



Tailoring genetic diversity of mungbean [*Vigna radiata* (L). Wilczek] germplasm through principal component and cluster analysis for yield and yield related traits

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Article published on August 12, 2014

Key words: *Vigna radiata* L., Principal component analysis, cluster analysis, Pearson's correlation and genetic diversity.

Abstract

The goal of investigation is to determine the extent of variability existing among 374 mungbean genotypes through Principal Component Analysis (PCA), cluster analysis and the relationship existing between yield and other characters through Pearson's correlation analysis. According to principal component analysis, 4 principal components (PC) had eigen values more than unity and accounted for 65.76% of the total variance among 12 characters. Amongst first four PCs, PC1 was accounted high proportion of total variance (30.53%) and the remaining three principal components viz., PC2, PC3 and PC4 revealed 16.05, 10.05 and 9.13% of total variance respectively. 374 accessions were classified into 8 clusters through hierarchical cluster analysis method. Cluster I composed of 218 accessions and it has maximum number of genotypes under study, whereas Cluster II and Cluster III consisted of 55 and 85 accessions respectively. Based on the cluster analysis results it was recommended that crosses could be made between the genotypes of Cluster VI and VIII, Cluster V and VIII, Cluster III and VIII and Cluster V and VIII.

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Introduction

Studies on genetic diversity is the process by which variation among individuals or groups of individuals or populations is analyzed. Data often involves numerical measurements and in many cases, combinations of different types of variables (Sneath and Sokal, 1973). Evaluation of germplasm collection have the highest priority among germplasm functions. Germplasm enhancement embraces those activities required to aggregate useful genes and gene combinations into usable phenotypes. These aggregates could be considered as the feedstock for varietal development programmes (Evgenidis *et al.*, 2011).

Green gram (*Vigna radiata* (L.) Wilczek) is one of the important pulse crops of India. India alone accounts for 65% of its world acreage and 54% of the production. In India, it is grown on an area of 3.7 million hectares with production of 1.57 million tonnes and productivity of 406.98 Kg/ha (Anon., 2012). Mungbean has the potential to make up the gap of protein shortage, but its yield per hectare in the country is still low and there is a need for improvement.

Yield, being a quantitative trait is a complex character in any crop. Various morphological and physiological plant characters contribute to yield. These yield contributing components are inter-related with each other showing a complex chain of relationship and also highly influenced by the environmental conditions. In order to raise yield per unit area, new mungbean varieties must be developed along with the improved cultural practices. The crosses between the parents with maximum genetic divergence are generally the most responsive for genetic improvement (Arunachalam, 1981). To group the sets of germplasm accessions, Principal Component Analysis (PCA), cluster analysis (Dillon and Goldstein, 1984; Hair *et al.*, 1987) and Pearson's correlation analysis were used by taking into account several characters and relationship between them simultaneously. Recently PCA has been cited by various authors for the reduction of multivariate data

into a few artificial varieties which can be further used for classifying material. This approach is especially valuable for screening large number of genetic resources by a large number of descriptor variables (Golbashy *et al.*, 2010 and Beiragi *et al.*, 2011). The aim of present study was to find out the genetic variability among different plant traits, direct and indirect contribution of these parameters towards yield and to identify better combinations as selection criteria for developing high yielding fine mungbean genotypes. Such type of findings may help mungbean breeders and it could provide new opportunities for promoting the production of mungbean with better yield.

Material

A total of 374 mungbean genotypes were included in the experiment for the evaluation of yield and yield related traits at University of Agricultural Sciences, Bangalore, Karnataka, India during the late *kharif* season of 2010. The experimental material was collected from University of Agricultural Sciences, Bangalore, Tamil Nadu Agricultural University, Coimbatore and National Bureau Plant Genetic Resources, New Delhi.

Methods

The test entries were planted during mid August and harvested during the last week of October. Each test entry was planted in a single row sub-plot of one metre length in an augmented design with row to row and plant to plant spacing of 45 cm and 10 cm, respectively. Each entry was evaluated in comparison to the check variety, Chinamung. All the recommended package of practices was followed to establish good plant stand.

