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Estimation of variability parameter in m_3 generation of green gram [*Vigna radiata* (L.) R.Wilczek]

Ahir DK, Rajiv Kumar, Chetariya CP and Jalu RK

Abstract
Experiment carried out for the studied of "Variability in M_3 generation of green gram" by inducing quantitative variability through mutagen to know the status of genetic variability parameters like GCV, PCV, Heritability and Genetic advance percent of mean. The genotypes differed significantly for all characters. The analysis of variance in M_3 generation for progeny within family indicated highly significant difference for plant height and chlorophyll content for majority of progenies. For all characters, phenotypic coefficient of variation was higher than genotypic coefficient of variation indicating that there was environmental influence on these traits. The combined results for heritability showed that the high estimates of heritability and genetic advance were scored for seeds per plant indicating that these characters were under the control of additive genetic effects. Environmental influence was high for all the observed characters. Individual plant analysis revealed significant variation among plants for all the characters, except number of pods per cluster.

Keywords: M_3 generation, GCV, PCV, heritability, variability

1. Introduction
Green gram belongs to order Fabales consist composed of more than 200 species. *Vigna*is closely related to *Phaseolus*, which is composed of more than 20 species, a number of species previously placed in *Phaseolus*are now placed in *Vigna*. Green gram it is botanically recognized as *Vignaradiata* (L.). Green gram belongs to family Fabaceaewith chromosome number 2n=2x=22. The origin of crop is considered in India from where it had spread to Indo-china, Java, Eastern and Central Africa, West Indies, warmer parts of China and U.S.A. (Janoria *et al.*, 1984) [6]. Green gram was domesticated in Mongolia, where its progenitor (*Vignaradiata* subspecies *sublobata*) occurs in wild. Archaeological evidence has turned up carbonized mung beans on many sites in India. Areas with early finding include the eastern zone of the Harappan civilization in Punjab and Haryana, where finding date back about 4500 years, and South India in the modern state of Karnataka where finding date back more than 4000 years.

Green gram is rich in protein (24gm/100gm) which is nearly 2.5 times more than cereals. It is also good source of carbohydrate (60gm/100gm), fat (1gm/100gm), minerals (3gm/100gm) and fiber (1gm/100gm). Mungbean seeds contain about 124 mg Calcium/100 gm, 326 mg Phosphorus/100 gm, 1.3% Fat,7.3 mg Iron/100 gm,4.1 % Fiber and having 334 Kcal Calorific Value. In our country peoples are predominantly vegetarian and pulses are main sources of protein, thus its vital importance in daily diet (Anon. 2014) [3]. Mutation induced in self-pollinated crops are of great value in plant breeding (Hagberg *et al.*, 1963) [5] and recognized as a potential tool for crop improvement by the way of creating variability in the population. Induced mutagenesis plays an important role in improvement of crops like green gram, where a large part of genetic variability has been eroded due to its continuous cultivation in marginal and sub-marginal land and its adaption to survival fitness rather than yield. Further, hybridization in this crop is difficult due to its small cleistogamous flower. Mutation breeding requires handling of large population, as chances of induction and detection of mutation in a particular gene is rare. This increases the cost of breeding and makes the selection procedure time consuming and tedious. Detection of effective mutagenic treated population in the early generations, particularly in M_3generation would no doubt reduce the population load in subsequent generation and provide better scope for selection. However, study in this respect is limited and needs more investigation. Several approaches have been takenup for enhancement of genetic variability in greengram and induction of mutation is considered to be quite promising. Gamma rays, the physical mutagen are non-particulate ionizing radiations, having

high energy and penetrable capacity in biological tissues and make changes in base, disruption of hydrogen bonds between complementary strands of DNA. Majority of mutant varieties (64%) were developed by the gamma rays (Ahloowalia *et al.* 2004) [1]. The M_3 is considered to be the most crucial stage for selection to commence, as $M_3=F_3$. This ostensibly saves the experiment of his considerable resources because a great deal of (unnecessary) load (of unproductive plants) is shed right in the M_3 generation itself.

