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EXPLOITATION OF *VIGNA MUNGO* (L.) HEPPER GERMPLASM USING MULTIVARIATE ANALYSIS BASED ON AGRONOMIC TRAITS

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Abstract

Twenty one genotypes representing a broad based germplasm were selected for evaluation using multivariate analyses for two years. High genetic variance was observed for plant height, maturity, pods, seed weight, biomass, grain yield and harvest index. The genotypes, Mash 3, 9039, 9067, 9010, 9026, 9092, 9005, 9025 and 9020 were observed early and uniform in maturity. First four PCs contributed 80.0% of variation during 1998 and 80.9% during 1999. Five yield contributing traits, i.e., branches, pods, pod length, biomass and grain yield were observed important for first component during both the years. PC_2 was more related to maturity traits rather than reproductive traits. Five genotypes were separated from others during both the years. Cluster analysis revealed that only a portion of genetic diversity has been exploited, and it is suggested to broaden the genetic base of cultivated blackgram involving diverse parents in breeding programme for future use.

Introduction

Blackgram or mash [*Vigna mungo* (L.) Hepper] has been cultivated on marginal lands with least inputs in Asia since ancient times (Paroda & Thomas, 1987). During the last two decades, mungbean cultivation has spread widely mainly because of availability of superior germplasm developed by Asian Vegetable Research and Development Centre (AVRDC) which is better adapted to a wide range of ecological zones. Although blackgram has been identified as a high yielding crop in many Asian countries, but due to its sensitivity to environmental fluctuation, high yielding and stable genotypes are yet to be explored/ identified. Various numerical taxonomic techniques have been successfully used to classify and measure the pattern of genetic diversity in legume crops by many scientists, as in blackgram (Shanmugam & Shreerangaswamy, 1982; Dasgupta & Das, 1984 & 1985, Ghafoor *et al*., 2000), mungbean (Singh, 1988; Bisht *et al*., 1998), pea (Amurrio *et al*., 1995); soybean (Perry & McIntosh, 1991); alfalfa (Smith *et al*., 1995) and lentil (Ahmad *et al*., 1997).

In blackgram, the availability of scientific literature is limited because this crop is not much researched and no systematic study has been carried out to investigate the extent of genetic variation and identification of high yielding and stable genotypes along with relationship between various groups for future utilisation. In order to develop high yielding cultivars resistant to stresses, exploitation of genepool is of paramount importance. Present study was thus designed to investigate the extent of genetic diversity in blackgram germplasm and to identify promising germplasm for future utilization.

Materials and Methods

Blackgram germplasm consisting of 484 accessions was characterised and evaluated for 18 different traits (Table 1). The germplasm was classified into 5 clusters using average distance (Ghafoor *et al*., 2001). Out of these, 21 genotypes including one exotic cultivar (PL-2 from AVRDC) and three approved varieties (Mash 1, Mash 2 and Mash 3) were selected on the basis of their performance. Although, blackgram is highly self-pollinated plant (flower cleistogamy in nature) but to ensure homozygosity, the selected genotypes were self-pollinated for 2 years prior to experimentation. The experiments was carried out during summer seasons of 1998 and 1999 in randomised complete block design (RCBD) in four replications at National Agricultural Research Centre, Islamabad, Pakistan (33.40 ^{o} N and 73.07^{o} E). Two rows of 4 meter length for each genotype in each replicate were planted with 10 cm intra-row spacing, whereas inter-row distance was kept 50 cm. Pesticides were sprayed to save the crop from infestation by pests especially white fly (*Bemisia tabaci* Genn.), a vector for Mungbean Yellow Mosaic Virus (MYMV). For evaluation, data were recorded following descriptors for *Vigna mungo* and *V. radiata* (Anon., 1985). The data for days to flowering and maturity were recorded on line basis at 50% of flowering and 90% pod maturity and each genotype was represented by single value. Other quantitative data, i.e., branches, pods, grain yield (g) and biomass (g) were recorded on 10 plants sampled randomly. Pod length (cm) and seeds per pod were recorded on 10 pods sampled at random within each genotype. Pods per branch were calculated, whereas seed weight was recorded after counting 100 seeds in grams. Harvest index was determined as economic yield expressed in percentage over total biomass. The disease data presented in the Table 1 were recorded in a different set of experiments under greenhouse conditions where crop was not sprayed with any pesticide.

