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MULTIVARIATE ANALYSES FOR QUANTITATIVE TRAITS TO DETERMINE GENETIC DIVERSITY OF BLACKGRAM [*VIGNA MUNGO* (L.) HEPPER] GERMPLASM

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Abstract

Thirty-seven pure-lines selected at random from a broad based germplasm were studied for quantitative traits to investigate the impact of selection on diversity in relationship to agronomic performance. Multivariate approach proved its validity to classify blackgram genotypes on the basis of agronomic performance and geographic origin. Five yield contributing traits (branches per plant, pods per plant, biomass per plant, grain yield per plant, harvest index) were important for first PC three years, hence populations in this component were categorized as high yielding with maximum number of reproductive organs. Cluster analysis grouped high yielding genotypes that indicated the importance of selection from a large set of germplasm. The breeding program is suggested to broaden involving diverse parents from various clusters.

Introduction

Blackgram or mash [*Vigna mungo* (L.) Hepper] is an important summer pulse crop of many South Asian countries including Pakistan, India, Nepal, Bangladesh, Thailand, Philippines and Korea. It is cultivated under a wide range of agro-ecological zones mainly of rainfed nature. Among pulses, it is the least researched crop and no international centre of CGIAR system has this crop on its mandate (Anon., 1976). Although it has been identified as a potential crop in a number countries, but no systematic research information is available on crop improvement using biometrical techniques except few reports in the recent years (Ghafoor *et al*., 2003). Quantitative traits provide an estimate of genetic diversity and numerical taxonomic techniques including principal component and cluster analyses have been successfully used to classify and measure the pattern of genetic diversity in germplasm, as in blackgram (Ghafoor *et al*., 2001), pea (Amurrio *et al*., 1995); soybean (Perry & McIntosh, 1991); alfalafa (Smith *et al*., 1995), chickpea (Naghavi & Jahansouz, 2005) and lentil (Sultana *et al*., 2006). The objectives of this study were to determine genetic diversity in blackgram using multivariate techniques on the basis of agronomic characters, and to identify the best genotypes for future use.

Materials and Methods

Thirty-seven pure-lines of blackgram including one check (Mash 1) were selected from five clusters constructed in a broad based germplasm (Ghafoor *et al*., 2001) to study the impact of selection on genetic variation for quantitative characters. Although, blackgram is highly self pollinated crop yet selected genotypes were self pollinated for two years prior to evaluation to ensure homozygosity. The experiment was planted with three replications consecutively from 2003 to 2005 during summer seasons (July to

October) at National Agricultural Research Centre, Islamabad, Pakistan (33.40° N and 73.07° E, 540 masl). Two rows of 4 meter length for each genotype were planted with 10 cm intra-row spacing, whereas inter-row distance was kept 50 cm. Pesticide "*karate*", 2.5% w/v Lambda-cyhalothrin was sprayed to save the crop from infestation of pests especially white fly, a vector for Mungbean Yellow Mosaic Virus (MYMV). For characterization and evaluation, data were recorded following IBPGR descriptors for *Vigna mungo* and *V. radiata* (Anon., 1985). Days to flowering, first pod matured and maturity were recorded at 50% of flowering, days taken to mature first pod and 90% maturity and these variables were represented by a single value for each genotype. Plant height, branches per plant, pods per plant, grain yield per plant and biomass per plant were recorded on 10 plants sampled randomly within each replication. Pod length (cm) and seeds per pod were recorded on ten pods sampled at random within each genotype. Hundred-seed weight was recorded in grams and harvest index was determined as economic yield expressed in percentage over total biomass.

The data were analyzed for 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. Means of each character were standardized prior to analyses to avoid the effect due to difference in scale. The resulting similarity coefficients were used to evaluate relationships among the genotypes with a cluster analysis using an unweighed pair-group method with arithmetic averages (UPGMA) and then plotted in the form of a dendrogram using computer software "STATSTICA" for windows. The first two factors with high proportion of variation in principal component analysis were plotted to investigate diversity pattern for all the three years using computer software "SPSS" for windows.

