



Genetic Variability and Diversity for Yield and Yield Attributing Traits in M_3 and M_4 Generations of Butter Bean (*Phaseolus lunatus* L.)

G. Ashokkumar¹, S.T. Bini Sundar¹, B.K. Savitha¹, D. Rameshkumar²,
P. Balasubramanian³, T. Sampathkumar⁴, D. Sathiyaraj⁵

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ABSTRACT

Background: Butter beans (*Phaseolus lunatus* L.) belong to the family *Leguminaceae*, is one of the oldest cool season food legume crops. A throughout knowledge of existing genetic variation among yield and its yield component traits is essential for developing high yielding varieties in butter bean. Realizing the importance of genetic diversity and variability in key economic characters, the present investigation had been planned to assess the genetic diversity and variability in for yield improvement in butter bean breeding programme.

Methods: Investigation was carried at Horticultural Research Station, Tamil Nadu Agricultural University, Kodaikanal, Tamil Nadu during 2019-2021. A total of 2734 M_2 mutant lines were evaluated as individual progenies in the previous study. Stringent selection was made based on yield, earliness and plant habit and a total of 35 mutant lines were identified and forwarded to M_3 and a total of 12 mutant lines for M_4 generations in present study. The data on eleven quantitative were analyzed using Mahalanobis D^2 statistic.

Result: The present study has assessed the genetic variation among studied traits of butter bean mutants for yield and its component traits. Thus, a total of five mutant lines namely BBMG-7-9-7, BBMG-9-10-6, BBMG-18-9-6, BBMG-25-13-3, BBMG-35-7-2 were identified to be forward to the M_5 generation as promising mutant lines for developing high yielding varieties and to test its homogeneity and stability.

Key words: Butter bean, Genetic analysis, Genetic diversity, Mahalanobis D^2 .

INTRODUCTION

Butter beans or Lima beans (*Phaseolus lunatus* L.), is an herbaceous plant of the *leguminaceae* family, native of Central and South America. It is cultivated in warm and semitropical regions throughout the world for its beans, which can be eaten fresh, generally without the bean pods or dried. Butter beans are high in protein, vitamins B and C and various minerals including iron, magnesium, phosphorus and potassium. A large seeded butter bean is considered to be a high value crop in the hilly regions of Tamil Nadu viz., Kodaikanal and Nilgiris. However, the genetic base of the present-day collection of butter beans remains poor due to lack of genetic variability owing to their autogamous nature. It is reported that beans, in general, are cleistogamous which means that they are already self-pollinated when the flower bud opens.

As a result, creation of variability is difficult through hybridization as it is a completely self-fertile crop with very less percentage of outcrossing under natural conditions. Under these circumstances mutation breeding has been effectively utilized to improve the yield and other polygenic characters (Deepalakshmi and Anandakumar, 2004). Induction of mutation forms an important part of breeding programme as it widens the genetic pool through creation of genetic variability. Therefore, the genetic variability is the basic requirement for making progress in plant breeding (Appala Swamy and Reddy, 2004). For effective breeding

¹Tamil Nadu Agricultural University, Coimbatore-641 003, Tamil Nadu, India.

²SRM College of Agricultural Sciences, Chengalpattu-603 001, Tamil Nadu, India.

³Department of Agronomy, Tamil Nadu Agricultural University, Coimbatore-641 003, Tamil Nadu, India.

⁴Department of Agronomy, Agricultural College and Research Institute, Tamil Nadu Agricultural University, Madurai-625 104, Tamil Nadu, India.

⁵Assistant Director of Agriculture, Govt. of Tamil Nadu, Horticultural Research Station, Tamil Nadu Agricultural University, Kodaikanal-624 101, Tamil Nadu, India.

Corresponding Author: D. Sathiyaraj, Assistant Director of Agriculture, Govt. of Tamil Nadu, Horticultural Research Station, Tamil Nadu Agricultural University, Kodaikanal-624 101, Tamil Nadu, India. Email: sathiyahorti@gmail.com

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strategy, variability, heritability and genetic advance is in prime importance for analysing the relative contributions of genetic and non-genetic factors to total phenotypic variances

in a population. The degree of genetic variability can reflect the level of genetic progress in crop breeding. There is constant search for new diverse genetic resources for improvement and stabilization of butter bean yield as well as quality of produce. From above point of view, to assess the genetic diversity in butter bean mutant lines.

