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Genetic Evaluation and Identification of Genetic Donors in Blackgram (*Vigna mungo*) Revealed by Agro-Morphological Traits and Seed Storage Protein Analysis

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Abstract: Sixty blackgram accessions, collected from diversity rich zones of India, were evaluated in field for nine quantitative characters as per IBPGR description, which classified accessions into eight non-overlapping clusters based on principal component and non-hierarchical euclidean cluster analysis. The maximum inter cluster distance (10.270) was present between cluster number VIII and III while minimum inter cluster distance (1.656) was observed between cluster I and V. The maximum numbers of accessions were grouped in cluster V, while minimum number of accession (1) was found in cluster VIII. SDS-PAGE for seed storage proteins of accessions classified them into eight groups. Total number of bands ranged from 17 to 28, spread over four zones (A B, C and D). Maximum (10) genotypes were grouped in 28 band group while minimum (2) genotypes were sorted in 17 band group. Based on euclidean distance and dissimilarity coefficient obtained for SDS-PAGE of seed proteins, dendrogram was constructed which categorized genotypes into nine clusters. Present study resulted in the evaluation of germplasm and listing of genetic donors for various agronomic characters, suitable for future breeding and biotechnology programme which will facilitate the use of this germplasm for blackgram improvement.

Key words: Blackgram · Euclidean cluster analysis · Dendrogram · PCA · Seed proteins · Gel electrophoresis

INTRODUCTION

Pulse crops have an eloquent role in the agricultural economy by virtue of their ability to fix atmospheric nitrogen in symbiotic association with *Rhizobium* spp. Blackgram, *Vigna mungo* (L.) Hepper, popularly known as urdbean or mash, is a legume domesticated from *V. mungo* var. silvestris [1-3] and widely cultivated in Indian subcontinent and to a lesser extent in Thailand, Australia, Asian and South Pacific countries. In India, blackgram was cultivated over an estimated area of 3.1693 mha and its production was estimated 13.266 metric tones with an average yield (productivity) of only 419 kg/ ha, during the year 2004-05 [4]. Major constraints in achieving high yield of this crop are the lack of genetic variability, absence of suitable ideotypes for different cropping systems, poor harvest index and susceptibility to diseases. Research on this species has lagged behind that of cereals and other legumes, therefore, the improvement of this crop is required through the utilization of available genetic diversity. The extent of genetic diversity in germplasm can be assessed through morphological characterization and genetic markers which will help plant breeders to select suitable genotypes for further hybridization programme and genetic engineering.

It is noteworthy to classify germplasm into homogenous group on the basis of multivariate parameters instead of using univariate parameters to know the structure of germplasm for the improvement of population [5] and to avoid duplicacy of accessions. Multivariate analysis based different classification approaches viz. Mahalanobis D^2 statistics [6], clustering by Tocher's method [7], a nonhierarchial cluster analysis [8] elaborated by Spark [9], Canonical analysis [10,11] and

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metroglyph analysis [12] are foremost multivariate analysis methods to classify the germplasm into cardinal groups. Characterization by morphological traits cannot be replaced by any of the molecular techniques and results of molecular or biochemical studies should be considered as complementary to morphological characterization [13]. Various numerical taxonomic techniques have been successfully used to classify and measure the pattern of genetic diversity in blackgram [14,15], mungbean [16,17], pea [18] and lentil [19].

Dasgupta and Das [20] appraised blackgram varieties, collected from India and Nepal but they did not find any correlation between genetic and geographic diversity while Renganayaki and Rengaswamy [21,22] evaluated genotypes of mungbean, blackgram and cowpea. Singh and Shukla [23] used euclidean cluster analysis to evaluate accessions of blackgram while D^2 analysis based study was carried out by Singh and Singh [24]. Similarly, Ram *et al.* [25] also assessed strains of blackgram, collected from different geographic regions of India however Verma and Katna [26] observed significant differences of genotype-cropping system interaction among diverse genotypes of blackgram. Principal component strategy was found to more useful in maximizing selection criteria than strictly random sampling in all individual groups. Genetic diversity analysis was carried out for 30 genotypes of blackgram by Manivannan *et al.* [27] while Ghafoor *et al.* [15, 28] evaluated mungbean lines and blackgram accession for quantitative trait using cluster and principal component analysis. Multivariate analysis was also carried out for quantitative traits in blackgram genotypes [29] and Mishra *et al.* [30] perceived significant differences between genotypes in mungbean and urdbean by evaluating quantitative traits.

Seed storage proteins have been used as genetic markers in genetic diversity, genetic resource conservation, genome relationship and in crop improvement. Seed protein pattern revealed by electrophoresis have been successfully utilized to resolve taxonomic and evolutionary problems of several crop plants [31-33] and SDS-PAGE is considered as a practical and reliable method for species identification because seed storage proteins are largely independent to environmental fluctuation [34].

Sixty blackgram accessions collected form diversity rich zones of India and some accessions acquired from National Bureau of Plant Genetic Resources (NBPGR), New Delhi. The data were recorded on different quantitative and visual characters in field. Nine quantitative characters were used to classify accessions into several diverse clusters based on principal component and non-heirarchial euclidean cluster analysis. These accessions were subjected to biochemical characterization through SDS-PAGE of total seed proteins.

MATERIALS AND METHODS

Present investigation was carried out to evaluate sixty germplasm accessions collected from diversity rich zones of India [35], at Crop Research Center, Pantnagar (29.5° North latitude and 79.2° East longitude and at an altitude of 243.83 meters above the sea level), located in foot hills of the Himalayan range (Shivalik hills) and falls under humid subtropical climate zone in a narrow belt called, Tarai.

Experimental Material: The experimental materials comprised of 60 germplasm accessions of blackgram [35] agglomerated from different diversity pockets of Uttar Pradesh and Uttaranchal state of India and some accessions acquired from National Bureau of Plant Genetic Resources (NBPGR), New Delhi (Table 1).

