RESEARCH ARTICLE Legume Research- An International Journal, Volume 43 Issue 4: 480-487 (August 2020)

Genetic diversity analysis of advanced breeding lines derived from interspecific and intervarietal crosses of black gram based on morphological and molecular markers

R. Devi, R.K. Mittal, V.K. Sood, P.N. Sharma

10.18805/LR-3999

ABSTRACT

The main objective of this study was to assess the genetic diversity of 34 genotypes comprising of 25 advanced derivatives of interspecific cross between *V. mungo* x *V. umbellata*, five intervarietal crosses along with four checks (including blackgram parents) using 11 morphological traits and molecular markers (RAPD and ISSR) and for their resistance towards *Cercospora*, anthracnose and MYMV. The lines Palampur-93 x BRS-1 (236-A-L-1-4), HPBU-35 and HPBU-111 were found statistically at par to the best check Him Mash-1 for seed yield. Correlation studies revealed that the selection should be based on large seeds, more number of pods per plant and more number of seeds per pod indicating the importance of these traits in yield determination. The cluster analysis on molecular basis grouped the blackgram parents and advance lines differently than at morphological level which revealed genetic variation among genotypes and also confirmed that rigorous selection had been made for blackgram types in segregating generations rather than ricebean types as most of the advance derivatives of *V. mungo x V. umbellata* were found to be concentrated near blackgram parents. The lines PDU-1 x PRR-1 (62-3-L-10-1) and Palampur-93 x PRR-1 (258-1L-2-5) were found resistant to both *Cercospora* and anthracnose while, Palampur-93 x BRS-1 (236-A-L-3-2) for MYMV. The information generated from this study would be helpful in characterizing the advanced derived lines and parents in the selection and utilization of diverse genotypes to enhance variability and productivity along with resistance breeding of *V. mungo.*

Key words: Black gram, Genetic diversity, Inter simple sequence repeat, Random amplified polymorphic DNA, *V. mungo* x *V. umbellate*.

INTRODUCTION

Blackgram, *Vigna mungo* (L.) Hepper, popularly known as urdbean or mash in India is a grain legume domesticated from *V. mungo* var. *silvestris*. It is the third most important crop among the various pulse crops and is being cultivated during summer, rainy (*kharif*) and winter (*rabi*) seasons in different parts of India. It has a special nutrition status and is an important source of protein in the diet of vegetarian masses. Among pulses, blackgram occupies a prominent place in India, with a production of 2.89 million tones. But its percent share to the total pulse production is less than other crops like gram and tur because of decrease in area and production of this crop (Anonymous, 2017).

The major constraints in achieving higher yield of this crop are lack of genetic variability, absence of suitable ideotypes for different cropping systems, poor harvest index and susceptibility to diseases (Singh *et al.* 2013). As with other crop species, the genetic improvement of *V. mungo* can be potentially achieved through use of related species like *V. umbellata* to diversify the primary gene pool by the incorporation of novel allele content. Under-utilized related species of black gram i.e. *V. umbellata* commonly known as ricebean, is a neglected crop, but is found to be nutritive, free from anti-nutritional factors, high yielding and resistant to various diseases like *Cercospora* leaf spot (Singh *et al.* 2013, Marappa 2008). Thus, it may be advantageous to transfer desirable genes from *V. umbellata* into *V. mungo* to enhance the genetic variability of *V. mungo*. Interestingly, traits such as high pod number, seed weight, productivity and resistance to diseases have been introgressed successfully into black gram from ricebean. The advanced lines derived from these hybrids could be further evaluated for disease resistance and other economic traits based on morphometric and molecular markers which would help to select superior derivatives with high yield and disease resistance/tolerance.

Morphological traits can be used to assess phenotypic variation in growing environments and are also used as tools for the indirect analysis of genetic variability and diversity.

Department of Crop Improvement, College of Agriculture, CSK Himachal Pradesh Agriculture University, Palampur-176 062, Himachal Pradesh, India.

Corresponding Author: R. Devi, Department of Crop Improvement, College of Agriculture, CSK Himachal Pradesh Agriculture University, Palampur-176 062, Himachal Pradesh, India.
Email: rajnidevi037@gmail.com

How to cite this article: Devi, R., Mittal, R.K., Sood, V.K. and Sharma, P.N. (2020). Genetic diversity analysis of advanced breeding lines derived from interspecific and intervarietal crosses of black gram based on morphological and molecular markers. Legume Research. 43(4): 480-487.

Submitted: 02-02-2018 **Accepted:** 16-08-2018 **Published:** 16-10-2018

However, morphological characterizations are susceptible to phenotypic plasticity allowing the assessment of diversity in the presence of environmental variation (Modini *et al.* 2009). In contrast, molecular markers not influenced by environmental conditions, have been successfully used to assess the genetic diversity among various crops (Belaj *et al.* 2007). Among the various molecular markers used, RAPD markers are very useful due to their simplicity, low cost and throughput capabilities; however, this technique can have problem with reproducibility (Waugh and Powell 1992, Sood *et al.* 2014). In contrast, ISSR markers target microsatellite motifs, which require no specific gene sequence information, have been used widely for genetic diversity analyses of blackgram (Souframanien and GopalaKrishna 2004) and both (RAPD & ISSR) have been useful for identifying relationships at the cultivar and species level (Rao *et al.* 2007, Sharma *et al.* 2008, Goyal *et al.* 2015). Therefore, RAPD and ISSR techniques are widely applicable because they are rapid, inexpensive and simple to perform. Moreover, they do not require prior knowledge of DNA sequence and require very little starting template (Karuppanapadian *et al.* 2010).

