Morphological and Nutritional Diversity of Some Mungbean Genotypes from Indian Subcontinent

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Seventy eight genotypes of mungbean [*Vigna radiata* (L.) Wilczek] originated from Indian subcontinent were found genetically diverse for morphological characters, level of seed protein and anti-nutritional factors. Genotypes, like, IC103179 and 'Kopergaon' with high protein yield along with low content of trypsin inhibitor were screened. Potential parental combinations for hybridization programme, namely, 'Kopergaon' x TRCM-5-1, 'Kopergaon' x IC103993 were identified through D^2 analysis for breeding high yielding genotypes with superior quality.

Key Words: Mungbean, Descriptors, D^2 analysis, Protein content, Trypsin inhibitor content

One of the constraints listed for lack of breakthrough in mungbean production has been non-availability of genetic variability for high yield potential. This was probably due to the utilization of only a few selected genotypes of mungbean in cultivar development programme and underutilization of the genepool of the Indian subcontinent (Gupta *et al.*, 2004). This was supported by the DNA fingerprinting information of Indian cultivars emanated from the investigations of many researchers (Lakhanpaul *et al.*, 2000; Chattopadhyay *et al.*, 2008). Therefore, identification and deployment of diverse genotypes is required for breeding new cultivars in the point of view of food and nutritional security. Total seed protein of mungbean was found to be around 24-25% while soluble protein was reported to be around 20% with huge variability. But protein rich genotypes of pulses may not be always useful to fulfill our nutritional requirements, as it may be associated with some anti-nutritional factors like trypsin inhibitor. The content of trypsin inhibitor in mungbean was found to be low as compared to other pulses (Rosario *et al.*, 1980). But a wide range of variation was found in respect of trypsin inhibitor content.

The present study was aimed at the estimation of genetic diversity on the basis of data of plant seed yield and yield contributing characters among the mungbean lines for two seasons (environments) and to find out the seasonal (environmental) effects on clustering pattern and identify suitable parental combination for improvement programme. Another objective was to identify high yielding and high protein genotypes with minimum level of trypsin inhibitor. Those lines would be regarded as ideal genotypes which could be either used directly as nutritionally superior varieties or in breeding for quality improvement in mungbean.

Seventy eight accessions of mungbean having diverse origin and nature (Table 1) were grown in the experimental blocks at D-Block Farm, Kalyani of Bidhan Chandra Krishi Viswavidyalaya, West Bengal, India. Those lines belonged to Rajasthan, Gujarat, Delhi, Punjab, Maharastra, Uttar Pradesh, West Bengal, Tripura and exotic genotypes from Myanmar. The materials were sown in the month of February of the year 2004 and 2005 in Randomized Black Design (RBD) with three replications. The plot size was 5 m^2, where row to row and plant to plant distances were maintained by 30 cm and 10 cm, respectively.

Two sets of data of seven quantitative characters, collected in two years were subjected to analysis of variance and test of homogeneity was done for each character. Genetic diversity was calculated among germplasm, using data of quantitative characters under two environments, separately, by D^2 analysis (Mahalanobis, 1936). Clustering of genotypes in the present study was done by Tocher's method (Rao, 1952) where groups were constructed by 'adding' genotypes using D^2 values. Soluble protein was estimated following Bradford's method (Bradford, 1976) from 50 mg powder of crushed seeds of each sample and colour absorbance reading was taken from Spectrophotometer in 460 nm and protein concentration was measured from standard graph. On the other hand, trypsin inhibitor was estimated from a few selected genotypes, representing all clusters generated by D^2 analysis from data taken in year 2004.

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100 mg of crushed seed powder of each sample was homogenized with Tris-$CaCl_2$ buffer having pH 8.1-8.2 for 5 minutes and centrifuged for 30 minutes at 8000 rpm. Aliquot of supernatant was diluted with buffer solution and five different concentration of solution were taken in five tubes. Subsequent analysis of trypsin inhibitor activity (in TIU) was done following the method given by Kakade *et al.* (1974).

Large observable variation was found among genotypes in respect of qualitative characters, like, flowering synchronization, pod and seed colour, etc. IC103788, a landrace from Gujarat was found to be early maturing with synchronous flowering habit. 'Kopergaon', a variety from Maharastra showed intermediate synchrony and profuse flowering in first flush of flowering. IC39566 with brown colour mature pod, yielded high. On the basis of high GCV, PCV, high heritability and genetic advance over mean and significant positive correlation with seed yield (data not shown), 1000-seed weight and number of pods per plant could be considered as useful selection criteria for identifying pure line or segregating progenies with superior productivity. Among the quantitative characters, the number of branches per plant and plant height were substantially influenced by the changes of environment. 'Kopergaon' was observed to bear highest number of pods while TRCM-5-1 showed largest pod length with larger seed size.