Field Experiment and Observations: Sixty genotypes were sown in 6 blocks, each having 10 plots, along with four checks, Narendra Urd-1 (NU-1), Type-9, Pant Urd-19 (PU-19) and Pant Urd-35 (PU-35). The checks were randomly allocated along with the test genotypes within the blocks and replicated as many times as the number of blocks. Each plot comprised of 4 m long two rows and row to row distance was 30 cm while plant to plant distances were kept 10 cm. Weeding operations including recommended package and necessary practices were carried out under controlled condition as well as crop was also protected from insects.

Observations were taken on plant and plot basis as per descriptor's list published by IBPGR, which included quantitative and visual characters both. Nine quantitative characters, Days to 50 per cent flowering, Days to maturity, Plant height (cm), Pods per plant, Pods per cluster, Pod length (cm), Seeds per pod, 100-seed weight (g) and Seed yield per plant (g) as well as qualitative characters viz. Hypocotyl color, Seedling vigour, Growth habit, Growth pattern, Branching pattern, Primary leaf shape, Leaf pubescence, Leaf color, Leafiness, Terminal leaflet shape, length and width, Leaf senescence, Petiole color, Stem color, Pod attachment to peduncle, Immature pod color, Mature pod color, Pod pubescence, Pod shattering in the field, Seed shape, Seed color, Hilum and Disease susceptibility were observed.

Table 1: Mean (adjusted) of different quantitative characters in blackgram genotypes

EntryNo.	Genotypes	Days to 50% flowering	Days to maturity	Plant height (cm)	Pods per plant	Pods per cluster	Pod length (cm)	Seeds per pod	100-seed weight (g)	Seed yieldplant (g)
1.	Shu-9503	52	85	50.2	41.8	3.8	4.7	6.2	5.2	55.9
2.	Shu-9505	61	92	65.4	49.8	3.2	4.2	6.2	5.16	86.3
3.	Shu-9508	58	93	75.8	45.6	2.6	4.9	6.6	4.88	50.56
4.	Shu-9511	55	88	72.6	26.4	4.0	4.3	6.3	4.68	30.12
5.	Shu-9519	49	81	50.8	46.4	3.6	5.0	7.4	5.4	63.98
6.	Shu-9525	56	89	66.7	65.8	3.0	4.6	6.0	4.86	72.06
7.	Shu-9532	52	88	50.4	38.8	3.6	4.5	6.8	4.94	52.24
8.	Shu-9536	51	81	54.6	28.2	3.8	4.4	6.8	5.44	40.42
9.	Shu-9603	50	87	58.4	41.0	5.4	4.6	7.2	4.58	49.06
10.	Shu-9609	53	89	54.0	51.6	3.3	4.5	6.4	4.92	54.92
11.	Shu-9612	49	83	64.8	54.8	3.6	4.7	6.8	5.26	72.22
12.	Shu-9626	50	84	74.0	63.8	3.15	4.6	6.6	5.34	84.34
13.	Shu-9614	52	83	53.8	41.0	2.8	4.8	6.2	5.12	40.16
14.	Shu-9619	50	91	78.8	95.8	4.0	4.6	6.4	4.2	90.02
15.	Shu-9601	48	87	66.4	69.8	5.2	5.0	7.2	4.98	78.66
16.	Shu-9621	50	81	44.8	53.2	3.6	4.7	6.8	5.12	58.56
17.	Shu-9632	53	95	116.4	58.6	3.0	5.3	7.2	4.96	69.38
18.	Shu-9633	51	89	68.0	49.8	5.0	4.8	6.8	5.5	68.9
19.	Shu-9636	50	88	62.4	51.6	3.0	5.4	7.6	5.5	65.3
20.	Shu-9641	52	89	67.8	44.6	3.0	5.5	8.4	5.58	59.64
21.	Shu-9642	50	85	48.8	48.8	3.2	4.7	7.0	5.7	72.28
22.	Shu-9682	53	89	35.6	35.6	3.2	5.2	7.4	4.54	14.48
23.	Shu-96110	61	90	73.0	19.2	1.6	4.2	5.8	5.34	39.08
24.	Shu-9720	55	88	41.8	10.9	1.6	4.1	5.0	5.1	19.78
25.	Shu-9725	60	96	99.4	61.2	3.4	4.5	6.2	5.5	90.88
26.	Shu-9737	60	94	41.8	21.8	2.0	3.8	5.6	5.2	28.82
27.	Shu-9797	52	95	92.4	80.2	3.4	3.4	6.8	4.16	82.3
28.	Shu-9901	63	98	49.8	84.0	3.4	4.8	5.6	4.9	84.9
29.	PLU-184	62	99	95.8	64.4	3.0	3..94	6.6	4.7	94.0
30.	PLU 195	54	96	111	52.8	2.8	4.0	6.4	4.7	60.63
31.	PLU-199	58	97	78.6	42.4	2.8	4.4	6.8	5.12	58.26
32.	PLU-289	52	90	49.4	59.4	3.8	4.5	7.2	3.96	65.5
33.	PLU-305	53	96	57.2	74.4	3.0	5.0	7.6	4.94	91.6
34.	PLU-309	59	97	75.8	59.0	3.0	4.6	6.0	5.18	54.5
35.	PLU-329	63	98	115.3	76.0	3.0	3.9	6.6	3.9	67.7
36.	PLU-342	61	100	82.8	70.2	3.2	4.3	6.8	4.52	94.92
37.	PLU-347	63	99	78.2	43.8	3.2	4.25	6.0	4.72	47.66
38.	PLU-433	53	91	67.0	73.4	3.0	3.0	8.0	5.28	134.98
39.	PLU-730	62	97	50.6	85.8	3.2	4.8	6.6	5.4	56.26
40.	PLU-820	51	89	56.2	51.8	3.4	4.0	6.6	5.56	71.26
41.	IC-292	62	97	53.2	55.8	3.3	4.3	6.8	4.3	50.4
42.	IC-37176	54	96	60.8	78.0	3.8	4.8	7.2	5.34	82.08
43.	IC-73264	62	98	105	52.4	4.0	4.5	7.4	4.4	69.4
44.	IC-110664	55	89	55.2	57.6	2.0	4.2	5.8	5.9	53.4
45.	IC-201886	50	85	44.2	54.8	3.0	4.06	6.4	5.72	56.7
46.	IC-201887	55	90	71.8	37.4	3.2	4.6	6.2	4.9	42.24
47.	IC-201889	52	88	37.6	48.2	3.3	4.5	6.6	5.56	47.24
48.	IC-201892	52	89	34.6	50.2	2.6	3.7	6.0	4.96	51.78
49.	IC-201893	50	87	72.6	30.2	3.06	5.04	6.14	2.5	10.5
50.	IC-208468	61	97	56.4	44.6	2.6	4.8	6.6	6.46	50.38
51.	NIC-8190	62	98	71.2	67.4	2.0	4.6	6.4	5.2	58.14
52.	NIC-15266	50	88	56.2	46.0	3.0	4.3	6.0	4.38	52.94
53.	NIC-15274	53	91	86.8	56.6	2.4	4.0	6.4	5.24	63.7
54.	NIC-23231	52	97	69.4	52.6	3.0	4.8	6.4	4.3	70.8
55.	NC-73203	52	88	33.6	63.8	2.2	3.9	5.6	4.4	69.44
56.	JBT-9193	52	92	57.4	46.4	3.4	4.4	6.4	4.98	62.44
57.	SDI-29	51	90	68.0	52.6	4.0	4.4	6.8	5.0	51.73
58.	Pusa-105	62	99	83.0	67.8	3.4	4.0	7.0	5.32	98.90
59.	VL-310	52	86	50.2	68.4	3.8	4.4	6.6	5.6	71.9
60.	NKG-43	59	96	50.2	64.8	2.4	4.1	6.0	5.2	6.3.18
61.	PU-19	50	86	54.2	49.0	2.5	4.2	6.6	5.04	49.04
62.	Type-9	51	87	61.8	5.3	3.0	4.5	6.4	4.86	56.34
63.	NU-1	49	86	57.0	53.6	2.6	4.4	7.0	4.9	61.5
64.	PU-35	50	88	81.2	62.8	2.4	4.7	7.0	6.36	82.5