Observations of twelve characters namely days to 50 per cent flowering, days to harvest, plant height, number of branches plant⁻¹, number of clusters plant⁻¹, number of pods plant⁻¹, pod length, number of seeds pod⁻¹, test weight, pod yield plant⁻¹, threshing per cent and seed yield plant⁻¹ were recorded. Principal Component Analysis (PCA) was used as a data reduction technique to summarise the information of

different phenotypic observations. The mean observation for each accession was standardized by subtracting from each observation the mean value of the character and subsequently dividing by its respective standard deviation. This resulted in standardized values for each trait with average 0 and standard deviation of 1 or less (Upadhyaya *et al.*, 2002). The standardized values were used to perform principal component analysis (PCA) using XLSTAT to know the importance of different traits in explaining multivariate polymorphism. The principal component analysis was performed in order to confirm the diversity pattern brought out by cluster analysis.

Results and discussion

The analysis of variance revealed the presence of significant differences among the genotypes for nine characters and among the checks, except number of seeds pod⁻¹ all other characters showed significant difference (Table 1). The mean, standard deviation and minimum and maximum values of different

characters are presented in Table 2. The stability of performance of each genotype was defined by the mean and standard deviation of all the traits. Accessions IC 103316 and PLM 214 recorded the earliest days (29 days) for 50 per cent flowering while the accessions that took the longest number of days (50 days) to attain 50 per cent flowering were PBM 51-90, IC 39558 and Thoppakunda. The genotype PBM 51-90 has taken more number of days to harvest (90 days after sowing) whereas 42 accessions attained maturity at 67 days. The genotypes IC 39520, Kangayan, IC 29789, SML 331, TB-7 and IC 103993 were the shortest (19.3 cm) accession in height and while the accession with the height of 34 cm were Bapatla, Pusabaisaki, Pant-M103, Velampatti, IC 8961-5 and MS 9384 were tallest. Only 2 clusters plant⁻¹ were exerted by the accession E C314286, Vellurior, and IC 39559 while the accessions *viz.*, MDU 3397, IC 39810, Hyb-12, T3485 and Thoppakunda were exerted 15 clusters plant⁻¹.

Table 1. Mean sum square from ANOVA for augmented randomized complete block design for 12 growth and yield parameters in 374 mungbean germplasm accessions.

Source of variation	df	50% FL [†]	DH	PH	NBR	NCL	NPD	PdL	TNSd/Pd	TWt	PY	THR%	SdY
Blocks	21	0.61	1.37	4.9	0.23	1.07	8.4	0.16	1	0.34	0.9	7.26	0.42
Genotypes + Checks	375	5.48**	5.35*	8.66	0.23	7.86**	69.65**	0.86**	1.68*	3.64**	21.34**	86.21**	11.19**
Checks	1	20.45**	19.11*	66.03**	0.09	56.82**	202.53**	1.24*	1.11	715.97**	26.35**	30.68*	108.14**
Genotypes	373	5.43**	5.27*	8.31	0.23	7.61**	69.27**	0.86**	1.68*	0.07	21.22**	83.37**	10.71**
Checks Vs. Genotypes	1	8.62**	21.52**	82.36**	0.25	50.84**	78.64**	3.13**	1.32	624**	60.93**	1201.86**	92.31**
Error	21	1.07	2.54	7.55	0.38	0.96	6.26	0.18	0.78	0.47	0.92	5.46	0.41

**Significant @ P= 0.01 & *Significant @ P= 0.05

[†] 50% FL, Days to 50% flowering; DH, Days to harvest; PH, Plant height; NBR, Number of branches plant⁻¹; NCL, Number of clusters plant⁻¹; NPD, Number of pods plant⁻¹; PdL, Pod length; TNSd/Pd, Number of seeds pod⁻¹; TWt, Test weight; PY, Pod yield; THR%, Threshing percentage and SdY, Seed yield plant⁻¹.

Table 2. Mean, range and standard deviation for 12 growth and yield parameters in 374 mungbean germplasm accessions.

