Himachal Journal of Agricultural Research 47(2): 156-162 (2021)

Studies on induced chlorophyll mutants in black gram (*Vigna mungo* L. Hepper)

Navdeep Kaur*, R.K. Mittal, V.K. Sood and Alka Soharu

Department of Genetics and Plant Breeding

CSK Himachal Pradesh Krishi Vishvavidyalaya, 176 062, India.

*Corresponding author: navib3455@gmail.com

Manuscript received: 13.09.2021; Accepted: 28.09.2021

Abstract

The investigation was undertaken to score and identify the chlorophyll mutants in M_2 generation of two genotypes of black gram *viz*., HM-1 and PLP-93 during *Kharif* 2019. M_1 generation was raised in *Kharif* 2018 from the seeds of both the genotypes treated with gamma-rays (100, 200, 300 and 400 *Gy*), EMS (0.1, 0.2, 0.3 and 0.44%) and their possible combinations. Five types of chlorophyll mutants were detected. Most frequent chlorophyll mutant was chlorina (31) followed by albino (15), xantha (12), variegated (7) and striata (6). The frequency of mutants showed gradual increase with increase in dose/concentration of mutagens in both gamma rays and EMS, while the combination treatments showed an irregular trend with increasing dose in both the genotypes of black gram. Combined treatments produced more mutant frequency than gamma-irradiation and EMS treatments for both genotypes of black gram. Genotype HM-1 was observed more responsive to mutation changes than the genotype PLP-93.

Key words: Chlorophyll mutants, EMS, gamma-rays, mutant frequency, black gram.

Mutagenesis has been used as an important supplement to plant breeding. Plant breeders are not constrained by insufficient allelic variation at one or more loci because of mutations, which contribute to creating previously undiscovered alleles at one or the more gene loci of interest. Mutations may occur spontaneously or can be induced artificially. When physical and chemical mutagens are used in combination treatments, antagonistic and synergistic effects may ensue. The choice of mutagen and its different doses is one of the important steps for creating new variability in the population which is the need of time for crop improvement.

Chlorophyll mutants are employed as markers in plant breeding programmes for the evaluation of gene action of mutagenic factor in inducing mutation studies. The chlorophyll mutation study could be useful for determination of the threshold dose of a mutagen that would enhance the genetic variability because chlorophyll mutants do not have any economic value due to their lethal nature. The scoring of chlorophyll mutation frequency in M_2 generation is primary index for evaluating the mutagenic induced genetic alterations of the mutagen treatments used on the plant ideotype. Mutation sensitivity is influenced by an array of factors, such as type of mutagen and their concentration used. Various classes of physical and chemical mutagens have diverged effects in their efficiency in inducing mutations and in the spectrum of mutations induced. The experiment was conducted to estimate the efficacy of physical mutagens, chemical mutagens & their combinations, and study the induced genetic variability for anthracnose resistance and yield traits in black gram.

Materials and Methods

One hundred mutagen treated seeds of two well adapted but susceptible to anthracnose genotypes of black gram *viz.,* Him Mash-1(HM-1) and Palampur-93 (PLP-93) were sown along with control at the Experimental Farm, Department of Genetics and Plant Breeding, CSK HPKV, Palampur during *kharif* 2018 and *kharif* 2019, the M_2 generation was evaluated.

For gamma irradiation, dry seeds of HM-1 and PLP-93 were exposed to varying doses of gamma-rays *viz*., 200, 300,400 and 500 Gy from Cobalt-60 (Co^{60}) at Gamma Irradiation Chamber, Punjab Agriculture University, Ludhiana. For treatment with chemical mutagen EMS, individually and in combination with gamma-rays (Table 1), dry seeds of each variety were first pre-soaked in distilled water for about 6 hours at room temperature and then treated with 0.1, 0.2 and 0.3% of freshly prepared EMS solution for 4 hours followed by post treatment of washing under running tap water. The screening and identification of the chlorophyll mutants was done throughout the plant growing period in M_2 generation, as described by Gustaffson (1940).