All sixty accessions and 4 checks in augmented design were analyzed using method given by Feeder [36,37] and elaborated by Feeder and Raghavarao [38] and Peterson [39], for nine quantitative traits and value of genotypic means were adjusted for the block effects measured by check's plots, which occurred in every block.

Principal Component Analysis (PCA): Adjusted mean values for nine quantitative characters of 60 accessions were analyzed using the concept of principal component (PC) based on multivariate technique [40,41]. For principal component analysis, accessions were identified on the basis of nine metric traits on a single point in a standardized multidimensional space. The axis of this space was principal components, obtained from the original data as orthogonal transformation of original varieties. In this way, each principal component becomes a linear combination of varietal scores corresponding to the original variables.

Non-hierarchical Cluster Analysis: The corresponding individual genetic distances between each accession serve as a basis for clustering of accessions with relation similarity within a cluster or relative dissimilarity between clusters. Genetic divergence among genotypes was studied using method of non-hierarchical euclidean cluster analysis described by Beale [8] and elaborated by Spark [9]. The principal component scores obtained from original variables were utilized for the analysis.

Isolation of Total Seed Storage Proteins and SDS-PAGE: Seeds of each accession were dehulled and ground to fine powder, 20 mg of this powder was mixed with 200 µl of sample buffer, containing 150 mM Tris (pH 6.8), 1% w/v SDS, 30% v/v glycerol, 15% v/v β-mercaptoethanol and 0.002% w/v Bromophenol blue and incubated at room temperature for overnight. Next day, samples were heated at 100°C in water bath for about 2-3 minutes thereafter centrifuged at 10000 rpm for 15 minutes. Supernatant (25 µl) was electrophoresed on SDS-PAGE (12% separating and 5% stacking gel) at 80 volts [42]. Protein bands of different intensities and positions were obtained after staining (in coomassie brilliant blue R) and relative mobitilies (Rm values) of different bands were calculated. A zero-one (presence or absence of bands) pattern was followed to study the diversity between genotypes by applying UPGMA (unweighted paired group method with arithmetical averages) method. Euclidean distance matrix was measured by the data acquired from SDS-PAGE and a dendrogram was constructed, which defines the clustering pattern of blackgram genotypes.

RESULTS

Agro Morphological Traits Evaluation: The germplasm showed enough range of variation for all nine quantitative (Table 1) and twenty four qualitative visual characters. Many accessions exceeded the limit of mean values obtained for four high yielding currently cultivated varieties used as checks (Table 2). On the basis of block errors, adjusted mean values for characters were 54.75 days, 91 days, 65.23 cm, 53.80, 3.20, 4.53 cm, 6.75, 4.99 and 62.49, computed for days to half flowering, maturity, plant height, pods per plant, pods per clusters, pod length, seeds per pod, 100-seeds weight and seed yield per plant, respectively.

Principal Component Analysis: Sixty blackgram accessions along with four checks were subjected to principal component analysis. Based on correlation matrix, eigen roots (eigen values) and eigen vectors were computed which represent eigen vectors, eigen roots, associated calculative vectors (Table 3) and cattle scree graph for variation shown by various principal components. The eigen vector for all nine components have been scaled (Table 3) in a manner that the largest value in each vector is unity. The elements were interpreted as the relative weight given to the variables in each component and those having the highest positive and negative value are important variables.

The first principal component had highest eigen root of 2.811 followed by eigen roots of 1.689, 1.235, 1.111, 0.904, 0.499, 0.438, 0.188 and 0.125 respectively. The first principal component accounted for 31.23 percent of total variation present in the original data followed by second to ninth which had the values of 18.76, 13.72, 12.35, 10.05, 5.54, 4.87, 2.09 and 1.39 respectively. The first two components together, accounted for 49.99 percent of cumulative variation while the first three components together constituted 63.71 percent of total variation. The first four components together had 76.24 percent, first fifth together had 86.29 percent, first sixth had 91.83 percent and first seventh had 96.70 percent of total variation present in the original data units. The first eight principal components which together explained the 98.79 percent of variance present in original data were utilized for non-hierarchical euclidean cluster analysis based on principal component analysis.