Test of homogeneity revealed that out of seven quantitative characters, five were homogeneous but rest two characters, namely, plant height and number of branches per plant were heterogeneous in nature. Therefore, taking all the characters, pooled D^2 analysis over the environments could not be done. Under that circumstance, D^2 analysis on separate data sets have been carried out and tried to identify some distant clusters or genotypes for making potential cross combinations.

Analysis of variances for dispersion and x^2 test using Wilk's criteria signified the variability among genotypes tested in 2004. The contribution of seed yield per plant was highest (27.87%) followed by 1000-seed weight (25.34%) and number of pods per plant (21.25%) in the estimates of divergence of genotypes. Seven clusters were generated through Tocher's method. Large number of genotypes (46) belonged to cluster-I while other clusters (with suffix II, III, IV, V, VI and VII) contained 16, 8, 2, 1 and 4 genotypes, respectively. Cluster VII possessed genotypes with highest mean seed yield (7.38 g/plant) while cluster V revealed highest mean 1000-seed weight (46.25 g) with lowest mean plant height (35.7 cm). Cluster V and cluster VI revealed highest inter-cluster distance (17.75) followed by cluster V and VII (16.39). D^2 value was calculated high between TRCM-5-1 (M-72) belonged to cluster V and S1 (BL x 444D1) (M-63) belonged to cluster VI. Therefore, this combination could be the best in generating useful transgressive segregants. On the other hand 'Kopergaon' (M-66) belonging to cluster VII showed highest D^2 value against TRCM-5-1 (M-72), belonging to cluster V. That combination also similarly had potentiality to generate enormous beneficial segregates in hybridization.

ANOVA of dispersion revealed the significant level of variability among the same set of genotypes grown in 2005 which was supported by x^2 test using Wilk's criterion. 1000-seed weight (32.4%) contributed largely to D^2 values. Again seven clusters were found through Tocher's method. Cluster IV, V, VI and VII contained one each genotype. Cluster II and cluster III contained 14 and 5 genotypes, respectively. Rest 55 genotypes belonged to cluster I. Cluster mean of seed yield per plant (6.37 g) and number of pods/plant (27.1) were highest in cluster V. Cluster III with highest pod length (8.11 cm) and highest 1000 seed weight (42.9 g) revealed the highest inter-cluster distance (12.09) with cluster VII, with lowest 1000-seed weight (6.33 g) and lowest pod length (22.5 cm). Considering the D^2 values, it was observed that, IC103984 (M-43), in cluster VII and TRCM-5-1 (M-72) in cluster III was most diverse. The second highest inter-cluster distance (11.35) was noted between cluster II and cluster VI. IC103993 (M-45) in cluster VI and 'Kopergaon' (M-66) in cluster II were also found diverse with high D^2 value. Those two combinations could be efficient for generating variation through hybridization. 'Kopergaon' and Sel1 (Mush x mung) were high yielding and consistently belonged to same group while evaluation for two years. Therefore, crossing between IC103993 and Kopergaon or Sel1 (Mush x mung) would likely to produce diverse segregates.

Significant variability was observed among the genotypes in respect to percent of soluble protein content. The range of the soluble protein content varied from 15% (IC39501) to 25.9% (IC103213) with a mean (20±1.82%) (Table 1). Working with 37 land races of Orissa, Naik *et al.* (2000) found the range of protein content varied from 17.2 to 29.9%. In the present investigation, the per plant protein yield was varied from 0.28 in IC103993 to 1.55 in Kopergaon. The average soluble protein harvest

Table 1. List of mungbean germplasm with their nature, origin, cluster name, estimation of soluble protein content, seed yield, trypsin inhibitor level in selected entries and top ten rankings based on protein yield per plant

