



Towards Quest for Early Maturing Mungbean [*Vigna radiata* (L.) Wilczek] Lines with Improved Nutritional Quality and High Yield

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ABSTRACT

Background: Mungbean [*Vigna radiata* (L.) Wilczek], a rich source of protein, carbohydrate and different micronutrients, can play an important role in food security programmes. Enhancement of nutritional qualities via biofortification of existing varieties is a promising area of research.

Methods: In the current study, sixty one mungbean were taken to study different biochemical parameters and also to establish an inter-relationship of each parameter with yield and maturity.

Result: Results show that, nine lines, which included two germplasms namely, B1 and Pusa-9632 along with four mutants as well as three hybrids were found to have higher nutritional quality especially carbohydrate and protein content. Moreover, the mutants and segregants showed a higher nutritional value (% increase) than the germplasms. In addition, correlation coefficient study ensured an interrelationship between agronomic and biochemical characters by reporting that the total starch and nitrogen content of mungbean seeds can serve as an excellent selection marker for improving seed yield.

Key words: Biochemical characterization, Germplasms, hybrids lines, Mungbean [*Vigna radiata* (L.) Wilczek], Mutant lines, Segregating lines.

INTRODUCTION

Food security has become a global concern. A recent conservative estimate indicates that as high as two billion people suffer globally from hunger and malnutrition (WHO 2017). Not only the availability of the right quantity of food is important, but also the quality of the food available needs due consideration. Important dietary proteins and other essential nutrients often lack in the food intake amongst a large section of the population. Grain legumes also known as pulses, have high levels of protein content which are rich in lysine as well as other minerals and nutrients which can supplement dietary deficiencies in undernourished populations (Singh 2017). In India, which on Global Hunger Index is positioned at 103rd out of 119 countries, presents a dismal picture of hunger and malnutrition (Global Hunger Index 2018). Among Indians in general and specifically amongst the rural female population, the consumed food is deficient in essential dietary nutrients. This leads to malnourishment and causes different kinds of health issues (Kshatriya and Acharya 2016).

Mungbean [*Vigna radiata* (L.) Wilczek], is one of the most important grain legumes in Asia and especially India. It is a cheap source of easily digestible lysine rich proteins, as well as rich in other essential dietary nutrients. The protein quality of mungbean is better than other legumes (Anisha and Prema 2008). Apart from its protein content, mungbean seeds also contain fat, fibre, ash and carbohydrate (Grewal *et al.*, 2006). A major carbohydrate constituent of mungbean is starch and amylose is the major component of this starch content of mungbean (Nair *et al.*, 2013). From this, it is evident that improvement of mungbean crop both from an agronomic perspective as well as from a nutritional

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perspective is essential. Despite its importance, progress in production and productivity of mungbean has been slow. The breeding programmes applied to mungbean so far have been purely conventional which has not been able to keep up with the ever increasing demands. In Indian agricultural systems, more importance is levied on increasing grain yield and crop productivity rather than enrichment of the nutrient quality, which will help to improve human health. This imbalance often gives rise to micronutrient malnutrition among the population. Hence, methods outlining the development of high yielding mungbean varieties with high nutritional quality have great relevance in fighting "micronutrient malnutrition" especially in poor and developing countries (Garg *et al.*, 2018).

Biofortification is a process which refers to the development of nutritionally improved food crops (Shobha

Rani *et al.*, 2019). This may be achieved in existing varieties through agronomic practices, conventional breeding techniques, or modern biotechnological tools. In this study we aim to evaluate the nutritional quality of sixty one high yielding (seed yield > 20g plant⁻¹) extra short duration (< 60 days) (Sarkar and Kundagrami 2017) mungbean accessions comprising of twenty mutants (Sarkar and Kundagrami 2018) and sixteen hybrids lines (Sarkar 2017) along with twenty five germplasms (which were the parental stock for the mutant and segregating lines, collected from all over India).

MATERIALS AND METHODS

Experimental materials

The experimental materials comprises of a total number of sixty one high productive short duration mungbean accessions (Table 1). The germplasm evaluation, mutation breeding and hybridization were conducted through Department of Genetics and Plant Breeding, Institute of Agricultural Science, University of Calcutta during 2016-2018 (Sarkar and Kundagrami 2017, 2018). First three generation of mutants (M₁-M₃) were evaluated at Baruipur experimental farm of University of Calcutta and fourth (M₄) generation was at a farmer's field in Udaynarayanpur, Howrah, West Bengal. On the other hand crossing and evaluation of first generation hybrids were done at Baruipur, second generation (F₂) was evaluated at Udaynarayanpur and third (F₃) generation was at another farmer's field at Arambagh. From M₄ generation twenty high yielding early maturing plants were selected to procure M₅ seeds and from F₃ generation sixteen high yielding early maturing plants were selected to procure F₄ seeds. The mutant and hybrids lines were considered to be stable lines after multilocation field trial followed by stability analysis (Sarkar 2017).

Experimental methods

Field study

Exhaustive field trials for the character evolution of all the 61 accessions were conducted in 2018 following RBD design with three replications at three different locations in the state of West Bengal, India. Information pertaining to each of the field is elaborated in Table 2. Seeds were sown in 3 cm deep pits along a line with pit to pit 20 cm spacing and line to line 30 cm. Plants were raised and maintained according to the standard agronomic practices for mungbean cultivation (Department of Agriculture, Forestry and Fisheries 2010). At the time of maturity, days to maturity and seed yield plant⁻¹ (weight of total seeds of a single plant) were recorded from five randomly selected healthy plants from each replication of each accession.