Non Hierarchical Euclidean Cluster Analysis: In present investigation, all variables were converted into single index of similarity in the form of principal component and a non-hierarchical euclidean cluster analysis carried out to estimate genetic divergence among 64 accessions of

Table 2: Mean, range and least significant differences in blackgram checks

Characters	Adjusted mean values*	Checks				Coefficient of variation (CV %)	Least significant difference			
		C1PU-19	C2NU-1	C3Type-9	C4 PU-35		CM	AVSB	AVDB	AVAC
Days to 50 percent flowering	54.75(48-63)	49.66	49.50	49.66	50.66	1.47	0.905	2.218	2.480	1.894
Days to maturity	91.00(81-100)	85.50	84.66	84.50	86.16	2.05	2.153	5.276	5.898	4.505
Plant height (cm)	65.23(33.6-116.4)	52.20	45.70	47.80	48.20	12.90	7.696	18.852	21.077	16.098
Pods per plant	53.80(10.9-95.8)	54.90	44.63	43.96	46.13	24.40	14.239	34.878	38.995	29.783
Pods per cluster	3.20(1.6-5.4)	3.73	3.76	3.93	3.53	17.35	0.799	1.957	2.188	1.677
Pod length (cm)	4.53(3.0-4.9)	4.66	4.48	4.38	4.40	7.52	0.415	1.016	1.136	0.868
Seed per Pod	6.57(5.0-8.4)	6.53	6.50	5.73	6.53	5.66	0.440	1.076	1.207	0.921
100 seed weight (g)	4.99(2.5-6.4)	5.02	4.97	4.82	5.75	4.33	0.274	0.671	0.750	0.573
Seed yield per plant (g)	62.49(10.5-134.9)	59.93	50.90	57.26	59.97	15.57	10.927	26.766	29.926	22.856

*Adjusted mean values for characters on the basis of block errors, Range given in Parentheses (n1-n2), CM= between check means, AVSB= between adjusted mean of two test genotypes in the same block, AVDB = between adjusted mean of two test genotypes in different blocks, AVAC= between an adjusted mean of a test genotypes against check 'n'

Table 3: Eigen vectors, eigen roots and associated variation for different principal components in blackgram germplasm

VARIABLES	EIGEN VECTORS Components→								
Characters ↓	1	2	3	4	5	6	7	8	9
Days to 50 percent flowering	0.467	0.536	0.418	0.364	-0.174	0.126	-0.056	0.335	-0.160
Days to maturity	-0.315	-0.170	0.030	-0.495	0.535	0.127	0.123	0.501	0.239
Plant height (cm)	0.170	0.042	-0.235	0.027	-0.220	0.621	0.583	-0.127	0.352
Pods per plant	0.107	0.148	0.211	-0.066	0.321	0.304	-0.556	-0.365	0.529
Pods per cluster	0.025	0.066	0.427	-0.180	-0.066	-0.548	0.389	0.033	0.569
Pod length (cm)	-0.622	0.749	-0.097	-0.163	0.050	0.045	0.091	-0.034	-0.050
Seeds per pod	-0.418	-0.155	0.081	0.385	-0.706	0.028	-0.296	0.002	0.246
100 seed weight	-0.277	-0.265	0.704	-0.310	-0.003	0.406	0.131	0.002	-0.282
Seed yield per plant (g)	0.060	-0.016	-0.181	-0.561	-0.165	0.147	-0.261	0.697	0.217
Eigen roots	2.811	1.689	1.235	1.111	0.904	0.499	0.438	0.188	0.125
Variation (%)	31.23	18.76	13.72	12.35	10.05	5.54	4.87	2.09	1.39
Cumulative Proportion of variation (%)	31.23	49.99	63.71	76.24	86.29	91.83	96.70	98.79	100.00

Table 4: Cluster mean, standard deviation (SD), coefficient of variability (CV) for different quantitative characters in blackgram germplasm

		NON-HIERARCHICAL CLUSTER NUMBER							
		I	II	III	IV	V	VI	VII	VIII
	Accessions (Entry Number) →	7,10,17,38, 42,44,45, 48,50,51, 57,60,64	24,33, 56,61, 62	5,8,11, 12,14,18, 19,30,58	21,23,25, 26,31,32, 34,54	1,3,4,6,16,20, 27,28,36,37, 39,40,41,43,47, 52,53,55,59	22,46	2,9, 13,15, 29,49, 63	35
CHARACTERS↓	Number of Accessions	13	5	9	8	19	2	7	1
Days to 50 percent flowering	Mean	51.31	59.09	49.33	58.78	50.83	58.40	51.70	62.00
	SD	1.78	3.75	1.00	4.44	1.19	3.13	2.26	0.00
	CV	3.46	6.34	2.02	7.55	2.34	5.35	4.37	0.00
Days to maturity	Mean	87.12	95.91	84.56	96.89	85.83	91.60	90.30	97.00
	SD	1.86	2.66	2.79	2.76	3.20	3.78	4.11	0.00
	CV	2.13	2.77	3.29	2.84	3.72	4.12	4.54	0.00

