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Multivariate Analysis among the Nepalese and Exotic Mungbean (Vigna Radiata L. Wilczek) Genotypes Based on the Qualitative Parameters

K. M. Basnet[1,*], N. R. Adhikari[2], M.P. Pandey[2]

[1]Local Initiatives for Biodiversity, Research and Development, Gairapatan, Pokhara, Kaski, Nepal
[2]Department of Plant Breeding and Genetics, Institute of Agriculture and Animal Science, Rampur, Chitwan, Nepal
*Corresponding Author: kiranmbasnet@gmail.com

Abstract An experiment was conducted in 2008 at the horticultural farm of the Institute of Agriculture and Animal Science (IAAS), Rampur, Chitwan, Nepal to evaluate the collected Nepalese/ local and exotic mungbean genotypes based on eight qualitative traits. The genotypes were grouped into 6 clusters according to Unweighted Pair Group Method with Arithmetic mean (UPGMA) hierarchical techniques. Cluster analysis grouped genotypes together with greater genetic similarity; the clusters did not necessarily include all genotypes from the same origin. Some cluster consisted only of the local or the exotic genotypes while in others, both categories were grouped under the same cluster. This was primarily due to similarity in the different genotypes for the qualitative traits observed. Although Principal Component Analysis (PCA) did not form robust group as outlined by the cluster analysis, it surely supported the groups formed in the dendogram. In general, the clusters formed displayed the closeness of the local and exotic genotypes among themselves than for the mixed population consisting of both genotypes. Principal component analysis showed that five Principal Components (PCs) together accounted for 92.30% of the total phenotypic variability observed in the mungbean genotypes. The first three PCs had nearly 78% of the total variation with individual share of 40.60%, 22.30% and 14.70% respectively.

Keywords Mungbean (Vigna radiata L. Wilczek), Qualitative traits, Cluster analysis, PCA

1. Introduction

Mungbean is an important legume crop for the world. It is a short duration grain legume crop grown primarily for their dry seeds. It contains about 25 percent protein, which is nearly three times than that of cereals (Thirumaran and Seralthan, 1988). It is particularly rich in leucine, phenylalanine, lysine, valine and isoleucine (Yimram et al., 2009). Mungbean also has an important role sustaining soil fertility by improving soil physical properties and associating with *Bradyrizobium* bacteria which in turn can fix atmospheric nitrogen (Joshi et al., 2003a,b).

The mungbean crop is grown in many parts of the world ranging from the Asian Sub-continent to the African, European and the American with its superior digestibility and recognition as high yielding pulse crop (Smartt, 1990). The crop grows better to different cropping systems in the tropics and subtropics (Yimram et al., 2009). Being drought resistant crop, it is suitable for dryland farming and mostly used as an intercrop or as a green manure or cover crop (Joshi et al., 2003a,b). In the world context, while the areas for cereals and other pulses have decreased, that for mungbean it has doubled in the last two decades (Tomooka et al., 2005). In Nepal, it is an emerging legume crop where approximately 12,000-hectare of land is under mungbean cultivation with an average annual production of 6,500 metric tons and with an average yield of 0.5 t/ha (Joshi et al., 1997).

Evaluation of germplasm is useful not only in selection of core collection but it is also important for breeding programs. In order to develop high yielding cultivars resistant to various stresses, exploitation of the gene pool is highly rewarding (Anishetty and Moss, 1988). Sometimes, the set of values of one variable will be found to be closely related to the set of values of one or more other variables and sometimes there will be no such relationship. The study of such joint variation (or lack of it) is one aspect of multivariate analysis. Multivariate statistics help the researcher to summarize data and reduce the number of variables necessary to describe it (Anderson, 1972). Multivariate analysis, and in particular, cluster and principal component analysis have been found appropriate for the evaluation of the germplasm while studying various traits (Mardia et al., 1979; Cruz and Rezzagi, 1994). The present study was undertaken to find the similarity indices and

divergence status among the collected local and exotic mungbean genotypes based on the observed qualitative parameters using multivariate analysis techniques- cluster and principal component analysis.