Nutritional characterization

Freshly harvested seeds of all the experimental materials were cleaned, washed and dried to remove the extraneous material and then ground to form fine flour. The flour of each of the lines was analysed for total carbohydrate (Dubois *et al.*, 1956), total starch (Hodge and Hofreiter 1962),

amylose (Sadasivam and Manickam 1996), crude protein (Lowry *et al.*, 1951), total free amino acid (Moore and Stein 1948) and total nitrogen content (AOAC 2000). 100mg flour was taken for each analysis for each accession. All the analysis was done with three replications and average value for each analysis was calculated for statistical analysis.

Statistical analysis

Data for field study and laboratory experiments were subjected to one way analysis of variance (ANOVA) and post hoc Duncan's Multiple Range Test using the statistical package of SPSS ver 21.0. Genetic variability estimates were calculated between all pairs of germplasms and lines by the Cluster analysis using SPSS ver 21.0 software. Principal component analysis (PCA) was done using Minitab ver 18.0 and Correlation coefficient study was done using SPAR 2.0 software.

RESULTS AND DISCUSSION

Field study

Agronomic parameters study

Days to maturity

Of all the Mungbean germplasms screened, Baruipur Local 2 (59 days) and B1(59 days) showed maximum significant reduction in days to maturity (Fig 1A). At the same time all the selected lines recorded maturity duration of less than 55 days. Among the mutants M2, M6, M7, M8, M9, M11, M12, M16 and M17 showed maturity duration of 53 days (Figure 1B) and of all the hybrids lines, H10 had the shortest maturity duration of 52 days (Fig 1C).

Seed yield plant⁻¹

Evaluation of seed yield plant⁻¹ revealed that among the germplasms, UPM-99-3 (22.55g) showed significantly ($p<0.05$) higher seed yield (Figure 1D) than others. Mutants namely M19 and M12 (34 g each respectively) along with hybrids plant H5 (32 g) registered maximum seed yield plant⁻¹ (Fig 1E and F).

Biochemical parameters

Quantification of total carbohydrate

All the accessions studied showed significant ($p<0.05$) variation in the total carbohydrate content. Amongst the tested germplasm Shonamung-1 (64%), amongst the mutants M8 (65%) and amongst the hybrids lines H10, H11, H1, H2 (65% each respectively) had higher carbohydrate content than the rest (Fig 2 A, 3A, 4A).

Quantification of total starch

The total starch content among accessions showed little significant ($p<0.05$) variation. Shonamung1 showed maximum content of (58%) starch (Fig 2B). M8, M17, M9, M7 and M2 along with H11, H1 and H2 recorded the highest starch content (59% each respectively) (Fig 3B and 4B).

Quantification of amylose content

The amylose content of the accessions varied significantly

Table 1: Description of experimental material.

Sl. No.	Name of the germplasm	Sl. No.	Name of Mutant lines	Parents	Gamma radiation doses	Sl. No.	Crosses	Name of segregating lines
G1	APDM-84	M1	M ₅₃ P ₃	B1	10kR	H1	B1 x Shonamung1	C ₁ F ₃ L ₂ P ₆
G2	MH-98-1	M2	M ₅₅ P ₈		10kR	H2		C ₁ F ₃ L ₃ P ₈
G3	B1	M3	M ₅₆ P ₂		10kR	H3	UPM-99-3 x Baruipur Local 2	C ₂ F ₃ L ₁₃ P ₁
G4	PS-16	M4	M ₅₇ P ₁₀		10kR	H4		C ₂ F ₃ L ₂₁ P ₅
G5	PTM-11	M5	M ₅₈ P ₉		20kR	H5		C ₂ F ₃ L ₂₁ P ₉
G6	SML-302	M6	M ₅₁₀ P ₁₀		20kR	H6	Pusa-9632 x Baruipur Local 2	C ₃ F ₃ L ₂₅ P ₂
G7	ML-5	M7	M ₅₁₀ P ₈		20kR	H7		C ₃ F ₃ L ₃₄ P ₆
G8	APDM-116	M8	M ₅₁₄ P ₉	Pusa-9632	10kR	H8		C ₃ F ₃ L ₃₆ P ₉
G9	UPM-99-3	M9	M ₅₁₄ P ₂		10kR	H9		C ₃ F ₃ L ₃₇ P ₄
G10	24-Pargana Local	M10	M ₅₁₅ P ₁		10kR	H10	UPM-99-3 xK-851	C ₄ F ₃ L ₄₀ P ₄
G11	Pusa Baisakhi	M11	M ₅₁₆ P ₉		10kR	H11		C ₄ F ₃ L ₄₀ P ₆
G12	Pusa-9632	M12	M ₅₁₇ P ₅		10kR	H12		C ₄ F ₃ L ₅₁ P ₈
G13	K-851	M13	M ₅₁₇ P ₃		10kR	H13		C ₄ F ₃ L ₅₂ P ₉
G14	Shonamung1	M14	M ₅₁₉ P ₈		20kR	H14	Pusa-9632 xK-851	C ₅ F ₃ L ₅₇ P ₅
G15	PM-2	M15	M ₅₂₇ P ₉	K-851	10kR	H15		C ₅ F ₃ L ₅₈ P ₉
G16	Baruipur Local 1	M16	M ₅₂₇ P ₅		10kR	H16		C ₅ F ₃ L ₆₃ P ₄
G17	Baruipur Local 3	M17	M ₅₂₉ P ₃		10kR			
G18	Shonamung 2	M18	M ₅₃₀ P ₈		20kR			
G19	Panna	M19	M ₅₃₂ P ₈		20kR			
G20	Baruipur Local 2	M20	M ₅₃₄ P ₅		30kR			
G21	Howrah local							
G22	Purulia Local							
G23	Bankura Local							
G24	WBM-29							
G25	Samrat							

($p < 0.05$) with an average value of 39.07%. Shonamung1 (45%) showed a higher amylose content (Fig 2C). The average amylose content of both the hybrids lines (44%) and the mutants (44%) was more than the germplasms. Among the hybrids lines the highest amylose content (45%) was recorded in H2, H1, H10 and H11 (Fig 4C) and in mutants the highest amylose content (45%) recorded in M10, M17, M13, M10, M8 and M2 (Fig 3C).