The data recorded were averaged and analyzed for analysis of variance and genetic advance using standard techniques described by Singh & Chaudhry (1985). The averaged data were analyzed by numerical taxonomic techniques using the procedure of cluster and principal component analyses (Sneath & Sokal, 1973) with the help of computer software "Statistica" and "SPSS" for Windows 95.

Results

Table 1 presents the evaluation and characterization data of the germplasm evaluated during summer season of 1995. High genetic variance was observed for plant height, maturity, pods, biomass, grain yield and harvest index on the basis of 2 years' analyses (Table 2). The characters which were more influenced by environmental variation were plant height, days to flowering, branches, pods/plant, seeds/pod, biomass, grain yield and harvest index, whereas maturity, pod length and seed weight were less affected by environmental variation. Although approved varieties, Mash 2 and Mash 3 were high yielding, both of these were more affected by environmental variation, whereas cultivars 9010, 9093, 9086 and 9020 were high yielding and less affected by environmental fluctuation. The genotypes, Mash 3, 9039, 9067, 9010, 9026, 9092, 9005, 9025 and 9020 were observed uniform in maturity and it was also noticed that all of these were early maturing.

Table 1. Range of variation in quantitative characters and predominance of qualitative descriptors status of the whole blackgram germplasm evaluated during 1995.

Evaluation Traits	Mean±SE	σ^2	h^2
Plant height	28.6±1.21	98.81	0.56
Days to flowering	47.2±0.46	101.91	0.65
Days to maturity	85.4±0.60	175.38	0.64
Branches/plant	15.2±0.38	68.59	0.46
Pods/plant	43.8±1.21	704.12	0.51
Pods/branch	3.0±0.07	2.26	0.40
Pod length (cm)	4.5±0.02	0.21	0.47
Seeds/pod	6.2±0.03	0.52	0.43
Seed weight (g)	4.77±0.03	0.31	0.33
Biomass (g)	44.44±1.30	812.13	0.61
Grain yield/plant (g)	10.02±0.32	48.15	0.49
Harvest index (%)	23.28±0.41	79.99	0.30

SE- standard error, σ^2- variance and h^2- broad sense heritability

Characterisation	
Hairiness	Pubescence occurs predominantly as 78.7 % of the germplasm was having hairs on plants.
Growth habit	About 80 % of the germplasm was observed semi-erect, 15 % was erect and 5 % was of spreading nature.
Leaf shape	Deltate leaf shape was predominant, as it was in 342 accessions, ovate in 113, lanceolate in 20, rhombic in 3 and obvate in 6 accessions.
Seed colour	Brown seed coat colour was highest in frequency as it was in 99.7 % of the germplasm and only 6 accessions were having green seed coat colour.
Seed spots	Similarly 99.5 % of the germplasm were having spots on seed testa, only 7 accessions were without spots.
Mungbean Yellow Mosaic Virus (MYMV)	The disease was recorded in a separate set of experiment under greenhouse conditions where crop was not sprayed with any pesticide, which enhance white fly population that ultimately increased disease incidence. 331 accessions were observed tolerant to MYMV and rest of the germpalsm was susceptible.

Principal component analysis

First four components with eigenvalues >1 contributed 80.0% during 1998 and 80.9% during 1999 of the variability amongst 21 genotypes evaluated for 13 quantitative traits (Table 3). Characters contributing more positively to PC_1, during 1998 were branches, pods, pod length, seed weight, biomass and grain yield, whereas plant height, branches, pods, pod length, seeds/pod, biomass and grain yield contributed more during 1999. Five yield contributing traits (branches, pods, pod length, biomass and grain yield) were observed important for first component during both the years, and the populations contributing to this PC were more related to reproductive traits rather than vegetative traits. The PC_2 was more related to maturity traits rather than reproductive traits. Plant height contributed maximum for PC_3 during 1998, whereas it was assessed

Table 2. Mean±SD, analysis of variance and genetic parameters in 21 genotypes of blackgram evaluated for two years.