2. Materials and Methods

The experiment was conducted at the horticulture farm of Institute of Agriculture and Animal Science (IAAS), Tribhuvan University (TU), Rampur, Chitwan, Nepal, from September, 2008 to November, 2008. The precise latitude, longitude and altitude as recorded by GPS of IAAS was $N27^0$ 39′ 0.45″ latitude, $E84^0$ 21′ 9.1″ longitude and 228 masl, respectively.

2.1. Collection of Experimental Materials

The germplasm used in the study consisted of fifteen Nepalese/ local and fifteen exotic mungbean genotypes. The exotic lines were obtained from the Asian Vegetable Research and Development Center (AVRDC), Shanhua, Taiwan and National Grain Legume Research Program (NGLRP), Rampur, Chitwan while the local lines were received from the Agriculture Botany Division (ABD), Nepal Agricultural Research Council (NARC), Khumaltar, Lalitpur, Nepal (Table 1).

2.2. Experimental Design and Layout

The layout of the field was in Randomized Complete Block Design (RCBD) with 30 treatments as mungbean genotypes and under three replication blocks. Individual plot size was 2 m^2 (2 m×1 m) where 3 continuous rows in each block represented a plot. The distance between two replications was maintained 1 m. Spacing between the individual plants was 10 cm and that between each row was 50 cm adjusting a total of 20 plants in a row and 60 plants in a plot. Thus, the total area of the research plots was 300 m^2 (30.0 m×10.0 m). The field was prepared for sowing by two harrowing and concurrent leveling. Fertilizers were applied as compost before one week of sowing and Nitrogen, Phosphorus, Potassium (N, P, K) at the rate of 20:40:20 prior to sowing. The seeds were soaked in *Rhizobium* culture and then sown after 2 hours maintaining the proper spacing. First weeding was done in two weeks of sowing and second before the unset of flowering. To protect the plants from insects and pests, a systemic insecticide, Thiodan (2 ml/litre of water) was applied thrice during the entire growth period. A local fungicide was applied twice within 20 days interval to protect the plants from prevalent fungal diseases. Other intercultural operations were performed as per the standard practice. Standard principles and procedures as outlined by Gomez and Gomez (1984) were followed to design the experiment and statistically interpret the results.

Eight different qualitative traits were observed during the research period viz. growth habit, raceme position, leaf color, terminal leaf shape, stem color, seed luster and seed stature and were considered for the multivariate analysis. The qualitative traits observed as designated with their respective codes and categories (IBPGR, 1985) are presented in Table 2.

Table 1. Source and status of the local and exotic mungbean genotypes used in the experiment at IAAS, Rampur, 2008

En. No.	Name	Status	Source	En. No.	Name	Status	Source
1.	NPGR-08711	Local	Dhanusha	16.	VC 6173A	Exotic	AVRDC, Taiwan
2.	NPGR-08705	Local	Dhanusha	17.	VC 6173B-10	Exotic	AVRDC, Taiwan
3.	NPGR-09791	Local	Rautahat	18.	VC 6153B-20G	Exotic	AVRDC, Taiwan
4.	NPGR-08709	Local	Dhanusha	19.	VC 3960A-88	Exotic	AVRDC, Taiwan
5.	NPGR-08057	Local	Siraha	20.	NM-92	Exotic	AVRDC, Taiwan
6.	NPGR-08055	Local	Siraha	21.	Pratikshya	Exotic	AVRDC, Taiwan
7.	NPGR-05232	Local	Chitwan	22.	VC 6368 (46-40-4)	Exotic	AVRDC, Taiwan
8.	NPGR-08707	Local	Dhanusha	23.	VC 6370 (30-65)	Exotic	AVRDC, Taiwan
9.	NPGR-08702	Local	Dhanusha	24.	VC 6370 (21-16)	Exotic	AVRDC, Taiwan
10.	NPGR-09351	Local	Morang	25.	VC 6173B-6	Exotic	AVRDC, Taiwan
11.	Saptari local	Local	Saptari	26.	VC 6173B-11	Exotic	AVRDC, Taiwan
12.	NPGR-09350	Local	Morang	27.	VC 6173 (B-10)	Exotic	AVRDC, Taiwan
13.	NPGR-08714	Local	Mahottary	28.	NIMB 101	Exotic	AVRDC, Taiwan
14.	NPGR-08060	Local	Saptari	29.	BARI-MUNG	Exotic	AVRDC, Taiwan
15.	NPGR-09347	Local	Mahottary	30.	KALYAN	Exotic	AVRDC, Taiwan