Quantification of crude protein

Crude protein content showed a significant ($p < 0.05$) variation among the accessions with an average of 26% (Fig 2D). PM2 (29.85%) showed the highest crude protein content among the germplasms. The average crude protein in mutants and hybrids lines was 27% (Fig 3D and 4D). The highest crude protein was recorded in M17, M13 and M18 among the mutants, whereas similar values were seen in H10, H1 and H2 among the hybrids lines.

Quantification of total free amino acid

The present study showed that the average value of total free amino acid content in studied germplasms was 0.89% of fresh weight of cotyledons which was lower than that in hybrids (1.02%) and mutant (1.03%) lines. With 0.98% content of total free amino acid, both Shonamung-1 and B1 showed higher free amino acid than the others (Fig 2E). For hybrids lines the highest total free amino acid was seen in H9 and H16 (1.11%) (Fig 4E) and that for mutants in M16 (1.20%) for every 100g cotyledon fresh weight (Fig 3E).

Quantification of total nitrogen

A genotypic variation of the total nitrogen content among the germplasms showed that Shonamung 1 (3.84g 100g⁻¹) had the highest nitrogen content. The average nitrogen content of the germplasms (3.1%) was lower than that of hybrids lines (3.75%) and mutants (3.66%) (Fig 2F). M8 (3.9%) among the mutants and H9, H15, H1 (4% each respectively) among the hybrids lines showed the highest nitrogen content (Fig 3F and 4F).

Multivariate analysis

Principal component study

In the present investigation, first two principle components with eigen values < 1 contributed 81.32 per cent of the cumulative variability amongst all the mungbean accessions evaluated for 6 qualitative and 2 quantitative traits (Fig 5). The first principal component contributed maximum towards variability (71.17%). Characters recorded the maximum variance in first principal component (PC1) reflects high carbohydrate and seed yield/plant of each accession. The second principal component (PC2) which described 10.14% of the total variance reflected significant loadings of crude protein (1.034) and total nitrogen (0.059), which were positively correlated that reflects PC2 describes crude protein and total nitrogen content of each accession. The PCA scores for eight parameters in the first two principal components were computed and plotted in graph to get the 2D scatter diagram (Fig 5). Moreover, depending on the PCA scores for sixty one accessions in the first two PCs with eigen value > 1 were also plotted in the above mentioned 2D graph (Fig 5), which confirms the distribution of different accessions on the basis of each parameters studied. Accessions belong to the first and second quadrante was reported to be high yielder and carbohydrate rich. On the other hand, accessions of third quadrante were early maturing protein rich and of fourth quadrante was nitrogen and free amino acid rich.

Cluster analysis

Dendrogram based on all the studied parameters grouped the sixty one experimental materials into four clusters based on similarity of coefficients within 0 to 10 rescaled distances (Fig 6). Mean values of each cluster revealed that the accessions belong to the cluster III and IV is improved quality lines with early maturity duration and high yield.

Estimates of correlation matrix

The genotypic correlation matrix of the six biochemical

Table 2: Details of experimental locations.

State: West Bengal, India	Locations		
	Baruipur	Arambagh	Udaynarayanpur
Date of sowing	12.3.2018	15.3.2018	20.3.2018
District	24 Parganas (south)	Hooghly	Howrah
Latitude	22°N	22.88°N	22.72°N
Longitude	88.26°E	88.78°E	87.98°E
Altitude (above sea level)	9.75 m	12 m	7 m
Avg temp. (°C)			
March	26.1	26.5	25.8
April	28.4	28.6	27.4
May	30.2	30.3	31.6
Avg rainfall (mm)			
March	26.5	18.6	20.5
April	46.2	38.5	31.6
May	100.5	81.3	49.2
Soil pH	6.9	7.1	6.6

constituents with seed yield plant⁻¹ and days to maturity were studied in all the experimental materials (Table 3). Correlation study showed that total carbohydrate had significant positive correlation with amylose (0.870), total free amino acid (0.491) and total starch (0.482) contents. Total starch showed positive significant correlation with amylose (0.582), total free amino acid (0.555) and seed yield (0.776), whereas, amylose content had significant positive correlation with total free amino acid (0.632), total nitrogen

(0.398) and seed yield (0.427). But both of them showed significant negative correlation with days to maturity (-0.656 and -0.438 respectively). Interestingly, days to maturity also showed significant negative correlation with seed yield plant⁻¹ (-0.718).