Results

Figure 1 revealed that cluster I consisted 13 genotypes, cluster II consisted 21 and cluster III 3 genotypes during 2003. During 2004, cluster I consisted 11 genotypes, cluster II 14 and cluster III 12 genotypes. Similarly during 2005, three clusters were observed i.e., 18 genotypes were in cluster I, 13 in cluster II and 6 in cluster III. Principal component analysis exhibited that first four components with eigenvalues >1 contributed 82.9% of variability during 2003, 84.4% during 2004 and 70.5% during 2005 amongst genotypes evaluated for quantitative traits (Table 1). Characters that contributed more positively to PC_1 during 2003, were branches per plant, pods per plant, pods per branch, seeds per pod, biomass per plant, grain yield per plant and harvest index (Table 2). During 2004, branches per plant, pods per plant, pods per branch, biomass per plant, grain yield per plant and harvest index contributed maximum, whereas plant height, branches per plant, pods per plant, pod length, biomass per plant, grain yield per plant and harvest index contributed more positively to PC_1 during 2005. Five yield contributing traits i.e., branches per plant, pods per plant, biomass per plant, grain yield per plant and harvest index were observed more important for first principal component during all the three years, hence the populations in this component were categorized as high yielding with maximum number of reproductive organs. The phenological traits were significant for PC_2 and 100-seed weight more related to PC_3. These findings suggest that PC_2 reflects the tendency of each genotype to emphasize vegetative, as opposed to reproductive growth and tend to have few large reproductive organs. First 2 PCs contributing more than half of the variance were plotted to observe the relationships between genotypes (Fig. 2). Genotypes originated from India, Korea and AVRDC were grouped together, whereas genotypes from Afghanistan grouped together only during 2003, whereas

performed inconsistent grouping during other two years. The pure-lines from Pakistan were scattered that exhibited the extent of diversity. The results obtained by cluster and PCA were similar that indicated the validity of multivariate approach for resolving intra-specific genetic diversity in blackgram.

The selection scores were calculated using average values of each character within each cluster for quantitative traits. The first three average values were ranked and multiplied in reverse order i.e., top value was multiplied with 3, second with 2 and third with 1. To minimize error, the product was multiplied with communality value as observed in principal component analysis for individual trait and the sum of these values were termed as selection score using the formula published by Ghafoor *et al*., (2001). The following is an illustration for calculating selection score for cluster I during 2003;

Traits ranking first	Plant height, seed weight
Traits ranking second	Branches per plant, pod length, biomass per plant
Traits ranking third	Days to flowering, days to first pod maturity, days to 90% maturity, pods per plant, pods per branch, seeds per pod, grain yield per plant, harvest index

$$\text{Selection score} = \sum [(3 \times 0.77) + (1 \times 0.82) + (1 \times 0.92) + (1 \times 0.94) + (2 \times 0.94) + (1 \times 0.93) + (1 \times 0.81) + (2 \times 0.73) + (1 \times 0.73) + (3 \times 0.58) + (2 \times 0.94) + (1 \times 0.94) + (1 \times 0.71)] = 16.1$$

The selection scores presented in Fig. 3 revealed that the members of cluster 3 gave higher agronomic performance for all the three years, hence these genotypes are suggested to test under wide range of environments to select the best one/s or selected genotypes could be utilized in hybridization program to develop pure lines.

Table 1. Eigen values > 1 for the first four principal components in blackgram genotypes.

Factor	2003	2004	2005
PC_1	4.49 (34.6)†	4.51 (34.7)	3.74 (30.8)
PC_2	3.44 (26.5)	3.68 (28.3)	3.64 (21.00)
PC_3	1.63 (12.6)	1.49 (11.5)	1.59 (12.3)
PC_4	1.19 (9.2)	1.04 (7.98)	1.09 (8.4)

†The values are eigen factors and percent contribution in parenthesis for various principal components for three years

Table 2. Contribution of first four PCs for quantitative characters in blackgram genotypes evaluated for three years.

Factor	2003	2004	2005
PC_1	Branches per plant, pods per plant, pods per branch, seeds per pod, biomass, grain yield per plant, harvest index	Branches per plant, pods per plant, pods per branch, biomass, grain yield per plant, harvest index	Plant height, branches per plant, pods per plant, pod length, biomass, grain yield per plant, harvest index
PC_2	Pod length, days to flowering, days to maturity (first pod), days to 90% maturity	Plant height, days to flowering, days to maturity (first pod), days to 90% maturity, pod length	Days to flowering, days to maturity (first pod), days to 90% maturity, seeds per pod
PC_3	100-seed weight, plant height	100-seed weight	100-seed weight
PC_4		Seeds per pod	