Table 2. Qualitative traits observed in mungbean genotypes with their codes and category

Traits	Code	Category
Growth habit	1	Erect
	2	Semi-erect
	3	spreading
Raceme position	1	Mostly above canopy
	2	Intermediate
	3	No pods visible above canopy
Leaf color	3	Light green
	5	Intermediate green
	7	Dark green
Terminal leaflet shape	1	Deltoid
	2	Ovate
	3	Narrowly ovate
	4	Lanceolate
	5	Rhombic
	6	Obovate
	7	Lobed
	8	Other (specify)
Stem color	1	Light green
	2	Dark green
	3	Light purple
	4	Dark purple
	5	Other (specify)
Seed lustre	1	Dull
	2	Shiny
Seed color	1	Light green
	2	Dark green
Seed stature	1	Small
	2	Medium
	3	Bold

Cluster analysis was performed based on the Euclidean distance matrix using the Unweighted Pair Group Method with Arithmetic mean (UPGMA) (Sneath and Sokal, 1973). Cluster analysis is a generic name for a group of techniques that place objects or units in groups or clusters in an "objective" manner based only on similarities in the data rather than any a *priori* groupings (Mead et al., 2002). It allocates a set of individuals to a set of mutually exclusive groups such that individuals within groups are similar to one another, while individuals in different groups are dissimilar (Cruz et al., 1999). Here one of the most widely used clustering techniques, hierarchical clustering was used. Hierarchical clustering method is agglomerative; the genotypes are started in different cluster (each = one genotype) and then the two closest genotypes are merged into single cluster. The measures of similarity and dissimilarity were derived by calculating the Euclidean distance between pairs of objects using average linkage method (Mead et al., 2002). Average linkage treats the distance between two clusters as the average distance

between all pairs of items where one member of a pair belongs to each cluster. The Euclidean distance is a multivariate generalization of the Pythogorean Theorem. For the r^{th} and s^{th} objects measured on variable $X_1 \ldots X_J$, it is defined as:

$$D_{rs} = \sqrt{\sum_j \left(X_{rj} - X_{sj}\right)^2}$$

Finally, the dissimilarity matrix are converted to a clustering of objects using hierarchical clustering method and presented as dendogram.

Principal Component Analysis (PCA), the next multivariate technique was used for analyzing relationships among several qualitative variables observed. It provides information about the relative importance of each variable in characterizing the objects (Mutsaers et al., 1997). The relative magnitude of the coefficient of each trait to the corresponding principal components from the component analysis can often provide an agronomic interpretation for each component axis. The sign of the coefficient is irrelevant and is in fact arbitrary, though negatively correlated traits will generally have opposite signs on a given axis (Brown, 1991). Though clear guidelines do not exist to determine the significance of a trait coefficient, one role of thumb is to treat coefficients >4 as having a large enough effect to be considered important (Cruz and Rezzagi, 1994).

The importance of the principal components is calculated from their eigenvalues and their contribution in explaining the overall variance. Here, eight qualitative trait determining variables were transferred to five principal components but only the components with eigenvalue greater than 1.0 were considered as they accounted for almost total variability.

3. Results and Discussion

Histograms showing the frequencies of the qualitative traits for the mungbean genotypes studied are presented in Figure 1. The breeders' major preferences are generally high grain yield, disease tolerance, attractive seed luster, seed color and seed stature. The interpretation and discussion of the individual qualitative traits observed is as follows.

Growth habit- The graph plotted from the data collected in the mungbean research shows that about 53% genotypes were having the erect growth habit (Figure 1a). Forty percent genotypes were having semi-erect growth habit. Altogether 93% genotypes were having erect and semi-erect growth habit. Only 6 % genotypes were of spreading growth habit. It means that the high yielding, or yield attributing or disease tolerance traits might have linked to the erect and semi-erect growth habit determining gene. It can also be concluded that most of the genotypes were having the traits of erect and semi-erect growth habit.