Table 4: Continued

Plant height (cm)	Mean	51.25	68.58	53.71	94.63	52.10	53.64	72.55	50.60
	SD	13.81	12.55	10.51	15.29	7.51	12.90	17.40	0.00
	CV	26.94	18.29	19.56	16.15	14.41	24.04	23.98	0.00
Pods per plant	Mean	40.08	56.53	51.78	66.49	47.76	30.82	66.94	85.80
	SD	14.51	12.21	9.33	9.75	8.74	13.50	14.50	0.00
	CV	36.20	21.59	18.01	14.66	18.29	43.80	21.66	0.00
Pods per cluster	Mean	3.18	2.85	4.80	3.24	3.42	1.96	3.14	3.20
	SD	0.53	0.45	0.48	0.36	0.42	0.41	0.45	0.00
	CV	16.66	15.78	10.00	11.12	12.28	20.91	14.33	0.00
Pod length (cm)	Mean	4.31	4.45	4.71	3.95	4.55	4.22	4.94	4.80
	SD	0.43	0.31	0.23	0.49	0.25	0.36	0.35	0.00
	CV	9.97	6.96	4.88	12.40	5.49	8.53	7.08	0.00
Seeds per pod (g)	Mean	6.22	6.30	6.78	6.87	6.61	5.76	7.11	6.60
	SD	0.67	0.38	0.45	0.55	0.40	0.57	0.68	0.00
	CV	10.77	6.03	6.63	8.00	6.05	9.89	9.56	0.00
100 seed weight (g)	Mean	4.55	4.93	5.05	4.72	5.31	5.60	5.26	5.40
	SD	063	0.35	0.51	0.55	0.35	0.57	0.57	0.00
	CV	13.84	7.09	10.09	11.65	6.59	10.17	10.83	0.00
Seed yield per plant (g)	Mean	46.34	62.58	64.67	88.19	56.54	38.29	76.35	56.26
	SD	16.29	13.23	8.91	22.24	10.14	14.21	11.14	0.00
	CV	35.15	21.14	13.77	25.21	17.93	37.11	14.59	0.00

SD: Standard Deviation; CV: Coefficient of Variation

blackgram. Cluster analysis coincided 60 genotypes with four checks, into eight non-overlapping clusters (Table 4) which was an appropriate cluster arrangement determined by F-test. Distribution of accessions in different cluster have been denoted by entry number as of Table 1. The cluster mean, standard deviation and co-efficient of variation is given in Table 4. Thirteen genotypes were grouped in cluster I, five in cluster II, nine in cluster III, eight in cluster IV, nineteen in cluster V, two in cluster VI, seven in cluster VII and only one genotype in cluster VIII (Table 4).

Genetic Donors for Different Characters: Based on screening for different quantitative traits some accessions were identified as genetic donors as these were significantly superior over the best checks, used in the study. For days to 50 percent flowering, accessions ShU 9519, ShU 9601, ShU 9603, ShU 9612, ShU 9619, ShU 9621, ShU 9626, ShU 9636, ShU 9642, IC 201886, IC 201893 and NIC 15266 were found to be early types while accessions ShU 9505, ShU 96110, ShU 9901, PLU 184, PLU 329, PLU 342. PLU 347, PLU 730, IC 292, IC 73264, IC 208468, NIC 8190 and Pusa 105 were observed to be late type. However, in terms of days to maturity, ShU 9519, ShU 9536, ShU 9612, ShU 9614, ShU 9621 and ShU 9626 were early maturing type while ShU 9901, PLU 184, PLU 329, PLU 342, PLU 347, IC 73264, NIC 8190 and Pusa 105 were late maturing accessions.

In concern to plant height, ShU 9632, ShU 9725, ShU 9797, PLU 184, PLU 195, PLU 329 and IC 73264 were tall accessions while ShU 9682, IC 201889, IC 201892 and NC 73203 were dwarf genotypes. For number of pods per plant, accessions ShU 9619, ShU 9797, ShU 9901, PLU 305, PLU 329, PLU 342, PLU 433, PLU 730 and IC 37176 had higher number of pods per plant however accessions ShU 9511, ShU 9601, ShU 9603, ShU 9619, ShU 9633, IC 73264 and SDI 29 had higher number of pods per cluster. Longer pods were observed in accessions ShU 9508, ShU 9519, ShU 9601, ShU 9632, ShU 9636, ShU 9641, ShU 9682, PLU 305 and IC 201893 whereas higher seeds per pod were notified in accessions ShU 9519, ShU 9601, ShU 9603, ShU 9632, ShU 9641, ShU9682, PLU 289, PLU 305, PLU 433, IC 37176, IC 73264 and Pusa 105. Accessions ShU 9505, ShU 9619, ShU 9626, ShU 9725, ShU 9901, PLU 184, PLU 305, PLU 433, PLU 342 and Pusa 105 yielded higher seeds per plant in comparison to check accessions. However in terms of 100-seeds weight, only one accession IC 208468 was observed to be superior over checks.

Variability in Seed Storage Proteins: Total seed storage proteins of sixty blackgram genotypes along with four checks, used in field experiments, were analyzed by

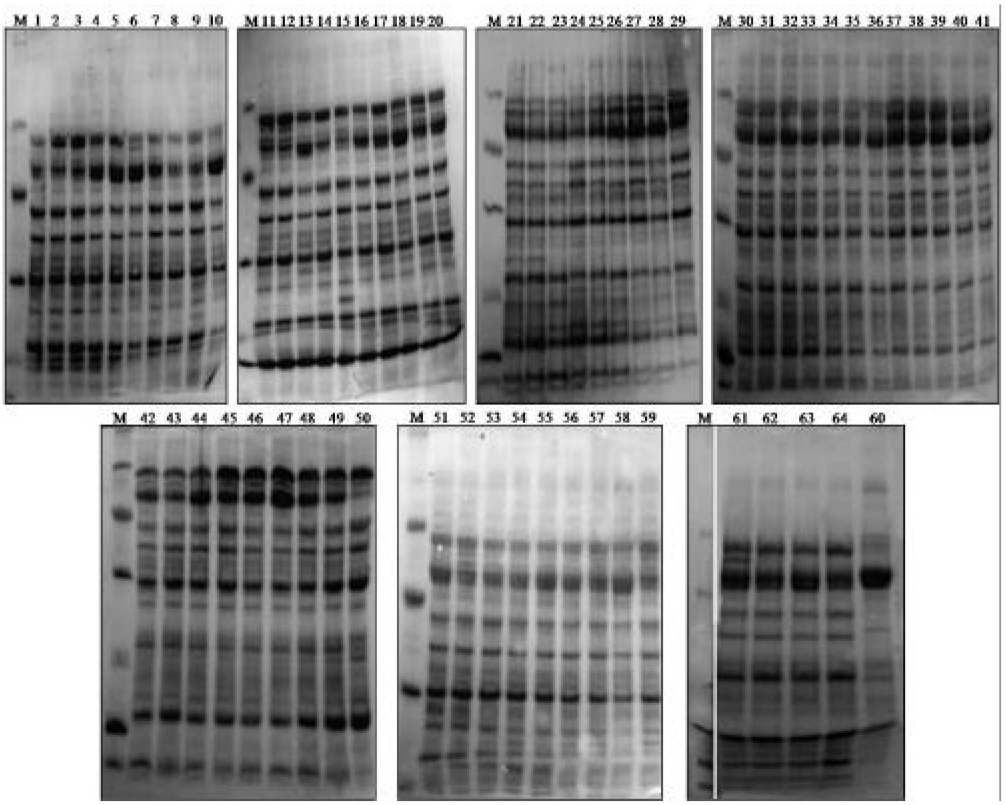

Fig. 1: Seed storage protein profile (SDS-PAGE) of blackgram (*Vigna mungo*) accessions; M: Marker and 1-60: entry no. of accessions (Table 1)

