Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2019; 8(6): 1246-1249
Received: 06-09-2019
Accepted: 10-10-2019

Rakhi Kumari
Department of Horticulture, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, Uttar Pradesh, India

Dr. AK Pal
Department of Horticulture, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, Uttar Pradesh, India

Sunil Singh
Plant Protection Officer (WS) Directorate of Plant Protection Quarantine & Storage, NH-IV, Faridabad, Haryana, India

Corresponding Author:
Rakhi Kumari
Department of Horticulture, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, Uttar Pradesh, India

Induced chlorophyll mutation in cowpea (*Vigna unguiculata* L. Walp.)

Rakhi Kumari, Dr. AK Pal and Sunil Singh

Abstract
Seeds of cowpea variety Kashi Kanchan were treated with gamma rays, EMS and combination treatment of gamma rays with EMS to obtain the spectrum and frequency of chlorophyll mutations in M2 generation in the present investigation. The individual treatment of Gamma rays was found to be more efficient than EMS to induce chlorophyll mutants. A progressive increase in mutation frequency of chlorophyll mutations was observed with increasing doses. Four different types of chlorophyll mutants, such as, albino, xantha, chlorina and viridis were induced with effect of mutagens. The highest frequency of chlorophyll mutations (2.87) was reported in the Gamma rays. There was a dose dependent increase in the spectrum and frequency of chlorophyll mutations whether mutagens were employed singly or in combination up to a certain dose / concentration level beyond which it decreased at higher frequency.

Keywords: Cowpea, gamma rays, EMS, chlorophyll mutants

Introduction
Cowpea (*Vigna unguiculata*) is an essential food legume and an important component of cropping systems in the drier regions of the tropics covering parts of Asia and Oceania, the Middle East, Southern Europe, Africa, southern USA, and Central and South America (Singh *et al*., 2002) [42]. Its vegetable utilization is fully exploited. In India Cowpea is cultivated in Rajasthan, Gujarat, Punjab, Maharashtra and Tamil Nadu. In India, cowpea is developed in a territory of 3.9 million hectares with a generation of 2.21 million tons. Mutation breeding is accomplished by physical or chemical treatments followed by selection for heritable changes of specific genotypes, and this method has been used successfully in the genetic improvement of crop plants (Mick *et al*., 1985). Mutagenesis has been widely used as a potent method of enhancing variability for crop improvement (Subuthi *et al*., 1991) [47]. Gene mutations influencing the green coloration of photo synthetically active parts are among the most common spontaneous or induced alterations arising in higher plants. The chlorophyll mutation frequency is an indicator to predict the frequency of factor mutations and thus an index for evaluation of genetic effects of mutagens (Gustafsson, 1951; D'Amato *et al*., 1962; Gichner and Veleminsky, 1965) [14, 6, 11]. Nuclear gene mutations or extra chromosomal mutations might result in chlorophyll deficient mutations (Levine, 1972; Walles, 1973) [52]. Chlorophyll mutations are considered as the most dependable indices for evaluating the efficiency of different mutagens in inducing the genetic variability for crop improvement and are also used as genetic markers in basic and applied research. The occurrence of chlorophyll mutations after treatments with physical and chemical mutagens have been reported in several crops (Swaminathan *et al*., 1962; Prasad and Das, 1980; Sharma and Sharma, 1981; Reddy and Gupta, 1989; Kharkwal, 1998; Mitra and Bhowmik, 1999) [50, 30, 39, 33, 18, 22]. In the present study an attempt was undertaken to understand the comparative response of physical and chemical mutagens singly and in combination on cowpea to determine the mutagen and treatment causing genotypic variations in response to induction of chlorophyll mutations in terms of frequency of induced chlorophyll mutations in M2 generation.