Considering potential nutritive value of *V. mungo* and lack of genetic divergence in primary gene pool, present study was aimed to assess the genetic diversity of 25 advanced derivatives of interspecific crosses between *V. mungo* x *V. umbellata* and 5 intervarietal crosses along with parental lines and checks using molecular and morphological characterization.

MATERIALS AND METHODS

Plant material and Experimental layout

The material for the present investigation included 25 advanced derivatives of interspecific crosses of *V. mungo* x *V. umbellata* along with two blackgram parents and 5 promising intervarietal crosses of *V. mungo* plus checks and five rice bean parents (Local, Naini, BRS-1, BRS-2 and PRR-1 which were used for disease study and molecular analysis) (Table1). The lines used were evaluated in randomized complete block design with three replications. Two rows of each line were grown in 2m length with row to row and plant to plant distance of 30cm and 10 cm, respectively. The genotypes were raised as per recommended package of practices.

Morphological evaluation

The data were recorded on 11 agro-morphological quantitatively measured traits for 2 years (2012-2013). The 11 agro-morphological traits recorded include days to 50 per cent flowering (DFM), days to 75 per cent maturity (DSM), plant height (cm) (PH), branches per plant (BPP), pods per plant (PPP), pod length(cm) (PL), seeds per pod (SPP), 100-seed weight (g) (SW), biological yield per plant (g) (BYPP), seed yield per plant (g) (SY) and harvest index (%) (HI). Traits were measured as per standard protocol on 5 randomly selected plants from each genotype and the mean from each genotype was used for analysis except days to 50 per cent flowering and days to 75 per cent maturity, which were recorded on plot basis. The genotypes were also screened under natural epiphytotic condition against *Cercospora* leaf spots (*C. canescens*, *C. cruenta*), anthracnose leaf spot (*Colletotrichum truncatum*) and mungbean mosaic disease (MYMV). The genotypes were classified for disease reaction according to scale given by Mayee and Datar (1986). For the analysis of morphological data mean values of 2-year observations were taken. The data were analyzed for descriptive statistics, ANOVA, correlations and cluster analysis (Tocher method). The statistical analysis was carried out using PROC GLIMMIX in SAS statistical software (SAS 2012).

Molecular marker analysis: DNA isolation and polymerase chain reaction

For molecular analysis, genomic DNA was isolated from young leaf tissue (0.5-1g) of total 34 genotypes used for morphological evaluation along with six ricebean parents using CTAB method (Murray and Thompson 1980). Polymerase chain reaction was performed in final volume of 12.5 µl containing 7.15µl of sterilized distilled water, 1.0µl template DNA (25ng/µl), 1.0 µl of primer (5µM), 1.0µl $MgCl_2$ (25mM), 1.25µl 10 X PCR buffer, 1.0µl dNTP mix (0.2mM each of dATP, dGTP, dCTP and dTTP) and 0.1µl *Taq* polymerase (5U/µl).The amplifications were carried out in eppendorf thermocycler. The PCR conditions for RAPD were optimized with 5 min initial denaturation at 94°C followed by 36 cycles of 94°C for 1 minute, with the annealing temperature of 37°C for 1 minute, extension at 72°C for 2 minutes and final extension step at 72°C for 10 minutes before cooling at 4°C. Forty one RAPD primers were screened for analysis.

Similarly, PCR conditions for ISSR were optimized with 5 min initial denaturation at 94°C followed by 36 cycles of 94°C for 1 minute, with the annealing temperature of 52°C for 1 minute, extension at 72°C for 2 minutes and final extension step at 72°C for 10 minutes before cooling at 4°C. Eighteen ISSR primers were screened for analysis. The gels were stained using ethidium bromide solution and docu mented in Gel Doc system (Biovis).

Scoring and data analysis

For diversity analysis, the amplified products of polymorphic primers, that generated ISSR and RAPD marker profiles, the presence or absence of each sharp and precise bands of a particular molecular weight was scored manually. A binary data matrix with '1' indicating the presence of particular molecular weight and '0' indicating its absence was generated separately for each primer. Initially, the potential of the suitable amplified markers for estimating genetic variability was examined by measuring the marker informativeness through the counting of bands. Primer banding characteristics such as number of total bands (TB), number of polymorphic bands (PB) and polymorphic bands % (PB) were obtained. The binary data generated was used

Table 1: Estimates of the mean values of blackgram genotypes.