Raceme position- Intermediate raceme position was present in more than 66% genotypes. Remaining 34 % genotypes were having the raceme position below and above the canopy Figure 1b). From the data, it can be concluded that the intermediate raceme position determining gene might be linked to the high grain yielding, or yield attributing or disease tolerance determining genes; it is one possibility. So, more genotypes having the trait of intermediate raceme position might have been selected during breeding work.

Leaf color- Out of 30 mungbean genotypes tested in the experiment, the trait of intermediate green leaf was present in 15 genotypes and the trait of dark green leaf was present in 15 genotypes (Figure 1c). There was no any genotype deviated to the trait of the dark green and intermediate green leaf color. The data indicates that these dark green and intermediate green leaf colors are important for the high yield or disease tolerance. These both traits might are equally important for grain yield, and selection so happened. Consequently, the frequencies of the genotypes possessing these two traits were in equal proportion.

Terminal leaf shape- More than 66% genotypes were having rhombic terminal leaf shape whereas 33% were having deltoid terminal leaf shape (Figure 1d). The data indicates that these traits were selected in the genotypes or transmitted to the genotypes in higher frequency. Or the rhombic terminal leaf shape arrived in many genotypes since the trait might have side effect to cause the yield improvement in any way. It can also be said that the rhombic terminal leaf shape determining gene might be vital and its contrasting allele might be lethal or sub lethal.

Stem color- Out of 30 genotypes observed, 24 genotypes were having the trait of light green stem and only 6 genotypes were having the dark green and light purple stem (Figure 1e). The data of genotypes based on the three categories of the traits show that the trait of light green stem might be favorable with respect to the grain yield improvement or disease tolerance, so that the breeders selected the trait of light green stem. Purple and dark green stem might not be transmitted equally in segregating generation while doing breeding work due to the lethal or sub-lethal nature of the alleles.

Seed luster- Out of 30 mungbean genotypes studied, 13 genotypes (43%) were having dull luster in the seed surface whereas 17 genotypes (56.66%) were having seeds with shiny seed surface (Figure 1f). Breeders might have selected genotypes which were high yielding and disease tolerant. So while undergoing selection for such traits, the trait of dull and shiny seed luster might have transmitted since the traits might cause yield enhancement.

Seed color- Twenty one genotypes were producing dark green seeds whereas only nine were producing light green seeds out of 30 genotypes (Figure 1g). The higher frequency of genotypes was having dark green seed color. It can be concluded that mungbean breeders were biased to select the seed color trait according to the consumer's preference. It can also be that dark green seed color might be of breeders' priority.

Seed stature- With seed stature character, trait of small sized grain were expressed in 10 genotypes (33%), medium

sized grain in 8 genotypes (26.6%) and bold sized grain in 12 genotypes (40%) (Figure 1h). It shows that bold sized seeds may be the primary interest of the breeders for selection because of such genotypes might be high yielding or possessing greater disease tolerance. But it should also be noted that the selection of the genotypes is also affected by the consumers' preference for the different statures of seed size.

For mungbean and other pulse crops, qualitative traits, particularly seed appearance such as color and luster are important breeding objectives since they are influenced by consumer preference. For example, black mature pods can protect green seeds from discoloration while the plants are standing for harvesting in the field. Tomooka et al. (1991) studied seed characters in 651 mungbean accessions collected from several countries and found that germplasm with shiny green seed coat is the most dominant trait, over dull green, brown, black and yellow types. According to Shanmugasundaram (1988), almost all mungbean cultivars released in India had green with dull or shiny seed coat; however, a few cultivars with yellow and black mottled seed coat also exist. Mungbean varieties in Southeast Asia and China normally have a shiny green seed coat, but never with black, brown or mottled ones (Srinives and Yang, 1988).