Improved nutritional value of mungbean along with enhanced agronomic parameters (such as shorter maturity duration and higher seed yield) can have important implications in the food security programmes in Asia and

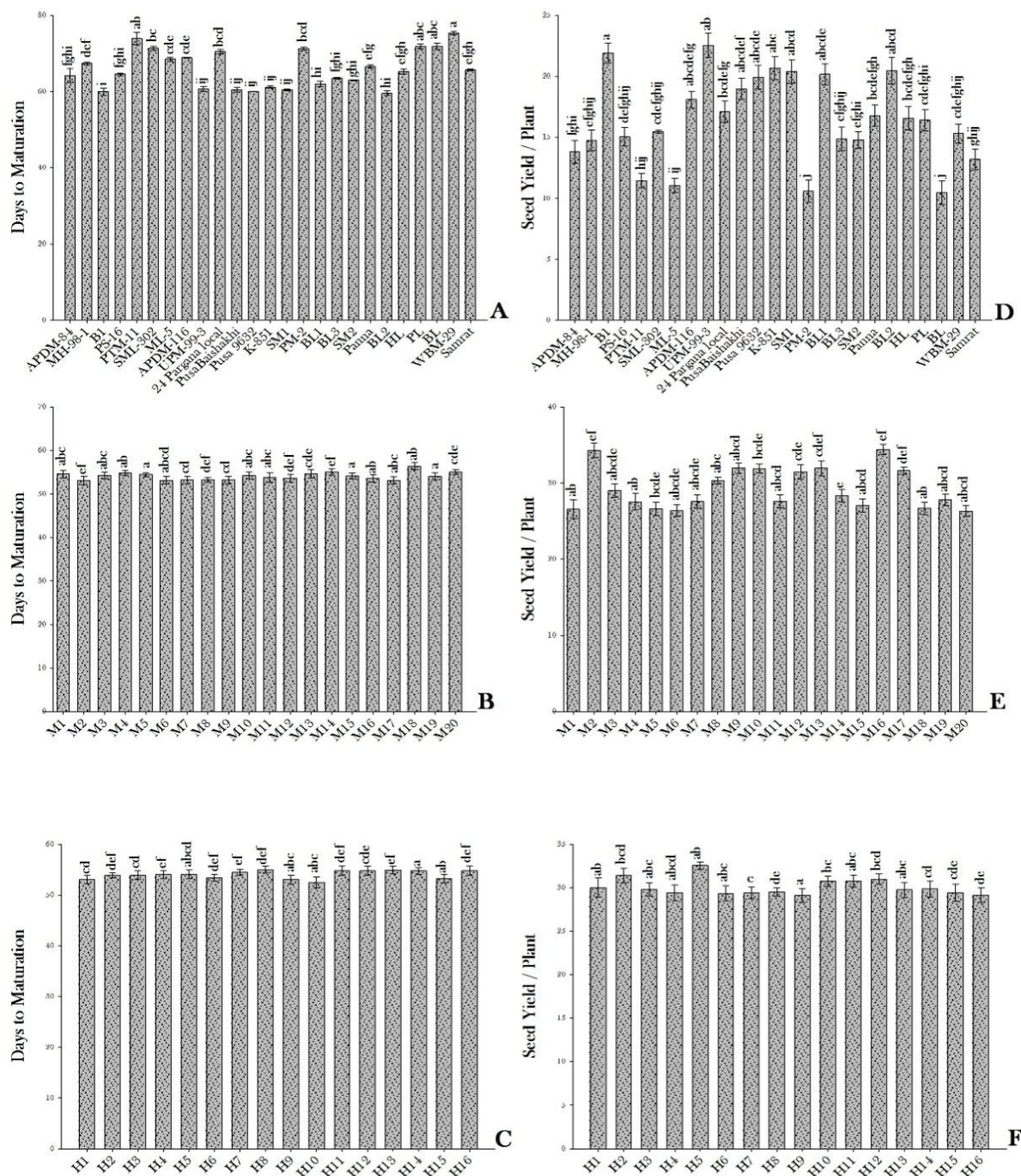


Fig 1: Evaluation the days to maturity and seed yield of germplasm (A and D), mutants (B and E) and hybrids (C and F). Small case alphabets represent significant differences between the samples at $p > 0.05$.

