

## **Analysis of Agronomically Important Morphological Traits Diversity in *Vigna radiate***

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### **Abstract**

Pulses constitute the most versatile and nutritious food available for vegetarian population of the country. Among all of the pulses green-gram is one of the most economically important pulse crops, in the present study made an attempt to characterize the germplasm of green-gram using economically important agronomic traits. Assessment of genetic diversity of crops is an important step in a program aimed at improving crop yields. We measured most diverse morphological traits of green-gram for three consecutive seasons in the field, such as; days to flowering, plant-height, pod-length, pod girth, number of seeds per pod, and 100 seed-weight. Various statistical tests were performed to check the distribution and the fitness of data. Out of 192 samples 8 samples were found as the best for at least two characters. we could find an accession (EC- 450447) which was found to be the best for 3 agronomic traits. This accession produced maximum number of seeds per-pod, maximum hundred seed-weight, and was resistant to MYMV infection. This study was done to select the accessions of most economically important traits, and we could find many of such accessions which can be used

for breeding program to produce high quality green-gram breeds.

**Keywords:** Legume, Morphological markers, Agronomy, genetic Diversity, Mung bean.

### **Introduction**

Systematic characterization and evaluation of plant genetic resources are necessary for the efficient use of the material through conventional methods or modern techniques, a number of methods and techniques are available to analyze the genetic diversity (Sajid Mehmood et. al, 2008). Analysis of genetic diversity is prerequisite to monitor genetic erosion, guidance for collection priorities, to investigate the evolutionary history of crops to support breeding strategies and methodologies, and to guide the management of germplasm collections (Engels 2003). Molecular and agronomic characters have been often used for advanced characterization (Williams *et al.*, 1990, Laurentin and Karlovsky, 2007 Dikhshit *et al.* 2007, 2009, Rajora and Rahman, 2003, Noor et al., 2003, Siddique et al., 1996, Hassan *et al.*, 1995). The importance for studying characterization traits in the collection of plant genetic resources (PGR) becomes more relevant in the changing scenario of PGR (Rao 2004).

Characterization of genepool is an important aspect for the management of germplasm collected for the future use. Many social and ethical issues are also associated with the characterization of accessions for their proper management, as it is the only way to stop biopiracy (Esquinas-Alcázar 2005).

*Vigna radiata* (2n=22), also known as green gram or mungbean, is an economically important pulse crop supplying the much-needed proteins for the predominantly vegetarian population. Green gram is an essential crop in developing countries of Asia, Africa and Latin America. Because of presence of high proteins, vitamins and minerals, green gram plays an important role in human consumption as well as animal feed. They are typically low in fats, contain no cholesterol and are high in proteins, folate, potassium, iron and magnesium (Andersan et al,1984, Grusak 2002a,b). Because of their ability to fix atmospheric nitrogen, they play a key role in maintaining soil fertility and ensuring sustainability of production system, particularly in low input, small-scale agriculture, so is the choice of breeders for developing new varieties. The accessions of Mungbean could be important source of genetic variability particularly to develop new varieties suitable for sustainable agriculture, crop diversification, and varieties fitting into the changing cropping pattern and for efficient use of marginal land, if systematic evaluation for important traits is undertaken. This justifies the continued characterization and evaluation of different collections. In the present study we focused on the characterization of important pulse crops i.e. green gram and black gram accessions by using molecular and morphological traits as diverse factors.

Morphological and agronomic characters have been often used for basic characterization. The

major advantages of phenotypic characterization data are easy scoring, less time consuming and cost effectiveness (Iftikhar Ahmad 2004). The present study revealed the overall picture at a glance of the variability pattern for these characterization traits in a collection of green gram accessions. The importance for studying characterization traits in the collection of plant genetic resources (PGR) becomes more relevant in the changing scenario of PGR regime where we can act on “The protection of plant varieties and Farmer’s Rights” (has been enacted by the Government of India 2001). According to this act a registrable variety must confirm the criteria of novelty, distinctiveness, uniformity and stability (DUS). Presently, DUS criteria can be justified by specifying a combination of characterization (qualitative) traits (Ramanna 2003). Thus the importance of accessions and to promote their utilization in crop improvement programs, may be facilitated by assessing the genetic (morphological and molecular) diversity for different traits of agronomic importance.

