Available online at www.pelagiaresearchlibrary.com

Pelagia Research Library

Advances in Applied Science Research, 2013, 4(2):134-139

ISSN: 0976-8610
CODEN (USA): AASRFC

Gamma rays and EMS induced flower color and seed mutants in cowpea (*Vigna unguiculata* L. Walp)

Girija M.*, Dhanavel D and Gnanamurthy S

Division of Cytogenetics and Plant Breeding, Department of Botany, Annamalai University, Annamalai Nagar, Tamil Nadu, India

---

ABSTRACT

The present study was carried out to induce flower color and seed mutants in cowpea. Seeds were subjected to different treatment levels of gamma rays and ethyl methane sulphonate (EMS). The treated and untreated plants were self-fertilized for five generations to observe different morphological characters in M6 generation. Several unique and interesting mutants were isolated in this investigation. These independent mutants have different phenotypes from control. Highest mutation frequency for flower color and seed characteristics was induced by 25mM EMS. The most distinct mutants included were different flower color and altered size, shape and coat color of seeds were also observed.

Key words: Cowpea, Gamma rays, EMS, mutation frequency.

INTRODUCTION

Cowpea is an important food crop throughout Sub- Saharan Africa. Its grain and leaves are rich sources of high-quality protein and vitamins which provide an excellent supplement to the lower quality cereal or root and tuber protein consumed in much of this region (16). Cowpea seeds vary markedly in size, shape, and color. They are 2-12mm in long, globular or kidney shaped (8). Seed shape is correlated with that of the pod. Where individual seeds are separated from adjacent ones during development, they become reniform, but as crowding within the pod increases, the seeds become globular and rounded (7).

Mutation induction is an important complementary method of breeding crop species. The utilization of induced mutations for the improvement of crop plants has yields several mutants which have been used directly as new cultivars (12). Mutation breeding is accomplished by chemical or physical treatments followed by selection for heritable changes of specific genotypes, and this method has been used successfully in the genetic improvement of crop plants (19). Mutagenesis has been widely used as a potent method of enhancing variability for crop improvement (27).

The induced mutations are of considerable value for comprehensing evolution and accelerating the process of plant improvement. Induced mutants constitute a valuable resource for research aimed at understanding the process in governing plant development (5). One of the most important breakthroughs in the history of genetics was discovered that mutations can be artificially induced in organisms (29). Present paper reports data on flower color and seed mutants induced by different doses of gamma rays and EMS in cowpea.

MATERIALS AND METHODS

Cowpea variety CO-7 was obtained from Millet Breeding Station, Tamilnadu Agricultural University, Coimbatore. Healthy and uniform seeds of cowpea were dried in sunlight to decrease the moisture content below 13%. Physical mutagen namely, gamma rays and chemical mutagens namely, Ethyl Methane Sulphonate (EMS) were used at various concentrations to induce mutagenesis. The seeds were treated with gamma rays (20, 25 and 30 KR) and EMS (20, 25 and 30mM).

The physical treatments were induced at sugarcane breeding institute (ICAR), Coimbatore. The chemically treated seeds were presoaked in distilled water for 6 hrs to ensure complete hydration of the seeds. The treated seeds were planted in the field according to randomized block design with three replications. Each plot (10×10 feet) consisted of 6 rows with a distance of 45cm between the rows and 20cm between the plants. Each M_1 plant was harvested separately and the seeds were sown in the next season in plant progeny rows, to raise M_2 generation in a randomized block design with three replications followed by M_3, M_4, M_5 and M_6 generation. Mutants affecting flower color were detected by observing the plants from budding to flowering stages in all generation. Mutants affecting color, size and shape of seeds were determined by opening six to eight pods from each M_5 plants.

RESULTS AND DISCUSSION

Mutations are phenotypically classified into two groups (10); **macro mutations:** These are easily detectable in individual plants, phenotypically visible and morphologically distinct and they are qualitatively inherited genetic changes, and occur in major genes or oligogenes; and **micro mutations:** These result in a small effect that, in general, can be detected only by help of statistical methods and quantitatively inherited genetic changes, and occur in minor genes or polygenes.

In the present investigation, some of the morphological (viable) mutants were observed in M6 generation with different dose/concentration of mutagens. Increase in the dose/concentration of gamma rays and EMS treatments increase in the number of viable mutants were realized in the present study. 25mM of EMS treatment was produced by more number of viable mutants than compared to other mutagens. Flower mutants such as early flowering, white color flower, blue color and pink color flower were also observed in all mutagenic treatments (Fig 1). Similar results were observed in chick pea (13); chrysanthemum (17); Artemisia (24); sesame (6); chickpea (3); sesame (4); French bean (18).