Entries	Pedigree/Source	Days to 50% flowering	Days to 75% Maturity	Plant height (cm)	Branches per plant	Pods per plant	Pod length (cm)	Seeds per pod	Seed yield per plant (g)	100-Seed weight (g)	Biological yield per plant(g)	Harvest Index (%)
62-3-L-1-4	PDU-1 x PRR-1	47.33	75.33	41.40	2.00	22.67	4.19	6.00	2.27	3.99	9.17	24.76
257-2-L-7-2	Palampur-93 x BRS 2	45.33	74.67	52.07	2.00	17.00	4.06	5.67	2.91	3.82	13.17	22.10
237-1L-1-4	Palampur-93 x BRS-1	46.00	75.00	58.20	3.00	18.00	4.26	6.33	1.58	3.66	14.00	11.29
236-A-L-3-2	Palampur-93 x BRS-1	46.00	75.67	60.07	1.00	17.00	4.45	6.67	2.09	4.09	12.17	17.18
62-3-L-10-1	PDU-1 x PRR-1	59.00	74.33	102.13	2.00	19.00	4.17	6.33	2.46	3.83	17.50	14.06
257-1-L-15-2	Palampur-93 x BRS 2	46.67	76.33	53.87	1.33	27.00	4.36	5.67	2.82	3.75	15.93	17.70
257-2-L-2-5	Palampur-93 x BRS 2	46.67	76.33	54.93	1.00	14.33	4.47	6.67	2.98	3.68	10.83	27.51
236-A-L-1-4	Palampur-93 x BRS-1	47.00	77.67	54.93	1.00	17.33	4.36	6.33	3.49	3.95	11.43	30.53
122-L-9	PDU-1 x Local-1	50.67	76.67	54.07	1.33	16.67	4.17	6.00	2.61	3.74	10.00	26.10
62-3-L-2-3	PDU-1 x PRR-1	45.67	73.67	53.13	2.00	18.00	4.15	5.67	2.11	3.97	7.47	28.26
257-1-L-11-1	Palampur-93 x BRS 2	46.67	77.33	50.53	1.33	11.67	4.20	6.33	0.81	3.47	9.33	8.68
221-1L-12-4	Palampur-93 x Naini	46.33	76.67	54.20	2.00	11.67	4.11	6.00	1.36	3.19	13.53	10.05
12-2L-7-4	PDU-1 x Naini	51.33	78.33	56.23	1.33	9.33	4.39	6.00	0.93	3.68	13.80	6.74
244-L-19-1	Palampur-93 x BRS-1	45.33	75.67	57.80	1.33	12.00	4.36	6.33	1.55	3.62	11.10	13.96
244-L-9-4	Palampur-93 x BRS-1	46.33	76.67	60.53	2.00	11.00	4.19	6.00	2.65	4.04	12.63	20.98
258-2L-2-1	Palampur-93 x PRR 1	47.33	76.00	57.13	1.33	15.33	4.08	6.00	1.34	3.16	13.50	9.93
257-1-L-7-1	Palampur-93 x BRS 2	48.00	77.33	57.10	2.33	8.67	4.18	6.00	1.24	3.16	11.33	10.94
244-L-1-5	Palampur-93 x BRS-1	48.00	77.67	61.13	1.33	12.33	4.10	5.67	1.41	3.25	11.83	11.92
257-2-L-4-1	Palampur-93 x BRS 2	44.33	76.00	60.73	2.00	12.33	4.27	6.67	2.53	4.07	13.00	19.46
258-1L-2-5	Palampur-93 x PRR 1	46.33	76.33	62.67	3.00	14.33	4.10	6.00	2.62	4.16	13.70	19.12
257-2-L-3-3	Palampur-93 x BRS 2	45.33	76.33	60.00	1.33	17.33	4.47	6.33	2.78	4.42	12.27	22.66
257-2-L-12-1	Palampur-93 x BRS 2	44.00	75.67	62.47	2.00	13.67	4.18	6.00	2.07	4.01	12.50	16.56
258-2L-4-3	Palampur-93 x PRR 1	46.67	77.33	53.33	1.33	13.33	4.25	6.00	2.57	4.44	10.57	24.32
221-2L-3-5	Palampur-93 x Naini	45.67	76.00	61.20	1.33	13.33	4.27	6.33	1.98	3.51	13.07	15.15
221-1L-1-3	Palampur-93 x Naini	44.67	77.00	60.63	2.00	17.00	4.29	6.33	1.69	3.60	12.33	13.70
HPBU-35	CSK HPKV, Palampur	48.67	77.67	61.27	2.00	15.67	4.48	6.67	3.85	3.93	11.90	32.35
HPBU-111	CSK HPKV, Palampur	45.67	74.33	55.33	1.00	19.33	3.97	5.67	3.59	3.93	12.07	29.75
HPBU-124	CSK HPKV, Palampur	51.33	79.00	54.27	2.00	19.67	4.29	6.33	3.00	3.51	11.83	25.35
HPBU-126	CSK HPKV, Palampur	48.67	77.00	62.53	2.00	19.67	4.22	6.00	2.11	3.61	14.67	14.39
HPBU-194	CSK HPKV, Palampur	50.00	78.67	62.93	2.00	16.33	4.49	6.33	1.66	3.70	12.27	13.53
Him Mash-1(C)		41.33	73.67	46.33	3.00	23.00	4.59	7.33	3.84	3.81	14.23	26.98
PDU-1(C)		51.67	79.00	59.47	1.00	8.33	4.43	6.33	1.29	3.79	12.07	10.69
Palampur-93(C)		47.33	78.00	58.53	2.00	14.00	4.25	6.00	2.34	3.55	13.00	18.00
UG-218(C)		49.00	77.67	61.53	2.00	15.67	4.67	6.33	2.10	4.15	14.30	14.69
Mean		47.36	76.50	58.31	1.75	15.65	4.28	6.19	2.25	3.77	12.43	18.67
Range		41.00-59.00	73.00-79.00	41.40-102.13	1.00-3.00	8.33-27.00	3.97-4.67	5.67-7.33	0.93-3.85	3.16-4.44	7.47-17.50	6.74-32.35
C.V (%)		6.63	2.67	15.48	18.97	17.41	6.09	8.05	15.23	3.29	11.00	19.32
S.E. (m)±		1.81	1.18	5.21	0.19	1.57	0.15	0.29	0.20	0.34	0.79	2.08
C.D. 5%		5.12	NS	14.71	0.54	4.44	NS	NS	0.56	0.20	2.23	5.88

*C: Check variety.

Table 2: Analysis of variance for all the agro-morphological traits of all genotypes.