Sr.	Genotype	Plant height	Days to flowering	Days to 1st flower	Days to maturity	Branches/ plant	Pods/plant	Pod length	Seeds/pod	Seed weight	Biological yield	Grain yield	Harvest index
1	Mash 3	41.0±1.41	39±7.07	63±1.41	67±2.12	27.8±13.79	70.0±53.74	4.70±0.40	6.3±0.35	4.41±0.33	47.88±16.64	15.13±12.20	29.31±15.99
2	9006	52.5±3.54	36±2.47	62±2.83	71±9.55	1±3.421.6	34.3±2.36	4.52±0.14	6.2±0.28	4.06±0.06	28.98±17.41	5.83±0.98	23.26±10.57
3	9039	33.0±2.83	39±5.30	60±0.71	65±2.47	15.4±3.42	32.1±2.71	4.56±0.08	6.3±0.07	4.23±0.05	21.47±7.36	6.64±1.24	31.71±5.25
4	9047	34.5±3.54	41±2.47	57±1.41	67±3.89	10.8±5.30	27.2±10.14	4.37±0.07	6.0±0.08	4.31±0.72	16.98±3.94	4.62±2.38	26.27±7.87
5	9067	29.0±1.41	38±0.00	66±2.12	70±0.00	8.0±0.00	16.0±0.00	4.26±0.00	6.8±1.84	4.10±0.00	8.94±1.05	3.50±0.56	39.15±1.99
6	9010	33.5±2.12	41±4.60	60±0.71	66±1.06	10.8±4.48	74.8±4.48	4.37±0.07	6.2±0.35	4.83±0.81	42.41±7.62	14.60±2.82	37.84±1.09
7	Mash 1	23.0±1.41	39±1.77	63±3.54	73±1.77	13.0±7.07	41.4±28.87	4.44±0.06	5.9±0.07	4.46±0.28	22.44±10.87	6.80±1.98	32.26±6.15
8	9093	31.5±2.12	38±0.00	56±5.66	77±0.00	13.0±0.00	43.0±0.00	4.62±0.00	9.2±1.10	4.38±0.00	27.28±1.56	9.29±1.01	34.05±1.05
9	PL-2	30.0±5.66	47±0.00	61±1.41	74±2.85	9.0±0.00	23.0±0.00	4.30±0.00	6.8±1.84	3.80±0.00	14.90±1.97	4.95±0.95	33.22±0.99
10	9026	32.0±2.83	41±3.18	61±2.12	66±1.09	23.5±10.61	36.3±10.25	4.44±0.03	6.1±0.14	3.97±0.38	20.86±11.33	6.17±2.82	30.44±2.98
11	Mash 2	41.0±1.41	39±7.07	59±1.41	68±2.83	22.7±6.58	67.6±44.72	4.48±0.04	5.8±0.25	4.28±0.06	35.17±15.61	12.74±5.84	36.12±0.62
12	9086	26.0±4.24	41±1.41	63±1.41	73±6.72	21.0±18.38	75.4±20.39	4.59±0.21	6.0±0.21	3.96±0.13	50.65±14.63	17.14±4.62	35.55±2.82
13	9080	20.5±4.95	49±0.00	71±1.41	80±0.71	4.0±0.00	5.0±0.00	3.92±0.00	6.8±2.41	3.90±0.00	2.23±2.05	0.82±1.01	36.77±0.94
14	9092	18.0±2.83	36±3.18	59±1.46	63±0.35	21.5±19.09	31.8±13.91	4.35±0.04	6.0±0.00	4.32±0.06	19.32±11.33	5.61±1.53	32.27±11.06
15	9005	21.0±1.41	36±3.18	60±0.72	64±1.08	15.50±0.71	35.7±6.60	4.08±0.20	5.8±0.28	4.09±0.21	20.52±5.21	6.59±1.41	32.44±1.18
16	9025	19.5±0.71	36±3.89	59±1.49	63±1.77	13.8±0.35	33.2±6.84	4.37±0.06	5.8±0.00	4.03±0.47	18.64±5.17	5.33±0.85	30.00±13.52
17	9056	48.0±2.83	41±1.06	51±1.89	58±2.85	15.3±3.89	42.7±25.83	4.48±0.08	6.0±0.00	4.16±0.02	25.50±15.00	8.14±6.68	28.50±10.08
18	45737	39.5±0.71	39±9.90	65±2.54	78±3.54	13.0±8.49	19.7±15.98	4.32±0.42	5.8±0.85	4.47±0.33	23.93±9.30	4.30±4.17	15.77±11.30
19	9020	39.0±1.41	41±0.92	63±2.04	68±2.33	16.6±5.02	40.1±5.85	4.56±0.14	6.9±1.00	4.63±0.04	22.98±6.89	7.94±1.71	35.20±2.77
20	9014	30.0±1.41	42±2.47	65±2.56	76±3.89	21.3±17.32	34.8±18.15	4.30±0.11	5.8±0.28	4.12±0.14	19.14±10.03	5.05±1.47	29.07±6.00
21	9029	27.5±0.71	47±11.67	55±1.41	65±1.06	13.6±5.07	21.0±0.00	4.36±0.16	5.7±0.07	4.33±0.07	13.24±1.07	4.17±0.74	32.49±1.53
MS (g)		102.28**	28.04**	23.83**	58.94**	88.06**	588.52**	0.06**	62.53**	0.19**	105.82**	21.22**	75.62**
MS (r)		9.02	16.56	6.42	18.42	62.43	356.95	0.03	35.42	0.07	109.82	18.52*	56.52
MS (y)		38.09**	111.27**	0.02	21.67	288.47**	1134.12**	0.04	106.86**	0.06	244.24*	19.88**	81.55*
MS (g x y)		69.24	53.24	12.24	34.24	94.42	504.68	0.05	91.15	0.06	86.42	16.42	62.42
MS (e)		5.89	12.65	4.97	10.04	41.01	200.95	0.02	24.29	0.04	61.53	5.20	20.19