#### **Material**

A set of 192 samples (Table 1) was selected for the morphological agronomic trait diversity analysis; those samples were grown in the National bureau of plant genetic resources (NBPGR) New Delhi, fields in three consecutive seasons (2008-2009). Out of 192 samples 176 samples produced significant data as few of the seeds did not show growth and few of them were not able to produce flowering, so those samples were discarded from the data set. Only 176 samples were used for further analysis. In this study we present the relation between agronomic traits of the samples (core collection) collected from different part of the country and from other Asian, European and American countries. the samples collected from India were mostly from, Gujarat, Maharashtra,

Rajasthan, Punjab, Bihar, Madhya Pradesh, Haryana, J & K, Uttar Pradesh and Himanchal Pradesh. The Exotic samples were mostly collected from Pakistan, Taiwan, Hungary, Philippines, Denmark, USA, USSR, Surinam and Italy.

### Methods

The data produced by the regular observation in the field for various agronomic traits such as days to flowering, plant-height, pod-length, pod-girth, number of seeds per pod, and hundred seed weight, was then analyzed using the software SYS-stat. the data observed in all the three seasons was used to calculate the mean, and the mean value for the particular trait and particular individual was then used for SYS-stat analysis. Various statistical parameters such as probability distribution, Chi-square test, Kolmogorov–Smirnov test, and Shapiro–Wilk test were used for analysis.

Many other simple statistical parameters were also used for the analysis viz. maximum, minimum, range, Median, Arithmetic Mean, Trimmed Mean (10%, Two Sided), Geometric Mean, Harmonic Mean, Standard Deviation, Coefficient of Variation, Skewness(G1), Kurtosis(G2), and Anderson-Darling Statistic (Table 2). Pearson correlation matrix was also calculated to identify the linear correlation between the various traits used for the study (Table 3)

### Results

The results obtained (Table 2 and 3) were carefully analyzed and used to create graphs to compare the data more accurately (Figures; A, B, C, D, E, F, G & H).

#### Pearson Correlation Matrix:

This statistical test was performed to check the relation between all the agronomic traits selected for the present study. By analyzing the data (Table 3) it was observed that, MYMV infection and days to flowering are the

independent traits and did not show any significant correlation with the other agronomic traits. The other traits such as pod length, pod girth, number of seeds per pod, and 100 seed weight, were found to be linearly dependent on one another the correlation value for plant height with pod girth and seeds per pod was 0.12 and 0.31 respectively which shows only small correlation on correlation matrix scale. Now the correlation between the pod length with 100 seed weight, pod girth and seeds per pod was found to be 0.394, 0.582 and 0.678 respectively which shows the medium to large colinearity between the traits and suggests that the pod length and seeds per pod are highly associated traits which was in accordance with the expected value similarly pod girth with seeds per pod and 100 seed weight showed the medium collinear relation with the value 0.32 and 0.37 respectively.

#### Days to flowering

All the 176 samples were checked regularly for the days to flowering and the dates were noted down for all the samples. A very immense range (36 days) of variation was observed from the minimum 29 days (**PLM- 734, PLM- 953, IC- 8961-5A IC- 10184, IC- 2056-2, STV -2635, and STV- 2765**) to maximum 65 days (**PLM- 184-1 and IC- 39232**). The median, arithmetic mean, geometric mean and harmonic mean calculated were 47, 47.39 ( $\pm 0.41$ ) 47.09, and 46.79 respectively. Standard deviation was found as 5.37 with variance of 288 and coefficient of variation of 0.113. Skewness and kurtosis was also calculated by using the mean (47.39) and standard deviation (5.357) values for measuring the asymmetry of the probability distribution of data, and the value of skewness and kurtosis was found to be 0.351( $\pm 0.183$ ) and 0.533( $\pm 0.364$ ) respectively. The value of skewness shows that the data is little asymmetric. The data was found to be little

skewed towards the right. The value of kurtosis suggests the peaked curve near the mean and the value was found to be little more than zero which also supports the result of skewness, but also supports the formation of nearly bell shaped curve (Fig. A).