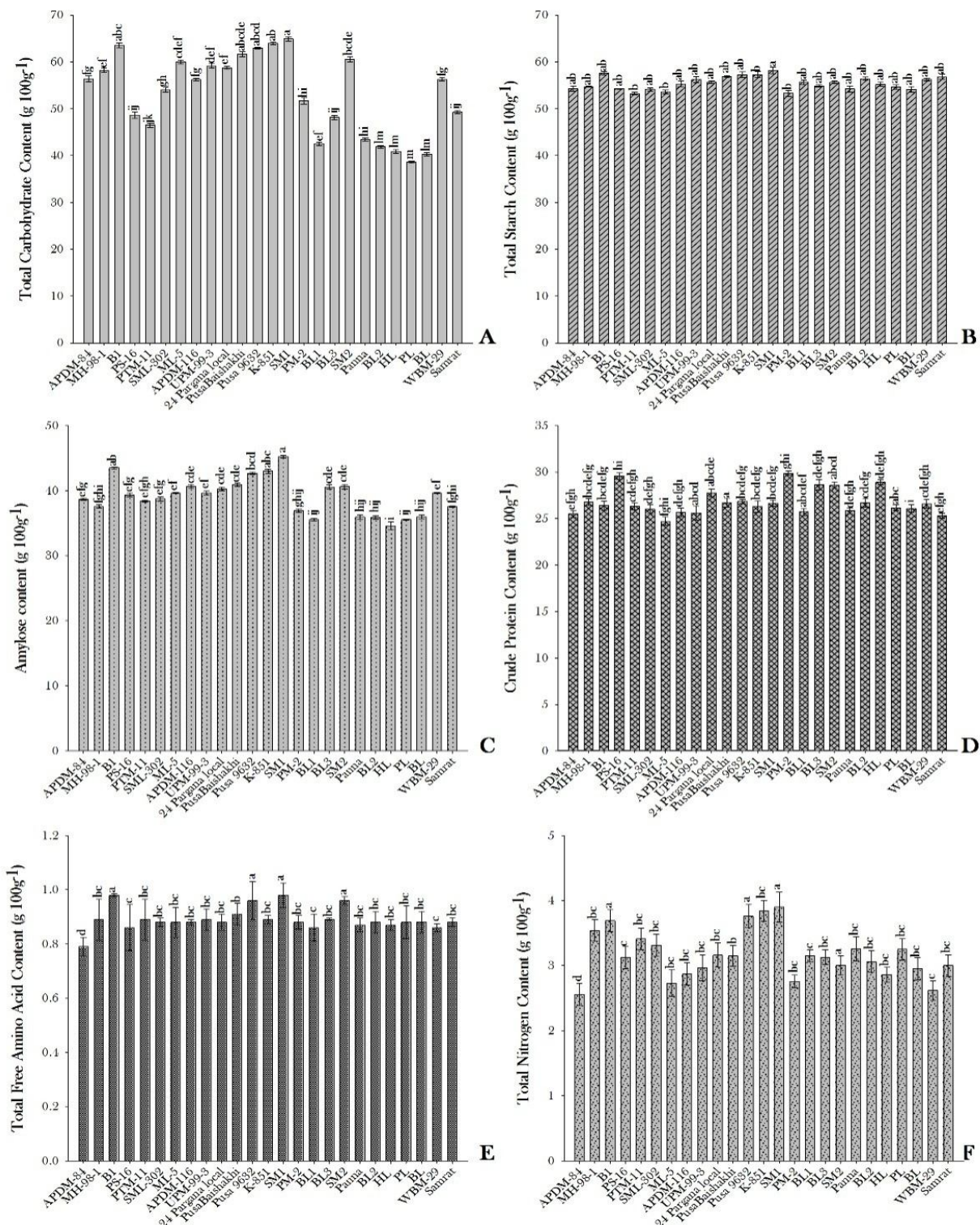


Fig 2: Estimation of the total carbohydrate (A), starch (B), amylose (C), crude protein (D), free amino acid (E) and total nitrogen (F) content in 25 mungbeangermplasms studied. Small case alphabets represent significant differences between the samples at $p > 0.05$.

especially India. Induced mutation as well as hybridization programmes for crop improvement is an established practice worldwide. During the past seventy years, more than 2,252 mutant varieties have been officially released (Maluszynski *et al.*, 2000). Significant improvements in mungbean have

been made either through the use of mutation breeding or conventional hybridization programmes as seen in earlier works, but most of them were for quantitative characters (Lal and Mishra 2006). The objective of our present work was to screen a large population of mungbean accessions