Entry No.	Accession number/ name	Nature of germplasm	Origin/place of collection	Cluster by D^2 analysis in 2004	Cluster by D^2 analysis in 2005	Percentage of protein content	Seed yield per plant (g)	Protein yield per plant (g)	Rank	Trypsin inhibitor (TIU) (selected entries)
M-1	IC39483	Landrace	Kachchh, Gujarat	I	I	17.60	3.015	0.531		–
M-2	IC39484			I	I	21.20	3.96	0.84		1428.57
M-3	IC39486			I	I	17.20	4.135	0.711		–
M-4	IC39487			I	I	17.00	2.21	0.376		–
M-5	IC39488			I	I	21.44	3.38	0.725		–
M-6	IC39489			I	I	21.48	3.025	0.65		–
M-7	IC39496			I	I	17.12	3.735	0.639		–
M-8	IC39499			I	I	24.20	2.365	0.572		–
M-9	IC39501			I	I	15.00	3.853	0.578		–
M-10	IC39503			I	I	15.20	2.705	0.411		–
M-11	IC39504			I	I	20.00	2.975	0.595		–
M-12	IC39525			I	I	18.24	2.395	0.437		–
M-13	IC39534			I	III	15.96	3.215	0.513		–
M-14	IC39535			I	I	21.08	2.978	0.628		–
M-15	IC39538			I	I	17.08	3.375	0.576		–
M-16	IC39539			I	I	18.44	3.86	0.712		–
M-17	IC39540			I	I	21.40	2.563	0.548		–
M-18	IC39541			III	I	16.72	2.84	0.475		–
M-19	IC39544			I	I	19.60	3.563	0.698		–
M-20	IC39547			I	I	15.90	2.945	0.468		–
M-21	IC39548			I	I	17.72	3.1	0.549		–
M-22	IC39549			VII	I	24.00	5.56	1.334	II	–
M-23	IC39552			I	I	18.00	2.455	0.442		–
M-24	IC39561			II	I	19.00	3.575	0.679		–
M-25	IC39566		Patan, Gujarat	II	V	24.92	4.97	1.239	III	–
M-26	IC39568			I	I	18.52	3.225	0.597		–
M-27	IC103014		Keda, Gujarat	I	I	18.24	3.82	0.697		–
M-28	IC103154		Pali, Rajasthan	II	I	17.56	4.365	0.766		–
M-29	IC103177		Bhilwara, Rajasthan	II	I	23.60	4.538	1.071	VII	–
M-30	IC103179		Bhilwara, Rajasthan	II	I	20.40	4.92	1.004	X	1250.0
M-31	IC103196		Bhilwara, Rajasthan	I	I	22.52	3.37	0.759		–
M-32	IC103213		Chitorgarh, Rajasthan	II	I	25.90	4.505	1.167	V	–
M-33	IC103224			I	I	19.28	2.595	0.50		–
M-34	IC103238		Banswara, Rajasthan	II	I	20.00	4.635	0.927		–
M-35	IC103300		Kachchh, Gujarat	I	I	22.88	3.165	0.724		–
M-36	IC103788			II	I	17.20	3.758	0.646		–
M-37	IC103861			II	I	18.20	2.44	0.444		–
M-38	IC103878			II	I	16.72	2.935	0.491		–
M-39	IC103880			I	I	18.68	3.07	0.573		–
M-40	IC103973		Valbhipur, Gujarat	II	IV	22.36	2.14	0.479		–
M-41	IC103974			III	I	23.24	2.175	0.505		–