Materials and Methods

The Field experiment was conducted at Instructional Farm, College of Agriculture, Junagadh Agricultural University, Junagadh during the Summer-2015.The experimental material consisted of M_2 derived M_3 seeds. Which consists of sixty six M_3 progeny lines (64 mutant + 2 controls). The progeny lines were selected on the basis of yield and its associated characters from M_2 generation. Each progeny line is an individual's plant seed. M_2 generation consists of two base varieties (GM-4 and meha) and two base mutagens (gamma rays and EMS). Both the mutagens with four levels *viz*,(gamma rays: 200, 400, 600, 800 Gy and EMS: 0.15, 0.20, 0.25, 0.30 %). From each mutagenic family [Sixteen families (GM-4; 200, 400, 600, 800 Gy and 0.15, 0.20, 0.25, 0.30 %) and (Meha;200, 400, 600, 800 Gy and 0.15, 0.20, 0.25, 0.30 %)] four progeny lines were selected, which leads to sixty four mutagenic progeny lines in M_3 generation. The M_3 generation was grown with three replications of randomized block design. Biometric observations were recorded and individual plant data were used for statistical analysis in order to asses extends of induced variation. Study carried out for various character which are days to flowering, days to maturity, plant height, numbers of primary branches per plant, number of clusters per plant, number of pod per cluster, number of pods per plant, pod length, number of seeds per pod, seed yield per plant, test weight and chlorophyll content. Significant differences were identified using the least significance difference estimated from the error mean square and tabulated values at the 5% level of significance. The analysis of variance for M_3 generation was carried out for Compact Family Block Design as per analysis suggested by Panse and Sukhatme (1978). Parameters estimated were the phenotypic co-efficient of variation (PCV), genotypic co-efficient of variation (GCV), broad-sense heritability (h^2) and expected genetic advance (GA). The estimate of the expected genetic advance as percentage of the mean values were assumed and computed by the formula Johnson *et al*. (1955) [7]. Heritability in broad sense was calculated by the following formula suggested by Allard (1960) [2]. Phenotypic variation and genotypic variation calculated by the formula suggested by Burton and DeVane.

Results and Discussion

The analysis of variance between families revealed that families differed significantly for days to flowering, days to maturity, plant height, numbers of primary branches per plant, number of pod per cluster, pod length, number of seeds per pod, test weight and chlorophyll content (Table-1.1). The analysis of variance in M_3 generation for progeny within family indicated significant difference for plant height and chlorophyll content for majority of progenies. However, few progenies were found to be significant for days to flowering (Family 2 and 3), days to maturity (Family 1, 2,4 and 8), number of primary branches per plant (Family 11), number of cluster per plant (Family 8 and 11), number of pods per plant (Family 8, 10 and 11), number of seeds per pods (Family 4, 10 and 11), pod length (Family 9 and 10), seed yield per plant (Family 3, 13 and 15) and test weight (Family 1, 2, 10, 12, 13 and 16), rest of the trait were found non-significant. In M_3 generation, family mean of different mutagenic treatments were in positive direction for numbers of primary branches per plant, pod length, numbers of seeds per pods and seed yield per plant. This indicated accumulation of positive gene. However, rest of the characters like days to flowering, days to maturity, plant height, numbers of clusters per plant, numbers of pods per plant, test weight and chlorophyll content had mean values in both the directions.

Most of the mutagenic treatments had wide range as compared to their respective control for the studied characters. This expended range of variation for different measurements generated in progenies of M_3 generation could be attributed to substantial changes in the genetic background comprising modifies complexes during mutation process.

For seed yield per plant wide genotypic and phenotypic difference was observed which indicates that there was significance influence of environment. All the mutagenic treatments show its superiority over control in phenotypic range, GCV, PCV, heritability and genetic advance. In case of GM-4 Higher PCV, GCV and genetic advance were observed in all mutagenic families as compared to control. Highest heritability (56.9 %) and highest genetic advance (44.2 %) were observed in family-3. The PCV is higher than GCV indicates significant influence of environment. While in case of meha Treatment means was higher in all mutagenic treatment, indicating shifting of means of positive direction. PCV and GCV were higher in all the mutagenic family with respect to control. The PCV is higher than the GCV indicates significant influence of environment. Heritability exhibited higher in all the mutagenic families. Higher genetic advance was observed in family-15 (65.2 %).