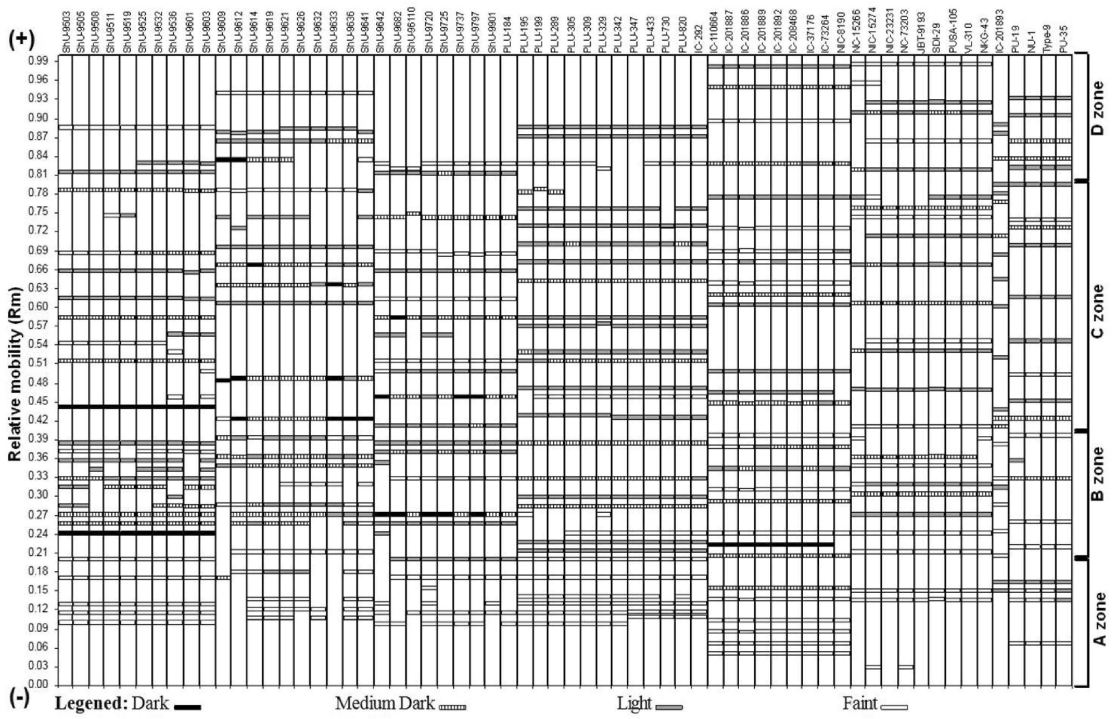

Fig. 2: Zymogram of electrophoretic banding patterns for SDS-PAGE of total seed proteins of sixty blackgram genotypes along with four checks

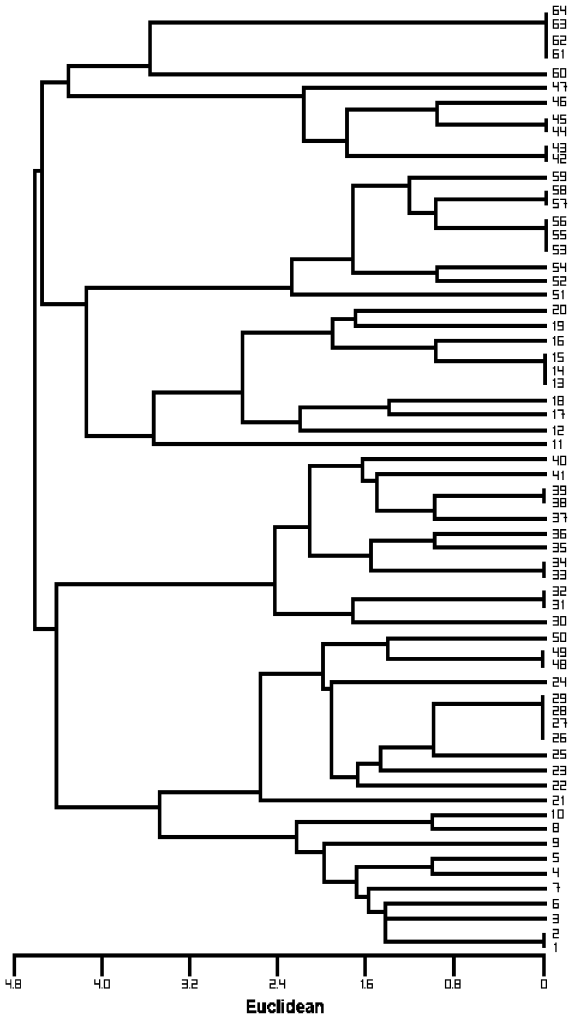

Fig. 3: Dendrogram for Seed storage protein profile (SDS-PAGE) of blackgram accessions (entry no. 1-64 as per Table 1) constructed by using UPGMA based Jaccard's similarity coefficient

SDS-PAGE (Figure 1). Banding pattern obtained on gel was utilized to illustrate zymogram, representing the relative position and intensities of bands (Figure 2). The whole zymogram was divided into four zones (A, B, C and D) on the basis of bands intensity viz. dark, medium dark, light and faint bands. The detailed study of zymograms revealed total number of protein bands, ranging from 17 to 28, spread over four zones (A, B, C and D), into eight groups based on number of bands and their intensity. Both high and low molecular weight proteins were included in these zones. In general, it was seen that genotypes varied greatly for the number of high molecular weight proteins, however their intensities were similar while in low molecular weight proteins, the differences recorded were marginal. Maximum genotypes included in this group had almost equal number of bands in each zone with maximum bands in zone C. Most of the bands among genotypes included in this group had light to faint intensity bands. There were two genotypes in seventeen band group, nine genotypes in twenty band group, eight genotypes in twenty two band groups, eleven genotypes in twenty three band group, six genotypes in twenty four band group, three genotypes in twenty five band group, six genotypes in twenty six band group and nineteen genotypes clustered in twenty eight band group.