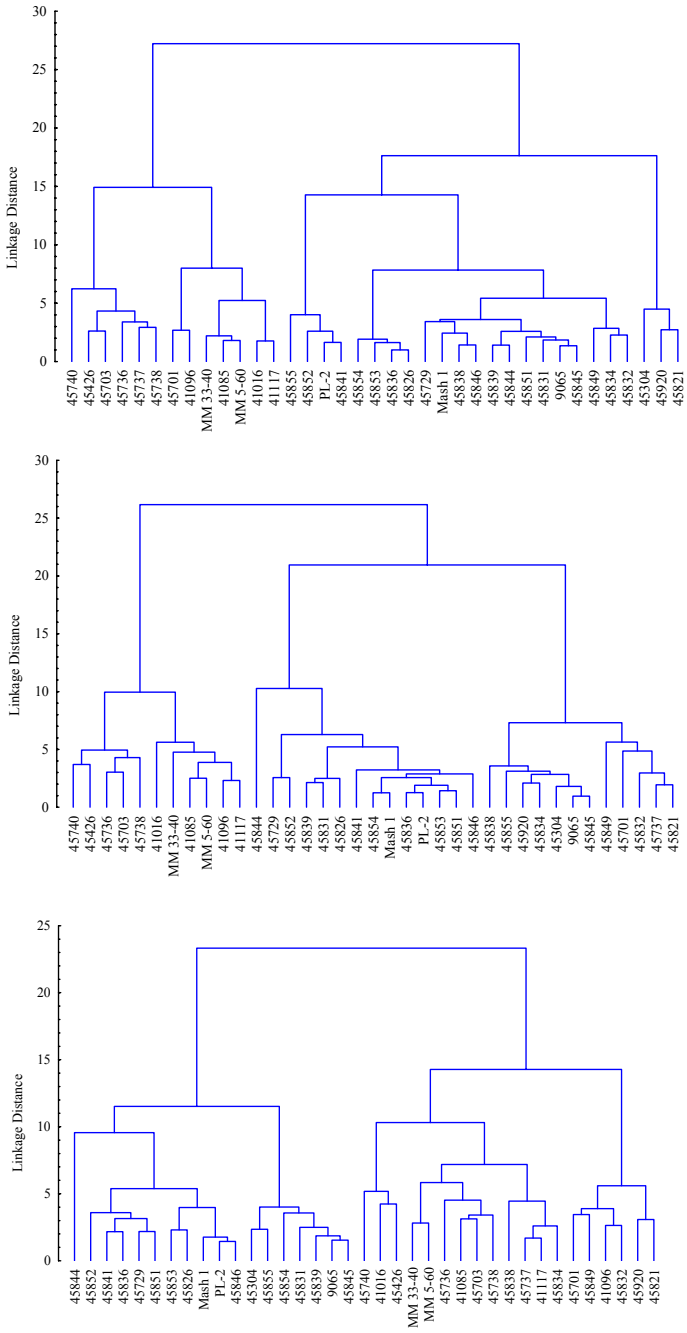

Fig. 1. Phenogram based on quantitative traits during 2003 (upper), 2004 (middle) and 2005 (lower) in *Vigna mungo*.

