➔DOI : 10.15740/HAS/AJBS/9.2/204-207
**e ISSN-0976-8343** | ■ Visit us : ***www.researchjournal.co.in***

Asian Journal of Bio Science, Volume **9** | Issue 2 | October, 2014 | 204-207
**Received :** 02.05.2014; **Revised :** 19.08.2014; **Accepted :** 30.08.2014

RESEARCH PAPER

# Frequency and spectrum of chlorophyll mutations in greengram [*Vigna radiata* (L.) Wilczek]

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Greengram [*Vigna radiata* (L.) Wilczek], popularly known as mungbean, is third most important pulse crop of India. Mungbean is a cheap source of dietary protein for the poor, with high levels of folate and iron compared with many other legumes. Variability is low available in mungbean and hence, to replace conventional breeding, mutation breeding has gained its momentum. Induced mutagenesis thus seems to be an ideal methodology for the induction of desirable genetic variability. Chlorophyll mutations, an important index in the estimation of induced genetic changes in mutagen treated population are most widely employed for assessing the potentialities of mutagens in creating genetic variability. An investigation was carried out in two mungbean genotypes CO (Gg) 7 and NM 65 treated by two mutagens *viz.*, gamma rays and ethyl methane sulphonate. A wide range of chlorophyll mutations was observed and scored in $M_2$ generation. The highest frequency rate was noted at 300 Gray and 10 mM on $M_1$ plant basis and $M_2$ seedling basis in both the genotypes. The mutant chlorina and xantha occurred in all the treatments of gamma rays and EMS at higher proportions.

**Key words :** Greengram, Chlorophyll mutation, EMS, Gamma rays, Variability

**How to cite this paper :** Vairam, N., Ibrahim, S.M. and Vanniarajan, C. (2014). Frequency and spectrum of chlorophyll mutations in greengram [*Vigna radiata* (L.) Wilczek]. *Asian J. Bio. Sci.*, **9** (2) : 204-207.

## INTRODUCTION

Greengram [*Vigna radiata* (L.) Wilczek], popularly known as mungbean, is third most important pulse crop of India (Grover, 2011). Among the various pulses grown in India, mungbean, a self-fertilized crop, occupies unique position in Indian agriculture and has been grown under various agro-ecological conditions. Mungbean is a cheap source of dietary protein for the poor, with high levels of folate and iron compared with many other legumes (Keatinge *et al*., 2011). Its grains are used as dal, soup and feed for animals. Its straw is used as fodder and fuel. Variability is low available in mungbean and hence, to replace conventional breeding, mutation breeding has gained its momentum. Induced mutations have been used to generate genetic variability and have been successfully utilized to improve yield and yield components of various crops. Induced mutation is a suitable source of producing variation through mutation breeding procedure (Domingo *et al*., 2007).

Chlorophyll mutations are most widely employed for assessing the potentialities of mutagens in creating genetic variability. It serves as an important index in the estimation of induced genetic changes in mutagen treated population (Singh and Rao, 2007). The frequency of chlorophyll mutation in $M_2$ generation were considered to be a standard measure of rates of induced mutations which helps in determination of effectiveness and efficiency of mutagens and their doses or concentrations (Kumar *et al.*, 2009). In mungbean, chlorophyll mutations can be classified into albina, xantha, viridis, maculate, chlorina, tigrina, lutercent, striata, virescent, coppery, light-green, variegated and white streak leaf (Sangsiri *et al.*, 2005). Occurrence of chlorina mutants have been attributed to different causes such as impaired chlorophyll biosynthesis, further degradation of chlorophyll and bleaching due to deficiency of carotenoids. The usefulness of any mutagenic agent depends on its ability to induce high frequency of desirable changes as compared to undesirable ones. Hence, the present investigation was carried out to calculate the spectrum and frequency of chlorophyll mutations induced by gamma rays and EMS for the genotypes studied.

## RESEARCH METHODOLOGY

Two greengram genotypes *viz.*, CO (Gg) 7 and NM 65 obtained from the Department of Pulses, Centre for Plant Breeding and Genetics, Tamil Nadu Agricultural University, Coimbatore. The genotypes were subjected to gamma irradiation at the doses of 300, 400, 500 and 600 Gy and EMS treatments of 10, 20 and 30 mM. Gamma irradiation was done using cobalt 60 sources in the Gamma chamber, installed at Centre for Plant Breeding and Genetics, Tamil Nadu Agricultural University, Coimbatore. The chemical mutagen, ethyl methane sulphonate ($CH_3SO_2OC_2H_5$) with molecular weight 124.16, from the sigma chemical company, USA was used for treating the seeds. In respect of EMS treatment, the seeds were presoaked in distilled water for six hours. Appropriate quantities of EMS were dissolved in distilled water to have the concentrations envisaged in the program. The treatment was performed at room temperature 22 +2$^0$C early morning hours with intermediate shaking during the treatment period of 6 hours. After the chemical treatment, the seeds were washed thoroughly with running tap water for half an hour to remove the residues of the chemical, if any and the excess moisture in seed coat was removed by using folds of blotting paper. The treated seeds were sown with a spacing of 30 x 10cm in a Randomized Block Design. The trial was conducted in the research farm of Agricultural College and Research Institute, Madurai during *Kharif* 2013. In $M_2$ generation the gamma rays doses 300, 400 and 500 Gy and EMS treatments 10, 20 and 30 mM were raised based on $LD_{50}$ value obtained in $M_1$ generation. The $M_2$ generation was raised from individual $M_1$ plant (*i.e.*) $M_1$ plant basis following plant to progeny method. The treated and control population of $M_2$ generation was carefully screened and classified as per Gustafsson (1940) for chlorophyll mutations after 5$^{th}$ day onward of sowing. Mutation frequency was estimated as per centage of segregating $M_1$ plant progenies and number of mutants per 100 $M_2$ families (Gaul, 1964). The frequency and spectrum of chlorophyll mutations for different treatments were computed.