The macro mutations of seed color were induced in the M_6 generation. Such seed mutations for different seed color were reported in pulse crops, for example, buff and black in Arhar (1), Yellow in Soybean (28) and golden yellow (26) in mung bean.

The brown color, white color and bold size seed mutant was observed at 25mM of EMS and 25KR of gamma rays respectively (Fig 2). The maximum number of seed mutant was observed at 25mM of EMS treatment. Similar reports of seed color were isolated by sesame (22), (23) and (9); Chick pea (13), Mustard (30), French bean (18).

In plants, EMS usually causes point mutations, but loss of a chromosome segment or deletion can also occur (21). Therefore, EMS has the potential of altering loci of particular interest with inducing a great number of closely linked mutations. The use of radiation and chemical mutagens for inducing variation is well established. Although desired variation is often lacking, mutagens can be used to induce mutations and thereby generate genetic variation. Morphological mutations affecting different plant parts can be of enormous practical utility and many of them have been released directly as crop varieties (25).

Gamma rays are belonging to ionizing radiation and interact with atoms or molecules to produce free radicals in cells. These radicals can damage or modify important components of plant cells and affect different morphology, anatomy, biochemistry and physiological characters in plants, mainly depending on the level of irradiation. These effects could changes in plant, the cellular structure and metabolism, like dilation of thylakoid membranes, alteration in photosynthesis, modulation of the antioxidative and accumulation of phenolic compounds (15 and 31).

Among the chemical mutagen, EMS is widely used as efficient one since they form adducts with nucleotides, causing them to mispairing with their complementary bases, thus introducing base changes after replication. Many of these chemicals have clastogenic (chromosome damaging) effects on plants via reactive oxygen-derived radicals (33). These effects can occur both spontaneously and artificially following induction by mutagens.

The mutation frequency is one of the most dependable indexes for evaluating the genetic effects of mutagenic treatments (14 and 32). It was estimated by dividing the number of segregating M_1 plant progeny with the determinate character by the total number of progeny. For the material grown in bulk, the mutant frequency was estimated by dividing the total number of mutants confirmed by the total number of M_2 plants in the bulk population studied (10). Most of the mutants bearing multi mutational events thus may be lethal in the first generation, affecting the frequency occurrence of multi mutations in M_2 and future generations (32).

In the present results, the frequency of M_6 progenies segregating for flower color and seed mutants is presented in Table 1 and 2. The highest mutation frequency was recorded in 25 mM EMS (9.77%) treatment followed by gamma rays treatment at 25KR (8.48%). The mutation frequency was independent of genotype and mutagens. In general, the mutation frequency was high in EMS than compared to gamma rays treatment. The results presented here show that gamma irradiation and EMS of bean seeds may be efficiently used to induce changes in flower color and seed characteristics, supporting previous results (11, 20, 2, and 12).

Table1. Frequency of M_2 progenies segregating for flower color and seed mutants

Mutagen		No. of M_5 seeds sown	No. of M_6 plants observed	No. of M_6 plants segregating for flower color and seed mutants	% Frequency
Gamma rays (KR)	**20**	200	172	11	6.39
	25	200	165	14	8.48
	30	200	153	6	3.92
EMS (mM)	**20**	200	180	13	7.22
	25	200	174	17	9.77
	30	200	160	9	5.62

Table 2. Phenotype of flower color and seed mutants in M_6 progenies

Mutagen		M_6 progenies segregating for flower color and seed mutants	Mutant phenotype	No. of plants
Gamma rays (KR)	20	11	Purple color flower	4
			Pink color flower	4
			Dark Brown color seed	3
	25	14	Light blue color flower	3
			White color flower	2
			Brown color seed	2
			White color seed	3
			Bold size seed	4
	30	6	Dark blue color flower	3
			Light pink color flower	2
			Wrinkled seed	1
EMS (mM)	20	13	Purple color flower	4
			Light blue color flower	3
			Wrinkled seed	3
			Light brown color seed	3
	25	17	Dark blue color flower	3
			White color flower	3
			Dark brown color seed	4
			Bold size seed	4
			White color seed	3
	30	9	Pink color flower	2
			Light blue color flower	4
			Brown color seed	3