Entry No.	Accession number/ name	Nature of germplasm	Origin/place of collection	Cluster by D^2 analysis in 2004	Cluster by D^2 analysis in 2005	Percentage of protein content	Seed yield per plant (g)	Protein yield per plant (g)	Rank	Trypsin inhibitor (TIU) (selected entries)
M-42	IC103976		Sihor Road, Gujarat	II	I	19.64	3.66	0.719		–
M-43	IC103984		Talaja Road, Gujarat	III	VII	23.92	2.53	0.605		–
M-44	IC103986		Mahuwa Road, Gujarat	III	I	22.72	2.2	0.50		–
M-45	IC103993		Khambhat Road, Gujarat	III	VI	18.64	1.525	0.284		–
M-46	EC16563	Exotic collection	Myanmar	I	I	17.32	2.973	0.515		–
M-47	EC16566			I	II	20.32	2.335	0.474		–
M-48	EC19732			I	III	17.64	4.09	0.721		1500.0
M-49	EC27515			I	I	22.52	3.5	0.788		–
M-50	EC33351			IV	I	23.76	4.065	0.966		1304.34
M-51	Sonamung	Landrace	West Bengal	I	II	20.92	4.335	0.907		–
M-52	Sub-2	Wild (*V. radiata* var *sublobata*)	Rajasthan	III	II	19.64	2.95	0.579		1411.76
M-53	Midnapur local	Landrace	West Bengal	I	II	19.32	3.96	0.765		–
M-54	Malda-95-13			I	I	20.74	3.98	0.825		–
M-55	Radiata-5			I	II	22.48	3.295	0.741		–
M-56	BM-18	Variety	Maharastra	I	I	19.10	4.27	0.816		–
M-57	PDM-19-257		Uttar Pradesh	I	II	17.80	2.615	0.465		–
M-58	83-m-selection	Selection line	West Bengal	I	II	21.96	2.76	0.606		–
M-59	SML-264	Variety	Punjab	I	II	18.24	3.393	0.619		–
M-60	NP-28		New Delhi	I	II	21.68	4.17	0.904		–
M-61	B-27		West Bengal	I	I	22.32	3.85	0.859		1363.64
M-62	SML-286		Punjab	I	I	24.48	4.22	1.033	IX	–
M-63	S1 (BL x 444D1)	Selection line	West Bengal	VI	II	21.60	5.455	1.178	IV	1463.41
M-64	PS-16	Variety	New Delhi	I	II	17.12	3.778	0.647		–
M-65	Dantan sonamung	Landrace	West Bengal	I	II	18.00	3.84	0.691		–
M-66	Kopergaon	Variety	Maharastra	VII	II	22.52	6.88	1.549	I	1361.0
M–67	Sel1 (Mush x mung)	Selection line	West Bengal	VII	II	19.90	5.3	1.055	VIII	1445.78
M-68	Sub-14	Wild (*V. radiata* var. *sublobata*)	Rajasthan	III	I	19.40	2.575	0.50		–
M-69	TRCM-1-1	Selection line	Lembucherra, Tripura	II	III	22.00	3.943	0.867		–
M-70	TRCM-3-1			VII	I	19.64	5.895	1.158	VI	–
M-71	TRCM-4-1			II	I	19.16	3.865	0.741		–
M-72	TRCM-5-1			V	III	21.60	3.335	0.720		1333.3
M-73	TRCM-7-1			V	III	21.88	2.94	0.643		–
M-74	TRCM-10-1			II	I	17.24	2.765	0.477		–
M-75	TRCM-16-1			II	I	17.60	4.39	0.773		–
M-76	TRCM-17-1			II	I	20.64	3.69	0.762		–
M-77	TRCM-21-1			I	I	22.48	4.23	0.951		–
M-78	TRCM-22-1-1			I	I	21.24	3.415	0.725		–
Mean						20±1.8	3.54± 0.5			–

per plant was 0.71. The other high protein containing genotypes were IC39566 (24.9), SML-286 (24.48), IC39549 (24), IC39499 (24.2). In respect of high protein yield per plant, the genotype identified were Kopergaon, followed by IC39549 (1.33), IC39566 (1.23), S1 (BL x 444D1) (1.18), IC103213 (1.17), TRCM-3-1 (1.16). On the other hand, the range of trypsin Inhibitor in present study, varied from 1250 to 1500 TIU with an average of 1386 TIU. The content of Trypsin inhibitor level was judged and two genotypes, namely, Kopergaon (1361 TIU) and IC103179 (1250 TIU) were detected to have high protein yield as well as moderate to low level of trypsin inhibitor.

In the research programme, many genotypes were taken from Rajasthan and Gujarat, being one of the probable areas of origin of the species, which were not included earlier in mungbean breeding programme. The germplasm tested in the present study were found highly diverse as revealed from test of significance (X^2 test) and high D^2 values based on phenotypes. Difference in clustering pattern was observed under two environmental situations may be attributed to the change of D^2 values among the genotypes. Variation in clustering pattern in different environments was also observed in mungbean by many workers (Raje and Rao 2001; Patil *et al.*, 2003). The change in the contribution of traits towards the total divergence could be responsible mostly to alter the relationship among the genotypes of mungbean. No relation could be drawn between geographical diversity and morphological diversity. Genotypes with similar origin or place of collection, were found to be placed in different clusters, as observed also in earlier studies in mungbean (Raje and Rao, 2001).

Genotypes were identified for their higher protein yield per plant with low level of antinutritional factor. Those genotypes could be incorporated in the mungbean breeding programme for quality improvement. They were the desirable parents for breeding better quality lines of mungbean. In future, the work on the development of diversity-analysis-model can be taken up where simultaneous incorporation of information on genotypic and environmental parameters make it able to show the best parental combination for hybridization followed by selection of desired segregants.

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