Environmental influence was high for all the observed characters *viz*. days to maturity, plant height, numbers of primary branches per plant, numbers of clusters per plant, numbers of pods per clusters, numbers of pods per plant, pod length, numbers of seeds per pod, seed yield per plant, test weight and chlorophyll content. Genetic parameters such as heritability & genetic advance increased many fold in almost all characters, as compared to control. The phenotypic and genotypic coefficients of variation increased in all the mutagenic treatments for all the characters except 600 Gy in meha for days to flowering for GCV, 400 Gy in meha for days to maturity for GCV, 400 Gy in GM-4 for number of cluster per plant for GCV, 400 Gy in meha for number of pods per cluster for PCV, 0.25 % EMS in meha for pod length for GCV.

Individual plant analysis revealed significant variation among plants (314 mutants + 30 controls) for all the characters, except number of pods per cluster. Controls (were significantly different from each other for four character *viz.*, days to flowering, days to maturity, plant height and chlorophyll content. Variation among mutant progenies were significantly in all the character except number of pods per cluster, that indicating the impact of mutation events. Variance due to controls vs. mutants was also significant for plant height, number of primary branches, number of seeds per pod, pod length, seed yield per plant and chlorophyll content. This implies that considerable change in the means of controls have been brought about by mutagenesis. It was also revealed that most of the families and progeny lines were homogeneous for most of the characters.

Table 1.1: Analysis of variance (mean square) between families for different characters in M3 generation of green gram

Source	Mean sum of square			
	Between Family		Among Family	
	Families	Error	Progenies	Error
d.f	17	17	65	65
1. Days to flowering	6.50**	0.82	8.32*	2.28
2. Days to maturity	27.86**	0.38	31.87*	1.85
3. Plant height	24.16**	3.93	49.00*	8.12
4. Number of primary branches per plant	0.10*	0.05	0.19*	0.12
5. Number of clusters per plant	2.74	3.11	7.42	6.70
6. Number of pods per cluster	0.04*	0.02	0.10*	0.05
7. Number of pods per plant	28.77	23.55	69.82	54.41
8. Pod length	0.16**	0.04	0.23*	0.08
9. Number of seeds per pod	0.45**	0.07	0.84*	0.26
10. Seed yield per plant	2.51	2.01	5.53	3.95
11. Test weight	0.12**	0.04	0.33*	0.10
12. Chlorophyll content	57.18**	0.44	86.09*	2.65

Table 1.2: Estimation of variability parameter GCV, PCV, H^2, GA% for different character in GM-4 variety

Traits	Doses	GM-4 varieties - Mutagens Dose/conc.											
		Days to flowering				Days to maturity				Plant height			
		PCV (%)	GCV (%)	H^2 (BS)	GA (%)	PCV (%)	GCV (%)	H^2 (BS)	GA (%)	PCV (%)	GCV (%)	H^2 (BS)	GA (%)
Gamma rays (kR)	T_1-200	3.23	1.75	29.3	2.0	4.11	3.92	91.0	7.7	14.20	11.20	62.2	18.2
	T_2-400	3.97	3.33	70.3	5.7	2.11	1.61	58.1	2.5	24.57	23.57	92.0	46.6
	T_3-600	4.03	3.10	58.9	4.9	-	-	-	-	-	-	-	-
	T_4-800	4.22	1.33	10.0	0.9	3.48	3.17	83.2	6.0	5.70	4.14	52.6	6.2
EMS %	T_9-0.15	-	-	-	-	2.97	1.92	41.7	2.5	8.66	4.61	28.3	5.0
	T_{10}-0.20	-	-	-	-	-	-	-	-	10.96	9.38	73.1	16.5
	T_{11}-0.25	5.04	0.80	2.5	0.3	2.24	1.07	22.9	1.1	15.14	14.69	94.1	29.4
	T_{12}-0.30	-	-	-	-	2.00	0.81	16.5	0.7	13.34	9.30	48.6	13.4
Control	T_{17}	0.80	0.32	16.3	0.3	0.85	0.52	38.3	0.7	2.94	1.43	23.5	1.4