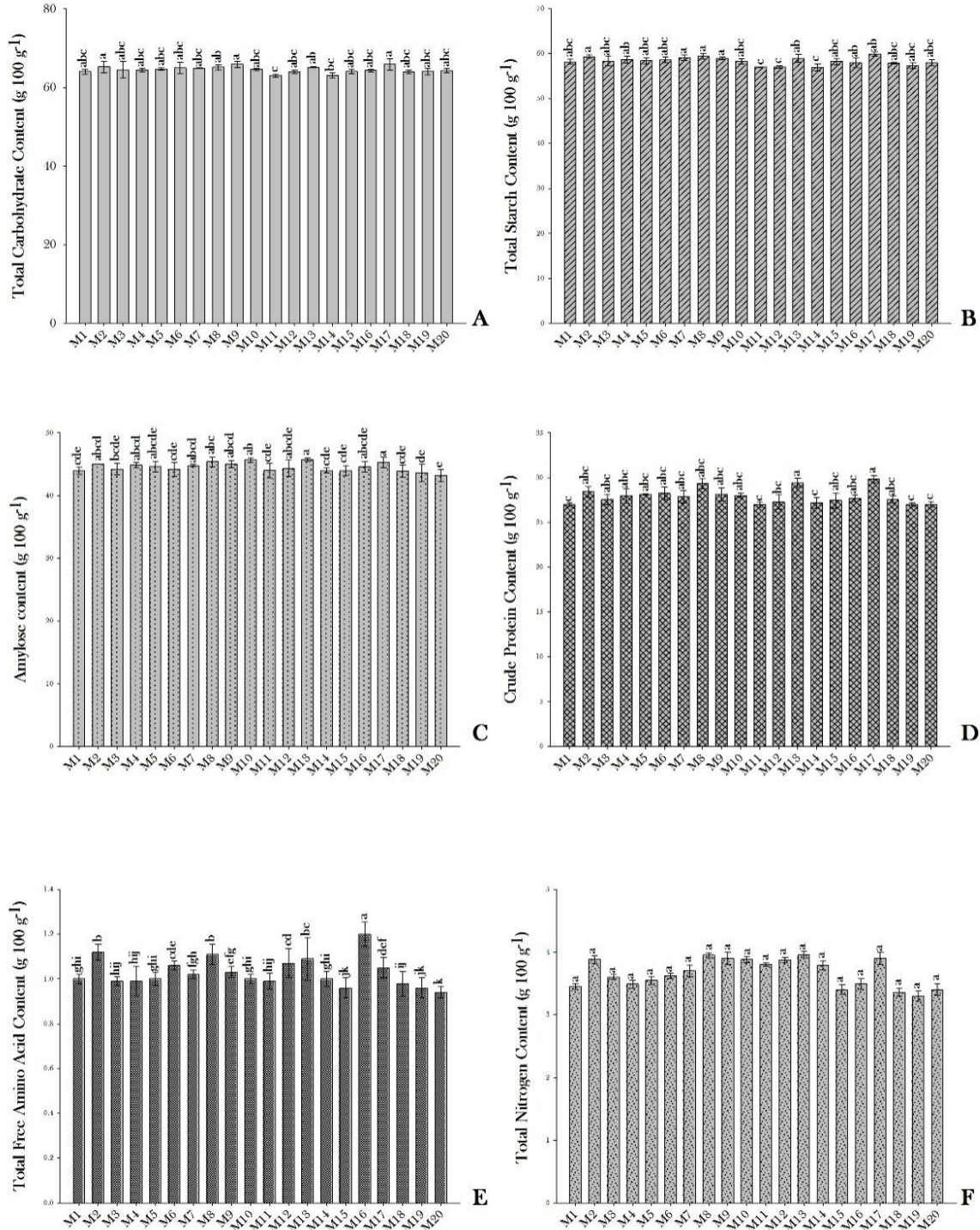


Fig 3: Estimation of the total carbohydrate (A), starch (B), amylose (C), crude protein (D), free amino acid (E) and total nitrogen (F) content in 20 mutant lines studied. Small case alphabets represent significant differences between the samples at $p > 0.05$.

based on nutritional qualities, collected from different geographical locations or derived from induced mutation or hybridization programmes.

In the present study, a total of sixty one accessions was studied, out of which twenty five were high productive short

duration mungbean germplasms collected from different parts of India, twenty mutant lines (M_5) derived through gamma radiation induced mutation breeding and sixteen shybrid lines (F_3) derived through the hybridization program were assessed for their maturity duration, seed yield and a

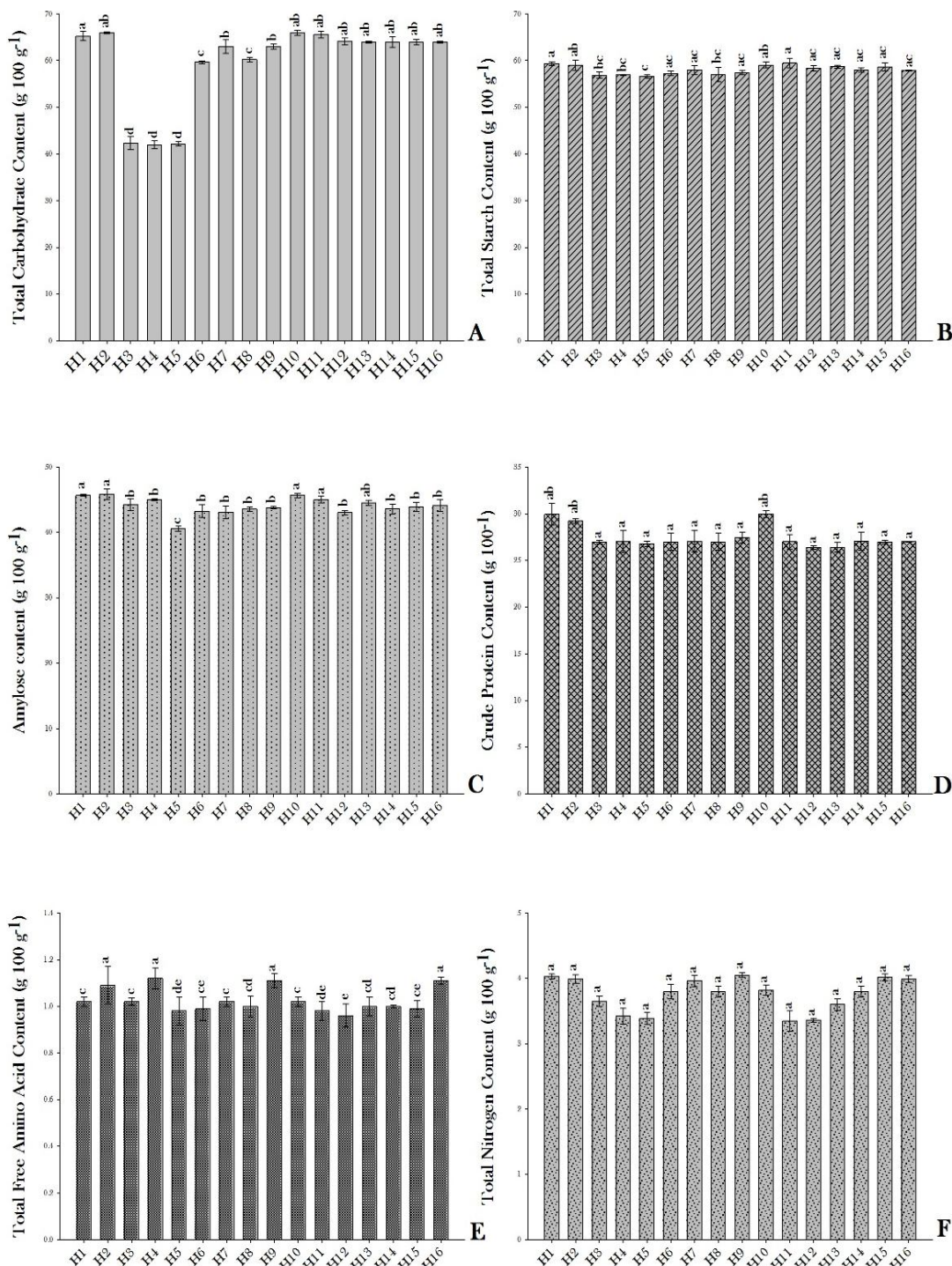
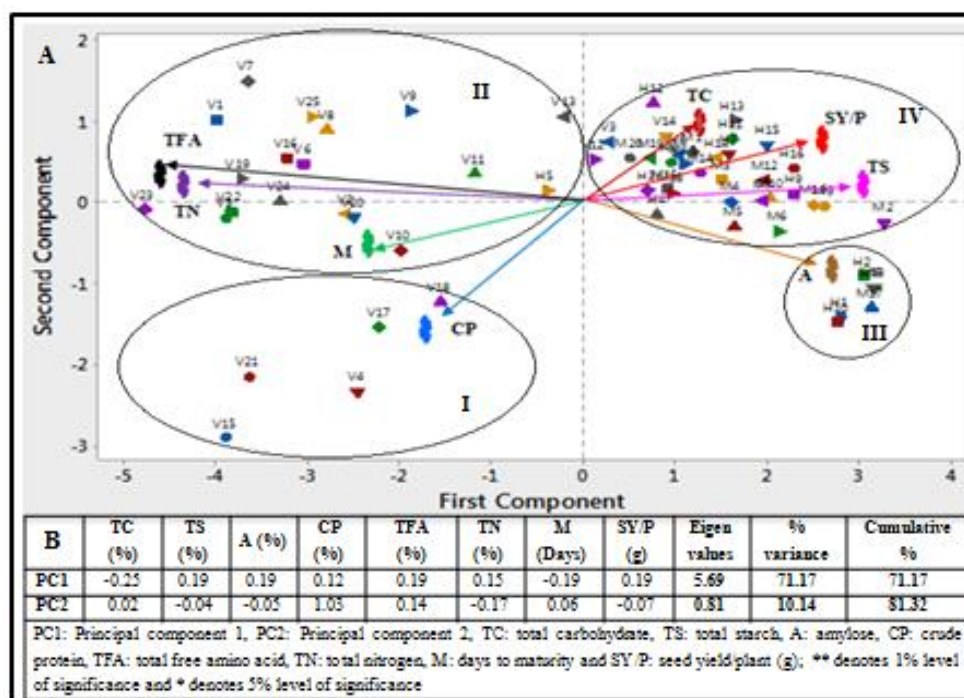


