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Research Article

Study of chlorophyll deficit types through induced mutagenesis in blackgram (*Vigna mungo* L. Hepper)

M. Tamilzharasi [1], D. Kumaresan[2], J. Souframanien[3] and P. Jayamani[4]

[1]Ph.D., Scholar, Department of Pulses, Centre for Plant Breeding and Genetics, Tamil Nadu Agricultural University, Coimbatore 641 003, India.

[2] Associate Professor, Centre for Plant Breeding and Genetics, Tamil Nadu Agricultural University, Coimbatore 641 003, India.

[3] Scientific Officer, Nuclear Agriculture and Biotechnology Division, BARC, Mumbai-400 085, India.

[4] Professor and Head, Department of Pulses, Centre for Plant Breeding and Genetics, Tamil Nadu Agricultural University, Coimbatore 641 003, India.

E-Mail: tamilsadursen@gmail.com

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Abstract

The investigation was carried out to study the phenotypic reaction of seeds of CO 6 blackgram variety exposed to Gamma rays doses at 200 Gy, 300 Gy, 400 Gy and combination treatments with 200 Gy + 20 mM EMS, 200 Gy + 30 mM EMS, 300 Gy + 20 mM EMS, 300 Gy + 30 mM EMS, 400 Gy + 20 mM EMS, 400 Gy +30 mM EMS. It was observed that different mutation frequencies and span of mutation spectra were induced under the action of different concentrations of the applied mutagens in the M_2 generation of blackgram. Five different types of chlorophyll mutants namely *albino*, *xantha*, *chlorina*, *viridis,* and *maculata* type were observed in the M_2 populations and chlorophyll mutation frequency was calculated on a plant population basis. In Gamma treated population, the frequency of xantha mutants was high followed by chlorina whereas in combination treatments of Gamma ray + EMS, the highest frequency was observed in chlorina followed by xanthan mutants. Maculata type of chlorophyll mutant was observed in the treatment 200 Gy + 20 mM EMS. The combination treatments of Gamma ray + EMS were found to be more efficient than gamma rays alone in inducing chlorophyll mutations. The frequency of chlorophyll mutations (2.51%) was high in the combination treatment 400 Gy+ 30 mM EMS.

Key words

Combination treatments, Chlorophyll mutants**,** EMS**,** Gamma ray, Mutation frequency.

Introduction

Vigna mungo (L.) Hepper, commonly known as blackgram, urdbean or mash bean, is a leguminous crop domesticated from *Vigna mungo* var. *silvestris*. It is an economically important pulse crop not only in India but also in several other Asian countries *viz*. Pakistan, Bangladesh, Myanmar, and Thailand. In India, it occupies about 4.48 million hectares area with a production of 2.83 million tonnes during 2016-17 (www.indistat.com). The significant hindrance in realizing higher yield in blackgram is due to lack of genetic variability, canopy architecture, poor harvest index and susceptibility to insects and diseases. In addition to available natural genetic variability present in the germplasm of blackgram, mutation techniques are proven as an effective and efficient tool for supplementing the existing germplasm (Dubinin, 1961) for cultivar development in blackgram. This approach has ample contribution in crops like blackgram where a larger part of the natural variability has been annihilated in the process of adaptation to the environmental stress. Induced mutations have played an exceptional role in the introduction of new genetic variation of useful traits for crop improvement. The chlorophyll mutation frequency in the M_2 generation is the most dependable index for determining the extent of genetic changes in mutagen induced populations (Gustaffson, 1952). Chlorophyll mutants are used as markers in genetic studies which are easily and frequently observed in M_2 generation.

The present investigation was carried out to generate a wide array of desirable mutants by treating blackgram seeds with gamma rays and a combination of the gamma rays with EMS mutagens to produce spectrum and frequency of chlorophyll mutants in blackgram.

