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Research Article

Genetic analysis and variability studies in mutants induced through electron beam and gamma rays in mungbean (*Vigna radiata* L. Wilczek)

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Abstract

Induced mutation is useful to create genetic variability in mungbean. The present study was conducted to assess the amount of genetic variation present among 17 mutants developed through electron beam and gamma rays. The experiment was carried out in two replications and observations were recorded for ten different traits. The genotypic coefficient of variation (GCV), heritability, genetic advance (GA) and correlations were estimated by standard methods. Highest GCV was recorded for seed yield plant^{-1} followed by pods cluster^{-1} and clusters plant^{-1}. Heritability was highest for seeds pod^{-1} (0.92), followed by pod length (0.89), branches plant^{-1} (0.88), 100- seed weight (0.88). Highest GA as per cent of mean was estimated for seed yield plant^{-1}. Seed yield plant^{-1} showed significant positive correlation with cluster plant^{-1}, pods cluster^{-1} and seeds pod^{-1}. A cluster analysis grouped 17 mutants into six clusters. Considerable genetic variability was present in mutants which can be used in mungbean improvement.

Keywords
Mungbean, induced mutation, heritability, correlation, genetic variability

Introduction

Mungbean (*Vigna radiata* L. Wilczek) is an important grain legume food for vegetarian population of India due to its good quality protein and minerals. It is well suited for different cropping systems as a catch crop and intercrop due to its short duration, low input requirements and nitrogen fixing ability. Mungbean was grown in 3.83 million ha area with 1.61 million tons of production (Anonymous, 2017). The productivity of mungbean is around 418 kg/ha, which is very less as compared to other pulse crops. The productivity can be increased by manipulating genetic potential and existing variability present in the germplasm to develop high yielding varieties with resistance to various biotic and abiotic stresses. Genetic variability is less in mungbean as compared to other crops. Use of very few genotypes to breed most of the mungbean cultivars resulted in the narrow genetic base and low buffering capacity of present day cultivars to various biotic and abiotic stresses (Gupta *et al*., 2004, Katiyar *et al*., 2007). Variability can be created through hybridization with wild species and induced mutations. Induced mutation is an important tool which can create variability with novel traits in self pollinated crops like mungbean. Genetic variability was generated through induced mutation in mungbean for various traits like root length, seed size, plant height, leaf shape and size, flower structure, branching pattern, and chlorophyll content *etc.* (Malik *et al*., 1986; Tah 2006; Tah and Saxena 2009; Khan and Goyal 2009; Dhole and Reddy 2010; Azad 2012; Sengupta *et al*., 2013; Gandhi *et al*., 2014). We developed 17 different types of mutants for plant height, days to flowering and maturity, seed coat and pod colour, seed size, pod thickness, leaf size and shape *etc.* by using electron beam and gamma rays in mungbean variety, TM-96-2. Such variability created through induced mutation should be studied thoroughly so that we can use it for crop improvement. Mutation in one trait can affect other traits due to pleiotropic effect and linkage between such traits, which can be broken by mutation followed by recombination. Correlation studies can clarify the possibilities of linkage break between positively or negatively correlated traits due to mutations. Genetic parameters like genetic coefficient of variation will help to compare the traits and identify the trait in which maximum variability has been created through induced mutation, while heritability and genetic advance is useful to understand environmental influence and expected progress under selection for mutant traits. Apart from genetic analysis, diversity studies in such mutants will help to understand the extent of variability created through induced mutation, similarities and differences among these mutants based on morphological traits. Hence, the present study was conducted in 17 mutants induced through electron beam and gamma rays along with parent to estimate genotypic coefficient of variation, heritability, genetic advance and correlations in different yield contributing traits and to find out the genetic variability among mutants based on cluster analysis.