Chi-square test was performed to check the goodness of the data produced (Table 4). The value of Chi-square was calculated as 27.88 with 3 degree of freedom and <0.01 p-value. The value obtained signify that the value of chi-square is very higher than the expected value for the 3 degree of freedom and <0.01 p-value which suggests that the test data was found to be significant. Kolmogorov-Smirnov Test, Shapiro-Wilk Test and Anderson-Darling Statistic test were also performed to measure the distance between the empirical distribution function of the sample and the cumulative distribution function of the reference distribution, And the values obtained at 1% (p value 0.01) were 0.143, 0.960, and 2.588 respectively.

#### **MYMV resistance**

For the MYMV infection data was observed and analyzed for the same parameters as we used to analyze the data for days to flowering, the infection was checked on a scale of 0 to 9. The minimum infection found was considered as 0 (PLM- 21, PLM- 37-A, PLM- 88, PLM- 88-A, PLM- 226, PLM- 11, PLM- 334-2, PLM- 350, PLM- 380, PLM- 410, PLM- 416, PLM- 380, UGG- 37, PLM- 720, PLM- 734, PLM- 775, PLM- 782, PLM- 818-A, MCV- 1, PLM- 962, PLM- 1037, PLM- 1057, IC- 8917, IC- 9923, IC- 8961-1, IC- 9886-B-2, IC- 10184, IC- 10451, WGG- 37, IC- 39275, IC- 39480, IC- 73465, IC- 73430, LGG- 450, EC- 450447, EC- 450448, EC- 450449, EC- 450450, ET- 52186, ET- 52187, ET- 52188, ET- 52189, ET- 52190, ET- 52198, ET- 52199, ET- 52200, PDM- 11-3R, STV- 2669, PLM- 100, PLM- 93) and maximum 9 (PLM- 231, PLM- 256, PLM-

334-1, PLM- 340-A, PLM- 427, PLM- 619, PLM- 625, PLM- 629-B, PLM- 660, PLM- 666, PLM- 884, PLM- 891, PLM- 904, PLM- 941, IC- 25789, IC- 25133, EC- 130722, IC- 25997, EC- 27853, EC-245952EC- 261790-A, STV- 2765, STV- 2766, EC- 27514, EC- 27185, EC- 13074-2, EC- 8837-2, ) scale and the values lies between the range from minimum zero to maximum 9. Median and arithmetic mean, was found as 3, and 6.76 ( $\pm 0.258$ ), respectively. The standard deviation value calculated was 3.42, which is very high for the range selected thus indicates that the data is not normally distributed around the mean value.

The values for Kolmogorov-Smirnov Test, Shapiro-Wilk Test and Anderson-Darling Statistic test, obtained were 0.204, 0.844 and 9.565 respectively which suggests the discreet distribution of the data. The graphical presentation of the data also supports the results obtained from various statistical analyses; most of the data lies at the corners of the graph. The skewness and kurtosis values were also calculated which were 0.403 and -1.408 respectively. The high value of skewness indicates the skewness of data and the negative value of kurtosis suggests the flat curve for the data which is clearly visible in the graph (Fig B). All of the result values obtained rejects the null hypothesis and suggests that the data is not normally distributed. The chi square test was performed to check the goodness of fit of the test for the given data set. The chi-square value 149.746 with Degrees of Freedom 9 and p-value 0.001 suggests that the test performed was significant (Table 5).

