Indian Journal of Biochemistry & Biophysics
Vol. 58, February 2021, pp. 91-99

Revealing genetic variation in mini core germplasm of urdbean (*Vigna mungo* (L.) Hepper)

B Baisakh[1], Swapan K Tripathy[2]*, Jagadeesan Souframanien[3], Digbijaya Swain[1] & Preeti Tripathy[1]

[1]Department of Plant Breeding and Genetics; & [2]Department of Agricultural Biotechnology, College of Agriculture, Odisha University of Agriculture & Technology, Bhubaneswar-751 003, Odisha, India

[3]Nuclear Agriculture & Biotechnology Division, Bhabha Atomic Research Centre, Mumbai-400 085, Maharashtra, India

Received 06 January 2020; revised 21 August 2020

A set of 47 urdbean genotypes including 12 promising varieties and 34 popular land races and a popular OUAT variety "Ujala" (standard check) were characterized for genetic variation based on molecular markers and morpho-economic traits. The molecular marker- based genotyping revealed a tremendous higher level of polymorphism (97.05%) with high average PIC (polymorphic information content) value (0.75). ISSR 1357 was considered highly informative that revealed the highest PIC (0.87) and marker index value (MI:5.25). An 840 bp allele (band) was characteristic to Kantapada local - A, Kendrapada local-D, and Nayagarh local - C. Such genotype- specific finger-printing may serve for reliable varietal characterization and elimination of duplicates. The test genotypes were grouped into six distinct clusters. TU 10-13, LBG 623, TAU 1, OBG 33, LBG 17 and PU 31 were highly divergent. PU 31 had inherent high yield potential (>5.0q/ha) with known YMV resistance. The above divergent high yielding test genotypes may serve as candidate varieties for further genetic improvement using recombination breeding.

Keywords: Genetic variation, Mini core germplasm, Molecular markers, Morpho-economic traits, Urdbean (*Vigna mungo* (L.) Hepper)

Urdbean (*Vigna mungo* (L.) Hepper) is an important legume crop of rainfed agriculture among Asian countries[1]. It is a good source of calorific value (341 Kcal), dietary fibre (18.3 g), calcium (138 mg), phosphorous (379 mg), iron (7.57 mg), easily digestible protein (25.21 g), essential amino acids and vitamins per 100 g of seeds (nutrition-and-you.com/black-gram.html). High values of lysine make urdbean an excellent complement to rice in terms of balanced human nutrition. The crop withstands adverse climatic conditions and improves the soil fertility by fixing atmospheric nitrogen (22.10 Kg N/ha) through root nodules[2]. Besides, it is also used as nutritive fodder for milch animals. But, urdbean continues to be a slow runner in productivity (585 kg/ha) in India (vikaspedia.in/agriculture/crop) owing to the usual practice of minimal cultivation in marginal and sub-marginal lands, sensitivity to biotic (YMV, Cercospora leaf spots, powdery mildew, root rot, and fruit fly) and abiotic stresses (drought, salinity and water logging), and insufficient irrigation facility. Germplasm is an important source of valuable genes. But, the crop is at high risk of genetic erosion[3] because of the high selection pressure for the desired trait (seed yield) and ignoring the other economically less important traits. Therefore, genotypic characterization is essential to search for diverse parents in hybridization programme to assemble favourable genes in a single genotype. Morphological characterization of germplasm collections is an important step in this regard. Many often biochemical and molecular markers are sought to elucidate the extent of genetic variation. In this regard, restriction fragment length polymorphism (RFLP), DNA amplification fingerprinting (DAF), amplified fragment length polymorphism (AFLP), Inter simple sequence repeats (ISSR), simple

*Correspondence:
Phone: +91-9937416867 (Mob)
E-mail: swapankumartripathy@gmail.com

Abbreviations: EDTA, Ethylenediaminetetraacetic acid; CTAB, Cetyl trimethylammonium bromide; NaCl, Sodium chloride; β-ME, Beta mercaptoethanol; PCR, Polymeric chain reaction; RFLP, Restriction fragment length polymorphism, RAPD, Random amplified polymorphic DNA; DAF, DNA amplification fingerprinting, AFLP, amplified fragment length polymorphism, ISSR, Inter simple sequence repeats, SSR, simple sequence repeats, STS, sequence tagged sites; Polymorphism information content (PIC) and Resolving power (Rp)

sequence repeats (SSR), sequence- tagged sites (STS) and random amplified polymorphic DNA (RAPD) serve as excellent tools for the characterization of germplasm lines. In the present investigation, an attempt has been taken to study genetic variation and to critically examine genetic diversity among a set of selected urdbean genotypes using molecular markers.

Materials and Methods

Experimental materials

The experimental materials comprised of 47 genetically pure genotypes (mini core germplasm) including 12 promising varieties and 34 popular locally adapted landraces of Odisha (India) and a popular OUAT urdbean variety "Ujala" (standard check). The test genotypes were grown in the field following Randomized Block Design (RBD) with three replications over four years (2014-15 to 2017-18) to assess yield and ancillary traits during Winter season at Department of Plant Breeding and Genetics, College of Agriculture, Odisha University of Agriculture and Technology, Bhubaneswar (INDIA). Standard statistical analysis was followed for agro-morphological traits for analysis of variance as per SPAR 2.0 (http://www.iasri.res.in/spar).

Isolation of genomic DNA

Genomic DNA was isolated from two gm. tender young leaves of aseptically grown seedlings on the same day of collection using standard CTAB (cetyl trimethyl ammonium bromide) method with minor modification[4]. The contaminating RNA was removed by treatment with 6 µL RNase (10 mg/mL) for each ml of DNA extract followed by incubation in a water bath at 37°C for 1 h and then the addition of an equal volume of chloroform: isoamyl alcohol (24:1). The content was centrifuged for 10 min at 10000 rpm and the upper aqueous phase was pipetted out. Starting from the addition of chloroform- isoamyl alcohol, the entire process was repeated twice. After the final centrifugation, $1/10^{th}$ volume of 3 M sodium acetate, pH 4.8 was mixed to the upper aqueous phase- separated to a fresh centrifuge tube. DNA was precipitated by adding 2.5 volumes of chilled iso-propylalcohol and pellated by spinning. The DNA pellet was washed twice with 70% ethanol and dried under vacuum. The dried DNA was dissolved in TE buffer (10mM Tris-HCl, pH 8.0 and 1mM EDTA) and stored in a deep freezer at −20°C for future use.