Materials and Methods
The dry, dormant and uniform seeds of the cowpea (*Vigna unguiculata* (L) Walp.) variety Kashi Kanchan were treated with gamma rays, EMS and their combination treatments were used in the present investigation. Gamma irradiation was used in 150 dry, uniform sized and healthy seeds of Cowpea in the present investigation as a physical mutagen. Different doses of gamma-rays were used for irradiation of seeds of cowpea of two varieties i.e. 10, 20, 30, 40 and 50 kR doses. The gamma irradiation was carried out at National botanical Research institute (NBRI), Lucknow, India. Similarly, in case of EMS treatment individually and in

combination with gamma rays. 150 healthy seeds each were pre-soaked in distilled water for 6 hours at room temperature.

Subsequently, the pre-soaked seeds were treated with 0.01, 0.02, 0.03, 0.04 and 0.05 M EMS concentrations separately for 6 hrs. The EMS treated seeds were washed thoroughly in running tap water to eliminate the residual effect of chemical. For combination treatments 150 seeds each were first irradiated with 10, 20, 30, 40 and 50 kR doses of gamma rays and then followed by EMS treatment of single concentration (0.02 M). After the completion of the treatment the treated seeds were sown immediately in the field along with their respective controls to raise the M1 generation in a randomized block design with three replications. All the recommended cultural measures namely, irrigation, weeding and plant production methods were carried out during the growth period of the crop. Pods from individual plant in each treatment of cowpea was harvested separately and stored to raise the M_2 generation. The M2 seedlings were screened from 6th to 15th day after germination to record the various chlorophyll mutants periodically. The classification and identification of the chlorophyll mutants was done based on the nomenclature adopted by Gustafson (1940) [12]. The mutation frequency was estimated on M2 seedling basis.

Results and Discussions

Mutations in leaf colour are most frequently observed mutation in both spontaneous and induced mutant populations, and generally used as an indicator of mutagenic effects and efficiency of various mutagens. Chlorophyll development seems to be controlled by many genes located on several chromosomes, which could be adjacent to centromere and proximal segment of chromosomes (Swaminathan, 1964) [49]. Chlorophyll mutations provide one of the most dependable indices for the evaluation of genetic effects of mutagenic treatments which have been reported in many legume crops by several workers including Gautam *et al*. (1992) [10]. The frequency of chlorophyll mutation in M2 generation is the most effective and reliable index for evaluating the genetic effects of mutagenic treatments (Mesken and Vander Veen, 1968) [28]. On the seedling basis of M2 generation, chlorophyll mutations frequency increase with increase in dose/ concentration upto a certain concentration/dose level beyond which it decreased. Albino-The seedlings on germination were white in colour due to absence of all pigment. They were least frequent and did not survive. Xantha-The leaves turned yellow in colour due to the absence of xanthophylls. Viridis-The seedlings were light green in colour and found to be lethal or semi lethal. Chlorina-The seedlings were yellowish green in colour. Among, four different types of chlorophyll mutations i.e. Albino, xantha, chlorina and viridis, viridis was recorded at highest frequency followed by xantha, chlorina and albino which were observed at lower frequencies. One or more mutants were observed at all mutagenic treatments. Chlorophyll mutations were found in almost all the mutagenic treatments. High frequency of chlorophyll mutations were found in the gamma rays, EMS and combination treatment of gamma rays with EMS. The highest frequency of chlorophyll mutations (2.87%) was reported in the 40 kR of gamma rays, whiles the lowest (0.40) frequency of chlorophyll mutations was found in 0.01 M of EMS. Gamma ray was found to be more effective for inducing chlorophyll mutations in comparison to EMS and their combinations (Table-1).

