International Journal of Genetics
ISSN: 0975-2862 & E-ISSN: 0975-9158, Volume 9, Issue 6, 2017, pp.-284-286.
Available online at http://www.bioinfopublication.org/jouarchive.php?opt=&jouid=BPJ0000226

Research Article

INDUCED GENETIC VARIABILITY AND DIVERGENCE THROUGH PHYSICAL AND CHEMICAL MUTAGENS IN M_3 GENERATION OF GREENGRAM (*Vigna radiata* L. WILCZEK)

PAUL PRONOB J., LAVANYA G. ROOPA*, BABU G. SURESH, YADAV YUVRAJ AND VERMA UMAKANT

Department of Genetics and Plant Breeding, Sam Higginbottom University of Agriculture, Technology and Sciences, Allahabad-211007, Uttar Pradesh, India
*Corresponding Author: Email- lavanya.roopa@gmail.com , pronobjpaul@gmail.com

Received: June 16, 2017; Revised: June 24, 2017; Accepted: June 25, 2017; Published: June 28, 2017

Abstract- An experiment was carried out to evaluate mutant population for variability and divergence in M_3 generation during *kharif* 2009. High magnitude of phenotypic coefficient of variation (PCV), as well as genotypic coefficient of variation (GCV), high heritability coupled with high genetic advance as percent of mean, were recorded for number of clusters per plant, number of primary branches, seed yield per plant and number of pods per plants, suggesting additive gene effects and selection may be effective for these characters for yield improvement. Fifty mutant lines along with a parent, formed eight clusters where cluster VII emerged as the largest one comprising 10 mutant lines and the cluster VIII as the smallest one containing three mutant lines. The maximum inter-cluster distance was observed between clusters I and VIII, suggesting that the selection of parents for hybridization from the diverse clusters to get a broad spectrum of variability. Estimation of genetic divergence among mutant lines in M_3 generation has immense bearing on identifying potential mutant lines, which may produce a broad spectrum of variability with transgressive segregants following hybridization.

Keywords- Greengram, Variability, Genetic divergence, Inter-cluster distance

Citation: Paul Pronob J., *et al*, (2017) Induced Genetic Variability and Divergence through Physical and Chemical Mutagens in M_3 Generation of Greengram (*Vigna radiata* L. Wilczek). International Journal of Genetics, ISSN: 0975- 2862 & E-ISSN: 0975-9158, Volume 9, Issue 6, pp.-284-286.

Academic Editor / Reviewer: Ketan Govindbhai Kanjariya, Shilpa Gupta

Introduction

Greengram (*Vigna radiata* L. Wilczek) occupies a prominent position in meeting the protein requirement of the masses and is grown all year round in multiple and inter cropping system because of its short growth duration and better adaptability. But the production and productivity of this important crop are much lower than cereals and other food legume crops. Improvement of productivity of a genotype/ cultivar can be achieved by creating variation and genetic divergence in a crop population. When genetic variability is essential for effective selection of genotypes, genetic divergence is important for selection of promising parents for hybridization. Induced mutagenesis serves as an important tool for creating genetic variability in crop plants and significant achievements in crop improvement have been made through mutation approach. A mutation breeding programme for an autogamous crop like greengram implies the creation of additional variability in yield component characters and selection of productive mutant lines. Thus, in the present study, an attempt was made to study diversity following mutagenesis with gamma rays alone and its combination with sodium azide in greengram. Multivariate analysis of genetic divergence (D^2) takes into account several quantitative characters simultaneously would be a more dependable method of determining stable differences among the genotypes [1].

Materials and Methods

The experiment was carried out to evaluate M_3 generation at the Field Experimentation Center, Department of Genetics and Plant Breeding, SHUATS, Allahabad. Uniform and healthy seeds of greengram *cv.* KM7-180 were treated with two mutagens, gamma rays, a physical mutagen and sodium azide, a chemical mutagen as well as in combination. For physical treatment, one hundred uniforms, healthy and dry seeds of mungbean genotype KM7-180 with 12 percent moisture were treated with 10, 20, 30, 40 and 50 kR of gamma rays for each dose (Source- Cobalt 60) at National Botanical Research Institute, Lucknow. For combination treatment, genetically pure one hundred uniforms, healthy and uniform seeds were first exposed to gamma rays (Source-Cobalt-60) of 20kR, 30kR and 40kR for each dose at NBRI, Lucknow. The three hundred seeds of three doses were then soaked in distilled water for 6 hours in the laboratory of Department of Genetics and Plant Breeding, SHUATS, Allahabad.