Test for quality and quantity of the purified DNA

The amount of DNA was determined through the UV-VIS spectrophotometer. The absorbance at 260 nm wavelength gave the quantity of the total DNA, and the ratio of the absorbance at 260 nm and 280 nm indicated the quality of the purified DNA. The quantification was done in comparison with the known standard. After quantification, the DNA was diluted in TE buffer to a working concentration of 25 ng/µL for PCR analysis. The DNA was also loaded in 0.8% agarose gel alongside diluted uncut lambda DNA as standard to recheck the quality and quantity of DNA.

DNA amplification and agarose gel electrophoresis

Nine SSR, one ISSR, two SCAR, and one RGA primer were used for amplification of genomic DNA. The amplification was performed in a reaction volume 25 µL containing 1X reaction buffer (10 mM Tris HCl, pH 9.0, 1.5 mM $MgCl_2$, 50 mM KCl, 0.01% gelatin), 2.5 mM each of dNTPs, 10 ng of each primer, 50ng of genomic DNA and 1 unit of Taq polymerase (Genei, Bangalore). DNA amplification was performed in the Gene-Pro Thermocycler (Bioer Tech. Co., Ltd, Japan), programmed for 5 min at 95°C, 40 cycles of 1 min at 94°C, 30 sec at respective annealing temperature for each primer and 2 min at 72°C and final extension for 7 min at 72°C followed by storing at 4°C till loading to the agarose gel. The amplified products were loaded in 2% agarose gel containing 1 µg/mL ethidium bromide and electrophoresed in a constant voltage at 50 V. The amplifications were checked for their reproducibility.

The gels were documented by gel doc system (Fire Reader-Uvtec, Cambridge, UK) for scoring the bands. The sizes of the amplicons was determined by comparing with the lambda DNA ladder (500 bp) with known size fragments. Gels were scored for the presence (1) or absence (0) of bands. Polymorphism information content (PIC) and resolving power (Rp) of each primer was estimated as per Prevost and Wilkinson[5]. The marker index for each primer was calculated as the product of PIC and number of polymorphic bands. The binary data matrix of the Unweighted Paired 1/0 score was analysed to estimate Jaccard's similarity coefficient values[6]. The dendrogram was constructed using the Unweighted Paired Group Method with Arithmetic Averages (UPGMA) employing Sequential agglomerative Hierarchic and Non-overlapping Clustering (SAHN).

Results and Discussion

Genetic variation in days to flowering and days to maturity was narrow. However, plant height, branches/plant, fruiting clusters/plant, pods per plant, pod length, and seeds per pod had shown appreciable genetic variation. Similarly, Ozukum and Sharma[7] revealed sufficient genetic variability to justify selection for genetic improvement in this crop. Among these, branches per plant, clusters per plant, and pod number per plant recorded a wider range indicating enough scope for selection of test genotypes based on such component traits. Among the test genotypes, PU 30 followed by Kenderapada local B bore more branches per plant (Table 1). LBG 17 recorded the highest number of pods/ clusters (8.2) followed by PU 30, Soroda local and Kenerapada local B. Pods per plant, seeds per pod, and 100-seed weight reflect direct bearing on seed yield. The number of pods per plant was shown to be very high in PU 30(29.56) and PU 31(29.03). PU 31, TU 94-2, TU 40, PU 30, and among local land races; Bawanipatna local and Keonjhar local bore bold seeds. The superiority of PU 31 for seed yield and yield attributes was also reported by Kumar *et al*[8]. Variation for seeds/pod was shown to be negligible and hence, selection for the trait may not be effective.

Seed yield ranged from 1.70 to 5.27q/ha. Among the test genotypes tested over four years; PU 31 and PU 30 performed better with seed yield performance to the tune of more than 5.0 q/ha. Besides, PU 40, PU 19, TU 94-2, and B 3-8-8 had shown high yield potential as compared to the check variety "Ujala"(4.57 q/ha). Among local landraces, only two test genotypes *e.g*., Keonjar local (Pejua Biri) and Kenderapada local -A managed to have yield performance (4.0 q/ha) at par with "Ujala".

The success in generating a wide range of polymorphic loci depends on the proper choice of primers for DNA amplification. The optimum number of primers required to differentiate two or more cultivars may vary with the test materials used. When the variation in the cultivars is high the use of few primers can serve the purpose of generating useful information. Hu and Quaros[9] revealed that 2-3 primers were sufficient to distinguish between cultivars of broccoli. However, more primers needs to be involved in a case where there is low germplasm diversity. In the present investigation, twenty- five primers were screened for the detection of amplified products and identification of polymorphic profile. Finally, only thirteen primers were selected for genomic DNA amplification of 47 test genotypes of urdbean (Table 1). The list of such informative primers used in this investigation is presented in (Table 2). In the present investigation, bands with similar mobility to those detected in the negative control if any, were not scored. The PCR amplification with the present set of primers resulted in total of 68 amplicons. The number of bands per primer ranged from 4-6 with an average of 5.23 bands. Primer CEDG 245, CP 24, and ISSR 1357 produced the maximum number of polymorphic amplicons (six each) (Fig. 1B & C). However, Kaewwongwal *et al.* (2015)[10] detected 9.95 alleles (bands) per SSR locus in this crop. In the present investigation, fragment size variation was maximum (250-2600 bp) in the case of CEDG 013 followed by CEDG 245(90-2100 bp), while AB 6 revealed narrow range of fragments. All primers revealed 100% polymorphic alleles(bands) totalling 66 bands except the primer CEDG 013 and CEDG 067 which produced one monomorphic band each (Fig. 1A). Thus, the present set of materials revealed a tremendous higher level of polymorphism (97.05%). In contrast, Souframanien and Gopalakrishna[11] reported 57.4% and 42.7% polymorphism in this crop using 16 ISSR and 25 random (RAPD) primers, respectively. The level of polymorphism obtained in the present investigation may be ascribed to higher genetic variation in the selected 47 test genotypes. Tondonba *et al.* (2018)[12] studied genetic variation using 30 SSR markers that revealed an overall lower level of polymorphism (32.97%). However, Das *et al.* (2014)[13] reported a moderately higher level of polymorphism (65.05%) in this crop using 10 ISSR markers.