Material and Methods

Material for the present study consists of 17 stable mutants (M_6) of mungbean developed through electron beam and gamma rays along with the parent, TM-96-2. The seeds of mungbean variety TM-96-2 were irradiated with 200, 300, 400 and 500 *Gy* gamma rays and electron beam and M_1 generation was grown in kharif 2013. Mutants were identified in M_2 generation raised in summer 2014. The mutants were found true breeding in M_3 generation grown in kharif 2014. The mutants were advanced to M_4 and M_5 generation in summer 2015 and kharif 2015 respectively. The mutants with their origin are listed in Table 1. The experiment was carried out at experimental and Gamma Field Facility Section, Bhabha Atomic Research Centre, Trombay, Mumbai during summer 2016 in randomized block design with two replications. Each plot consists of two rows of 3m length with spacing of 30 cm between rows and 10 cm between plants. All the recommended cultural practices were followed for normal growth and development of the crop. Data was recorded for ten different traits *viz.* days to flowering and maturity, plant height (cm), number of branches^{-1}, number of clusters^{-1}, number of pods^{-1}, number of seeds^{-1}, pod length (cm), 100 seed weight (g) and seed yield^{-1}(g). Data was recorded on five randomly selected competitive plants from each mutant and replication. Mean values of five randomly selected plants were subjected to analysis of variance. Genotypic and phenotypic variances were estimated by using mean of two replications. Genotypic coefficient of variation, broad-sense heritability (h^2_{BS}) and genetic advance were estimated according to the standard method (Falconer and Mackey, 1996). Pearson's correlation coefficients, cluster analysis and dendrogram were estimated by using SAS 9.3.1 (SAS Institute Inc., Cary, NC).

Result and Discussion

Analysis of variance showed significant differences among mutants, while differences due to replications were non-significant for all ten traits (Table 2). This revealed that the observed variations for these traits were genetic and generated through induced mutation. Among mutants, lowest mean values were recorded for plant height, pods cluster^{-1}, pod length, seeds pod^{-1} and seed yield plant^{-1} in yellow seed coat colour mutants which were late maturing also. Small leaf mutant was late flowering, having less number of branches and clusters plant^{-1}. As expected, early dwarf mutant showed early flowering and maturity. Highest number of pods cluster^{-1} and lowest 100-seed weight was observed in thin pod mutant. Highest cluster plant^{-1}, pod length and 100- seed weight were recorded in large seed and pod mutant (Table 1). Most probable reason for lowest mean values for most of the traits in yellow seed coat colour mutant was that this mutant was weak, having less chlorophyll content and shy bearer. In this mutant, mutation might be occurred in major gene which controls the chlorophyll content in leaf which might be responsible for accumulation of green pigments in seed coat. We observed that mutation related to leaf morphology like lanceolated, small leaf, chlorophyll mutants resulted in change in other traits like pod length and seed size. Amount of variability of different traits can be compared with the help of genotypic coefficient of variation. Highest genotypic coefficient of variation was recorded for seed yield plant^{-1} (42.05%) followed by number of pods cluster^{-1} (28.09%), number of clusters plant^{-1} (27.21%), plant height (20.52%), number of branches plant^{-1} (19.11%) and number of seeds pod^{-1}(14.75%), while lowest genotypic coefficient of variation was observed for days to maturity (2.70 %) and days to flowering (5.02 %). Heritability (broad sense) was highest for number of seeds pod^{-1}(0.92), followed by pod length (0.89), number of branches plant^{-1} (0.88), 100 seed weight (0.88), plant height (0.86) and number of pods cluster^{-1}(0.84), while it was lowest for seed yield plant^{-1} (0.77) (Table 3). High heritability values for these traits were also reported in earlier studies on mungbean (Bisht *et al*., 2014; Tabasum *et al*., 2010; Kumar *et al*., 2010, Singh *et al*., 2014). Genetic advance (as % of mean) was found to be highest for seed yield plant^{-1} (66.79) followed by number of pods cluster^{-1} (48.42), number of clusters plant^{-1} (46.23), plant height (36.27) and number of branches plant^{-1}(34.56). The highest genetic advance for seed yield plant^{-1} was attributed to high genetic variance although heritability was less. It suggested the importance of genetic variability for effective selection in mungbean. High genetic advance for these traits was also reported in earlier studies in mungbean (Keerthiga *et al*., 2017, Bisht *et al*., 2014). Seed yield plant^{-1} showed significant positive correlation with number of cluster plant^{-1}, number pods cluster^{-1} and number of seeds pod^{-1}, while number of cluster plant^{-1} was significantly and positively correlated with number of branches plant^{-1}. Significant and positive correlation was also noticed between days to flowering and days to maturity; number of seeds pod^{-1} and number of pods cluster^{-1}; number of seeds pod^{-1} and pod length (Table 4). Correlation between yield and yield contributing traits were also reported in earlier studies (Kumar *et al*., 2010, Bisht *et al*., 2014, Canci and Toker, 2014, Singh *et al*., 2014, Garje *et al*., 2014, Divyaramakrishnan and Savithramma 2014, Dhoot *et al*., 2017). Significant negative correlation between seed yield and 100- seed weight was reported in earlier studies (Singh *et al*., 2014), while in the present study, we observed positive but non-significant correlation between seed yield and 100-seed weight. Cluster analysis grouped seventeen mutants and their parent into six clusters (Fig. 1). Cluster-I, cluster-II and cluster –III involved four mutants each, while rest of three clusters had two mutant each. Cluster-VI was the most distantly separated from other clusters and had two yellow seed coat colour mutants. The mean values of cluster-VI were highest for days to flowering and maturity while lowest for plant height, number of clusters plant^{-1}, number of pods cluter^{-1}, pod length, number of seeds pod^{-1}, 100- seed weight and seed yield plant^{-1} as compared to other clusters (Table 5). Yellow seed coat colour mutants in cluster-VI were most distantly related to other mutants. The leaf colour of these mutants was pale green with less chlorophyll content which affected normal growth and development. This resulted in lowest mean for seed yield and other yield contributing traits in yellow seed coat colour mutants.