Fig 4: Estimation of the total carbohydrate (A), starch (B), amylose (C), crude protein (D), free amino acid (E) and total nitrogen (F) content in 16 hybrid lines. Small case alphabets represent significant differences between the samples at $p > 0.05$.

Table 3: Correlation matrix for biochemical constituents of sixty one tested entries.

	TC (%)	TS (%)	A (%)	CP (%)	TFA (%)	TN (%)	M (Days)	SY/P (g)
TC (%)	1.000	0.482*	0.870**	-0.143	0.491*	0.218	-0.381	0.337
TS (%)		1.000	0.582**	-0.312	0.555**	0.345	-0.656**	0.776**
A (%)			1.000	-0.203	0.632**	0.398*	-0.438*	0.427*
CP (%)				1.000	-0.052	0.078	0.427*	-0.054
TFA (%)					1.000	0.647**	-0.432*	0.427*
TN (%)						1.000	-0.302	0.409*
M (Days)							1.000	-0.718**
SY/P (g)								1.000

TC: Total carbohydrate, TS: Total starch, A: Amylose, CP: Crude protein, TFA: Total free amino acid, TN: Total nitrogen, M: Days to maturity and SY/P: seed yield/plant (g); ** denotes 1% level of significance and * denotes 5% level of significance.

**Fig 5:** A. Two-dimensional plot showing relative position of germplasms of mungbean and tested parameters based on PCA scores.**B. principal components study.**

number of quality parameters. Among the tested germplasms, B1, Pusa Baisakhi, Pusa 9632, K-851, Shonamung-1 and Shonamung-2 outperformed others, in having increased carbohydrate, starch, amylose, crude protein, free amino acid and nitrogen content. Based on the two important agronomic parameters, i.e., days to maturity and seed yield plant⁻¹, three germplasms B1, Pusa 9632 and K-851, were taken up for mutation breeding and raising of mutant generations (Sarkar and Kundagrami 2018). Additionally, hybridization was done between some outperforming germplasms to produce hybrid lines. In terms of maturation days, mutants showed maturity duration of less than 54 days, whereas seed yield plant⁻¹ increased to over 30 gm seeds plant⁻¹. Therefore the mutation decreased the maturity duration and increased seed yield, in comparison to the respective controls. Early maturing and with increased yield was also seen in earlier studies, with

gamma induced mutant cultivars of mungbean in India and Pakistan (Khan and Wani 2006; Sarkar and Kundagrami 2018). hybrid lines also showed decreased maturity days and among them H5, H11 and H12 showed increased number of seed yield plant⁻¹.