Characters	Mean±SD	Range	
		Minimum	Maximum
Days 50% flowering	32.76 ± 2.33	29	50
Days to harvest	70.03 ± 2.29	67	90
Plant height (cm)	26.42 ± 2.88	19.30	34
Number of branches plant ⁻¹	2.81 ± 0.47	1	3
Number of clusters plant ⁻¹	6.45 ± 2.76	2	15
Number of pods plant ⁻¹	19.49 ± 8.32	6	45
Pod length (cm)	6.64 ± 0.93	3	14.8
Number of seeds pod ⁻¹	11.20 ± 1.30	5	15
Test weight (g)	3.19 ± 0.26	2.43	4.25
Pod yield plant ⁻¹ (g)	9.73 ± 4.61	1.76	27.69
Threshing percentage	62.48 ± 9.13	26.58	85.83
Seed yield plant ⁻¹ (g)	6.19 ± 3.37	1.00	22.63

MAVT 801 had the least number of pods plant⁻¹ (6 pods) whereas LM 192 had the highest number of 45 pods plant⁻¹. The accession with least pod length was MUM 6 with 3 cm and the accession PBM 51-90 recorded more pod length (14.8 cm). The least number of seeds pod⁻¹ was recorded in IC 370489 (5 seeds) and PBM 51-90 recorded highest number of 15 seeds pod⁻¹ and also more test weight of 4.25 g respectively. Highest threshing percentage was exerted by the accession ADT 1 (85.83%) and lowest by T 2272 (26.58%). The accession AC 5 exhibited highest pod and seed yield plant⁻¹ (27.69 g and 22.63 g respectively) and EC 496841 exhibited lowest yield of 1.76g per plant with 1.0g of seed yield per plant.

Principal component analysis

The progress in breeding programme for economic characters often depends on the availability of a large germplasm representing a diverse genetic variation. In order to ensure the efficient and effective use of crop germplasm, its characterization 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 and Das (1984) considered multivariate analysis best for choosing parents for hybridization. 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 and Damania, 1996). According to principal component analysis, 4 principal components (PC) had eigen values more than unity and accounted for 63.87 per cent of the total variance in the data (Table 3). Amongst first four PCs, PC1 accounted high proportions of total variance (28.02%) and the remaining three principal components *viz.*, PC2, PC3 and PC4 recorded 16.66, 10.11 and 9.08 per cent of

total variance respectively. Eigen values of 12 principal components have been shown in the scree plot (Fig. 1).

Table 3. Eigen values and percentage of variation in respect of 12 growth and yield parameters in 374 germplasm accessions of green gram.

PC	Eigen value	Variability (%)	Cumulative %
1	3.36	28.02	28.02
2	2.00	16.66	44.67
3	1.21	10.11	54.79
4	1.09	9.08	63.86
5	0.91	7.61	71.48
6	0.84	6.99	78.47
7	0.80	6.63	85.10
8	0.77	6.40	91.50
9	0.63	5.22	96.71
10	0.29	2.44	99.15
11	0.09	0.76	99.91
12	0.01	0.09	100.00

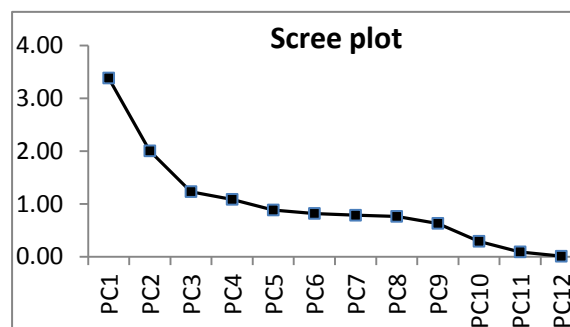


Fig. 1. Scree plot constructed using 12 principal components.

The criterion of Raji (2002) was chosen to determine the cut off limit for the coefficients of the proper vectors, this criterion treated coefficients was greater than 0.3 as having large effect to be considered important while traits having a lesser coefficient value than 0.3 were considered not to have important effects on the overall variation observed in the present study. Eigen vectors (loadings) of the first four principal components were presented in Table 4.

Table 4. Eigen vectors (loadings) of the first four principal components.