## RESEARCH FINDINGS AND ANALYSIS

The occurrence of chlorophyll deficient mutant might be attributed due to change in gene, a set of genes responsible for chlorophyll mutation. Number of $M_1$ families segregating for such chlorophyll mutation was also recorded. Four types of chlorophyll mutants *viz.*, albino, xantha, chlorina and viridis were observed in $M_2$ seedlings. Similar result was obtained by Ashok Kumar *et al.* (2009) in cowpea, Mahamune and Kothekar (2012) in French bean.

The spectrum and frequency of chlorophyll mutants is being used as primary index of effectiveness of mutagens and mutability of the genotypes towards the mutagen which in turn would be useful to generate the wide array of desirable mutants in the treated population (Gaul, 1964).

**Frequency of chlorophyll mutants :**

The frequency of chlorophyll mutants was estimated by periodical scorings starting from 3$^{rd}$ to 15$^{th}$ day. In CO (Gg) 7, the chlorophyll mutants occurred in all the treatments. In gamma irradiated population, the frequency of chlorophyll mutation varied from 21.53 per cent (500 Gy) to 24.79 per cent (400 Gy) on $M_1$ plant basis and from 1.10 per cent (500 Gy) to 1.64 per cent (300 Gy) on $M_2$ seedling basis. The treatment 400 Gy recorded the maximum frequency rate on $M_1$ and 300Gy for $M_2$ plant basis. For EMS treatments, the frequency ranged from 18.91 per cent in 30 mM to 24.61 per cent in 10 mM on $M_1$ plant basis and 1.09 per cent in 30 mM to 1.70 per cent in 10 mM on $M_2$ plant basis. The treatment 10mM exhibited the maximum frequency of chlorophyll mutants on both $M_1$ and $M_2$ plant basis. The frequency of different types of chlorophyll mutation for CO (Gg) 7 is presented in Table 1.

In NM 65, the frequency of gamma ray mutants ranged from 17.00 per cent in 500 Gy to 23.82 per cent in 400 Gy and from 1.05 per cent in 500 Gy to 1.61 per cent 300 Gy on

**Table 1 : Chlorophyll mutation frequencies in $M_2$ generation – CO (Gg) 7**

| Mutagen dose | No. of $M_1$ plants | | No. of $M_2$ plants | | Mutation frequency (%) | |
|---|---|---|---|---|---|---|
| | Plants forwarded | Segregating | Studied | Chlorophyll mutants | $M_1$ plant basis | $M_2$ plant basis |
| **Gamma - ray (Gy)** | | | | | | |
| Control | 50 | - | 1000 | - | - | - |
| 300 Gy | 320 | 75 | 8200 | 135 | 23.43 | 1.64 |
| 400 Gy | 242 | 61 | 7300 | 100 | 24.79 | 1.34 |
| 500 Gy | 195 | 42 | 6400 | 71 | 21.53 | 1.10 |
| **EMS (mM)** | | | | | | |
| Control | 50 | - | 1000 | - | - | - |
| 10 mM | 325 | 80 | 8495 | 145 | 24.61 | 1.70 |
| 20 mM | 220 | 53 | 6970 | 90 | 24.09 | 1.29 |
| 30 mM | 185 | 35 | 5300 | 58 | 18.91 | 1.09 |

$M_1$ and $M_2$ plant basis, respectively. For EMS, the frequency rate varied from 13.75 per cent in 30 mM to 22.32 per cent in 20 mM on $M_1$ plant basis and from 0.80 per cent in 30 mM to 1.48 per cent (10 mM) on $M_2$ seedling basis. The highest frequency rate was noted as 22 per cent on $M_1$ Plant basis and 1.46 per cent (10 mM) on $M_2$ seedling basis. The frequency of different types of chlorophyll mutants for the genotype NM 65 is presented in Table 2.

Results indicated that lower doses of mutagens induced high frequency of chlorophyll mutations. This was in contrast with the findings of Kumar *et al.* (2009). In both the genotypes, EMS (10mM) was very effective in inducing chlorophyll mutants. Similar findings were repoted by Girija and Dhanavel (2009) in cowpea. The high incidence of chlorophyll mutations induced by EMS may be due to its specificity to affect certain regions of the chromosome (Natrajan and Upadhya, 1964).