MATERIALS AND METHODS

To study the mutant population of M₃ and M₄ generation induced through gamma rays in butter beans (*Phaseolus lunatus* L.) variety KKL-1. A total of 2734 M₂ mutant lines were evaluated as individual progenies in the previous study. Stringent selection was made based on yield, earliness and plant habit and a total of 35 superior mutant lines were identified and forwarded to M₃ and a total of 12 mutant lines forwarded to M₄ generation. The main objective of the study is to identify beneficial mutants with early duration and high yield. The experiments were conducted at Horticultural Research Station, TNAU, Kodaikanal, Tamil Nadu during 2019-2021 in a Randomized Block Design. The eleven quantitative traits were used to assess the genetic variability parameters and genetic divergence viz., Plant height (cm), Number of leaves per plant, Days to first flowering, Number of pods per plant, Pod length (cm), Pod girth (cm), Pod weight (g), Number of fresh ovules per pod, 100 seed weight (g), Shelling percentage (%) and Pod yield per plant. Genotypic and phenotypic coefficients of variation (GCV and PCV) were estimated as per the method suggested by Burton and Devane (1953); heritability and genetic advance by Hanson *et al.* (1956) and Johnson *et al.* (1955), respectively; Hierarchical Euclidean cluster analysis by Mahalanobis D² statistic (Mahalanobis, 1936) based on minimum genetic distance using Tocher's method as described by Rao (1952). An average intra- and inter-cluster distances were determined using INDOSTAT software as suggested by Singh and Chaudhary (1977).

RESULTS AND DISCUSSION

A total of 35 mutant lines were selected from the 2734 mutant lines of M₂ generation maintained at HRS, Kodaikanal. The

35 M₃ mutant lines encompass 12, 15 and 8 lines with 50Gy, 100Gy and 150Gy gamma treatments respectively. Eleven biometric traits were analysed in the selected thirty-five mutant lines with the view to identify elite mutant lines and a totally 12 mutant lines were forwarded to M₄ generations and eleven quantitative traits analysed.

Genetic variability studies in M₃ and M₄ generations

The GCV, PCV, heritability (h²) and genetic advance (GA) as % of mean were worked out for eleven biometric traits in the M₃ and M₄ generations and results were presented in Table 1 respectively. Plant height in M₃ progenies registered a moderate PCV of 19.65% and a moderate GCV of 18.52%. This trait also expressed high heritability of 88.83% followed by low GA of 4.52% (Table 1). In M₄ progenies plant height registered high PCV value of 28.90% and high GCV value of 28.51%. This trait also expressed high heritability value of 90.29% and followed by low GA of 5.8% (Table 1). Mathew and Palmer (2005) reported high PCV and GCV plant height indicating greater role of environment interacting with genetic factors in the expression of these characters. The reason for high level of PCV and GCV observed for morphological traits could be because of the expression of limited mutant loci, as a single mutation can have marked influence on the plant traits (Gaur and Gour, 2003).

The PCV in M₃ for leaves per plant was found to be high with the value of 20.18% and it was recorded a moderate GCV of 18.53%. This trait also expressed high heritability of 84.36% and high value (37.91%) of GA (Table 1). The PCV in M₄ for leaves per plant was found to be high with the value of 23.75% and it was recorded a high GCV of 23.27%. This trait also expressed high heritability value of 85.97% and high value (43.38%) of GA (Table 1). Leaves per plant showed high heritability and GA. Similar results reported by Kalaichelvi (2007). Johnson *et al.* (1955) stated, high heritability and GA for a character would indicate the predominance of additive gene action for that trait and as such, the trait is likely to respond effectively to phenotypic selection. Thus, these traits were governed by additive genes.

Table 1: Genetic parameters in M₃ and M₄ generation mutants of butter bean var. KKL-1.

Generations	M ₃					M ₄				
Traits	PCV	GCV	h ²	GA	GA %	PCV	GCV	h ²	GA	GA %
Plant height (cm)	19.65	18.52	88.83	8.61	4.52	28.90	28.51	90.29	10.92	5.82
Leaves per plant	20.18	18.53	84.36	8.50	37.91	23.75	23.27	85.97	9.84	43.38
Days to first flowering	8.93	8.33	87.00	5.74	14.13	11.46	10.99	92.02	6.69	16.27
Pods per plant	23.14	22.08	91.03	9.45	30.63	16.61	14.96	81.10	7.56	29.79
Pod length (cm)	19.69	16.46	69.90	7.64	61.79	22.96	22.59	86.77	9.71	65.36
Pod girth (cm)	31.89	24.27	57.93	8.85	196.50	27.41	26.04	70.29	10.25	253.89
Pod weight (g)	19.69	17.94	83.04	8.33	123.30	58.42	57.96	88.43	15.62	133.20
Fresh ovules per pod	24.74	20.31	67.38	8.41	156.23	17.41	13.02	65.94	6.43	99.06
100 seed weight (g)	15.96	14.48	82.34	7.47	12.64	16.04	15.48	93.18	7.96	12.54
Shelling percentage	12.31	11.63	89.35	6.83	10.07	11.88	11.51	93.84	6.88	10.93
Pod yield per plant (g)	34.15	28.03	67.35	9.88	4.64	48.44	47.34	70.58	14.01	4.76