After pre-soaking, the seeds were blotted dry and treated with freshly prepared chemical mutagen solution of sodium azide of concentration of 0.02%. The seeds were kept in the mutagenic solution for 6 hours at room temperature $26^0C \pm 2^0C$ with intermittent shaking for providing uniform treatment to the dipped seeds after the treatment time is over, the seeds were thoroughly washed in running tap water for two hours and then sown in the field. The fifty M_2 family progeny in M_3 generation was laid in randomized block design with three replications during *kharif* 2009. Test plots were managed following the recommended site-specific standard agronomic practices. The plot size was $4m^2$ and spacing between rows and plants was 30 and 10 cm, respectively.

Observations were recorded on five randomly selected plants from each plot for 10 characters *viz*., days to 50% flowering, plant height (cm), number of primary branches $plant^{-1}$, number of clusters $plant^{-1}$, number of pods $plant^{-1}$, days to maturity, pod length, number of seeds pod^{-1}, 100-seed weight (g) and seed yield $plant^{-1}$ (g). Means were computed and data were subjected to Analysis of Variance [2], phenotypic and genotypic coefficients of variation [3] and heritability (broad sense) as the ratio of genotypic to phenotypic variance [4]. Expected genetic advance and genetic advance as percent of mean was calculated following the procedure described in the paper [5]. The mean data was also used for estimating genetic distance among genotypes using D^2 statistics [6] and the genotypes were distributed into clusters according to Tocher's method [7].

Results and Discussion

Analysis of variance for ten quantitative characters in M_3 generation revealed highly significant differences among 50 mutant lines for all the characters except for days to maturity and 100-seed weight, indicating the presence of considerable genetic variability among the mutant lines which can be further utilized for the evolution of desirable mutant lines [Table-1]. These findings are in accordance with the findings of [8,9] who also observed significant variation for yield and its component characters in greengram. Increased variability could be explained as due to mutations of polygene governing the quantitative characters. The presence of a small amount of variation for days to maturity and 100-seed weight might be due to all mutant lines were developed from single parent.

Table-1 *Analysis of variance for different quantitative characters in M_3 generation of greengram*

S. No.	Characters	Mean sum of squares		
		Replications d.f.=2	Treatments d.f.=80	Error d.f.=160
1	Days to 50% flowering	0.37	21.37**	0.74
2	Plant height	28.85	233.76**	0.99
3	Number of primary branches	0.15	1.45	0.28
4	Number of clusters plant^{-1}	0.76	56.46**	0.80
5	Number of pods plant^{-1}	3.24	147.60**	1.27
6	Days to maturity	2.44	20.04**	0.76
7	Pod length	2.25	0.58	0.07
8	Number of seeds pod^{-1}	3.15	5.15**	0.30
9	100 seed weight	0.05	0.45	0.02
10	Seed yield plant^{-1}	1.67	19.75**	0.15

**Significant at 1% level of significance

Mutations affecting quantitative characters can best be inferred by the estimation of genetic parameters in the mutagen treated populations. High magnitude of phenotypic coefficient of variation (PCV) as well as genotypic coefficient of variation (GCV) were recorded for number of clusters plant^{-1} (39.99% and 39.16%), number of primary branches (41.24% and 31.58%), seed yield plant^{-1} (24.34% and 24.07%) and number of pods plant^{-1} (23.72% and 23.42%) and moderate estimates recorded for plant height (15.17% and 15.08%), number of seeds pod^{-1} (12.67% and 11.65%), 100-seed weight (12.29% and 11.70%) whereas low estimates for pod length (6.91% and 5.77%), days to 50% flowering (8.18% and 7.78%) and days to maturity (5.22% and 4.94%) [Table-2].