The presence or absence of a specific band is the inherent status of each genotype. A 90 bp band was specific to Ranipeta local (V28). Two distinctly conspicuous bands at 800 and 840 bp were produced by CEDG 067. The former amplicon was present in 23 varieties while the 840 bp band was characteristic to Kantapada local - A (V13), Kendrapada local-D (V20), and Nayagarh local - C (V26) only. Similarly, no amplicon was produced in PU 31 (V40), OBG 33 (V42), TAU 1(V43), LBG 17(V46) and LBG 623 (V47) by AB6; Bhawanipatna Local (V1) and Badamba local – 2(V7) by CEDG 180; Berhampur local (V4), Banki local (V6), Kothagarh local (V16), Mohana local A(V21), PU 40 (V39), TAU 1 (V43), LBG 623 (V47); Badamba local-2 (V7), Dayapali local

Table 1 — Performance of 47 urdbean genotypes pooled over four years for seed yield and ancillary traits (*Contd.*)

Sl. No.	Variety Name	DF (days)	DM (days)	PHT (cm)	BP	CP	PP	PL (cm)	SP	SW (g)	SY (q/ha)
1	Bhawanipatna Local	37.06	77.5	19.23	1.91	4.66	17.68	4.76	6.7	4.35	3.74
2	Bilipara Local	38.00	76.5	21.92	2.40	5.27	19.98	4.39	6.18	3.68	3.41
3	Badamba local - 1	36.36	78.5	19.13	2.51	3.48	14.27	4.68	6.16	3.34	2.56
4	Berhampur local	36.12	79.0	20.7	2.76	4.28	18.33	4.76	6.23	3.80	3.32
5	Badachana local	40.76	79.5	18.51	2.23	3.40	16.53	4.29	5.56	3.32	2.81
6	Banki local	39.87	78.5	20.61	2.17	4.65	15.86	4.41	5.90	3.45	3.29
7	Badamba local - 2	40.45	76.0	18.06	2.12	3.78	14.30	4.15	5.78	3.31	2.89
8	Balangir local	37.61	78.0	15.42	2.42	3.47	14.36	4.27	6.05	3.81	2.83
9	Cheripali local	38.05	78.0	18.67	2.57	4.51	17.95	4.44	5.95	3.57	3.25
10	Dayapali local	37.82	76.5	19.97	2.06	4.95	17.71	4.40	6.28	3.42	3.30
11	Deogaon local	37.42	78.5	17.50	2.11	4.41	15.86	4.17	6.26	2.99	2.88
12	Keonjhar local	38.80	77.0	17.95	2.13	4.95	21.12	4.82	6.60	4.62	4.00
13	Kantapada local - A	38.88	77.0	18.26	2.47	4.76	16.06	4.84	6.27	3.87	3.18
14	kantapada local - B	38.87	78.0	18.98	2.11	3.67	14.16	4.39	5.90	3.51	2.89
15	Aska local	38.87	76.0	16.56	1.78	3.28	13.98	4.73	6.31	2.97	2.60
16	Kothagarh local	37.82	77.0	15.00	1.51	4.65	18.30	4.46	5.92	3.26	3.02
17	Kendrapada local - A	38.67	77.0	24.26	2.15	5.43	22.20	4.42	6.36	3.92	3.83
18	Kendrapada local - B	38.42	78.5	20.96	4.26	7.63	20.41	4.71	6.43	3.71	4.05
19	Kendrapada local - C	39.50	79.0	18.35	2.05	5.13	17.06	4.11	5.82	3.29	3.14
20	Kendrapada local - D	40.50	77.0	24.57	3.06	4.82	21.43	4.38	6.37	3.44	3.52
21	Mohana local - A	39.43	77.0	16.46	1.83	3.76	13.30	4.58	6.01	3.56	2.81
22	Mohana local - B	38.45	77.0	18.77	2.22	6.25	23.68	4.61	6.36	3.85	3.35
23	Mahimunda local	38.12	78.5	18.38	2.83	4.93	23.56	4.61	6.65	3.42	3.54
24	Nayagarh local - A	38.05	78.0	21.03	2.47	4.81	25.41	4.35	6.05	3.43	3.47
25	Nayagarh local - B	36.92	77.0	21.53	2.61	5.37	19.87	4.78	6.57	3.25	3.02
26	Nayagarh local - C	38.68	76.0	21.26	2.62	4.45	17.82	4.33	6.23	3.33	2.77
27	Pendibari local	37.75	78.0	21.05	3.26	4.63	20.03	4.48	6.71	3.43	3.20
28	Ranipeta local	36.57	77.5	22.31	2.95	5.57	20.05	4.50	6.28	3.64	3.57
29	Similiguda local	37.82	78.0	20.32	2.83	6.41	22.82	4.41	6.31	3.51	3.33
30	Soroda local	37.62	77.5	27.13	2.48	7.86	21.15	4.79	6.38	4.35	3.65
31	Sudhasarangi local	38.05	76.5	22.68	2.47	5.57	18.48	4.67	6.41	3.56	3.40
32	Subarnagiri local	37.87	76.0	19.21	1.88	4.87	20.18	4.69	6.17	3.82	3.30
33	Sheragarh Local	38.12	77.0	21.27	2.35	4.28	19.95	4.54	6.47	3.76	3.13
34	Tigiria local	39.05	76.5	23.08	2.88	4.51	21.11	4.61	6.13	3.65	3.29
35	Ujala (Check)	37.55	77.0	22.08	2.57	6.88	25.46	4.99	6.82	4.10	4.57
36	PU 30	38.25	78.5	24.80	4.47	7.83	29.56	4.88	6.41	4.58	5.10
37	B 3-8-8	37.18	79.0	23.33	2.25	6.38	23.28	4.97	6.33	4.32	4.78
38	PU 19	37.07	78.5	21.18	2.52	5.36	22.83	4.66	6.15	4.57	4.84
39	PU 40	37.20	76.0	22.41	2.63	5.73	26.31	4.73	6.40	4.26	4.96
40	PU 31	37.82	78.5	22.10	2.41	7.02	29.03	4.93	6.48	4.84	5.27
41	Mahuri	37.92	79.0	20.33	2.56	6.11	21.03	4.84	6.47	3.95	4.19
42	OBG 33	37.06	77.0	20.21	1.88	4.26	17.67	4.43	5.90	3.70	3.92
43	TAU 1	37.00	76.5	23.10	1.50	2.50	7.40	5.35	6.90	3.70	1.70