MS - mean squares for genotypes (g), replications (r), years (y), genotype-year interaction (g x r) and error (e).

Table 3. Principal Components for two years based on quantitative traits in *Vigna mungo*.

	1998				1999			
	PC_1	**PC_2**	**PC_3**	**PC_4**	**PC_1**	**PC_2**	**PC_3**	**PC_4**
Eigen value	5.20	2.57	1.62	1.02	5.27	2.46	1.44	1.35
Proportion of variance	40.0	19.8	12.4	7.8	40.5	18.9	11.1	10.4
Commulative variance	40.0	59.8	72.2	80.0	40.5	59.4	70.5	80.9
	Eigen vectors				**Eigen vectors**			
Plant height (cm)	0.329	-0.386	0.628	0.139	0.716	0.501	-0.046	-0.173
Days to flowering	-0.474	0.419	0.108	-0.434	-0.270	0.380	-0.567	0.336
First pod matured	-0.212	0.757	-0.354	0.208	-0.504	0.677	0.234	0.014
Days to maturity	-0.473	0.668	-0.126	0.418	-0.167	0.736	0.398	-0.026
Branches/plant	0.741	0.116	-0.529	-0.268	0.773	0.096	-0.475	0.036
Pods/plant	0.922	0.299	-0.004	0.090	0.906	-0.295	-0.109	-0.001
Pods/branch	0.472	0.247	0.698	0.303	0.471	-0.577	0.544	-0.098
Pod length (cm)	0.819	0.055	0.089	-0.373	0.727	0.204	0.424	-0.47
Seeds/pod	0.501	0.638	0.267	0.012	0.591	0.235	0.372	0.424
Seed weight (g)	0.543	-0.090	-0.148	0.321	0.380	0.319	0.056	0.767
Biomass (g)	0.935	0.193	-0.183	0.156	0.890	0.266	-0.133	-0.230
Grain yield (g)	0.918	0.352	0.002	0.039	0.900	-0.200	-0.127	0.162
Harvest index (%)	0.208	0.696	0.410	-0.393	-0.363	-0.570	0.118	0.585

Table 4. Mean and SD for clusters based on agronomic characters.