Characters	PC1	PC2	PC3	PC4
Days to 50% flowering	0.029	0.307	-0.278	0.427
Days to harvest	-0.056	0.298	-0.056	0.508
Plant height (cm)	-0.006	-0.123	-0.603	0.242
Number of branches plant ⁻¹	0.057	0.031	0.643	0.167
Number of clusters plant ⁻¹	0.354	-0.055	-0.032	-0.064
Number of pods plant ⁻¹	0.511	-0.038	0.026	0.040
Pod length (cm)	-0.039	0.614	-0.095	-0.210
Number of seeds pod ⁻¹	-0.043	0.530	0.020	-0.438
Test weight (g)	0.050	0.339	0.264	0.404
Pod yield plant ⁻¹ (g)	0.514	0.016	0.041	0.065
Threshing percentage	0.245	0.138	-0.243	-0.254
Seed yield plant ⁻¹ (g)	0.526	0.062	-0.029	-0.016

The results showed that seed yield had the highest positive loadings (0.526) followed by pod yield (0.514), number of pods plant⁻¹ (0.511) and number of clusters plant⁻¹ (0.354) and other characters showed positive loading in first principal component (PC1) were days to 50 per cent flowering, number of branches and test weight. In PC2, pod length (0.614) and number of seeds pod⁻¹ (0.530) exhibited highest positive loadings except plant height, number of clusters plant⁻¹ and number of pods plant⁻¹. Other characters showed positive loadings. Number of branches plant⁻¹ recorded highest positive loadings (0.643), while plant height registered highest negative loading (-0.603) in PC3. Days to 50 per cent flowering and threshing percentage had high negative loadings in PC3. Days to harvest, days to 50 per cent flowering and test weight registered highest positive loadings of 0.508, 0.427 and 0.404, while number of seeds pod⁻¹ showed highest negative loadings (-0.438) in PC4, plant height, days to 50 per cent flowering, days to harvest and days to harvest, number of branches and test weight showed positive loading respectively.

Ghafoor *et al.* (2002) have taken first four components of PCA with eigen values >1 contributed 78.7 per cent of the total variance amongst 40 mungbean genotypes studied. Yimram *et al.* (2009) evaluated 9 qualitative and 21 quantitative traits in 340 diverse cultivated mungbean accessions collected from AVRDC and principal component analysis revealed that the first three PCs explained 74.9 per cent of the total variation. Eighteen quantitative and 37 qualitative characters for 646 greengram accessions were subjected to multivariate analysis and 63.79% variation was explained by first 3 principal components by Pandiyan *et al.*, 2012.

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Pearson's correlation

To know the extent of relationship between yield and its various components, it is important for the plant breeder to select plants which consists of desirable characteristics. The knowledge of relationship between yield and yield components has been successfully exploited in crop improvement. In character association analysis (Table 5), 50 per cent flowering exhibited significant positive association with days to harvest, pod length and test weight. Days to harvest showed significant positive correlation with pod length and test weight. Number of clusters plant⁻¹ corresponded positively and it showed significant association with pod yield plant⁻¹ ($r = 0.471$), threshing percentage ($r = 0.240$) and seed yield ($r = 0.479$). These results are in agreement with the findings of Rajan *et al.*, 2000; Ahmad *et al.*, 2013 and Narasimhulu, *et al.*, 2013. Number of pods plant⁻¹ exhibited positive and significant correlation with pod yield ($r = 0.923$), threshing percentage ($r = 0.266$) and seed yield ($r = 0.892$). Similar results were also observed by Makeen *et al.* (2007), Kumar *et al.* (2010), Srivastava and Singh, 2012 and Ahmad *et al.*, 2013. The trait pod length exhibited significant positive association with number of seeds pod⁻¹ ($r = 0.655$) and test weight ($r = 0.288$). Number of secondary branches plant⁻¹, number of pods plant⁻¹, number of seeds pod⁻¹, pod length and 100 seed weight exhibited positive and significant correlation along with high positive direct effect with seed yield (Rajan *et al.*, 2000; Makeen *et al.*, 2007; Srivastava and Singh, 2012; Kumar *et al.*, 2013; Narasimhulu, *et al.*, 2013 and Thippiani *et al.*, 2013). Pod yield recorded significant positive association with threshing percentage ($r = 0.270$) and seed yield ($r =$

0.964). Threshing percentage showed positive significant correlation with seed yield ($r = 0.477$). Among the association between characters, pod yield showed highest positive significant correlation with seed yield ($r = 0.964$) followed by total number of

pods per plant with pod yield ($r = 0.923$), number of pods plant⁻¹ with seed yield ($r = 0.892$) and pod length with number of seeds pod⁻¹ ($r = 0.655$).