Source \ Traits	DFM	DSM	PH	BPP	PPP	PL	SPP	BYPP	SW	SY	HI
MSS	27.66*	6.03	249.59*	0.96*	51.33*	0.08	0.39	11.08*	0.31*	1.92*	153.79*
EMS	9.85	4.17	81.48	0.11	7.42	0.07	0.25	1.87	0.35	0.12	12.98
F value	2.81	1.44	3.06	8.62	6.92	1.15	1.56	5.93	0.89	16.35	11.85

* $P \leq 0.05$.

Table 3: Scoring of different blackgram genotypes to *Cercospora* leaf spots, anthracnose leaf spots and mungbean yellow mosaic virus under natural epiphytotic conditions.

Entries	Disease Score					
	Cercospora	Reaction	Anthracnose	Reaction	MYMV	Reaction
62-3-L-1-4	5	MS	5	MS	5	MS
257-2-L-7-2	3	MR	1	R	7	S
237-1L-1-4	3	MR	1	R	5	MS
236-A-L-3-2	5	MS	3	MR	1	R
62-3-L-10-1	1	R	1	R	7	S
257-1-L-15-2	5	MS	3	MR	3	MR
257-2-L-2-5	3	MR	1	R	7	S
236-A-L-1-4	3	MR	1	R	5	MS
122-L-9	3	MR	3	MR	7	S
62-3-L-2-3	3	MR	5	MS	9	HS
257-1-L-11-1	3	MR	1	R	7	S
221-1L-12-4	3	MR	3	MR	7	S
12-2L-7-4	1	R	3	MR	9	HS
244-L-19-1	3	MR	5	MS	7	S
244-L-9-4	5	MS	1	R	3	MR
258-2L-2-1	5	MS	3	MR	7	S
257-1-L-7-1	3	MR	3	MR	9	HS
244-L-1-5	3	MR	3	MR	7	S
257-2-L-4-1	3	MR	1	R	7	S
258-1L-2-5	1	R	1	R	3	MR
257-2-L-3-3	5	MS	5	MS	3	MR
257-2-L-12-1	3	MR	3	MR	3	MR
258-2L-4-3	3	MR	3	MR	5	MS
221-2L-3-5	5	MS	5	MS	5	MS
221-1L-1-3	3	MR	5	MS	5	MS
HPBU-35	3	MR	3	MR	3	MR
HPBU-111	5	MS	3	MR	3	MR
HPBU-124	5	MS	5	MS	5	MS
HPBU-126	3	MR	1	R	3	MR
HPBU-194	3	MR	3	MR	3	MR
Him Mash-1	5	MS	1	R	5	MS
PDU-1	5	MS	5	MS	9	HS
Palampur-93	3	MR	3	MR	9	HS
UG-218	3	MR	3	MR	3	MR
Local	0	HR	0	HR	0	HR
Naini	0	HR	0	HR	0	HR
PRR-1	0	HR	0	HR	0	HR
PRR-9301	0	HR	0	HR	0	HR
BRS-1	0	HR	0	HR	0	HR
BRS-2	0	HR	0	HR	0	HR

to calculate a genetic dissimilarity matrix using the Jaccard dissimilarity index (dij) between pairs of accessions (units).

$$d_{ij} = \frac{(b + c)}{a + (b + c)}$$

Where,

d_{ij} represents the dissimilarity between units i and j.

From the dissimilarity matrix, a Neighbor-Joining tree (UnWeighted Neighbor-Joining) was computed using the DARwin software version 5.0 (Perrier and Jacquemoud-Collet 2006).

The Polymorphic information content (PIC) values provide an estimate of the discriminatory power of the locus or loci by taking into account not only the number of alleles that are expressed, but also the relative frequency of those alleles. It measures the informativeness of a given DNA marker and these were calculated according to Anderson *et al.* (1993):

$$PIC = 1 - \sum_{i=1}^{k} P_i^2$$

Where,

k is the total number of alleles detected for a given marker locus and P_i is the frequency of the i^{th} allele in the set of genotypes investigated. The PIC value ranges from 0 (monomorphic) to 1 (very highly discriminative, with many alleles each in equal and low frequency).

RESULTS AND DISCUSSION

Utilization of wild relatives like rice bean for the introgression of desirable alleles into black gram could be rewarding to broaden the genetic base for yield and disease resistance. However, success of gene introgression to a large extent depends upon the genetic divergence of the parents and advanced lines derived after crossing. It is important that variability for traits must exist in the advanced lines for the possible exploitation following recombination breeding and selection. Therefore, it is necessary to characterize the available gene pool to assess the level of the genetic variation for the various characters and their genetic relationship (Mondini *et al.* 2009). With this aim, the present study was attempted to assess the genetic divergence of advanced interspecific and intervarietal derivatives of blackgram using morphological and molecular characterization.

Morphological characterization

The morphological data used in this study were based on the mean values recorded for 2 years (2013–2014). Based on analysis of variance, large and significant differences were found among all lines for the traits *viz.,* DFF, PH, BPP, PPP, BYPP, SY, SW and HI except for DSF, PL and SPP, suggesting the prevelance of wide range of genetic variability and scope of selection for these traits (Table 2). The summary statistics including mean, range, standard error and coefficient of variation (CV) for 11 agro-morphological characters are mentioned in Table 1. All the material used in the study revealed a large variation for most of the traits,

Table 4: Number of scorable and polymorphic RAPD and ISSR bands obtained in the PCR amplified DNA of blackgram genotypes generated by 25 primers.