Table 1. Combined treatments of gamma-rays and EMS

1	200 Gy + 0.1% EMS
2	200 Gy + 0.2% EMS
3	200 Gy + 0.3% EMS
4	300 Gy + 0.1% EMS
5	300 Gy + 0.2% EMS
6	300 Gy + 0.3% EMS
7	400 Gy + 0.1% EMS
8	400 Gy + 0.2% EMS
9	400 Gy + 0.3% EMS
10	500 Gy + 0.1% EMS
11	500 Gy + 0.2% EMS
12	500 Gy + 0.3% EMS

Results and Discussion

Five different types of chlorophyll mutants were identified and broadly grouped into lethal and viable ones (Figure 1 and Figure 2). The following are the primary characteristics of these mutations:

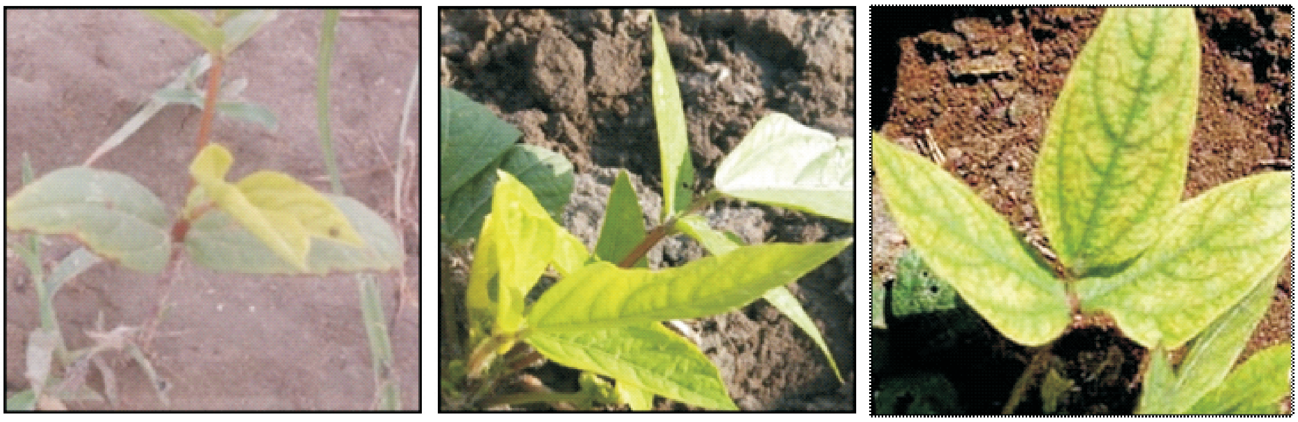
1. Lethal mutations:
   a) Albina: The seedlings exhibited colorless leaves. These mutants survived for about a week only.
   b) Xantha: In young seedlings, the leaves were predominantly yellow in color. These showed normal growth in the beginning, but withering after 8-10 days and ultimately died within two weeks.
2. Viable mutations:
   a) Chlorina: These mutants were characterized by the presence of yellowish green colored leaves.
   b) Striata: The striata mutants exhibited longitudinal white or yellow strips in their leaves.
   c) Variegated: The alternating stripes of yellow, white and green color on the lamina of leaves.

A similar result of maximum chlorina mutants followed by other viable mutants was obtained for by Dhanavel *et al.* (2008), Kumar *et al.* (2009) and Bind *et al*. (2016) in cowpea; Mahamune and Kothekar (2012) in french bean; Sagade and Lad (2008), Makeen *et al.* (2013), Souframanien *et al.* (2016) and Tamilzharasi *et al.* (2019) in black gram; Swain *et al.* (2019) in mungbean and Bhoi and Mishra (2021) in ricebean. Joshi *et al.* (2015) reported that electron beam and gamma-rays induced more number of 'chlorina' mutants in variety vijay of chickpea, while Khan and Tyagi (2009) reported that chemical mutagens induced high frequency of 'chlorina' mutants in M_2 generation of chickpea. Occurrence of maximum 'chlorina' mutants in large number of crops have been attributed to different causes such as degradation of chlorophyll, impaired chlorophyll biosynthesis and deficiency of carotenoids.