Apart from use of mutants for mungbean improvement, the novel mutants like yellow seed coat colour, brown and yellow pod colour, four primary leaves and lanceolated leaf mutants will be very useful for genetics and linkage studies. In the era of genomic studies, such mutants will play very important role in gene identification, gene mapping, identifying gene function and their effect on other traits. Mutants and parent will have single or few gene differences just like isogeneic lines, hence it is very easy to identify the cause of mutations at DNA level. Thus, these studies on induced mutants can add some important information on developmental biology of mungbean.

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Table 1. Mutants with their origin along with mean for yield and yield contributing traits in mungbean

S. No.	Mutant	Origin of mutant	Days to flowering	Days to maturity	Plant height (cm)	No. of branches plant^{-1}	No. of clusters plant^{-1}	No.of pods cluster^{-1}	Pod length (cm)	No. of seeds pod^{-1}	100- seed weight (g)	Seed yield plant^{-1} (g)
1	TM-96-2 (C)	Control/parent	34.7	64.7	49.4	2.1	6.8	4.65	8.1	10.46	5.15	3.97
2	Yellow seed-1	EB 200	34.8	65.15	25.05	1.95	4.3	2.05	5.45	6.3	3.79	1.09
3	Yellow seed-2	EB 200	34.95	64.95	28	2.1	5.25	2.05	6.2	7.6	3.525	1.275
4	Yellow pod	EB 200	32.75	62.7	55.5	2.1	6.35	5.1	7.55	10.25	5.18	6.87
5	Brown pod	EB 400	33.05	64.4	59.35	1.85	5.25	3.25	8.18	11.85	4.44	3.86
6	Thin pod-1	EB400	30.2	60.9	52.05	1.55	6.45	5.75	7.78	11.75	3.78	9.84
7	Thin pod-2	EB400	30.9	61.8	51.65	1.75	7.65	6.65	7.75	11.75	3.41	6.26
8	Lanceolated leaf	G 500	31.6	62.7	49.7	2.29	8.65	4.05	5.65	7.8	4.33	7.24
9	Four primary leaves	G 400	32.8	64.5	54.8	1.85	5.1	3.5	8	10.3	4.79	5.05
10	Small leaf	EB 400	35.5	64.8	53.6	1.9	6.5	3.5	8.06	10.8	4.51	5.66
11	Small leaf	G 200	32.6	62.5	46.5	1.45	4.1	4.1	7.79	10.6	4.49	4.24
12	Early maturity	EB 400	31.3	60.8	51.7	2.1	8.1	4.3	7.75	9.8	5.32	4.51
13	Dwarf branching	EB 500	32	61.8	43.5	2.75	6.7	5.15	8.45	10.6	4.24	6.23
14	Early dwarf	EB 500	29.8	59.1	35.8	2.05	6.25	3.45	6.68	9.08	4.23	4.79
15	Tall	EB 500	32.25	62.8	64.7	1.82	5.15	3.05	7.86	10.28	4.77	4.05
16	Basal branching	EB 200	34.9	64.6	52.4	2.15	6.4	4.45	8.14	11.15	4.99	7.44
17	Large pod and seed	EB 500	34.5	64.9	44.1	2.65	7.1	3.25	8.48	11.1	5.3	6.12
18	Large pod	EB 300	33.5	60.8	41.3	3.1	12.3	4.4	8.25	10.9	5.1	10.8

Table 2. Analysis of variance (ANOVA) for yield and yield contributing traits in 17 mutants of mungbean