SN	RAPD primers	Primer Sequence	N_f	N_p	Polymorphic bands (%)	PIC	SN	ISSR primers	Primer Sequence (5'-3')	N_f	N_p	Polymorphic bands (%)	PIC
1	GLA-04	AATCGGGCTG	13	7	53.85	0.83	1	ISSR 809	AGA GAG AGA GAG AGA GG	10	6	60.00	0.85
2	GLB-07	GGTGACGCAG	13	6	46.15	0.84	2	ISSR 810	GAGAGAGAGAGAGAGAT	10	6	60.00	0.79
3	OPA-7	GAAACGGGTG	13	7	53.85	0.80	3	ISSR 817	CACACACACACACAA	7	6	85.71	0.84
4	OPAB-10	CTGCTGGGAC	11	6	54.55	0.82	4	ISSR 818	CACACACACACACAG	10	6	60.00	0.78
5	OPAH-17	CAGTGGGGAG	10	7	70.00	0.87	5	ISSR 821	GTGTGTGTGTGTGTT	7	6	85.71	0.91
6	OPD-11	AGCGCCATTG	14	8	57.14	0.80	6	ISSR 823	TCTCTCTCTCTCTCC	7	4	57.14	0.80
7	OPG-10	AGGGCCGTCT	11	9	81.82	0.89	7	ISSR 832	AGA GAG AGA GAG AGA GYC	8	5	62.50	0.88
8	OPM-11	GTCCACTGTG	9	6	66.67	0.86	8	ISSR 833	AGA GAG AGA GAG AGA GYA	9	7	77.78	0.78
9	OPT-04	GTCCTCAACG	9	7	77.78	0.78	9	ISSR 838	GAG AGA GAG AGA GAGAYC	14	9	64.29	0.90
10	RAPD-1	GTTTCGCTCC	12	9	75.00	0.86	10	ISSR 845	CAC ACA CAC ACA CAC ARG	7	5	71.43	0.79
11	RAPD-11	AAGCCCCCCA	12	9	75.00	0.88							
12	RAPD-3	CCCGTCAGCA	11	8	72.73	0.88							
13	RAPD-6	AAGAGCCCGT	12	6	50.00	0.80							
14	RAPD-9	GGCAGTTCTC	12	9	75.00	0.81							
15	S-221	TGA CGC ATG G	10	7	70.00	0.84							
Mean			11.47	7.40	65.30	0.84				8.90	6.00	68.46	0.83

N_f- number of fragments; N_p- number of polymorphic fragments; PIC- Polymorphic information content

with mean values ranging from 41.40-102.13 for PH, 8.33 - 27.00 for PPP, 7.47- 17.50 for BYPP and 0.81-3.85 for SY. The traits BPP (35.35%), PPP (29.85%), HI (21.21%), PH (20.63%), BYPP (17.87%) and SY (17.27%) revealed the highest CV among all other traits. On the basis of mean performance PDU-1xPRR-1 (62-3-L-10-1) was found to be superior for PH and BYPP, Palampur-93 x BRS-2 (257-2-L-3-3) and Palampur-93 x PRR-1 (258-2L-4-3) for SW as compared to the best check UG-218 whereas, three genotypes namely, Palampur-93 x BRS-1 (236-A-L-1-4), HPBU-35 and HPBU-111 were found statistically at par to the best check Him Mash-1 for seed yield. Performance of different advanced derivatives for other characters were found to be at par as compared to their best checks *viz.,* Him-mash-1 and UG-218 depicting their superior performance as compare to their parental genotypes *viz.,* PDU-1 and Palampur-93.

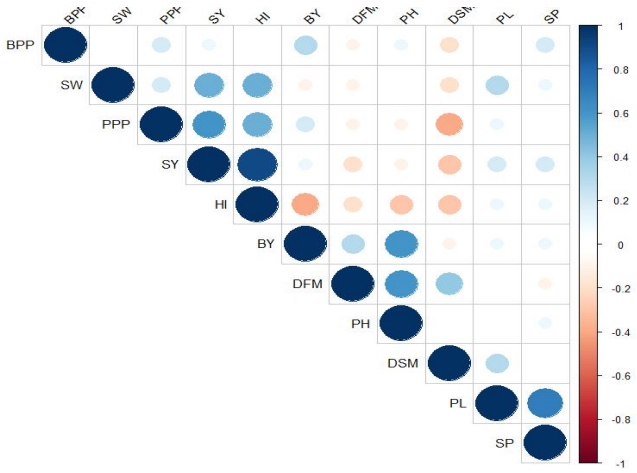

Fig 1: Correlations for 11 agro morphological traits in all genotypes.

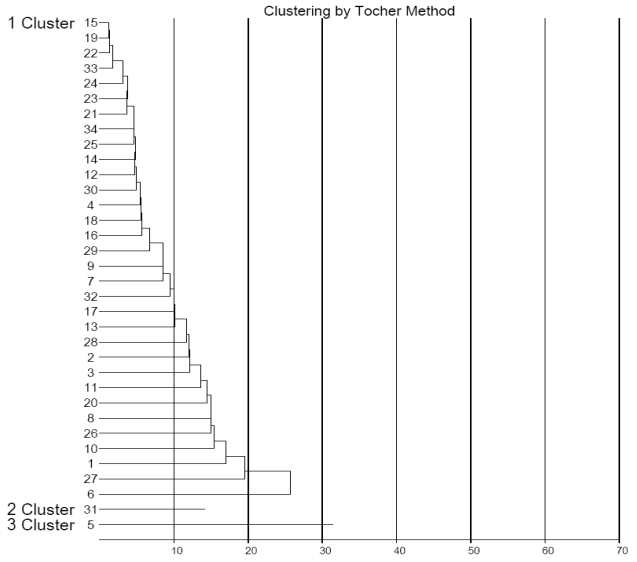

Fig 2: Cluster analysis of 11 agro-morphological traits in all lines based on Tocher method.