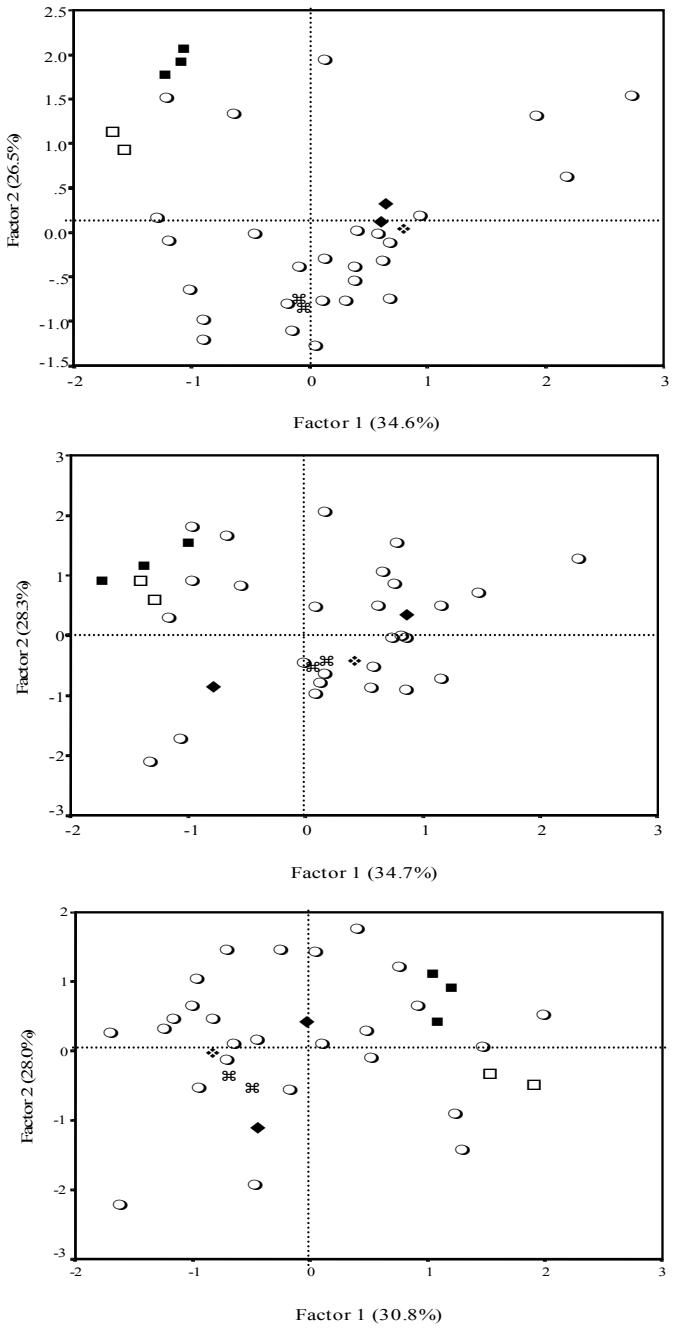

Fig. 2. Scattered diagram for 37 genotypes of black gram during 2003 (upper), 2004 (middle) and 2005 (lower) based on quantitative traits. The symbols represents as, ❍-Pakistan, ■-India, □-AVRDC, ⌘-Korea, ◆-Afghanistan, ❖-Variety

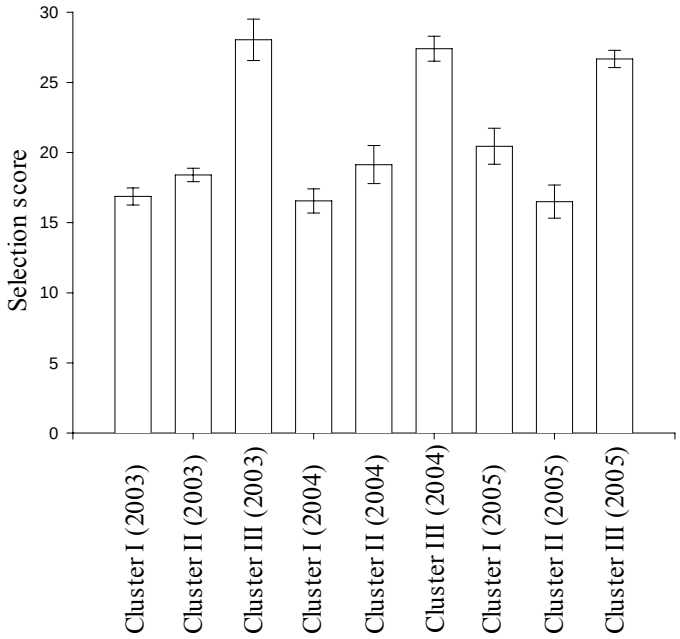

Fig. 3. Selection scores based on three years evaluation in *Vigna mungo*.

Discussion

Agronomic evaluation is an important step in description and classification of crop germplasm as improvement depends upon the magnitude of genetic variability and it enables researchers to plan use of appropriate gene pools in crop improvement for specific plant attributes (Peeters & Martinelli, 1989). Though cluster analysis depended upon genetic similarity, some of the clusters include genotypes from same origin or source. The agronomic performance was related towards genetic similarities, hence quantitative traits could be used to resolve genetic diversity in a selected sample from a diverse germplasm without loosing genetic diversity. The populations with high PC_1 values were characterised by high yield potential, whereas population with high PC_2 values were characterized by late flowering and maturity. Selective genotypes chosen from particular cluster for hybridization with other approved cultivars is suggested to broaden the genetic base of crop. Field evaluation adds information to taxonomy and should not be disassociated from morphological, anatomical and cytological observation (Sultana & Ghafoor, 2007).

Cluster analysis based on agriculturally important traits revealed that high selection scores for the genotypes in cluster 3 for all the three years might be due to selection pressure for yield potential and other related characters as these genotypes were selected from the results of a broad based germplasm reported earlier (Ghafoor *et al*., 2001). Hence these genotypes were selected from various clusters at random, the results could be of broader acceptance. It is suggested to broaden the genetic base of cultivated blackgram involving the best parents from various clusters in breeding program. Grouping of germplasm originated from Pakistan in different clusters may be because of frequent exchange of germplasm by the breeders.

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