#### **Plant height**

The plant height is a highly variable factor and a very high range (74) of variation was observed from the minimum 14cm (PLM- 334-2) to maximum 88cm (PLM- 748). the median, arithmetic mean geometric mean and

harmonic mean calculated for the data set and was found as 40, 41.92 (1.03), 39.64 and 37.28 respectively. Standard deviation was found as 13.74 which was found to be in proportion of mean the value of skewness was calculated as 0.500. This value is very close to zero and indicates only very small skewness of the data. The value of kurtosis was found to be 0.142 which cannot be considered as the significant value and is very close to zero thus it suggests that the distribution is not the normal distribution and the discreteness of the data is very low and will result in a nearly perfect bell shaped curve. The calculated chi-square value shows the significance of result data (Table 6). Shapiro-Wilk Test and Anderson-Darling Statistic test values 0.919 and 0.981 respectively suggest the nearly normal distribution of the data. The skewness value 0.500 and the kurtosis value 0.142 suggest that data is skewed toward the right but not much skewed, and the graph obtained was imperfect but nearly bell shaped (Fig C).

#### **Pod length**

In Vigna, pod length is a highly variable and very important agronomic trait so the variability in the pod length was observed and analyzed. The variation ranged (51.4 mm) from minimum 22.85mm (**TAP- 7**) to maximum 74.33mm (**EC- 450450, ET- 52196**). And the median, arithmetic mean, geometric mean, and harmonic mean was calculated as 47.58, 47.24, 45.88, and 44.45 respectively. The skewness value 0.18 is very near to zero and indicates the nearly equal distribution of data around the mean and suggests that the data is very less skewed from the mean so resulting in the formation of nearly perfect bell shaped curve (Fig D). Kolmogorov-Smirnov Test, Shapiro-Wilk Test and Anderson-Darling Statistic test were performed and values calculated and were found as 0.031, 0.993, and 0.189 respectively which indicates the nearly

normal distribution of the data. The chi-square value 5.232 at 6 degree of freedom and at 0.514 p value designates the significance of result (Table 7).

#### **Pod Girth**

Pod girth is also a very variable character found in Vigna. In the selected set of data it ranged (4.3mm), from minimum 2mm (**PLM- 1037**) to maximum 6.3 mm (**ET- 52196**). The median arithmetic mean and harmonic mean were calculated as 4.3, 4.33, and 4.23 respectively. The value of skewness and kurtosis were found to be -0.047 and 1.966 the negative value of skewness suggests that the data is skewed towards the left of the distribution curve, the value of kurtosis supports the formation of nearly bell shaped curve (Fig E).

Kolmogorov-Smirnov Test and Shapiro-Wilk Test resulted in the values 0.086 and 0.971, respectively and both of the test values support the formation of nearly bell shaped curve. The value of chi-square test was calculated as 6.071 at 4 degrees of freedom and a 0.194 p-value confirms the significance of the data results (Table 8).

#### **Seeds per pod**

Seeds were taken from at least 7-10 pods from each of the sample, and were counted manually. The average seeds per pod per sample were then calculated for the further analysis. Seeds per pod, is also a highly variable character and it was ranged from minimum 2 (**PLM- 713, STV -2635, IC- 73465**) seeds per pod to maximum 10 (**PLM- 829, PLM- 256, PLM- 76, IC- 118959, EC- 27185, PLM- 416, PLM- 468-A, EC- 450447, C- 22427, ET- 52188**) seeds per pod. From the data generated – Median, arithmetic mean and harmonic mean were calculated and were found as 5.5, 5.73(±0.13), and 5.08 respectively. The value of skewness and kurtosis were found to be 0.213 and -0.463 respectively. Which suggests that the data is

little skewed but supports the formation of nearly bell shaped curve (Fig; F). The values of

Kolmogorov-Smirnov Test and Shapiro-Wilk Test also support the results of above tests. The Chi-square test value was found as 14.49 at 7 degree of freedom and 0.043 p value, which suggests that the test data was found to be significant (Table 9).