Correlation coefficients of the 11 agro-morphological traits were evaluated to assess the association between the traits (Fig 1). Seed yield per plant showed significant and positive correlation with PPP, SPP, SW and HI. This indicated that selection should be based on large seeds, more number of pods per plant and seeds per pod. Rani and Rao (1981) also observed positive association of pods per plant, seeds per plant and 100-seed weight with seed yield. A significant negative correlation was found between PPP and DSM, and HI and BY. The cluster diagram constructed using tocher method based on agro-morphological traits resulted in the establishment of three distinct clusters (Fig 2). Cluster I ended with maximum number of genotypes. Cluster II and III genotypes ended with only one genotype each *viz.,* Him Mash-1 and 62_3_l_10_1. Him Mash-1 was found to be superior and also as best check for traits like SY, BPP, PPP and HI. So it should be included in future hybridization programmes to have desirable segregants as it formed separate cluster in clustering pattern *i.e.* cluster II.

Diseases reaction of genotypes

Data on reaction of blackgram genotypes to the diseases under natural epiphytotic conditions are presented in the Table 3. Results revealed that only three genotypes *viz.,* PDU-1 x PRR-1 (62-3-L-10-1), PDU-1 x Naini (12-2L-7-4), Palampur-93 x PRR-1 (258-1L-2-5) were resistant to the *Cercospora* disease. Ten blackgram genotypes with one check *viz.,* Palampur-93 x BRS-2 (257-2-L-7-2, 257-2-L-2-5, 257-1-L-11-1, 257-2-L-4-1), Palampur-93 x BRS-1(237-1L-1-4, 236-A-L-1-4, 244-L-9-4), PDU-1 x PRR-1 (62-3-L-10-1), Palampur-93 x PRR-1 (258-1L-2-5), HPBU-126, Him Mash-1 were resistant to the anthracnose leaf spots.

Only one genotype *i.e.* Palampur-93 x BRS-1 (236-A-L-3-2) was found resistant to Mungbean Yellow Mosaic Virus. However, nine genotypes with one check *viz.,* Palampur-93

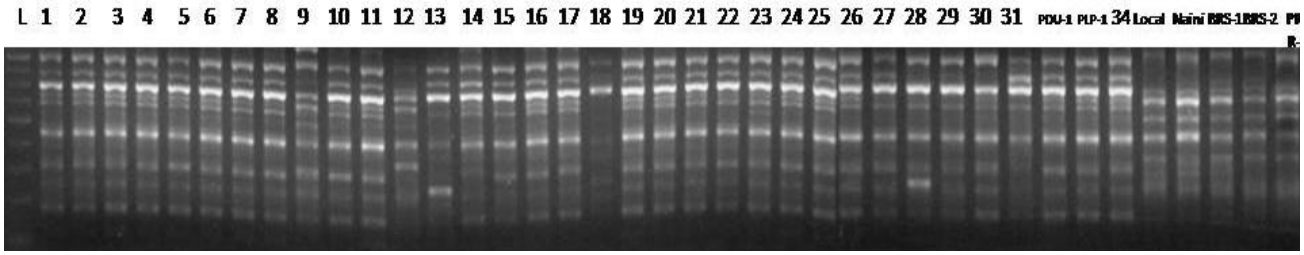

Fig 3: RAPD profile of advance derivatives of interspecific and intervarietal crosses/fixed lines along with blackgram and ricebean parents using primer OPD-11, L = 100 bp DNA ladder.

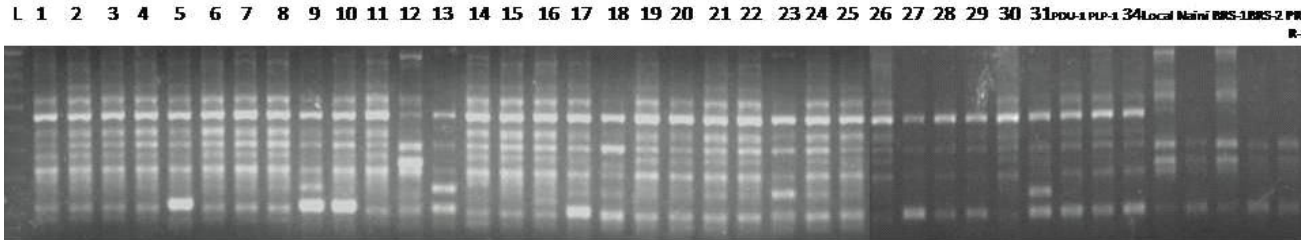

Fig 4: ISSR profile of advance derivatives of interspecific and intervarietal crosses with blackgram and ricebean parents using primer ISSR-838, L = 100 bp DNA ladder.