	1998					1999			
Character	**Cluster I**	**Cluster II**	**Cluster III**	**Cluster IV**	**Cluster V**	**Cluster I**	**Cluster II**	**Cluster III**	**Cluster IV**
Plant height	25.8±6.34	32.5±7.33	24.6±5.60	46.7±5.77	40.0±0.00	28.7±3.98	25.7±6.35	38.0±5.76	45.3±8.50
Days to flowering	42±5.98	40±1.85	37±3.40	36±4.92	34±0.00	42±4.72	42±6.63	42±2.40	42±4.16
First pod matured	64±4.32	60±1.63	60±2.33	57±6.43	62±2.83	66±3.93	57±1.97	57±4.63	64±2.52
Days to maturity	73±6.03	72±4.68	66±3.42	65±9.52	65±0.71	75±4.10	64±1.94	68±5.71	75±6.43
Branches/plant	7.2±2.19	16.4±3.24	24.6±9.51	9.6±2.77	32.4±7.21	7.7±1.86	11.8±6.02	14.8±2.48	17.0±2.65
Pods/plant	16.4±8.19	45.8±11.53	37.8±10.18	21.8±12.33	103.6±6.19	18.0±6.81	26.8±7.08	39.7±11.50	33.0±0.51
Pods/branch	2.2±0.71	2.9±0.57	1.7±0.49	2.3±1.31	3.6±0.78	2.3±0.54	2.4±0.46	2.7±0.61	2.0±0.51
Pod length (cm)	4.2±0.21	4.5±0.17	4.5±0.16	4.3±0.27	4.7±0.34	4.3±0.20	4.23±0.18	4.5±0.10	4.6±0.12
Seeds/pod	4.4±0.45	5.1±0.26	6.0±0.20	5.7±0.46	6.1±0.60	5.7±0.10	5.8±0.20	6.4±0.60	6.3±0.23
Seed weight (g)	3.86±0.19	4.63±0.18	4.22±0.12	4.14±0.11	4.44±0.29	4.03±0.19	3.98±0.31	4.41±0.25	4.33±0.33
Biomass (g)	8.27±5.24	26.26±4.50	24.89±5.67	16.31±1.27	52.93±9.50	10.53±4.70	14.03±1.88	23.28±7.58	35.97±5.39
Grain yield (g)	2.97±1.73	8.24±1.38	6.85±2.10	3.30±1.89	20.31±4.86	3.76±1.61	4.65±1.07	8.31±2.60	6.76±0.43
Harvest index (%)	36.93±2.68	31.76±2.73	27.83±4.40	19.96±11.54	38.59±2.86	36.10±2.37	33.29±6.99	35.84±1.16	19.18±4.12

significant for PC_1 during 1999. Pods/branch was assessed maximum for PC_3 during both the years. The PC_4 shared variance for all the traits both the years, except seed weight and harvest index which contributed maximum to this PC during 1999. First two PCs which exhibited about 60% of variance were plotted to observe the relationships between blackgram cultivars tested during 1998 and 1999 (Fig. 1). During 1998, genotypes 9080, PL- 2, 9067, 9093, Mash 3, Mash 2, 45737 and 9056 were separated from the major group which was concentrated to zero. During 1999 cultivars, 45737, 9006, Mash 3, 9080, 9093, 9005, 9092, 9025 and 9056) were separated from major group. In general five genotypes were common that separated from others during both the years.

Cluster analysis

During 1998, the Euclidean dissimilarity coefficients of 21 genotypes ranged between 1.83 and 11.50. The genotype 9039 and 9026 exhibited the lowest dissimilarity index, whereas Mash 3 and 9080 were observed with the highest genetic dissimilarity. In 1999, pairwise Euclidean dissimilarity estimates for same set of genotypes ranged from 1.90 (Mash 1 Vs 9080) to 11.20 (9080 Vs 9056). Five clusters were observed during 1998, whereas 4 during 1999. During 1998, cluster I consisted four genotypes and out of these 3 were grouped together in cluster I during 1999 (Fig. 2). On the basis of evaluation data of 1998, cluster II comprised of 4 genotypes and out of these 2 were grouped in cluster III during 1999. Five members (9029, 9025, 9005, 9092, 9026) of cluster III during 1998 were observed together in cluster II during 1999. Mash 2 and 3 were grouped together in cluster V during 1998, whereas separated in cluster III (Mash 2) and IV (Mash 3). Mash 2 and Mash 3 were high yielding genotypes, but both of these were more influenced by the environmental fluctuations (Table 4).

