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Research Note

Occurrence of chlorophyll deficient mutants in the mutated populations of greengram (*Vigna radiata* (L.)Wilczek)

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Abstract
In mutation breeding, occurrence of chlorophyll deficient mutants is the best index to assess efficient dose of mutagen in producing genetic variability. The seeds of three greengram varieties *viz*., CO(Gg)7, CO5 and VBN(Gg)3 were irradiated with 550Gy and 600Gy dose of gamma rays. In M_2 generation, totally 2957 mutant lines of three varieties were raised in plant to progeny basis. Five different types of chlorophyll deficient mutants *viz*., albino, xantha, chlorina, viridis and variegated leaf mutants were observed in mutated population of three greengram varieties in M_2 generation. Frequency of occurrence was more in lower dose, 550Gy irrespective of the varieties. Among different chlorophyll deficient mutants, albino occurred more frequently followed by xantha and variegated leaf mutants. Chlorophyll deficient mutants were observed only in M_2 and M_3 generation but not in further generations.

Key words
Chlorophyll deficient mutants, gamma irradiation, greengram, mutagenesis, genetic variation

One of the ways to induce genetic variation in crop plants is induced mutagenesis which has been long ago considered as an effective breeding methodology especially in self pollinated crops like greengram. However, success of mutation breeding in any crop improvement depends primarily on the effect of mutagen in inducing genetic variation. The occurrence of chlorophyll deficient mutants is the best indicator to predict the frequency of gene mutation and thus ultimately to evaluate the genetic effects of mutagen (Usharani and Ananda Kumar, 2015). Gene mutations either in nuclear or cytoplasmic genes might result in chlorophyll deficient mutants (Levine, 1972 and Walles, 1973). Several types of chlorophyll deficient mutants *viz*., albino, xantha, chlorina, maculata, virescent, viridis and striata have been reported in mutated population by many authors (Lal *et al*., 2009; Goyal and Khan, 2010; Wani *et al*., 2011). Not all the chlorophyll deficient mutants are economically used except variegated leaf mutants which is particularly desirable for ornamental purposes. The main objective of mutation breeding in greengram is to create genetic variation. In the present investigation, an attempt was made to assess the genetic effects of gamma rays based on the frequency and spectrum of chlorophyll deficient mutants in three greengram varieties.

In the present investigation, 200 g seeds of three greengram varieties *viz*., CO5, CO(Gg)7 and VBN(Gg)3 were exposed to 550Gray and 600 Gray dose of gamma irradiation emitted from ^{60}CO which was installed at Centre for Plant Breeding and Genetics, Tamil Nadu Agricultural University, Coimbatore as LD 50 value for this crop is 600 Gray. The treated seeds along with control (untreated seeds) were immediately sown in the field at Agricultural Research Station, Tamil Nadu Agricultural University, Vaigai Dam during *Rabi*, 2011. Normal agronomic practices were followed to raise a healthy crop. At the time of maturity, the seeds were harvested on single plant basis. All the seeds of each M_1 plants were sown in the field on plant to progeny basis in M_2 generation during *Summer*, 2012. The occurrence of different types of chlorophyll deficient mutants was examined in each and every M_2 lines and recorded immediately after germination up to one month old crop. Number of mutant lines studied in M_2 generation was given in Table 1. Spectrum of chlorophyll deficient mutants was recorded and the frequency of chlorophyll mutants was calculated as percentage of M_2 lines.

Occurrence of chlorophyll deficient mutants in a mutated population acts as an excellent visible index for assuring mutagenesis in crop plants and ultimately useful to predict the effect of mutagenic dose in creating genetic variation. In this present study, totally five different types of chlorophyll deficient mutants *viz*., albino, xantha, chlorina, viridis and variegated leaf mutants were observed in M_2 segregating population of greengram. The spectrum of chlorophyll deficient mutants observed and their description are given in Table 2.

The spectrum of chlorophyll deficient mutants is normally studied in M_2 and succeeding generation in mutation breeding. In this study chlorophyll deficient mutants were not observed in M_1 generation. In M_2 generation the above said five different types of chlorophyll deficient mutants were observed in some of the mutant lines. In these mutant lines not all the plants of a line were chlorophyll deficient mutants but occurred in proportion which means that normal and chlorophyll deficient mutants occurred simultaneously in a mutant line. The proportion of chlorophyll deficient mutants in a mutant line varied from 1 to 40 percent. Among these chlorophyll deficient mutants, albino and xantha died within a week of germination and hence they are inviable chlorophyll deficient mutants. Other three types *viz*., chlorina, viridis and variegated leaf mutants produced flowers and set seeds and hence they are called as viable chlorophyll deficient mutants. However, the chlorophyll deficient mutants are not economically used except variegated leaf mutants which are particularly desirable for ornamental purposes. Similar results were observed in chickpea by Bara *et al*., (2017) A normal plant from each mutant line observed with chlorophyll deficient mutants in M_2 was forwarded to M_3 and further generations on plant to progeny basis. The chlorophyll deficient mutants were observed in M_3 generation in lower frequency when compared to M_2 generation but not in further generations *viz*., M_4 and M_5.No evidence was viewed for the study of chlorophyll mutants in M_3 and further generations.