HM-1 (Control)

Chlorina
(HM1- 0.1%)

Xantha
HM-1 (400 Gy)



Albina (HM-1-0.2% EMS) | Striata (HM-1- 300 Gy+0.1%) | Variegated (HM-1- 500 Gy+ 0.3%)

Figure 1. Chlorophyll mutants for HM-1 genotype

PLP-93 (Control) | Chlorina (PLP-93- 0.3%) | Xantha PLP-93 (400 Gy)

Albina (PLP- 93- 0.2% EMS) | Striata (PLP-93- 300 Gy+0.1%) | Variegated (PLP-93- 500 Gy)

Figure 2. Chlorophyll mutants for PLP-93 genotype

Frequency of chlorophyll mutations

The frequency of chlorophyll mutations was calculated as the percentage of chlorophyll mutants isolated in the whole population of a particular treatment. The results obtained (Table 2 and Table 3) illustrated that the frequency of mutants showed gradual increase with increase in dose/concentration of mutagens in both gamma rays and EMS, while the combination treatments showed an irregular trend with increasing dose in both the genotypes of black gram. Combined treatments produced more mutant frequency than gamma-irradiation and EMS treatments for both cultivars.

The frequencies of chlorophyll mutations in different crops with different mutagens have been found to be markedly different. Chemical mutagens in combination with radiation are not only mutagenic themselves but are more effective in inducing chlorophyll mutations comparing to individual treatments (Khan and Tyagi, 2009). Most frequent chlorophyll mutant was chlorina followed by albino, xantha, variegated and striata. Chlorina type was the most frequent in both varieties suggesting high mutability of the gene controlling the phenotype. The highest frequency of chlorophyll mutation in different mutagenic treated population was 1.111% (400 Gy+ 0.2% EMS) in HM-1 and 1.261 Gy (500Gy) in PLP-93.

The most effective gamma radiation treatment at 500 Gy resulted in producing 1.48 % of mutation frequency followed 400 Gy (0.654%) and 300 Gy (0.585%) in HM-1, while 500 Gy was reported to produce 1.262% of mutant frequency in PLP-93 genotype. 400 Gy and 300 Gy treatment produced 0.784% and 0.692% of mutant frequency in PLP-93.

For EMS treatments, the highest mutations frequency was observed by treatment of 0.3% in both HM-1 (0.877%) and PLP-93 (0.882%). In case of combined treatments, the maximum chlorophyll mutation frequency was observed at combined treatment of 400Gy + 0.2% EMS in HM-1(1.111%) while 200 Gy+0.1% (0.929%) EMS reported maximum mutant frequency in PLP-93.

Pooled frequency (Table 4) and spectrum of different chlorophyll mutations in two black gram genotypes revealed that the gamma-rays + EMS combination treatments induced high mutation frequency than individual gamma raysand EMS treatment, which were also highlighted by Bhat *et al.* (2007), Khursheed and Khan (2016) and Tamilzharasi *et al.* (2019). Swaminathan *et al.* (1962) reported that EMS was more superior to gamma-rays as it induced higher mutation frequencies and wider spectrum of chlorophyll mutations in M_2 generation. High frequency of chlorophyll mutations in EMS treatment is perhaps due to preferential action of EMS on genes for chlorophyll development located near the centromere (Varghese and Swaminathan, 1966) or preferential effect of EMS on guanine in the GC rich chlorophyll genome.

Table 2. Frequency of chlorophyll mutants in M_2 generation for HM-1 genotype

Treatment	Chlorina	Albina	Xantha	Striata	Variegated	Mutant Frequency
Control	-	-	-	-	-	-
200 Gy	-	-	-	-	-	-
300 Gy	2 (0.585)	-	-	-	-	2 (0.585)
400 Gy	1 (0.327)	-	1 (0.327)	-	-	2 (0.654)
500 Gy	-	3 (1.111)	-	1 (0.370)	-	4 (1.481)
0.1% EMS	-	2 (0.213)	-	-	-	2 (0.427)
0.2% EMS	1 (0.231)	-	1 (0.231)	-	-	2 (0.463)
0.3% EMS	1 (0.292)	-	-	2 (0.584)	-	3 (0.877)
200 Gy + 0.1% EMS	1 (0.158)	-	1 (0.158)	-	-	2 (0.617)
200 Gy + 0.2% EMS	-	-	-	-	1 (0.292)	1 (0.292)
200 Gy + 0.3% EMS	2 (0.483)	1 (0.245)	-	-	1 (0.245)	4