Among all the mutagens tested Gamma rays induced maximum frequency of chlorophyll mutations indicating their greater effectiveness. Increase frequency of chlorophyll mutations with an increase in concentration/dose of the mutagens in cowpea except light decreasing frequency at higher concentration/dose were reported by Srivastava *et al.* (1973) [46], Nadarajan *et al.* (1982) [23, 24], Toker and Cagirgan (2004) [51], Yamaguchi *et al*. (2009) [53] & Pawar *et al*. (2010) [27] in different crops including chickpea. It is also observed that with increase in dose/concentrations beyond a certain level, the strong mutagens become more toxic than the higher doses of relatively weaker mutagens (Singh and Bandhu, 2003) [43]. Increase in the frequency of chlorophyll mutations with an increase in the concentration/dose of the mutagenic treatments was reported by John (1999) [15], Palaniswamy *et al.*, (1978) [25], Pandey and Pawar (1998) [26], Satpute (1996) [38], Ritakumari (1996) [35], Singh *et al.,* (1999) [41], Rybinsky (2003) [36], Arvindkumar *et al.*, (2007) [2], Barshile *et al.*, (2006) [3], Rangaiah *et al.* (2004) [31], Sagade (2008) [37], Mensah and Obadoni (2007) [20], Dhanavel *et al* (2008) [7]. However, Mehraj-ud-din *el al,* (1999) [19], Sharma *et al.*, (2006) [40], Solanki and Sharma (2001), Swaminathan (1961) [48], Gaul (1964) [8], Raveendran and Jayabalan (1997) [32], Chemma and Atta (2003) [5], Barshile (2006) [3], reported a decrease in the frequency of chlorophyll mutations at higher concentrations. Khan and Tyagi S, (2010) [17] reported gamma rays were found more effective than EMS and their combined treatments to induce chlorophyll mutation in the cultivars Pusa-16 and PK-1042 of Soy bean. Development of chlorophyll mutations in large number of crops have been recognized to different causes *viz*., impaired chlorophyll biosynthesis, further degradation of chlorophyll and bleaching due to deficiency of carotenoids and may be related to their preferential action on chlorophyll development genes (Reddy *et al.,* 1993) [34]. The decrease in chlorophyll mutation frequency as observed at the highest doses of mutagens may be attributed to chromosomal aberrations or saturation in the mutational events which may result in the elimination of the mutant cells at the highest doses/concentrations during growth. In the present studies, chlorophyll mutation of albino, xantha, chlorina and viridis observed in all mutagenic treatments. But predominant occurrence of viridis mutant was observed in all mutagenic treatments. The reason for the appearance of greater number of viridis may be attributed to involvement of polygenes in the chlorophyll formation (Gaul, 1964) [8]. Ambarkar (2005) [1] reported dominance of viridis among chlorophyll mutant types in chickpea. Sjodin (1962) [44] reported that viridis was most common in *Vicia faba* and xantha was the next most common mutant type, whereas albina was very rare mutant as in most leguminous. The viridis types were predominant than albina, xantha and chlorina types, irrespective of the cultivar in rice bean as reported by Prakash and Shambulingappa (1999) [28], Prakash and Khanure (2000) [29]. Higher efficiency of EMS in inducing chlorophyll mutations and highest frequency of viridis mutant was observed by Chary and Bhalla (1988) [4] in pigeon pea and by Khan and Tyagi (2009) [16] in Soybean.

Table 1: Effect of different combination of gamma rays and EMS on frequency and spectrum of chlorophyll mutations in M2 generation of Kashi Kanchan

Treatments (Dose/Conc.)		Number of plants observed	Spectrum of Chlorophyll mutants				Total no. of chlorophyll mutants	% of mutation frequency
			Albino	Xantha	Chlorina	Viridis		
Gamma rays	10 kR	485	-	-	1	2	3	0.61
	20 kR	446	1	2	1	2	6	1.35
	30 kR	408	2	3	1	2	8	1.96
	40 kR	383	2	2	3	4	11	2.87
	50 kR	368	1	3	2	3	9	2.44
EMS	0.01 M	496	-	1	-	1	2	0.40
	0.02 M	458	1	-	1	2	4	0.87
	0.03 M	417	2	2	1	1	6	1.44
	0.04 M	398	2	2	2	3	9	2.26
	0.05 M	376	3	1	1	2	7	1.86
Combined Treatments (Gamma rays + EMS)	10 kR + 0.02 M	464	-	1	-	1	2	0.43
	20 kR + 0.02 M	421	1	-	1	1	3	0.71
	30 kR + 0.02 M	382	1	2	1	1	5	1.31
	40 kR + 0.02 M	357	2	1	2	2	7	1.96
	50 kR + 0.02 M	332	2	1	1	2	6	1.81