### **Hundred Seed weight**

This is again a highly variable character in *Vigna*. The seeds harvested from the crop were counted manually and 100 seeds were taken individually from all the 176 samples and the weight was checked using weighing balance, the data obtained was then processed for various parameters analysis. The seed weight lies in between the range (4.45 g) from minimum 1.56 g (PLM- 119) to maximum 6.1g (ET- 52197, EC- 10732, EC- 450447, ET- 52195, ET- 52197-A, PLM- 334-1, ET- 52194, ET- 52196 , EC- 450448). Median, arithmetic mean, Geometric mean and harmonic mean were calculated as 3.16, 3.34 ( $\pm 0.065$ ), 3.23 and 3.13 with the standard deviation of 0.858 and coefficient of variation of 0.73. The values of skewness and kurtosis were calculated to be 0.785 and 0.681 respectively which suggests that the data is not perfectly normally distributed but indicates the nearly bell shaped curve (Fig G). The values of Kolmogorov-Smirnov Test and Shapiro-Wilk Test were found to be 0.115 and 0.959 respectively, which supports the formation of bell shaped curve. The Chi-square Test Statistic for 100 seed weight was found to be 21.28 at 5 degree of freedom and 0.001 p-values (Table 2).

### **Discussion**

Total yield is an important factor as it measures the economic productivity in mungbean, but its inheritance is extremely complex and is affected by so many factors and genotypic and phenotypic traits. The classical breeding

systems that make use of additive genetic variance will be effective breeding procedures for improving the mungbean seed yield, but very little basic information is available on all the agronomic traits which directly affect the yield in mung bean. (Khattak *et al.*, 1999 a, b and 2001a, c).

Present study was conducted to generate knowledge on genetic variability and character association for crop improvement. The information obtained from this study would be helpful to specify certain parameters that could be used as selection indices for identification of potentially high yielding mungbean genotypes

### **Analysis of agronomic traits**

Analysis of agronomic traits reveals the high genetic differentiation for all the phenotypic traits selected for the present study. This shows that the core collection (collected from different parts of India and from so many other different countries), can be used to fill up the gaps for variability of these traits in green gram germplasm resources. All the traits selected (days to flowering, MYMV resistance, plant height, pod length, seeds per pod and 100 seed weight) for the study showed high degree of variability. The range of variability found in days to flowering was 36 days, which is more than a month, so this can be considered as highly variable trait for the data set. The samples which show the early flowering will show the early maturity. So this character can be used to produce/ generate short period crops. The accessions (PLM- 734, PLM- 953, IC- 8961-5A IC- 10184, IC- 2056-2 and STV 2635) are few of the selected samples which showed the earliest flowering i.e. 29 days, so these accessions can be used as short period crop.

On field trial of all 176 accessions, MYMV resistance was checked and was scored on a scale of 0 to 9. The accessions which were

resistant to MYMV were scored as zero. Out of 176 samples 50 (**PLM- 21, PLM- 37-A, PLM- 88, PLM- 88-A, PLM- 226, PLM- 11, PLM- 334-2, PLM- 350, PLM- 380, PLM- 410, PLM- 416, PLM- 380, UGG- 37, PLM- 720, PLM- 734, PLM- 775, PLM- 782, PLM- 818-A, MCV- 1, PLM- 962, PLM- 1037, PLM- 1057, IC- 8917, IC- 9923, IC- 8961-1, IC- 9886-B-2, IC- 10184, IC- 10451, WGG- 37, IC- 39275, IC- 39480, IC- 73465, IC- 73430, LGG- 450, EC- 450447, EC- 450448, EC- 450449, EC- 450450, ET- 52186, ET- 52187, ET- 52188, ET- 52189, ET- 52190, ET- 52198, ET- 52199, ET- 52200, PDM- 11-3R, STV- 2669, PLM- 100, PLM- 93**) were found to be resistant to MYMV. This is agronomically very important trait for the individual sample, and can be used in the crop improvement program to produce new resistant varieties.

Plant height was also a very diverse trait in pulses. Plant height ranged from 14 cm to 88 cm the plant with shortest length was (PLM-748) and the plant with maximum height was (PLM- 334-2). Small plants are always the choice of farmers so can be used as dwarf crop variety. Similarly the pod length also had high range (51.4mm) of variability the pod with minimum length was TAP-7 and with maximum length were **EC- 450450 and ET- 52196**. The pod length is directly associated factor with number of seeds per pod and ultimately the yield, so the plant with the high pod length can be used as economically important high yielding crop. Pod girth ranged from 2mm to 6.3mm. The pod with minimum girth was PLM-1037 and the pod with maximum girth was **ET- 52196**. This trait is directly associated with the fact that the plant varieties with pod of high girth will produce large size grains and ultimately give the high yield value. So the accessions ET- 52196 can be used for the breeding programs to produce high yielding varieties.