Induction of chlorophyll deficient mutations in general is considered as a measure to assess the effect of mutagen in inducing genetic changes in crop plants. The frequency of chlorophyll deficient mutants observed in M_2 generation is mainly used as a dependable measure for assessing genetic effect of mutagen. The frequency of chlorophyll deficient mutants was calculated as percentage of

M_2 lines with respect to greengram varieties and irradiation doses which are given in Table 3.

Occurrence of chlorophyll deficient mutants was observed in all the three greengram varieties and in both doses of irradiation. However, the frequency of occurrence was varied with varieties and doses which suggested the differential response of gene controlling trait in varieties and doses of irradiation. In total, frequency of chlorophyll deficient mutants was found to be higher in VBN(Gg)3and CO(Gg)7 than CO5. This indicated higher sensitiveness of greengram varieties *viz*., VBN(Gg)3 and CO(Gg)7 towards gamma irradiation. The frequency of chlorophyll deficient mutants was found to be dose dependant also. It was found to be higher in lower dose, 550Gy than higher dose of 600Gy irrespective of the varieties. Hence, 550Gy dose was found to be efficient irradiation dose in inducing genetic changes in greengram. Decrease in mutation frequency in high dose may be due to increased lethality at higher dose of irradiation. The higher frequency of chlorophyll deficient mutants with lower mutagen dose was also reported by Nadarajan *et al* (1982) and Tah (2006). However, an increased chlorophyll deficient mutant with increased mutagenic dose was reported by Ignacimuthu and Babu (1988) and Lal *et al*. (2009). Singh and Rao (2007) observed no relationship between dose of mutagen and frequency of chlorophyll deficient mutants in greengram.

All the five types of chlorophyll deficient mutants were found to occur in CO(Gg)7 variety, whereas chlorina in VBN(Gg)3 and viridis and chlorinain CO5 were not observed. Among different types of chlorophyll deficient mutants, albino was found to occur in higher frequency in all the three greengram varieties which indicated high mutability of the gene controlling albino type. Of these five types of chlorophyll deficient mutants observed in the present study, albino and xantha died within a week of germination and hence they are inviable chlorophyll deficient mutants. Other three types *viz*., chlorina, viridis and variegated leaf mutants produced flowers and set seeds and hence they are called as viable chlorophyll deficient mutants.

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Table 1. Number of mutant lines studied in each generation

Sl.No.	Greengram variety	Dose	No. of mutants in M_2
1.	CO(Gg)7	550Gy	1053
		600Gy	693
2.	CO5	550Gy	390
		600Gy	27
3.	VBN(Gg)3	550Gy	470
		600Gy	324
	Total		**2957**

Table 2. Spectrum of chlorophyll deficient mutants and their description

Sl. No.	Spectrum of chlorophyll deficient mutants	Trait description
1	Albino	Totally white plants, No chlorophylls and carotenoids in leaves, non viable and observed in two leaf stage
2	Xantha	Yellow coloured plants, Carotenoids are present in leaves but not chlorophyll, non viable and observed in two leaf stage
3	Chlorina	Uniform light green coloured leaves with white patch on tip of leaves, viable and observed in two leaf stage
4	Viridis	Uniform light yellow green coloured leaves, viable and observed in two leaf stage
5	Variegated leaf	Persistent variegated yellow green leaves, viable and observed at 10 to 15 days after germination

Table 3. Frequency of chlorophyll deficient mutants in M_2 generation

Variety	Dose	Total no. of mutant lines	Chlorophyll deficient mutants in M_2 generation					Total
			Albino	Xantha	Viridis	Chlorina	Variegated leaf mutants	
CO(Gg)7	550Gy	1053	17 (1.61)	11 (1.04)	13 (1.23)	2 (0.19)	8 (0.76)	51 (4.84)
	600Gy	693	11 (1.59)	7 (1.01)	4 (0.58)	0 (0.00)	4 (0.58)	26 (3.75)
CO5	550Gy	390	3 (0.77)	1 (0.26)	0 (0.00)	0 (0.00)	2 (0.51)	6 (1.54)
	600Gy	27	1 (3.70)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	1 (3.70)
VBN(Gg)3	550Gy	470	10 (2.13)	8 (1.70)	2 (0.43)	0 (0.00)	7 (1.49)	27 (5.74)
	600Gy	324	7 (2.16)	5 (1.54)	1 (0.31)	0 (0.00)	3 (0.93)	16 (4.94)
	Total	2957	49 (1.66)	32 (1.08)	20 (0.68)	2 (0.07)	24 (0.81)	127 (4.78)

(Frequency of respective chlorophyll deficient mutants are given in parenthesis)

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