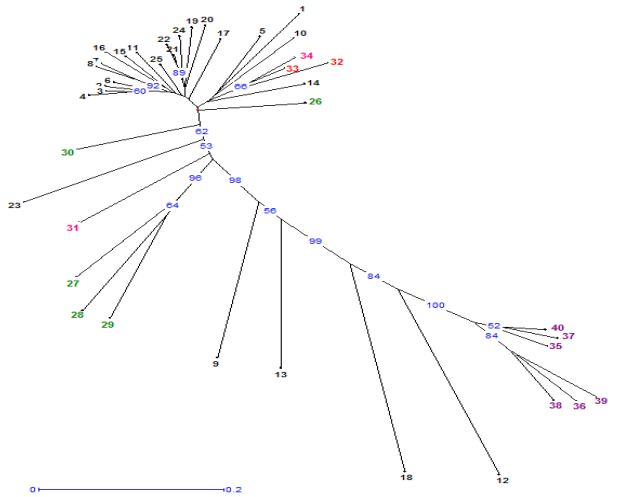

Fig 5: Neighbor-joining tree using RAPD+ISSR, where, Red colour represents Blackgram parents; Green colour epresents advance lines of intervarietal crosses/ fixed lines; Pink represents Checks; Purple represents Ricebean parents; Black represents advanced derivatives.

x BRS-2 (257-1-L-15-2, 257-2-L-3-3, 257-2-L-12-1), Palampur-93 x BRS-1 (244-L-9-4), Palampur-93 x PRR 1 (258-1L-2-5), HPBU-35, HPBU-111, HPBU-126, HPBU-194, UG-218 were found to be moderately resistant to the disease. Overall, 11 genotypes were found to be resistant to anthracnose leaf spots, 3 genotypes resistant to *Cercospora* leaf spot and 1 genotype resistant to Mungbean Yellow Mosaic Virus. On the basis of disease reaction the resistant genotypes identified could be exploited to develop high yielding varieties of blackgram and likewise the genotypes, PDU-1 x PRR-1 (62-3-L-10-1) and Palampur-93 x PRR-1 (258-1L-2-5) were found resistant to both *Cercospora* and anthracnose diseases could be utilized for this purpose. Singh *et al.* (2013) observed that all the progenies of *V. mungo* x *V. umbellate crosses* were found resistant to MYMV, *Cercospora* leaf spot and Bacterial leaf spot diseases.

Molecular characterization

A total of 59 primers *viz.,* 41 RAPD and 18 ISSR were screened for PCR amplification on 25 advance derivatives of *V. mungo x V. umbellata*, 5 advance lines of inter-varietal crosses of blackgram, two blackgram (parents), two checks and six ricebean (parents) genotypes. Out of these 59 primers, 15 RAPD and 10 ISSR produced amplified products and were found to be informative and polymorphic. In total, 15 RAPD primers produced 174 amplicons out of which 111 (65.30%) were polymorphic. On average 7.40 polymorphic fragments were generated per primer (Table 4). The number of amplified fragments with RAPD primers ranged from 6 (GLB-07, OPAB-10, OPM-11 and RAPD-6) to 9 (OPG-10, RAPD-1, RAPD-11 and RAPD-9). Percentage of polymorphism ranged from 46.15–81.82% with an average of 65.30%. The RAPD profile of a representative primer OPD-11 is shown in (Fig 3). The ISSR profiles of lines generated in total 89 amplicons of which 60 (68.46%) were polymorphic. On average 6 bands were generated per primer (Table 4). The primer, ISSR 838 generated maximum number of 9 bands and ISSR 823 produced minimum number of 4 bands. Percentage of polymorphism ranged from 57.14–85.71% with average of 68.46%. The ISSR profile of a representative primer ISSR-838 is shown in (Fig 4).

Based on RAPD+ISSR polymorphism data, a neighbor joining tree generated results in the formation of three clusters which were further subdivided into sub clusters (Fig 5). Cluster I was the largest cluster among the three consisted of 23 genotypes, which was further subdivided into six sub clusters. The cluster 1 includes most of the advanced derivatives with blackgram parents (32 and 33) and check UG-218. Cluster II with 16 genotypes was mainly consisted of intervarietal lines (27 to 30), all the ricebean parents (35 to 40) and five interspecific advanced derivatives. Cluster III consisted of only one genotype i.e. HPBU-35. In the phylogenetic tree generated using RAPD+ISSR, most of the advance derivatives of *V. mungo x V. umbellata* were found to be concentrated near blackgram parents which showed that rigorous selection has been made for blackgram types in segregating generations rather than ricebean types. Such comparative studies in legumes involving RAPD and ISSR markers have been successfully carried out previously in crops like Black gram (Souframanien and Gopalakrishna 2004), *Vigna* species (Dikshit *et al.* 2007) and Rice (Ravi *et al.* 2003). Also, RAPD markers has been used to study the genetic interraltionships among blackgram genotypes to develop multidisease resistance germplasm (Vishalakshi *et al.* 2017).

Based on analysis of variance, large and significant differences were found among all lines for all the measured traits, indicating high levels of variation in the lines. Presence of significant variation among lines and their superiority for

different traits and disease resistance may act as basis for the selection of lines in recombination breeding to improve various traits. Singh *et al.* (2013) found that desirable traits such as high pod number, seed weight, productivity and resistance to diseases can be introgressed successfully into black gram from ricebean.

Significant positive correlation between the traits infers that selection for one trait will have a positive effect on another and will increase correlated response to selection. Therefore, high positively correlated traits should be given utmost consideration in selection programs. Based on these estimates the breeders can decide the method of breeding to be followed to exploit the useful correlation. The cluster analysis based on molecular analysis grouped the blackgram parents and advance lines differently than at morphological level revealed genetic variation among genotypes. However, grouping of rice bean parents together by phylogenetic tree indicated less genetic divergence among the rice bean genotypes.