(*Contd.*)

Table 1 — Performance of 47 urdbean genotypes pooled over four years for seed yield and ancillary traits

Sl. No.	Variety Name	DF (days)	DM (days)	PHT (cm)	BP	CP	PP	PL (cm)	SP	SW (g)	SY (q/ha)
44	TU 10-13	36.00	76.5	25.90	3.00	7.40	25.00	4.90	6.70	4.03	4.20
45	TU 94-2	36.00	77.5	23.40	2.20	5.40	26.30	4.65	6.20	4.80	4.82
46	LBG 17	36.50	76.5	26.30	2.20	8.20	27.20	5.00	6.90	4.27	3.90
47	LBG 623	37.50	76.0	23.10	2.20	7.50	22.30	4.85	6.60	3.88	3.47
Mean		37.99	77.4	20.79	2.44	5.21	19.96	4.61	6.30	3.77	3.53
Range		36.00-40.76	76.0-79.5	15.00-27.13	1.50-4.47	2.50-8.20	7.40-29.56	4.11-5.35	5.56-6.90	2.97-4.84	1.70-5.27
$CD_{0.05}$		1.08	1.61	6.1	0.9	2.6	7.5	0.9	0.6	0.62	1.08

Note: DF -Days to 50% flowering, DM -Days to maturity, PHT - Plant height (cm.), BP -No. of Branches/plant, CP -No. of Clusters/plant, PP -No. of Pods/ plant, PL -Pod length(cm.),SP -No. of seeds/ pod, SW -100- seed weight (g), SY -Seed yield/ Plant (g)

Table 2 — Primers used for genomic DNA amplification in 47 urdbean genotypes

Primer Code	Primer type	Sequence(3′-5′)	Tm (°C)	Bands scored			PIC	Resolving power (Rp)	Marker Index (MI)	Fragment Size (bp)
				Monomorphic	PolyMorphic	Total				
AB6	SSR	F:AATTGCTCTCGAACCAGCTC R:GGTGTACAAGTGTGTGCAAG	55	0	5	5	0.72	4.42	3.60	170-490
CEDG 013	SSR	CGTTCGAGTTTCTTCGATCG ACCATCCATCCATTCGCATC	54	1	5	6	0.82	3.02	4.11	250-2600
CEDG 014	SSR	GCTTGCATCACCCATGATTC AAGTGATACGGTCTGGTTCC	58	0	4	4	0.71	3.45	2.85	120-610
CEDG 020	SSR	TATCCATACCCAGCTCAAGG GCCATACCAAGAAAGAGG	56	0	5	5	0.65	5.23	3.27	130-510
CEDG 067	SSR	AGACTAAGTTACTTGGGCAACC AGTGACGGCCCGGCTCTCC	62	1	4	5	0.67	4.55	2.67	140-840
CEDG 180	SSR	GGTATGGAGCAAAACAATC GTGCGTGAAGTTGTCTTATC	55	0	5	5	0.79	3.45	4.72	230-580
CEDG 245	SSR	GATAGAGCTTAAACCCTC CTTTTGATGACAAATGCCC	53	0	6	6	0.84	3.53	4.22	90-2100
CP 24	SSR	F:TCAACAACACCTAGGAGCCA AR:ATCGTGACCTAGTGCCCACC	59	0	6	6	0.76	4.89	4.59	115-820
DMBSSR 158	SSR	F:GTGATGCACACGGTTACGGTR: GGTGACGCAGTCCATACAAATTT	57	0	5	5	0.80	3.53	4.01	230-820
MYMVR 583	SCAR	F:GTGATGCACACGGTTACGGTR: GGTGACGCAGTCCATACAAATTT	62	0	5	5	0.64	5.49	3.19	140-950
ISSR 1357	ISSR	F: GAGAGAGAGAGAGAGAC	53	0	6	6	0.87	4.00	5.25	110-680
VMYR 1	RGA	F:AGTTTATAATTCGATTGCTR:A CTACGATTCAAGACGTCCT	47	0	5	5	0.73	3.49	3.65	90-510
YMV 1	SCAR	F:GAGAGAGAGAGAGAGAC AAAGR:GAGAGAGAGAGAG AGACAGGA	65	0	5	5	0.73	5.15	3.63	90-480
		TOAL		2	66		9.75	54.21	68	
		Average		0.15	5.07		0.75	4.17	5.23	

(V10), Kantapada local - B (V14), Subarnagiri local (V32), Mahuri (V41) by ISSR 1357; and Ranipeta local (V28) by the primer VMYR 1. Such genotype specific finger-printing may serve for reliable varietal characterization and elimination of duplicates. Ganguly and Bhat (2012)[14] used SSR markers to study genetic diversity in urdbean. Chattopadhyay *et al*. (2005)[15] reported the ISSR marker system as a more