Materials and Methods

Fresh and healthy seeds of CO 6 variety of blackgram were treated with gamma rays (3500 seeds per treatment) at 200 Gy, 300 Gy, and 400 Gy by irradiating seeds from the Cobalt 60 (^{60}Co) gamma source in the gamma chamber installed at Bhabha Atomic Research Centre, Mumbai;

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combination treatments of gamma rays and EMS at 200 Gy + 20mM EMS, 200 Gy + 30 mM EMS, 300 Gy + 20 mM EMS, 300 Gy + 30 mM EMS and 400 Gy + 20 mM EMS, 400 Gy + 30 mM EMS by subjecting 750 irradiated seeds of 200 Gy, 300 Gy and 400 Gy per treatment were presoaked for initially 5 h in distilled water. Then soaked seeds were treated with 20 mM to 30 mM EMS for 1 h with chemical mutagen, washed in running water before sowing. Two sets were used as control. The M_1 generation was raised in the field and individual plants were harvested and 600 M_1 individuals were raised as plant progeny rows to raise the M_2 generation. The treated and the untreated materials were screened for the frequency of chlorophyll mutations in M_2 generation. The mutagenic frequency was estimated as the percentage of segregating M_1 plant progenies. Chlorophyll mutations were classified into various types based on the method followed by Gustafsson,(1940).

Albino: White and relatively smaller than normal seedlings and survived normally up to 12 days.
Chlorina: Uniform Light green colour, viable.
Xantha: Yellow to yellowish white, carotenoids present but chlorophyll absent, These seedlings survived for 10-20 days only.
Viridis: Uniform light yellow green colour of leaves, viable.
Maculata: Leaves with whitish dots on an entire leaflet and may attain pod maturity.

Results and Discussion

Diverse enzymes and biochemical reactions are required to regulate the complex process of photosynthesis (Johnson, 2016). Reduced photosynthesis is owing to a mutation in any one of the steps *viz.*, genes that encode for an enzyme entangled in the biosynthesis of a pigment or in metabolic pathways. These mutants are designated as chlorophyll-deficient mutants (Emerson,1929). To find out the sensitivity and genetic effects of crop plants to various mutagens, easily detectable Chlorophyll mutations are used as an Index.

In the present study, the frequency of chlorophyll mutants calculated as per cent of M_1 plant progenies and M_2 plants basis were presented in Table 1 and Figure.1. It was observed that the frequency of induced chlorophyll mutants was increased with an increase in the dose of gamma ray *viz.,* 200 Gy (0.41%) < 300 Gy (0.57 %) < 400 Gy (0.78%)). The results were in agreement with several other workers indicated a dose dependent increase in chlorophyll mutation frequency (Kharkwal, 1998; Das and Kundagrami, 2000; Barshile *et al.*, 2006). In case of combination treatment of Gamma rays with EMS, the mutation frequencies were found in the following order: 400 Gy+ 30 mM (2.51%) > 400 Gy+ 20 mM (1.10%)> 200 Gy+ 30 mM (1.10%)> 200 Gy+ 20 mM (1.02%)> 300 Gy+ 30 mM (0.81%)> 300 Gy+ 20 mM (0.78 %). Among the treatments, the highest mutation frequency of 2.51% was recorded by the treatment of 400 Gy +30 mM EMS. It was observed that the mutation frequency (1.02%) was double in combination treatment of 200 Gy+20 mM EMS than gamma ray alone (0.42 %). This confirmed that EMS is a more potent chemical mutagen in inducing chlorophyll mutants. Similar results were earlier reported by Gautam *et al.* (1992) in *Vigna mungo*; Khan *et al.* (2005) in *Cicer arietinum*. EMS is supposed to be specific to certain chromosomal regions (Goud, 1967) containing genes for chlorophyll development and has been reported to induce a high frequency of chlorophyll mutations (Swaminathan *et al.,* 1962; Kawai and Sato 1969; Marki and Bianu 1970; Sharma and Sharma 1984; Bhattacharya 2003; Shah *et al.,* 2006).