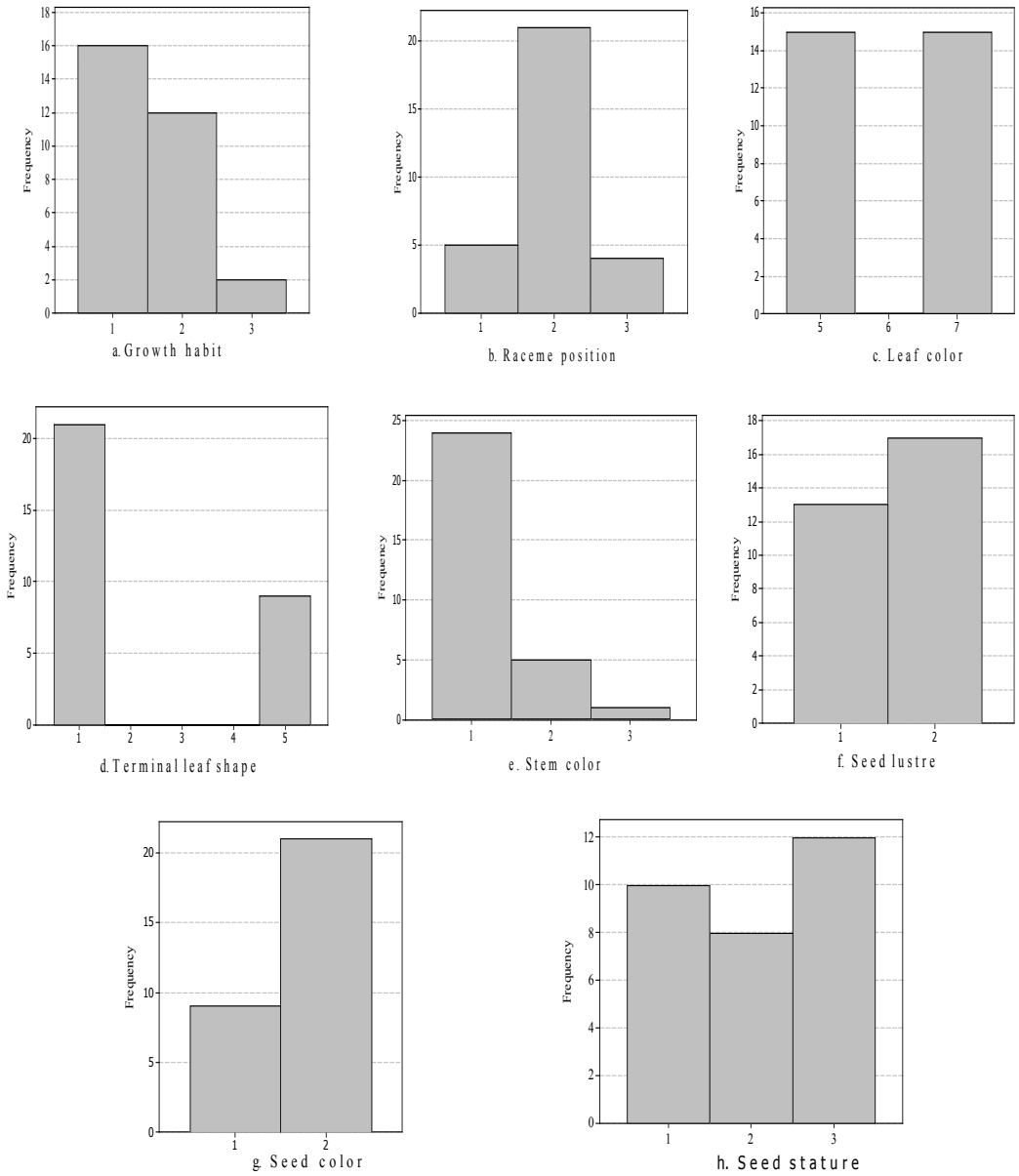

Figure 1. Histograms showing the frequencies of the qualitative traits in mungbean genotypes