Dendrogram, based on N-J trees clearly separated rice bean parents into one group and makes distinguishes in advanced lines and intra varietal crosses. Genetic divergence found in the advanced lines and intervarietal cross is important to select the lines and parents for future breeding programs. The blackgram parents and advanced lines that clustered separately from the rest of lines in the study based both on morphological and molecular data, suggested the singularity of these lines and these diverse genotypes may be utilized in hybridization programmes to enrich the genetic diversity of available primary gene pool. Interestingly, the inclusion of the advanced derivatives around the blackgram parents might be due to the fact that rigorous selection had been made for blackgram types in segregating generations rather than rice bean types.

CONCLUSION

In the present study, based on morphological and molecular characterization, great genetic variability among lines of blackgram, advanced lines and intervarietal crosses was revealed. The diverse genotypes identified for their superiority among different traits and resistance to diseases could be selected and utilized for enhancing the variability and productivity along with resistance breeding of *V. mungo.*

REFERENCES

Anderson, J.A., Churchill, G.A., Sutrique, J.E., Tanksley, S.D. and Sorrells, M.E. (1993). Optimizing parental selection for genetic linkage maps. Genome. 36:181-186.

Anonymous (2017). Commodity Profile for Pulses-March, 2017. http://agricoop.nic.in/sites/default/files/Pulsespdf.

Belaj, A., Munoz-Diez, C., Baldoni, L., Porceddu, A., Barranco, D. and Satovic, Z. (2007). Genetic diversity and population structure of wild olives from North-Western Mediterranean assessed by SSR markers. Ann Bot. 100: 449-458.

Dikshit, H.K., Jhang, T. Singh, N.K., Koundal, K.R., Bansal, K.C., Chandra, N., Tickoo, J.L. and Sharma, T.R. (2007). Genetic differentiation of *Vigna* Species by RAPD, URP and SSR Markers. Biologia Plantarum. 3: 451-457.

Goyal, P., Jain, R., Kachhwaha, S., and Kothari, S.L. (2015). Assessment of genetic diversity in *Pithecellobium dulce* (Roxb.) Benth. germplasm using RAPD and ISSR markers. Trees. 29: 637-653.

Karuppanapadian, T., Wang, H.W., Karuppudurai, T., Rajendhran, J., Kwan, M., Jang, G.S., Kim, S.H., Manoharan, K. and Kim, W. (2010). Efficiency of RAPD and ISSR markers in assessing genetic diversity and relationships in blackgram [*Vigna mungo* (L.) Hepper] varieties. Can J Plant Sci. 90: 443-452.

Marappa, N. (2008). Screening of mungbean genotypes and its wild relatives for resistant sources to *Cercospora* leaf spot disease. Asian Journal of Bio Science. 3: 324-326.

Mayee, C.D. and Datar, V.V. (1986). *Phytopathometry. Technical Bulletin-1* (Special bulletin-3), Marathwada Agricultural University, Parbhani, Maharastra, India. Pp 74-76.

Mondini, L., Noorani, A. and Pagnotta, M.A. (2009). Assessing plant genetic diversity by molecular tools. Diversity. 1:19-35.

Murray, M.G. and Thompson, W.F. (1980). Rapid isolation of high molecular weight plant DNA. Nucleic Acids Res. 8: 4321-4325.

Perrier, X. and Jacquemoud-Collet, J.P. (2006). DARwin software http://darwin.cirad.fr/.

Rani, Y.U. and Rao, J.S. (1981). Path analysis of yield components in blackgram. Indian J. Agril. Sci. 51: 378-381.

Rao, L.S., Usha Rani, P., Deshmukh, P.S., Kumar, P.A., Panguluri, S.K. (2007). RAPD and ISSR fingerprinting in cultivated chickpea (*Cicer arietinum* L.) and its wild progenitor *Cicer reticulatum* Ladizinsky. Genet Resour Crop Ev. 54: 1235-1244.

Ravi, M., Geethanjali, S., Sammeyafarheen, F. and Maheswaran, M. (2003). Molecular marker based genetic diversity analysis in rice (*Oryza sativa* L.) using RAPD and SSR markers. Euphytica. 133: 243-253.

SAS (2012) Base SAS® 9.4, SAS Institute Inc., Cary, NC, USA.

Sharma, K., Agarwal, V., Gupta, S., Kumar, R. and Prasad, M. (2008). ISSR marker-assisted selection of male and female plants in promising dioecious crops: jojoba (*Simmondsia chinensis*). Plant Biotechnol Rep. 2: 239-243.

Singh, I., Sandhu, J.S. and Singh, S. (2013). Introgression of productivity and other desirable traits from ricebean (*Vigna umbellata*) into black gram (*Vigna mungo*). Plant Breed. 132: 401-406.

Sood, V.K., Rana, I. and Hussian, W. (2014). Genetic diversity of genus Avena from North-Western Himalayas using molecular markers. In: Proceedings of National Academy of Sciences, India Section B: Biological Sciences. 86:151-158.

Souframanien, J. and GopalaKrishna, T. (2004). A comparative analysis of genetic diversity in blackgram genotypes using RAPD and ISSR markers. Theor Appl Genet. 109: 1687-1693.

Vishalakshi, B., Umakanth, B., Shanbhag, A.P., Ghatak, A., Sathyanarayanan, N., Madhav, M.S., Gopala Krishna, G. and Yadla, H. (2017). RAPD assisted selection of black gram (*Vigna mungo* L. Hepper) towards the development of multiple disease resistant germplasm. 3 Biotech. 7: 1.

Waugh, R. and Powell, W. (1992). Using RAPD markers for crop improvement. Trends Biotechnol. 10: 186-191.