Discussion

The genotypes possessing the best genes for traits of economic importance were identified and hence are recommended to be utilized directly or included in hybrid programme for varietal development. Blackgram germplasm displayed high genetic variance and breeding program mainly depends upon magnitude of genetic variability (Smith *et al*., 1991). Subdividing the variance into its components assists the genetic resources conservation and utilization and enables in planning for use of appropriate gene pools in crop improvement for specific plant attributes (Pecetti & Damania, 1996). The populations with high PC_1 values were characterised by high yield potential, late maturing and low harvest index, whereas population with high PC_2 values were characterised by vegetative traits rather than reproductive. Falcinelli *et al*., (1988) showed multivariate analyses to be a valid system to deal with germplasm collections. Grouping of germplasm by multivariate methods in the study is of practical value to the breeders of blackgram. Representative accessions may be chosen from particular groups for hybrid programme with other approved varieties. Several potentially important agronomic types have been identified which may be exploited for genetic potential to transfer the desirable genes (Singh, 1988, Clements & Cowling, 1994). Inclusion of genotypes from distinct clusters and their implication in breeding programme is suggested (Tawar *et al*., 1988). Moreover, if one of the goals is to bring together varieties with genetically similar characteristics, quantitative characters may be useful for grouping. Nevertheless, the qualitative traits must be often used for separating varieties when a limited range of quantitative traits if, found in certain groups (Sneedon, 1970; Amurio *et al*., 1995). In order to ensure the efficient and