Genotypes were differentiated from each other on the basis of Rm value and intensities of bands both. Some Rm values were common to all genotypes and many were not found in others, called specific Rm values. The genotypes were distinguished on the basis of differences in Rm values, calculated for each genotype and utilized to estimate distance coefficients ranging from 0.00 to 5.39 and represented the similarity index among the genotypes. On the basis of Euclidean distance matrix, dendrogram (Figure 3) was drawn, which classify genotypes into nine clusters. Clusters IV and VIII contained only one genotype and clusters V and VI had nine genotypes each. Clusters IX, VII and I had four, six and ten genotypes respectively while cluster II and III were the largest group including twelve genotypes each.

Cluster I and II, interrelated to each other with coefficient 3.45, was correlated to cluster III by 4.45, while cluster IV and V coinciding each other at coefficient 3.50, allied to cluster VI at coefficient 4.30. Cluster VIII and IX interlinked to each other with coefficient 3.65 and was coincided with cluster VII at coefficient 4.4. Major cluster, including clusters IV, V and VI coincided with another major cluster including clusters VII, VIII and IX at coefficient 4.60, which cumulatively concurred with group of clusters I, II and III at a coefficient 4.70.

Cluster I contained ten genotypes with maximum intra cluster distance of 2.83 between ShU 9519 and ShU 9603 and minimum intra cluster distance (0.00) between ShU 9503 and ShU 9505. Cluster II included twelve genotypes out of which 4 genotypes ShU 9737, ShU 9797, ShU 9901 and PLU 184 exhibited minimum intra cluster distance (0.00) to each other while rest displayed variability. Cluster III also included twelve genotypes in which three pair (ie. 6 no.) of genotypes exhibited cent-percent pair-wise similarity. Nine genotypes were coincided in cluster V with minimum genetic dissimilarity (0.00) for genotype ShU 9614, ShU 9619 and ShU 9621, however rest exhibited variability. Cluster VI contained nine genotypes including two set of genotypes (SDI 29-Pusa 29 and NIC 15274-JBT

9193-NC 73203) with minimum intra cluster distance. In cluster VII, zero distance was observed between genotypes IC 110664 and IC 201887 and genotypes IC 37176 and IC 73264, while cluster IX contained 4 genotypes and all exhibited zero euclidean genetic distance, however cluster IV and VIII had only one genotype.

DISCUSSION

Blackgram (*Vigna mungo* (L.) Hepper) is the fourth important legume crop after chickpea, pigeonpea and soybean. Therefore, the possibility to obtain superior genotypes for various morpho-agronomical traits needs to be explored through field evaluation and characterization of diversity, present among genotypes. It involves description of variation for morphological traits, particularly agro-morphological characteristics of direct interest to users. The variability present for agronomic as well as economic traits, abiotic and biotic stress in blackgram has been studied by various markers [43,44].

Genotype ShU 9601 flowered in 48 days, quite earlier than all the checks. Similarly, minimum duration of maturity (81d) was observed for ShU 9519, ShU 9536 and ShU 9621 and was significantly earlier than check varieties. For plant height, pods per plant, pods per cluster, pod length, seeds per pod and 100-seed weight, the upper limit of range were 116.4 cm, 95.8, 5.4, 4.9 cm, 8.4 and 6.4 g respectively which was again significantly higher than those of the check varieties. These accessions with such characters may be utilized either directly or for hybridization programme. Accession PLU 433 gave highest seed yield per plant (134.98 g) followed by Pusa-105 (98.90 g). Earliest maturity of 81 days was observed in three genotypes ShU 9519, ShU 9536 and ShU 9621 followed by ShU 9612 and ShU 9614 (83 days) while highest days to maturity were shown by PLU 342 (100 days).

The highest value for plant height was elicited by genotype ShU 9632 (116.4 cm) while the shortest height was obtained for NC 73203 (33.6 cm). ShU 9619 had highest number of pods per plant (95.8) whereas the lowest pods per plant (10.9) was counted for ShU 9720. The accession ShU 9603 had highest number of pods per cluster (5.4), However ShU 96110 and ShU 9720 have lowest number of pods per cluster (1.6). Largest pods (5.5 cm) were recorded for the accession ShU 9641 while shortest pods (3.0 cm) were obtained in PLU 433. Highest number of seeds per pod (8.4) was also elicited in ShU 9641 whereas lowest value (5.0) was recorded for ShU 9720. The highest 100-seed weight (6.46 g) was observed for IC-208468 while genotype IC-201893 had lowest 100-seed weight (2.5 g). For seed yield per plant, ShU 9619, ShU 9727, PLU 184, PLU 305, PLU 342, PLU 433 and Pusa 105 were found high yielding which are indicative of availability of high yielding germplasm and may be tested in wide areas. These results suggest the richness of variability in existing collection and scope of improvement over existing checks.

Principal component analysis is an ordination method and concerned with the variances and covariance of elements of a random vector. It provides a spatial arrangement for accessions in a geometric space. The objective of principal component analysis is to construct new variables from original characters. Principal component analysis is optimized in the sense that the information lost during the formation of new variables from original is kept to the minimum. With this objective of principal component analysis, the multidimensional data of present study was condensed into manageable numbers of new variables without loosing any vital information about the original data. This provides a multidimensional viewing of original data and discloses their nature of variation in a multivariate setup.