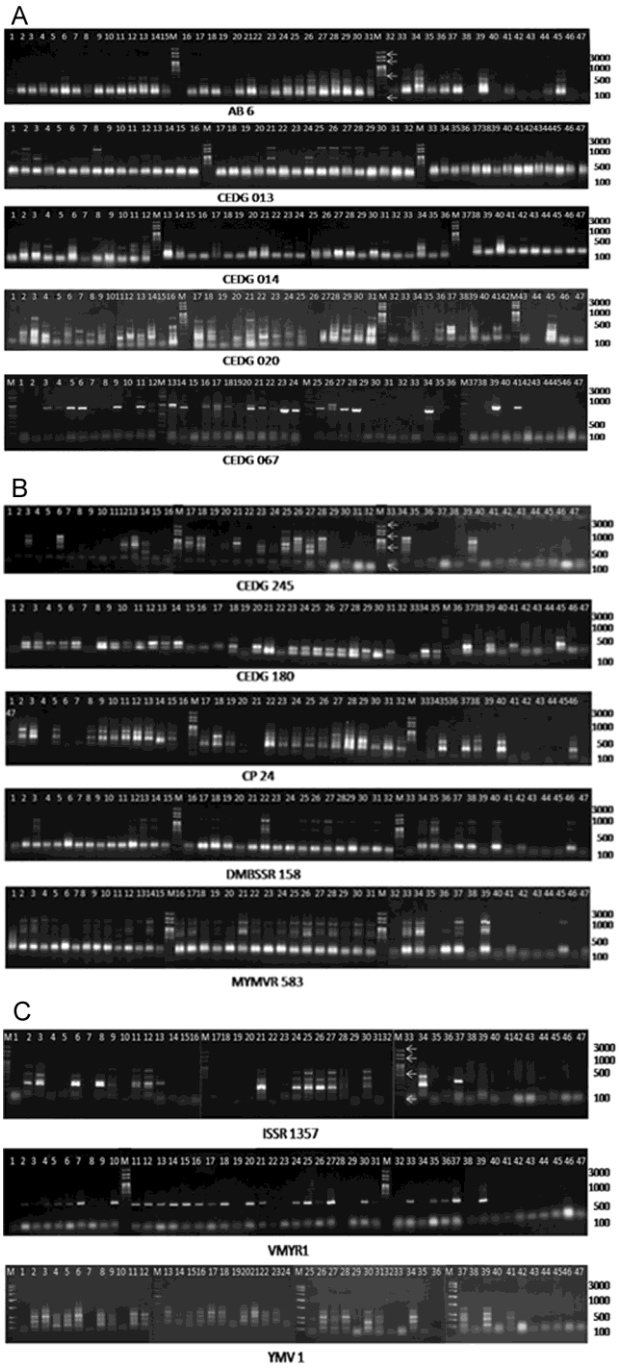

Fig. 1 — (A) DNA profiling of a set of 47 selected urdbean land races including popular improved varieties amplified by primer AB 6, CEDG 013, CEDG 014. CEDG 020 and CEDG 067; (B) CEDG 245, CEDG 180, CP 24. DM BSSR 158 and MYMVR 583; and (C) ISSR 1357, VMYR 1 and YMV 1

efficient technique than RAPD to generate polymorphic loci in this crop. In the present investigation, SSR and ISSR systems seem to be more efficient than either RAPD or SCAR markers. However, the AFLP technique is reported to be most useful in the genetic characterization of cultivars or germplasm in mung bean owing to its feasibility to fine- tune the amplification products[16]. RAPD based DNA fingerprinting has been also reported to differentiate genotypes with great success in many cereal crops including rice[17], barley[18], and pearl millet[19]. In addition, RAPD is suggested to be a potent technique for the identification of hybrids and use in marker- assisted selection.

The DNA banding pattern of each of the genotypes is expected to differ if they are genetically different. Even subtle difference at a genotypic level which other-wise some time could not be possible to differentiate by phenotyping can be confirmed by the use of markers. The DNA markers by use of most informative primers could pave the way for success. Polymorphism information content (PIC)- a measure of allelic diversity produced by a primer was estimated for all primers (Table 2). All primers revealed PIC value more than 0.5 with an average PIC value of 0.75; and the maximum value as high as ≥0.8 was recorded in the case of CEDG 013(0.82), CEDG 245(0.84), DMB SSR 158(0.80) and ISSR 1357(0.87). Further, it is worth to note that the all above primers except CEDH 013 also revealed 100% polymorphism across the test genotypes. Marker index (MI) is the primer capacity to detect polymorphic locus among different genotypes. MI values oscillated from 2.67 for CEDG 067 to 5.25 for ISSR 1357. Therefore, ISSR 1357 may be considered highly informative. Pyngrope *et al*. (2015)[20] also studied genetic diversity in urdbean using a set of CEDG primers that revealed PIC ranging 0.221-0.682 with the maximum value reported in the case of CEDG 180, CEDG 139 and CEDG 279. They sorted out three genotypes (WBB-1, VBG 09-005, and IPU 10-17) which showed a high level of genetic divergence from rest 28 urdbean genotypes.

The ability of primers to distinguish between genotypes was assessed by calculating their resolving power (Rp). Rp-value for different primers ranged from 3.02 in CEDG 013 to as high as 5.49 in MYMVR 583 with a mean value of 4.17 (Table 2). CEDG 020, MYMVR 583, and YMV 1 revealed higher resolving power (>5.0). All such primers also produced 100% polymorphism among the test genotypes indicating their immense discriminative power for varietal identification.