Traits	Doses	GM-4 varieties - Mutagens Dose/conc.											
		Number of primary branches per plant				Number of clusters per plant				Number of pods per cluster			
		PCV (%)	GCV (%)	H^2 (BS)	GA (%)	PCV (%)	GCV (%)	H^2 (BS)	GA (%)	PCV (%)	GCV (%)	H^2 (BS)	GA (%)
Gamma rays (kR)	T_1-200	-	-	-	-	-	-	-	-	9.14	2.81	9.5	1.8
	T_2-400	-	-	-	-	20.04	4.88	5.9	2.4	8.84	4.76	29.0	5.3
	T_3-600	20.17	7.93	15.5	6.4	46.24	31.89	47.6	45.3	12.06	6.10	25.5	6.3
	T_4-800	20.33	8.61	17.9	7.5	-	-	-	-	-	-	-	-
EMS %	T_9-0.15	-	-	-	-	-	-	-	-	-	-	-	-
	T_{10}-0.20	-	-	-	-	42.79	28.26	44.9	39.0	-	-	-	-
	T_{11}-0.25	24.24	19.86	67.2	33.5	32.52	29.27	81.0	54.3	-	-	-	-
	T_{12}-0.30	25.54	18.57	52.8	27.8	52.74	25.26	22.9	24.9	7.02	4.63	43.5	6.3
Control	T_{17}	8.17	4.93	36.4	6.1	9.70	5.59	33.2	6.6	2.19	1.34	37.5	1.7
Traits	**Doses**	**GM-4 varieties - Mutagens Dose/conc.**											
		Number of primary branches per plant				**Number of clusters per plant**				**Number of pods per cluster**			
		PCV (%)	**GCV (%)**	**H^2 (BS)**	**GA (%)**	**PCV (%)**	**GCV (%)**	**H^2 (BS)**	**GA (%)**	**PCV (%)**	**GCV (%)**	**H^2 (BS)**	**GA (%)**
Gamma rays (kR)	T_1-200	-	-	-	-	-	-	-	-	9.14	2.81	9.5	1.8
	T_2-400	-	-	-	-	20.04	4.88	5.9	2.4	8.84	4.76	29.0	5.3
	T_3-600	20.17	7.93	15.5	6.4	46.24	31.89	47.6	45.3	12.06	6.10	25.5	6.3
	T_4-800	20.33	8.61	17.9	7.5	-	-	-	-	-	-	-	-
EMS %	T_9-0.15	-	-	-	-	-	-	-	-	-	-	-	-
	T_{10}-0.20	-	-	-	-	42.79	28.26	44.9	39.0	-	-	-	-
	T_{11}-0.25	24.24	19.86	67.2	33.5	32.52	29.27	81.0	54.3	-	-	-	-
	T_{12}-0.30	25.54	18.57	52.8	27.8	52.74	25.26	22.9	24.9	7.02	4.63	43.5	6.3
Control	T_{17}	8.17	4.93	36.4	6.1	9.70	5.59	33.2	6.6	2.19	1.34	37.5	1.7
Traits	**Doses**	**GM-4 varieties - Mutagens Dose/conc.**											
		Seed yield per plant				**Test weight**				**Chlorophyll content**			
		PCV (%)	**GCV (%)**	**H^2 (BS)**	**GA (%)**	**PCV (%)**	**GCV (%)**	**H^2 (BS)**	**GA (%)**	**PCV (%)**	**GCV (%)**	**H^2 (BS)**	**GA (%)**
Gamma rays (kR)	T_1-200	39.55	14.92	14.2	11.6	16.35	15.89	94.5	31.8	20.24	19.84	96.1	40.1
	T_2-400	-	-	-	-	6.67	5.54	69.2	9.5	7.94	3.69	21.6	3.5
	T_3-600	37.74	28.46	56.9	44.2	8.24	4.43	28.9	4.9	5.21	3.80	53.0	5.7
	T_4-800	-	-	-	-	11.86	6.51	30.2	7.4	12.91	10.94	71.8	19.1
EMS %	T_9-0.15	-	-	-	-	6.42	1.44	5.0	0.7	5.93	1.60	7.3	0.9
	T_{10}-0.20	33.07	22.48	46.2	31.5	14.53	11.94	67.5	20.2	12.93	11.40	84.7	21.6
	T_{11}-0.25	21.98	13.86	39.8	18.0	9.83	3.18	10.4	2.1	21.83	20.15	85.2	38.3
	T_{12}-0.30	41.94	26.46	39.8	34.4	6.28	5.15	67.4	8.7	20.39	20.17	97.9	41.1
Control	T_{17}	1.62	0.81	25.0	0.8	2.01	0.53	6.8	0.3	2.40	1.20	24.9	1.2