High heritability was observed for all characters studied except the number of primary branches which showed moderate heritability [Table-2]. In the present study, high heritability coupled with high genetic advance as percent of mean was recorded for the characters- number of clusters plant^{-1} (95.8% and 78.97%), seed yield plant^{-1} (97.8% and 49.05%), number of pods plant^{-1} (97.5% and 47.63%) and plant height (90.7% and 30.86%). High heritability coupled with the high genetic advance for number of pods plant-1, seed yield plant^{-1} in mutant population of greengram was also reported in previous studies [10,11].

Table-2 *Estimates of genetic parameters for different characters in M_3 generation of greengram*

S.No.	Characters	Genetic parameters				
		Coefficient of Variation		Heritability (bs) %	Genetic Advance	GA as % of Mean
		GCV(%)	PCV(%)			
1	Days to 50% flowering	7.78	8.15	90.3	5.13	15.21
2	Plant height	15.08	15.17	98.7	18.03	30.86
3	Number of primary branches	31.58	41.24	58.6	98.7	49.82
4	Number of clusters plant^{-1}	39.16	39.99	95.8	8.69	78.97
5	Number of pods plant^{-1}	23.42	23.72	97.5	14.20	47.63
6	Days to maturity	4.94	5.22	89.5	4.94	9.62
7	Pod length	5.77	6.91	69.7	0.71	9.92
8	Number of seeds pod^{-1}	11.65	12.67	84.6	2.40	22.08
9	100 seeds weight	11.70	12.29	90.6	0.75	22.96
10	Seed yield plant^{-1}	24.07	24.34	97.8	5.21	49.05

It indicates that the heritability is most likely due to additive gene effects and selection may be effective for these characters for yield improvement. Low heritability coupled with high genetic advance as percent of mean for number of primary branches, indicating non-additive gene action [Table-2]. Genetic divergence is the measure of genetic distance among the variants (mutants). Fifty mutant lines and the parent variety were grouped into eight genetically diverse clusters following Non-Hierarchical Eucledian cluster analysis in M_3 generation [Table-3]. Thus, it was inferred that many mutant lines derived from same parental variety showed diversity from the parent and also among themselves. The divergence in mutants may be due to different genes being subjected to changes in different plants at the same time due to induced mutagenesis [1]. Similar findings of genetic divergence in irradiated populations in greengram studying eight yields component characters were also reported [12]. A close examination of clustering pattern revealed that seven lines grouped with parent variety in cluster II. Thus, it appears that these lines, which grouped with parent, do not possess enough genetic divergence from the parent for the ten characters to be classified as micro mutants.

Table-3 *Distribution of induced mutants of M_3 generation into different clusters in greengram*

Cluster No.	Name of mutant lines included	No. of mutant lines
I	KML 103, KMLC 303, KMLC 302, KML 101	4
II	KML 301, KMLC 308, KML 501, CONTROL, KML 207, KML 502, KMLC 301, KMLC 406	8
III	KML 201, KMLC 304, KMLC 201, KML 102	4
IV	KML 205, KMLC 408, KML 401, KMLC 306, KML 208, KMLC 3011, KMLC 401	7
V	KML 403, KMLC 206, KMLC 203, KMLC 202, KMLC 403, KMLC 305, KMLC 407	7
VI	KML 203, KML 503, KMLC 402, KML 402, KML 404, KMLC 204, KMLC 3013, KMLC 405	8
VII	KML 204, KML 209, KML 206, KMLC 3012, KML 202, KMLC 205, KMLC 404, KML 302, KMLC 3010, KMLC 208	10
VIII	KMLC 207, KMLC 307, KMLC 309	3

The remaining 43 mutant lines grouped into seven different clusters away from the cluster II. Thus, these mutant lines not only showed divergence in characters from the parent but also exhibited divergence among them to be classified into different clusters. The clustering pattern of the mutant lines revealed that most clusters included mutant lines derived from different mutagenic treatments. Conversely, lines derived from the same mutagen also often grouped into different clusters. So, the different mutagens did not show any definite pattern of induction of mutations for the characters under study. Cluster VII comprised 10 mutant lines, forming the largest cluster followed by cluster - II and VI with eight mutant lines each and then cluster IV and cluster V with seven lines each, cluster I and III with four mutant lines each while cluster VIII was the smallest one containing only three mutant lines [Table-3]. The pattern of group constellation proved the existence of the significant amount of divergence.