Similarity matrix (SI) generated by Jaccard's coefficient values (data not shown) revealed the

extent of relatedness between the test genotypes. SI ranged from 0.1304 to 0.80. The highest SI value of 0.80 was between Cheripali local (V9) and Deogarh local (V11) followed by that between Nayagarh local B (V25) and Pendibari local (V27) (0.796). Higher the inter-distance between the genotypes, the better is the scope of using them in hybridization to achieve transgressive segregates. In this context, TU 10-13(V44), LBG 623(V47), and TAU 1(V43) maintained negligible homology with the rest of the test entries. Similarity index values were used to construct dendrogram to a certain hierarchical relationships among the 47 test genotypes of urdbean. Broadly, all the test genotypes were grouped into six distinct clusters (Fig. 2). Initially, TU 10-13 (V44), LBG 623 (V47), TAU 1 (V43), OBG 33 (V42), LBG 17 (V46), PU 31(V40), and PU 19(V38) were separated from the rest of the materials (varieties) and these form two divergent clusters (Cluster I and Cluster II) at about 0.45 phenon level. Cluster I included TU 10-13 (V44), LBG 623 (V47), TAU 1 (V43) while, Cluster II clubbed OBG 33 (V42), LBG 17 (V46), PU 31 (V40) and PU 19 (V38) alongwith Ujala (V35). The rest of the genotypes were distributed into four groups. Among these, Cluster VI(V1:, V32: Subarnagiri local, V15:Aska local, V19: Kendrapada local-C, V41:Mahuri) and Cluster V(V45:TU94-2, V33: Sheragarh Local, V36: PU30 and V7: Badamba local - 2) were initially separated at about 0.45 phenon level, while the other two groups were subsequently separated at about 0.47 phenon level forming cluster III (V37:B 3-8-8, V31: Sudhasarangi local and V29: Similiguda local) and a large multi-variety Cluster IV which contained rest 27 urdbean genotypes. The genotypes included in this cluster maintained more inter se homology among themselves than with any of the members of the above five clusters. Cheripali local (V9) and Deogaon local

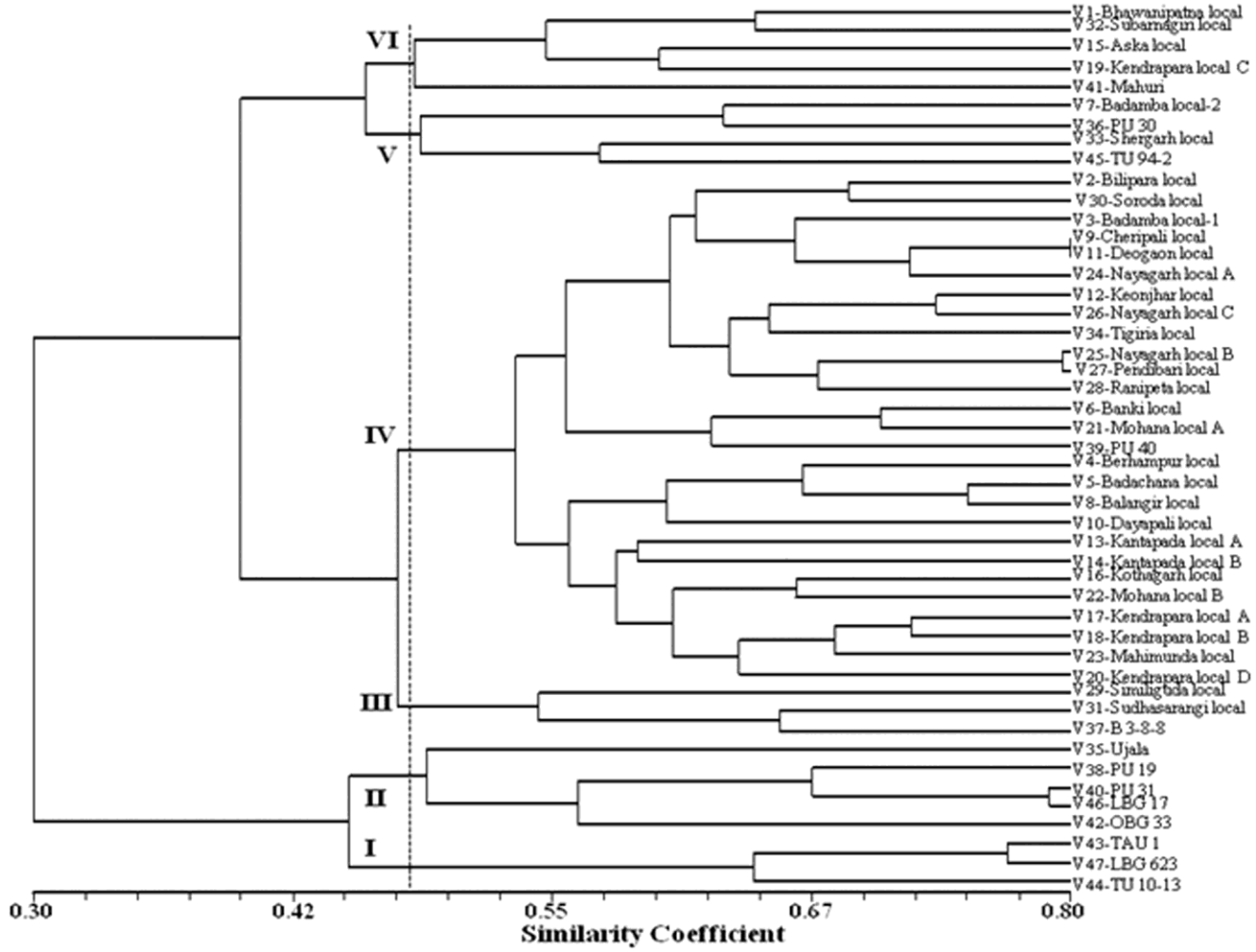

Fig. 2 — Dendrogram showing genetic diversity of 47 urdbean genotypes based on DNA profiles