Table 5. Pearson's correlation coefficient among seed yield and its attributing characters in 374 germplasm accessions.

	50% FL [†]	DH	PH	NBR	NCL	NPD	PdL	TNSd/Pd	TWt	PY	THR%	SdY
50% FL	1	0.144**	0.074	-0.023	0.049	0.015	0.278**	0.098	0.140**	0.049	0.073	0.070
DH		1	-0.007	0.004	-0.091	-0.104*	0.235**	0.073	0.180**	-0.055	0.005	-0.048
PH			1	-0.134**	-0.021	0.004	-0.103*	-0.087	-0.097	-0.014	0.026	-0.002
NBR				1	0.047	0.068	-0.061	0.046	0.144*	0.095	-0.075	0.077
NCL					1	0.527	-0.086	-0.067	-0.030	0.471**	0.240**	0.479**
NPD						1	-0.099	-0.090	0.065	0.923**	0.266**	0.892**
PdL							1	0.655**	0.228**	-0.032	0.124*	0.010
TNSd/Pd								1	0.138**	-0.056	0.087	-0.012
TWt									1	0.094	0.033	0.096
PY										1	0.270**	0.964**
THR%											1	0.477**

**Significant @ P= 0.01 & *Significant @ P= 0.05

[†] 50% FL, Days to 50% flowering; DH, Days to harvest; PH, Plant height; NBR, Number of branches plant⁻¹; NCL, Number of clusters plant⁻¹; NPD, Number of pods plant⁻¹; PdL, Pod length; TNSd/Pd, Number of seeds pod⁻¹; TWt, Test weight; PY, Pod yield; THR%, Threshing percentage and SdY, Seed yield plant⁻¹.

Cluster analysis

Through hierarchical clustering method, 374 genotypes were confined to 8 clusters. Maximum number of genotypes under study belongs to Cluster I and it has 218 accessions followed by Cluster III and Cluster II consists of 85 and 54 accessions respectively. Cluster V and Cluster VI comprised of 5 accessions each whereas Cluster IV comprised of 4 accessions. Cluster VII and Cluster VIII were found to be solitary clusters which includes genotypes IC 39558 and Thoppakunda respectively.

The mean performance of all the characters in different clusters is presented in Table 6. Late flowering genotypes were classified under Cluster VII and Cluster IX, remaining six clusters consisted of early flowering genotypes. Difference between cluster means of days to harvest, plant height, number of

branches plant⁻¹, pod length, number of seeds pod⁻¹ and test weight were almost equal in all eight clusters. Cluster IX comprised of only one genotype 'Thoppakunda' which exhibited high cluster mean for four important yield related characters viz., number of clusters plant⁻¹, number of pods plant⁻¹, pod length and number of seeds pod⁻¹. Cluster IV encompasses of five genotypes LM 192, AC 5, PS 16, ADT 1 and MAVT 836 and it showed high cluster mean for pod yield plant⁻¹, threshing percentage and seed yield plant⁻¹. Cluster V consists of five genotypes MDU 2268, Bapatla, MDU 1942, K. Pudur 1 and IC 118559. Cluster VI composed of five genotypes KM 1/1, EC 482908, NP 36, T 2272 and MS 9721. Cluster VII includes only one genotype IC 39558 and cluster VIII composed two genotypes namely MUM 5 and IC 103316.

Table 6. Cluster means for 12 growth and yield parameters in 374 germplasm accessions of green gram.