The genotypes were grouped into 6 clusters according to UPGMA hierarchical techniques as shown in the dendogram (Figure 2). There were five major groups and one outliner. Cluster I consisted of nine genotypes, cluster II of 6, cluster III of 6, cluster IV of 2, cluster 5 of 6 and cluster VI of 1 (Table 3).The genotypes of cluster I had semi-erect growth habit, intermediate raceme position, dark green leaf color, deltoid terminal leaflet shape, light green stem color, dull seed luster, light green seed color and small seed stature. In case of cluster II, traits like erect growth habit, no pods visible above canopy, dark green leaf color, deltoid terminal leaflet shape, light green stem color, shiny seed luster, dark green seed color and medium seed stature were prominent. That for cluster III, erect growth habit, intermediate raceme position, intermediate green leaf color, deltoid terminal leaflet shape, light green stem color, shiny seed luster, dark green seed color and bold seed stature traits were observed. Cluster IV genotypes were semi- erect, had intermediate raceme position, dark green leaf color, rhombic terminal leaf let shape, light green stem color, dull seed luster, light green seed color and small seed stature. In cluster V, erect growth habit, intermediate raceme position, intermediate green leaf color, rhombic terminal leaflet shape, light green stem color, shiny seed luster and dark green seed color and bold seed stature traits were found in the genotypes. Cluster VI consisted only one genotype which was unique amongst all with spreading growth habit, no pods visible above canopy, dark green leaf color, rhombic terminal leaflet shape, light purple stem color, shiny seed luster, light green seed color and bold seed stature.

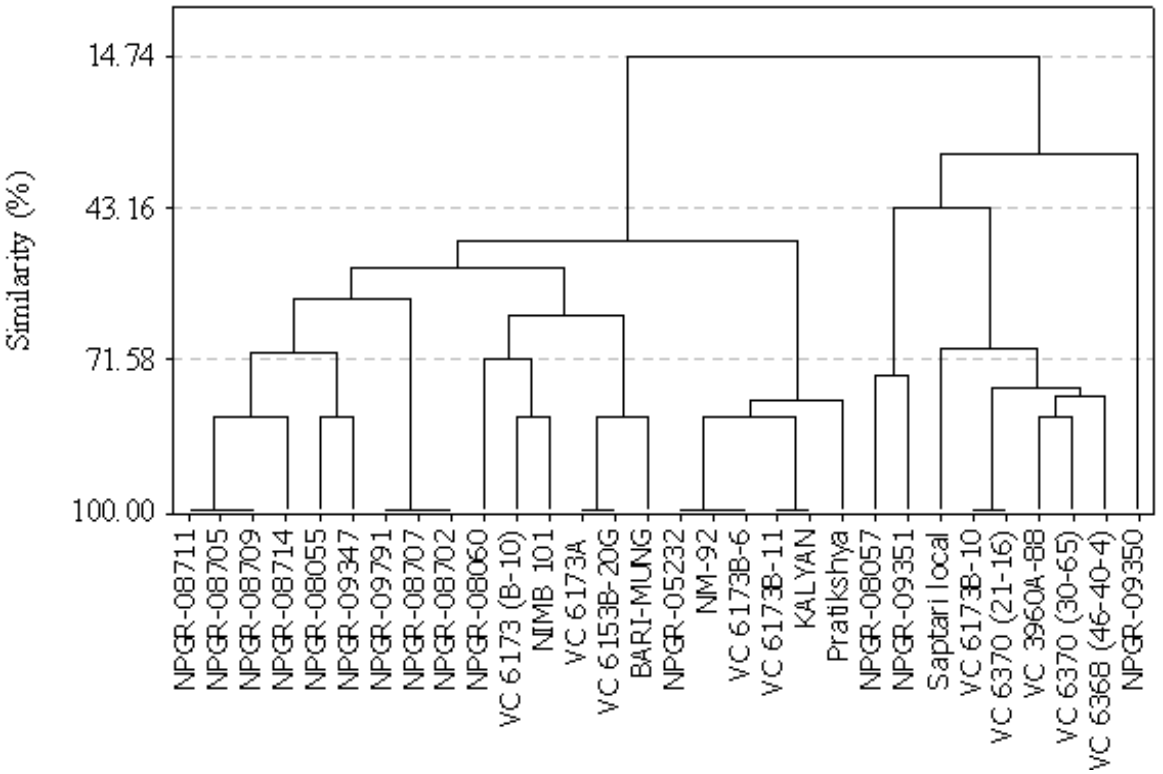

Figure 2. Dendogram based on 8 qualitative traits observed in mungbean genotypes

Table 3. Grouping of mungbean genotypes according to dendogram into six clusters

Clusters	Genotypes
I	NPGR-08711, NPGR-08705, NPGR-08709, NPGR-08714, NPGR-08055, NPGR-09347, NPGR-09791, NPGR-08707, NPGR-08702
II	NPGR-08060, VC 6173 (B-10), NIMB 101, VC 6173A, VC 6153B-20G, VC 6153B-20G, BARI-MUNG
III	NPGR-05232, NM-92, VC 6173B-6, VC 6173B-11, KALYAN, Pratikshya
IV	NPGR-08057, NPGR-09351
V	Saptari local, VC 6173B-10, VC 6370 (21-16), VC 3960A-88, VC 6370 (30-65), VC 6368 (46-40-4)
VI	NPGR-09350