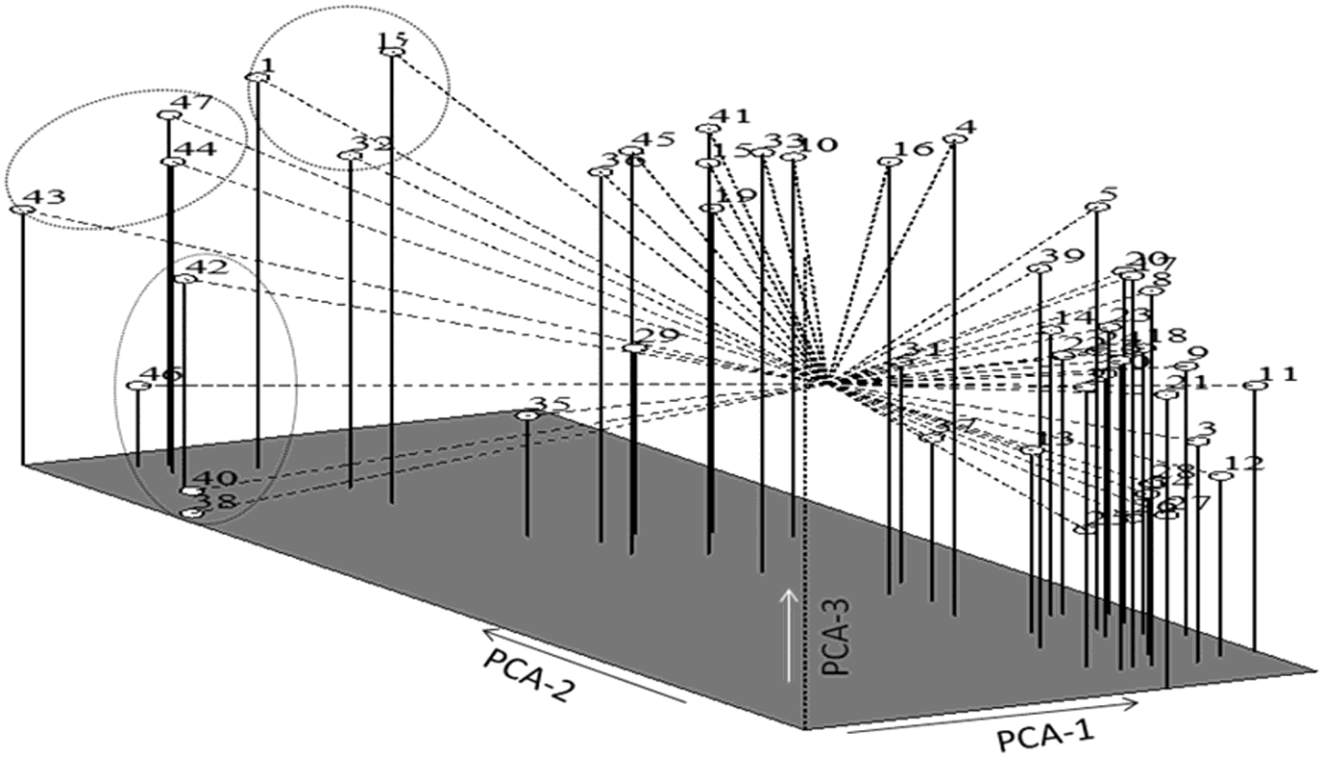

Fig. 3 — Three- dimensional representation showing genetic diversity based on polymorphism in DNA profiles of 47 urdbean genotypes.

(V11) included in the large multi-variety cluster, did not reveal any difference in DNA profiling even at 80% phenon level and therefore, recorded the highest homology (SI: 0.80). In contrast, all genotypes in Cluster I and Cluster II followed by Cluster VI and Cluster V seem to have a higher level of inherent genetic diversity. However, Ganguly and Bhat[14] revealed high homology (95% similarity) among 85 accessions of urdbean (*Vigna mungo*) indicating the narrow genetic base of the crop. Similar to urdbean, intra-specific diversity using DNA markers has been also studied in many legume crops *e.g.*, mungbean[16,21], rice bean[22], common bean[23], cowpea[24], soybean[25], and Chickpea[26-28].

The grouping of genotypes using three dimensional scalings based on PCA values (Fig. 3) was found to be more or less consistent with that of UPGMA analysis (Fig. 2). The divergent genotypes (TU 10-13, LBG 623, TAU 1, OBG 33, LBG 17 and PU 31) which were initially separated from rest of the test genotypes in case of UPGMA clustering (Fig. 2) were also seen to be screened out to diverse extreme positions in case of three- dimensional scaling with vectors (Fig. 3). PU 31 bore a higher number of pods, bold seeds, and had inherent high yield potential (>5.0 q/ha) with known YMV resistance. Besides, Keonjar local (Pejua Biri) and Kenderapada local - A with high seed yield (4.0 q/ha) need special attention. The above divergent and high yielding test genotypes may serve as valuable materials for further genetic improvement in urdbean using recombination breeding.

Conclusion

Urdbean breeding suffers a serious setback due to a narrow genetic base. The creation or presence of in situ genetic variabilites is a pre-requisite for any breeding programme or else the selection becomes ineffective. The knowledge of genetic diversity is a useful tool for efficient sampling and utilization of germplasm either by identifying and/or eliminating duplicates in the genetic stock ultimately resulting in the development of core collection of genotypes (prebreeding). Many often molecular markers are sought for the purpose owing to the paucity of morphological markers. Genotyping based clustering followed by an assessment of the merit of the genotypes based on morpho-economic traits seems to be an appropriate strategy for reliable selection of parents for hybridization. In this context, a few high yielding divergent genotypes identified in this investigation may serve as useful materials for a future breeding programmes in urdbean.

Acknowledgement

The authors highly acknowledge the financial assistance by the Board of Research in Nuclear Sciences, BARC, Trombay, India to carry out this work.

Conflict of Interest

All authors declare no conflict of interest.

References

1 Bansal R, Sharma S, Tripathi K, Gayacharan & Kumar A, Waterlogging tolerance in black gram [*Vigna mungo* (L.) Hepper] is associated with chlorophyll content and membrane integrity. *Indian J Biochem Biophys*, 56 (2019) 81.

2 Kachave RR, Indulkar BS, Vaidya PH, Ingole AJ & Patil NM, Effect of phosphorus and PSB on growth, yield and quality of blackgram (*Vigna mungo* L.) in inceptisol. *Int J Curr Microbiol Appl Sci*, 7 (2018) 3359.

3 Aftab N, Lal GM, Sheera A., Bose NC & Tripathy AM, Evaluation of genetic variability in Black gram (*Vigna mungo* (L.) Hepper) germplasm. *JPDS*, 10 (2018) 445.