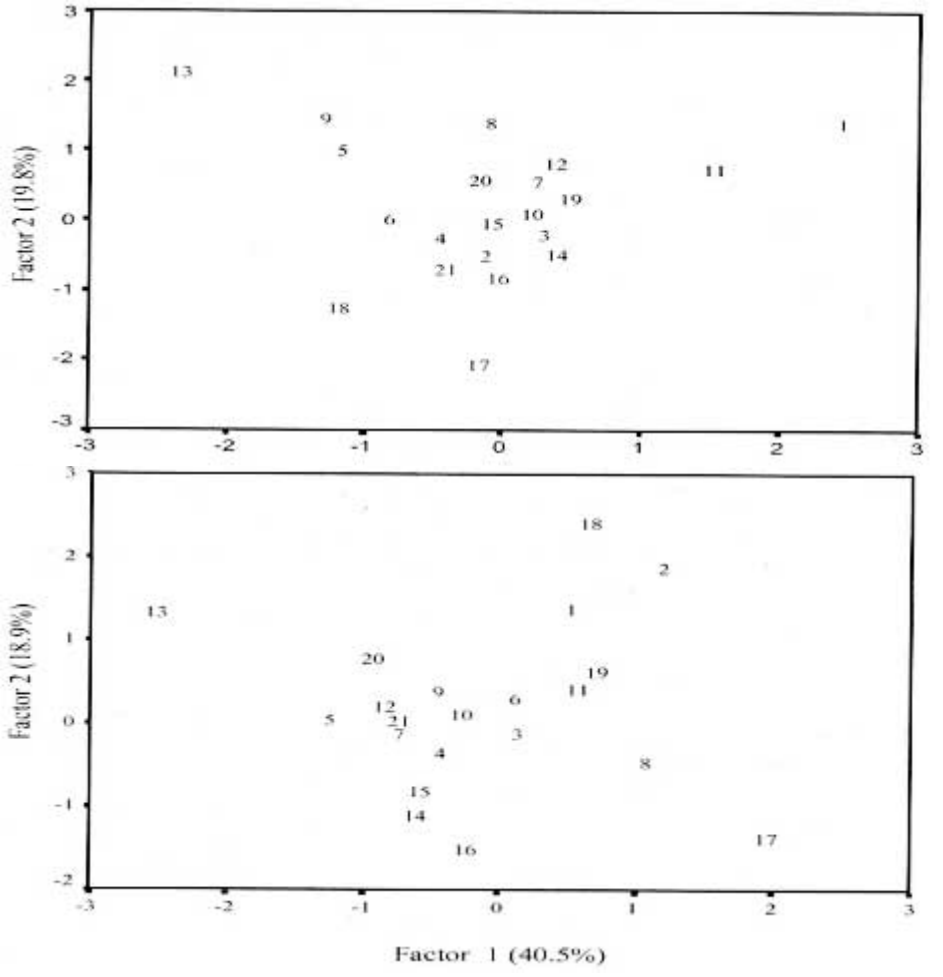

Fig. 1. Scattered diagram for the years 1998 (upper) and 1999 (lower) for first two principal components in blackgram. Name of genotypes are given in the Table 2.

effective use of crop germplasm, its characterisation is imperative and multivariate analysis provides a good evaluation of landraces by identifying those that should be further evaluated at the genetic level (Rabbani *et al*., 1998). Dasgupta & Das (1984) considered multivariate analysis best for choosing parents for hybridization. They investigated 12 characters on 40 strains of blackgram collected from India and Nepal, and grouped into 17 different clusters. Genetic divergence conducted in 38 genotypes of blackgram by Dasgupta & Das (1985) using D^2 statistics revealed no relationship between geographic distribution and genetic divergence. Similarly in the present findings, the genotypes were grouped together on the basis of agronomic performance rather than origin or source. Cluster analysis based on agriculturally important

characters revealed that advance breeding lines were categorised mostly in cluster II, III and V during 1998, whereas in clusters III and IV during 1999, which may be because of selection pressure for high yield potential by the researchers. This revealed that only a portion of genetic diversity has been exploited, and it is suggested to broaden the genetic base of cultivated blackgram involving diverse parents in breeding programme. In general the PCA results confirmed the findings obtained by those of cluster analysis, though it did not form robust groups. By utilizing genetic diversity, it is possible to improve reproductivity of existing blackgram cultivars.

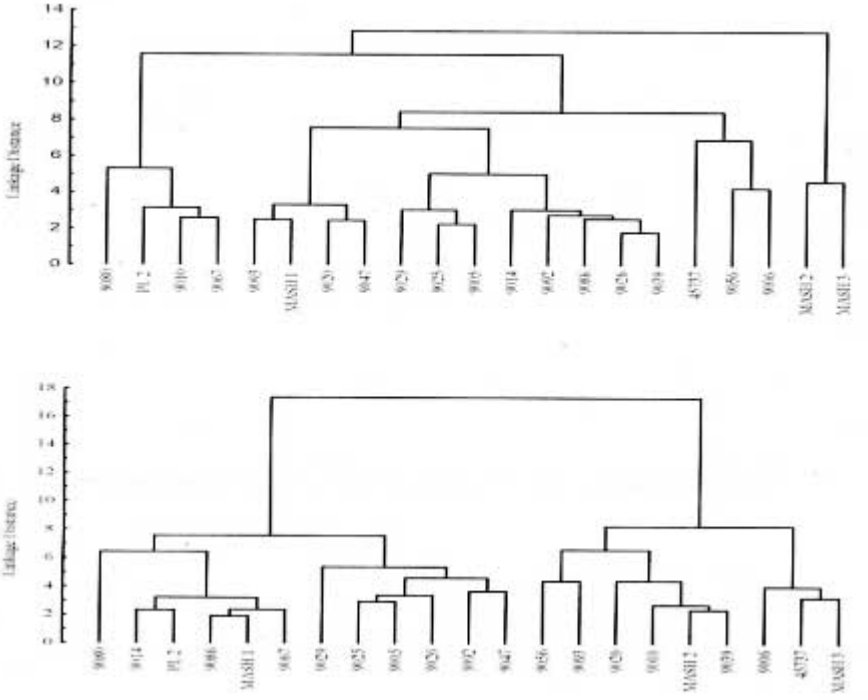

Fig. 2. Phenogram for the year 1998 (upper) and 1999 (lower) based on quantitative traits in blackgram.

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