Conclusion

It concluded that viable chlorophyll mutations, i.e., viridis were produced more at higher doses/concentrations of mutagens up to certain level of doses/concentrations after which frequency was decreases. Similarly, lethal/semi-lethal mutants, namely, albino, xantha and chlorina were also increases as per doses/concentration dependently except at higher doses/concentration where it slightly decreases. The chlorophyll mutations do not have any economic value due to their lethal scenery. Such a study could be useful in identifying the threshold dose of a mutagen that would increase the genetic variability and number of economically useful mutants in the segregating generations.

References

1. Ambarkar AS, Harer PN, Kulkarni RV. Radio sensitivity and visible mutations in chickpea (*Cicer arietinum* L.). Adv. Plant Sci. 2005; 18(2):559-563.
2. Arvind Kumar, Mishra MN, Kharkwal MC. Induced mutagenesis in blackgram [*Vigna mungo* (L.) Hepper], Indian J Genet. 2007; 67(l):41-46.
3. Barshile JD, Auti SG, Dalve SC, Apparao BJ. Mutagenic sensitivity studies in chickpea employing SA, EMS and gamma rays, Indian J Pulses Res. 2006; 19(1):43-46.
4. Chary NS, JK Bhalla. Mutagenic effectiveness and efficiency of gamma rays and EMS on pigeon pea (*Cajanus cajana* L. Millsp.) J Cytol. & Genet. 1988; 23:190- 193.
5. Cheema AA, Atta BM. Radiosensitivity studies in Basmati Rice, Pak. J Bot. 2003; 35:197-207.
6. D'Amato F, Scarascia GT, Monti LM, Bozzini A. Types and frequencies of chlorophyll mutations in durum wheat induced by radiation and chemicals. Rad. Bot. 1962; 2:217-239.
7. Dhanavel D, Pavadai P, Mullainathan L, Mohana D, Raju G, Girija M *et al*. Effectiveness and Efficiency of chemical mutagens in cowpea (*Vigna unguiculata* L. Walp.), Afri. J. Biotech. 2008; 7:4116-4117.
8. Gaul H. Mutation in plant breeding. Rad. Bot. 1964; 4:155-232.
9. Gautam AS, Mittal RK. Induced mutations in Black gram (Vigna mungo (L.) Hepper). Crop Res. 1998; 16(3):344-348.
10. Gautam AS, Sood KC, Richarria AK. Mutagenic effectiveness and efficiency of gamma rays, ethylmethanesulphonate and their synergistic effects in blackgram (*Vigna mungo* L.). Cytologia. 1992; 57:85-89.
11. Gichner, Veleminsky J. Induction of chlorophyll chimeras by X-rays and ethyl-methane sulphonate (EMS) in different heterozygous strains of Arabidopsis thaliana. Proc. Symp. Induction of Mutations and the Mutation Process Praha, 1963, 27-29. 1965, 54-56.
12. Gustafsson A. The mutation system of the chlorophyll apparatus. Lund Univ. Arasks. N.P. Adv. 1940; 36:1-40.
13. Gustafsson A. Mutations in agricultural plants. Hereditas. 1947; 24:3-93.
14. Gustafsson A. Induction of changes in genes and chromosomes II Mutations, environment and evolution Cold Spring. Harbour Symp Quant Biol. 1951; 16:263-281.
15. John SA. Mutation frequency and chlorophyll mutations in parents and hybrids of cowpea following gamma irradiation. Indian J. Genet. 1999; 59(3):357-361.
16. Khan MH, Tyagi SD. Studies on chlorophyll mutations in Soybean, *Glycine max* (L.) Merrill. Front. Agric. China. 2009; 3(3):253-258.
17. Khan MH, Tyagi SD. Induced morphological mutants in soybean [*Glycine max* (L.) Merrill]. Front. Agric. China. 2010b; 4(2):175-180.
18. Kharkwal MC. Induced mutations in chickpea (*Cicer arietinum* L.) II. Frequency and spectrum of chlorophyll mutations. Ind. J Genet. 1998; 58:465-474.
19. Mehraj-ud-din Bahar, Siddiqui A, Samiullah Khan, Mujeeb-Ur-Rehman. Induced mutations in mungbean (*Vigna radiata* (L.) Wilczek): Efficiency and effectiveness of chemical mutagens. Legume Research. 1999; 22(4):245-248.
20. Mensah JK, Obadoni B. Effect of sodium azide on yield parameters of groundnut (*Arachis hypogaea* L.), Afri. J Biotech. 2007; 6(6):668-671.
21. Mesken M, Van der Veen JH. The problem of induced sterility. A comparison between EMS and X-rays in Arabidopsis thaliana. Euphytica. 1968; 17:363-370.
22. Mitra PK, Bhowmik G. Studies on the frequency and segregation of induced chlorophyll mutations in *Nigella sativa* L. Adv. Plant Sci. 1999; 12:125-129.
23. Nadarajan N, Ramalingam RS. Mutagenic effectiveness and efficiency in *Cajanus cajan* (L.) Millsp. Madras agric. J. 1982; 69(2):71-75.