The eigen roots associated with nine vectors explained 100 percent of variation present in whole data set. These nine vectors were utilized for ordination. Kaisre [45] suggested that only first three components should be used since they have eigen roots more than unity but in present study, first three components accounted only for 63.71 per cent of total variance. Cattell's [46] approach of scree test method suggested that only those components should be kept which follow a large gap in variance on score graph. First 5 components in present study, accounted only for 86.29 percent of total variance as per Cattell's criteria. Thus, these two approaches tend to give few components and lost at least one third of the information, so inappropriate for this investigation.

However, Rao's approach [7] based on covering 90 percent of total variance seems to be more appropriate and as such or with some modifications, have been used by most of earlier workers. Thus, first eight components, which accounted for 98.79 percent of total variation, were taken for summarization of original data on blackgram genotype in reduced dimensions. Similar approach has also been used in classifying pasture data on posture [47], mungbean [48] and blackgram [15, 29].

Jeffers [49] suggested that these elements may be interpreted as the relative weights given to the variables in each component and important variables are those, which have high positive or negative weights.

The sixth component had all positive weight except pods per cluster, therefore represent an index of seed yield contributing characteristics. However, fifth component was found to have maximum of six negative weights. Highest positive weight for seed yield was found for eighth component (0.697), also having positive weight for seeds per pod and 100-seed weight.

The interpretation of scaled eigen vectors of principal component in present study were in agreement with those made by Jeffers [49] in winged aphid data, Dudzinsky and Arnold [47] in pasture and Ghafoor *et al.* [15] in blackgram. The principal component analysis summarized large multidimensional data into reduced dimensions utilizing categorization of genotypes into homogenous groups, based on genetic distance among genotypes. Euclidean non-hierarchical cluster analysis [8-9] based on principal component analysis [40-41] is a method of numerical taxonomy and found to be useful for estimating genetic divergence in this study. However, clustering of large number of genotypes from different places, in single cluster such as cluster V, indicated that some genotypes have moved with time to different regions from same locality. Clustering pattern also indicated no correlation between eco-geographical distribution of genotype and genetic divergence, as genotypes selected from diverse locations were clustered together. On the other hand, genotypes from same geographic region were distributed in different clusters. This type of genetic diversity may be due to differential adaptation, selection criteria, selection pressure and the environment which support that genetic drift and selection in different environment may produce greater diversity compared to geographic diversity [15, 20,23,26,27,30,48].

Cluster IV had eight genotypes and it showed maximum intra cluster distance (2.220). These genotypes were heterogenous and wider within group genetic distance and therefore it may be best for within group hybridization. On the other hand, cluster V, contained minimum intra cluster distance (1.362) with 19 genotypes, suggested that most of genotypes of this cluster were close to each other, either in traits or origin.

Cluster VIII showed maximum inter cluster genetic distance from cluster III (10.270) suggesting wide diversity between these two clusters. These clusters had maximum variability for days to 50 percent flowering, maturity and pods per plant. Cluster III contained all early maturing varieties while cluster VIII had late maturing variety. These cluster also had higher values for pods per cluster, pod length, seeds per pod and 100-seed weight. From study it may be concluded that hybridization between clusters III genotypes and the one present in cluster VIII will produce a variable progeny.

Cluster I and V had minimum genetic distance (1.656) between them which indicate these genotypes were somewhat similar in genetic constitution and hybridization between these groups may not result in sufficient variability or likely to give heterotic desirable segregate for the characters under classification. Consequently, a crossing programme may be formulated in such a way that the parents should belong to different clusters to yield heterotic and transgressive segregates.

Cluster III had early maturing genotypes while genotypes in cluster VIII were all late maturing. Taller genotype was present in cluster IV, whereas shorter types were present in cluster VIII. For pod length, cluster III, VII and VIII contained higher mean values while for pods per plant, higher mean values were observed in clusters IV, VII and VIII however genotypes with higher pods per cluster, higher seeds per pod and maximum 100-seed weight, were present in cluster III, cluster VII and cluster VI respectively. For seed yield per plant, genotypes of cluster IV had maximum mean value and PLU 433 yielded 134.98 g seeds per plant beside this it also had higher number of seeds per pod and an early maturity of 91 days. In present study genotypes of cluster IV are identified as promising accession for genetic donors.

Stability in seed storage protein profile made it an additional tool for species identification besides other traditional biosystematics approaches and it has been used for large number of genera, in order to resolve taxonomic and evolutionary problems. The clusters constructed by using seed storage protein profile (Figure 3) were related to each other at a certain co-efficient value, where they merged to form a major cluster. Farther the clusters, more diverse were the genotypes and vice-versa. All minor clusters enclosed in major cluster which is an indicative of some commonness among all the genotypes. In case of similarity index (SI), some genotypes had zero distance or 100 percent similarity to each other within a cluster but the same genotype exhibited a particular genetic distance with others comprised in another cluster and showing differences with them. Therefore, all thirty genotypes having zero distance or 100-percent similarity could not be considered as one cluster as existed in different clusters. Genotypes included in one cluster were considered to be similar but since they had different origin and geographical distribution, they too had some distance with each other and those having 100 percent similarity were supposed to have similar genetic constitution and hence should not be used in crossing programme.

CONCLUSION

In present study, it may be concluded that genotypes of clusters III and VIII were ideal for the group hybridization, in resultant, a variable progeny will be generated while genotypes of cluster IV were best for within group hybridization. Genotypes of cluster IV were distinguished as auspicious accession for genetic donors, however individually, accessions ShU 9619, PLU 305 and PLU 433 were identified very promising accessions and it can be explored for future improvement by breeding and genetic engineering. It was observed that the number of clusters formed and genotypes included among clusters by two different procedures, provided a different clustering pattern. It was revealed that overall eight clusters were formed with quantitative traits analysis whereas nine clusters were obtained with SDS-PAGE analysis of total seed storage protein. The number of clusters obtained by quantitative traits and SDS-PAGE of total seed proteins were almost the same but the genotypes included in each cluster varied, which was an indicative of environmental influence over the traits.

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