Characters	Cluster I	Cluster II	Cluster III	Cluster IV	Cluster V	Cluster VI	Cluster VII	Cluster VIII
Days to 50% flowering	32.74	32.40	32.64	33.50	33.80	32.40	50.00 [†]	50.00 [†]
Days to harvest	70.09	69.42	70.27	69.75	70.00	71.00	71.00	67.00
Plant height (cm)	26.40	25.71	26.64	27.63	27.84	27.68	29.30	29.30
Number of branches plant ⁻¹	2.78	2.91	2.84	3.00	2.40	2.80	3.00	3.00
Number of clusters plant ⁻¹	6.35	8.65	5.07	10.00	8.40	4.20 [‡]	5.00	15.00 [†]
Number of pods plant ⁻¹	17.04	32.89	15.51	38.75	35.20	11.20 [‡]	20.00	40.00 [†]
Pod length (cm)	6.69	6.50	6.57	7.35	6.36	6.40	6.30	7.40
Number of seeds pod ⁻¹	11.28	11.05	11.13	12.00	10.20	11.00	11.00	12.00
Test weight (g)	3.18	3.21	3.18	3.14	3.33	3.13	3.09	3.14
Pod yield plant ⁻¹ (g)	8.46	16.40	7.77	22.91	17.17	4.51 [‡]	9.46	20.52 [†]
Threshing percentage	66.71	65.24	51.39	81.96	51.15	31.82 [‡]	54.76	71.00 [†]
Seed yield plant ⁻¹ (g)	5.67	10.70	4.05	18.70	8.76	1.41 [‡]	5.18	14.57 [†]

‡ - denotes lowest value and † - denotes highest value

The inter-cluster distance value showed that the most diverse cluster was IV and VI (62.84) followed by Cluster VI and VIII (56.93), Cluster III and IV (44.11) (Table 7). The minimum inter-cluster values was observed between cluster II and V (14.70) and remaining all other inter-clusters exhibited less than 50% diversity from most diverse Cluster VI and VIII (62.84) which indicated that these groups were very less diverse. Studies conducted by Bisht *et al.*, 1998

showed that 111 mungbean accessions were grouped into six discrete and well-defined clusters. Pandiyan *et al.* (2012) subjected 646 greengram accessions into hierarchical cluster analysis which revealed eight distinct clusters. Similar results were reported by Rahim *et al.* (2008) and Abna *et al.* (2012). Fig. 2 depicts the clusters formed by hierarchical clustering method.

Table 7. Average inter-cluster distance between 8 clusters of 374 mungbean accessions.

	Cluster I	Cluster II	Cluster III	Cluster IV	Cluster V	Cluster VI	Cluster VII	Cluster VIII
Cluster I	0	18.661	15.599	33.147	25.814	35.957	21.522	34.103
Cluster II		0	25.048	20.699	14.704	42.916	26.331	22.048
Cluster III			0	44.115	22.661	20.534	18.572	40.955
Cluster IV				0	33.178	62.840	41.937	21.258
Cluster V					0	34.432	24.383	28.001
Cluster VI						0	30.911	56.936
Cluster VII							0	31.507
Cluster VIII								0

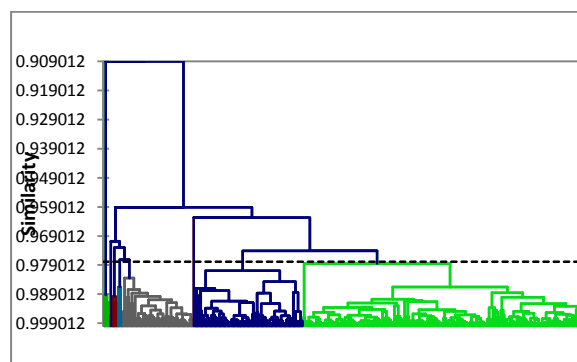


Fig. 2. Dendrogram based on Euclidean distance for 12 morphological characters of 374 germplasm accessions.

Conclusion

In the present study, the PCA and Pearson's correlation analysis suggested following important characters for genetic improvement of greengram *viz.*, seed yield plant⁻¹, pod yield plant⁻¹, number of clusters plant⁻¹, number of pods plant⁻¹ and threshing percentage. Hence more emphasize should be given to these characters during hybridization programme. Based on the cluster analysis it is recommended that crosses can be made in breeding programme between the genotypes from farthest clusters. Classifying genotypes according to their agronomic traits with sophisticated multivariate techniques can reduce the cost of time and money in crop improvement.

However, stability analysis of different traits on the already established groups of the current study requires further investigation based on sufficient data that cover different years and locations.

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