Pods per plant in the M_3 progenies registered a high PCV of 23.14% and a high GCV of 22.08%. The trait also expressed high heritability value of 91.03% followed by high GA of 30.63% (Table 1). The PCV in M_4 for pods per plant were found to be moderate with the value of 16.61% and it recorded a moderate GCV of 14.96%. This trait also expressed high heritability value of 81.10% and high value of 29.79% of GA (Table 1). Singh *et al.* (2000) reported, highest magnitude of variability for pods per plant in black gram due to induced mutagenesis.

Pod length in M_3 progenies registered a moderate PCV of 19.69% and a moderate GCV of 16.46% for pod length. This trait also expressed high heritability value of 69.90% followed by high GA of 61.79% (Table 1). For pod length in the M_4 progenies registered a high PCV of 22.96% and a high GCV of 22.59%. This trait also expressed high heritability value of 86.77% followed by high GA of 65.36% (Table 1). The PCV for pod girth was found to be high with the value of 31.89% and it was recorded a high GCV of 24.27%. This trait also expressed medium heritability value of 57.93% and high value (196.50%) of GA (Table 1). The PCV in M_4 for pod girth was found to be high with the value of 27.41% and it was recorded a high GCV of 26.04%. This trait also expressed high heritability value of 70.29% and high value of 253.89% of GA (Table 1). The PCV for pod weight in M_3 was found to be moderate with the value of 19.69% and it was recorded a moderate GCV of 17.94%. This trait also expressed high heritability value of 83.04% and high value (123.30%) of GA (Table 1). The M_4 progenies registered a high PCV of 58.42% and a high GCV of 57.96% for pod weight. This trait also expressed high heritability value of 88.43% followed by high GA of 133.20% (Table 1). The range of low to medium PCV and GCV were expressed for pod length, pod weight in M_3 generation. However, low values have indicated narrow range of variation for these characters and provides very least scope for selection (Roychowdhury and Tah, 2013).

The PCV for pod yield per plant in M_3 was found to be high with the value of 34.15% and it was recorded a high GCV of 28.03%. This trait also expressed high heritability value of 67.35% and low value (4.64%) of GA (Table 1). The pod yield per plant in the M_4 progenies registered a high PCV of 48.44% and a high GCV of 47.34%. This trait also expressed high heritability value of 70.58% followed by low GA of 4.76% (Table 1). Kalaichelvi (2007) also reported high PCV and GCV for yield per plant. High heritability and low GA as percentage of mean were recorded for pod yield per plant in M_3 generation. The same result obtains from M_4 generation for plant height, pod yield per plant in same generation. This result indicated high heritability and less stability of the mutant population were expressed. This was similar to the reports of Govindarasu *et al.* (1997). Combination of high heritability with low genetic advance is indicative of non-additive gene action (Manimaran and Raveendran, 2004).

The PCV for days to first flowering was found to be low with the value of 8.93% and it was recorded a low GCV of

8.33%. This trait also expressed high heritability value of 87.0% and moderate value (14.13%) of GA (Table 1). The M_4 progenies registered a moderate PCV of 11.46% and a moderate GCV of 10.99% for days to first flowering. This trait also expressed high heritability value of 92.02% followed by moderate GA of 16.27% (Table 1). For shelling percentage in the M_3 progenies registered a moderate PCV of 12.31% and a moderate GCV of 11.63%. The trait also expressed high heritability value of 89.35% followed by moderate GA as% of mean of 10.07% (Table 1). The PCV in M_4 for shelling percentage was found to be moderate with the value of 11.88 % and it was recorded a moderate GCV of 11.51%. This trait also expressed high heritability value of 93.84% and high value of 10.93% of GA (Table 1). High heritability and moderate GA as percentage of mean were recorded for days to first flowering and 100 seed weight, shelling percentage in M_3 and M_4 generation. High heritability with moderate to high genetic advance for days to first flowering, 100 seed weight and shelling percentage were observed by Awnindra and Kumar, (2009).