300 Gy + 0.1% EMS	1 (0.253)	-	-	-	-	1 (0.253)
300 Gy + 0.2% EMS	1 (0.231)	-	-	1 (0.231)	-	2 (0.463)
300 Gy + 0.3% EMS	-	2 (0.463)	-	-	-	2 (0.463)
400 Gy + 0.1% EMS	1 (0.370)	-	-	-	-	1 (0.370)
400 Gy + 0.2% EMS	2 (0.740)	-	1 (0.370)	-	-	3 (1.111)
400 Gy + 0.3% EMS	-	-	3 (0.833)	-	-	3 (0.833)
500 Gy + 0.1% EMS	3 (0.794)	-	-	-	1 (0.264)	4 (1.058)
500 Gy + 0.2% EMS	-	-	-	-	-	-
500 Gy + 0.3% EMS	-	-	-	-	1 (0.347)	1 (0.347)
Total	**16 (0.243)**	**8(0.212)**	**7 (0.106)**	**4 (0.060)**	**4 (0.060)**	**39 (0.593)**

Table 3. Frequency of chlorophyll mutants in M_2 generation for PLP-93 genotype

Treatment	**Chlorina**	**Albina**	**Xantha**	**Striata**	**Variegated**	**Mutant Frequency**
Control	-	-	-	-	-	
200 Gy	1 (0.294)	-	-	-	-	1 (0.294)
300 Gy	2 (0.692)	-	-	-	-	2 (0.692)
400 Gy	-	-	2(0.784)	-	-	2 (0.784)
500 Gy	2 (0.840)	-	1(0.420)	-	-	3 (1.261)
0.1% EMS	1 (0.245)	1 (0.245)	-	-	1 (0.245)	3 (0.735)
0.2% EMS	1 (0.255)	1 (0.255)	-	1 (0.255)	-	3 (0.767)
0.3% EMS	2 (0.588)	1 (0.294)	-	-	-	3 (0.882)
200 Gy + 0.1% EMS	-	1 (0.309)	1(0.309)	-	1 (0.309)	3 (0.929)
200 Gy + 0.2% EMS	-	-	-	-	-	-
200 Gy + 0.3% EMS	-	1 (0.294)	-	-	-	1 (0.294)
300 Gy + 0.1% EMS	2 (0.588)	-	-	-	-	2 (0.588)
300 Gy + 0.2% EMS	-	-	-	1 (0.267)	-	1 (0.267)
300 Gy + 0.3% EMS	1 (0.256)	-	-	-	-	1 (0.256)
400 Gy + 0.1% EMS	-	2 (0.784)	-	-	-	2 (0.784)
400 Gy + 0.2% EMS	1 (0.420)	-	-	-	-	1 (0.420)
400 Gy + 0.3% EMS	2 (0.654)	-		-	-	2 (0.654)
500 Gy + 0.1% EMS	-		-	-	1 (0.327)	1 (0.327)
500 Gy + 0.2% EMS	-	-	1(0.420)	-	-	1 (0.420)
500 Gy + 0.3% EMS	-		-	-	-	-
Total	**15 (0.255)**	**7 (0.119)**	**5(0.085)**	**2 (0.034)**	**3 (0.051)**	**32 (0.545)**

Table 4. Pooled frequency and spectrum of different chlorophyll mutations in M_2 generation of two black gram varieties

a) Relative frequency (no.) of chlorophyll spectrum on mutagen basis

Varieties	**Relative frequency (no.) of chlorophyll spectrum**					
	Chlorina	**Albina**	**Xantha**	**Striata**	**Variegated**	**Total**
HM-1	16	8	7	4	4	38
PLP-93	15	7	5	2	3	32
Total	31	15	12	6	7	71

b) Relative frequency (no.) of chlorophyll spectrum on variety basis

Mutagens	**Relative frequency (no.) of chlorophyll spectrum**					
	Chlorina	**Albina**	**Xantha**	**Striata**	**Variegated**	**Total**
Gamma rays	8	3	4	1	0	16
EMS	6	5	1	3	1	16
Gamma rays + EMS	17	7	7	2	6	38
Total	31	15	12	6	7	71

It can be concluded from the study that combination treatments (38) induced high mutation frequency than individual gamma rays (16) and EMS (16) treatment, indicating their greater effectiveness due to their cumulative effect.