4 Doyle JJ & Doyle JL, Isolation of DNA from plant tissue. *Focus*, 12 (1990) 13.

5 Prevost A & Wilkinson MJ, A new system of comparing PCR primers applied to ISSR fingerprinting of potato cultivars. *Theor Appl Genet*, 98 (1999) 107.

6 Jaccard P, Nouvelles recherches Sur la distribution florale. *Bulletin Society Vaud Science National*, 44 (1908) 223.

7 Ozukum C & Sharma MB, Variability among urdbean (*Vigna mungo* L. Hepper) for yield and yield components. *Int J Res Innov Appl Sci*, 2 (2017) 26.

8 Kumar A, Tiwari UK, Singh A, Jaiswal SK & Sahu MP, To study the performance of urdbean varieties for yield attributes and yield under rainfed condition. *Int J Pure Appl Biosci*, 6 (2018) 413.

9 Hu J & Quaros CF, Identification of broccoli and cauliflower cultivars with RAPD markers. *Plant Cell Rep*, 10 (1991) 505.

10 Kaewwongwal A, Kongjaimun A, Somta P, Chankaew S, Yimram T & Srinives P, Genetic diversity of the blackram (*Vigna mungo* (L.) Hepper) gene pool as revealed by SSR markers. *Breed Sci*, 65 (2015) 127.

11 Souframanien J & Gopalkrishna T, A comparative analysis of genetic diversity in blackgram genotypes using RAPD and ISSR markers. *Theor Appl Genet*, 109 (2004) 1687.

12 Tondonba SP, Khanna VK & Tejaswini VU, Crossability studies and genetic diversity analysis in blackgram (*Vigna mungo* L. Hepper) using molecular markers. *Agrotechnology*, 7 (2018) 179.

13 Das S, Das SS & Ghosh P, Analysis of genetic diversity in some black gram cultivars using ISSR. *Euro J Exp Bio*, 4 (2014) 30.

14 Ganguly PR & Bhat KV, Study of the pattern of variation for microsatellite markers in Black gram, *Vigna mungo* (L.) Hepper germplasm. *DHR Int J Biomed Life Sci*, 3 (2012) 168.

15 Chattopadhyay K, Ali MN, Sarkar HK, Mandal N & Bhattacharya S, Diversity analysis by RAPD and ISSR markers among the selected mungbean (*Vigna radiata* (L.) Wilczek) genotypes. *Indian J Genet Plant Breed*, 65 (2005) 173.

16 Bhat KV, Lakhanpaul S & Chadha S, Amplified fragment length polymorphism (AFLP) analysis of genetic diversity in Indian mungbean [*Vigna radiata* (L.) Wilczek] cultivars. *Indian J Biotech*, 4 (2005) 56.

17 Ko HL, Cowan DC, Henry RJ, Graham GC, Blakeney AB & Lewin LG, Random amplified polymorphic DNA analysis of Australian rice (*Oryza sativa* L.) varieties. *Euphytica*, 80 (1994) 179.

18 Fernandez ME, Fingueiras AM & Benito C, The use of ISSR and RAPD markers for detecting DNA polymorphism, genotype identification and genetic diversity among barley cultivars with known origin. *Theor Appl Genet*, 104 (2002) 845.

19 Chowdhary KV, Davierwala AP, Gupta VS, Ranjekar PK & Govila OP, Genotype identification and assessment of genetic relationships in pearl millet (*Pennisetum glaucum* (L.) R. Br) using microsatellites and RAPDs. *Theor Appl Genet*, 97 (1998) 154.

20 Pyngrope AH, Noren SK, Wricha T, Sen D, Khanna VK & Pattanayak A, Genetic diversity analysis of blackgram [*Vigna mungo* (L.) Hepper] using morphological and molecular markers. *Int J Appl Pure Sci and Agric*, 1 (2015) 104.

21 Saini M, Singh S, Hussain Z & Yadav A, RAPD analysis in mungbean(*Vigna radiate* (L.) wilczek): I. Assessment of genetic diversity. *Indian J Biotechnol*, 9 (2010) 137.

22 Muthusamy S, Kanagarajan S & Ponnusamy S, Efficiency of RAPD and ISSR marker system in accessing genetic variation of rice bean (*Vigna umbellata*) landraces, *Elect J Biotechnol*, 11 (2008) 1.

23 Galvan M Z, Bornet B, Balatti PA & Branchard M, Inter simple sequence repeat (ISSR) marker as a tool for the assessment of both genetic diversity and gene pool origin in common bean (*Phaseolus vulgaris* L.). *Euphytica*, 132 (2003) 297.

24 Fang J, Chao CT, Roberts PA & Ehlers JD, Genetic diversity of cowpea (*Vigna unguiculata* (L.) Walp.) in four west African and USA breeding programs as determined by AFLP analysis. *Gen Res Crop Evol*, 54 (2007) 1119.

25 Caetano-Anolles G & Gresshof PM, DNA amplification finger printing using arbitarily mini-hairpin oligonucleotide primers. *Biotechnology*, 12 (1994) 619.

26 Winter P, Benko-Iseppon AM, Huttel B, Ratnaparkhe M, Tullu A, Sonnante G, Ptaff T, Tekeoglu M, Santra D, Sant VJ, Rajesh PN, Kahl G & Muehlbauer FJ, A linkage map of the chickpea (*Cicer arietinum* L.) genome based on recombinant inbred lines from a *C. arietinum* X *C. reticulatum* cross: Localization of resistance genes for *Fusarium* wilt races 4 and 5. *Theor Appl Genet*, 101 (2000) 1155.

27 Benko-Iseppon A, Winter P, Huettel B, Staginnus C, Muehlbauer FJ & Kahl G, Molecular markers closely linked to *Fusarium* resistance genes in chickpea show significant alignments to pathogenesis related genes located on Arabidopsis chromosomes 1 and 5. *Theor Appl Genet*, 107 (2003) 379.

28 Rakshit S, Winter P, Benko-Iseppon AM, Muchlbauer FJ & Kahl G, DAF marker tightly linked to a major locus for Aschochyta blight resistance in chickpea (*Cicer arietinum* L.). *Euphytica*, 132 (2003) 23.