The PCV of M_3 for number of fresh ovules per pod was found to be high with the value of 24.74% and it was recorded a high GCV of 20.31%. This trait also expressed high heritability of 67.38% and high value of 156.23% of GA (Table 1). The PCV in M_4 for fresh ovules per pod was found to be moderate with the value of 17.41% and it was recorded a moderate GCV of 13.02%. This trait also expressed high heritability value of 65.94% and high value of 99.06% of GA (Table 1). High heritability indicates that the induced variability in mutant lines was fixed by selection (Khan and Goyal, 2009). In M_3 generation of butter beans mutants have high heritability coupled with high genetic advance was observed for traits *viz.*, fresh ovules per pod, pods per plant, pod length, pod weight in M_3 generation.

The M_3 progenies registered a moderate PCV of 15.96% and a moderate GCV of 4.48% for 100 seed weight. This trait also expressed high heritability value of 82.34% followed by moderate GA of 12.64% (Table 1). For 100 seed weight in the M_4 progenies registered a moderate PCV of 16.04% and a moderate GCV of 15.48%. This trait also expressed high heritability value of 93.18% followed by moderate GA as % of mean of 12.54% (Table 1). For the improvement of seed yield, selection may be based directly on these attributes (Sarwar and Haq, 2006). It indicates the lesser influence of environment in the expression of these characters and prevalence of additive gene action in their inheritance. Similar findings were reported by Jain *et al.* (2013) in M_3 and M_4 generation.

In the present study both M_3 and M_4 generations, among the traits evaluated, a high heritability coupled with high GA as percent of mean was noted for number of leaves per plant, number of pods per plant, pod length, pod weight, number of fresh ovules per pod. Plant height, leaves per plant, pod length, pod girth, pod yield per plant showed the highest PCV and GCV in M_3 generation. The highest PCV and GCV were observed in pods per plant, pod girth, fresh ovules per pod and pod yield per plant in M_4 generation.

Days to first flowering exhibited low PCV and GCV in M₃ generation for all the mutants. The yield attributing traits viz., pods per plant, fresh ovules per pod, 100 seed weight and shelling percentage, showed low to moderate PCV and GCV for all the doses of gamma rays for entire mutants in M₄ generation. This clearly denoted that these traits are governed largely by additive gene effect, which may be favorably exploited for further improvement of these characters by selection.

Diversity analysis in M₄ generation

The assessment of genetic diversity is an important component in any crop improvement programme. The accurate estimation of genetic diversity in the M₄ mutant population generated out of gamma irradiation will categorize the mutants based on genetic distances (Barratt and Kidwell, 1998). Increasing genetic variability in butter beans variety KKL-1 will enable the plant breeders to broaden the genetic base and to introgress desirable traits from diverse mutant into the available cultivars (Thompson *et al.*, 1998). This study could be particularly useful for precise identification of diverse mutant lines and their genetic distant from the check variety KKL-1 butter bean. This will also help the plant breeder to formulate future breeding programme. Diversity analysis was carried out using all the 13 characters and a generalized tree distance in NTSYSp dendrogram (Fig 1) tree plot was calculated for each pair of mutants among the possible combinations.

Group constellations

NTSYSp analysis grouped the 12 mutants into four clusters based on similarity co efficient. Cluster IV is the largest grouping comprised of seven mutants viz., BBMG-1-3-6, BBMG-13-10-7, BBMG-27-10-9, BBMG-21-14-5, BBMG-5-8-10, BBMG-7-9-7, BBMG-29-8-4 and the check variety KKL-

1 (Fig 1). The dwarf mutant line BBMG-35-7-2 and semi dwarf mutant line BBMG-33-11-8 formed separate cluster I and III respectively. Three mutants viz., BBMG-25-13-3, BBMG-18-9-6 and BBMG-9-10-6 (Fig 2) which has desirable pod characters, seed weight and increased yield constituted the cluster II. Table 2 showed the pattern of distribution of 12 mutants along with check variety KKL-1.