Conflict of interest: The authors declare that there is no conflict in this research paper.

References

Bhat TA, Khan AH and Sahba P. 2007. Spectrum and Frequency of Chlorophyll Mutations Induced by MMS, Gamma Rays and Their Combination in two varieties of *Vicia faba* L. Asian Journal of Plant Sciences **6** (3): 558-561.

Bhoi L and Mishra D. 2021. Mutagenic effects on frequency and spectrum of chlorophyll mutations in rice bean (*Vigna umbellata* Thunb, OHWI & OHASHI). Journal of Pharmacognosy and Phytochemistry **10** (2): 1511-1514.

Bind D, Dwivedi VK and Singh SK. 2016. Induction of chlorophyll mutations through physical and chemical mutagenesis in cowpea *Vigna unguiculata*. International Journal of Advanced Research **4** (2): 35-39.

Dhanavel D, Pavadai P, Mullainathan L, Mohana D, Raju G, Girija M and Thilagavathi C. 2008. Effectiveness and efficiency of chemical mutagens in cowpea (*Vigna unguiculata* (L.) Walp). African Journal of Biotechnology **7** (22): 4116-4117.

Gautam AS and Mittal RK. 1998. Induced mutations in black gram [*Vigna mungo* (L.) Hepper.]. Crop Research **16** (3): 344-348.

Joshi A, Reddy KS, Petwal VC and Dwivedi J. 2015. Identification of novel mutants through electron beam and gamma irradiation in chickpea (*Cicer arietinum* L.). Journal of Food Legumes **28** (1): 99-104.

Khan MH and Tyagi SD. 2009. Studies on induction of chlorophyll mutations in soybean, *Glycine max* (L.) Merril. Frontiers of Agriculture in China **253** (2): 009-058.

Khursheed S and Khan S. 2016. Screening of chlorophyll mutations in the mutagenized population of two cultivars of *Vicia faba* L. American Journal of Experimental Agriculture **11** (5): 1-7.

Mahamune SE and Kothekar VS. 2012. Induced chemical and physical mutagenic studies in M_1 generation of French bean (*Phaseolus vulgaris* L.). Current Botany **3** (3): 17-21.

Makeen K, Babu GS and Lavanya GS. 2007. Studies of chlorophyll content by different methods in black gram (*Vigna mungo* L.). International Journal of Agricultural Research **2** (7): 651-654.

Mullainathan Land Ambli K. 2015. Chlorophyll and morphological mutants of Pearl millet (*Pennisetum* *tphoides* (Burn.) stapf. Var. CO (cu) 9. European Journal of Experimental Biology **5** (3): 72-77.

Priyadharshni B, Mullainathan L and Tamizharasi G. 2021. Studies on effects of induced mutagenesis on quantitative characteristics of Proso millet (*Panicum miliaceum*) Var-CO3 in M_2 and M_3 generation. International Journal of Agriculture and Environmental Research **7** (1):92-101.

Souframanien J, Reddy K, Petwal VC and Dwivedi J. 2016. Comparative effectiveness and efficiency of electron beam and 60Co ã-rays in induction of mutations in black gram [*Vigna mungo* (L.) Hepper]. Journal of Food Legumes **29** (1): 1-6.

Swain D, Baisakh B, Tripathy SK and Lenka D. 2019. Mutagenic effect of gamma rays, EMS, NG and their combinations for induction of chlorophyll and macro-mutations in mungbean (*Vigna radiata* (L.) Wilczek). Journal of Pharmacognosy and Phytochemistry **8** (5): 2489-2495.

Swaminathan MS, Chopra VL and Bhaskaran S. 1962. Chromosome aberrations and the frequency and spectrum of mutations induced by ethylmethanesulphonate in barley and wheat. Indian Journal of Genetics and Plant Breeding **22**: 192-207.

Tamilzharasi M, Kumaresan D, Souframanien J and JayamaniP. 2019. Study of chlorophyll deficit types through induced mutagenesis in blackgram (*Vigna mungo* L. Hepper). Electronic Journal of Plant Breeding **10** (4): 1471-1476.

Varghese G and Swaminatnan MS. 1966. Changes in protein quantity and quality associated with a mutation for amber grain colour in wheat. Current Science **35**: 469-470.