The average inter- and intra-cluster D^2 values among eight clusters were computed and presented in [Table-4] and [Fig-1]. The average intra-cluster distance ranged from 122.14 to 281.00. The maximum intra-cluster distance was recorded for cluster V (281.00) followed by cluster III (248.75) and cluster VIII (247.96) while the minimum intra-cluster distance was recorded for cluster IV (122.14), indicating comparatively homogenous nature of the mutant lines within the cluster.

The inter- cluster D^2 value was maximum between cluster I and VIII (1312.61) followed by cluster I and V (1262.06), cluster III and VIII (1235.71) and V and VI (999.47) [Table-4], suggesting that the mutant lines present in divergent clusters may be used as parents for the cross breeding programme to develop desirable types. The mutant lines with reasonably good yield and showing divergence between them for different characters may be of breeding value for use in hybridization programme probably due to the complementary interaction of divergent genes in parents.

The clusters mean performance revealed wide differences for different characters. The clusters mean for various characters revealed the extent of diversity of groups of mutants from the parental cluster and among themselves [Table-5].

Table-4 *Intra (diagonal) and inter-cluster distance (D^2 values) among different clusters formed in M_3 generation of greengram*

	Cluster I	Cluster II	Cluster III	Cluster IV	Cluster V	Cluster VI	Cluster VII	Cluster VIII
Cluster I	**139.93**	251.59	456.76	548.72	1262.06	533.15	617.33	1312.61
Cluster II		**125.09**	383.32	251.21	701.14	285.03	255.95	660.39
Cluster III			**248.75**	364.36	639.70	871.19	749.23	1235.71
Cluster IV				**122.14**	395.42	501.44	292.96	563.40
Cluster V					**281.00**	999.47	669.13	659.03
Cluster VI						**174.24**	254.94	486.52
Cluster VII							**144.52**	338.46
Cluster VIII								**247.96**

Table-5 *Mean performance of eight different clusters for 10 different characters in M_3 generation of greengram*

Cluster	Days to 50% flowering	Plant height (cm)	Primary branches/ plant	Clusters/ plant	Pods/ plant	Days to maturity	Pod length (cm)	Seeds/ pod	100 Seed weight (g)	Seed Yield/ plant (g)
Cluster I	31.33	42.12	1.99	7.88	27.25	48.08	7.44	10.67	3.41	9.64
Cluster II	33.88	52.77	1.86	10.05	27.74	50.71	7.19	10.95	3.33	10.30
Cluster III	32.33	46.90	2.06	15.97	37.00	48.92	7.28	10.87	3.44	13.49
Cluster IV	32.71	59.10	2.00	11.32	36.10	50.76	6.82	9.85	3.32	11.39
Cluster V	34.95	65.61	2.21	17.30	38.41	53.52	7.20	11.99	3.29	14.80
Cluster VI	32.29	59.47	1.88	9.03	20.93	50.25	7.21	11.00	3.32	7.83
Cluster VII	35.60	62.84	2.15	8.29	27.60	53.53	7.08	10.66	2.98	9.03
Cluster V	35.78	74.72	1.32	9.87	25.64	52.56	7.13	11.74	3.27	10.10