Fig - 1. Flower color mutants in cowpea

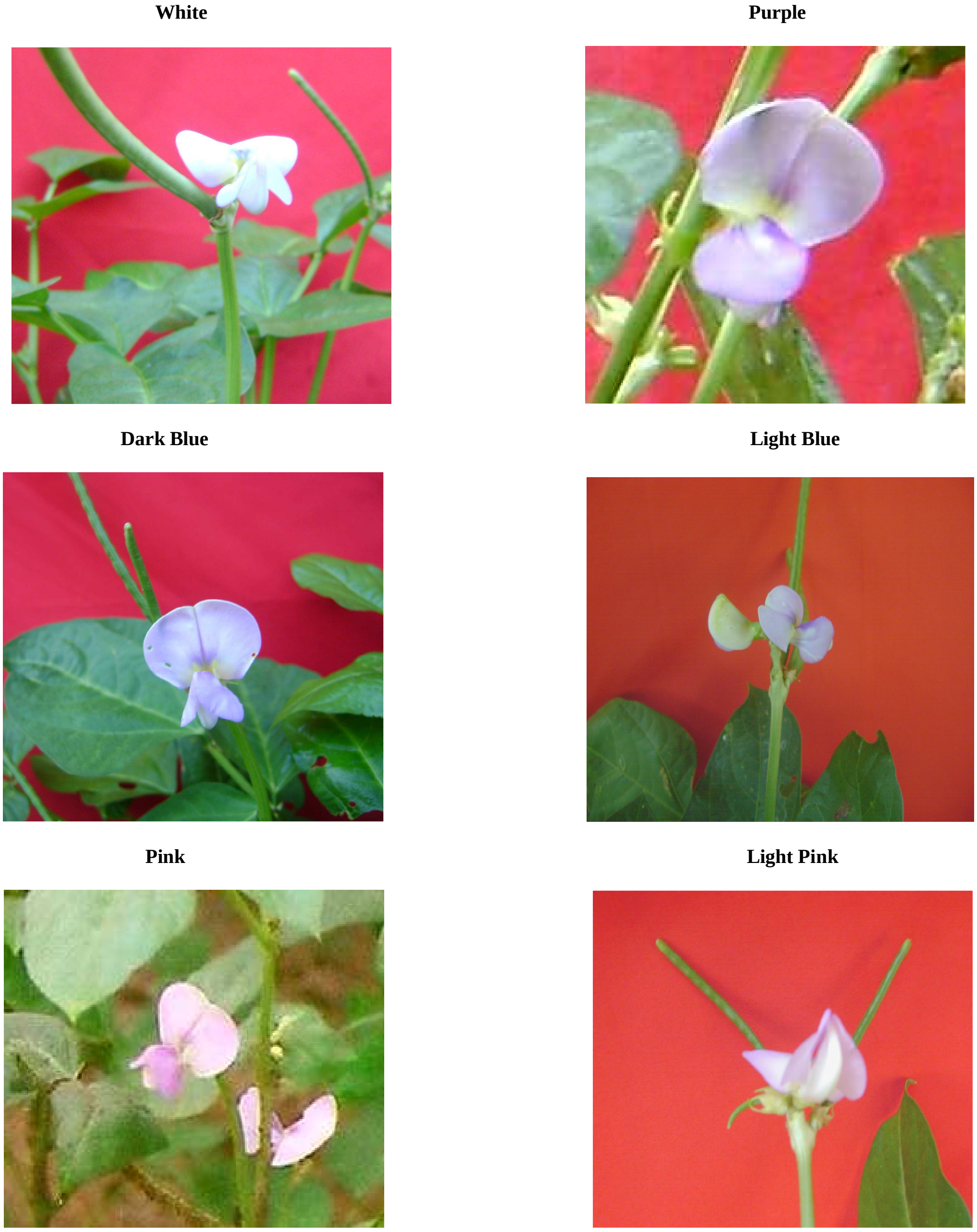

Fig - 2. Seed size, shape and coat color mutants in cowpea

CONCLUSION

The improved variety of cowpea variety CO-7 responded more and more number of viable and economic mutants for higher productivity in 25mM of EMS than the other mutagenic treatments in all generations. The morphological mutant characters studied can be utilized for identification and characterization of cowpea genotypes. The present investigation revealed that the isolation of flower color and altered size, shape and coat color of seeds is possible in 25mM of EMS. It provides greater chances for the selection of desired characters.

Acknowledgement
The authors are thankful to Head of the Department of Botany, and authorities of Annamalai University for providing the necessary facilities to carryout this work.