Clusters mean values of the characters

The mean values of 13 characters for four clusters are presented in the Table 3. Cluster II recorded the highest mean values for pod yield per plant (515.47 g) and cluster III recorded the lowest mean values for pod yield per plant (114.06 g) Cluster I recorded lowest mean value of 39.83cm for plant height and cluster II showed highest mean values of 214.28 cm for plant height. Cluster II recorded the highest mean value of 42.66 days for days to first flowering and followed by cluster IV (41.66 days). The mutant lines under cluster II exhibited highest values for plant height, leaves per plant, days taken for first flowering, pod length and pod weight, fresh ovules per pod, 100 seed weight shelling percentage. Mean performance studies also confirmed this finding and this mutant has been selected as one of the elite mutants which may be carried over future studies by way of stabilizing the traits and subjecting to introgression studies. Studies on finding the mutant alleles by way of TILLING analysis may also be initiated in the mutants. The lowest mean value of 36.33 was recorded for days to first flowering by cluster III. The highest mean value of 25.64 was recorded for leaves per plant by cluster II (25.64) and lowest mean value of 8.10 was recorded by cluster I.

The highest mean value of 33.77 for pods per plant was recorded by cluster I. and lowest mean value of 10.51 was recorded by cluster III. Cluster I recorded the lowest mean value of 8.29 cm for pod length. Cluster II showed the

Table 2: Clustering pattern of 12 butter bean mutants in M₄ generation mutants of butter bean var. KKL-1.

Cluster No.	Number of mutants	Mutants
I	1	BBMG-35-7-2
II	3	BBMG-25-13-3, BBMG-18-9-6, BBMG-9-10-6
III	1	BBMG-33-11-8
IV	8	BBMG-29-8-4, BBMG-7-9-7, BBMG-5-8-10, BBMG-21-14-5, BBMG-27-10-9, BBMG-13-10-7, BBMG-1-3-6, Check variety KKL-1.

Table 3: Cluster means of the eleven characters studied in M₄ generation mutants of butter bean var. KKL-1.

Traits/clusters	PH	LPP	DFF	PPP	PL	PG	PW	FOPP	100SW	SP	PYPP
Cluster I	39.83	8.10	37.67	33.77	8.29	1.97	4.65	4.00	47.41	57.47	157.02
Cluster II	214.28	25.64	42.66	27.21	16.79	5.59	18.91	7.00	73.85	69.19	515.47
Cluster III	109.80	15.37	36.33	10.51	13.33	7.38	7.38	5.93	57.41	62.47	114.06
Cluster IV	208.78	24.54	41.66	24.73	15.59	3.77	10.28	6.70	62.32	61.14	256.08

• Maximum mean value is indicated in bold.

• Minimum mean value is indicated in bold and underline.

(PH- Plant height, LPP-Leaves per plant, DFF- Days to first flowering, PPP-Pods per plant, PL- Pod length (cm), PG- Pod girth (cm), PW- Pod weight (g), FOP- Fresh ovules per pod, 100SW- 100 seeds weight (g), SP- Shelling percentage PYPP- pod yield per plant (g).

highest mean value of 16.79 cm for pod length. The highest mean value of 7.38 cm for pod girth was recorded by cluster III and the lowest mean value was recorded by cluster I (1.97 cm). The highest pod weight of 18.91 g was recorded by cluster II and the lowest pod weight of 4.65 g was recorded by cluster I. Cluster II recorded the highest mean value of 7.00 for fresh ovules per pod and cluster I recorded the least mean value of 4.00 for fresh ovules per pod. The highest and least mean value for 100 seed weight was possessed by cluster II (73.85 g) and cluster I (47.41 g). The highest shelling percentage of 69.19% was recorded

by cluster II and least shelling percentage was recorded by cluster I (57.47%).

Cluster I possessed a unique dwarf early mutant which recorded more pods with small seeds. This mutant is highly divergent from the original variety KKL-1 butter beans and attention has to be given to utilize this mutant in a pole free (without support) cultivation system. This will have great impact in the cost of cultivation of this crop. Introgression into the line by way of subjecting this to classical breeding programme will be beneficial in identifying elite dwarf types. Efforts may also be taken to identify the genes responsible