Enhancement in quality parameters of mutants and segregating lines over the respective controls was seen in the present study. Typically mungbeans show a starch content in excess of 30% (Singh *et al.*, 1989). All mutant and hybrid lines showed increase of total carbohydrate, total starch and total amylose content. Our study showed a protein content of 25% - 29% in the experimental materials, well in accordance with previous reported results (Butt and Batool 2010). The protein quality of a food depends on its digestibility and the concentration of essential amino acids. So, quantitative estimation of total free amino acid in mungbean seed is an important estimate. The average value

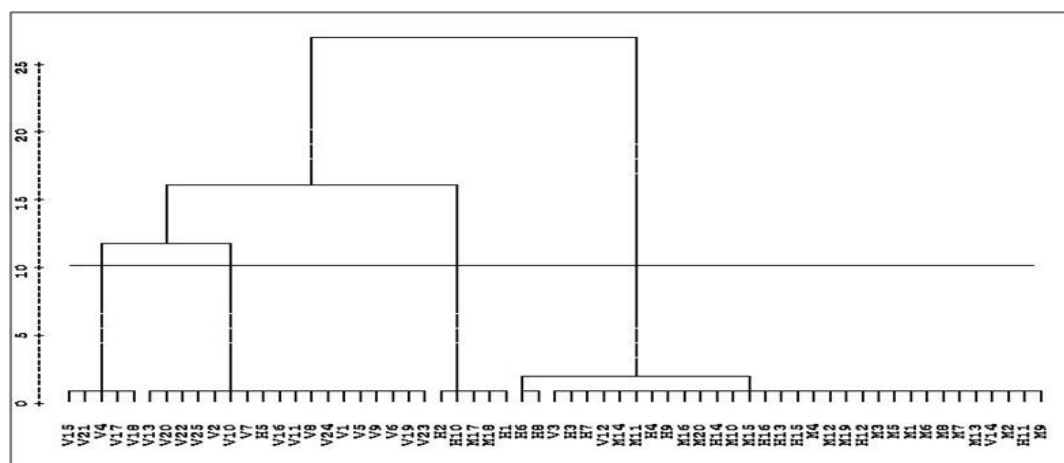


Fig 6: A dendrogram showing relationship between twenty five mungbean accessions and their selected M_5 and F_3 progenies based on unweighted pair group method with arithmetic mean (UPGMA) of Euclidean distances of their agronomic as well as biochemical parameters.

for total free amino acid of the germplasms, hybrids and mutants were 0.89%, 1.03% and 1.02% respectively, much higher than previously reported range of 0.88% - 0.76% (Banusha and Vasantharuba 2013). Gopalan *et al.* (2007) reported that the nitrogen acquisition in mungbean seed is about 3.84g per 100g seed.

Multivariate statistics help to summarize the data and reduce the number of variables necessary to describe it (Anderson 1972). Principal component analysis (PCA) reflects the importance of the largest contributor to the total variation at each axis of differentiation (Sharma 1998). Principal Components (PCs) are independent of each other (Mohammadi and Prasanna 2003) and they describe the variability which was not elucidated by others. In this investigation, the total variability was explained by two PCs (Fig 5). This may indicate the contribution of many traits with higher level of correlation to explain the gross diversity. These findings are in line with the earlier findings of (Caldo *et al.*, 1996; Chakravorty *et al.*, 2013). The twenty five germplasms were found together in two groups while the mutants and hybrid lines aggregated and remained together in two separate groups. This indicated that genotypically the mother germplasms and the derived mutants as well as hybrids were distinct based on the parameters studied. There was a significant improvement with regard to mutation or hybridization with respect to the original germplasms.

To group the accessions into various clusters, hierarchical cluster analysis was followed (Fig 6). Principal component scores were used as variables instead of parameters for clustering procedures, making the results equivalent to those from initially standardized data as the correlation matrix was used for principal component analysis. Based on the parameters studied, mean value of each cluster indicates that no single cluster, may be suitable for all the tested parameters. Dendrogram revealed that high yielding early maturing nutrient rich lines could be found in cluster III and IV. PCA and cluster analysis together allowed

a natural grouping of the genotypes (Kraic *et al.*, 2009). Khodadadi *et al.* (2011) showed that cluster analysis based on PCA is a more precise indicator of differences among genotypes than cluster analysis not based on PCA.

Correlation study revealed that protein contents were recorded slightly negative correlations with seed yield, but positive with maturity duration (Table 3). This indicates that the germplasms with high protein contents are not essentially high seed yielding but early maturing. The result also suggested that there is possibility of selection for increased protein content without detrimental effect on seed yield (Govindaraj *et al.*, 2009). Another report also registered negative correlation of protein with that of yield (Raturi *et al.* 2014). Interestingly, total nitrogen contents and total starch had highly significant positive correlation with seed yield suggesting that amount of starch and nitrogen of mungbean accessions can serve as an excellent selection marker for improving seed yield.

Induced mutation using physical and chemical mutagens is one way to create genetic variation resulting in new varieties with better characteristics. In our study we have found that both induced mutation and conventional hybridization had positive impact on the nutritional quality as well as seed yield and maturity duration of the mungbean crop. A previous report revealed that induced mutations in mungbean significantly affected yield and nutritional quality (Sandhu and Saxena 2003). Between the mutants and the hybrid lines, the mutant lines were superior to segregating lines. Mutant M2, M7 (Control: B1), M8, M9, M13 (Control: Pusa 9632) and M17 (Control: K-851) were found to have improved quality parameters as well as short maturity duration (<55 days) and increased seed yield (>30gm per plant).

CONCLUSION

This study successfully identified germplasms, mutants and hybrid lines with improved quality parameters. It was evident from the results that germplasms B1, Pusa 9632, K-851,

Shonamung-1 and Shonamung-2 can be excellent candidates for developing improved mungbean varieties through conventional and other breeding strategies. H1, H2, H10 and H11 hybrids as well as the mutants M2, M9, M12 and M17 showed promising results and can eventually be released as improved varieties after further multi-location trials in accordance with standard norms. Hence, it is beyond doubt that both conventional hybridization as well as induced mutation is highly successful in establishing mungbean varieties with improved agronomic and quality parameters.

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