REFERENCES

[1] Alikhain, W.M. and R. Veeraswamy, *Rad. Bot*., **1974**, 14: 237-242.
[2] Al-Rubeai, M.A.F. *Rev. Brasil. Genet*., **1982,** 5: 503-515.
[3] Atta, B.M., M. Ahsan ul Haq, T.M. Sadiq, M. Sadiq, Mahmud ul Hassan and H. Syed, *Int. Chickpea and Pigeon pea Newsl*., **2003,** 10: 6-7.
[4] Chowdhury, S., A. K. Datta and S. Maity, *Indian J. Sci and Tech*., **2009,** 2(5): 58-61.
[5] Cove, D.J., *Plant Cell*, **1993**, 3: 303-308.
[6] Diouf, M., S. Boureima, T. Diop and M.Cagirgan, *Turk. J. Field Crops.*, **2010,** 15(1): 99-105.
[7] Fery, R.L. Genetics of vigna, p311-394; in Horticultural Reviews (Ed. Janick. J.). AVI Publishing, USA, **1980.**
[8] Fox, F.W. and M.E.N. Young. Food from the Veld. Delta Books, Johannesburg South Africa, **1982,** Pp197.
[9] Ganesan, J., Induced mutations for sesame improvement. IAEA Res. Rept., **1996,** II, 33.
[10] Gaul, H., *Rad. Bot*., **1964,** 4: 155-232.
[11] Gotoh, K., *Gamma field symp*., **1968,** 3: 76-89.
[12] Gottschalk, W. and G. Wolf, Induced mutations in plant breeding. Monographs on theoretical and applied genetics, Berlin, Vol 7, Springer Verlag., **1983,** pp 238.
[13] Haware, M.P., Y.L. Nene, R.P.S. Pundir and R.J. Narayana., *Field Crops Res*., **1992,** 30: 147-154.
[14] Kharkwal, M.C., *Indian J. Gen. Pl. Breed*., **1999,** 59(4): 451-464.
[15] Kim, J.H., M.H. Baek, B.Y. Chung, S.G. Wi and J.S. Kim., *J. Plant Biol*., **2004,** 47: 314-321.
[16] Kitch, L.W., Boukar, O., Endondo, C. & Murdock, L.L., *Expl Agric*., **1998,** 34: 475–486.
[17] Lamseejan, S., P. Jompuk1, A. Wongpiyasatid, S. Deeseepan and P. Kwanthammachart, *J. Nat. Sci*., **2000,** 34: 417 – 422.
[18] Mahamune, S.E. and V.S. Kothekar, *Recent Res. Sci & Tech.*, **2011,** 3(5): 33-35.
[19] Mick, A., M. Maluszynski and B. Donin, *Mutat. Breed. Rev*., **1985,** 3: 1-92.
[20] Moh, C.C. *Euphytica.*, **1971,** 20: 119-125.
[21] Okazaki, A.T. **1991,** PASJ, 43: 75.
[22] Prabhakar, L.V., M.Sc. (Ag.) Thesis, Tamil Nadu Agricultural University, Coimbatore, India, **1985.**
[23] Pugalendi, N., M.Sc. (Ag.) Thesis, Annamalai University, Tamil Nadu, **1992.**
[24] Rekha, K. and A. Langer, *Genet. Resour. Crop. Evol*, **2007,** 54: 437–443.
[25] Shah, T. M, B. M. Atta, S. S. Alam, H. Ali, M. A. Haq and M. Hassan. *Pak. J. Bot.*, **2010,** 42(5): 3533-3545.
[26] Singh, V.P., R.D.S. Yadav, R.M. Singh, B.D. Singh and R.B. Singh, *Legume Res.*, **1982,** 5(2): 111-114.
[27] Subuthi, P.K., B.K. Mohapatra and S.K. Sinha, *Indian J. Genet.*, **1991,** 5 (1): 101-111.
[28] Tyagi, B.S. and P.K. Gupta, *Ind. J. Genet & Plant Breed*., **1991,** 51: 326–331.
[29] Van Harten, A.M., Mutation breeding, Theory and Practical Applications. Cambridge University Press, Cambridge, United Kingdom. **1998,** pp 127-140.
[30] Verma, V.D. and B. Raj, *Indian J. Agric. Sci*., **1980,** 50: 545-548.
[31] Wi, S.G., B.Y. Chung, J.H. Kim, M.H. Baek, D.H. Yang, J.W. Lee and J.S. Kim, *J. Plant Biol.*, **2005,** 48(2): 195-200.
[32] Waghmare, V.N. and R.B. Mehra. *Indian J. Genet*., **2001,** 61(1): 53-56.
[33] Yuan, H.Y. and Z.I. Zhang, *Acta Bot. Sin*., **1993,** 35: 20-26.