The Principal Component Analysis (PCA) in general confirmed the grouping obtained through cluster analysis though it did not form robust groups (Figure 3). PCA based on the data revealed that five principal components together accounted for 92.30% of the total phenotypic variability observed in the mungbean genotypes (Table 4). The first three Principal Components (PCs) explained nearly 78% of the total variation with individual contribution as 40.60%, 22.30% and 14.70% respectively. The variation explained by PC1 was due to variation in all the traits observed except raceme position and terminal leaf shape. The variance for PC2 was mostly accounted for growth habit, raceme position and stem color. The variance obtained from PC3 mostly resulted from traits like terminal leaf shape, stems color and seed color. PC1 had negative loading score for all traits except growth habit and leaf color. PC2 had negative loading score for all traits except terminal leaf shape and seed color. PC3 had negative loading score for all traits except growth habit, terminal leaf shape and stem color.

Table 4. The first five principal components of 8 qualitative traits, eigenvalues, proportions and cumulatives

Trait	PC1	PC2	PC3	PC4	PC5
Growth habit	**0.394**	**-0.404**	0.154	-0.140	0.273
Raceme position	-0.029	**-0.641**	-0.248	0.203	**0.532**
Leaf color	0.317	-0.273	-0.197	**-0.723**	-0.364
Terminal leaf shape	-0.166	0.066	**0.785**	-0.339	0.368
Stem color	-0.204	**-0.528**	**0.401**	0.119	**-0.504**
Seed lustre	**-0.499**	-0.199	-0.151	-0.004	-0.051
Seed color	**-0.407**	0.096	-0.271	**-0.530**	0.305
Seed stature	**-0.509**	-0.137	-0.042	-0.080	-0.158
Eigenvalue	3.2446	1.7846	1.1757	0.7570	0.4230
Proportion	0.406	0.223	0.147	0.095	0.053
Cumulative	0.406	0.629	0.776	0.870	0.923

Note: Bold numbers and values denote contribution of the corresponding traits for variation in the respective PCs