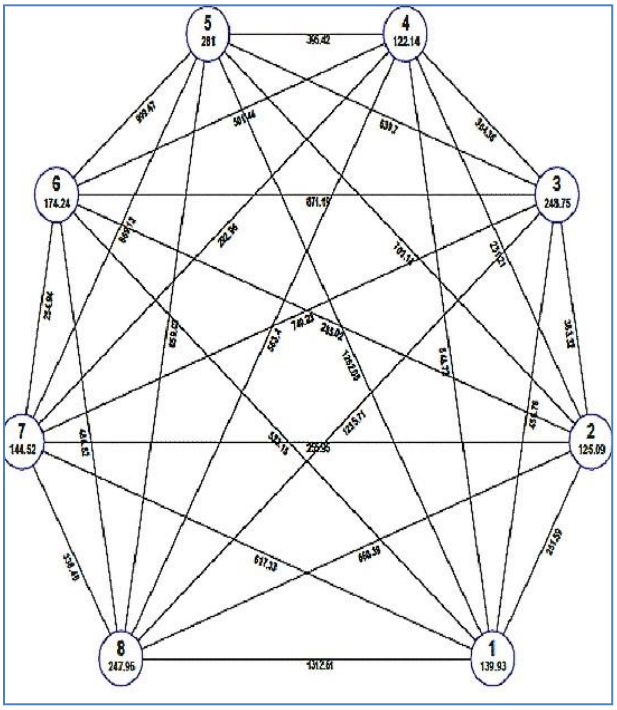

Fig-1 Intra-inter cluster diagram of M_3 population

Cluster I showed low mean value for days to flowering and days to maturity, indicating earliness and high mean for pod length and 100-seed weight. Cluster III recorded high mean value for number of clusters plant^{-1}, number of pods plant^{-1}, pod length, 100-seed weight and seed yield plant^{-1} and low value for days to maturity. Cluster V showed maximum high mean value for the characters-primary branches plant^{-1}, number of clusters plant^{-1}, number of pods plant^{-1}, number of seeds pod^{-1}, and seed yield plant^{-1}. Cluster VII showed high value for the characters- days to flowering, indicating late maturity and primary branches plant^{-1} and Cluster VIII showed highest mean value for plant height. The findings about the mean performance of clusters, including mutant lines for different characters were also reported by Momin and Mishra (2005) [13].

Conclusion

This study revealed the large genetic variation present in M_3 population which validates the effectiveness of the mutagens used in this study. The existence of the large genetic variation can be exploited in further breeding programme in lines development. The potential mutant lines present in different clusters can be used as parents, which may produce a broad spectrum of variability with transgressive segregants following hybridization.

Acknowledgement / Funding: The authors duly acknowledge the Gamma irradiation facility provided for the experimental materials by the CSIR- National Botanical Research Institute (NBRI), Lucknow, Uttar Pradesh 226001, India.

Author Contributions: GRL and PJP conceived this study. UV, YY, GRL helped PJP in field experiments and phenotyping. PJP, GRL and GSB interpreted the data. PJP and GRL wrote the manuscript and GSB, UV and YY contributed to it. All the authors reviewed and approved the final manuscript.
Conflict of interest: The authors declare no conflict of interest.

Ethical approval: This article does not contain any studies with human participants or animals performed by any of the authors.

References

[1] Mishra R.C. (1995) *Env. Eco.,* 13, 508-512.
[2] Fisher R.A. (1936) Statistical Method for Agricultural workers (2nd Ed.) ICAR publications, New Delhi.
[3] Burton G.W. (1952) Quantitative inheritance of grasses. Proc. 6th International Grassland Congress, 1, 277-283.
[4] Lush J.L. (1949) Heritability of qualitative characters in farm animals. Proceedings of 8th Genetic Congress. Heriditas (suppl.). pp 356-357.
[5] Johnson H.W., Robinson H.F. and Comstock R.E. (1955) *Agron J.,* 74, 477 - 483.
[6] Mahalanobis P.C. (1936) On the generalized distance in statistics. Proceedings of National Inst. Sciences, India. 2, 49-55.
[7] Rao C.R. (1952) *J. Rural Statistics Soc.,* 10(13), 159 – 203
[8] Matthew V., Lavanya G.R., Lal S.B. and Babu G.S. (2005) *Mysore J. Agri. Sci.,* 39(4), 462-465.
[9] Idress A., Sadiq M.S., Hanif M., Abbas G. and Haider S. (2006) *J. Agri. Res.,* 44 (3), 45-47.
[10] Ignacimuthu S. and Babu C.R. (1990) *J. Nucl. Agri. Biol.,* 19, 119-123.
[11] Sahu B.C. and Patra G.J. (1997) *Indian J. Agri. Sci.,* 67, 533-535.
[12] Sarma R.N. and Talukdar P. (1991) *J. of Nucl. Agri. and Biol.,* 20(3), 164-168.
[13] Momin B.W. and Misra R.C. (2005) *Andhra Agri. J.,* 52, 362-364.