Five different chlorophyll mutants were observed in M_2 generation when the seedlings were 7-15 days old. The spectrum of M_2 chlorophyll mutants included albino, xantha, chlorina, maculata, and viridis are presented in Table 2. In gamma treatment alone, the frequency of xantha mutants was the highest followed by chlorina, albino and viridis. The highest frequency of xantha mutants in the gamma ray treatment is due to the genes for xanthophylls development that are readily available for mutagenic action. Similar reports were already given by Lal *et al.* (2009), Khan *et al.* (2005), Haq (1990). With respect to gamma + EMS, chlorina recorded the highest frequency followed by xantha, albino, viridis, and maculata (Figure.2.). The occurrence of chlorina mutants in a large number of crops has been attributed to different causes such as impaired chlorophyll biosynthesis, further degradation of chlorophyll and bleaching because of a deficiency of carotenoids (Bevins *et al.,* 1992). The least frequency of maculata type of chlorophyll mutant (8%) was recorded only in the combination treatment of 200 Gy+20 mM EMS.

Among the treatments, combination treatments (Gamma ray + EMS) induced the highest frequency and widest spectrum of chlorophyll mutations. Akin synergistic effect of combination treatment was observed by Khalatkar and Bhatia, (1975) in Barley, Khursheed *et al.* (2015) in *Vicia faba* L., Bind *et al*. (2016) in cowpea. Synergistic effects of combined treatments have been attributed by Sharma, (1970) to either, the first mutagen treatment makes accessible otherwise nonavailable sites for reaction to the second mutagen; pre-mutational lesions induced by the first mutagen become fixed due to an inhibitory effect of the second mutagen on repair enzymes.

It is concluded that gamma ray alone and a combination of gamma +EMS induces rational chlorophyll mutations, thus every single treatment could be used in mutation breeding programs for identifying trait of interest in the mutant population.

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Table 1. Frequency of Chlorophyll mutations in M_2 generation of CO 6 variety

Mutagen dose	M_1 Plants Progenies forwarded	M_1 segregants for mutation	M_2 Plants scored	M_2 mutant	Mutation frequency (%)	
					M_1 Plant basis	M_2 plant basis
Gamma ray (Gy)						
Control	250	-	6740	-	-	-
200 Gy	600	38	17024	71	6.33	0.42
300 Gy	600	53	16531	94	8.83	0.57
400 Gy	600	61	15306	120	10.17	0.78
Combination [Gamma ray (Gy) **+ EMS (mM)]**						
Control	250	-	6572	-	-	-
200 Gy+20 mM	200	45	4910	50	22.5	1.02
200 Gy+30 mM	200	27	4367	48	13.5	1.10
300 Gy+20 mM	200	17	3832	30	8.5	0.78
300 Gy+30 mM	200	12	3453	28	6	0.81
400 Gy+20 mM	200	10	2281	25	5	1.10
400 Gy+30 mM	200	13	1115	28	6.5	2.51
Total	3500	276	82131	494		

Table 2. Spectrum and Frequency of chlorophyll mutations in CO 6 Blackgram variety in different mutagenic treatment

Mutagen Gamma ray (Gy)	Total Chlorophyll Mutants in M_2 generation	The relative percentage of chlorophyll mutants (%)				
		Albino	Xantha	Chlorina	Maculata	Viridis
Gamma ray (Gy)						
200 Gy	71	15.5	32.40	35.21	-	16.90
300 Gy	94	19.14	37.23	28.72	-	14.90
400 Gy	120	20.00	33.33	31.66	-	15
Combination [Gamma ray (Gy)**+ EMS (mM)]**						
200 Gy+20 mM	52	30.76	25	19.23	8	17.30
200 Gy+30 mM	48	10.42	31.25	41.67	-	16.67
300 Gy+20 mM	30	10	26.67	50	-	13.33
300 Gy+30 mM	28	17.86	28.57	42.85	-	10.71
400 Gy+20 mM	25	24	20	28	-	28
400 Gy+30 mM	28	25	14.29	42.86	-	17.85