**Spectrum of chlorophyll mutation :**

In CO (GG) 7, both gamma rays and EMS treatments recorded a maximum number of chlorina and xantha. The mutant albino occurred at 20mM EMS in minimum proportion. The occurrence of albino was very rare and could be clarified with the report of Sjodin (1971) who suggested that the occurrence of albino was a rare sequence in Leguminosae. In NM 65, chlorina and xantha occurred in higher frequency in 500 Gy and 300 Gy gamma ray treatments. The mutant viridis occurred at 400 Gy and 10mM for the genotype NM 65. The spectrum of chlorophyll mutants was different in EMS and gamma-rays. Nilan *et al.* (1964) also reported that the mutant spectrum of chemical mutagens differ from physical mutagens. The reason for such differences laid only to a small extent in different mutations induced in the chromosomes. It appeared that many of these differences were the result of different ratio between gene mutations, small deficiencies and chromosomal aberrations.

Among the different types of chlorophyll mutations, chlorina type was observed at highest frequency 66.66 per cent (500 Gy) for CO (Gg) 7 and 83.34 per cent (30mM) for NM 65, suggesting high mutability of the gene controlling the phenotype. Similar results were reported by Das and Baisakh (2009). The presence of certain chlorophyll mutants in some mutagenic treatments and absence in others indicate difference in the sensitivity of mutagenic loci to mutagen. The spectrum of chlorophyll mutation and the relative frequencies of different types of chlorophyll mutants for both the genotypes studied are presented in Table 3.

**Table 2 : Chlorophyll mutation frequencies in $M_2$ generation – NM 65**

| Mutagen dose | No. of $M_1$ plants | | No. of $M_2$ plants | | Mutation frequency (%) | |
|---|---|---|---|---|---|---|
| | Plants forwarded | Segregating | Studied | Chlorophyll mutants | $M_1$ plant basis | $M_2$ plant basis |
| **Gamma - ray (Gy)** | | | | | | |
| Control | 50 | - | 1000 | - | - | - |
| 300 Gy | 317 | 70 | 8000 | 129 | 22.08 | 1.61 |
| 400 Gy | 235 | 56 | 7190 | 87 | 23.82 | 1.21 |
| 500 Gy | 178 | 32 | 5200 | 55 | 17.97 | 1.05 |
| **EMS (mM)** | | | | | | |
| Control | 50 | - | 1000 | - | - | - |
| 10 mM | 300 | 65 | 8105 | 120 | 21.66 | 1.48 |
| 20 mM | 215 | 48 | 7000 | 80 | 22.32 | 1.14 |
| 30 mM | 160 | 22 | 5100 | 41 | 13.75 | 0.80 |

**Table 3 : Frequency of different types of chlorophyll mutants in $M_2$ generation of CO (Gg) 7 and NM 65**

| Mutagen | Total chlorophyll mutants in $M_2$ generation | | Relative percentage of chlorophyll mutants (%) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Albino | | Xantha | | Chlorina | | Viridis | |
| **Gamma ray (Gy)** | CO (Gg) 7 | NM 65 | CO (Gg) 7 | NM 65 | CO (Gg) 7 | NM 65 | CO (Gg) 7 | NM 65 | CO (Gg) 7 | NM 65 |
| 300 Gy | 135 | 129 | - | - | 50.00 | 45.23 | 25.00 | 41.96 | 25.00 | 12.81 |
| 400 Gy | 100 | 87 | - | - | 42.86 | 25.00 | 57.14 | 25.00 | - | 50.00 |
| 500 Gy | 71 | 55 | - | 25.00 | 33.34 | - | 66.66 | 50.00 | - | 25.00 |
| **EMS (mM)** | | | | | | | | | | |
| 10 mM | 145 | 120 | - | - | 41.67 | 45.00 | 16.66 | 12.81 | 41.67 | 42.19 |
| 20 mM | 90 | 80 | 25.00 | 25.00 | - | 25.00 | 75.00 | 50.00 | - | - |
| 30 mM | 58 | 41 | - | - | 42.86 | 16.66 | 57.14 | 83.34 | - | - |

Different pattern of chlorophyll mutations were reported in mungbean mutants. Our results are contrary to the findings of Haq (1990). Occurrence of chlorina mutants have been attributed to different causes such as impaired chlorophyll biosynthesis, further degradation of chlorophyll and bleaching due to deficiency of carotenoids. It means that the genetic differences in genotypes under reference for inducing chlorophyll mutation type have been observed and identified by many workers in Bengal gram (Nerkar and Mote, 1978) and lentil (Sharma and Sharma, 1981).

**Conclusion :**

Efficient mutagens and their treatments are essential for the economic use of the mutagen as a tool for the induction of useful mutations and their direct and indirect utilization. From the present investigation, it was concluded that EMS is very effective in inducing chlorophyll mutants in CO (Gg) 7 and NM 65 on $M_2$ plant basis. The genotype CO (Gg) 7 responded well by producing more chlorophyll mutants of gamma rays and EMS. Both the genotypes showed the high frequency of the mutant chlorina and xantha in all the treatments of gamma rays and EMS.

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