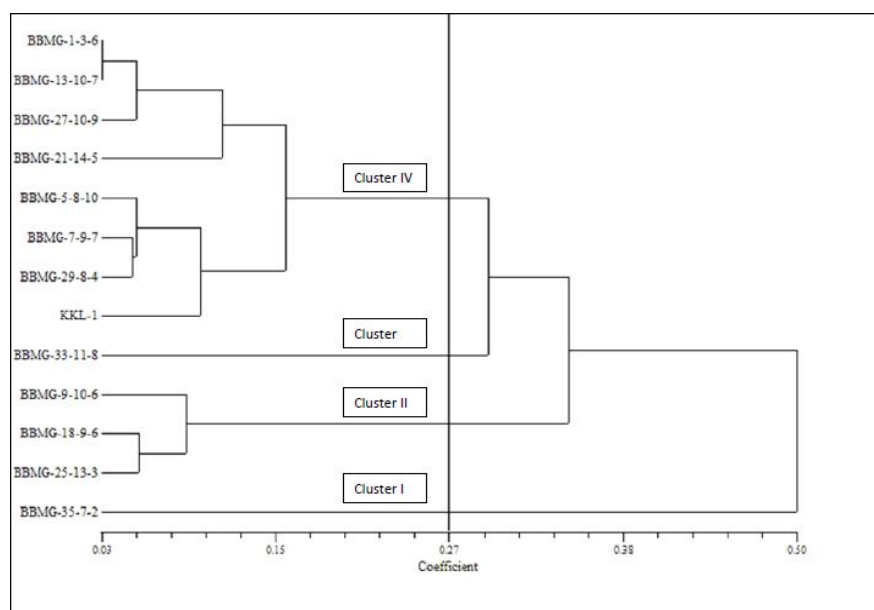


Fig 1: Dendrogram based on quantitative characters in M_4 generation.

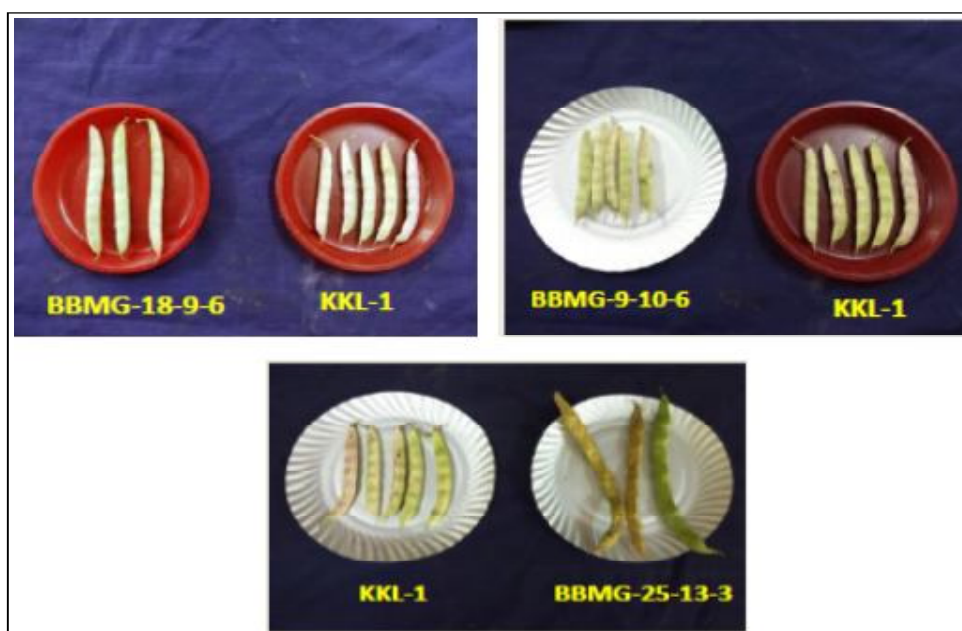


Fig 2: High yielding mutant lines.

for dwarfness using KKL-1 butter beans as template. Cluster IV which encompasses seven mutant lines and the check variety has traits similar to KKL-1.

CONCLUSION

The present investigation studied significant variation for different characters such as mutants with dwarf, semi dwarf, early maturity, blue striped pods with original seed colour, higher seeds per pod, pod length and higher pod length with high flesh content, vegetable moringa bean type, less pod length with bold seeded mutants offer greater scope for developing a line with high yield. The higher dose for creating variability in butter bean was at 150Gy and the beneficial changes that has occurred due to mutation breeding which plant architectural changes with lower duration. Increase of yield in the mutant BBMG-18-9-6 over the check was observed. A total of five mutant lines namely BBMG-7-9-7, BBMG-9-10-6, BBMG-18-9-6, BBMG-25-13-3, BBMG-35-7-2. The above butter beans accessions would be useful for further selection programmes (M₅ generation) to fix the desirable character to be exploited for evolving a suitable variety of butter bean.

Conflict of interest: None.

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