24. Nadarajan N, Sathupati R, Shivaswamy N. Investigation on induced macro mutations in *Cajanus cajan*. Madras Agric. J. 1982; 69:713-717.
25. Palanisamy GA, Nagarajan R, Loganathan NS. A note on chlorophyll mutation in gamma irradiated cowpea [*Vigna Sinensis* (L.) Savi] C-152. Madras Agric. J. 1978; 65(4):262-263.
26. Pandey RN, Pawar SE. Induced mutations for improving plant type, quality and yield in cowpea. (in) DAE-Symposium on Induced mutations and molecular techniques in improving crop productivity and quality, Chennai, 1998, 2.
27. Pawar N, Pai S, Nimbalkar M, Kolar F, Dixit G. Induction of chlorophyll mutants in *Zingiber officinale* Roscoe by gamma rays and EMS. Emir. J Food Agric. 2010; 22(5):406-411.
28. Prakash BG, Shambulingappa KG. Estimation of chlorophyll and viable mutation under M2 generation in rice bean (*Vigna umbellate* L. Thumb). Karnataka J. Agr. Sci. 1999; 12(1-4):58.
29. Prakash BG, Khanure SK. Isolation of mutants and their frequencies under M2 generation in rice bean (*Vignaumbellata* L. Thumb). Madras Agri. J. 2000; 86(10/12):568-572.
30. Prasad AB, Das AK. Studies on induced chlorophyll mutations in Lathyrussativus L. Cytologia. 1980; 45:335-341.
31. Rangaiah S, Manjunath A, Puttaramanaik. Mutagenic effects of gamma rays on the frequency and spectrum of chlorophyll and viable macro mutations on chilli {*Capsicum annum* L.). J Nuclear Agric. Biol. 2004; 33(2):119-122.
32. Raveendran P, Jayabalan J. Induced Chlorophyll mutation studies in cowpea [*Vigna unguiculata* (L) Walp]. J Indian Bot. Soc. 1997; 76:197-199.
33. Reddy VRK, Gupta PK. Induced mutations in Triticale. Frequency and spectrum of chlorophyll mutations. Indian J Genet. 1989; 49: 183-190.
34. Reddy VRK, Thresiamma PJ, Edwin R. A comparative study of microsperma and macrosperma lentils. I. Chlorophyll mutations. J Indian Bot. Soc. 1993; 72:25-28.
35. Rita K. Effectiveness and Efficiency of physical chemical and physico-chemical mutagens in M2 generation of *Vicia faba* L. var VH82- 1. J Nuclear Agric. Biol. 1996; 25(3):172-175.
36. Rybinski Wojciech. Mutagenesis as a tool for improvement of traits in grass pea (*Lathyrus sativas* L.) Lathyrus lathyrism Newsletter. 2003; 3:27-31.
37. Sagade AB. Genetic improvement of urdbean through mutation breeding, Ph. D. Thesis, University of Pune, 2008.
38. Satpute RA, Kothekar VS. Mutagenic efficiency and effectiveness in safflower. J Nuclear Agric. Biol. 1996; 25(4):230-234.