Source	DF	Days to flowering		Days to maturity		Plant height		No of branches plant^{-1}		No of clusters plant^{-1}		No of pods cluster^{-1}		Pod length		No seeds pod^{-1}		100- seed weight		Seed yield plant^{-1}	
		MS	F value	MS	F value	MS	F value	MS	F value	MS	F value	MS	F value	MS	F value	MS	F value	MS	F value	MS	F value
Replication	1	0.134	0.35	1.604	17.42	0.028	0.02	0.026	2.12	0.113	3.86	0.054	2.17	0.001	0.04	0.001	0.02	0.019	0.73	0.667	4.17
Genotypes	17	6.031*	15.7	6.775*	73.50	207.7*	149.71	0.338*	27.44	7.074*	245.99	2.813*	112.38	1.703*	58.92	4.652*	54.31	0.794*	29.36	12.33*	77.18
Error	17	0.583	-	0.692	-	15.88	-	0.022	-	0.68	-	0.25	-	0.09	-	0.186	-	0.047	-	1.59	-

Table 3. Components of genetic variance for yield and yield contributing traits in mungbean

S. N.	Traits	Mean	Range	GCV (%)	PCV (%)	Heritability	Genetic advance (GA)	GA (as % of mean)
1	Days to flowering	32.89	29.8 – 35.5	5.02	5.52	0.82	2.80	8.51
2	Days to maturity	62.99	59.1 – 65.15	2.70	3.06	0.81	2.93	4.65
3	Plant height (cm)	47.72	25.05 – 64.7	20.52	22.15	0.86	17.31	36.27
4	No. of branches plant^{-1}	2.08	1.45 – 2.75	19.11	20.39	0.88	0.72	34.56
5	No. of clusters plant^{-1}	6.57	4.1 - 12.3	27.21	29.96	0.82	3.04	46.23
6	No. of pods cluster^{-1}	4.03	2.05 - 6.65	28.09	30.07	0.84	1.95	48.42
7	Pod length (cm)	7.56	5.45 - 8.48	11.87	12.52	0.89	1.66	22.01
8	No. of seeds pod^{-1}	10.13	6.3 – 11.85	14.75	15.35	0.92	2.84	28.05
9	100- seed weight (g)	4.53	3.41 - 5.45	13.49	14.31	0.88	1.12	24.69
10	Seed yield plant^{-1} (g)	5.51	1.09 – 10.8	42.05	47.87	0.77	3.68	66.79

Table 4. Pearson's correlation coefficients for yield and yield contributing traits in mutants of mungbean

Characters	Days to flowering	Days to maturity	Plant height	No. of branches plant^{-1}	No. of clusters plant^{-1}	No. of pods cluster^{-1}	Pod length	No. of seeds plant^{-1}	100- seed weight	Seed yield plant^{-1}
Days to flowering	1	0.81**	-0.19	0.19	-0.13	-0.48	0.05	-0.15	0.25	-0.31
Days to maturity		1	-0.03	-0.09	-0.43	-0.48	-0.04	-0.16	0.08	-0.46
Plant height			1	-0.28	0.02	0.41	0.58	0.67	0.39	0.33
No. of branches plant^{-1}				1	0.69**	-0.02	0.15	-0.05	0.35	0.36
No. of clusters plant^{-1}					1	0.39	0.18	0.19	0.29	0.70**
No. of pods cluster^{-1}						1	0.43	0.60**	0.01	0.65**
Pod length							1	0.89**	0.49	0.41
No. of seeds plant^{-1}								1	0.27	0.54*
100- seed weight									1	0.21
Seed yield plant^{-1}										1

*Significance at 0.05 probability level; **Significance at 0.01 probability level

Table 5. Comparison of different clusters on the basis of cluster means for yield and yield contributing traits in mungbean

Cluster number	No. of mutant in each cluster	Cluster mean for different traits									
		Days to flowering	Days to maturity	Plant height (cm)	No. of branches plant^{-1}	No. of clusters plant^{-1}	No. of pods cluster^{-1}	Pod length (cm)	No. of seeds plant^{-1}	100- seed weight (g)	Seed yield plant^{-1} (g)
Cluster-I	4	33.99	64.15	54.08	2.00	6.09	4.14	7.94	10.63	4.87	6.26
Cluster-II	4	31.00	61.55	51.28	1.92	7.71	5.19	7.23	10.28	4.21	6.97
Cluster-III	4	33.45	63.48	45.88	2.24	6.18	4.29	8.19	10.69	4.87	5.14
Cluster-IV	2	32.65	63.60	62.03	1.84	5.20	3.15	8.02	11.07	4.61	3.96
Cluster-V	2	31.65	59.95	38.55	2.58	9.28	3.93	7.47	9.99	4.67	7.80
Cluster-VI	2	34.88	65.05	26.53	2.03	4.78	2.05	5.83	6.95	3.66	1.19

