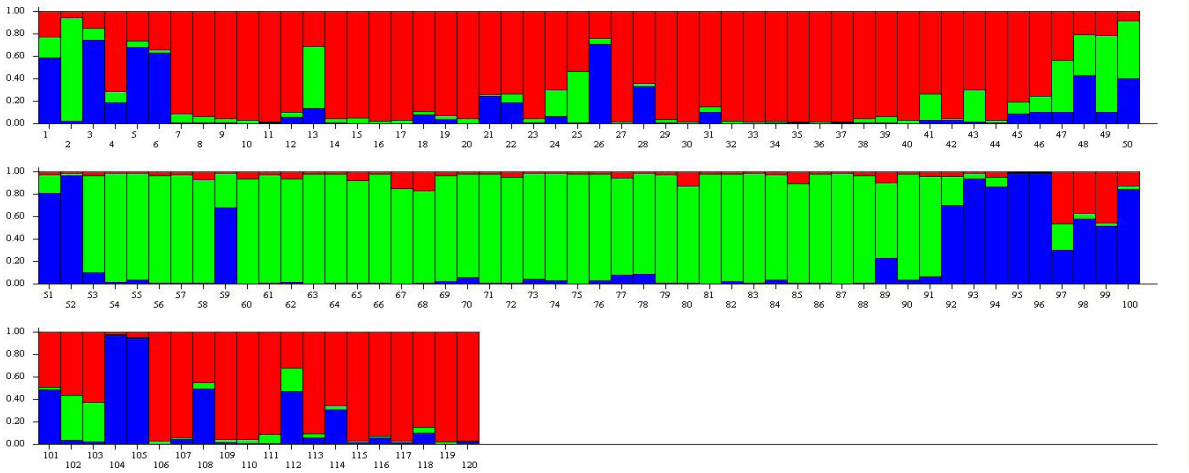

Fig. 3. Model-based cluster analysis as calculated by Structure. Each vertical represents one genotypes, length of colour fragment shows accession's estimated proportion of membership in that cluster

with the highest ΔK value (Fig. 2), indicating three genetic clusters, which was similar to UPGMA clustering with minor deviation. The presence of 30 admixture in the population (Fig. 3) suggested that the collected germplasms were ancestrally admixed to some extent.

Ewens-Watterson neutrality test compares the expected homozygosity with observed homozygosity. It was performed based on the pairwise genetic versus geographic distribution of the genotypes (Table 7). The loci will be neutral to selection pressure if the observed F-value is within the lower and upper 95% limit. Except for CEDG026 and c29169gC, all other loci were neutral to selection pressure. This may be understood as the SSR markers are present in conceding region of the genome, which is not much affected by selection pressure.

The SSR marker systems can be effectively used to look at the pattern of genetic variation and population structure among closely related species or individuals from the same species. It could be concluded that ricebean accessions collected from different North-Eastern states of India showed high genetic diversity and grouped into three distinct clusters regardless of their geographical distribution.

Authors' Contribution

Conceptualization of research (AP); Designing of the experiments (AP, MR, WT); Contribution of experimental materials

(AP); Execution of field/lab experiments and data collection (YSD, CA); Analysis of data and interpretation (AP, AK, YSD); Preparation of the manuscript (AP, YSD).

Acknowledgment

We sincerely acknowledge the Director, ICAR-RC for NEH Region, Umiam, Meghalaya 793 103 for providing the financial support (Project code: 1XX08769) and the facilities to carry out this research work.

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