Seeds per pod are also a very important agronomic trait, which can directly affect the total yield. In the present study it was found to be highly variable character, which ranged from 2 seeds per pod to 10 seeds per pod. Total 10 accessions (PLM- 829, PLM- 256, PLM- 76, IC- 118959, EC- 27185, PLM- 416, PLM- 468-A, EC- 450447, IC- 22427 and ET- 52188) were found to containing maximum number of seeds per pod. Those ten accessions can be used as high yielding crop, and the accessions which showed less no of seeds per pod can be avoided by the breeders and farmers. Hundred seed weight is also a very important agronomic trait, for the crop improvement programs. This factor directly affects the yield of crop. In the present study it was found to be highly variable trait, which ranged from 1.56g to 6.1 g. Out of 176 samples total nine accessions (**ET- 52197, EC- 10732, EC- 450447, ET- 52195, ET- 52197-A, PLM- 334-1, ET- 52194, ET- 52196, EC- 450448**) showed the maximum value for 100 seed weight, which indicate the production of large sized seeds, and finally the high yield. Those 9 accessions can be used for further crop improvement program.

Accession number PLM-734 and IC 8961 were the samples which showed the minimum flowering/maturity time and were also found to be resistant to MYMV infection. Accessions named PLM-334-2 was found to be short heighted and also resistant to MYMV infection. ET-52188 and PLM- 416 produced maximum number of seeds per pod and were also resistant to MYMV infection. EC- 450447 was the only accession which was found to be the best for three agronomic traits. This accession produced maximum number of seeds per pod, 100 seed weight calculated was also found maximum in that case, and was resistant to MYMV infection. The accession number ET- 52196 was found to be produced pods of

maximum length and also the maximum 100 seed weight. EC-450458 was found to produce maximum value for 100 seed weight and was resistant to MYMV infection. According to Arshad et al., 2006, the selection for genotypes having high yield potential may be based on days to flowering and plant height. Both these characters are easily measurable and could be used directly to increase mungbean seed yield.

#### **Correlation analysis**

On correlation analysis it was found that most of the variables were not dependent on each other just few of them showed considerable relation. The maximum correlation values was observed in seeds per pod and pod length, that was expected because as the pod length increases the chance of getting more seeds per pod also increases. Pod girth and pod length also showed good correlation, and for other variables low degree of correlation was found. Seeds per pod and plant height showed considerable correlation. Hundred seed weight was also found to be correlated with pod length and pod girth. In genetic system, most of the characters are associated with each other and such association may be the product of some pleiotropic effects of gene, existence of two genes on the same chromosome, chromosomal segmental affiliation or due to environmental influences (Rothwell, 1983). Therefore, information of character association between traits and yield component is important to select high yielding genotypes. However, Bhatti, 1972, contradicted that only correlation studies do not clearly disclose reliable information for selection and an inadequate knowledge of interrelationship of heritable traits may lead to negative results. Ahmed *et al.*, 1981 found number of pods and secondary branches per plant and number of seeds per pod had significant positive correlation with yield per plant while days to maturity and 100-seed

weight were negatively correlated with seed yield. Hassan *et al.*, 1995 reported positive direct effect on seed yield with days to flowering, days to maturity, number of pods per plant, number of seeds per pod and seed weight. The correlation between yield components has been extensively studied by so many researchers. Assady et al., 2005, reported that days of flowering had the highest significant positive correlation with seed yield and 100 seed weight had significant negative correlation with seed yield. In another study Siddique et al., (1996) observed inverse correlation of seed yield with days to flowering and podding, which was found opposite in our case study. Noor et al., (2003) noted that days to flowering and 100 seed weight exhibited high heritability.

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