Table 1.3: Estimation of variability parameter GCV, PCV, H^2, GA% for different character in MEHA variety

Traits	Doses	MEHA varieties - Mutagens Dose/conc.											
		Days to flowering				Days to maturity				Plant height			
		PCV (%)	GCV (%)	H^2 (BS)	GA (%)	PCV (%)	GCV (%)	H^2 (BS)	GA (%)	PCV (%)	GCV (%)	H^2 (BS)	GA (%)
Gamma rays (kR)	T_5-200	4.54	2.60	32.7	3.1	-	-	-	-	11.05	8.74	62.6	14.2
	T_6-400	1.96	0.74	14.3	0.6	2.06	0.48	5.5	0.2	14.36	13.16	84.0	24.8
	T_7-600	2.80	0.57	4.1	0.2	-	-	-	-	10.76	10.08	87.9	19.5
	T_8-800	-	-	-	-	1.87	1.66	78.5	3.0	14.31	12.28	73.6	21.7
EMS %	T_{13}-0.15	3.60	1.92	28.4	2.1	1.90	0.93	23.8	0.9	17.45	15.96	83.6	30.1
	T_{14}-0.20	2.99	1.98	44.1	2.7	1.58	1.06	44.9	1.5	8.72	5.00	32.9	5.9
	T_{15}-0.25	3.81	2.25	34.7	2.7	2.58	0.81	9.8	0.5	20.83	16.73	64.5	27.7
	T_{16}-0.30	3.59	2.07	33.4	2.5	1.73	1.07	38.3	1.4	-	-	-	-
Control	T_{18}	0.86	0.63	52.8	0.9	0.91	0.49	29.1	0.5	2.98	2.09	49.2	3.0