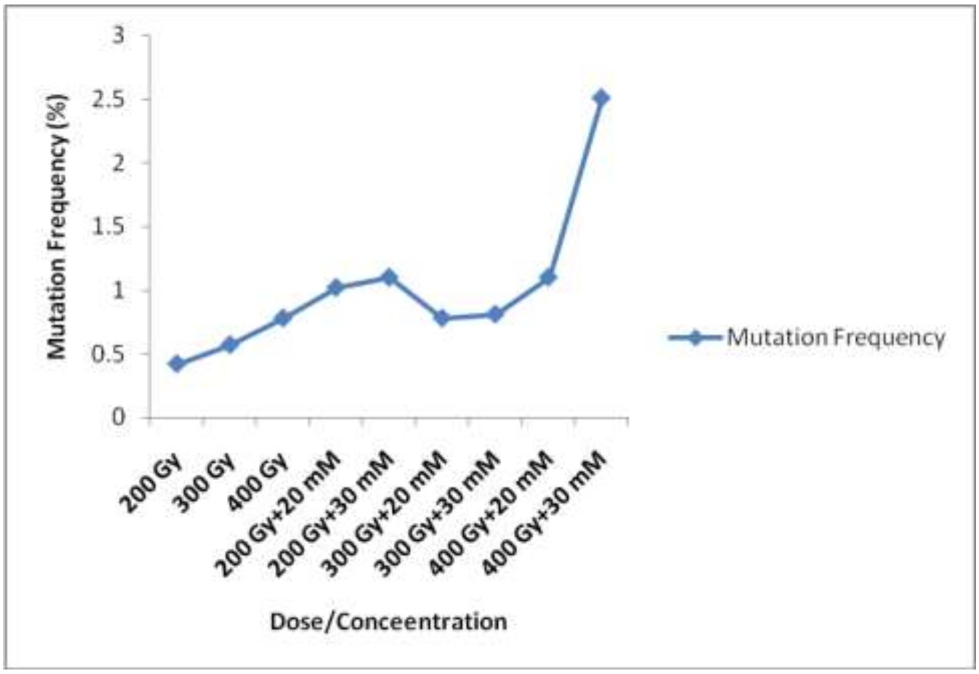

Fig. 1. Frequency of chlorophyll mutants in M_2 generation of CO 6 variety

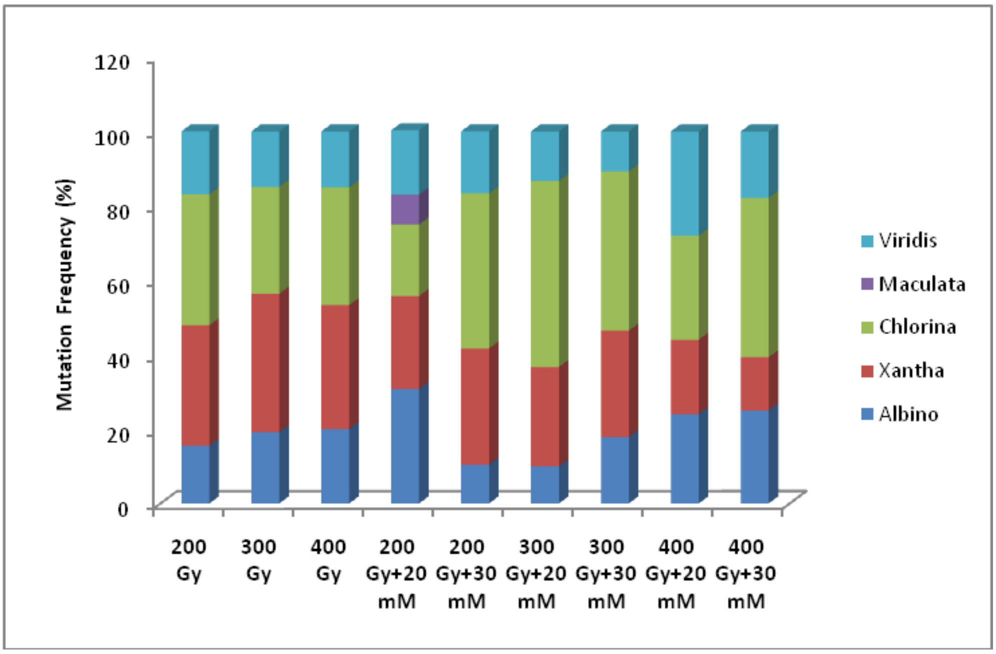

Fig. 2. Spectrum and Frequency of chlorophyll mutants in M_2 generation of CO 6 variety

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