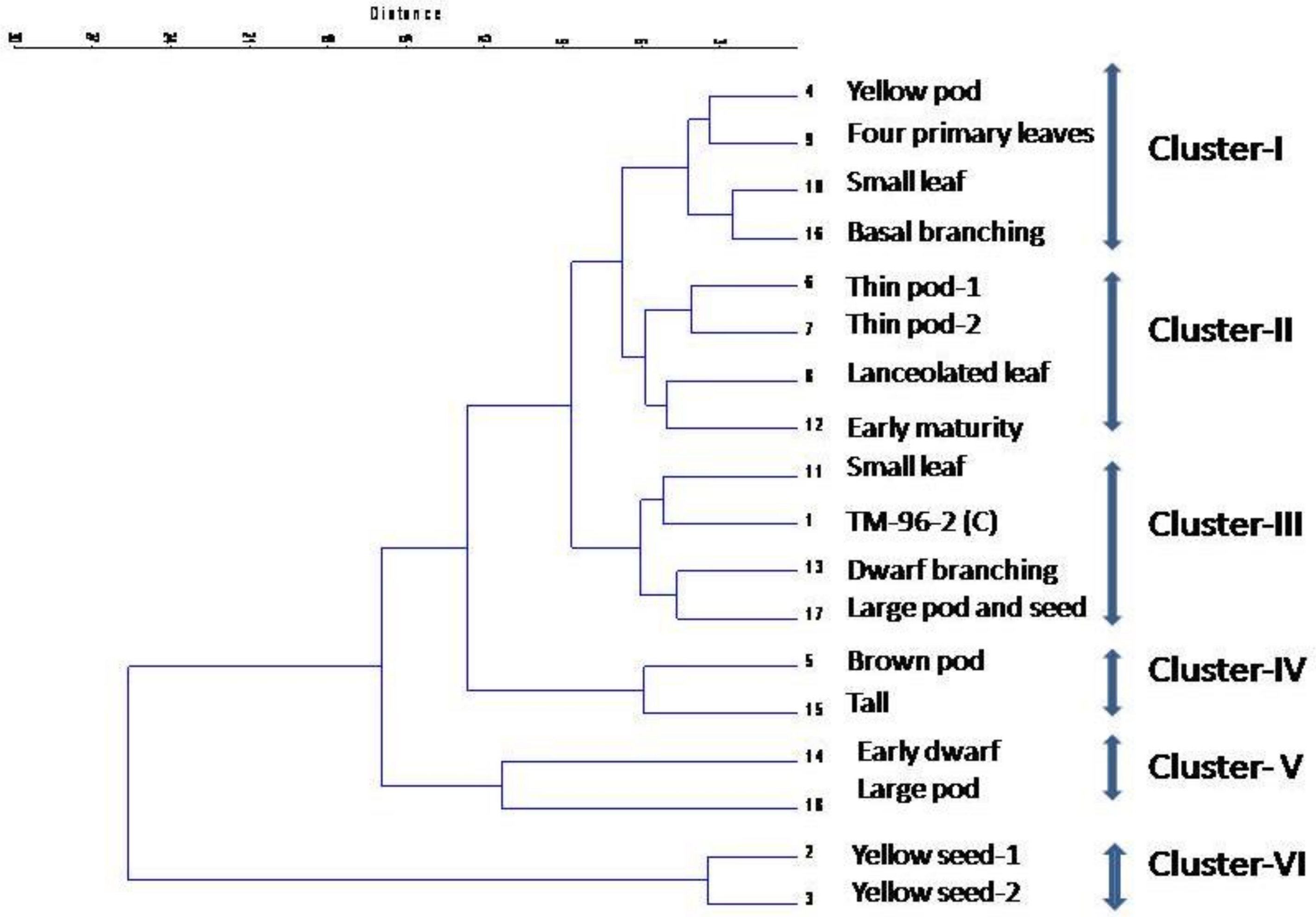

Fig. 1. Dendrogram based on cluster analysis of ten different traits in 17 mutants of mungbean