Traits	Doses	MEHA varieties - Mutagens Dose/conc.											
		Number of primary branches per plant				Number of clusters per plant				Number of pods per cluster			
		PCV (%)	GCV (%)	H^2 (BS)	GA (%)	PCV (%)	GCV (%)	H^2 (BS)	GA (%)	PCV (%)	GCV (%)	H^2 (BS)	GA (%)
Gamma rays (kR)	T_5-200	-	-	-	-	-	-	-	-	10.91	6.34	33.7	7.6
	T_6-400	-	-	-	-	16.92	5.84	11.9	4.2	4.68	2.59	30.5	2.9
	T_7-600	-	-	-	-	-	-	-	-	-	-	-	-
	T_8-800	14.73	9.91	45.2	13.7	33.61	29.03	74.6	51.7	8.05	5.80	51.8	8.6
EMS %	T_{13}-0.15	12.25	5.00	16.7	4.2	23.95	10.74	20.1	9.9	10.39	6.57	40.0	8.6
	T_{14}-0.20	-	-	-	-	21.89	7.59	12.0	5.4	10.24	7.42	52.5	11.1
	T_{15}-0.25	17.61	11.37	41.7	15.1	46.74	30.36	42.2	40.6	-	-	-	-
	T_{16}-0.30	-	-	-	-	-	-	-	-	-	-	-	-
Control	T_{18}	4.97	3.14	40.0	4.1	4.07	2.22	29.8	2.5	5.31	0.92	3.0	0.3
Traits	Doses	MEHA varieties - Mutagens Dose/conc.											
		Number of pods per plant				Pod length (cm)				Number of seeds per pod			
		PCV (%)	GCV (%)	H^2 (BS)	GA (%)	PCV (%)	GCV (%)	H^2 (BS)	GA (%)	PCV (%)	GCV (%)	H^2 (BS)	GA (%)
Gamma rays (kR)	T_5-200	-	-	-	-	6.34	3.75	34.9	4.6	-	-	-	-
	T_6-400	37.41	22.33	35.6	27.5	5.15	1.88	13.3	1.4	-	-	-	-
	T_7-600	-	-	-	-	5.06	2.34	21.4	2.2	10.29	6.92	45.2	9.6
	T_8-800	32.20	25.08	60.7	40.2	5.12	2.76	29.1	3.1	6.99	2.83	16.3	2.4
EMS %	T_{13}-0.15	21.20	12.07	32.4	14.2	4.48	2.17	23.5	2.2	8.99	7.32	66.1	12.3
	T_{14}-0.20	21.99	12.37	31.6	14.3	-	-	-	-	-	-	-	-
	T_{15}-0.25	42.92	31.50	53.9	47.6	6.33	1.28	4.1	0.5	15.38	9.60	38.9	12.3
	T_{16}-0.30	-	-	-	-	-	-	-	-	-	-	-	-
Control	T_{18}	7.88	5.08	41.5	6.7	2.57	1.17	20.7	1.1	3.74	0.88	5.5	0.4

Traits	Doses	MEHA varieties - Mutagens Dose/conc.											
		Seed yield per plant				Test weight				Chlorophyll content			
		PCV (%)	GCV (%)	H^2 (BS)	GA (%)	PCV (%)	GCV (%)	H^2 (BS)	GA (%)	PCV (%)	GCV (%)	H^2 (BS)	GA (%)
Gamma rays (kR)	T_5-200	-	-	-	-	-	-	-	-	12.89	11.82	84.2	22.3
	T_6-400	-	-	-	-	7.48	3.86	26.6	4.1	14.39	12.39	74.2	22.0
	T_7-600	-	-	-	-	9.96	7.35	54.5	11.2	6.86	4.95	52.1	7.4
	T_8-800	32.60	20.55	39.8	26.7	7.54	2.01	7.1	1.1	19.08	18.88	98.0	38.5
EMS %	T_{13}-0.15	28.60	22.64	62.7	36.9	12.31	9.48	59.3	15.0	11.17	10.50	88.4	20.3
	T_{14}-0.20	26.40	13.55	26.3	14.3	-	-	-	-	7.79	4.84	38.6	6.2
	T_{15}-0.25	51.40	40.33	61.5	65.2	12.20	4.59	14.2	3.6	-	-	-	-
	T_{16}-0.30	-	-	-	-	7.23	6.25	74.7	11.1	30.04	29.40	95.8	59.3
Control	T_{18}	1.70	0.97	32.4	1.1	1.86	0.39	0.01	0.2	4.96	2.84	32.8	3.4

Conclusion

From the results and discussion, it can be concluded that analysis of variance showed that the genotypes differed significantly for all the character under study indicating presence of adequate variability. Analysis of variance between families are significant for most of characters except number of clusters per plant, number of pods per plant and seed yield per plant but among families different characters showed maximum presence of diversity are plant height and chlorophyll content so the mutagenic families having presence in wide range of class intervals as compared to controls. The phenotypic and genotypic coefficients of variation increased in all the mutagenic treatments for all the characters compare to control. For all characters, phenotypic coefficient of variation was higher than genotypic coefficient of variation indicating that there was environmental influence on these traits. The outcome of the present investigation can be used directly as a variety or parental lines for future hybridization programme, as majority of progeny lines showed homogeneity for most of the characters and induced mutagenesis plays an important role in improvement of crops like green gram.

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