39. Sharma SK, Sharma B. Induced chlorophyll mutation in Lentil. Indian J Genet. 1981; 41:328-333.
40. Sharma AK, Singh VP, Singh RM. Efficiency and effectiveness of the gamma rays, EMS and their combinations in urdbean; Indian J Pulses Res. 2006; 19(1):111-112.
41. Singh VP, Man Singh, Pal JP. Mutagenic effects of gamma rays and EMS on frequency and spectrum of chlorophyll and macro mutations in urdbean (*Vigna mungo* L. Hepper). Indian J Genetics. 1999; 59(2):203-210.
42. Singh JV, Arora RN. Genetics and Breeding. In: Kumar D and Singh NB (eds.) Guar in India. Jodhpur: Indian Arid Legume Society, 2002, 817233-310-2.
43. Singh PP, Bandhu V. Induced macro mutations, mutagenic effectiveness and efficiency in urdbean. In: National Symposium on Pulses for Crop Diversification and Natural resources Management, at IIPR, Kanpur, (Abstract), 2003, 20-22, 94.
44. Sjodin J. Some observations in X1 and X2 of *Vicia faba* L. after treatment with different mutagens. Hereditas. 1962; 48:565-586.
45. Solanki IS, Phogat DS, Waldia RS. Frequency and spectrum of morphological mutations and effectiveness and efficiency of chemical mutagens in Macrosperma lentil. National J Plant Improvement. 2004; 6(1):22-25.
46. Srivastava LS, Chand H, Kumar S. Dose response studies on EMS and MMS treated gram. Sci. Cul. 1973; 39(8):345-347.
47. Subuthi PK, Mohapatra BK, Sinha SK. Use of pollen traits for early detection of induced micro mutations in wheat. Ind. J Genet. 1991; 5(1):101-111.
48. Swaminathan MS. Effect of diplontic selection on the frequency and spectrum of mutations induced in polyploids following seed irradiation.//i: Effects of ionizing Radiations on seeds, lAEA. Vienna, 1961, 279-288.
49. Swaminathan MS. A comparison of mutation induction in diploids and polyploids. In: The use of induced mutations in plant breeding. Rad. Mut. Organ. FAO/IAEA Vienna, 1964, 619-641.
50. Swaminathan MS, Chopra VL, Bhaskaran S. Chromosome aberrations frequency and spectrum of mutations induced by EMS in barley. Ind. J Genet. 1962; 22:192-207.
51. Toker C, Cagiranan MI. Spectrum and frequency of induced mutations in chickpea ICPN. 2004; 11:8-10.
52. Walles B. Plastid structures and mutations. In: Structure and Function of Chloroplasts, Gibba M (ed), Springer Verlag New York, 1973, 51.
53. Yamaguchi H, Hase Y, Tanaka A, Shikazono N, Degi K, Shimizo A *et al*. Mutagenic effects of ion beam irradiation on rice. Breed. Sci. 2009; 59:169-177.