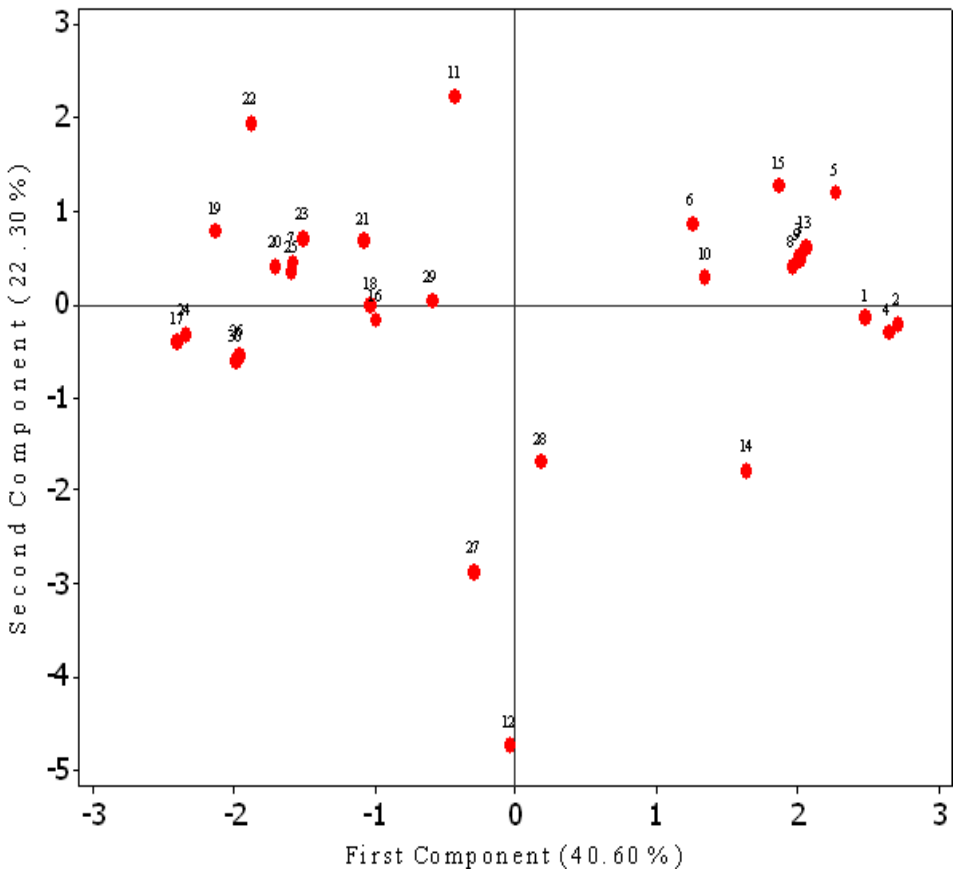

Figure 3. Score plot diagram of the mungbean genotypes for the first two PCs

The first two PCs were plotted against each other in score plot to observe the relation among the mungbean genotypes according to the observed traits (Figure 3). The genotypes were distinguished into four broad clusters or categories. Cluster I, lying in the left corner of the plot, consisted of the exotic genotypes, Kalyan, VC 6173B-11, VC 6173B-10, VC 6370 (21-16), VC 3960A-88, VC 6368 (46-40-4), NM-92, NPGR-05232, VC 6173B-6, VC 6370 (30-65), Pratikshya, VC 6153B-20G, VC 6173A, Saptari local and BARI-MUNG. Cluster II, aligned at the upper right corner of the plot, had the local genotypes in all viz. NPGR-08055, NPGR-09351, NPGR-09347, NPGR-08714, NPGR-08707, NPGR-08702, NPGR-08711, NPGR-08709, NPGR-08705, NPGR-08057 and NPGR-09791. Two exotic genotypes: VC 6173 (B-10) and NIMB 101 and one local genotype: NPGR-08060 made up the cluster III aligned at the lower middle portion. Lastly local genotype, NPGR-09350 alone remained at the bottom of the plot to form the cluster IV.

The multivariate analysis involving the qualitative traits clearly showed the alignment of the local and exotic genotypes into different clusters according to similarity indices. Cluster analysis grouped genotypes together with greater genetic similarity; the clusters did not necessarily include all genotypes from the same origin. Some cluster consisted only of the local or the exotic genotypes while in others, the genotypes of both genotypes were found together. This was primarily due to similarity in the different genotypes for the qualitative traits observed. Though PCA did not form robust group as outlined by the cluster analysis, it surely supported the groups formed in the dendogram. In general, the clusters formed by the genotypes of mungbean displayed the closeness of the local and exotic lines among themselves than for the mixed population consisting of both genotypes.

The importance of the multivariate analysis is high with respect to the grouping of the crop genotypes based on the traits considered under study. The genotypes grouped referring the similarity indices can be explored further at the genetic and molecular level for the crop improvement aspects employing appropriate breeding methodologies. 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).

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 mungbean and blackgram (Dasgupta and Das, 1984). Representative accessions may be chosen from particular groups for hybrid program 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 and Cowling, 1994). Inclusion of genotypes from distinct clusters and their implication in breeding program is suggested (Tawar et al., 1988).

4. Conclusions

Fifteen each of Nepalese and exotic mungbean genotypes were assessed using multivariate analysis techniques as Cluster analysis and PCA based on the qualitative traits. The genotypes were grouped into 6 clusters based on the Euclidean distance matrix using UPGMA hierarchical technique which was supported by PCA. The clusters formed had prominence for different qualitative traits observed. The local and exotic mungbean lines grouped themselves into distinctive clusters while some were found together in the same cluster. Multivariate analysis helps in identifying the genotypes to be further evaluated at the genetic level. The genotypes having preferable traits are used in the crop improvement program involving different breeding techniques.

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