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Mutagenic Effects of Gamma Rays and EMS on Frequency and Spectrum of Chlorophyll Mutations in Urdbean (*Vigna Mungo* (L.) Hepper)

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Abstract

Chlorophyll mutations act as a significant index in the judgment of induced genetic variations in mutagen treated populations. Different types of chlorophyll mutation have been observed in various crop plants. In the current study, the effect of different concentrations (40kR, 50kR and 60kR) of gamma rays, Ethyl Methane Sulphonate (50mM, 60mM and 70mM) in single and combination dose/concentration on the frequency and spectrum of chlorophyll mutation and the effect of VBN 4 urdbean variety to such irradiation dose was observed. Results showed induction of broad spectrum of chlorophyll mutations which included albina, xantha, chlorina and viridis. Among these chlorina type was predominant in all the mutagenic treatments. The albina type of chlorophyll mutants occurred very rarely and was found only at 60mM of EMS treatment and at 40kR+50mM, 60kR+70mM of combination treatments. Based on the chlorophyll mutation frequency, gamma rays were most effective followed by EMS and combination of treatments.

Keywords: Chlorophyll Mutation, EMS, Gamma Rays, Mutagenic Effects, *Vigna mungo*

1. Introduction

Blackgram or Urdbean (*Vigna mungo* (L.) Hepper) is a highly self pollinated crop with cliestogamy up to 42%[1]. Urdbean is grown all over the South East Asia. It is a rich source of protein (20.8 to 30.5%); its total carbohydrates range from 56.5 to 63.7%. It was used up in the form of split pulse as well as whole pulse, which is an important supplement of cereal based diet. In addition, being a significant source of human food and animal feed, it also acts significant role in sustaining soil fertility by enhancing soil physical properties. Being a drought resistant crop, it is appropriate for dry land farming and predominantly used as intercrop cultivation with other crops. Due to the lack of sufficient natural variability for yield and its components in urdbean, conventional methods have a limited scope. Gustafsson[2] advocated that mutation approach was superior to other methods of crop improvement. It has been observed that induced mutations can increase yield as well as other quantitative traits in plants. Many physical and chemical mutagens have been used for induction of useful mutants in a number of crops. Induction of chlorophyll mutations are act as the most reliable indices for evaluating the efficiency of various mutagens, besides inducing the genetic changes for crop development. It can also be used as genetic symbol in basic and applied research[3]. In induced mutation studies various types of chlorophyll mutation have often been reported in different crop plants including blackgram[4–6]. In the present investigation, an attempt was made to study the effect of three doses each of gamma rays and EMS and nine

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doses of combination treatment on the spectrum and frequency of chlorophyll mutations in the M_2 generation and the response of urdbean variety to such irradiation.

2. Materials and Methods

Seeds of VBN 4 blackgram were subjected to three different doses of gamma rays *viz*., 40kR, 50kR and 60kR, EMS 50mM, 60Mm and 70mM and combination treatments *viz*., 40kR+50mM, 40kR+60mM, 40kR+70mM, 50kR+50mM, 50kR+60mM, 50kR+70mM, 60kR+50mM, 60kR+60mM, 60kR+70mM. To raise M_1 generation, a total of fifteen treatments along with the control were sown in the field at the rate of 150 seeds for each treatment at a spacing of 30 x 15cm at Agricultural College and Research Institute, Madurai during August, 2010 in Randomized block design (RBD) with 3 replications. M_2 generation was raised on M_1 plant basis following plant to progeny method in a RBD with 3 replications during the month of January, in the year 2011. All the recommended agronomic practices were carried out during the growth period of the crop. Frequency and spectrum of chlorophyll mutations was worked out per 100 M_2 seedling basis. The chlorophyll mutants were differentiated in accordance with the system of Gustaffson[7]. Mutations frequency was worked out by the following methods given by Gaul[8]:

$$\text{Mutation frequency} = \frac{\text{No. of mutated plants}}{\text{Total number of plants}} \times 100$$

3. Results and Discussion

Micro-mutants play an important role to assess the dose/concentration of mutagens. Chlorophyll development has been suggested earlier to be controlled by several genes located on many chromosomes, which could be bordering to centromere and proximal part of chromosomes[9]. Mutations in these chlorophyll genes are expressed in the M_2 and the coming after generations in the form of various types of mutants. To estimate the chlorophyll mutation, periodical scoring was carried out starting from 5th day to 15th day after sowing[10]. The occurrence of chlorophyll mutations after treatments with physical and chemical mutagens have been reported in crops by different workers, *viz*., Deepalakshmi and Ananda Kumar[11], Hemavathy and Ravindran[12], Arvind Kumar et al.[13], Senapati et al.[4], Arulbalachandran et al.[14] and Anbu Selvam et al.[6] in blackgram, Lourdswamy and Rathnaswamy[15] in lab, Singh et al.[16], Singh et al.[17] and Chontira et al.[18] in greengram, Singh et al.[19] in lentil

3.1 Frequency of Chlorophyll Mutants

The mutagenic effectiveness of any given mutagen is fully explicit only from the frequency of induced mutations and induced chlorophyll mutation frequency is a very dependable index for evaluating the efficiency of mutagenic treatments, destined to generate wide array of variability. The chlorophyll mutation frequency was estimated as percentage of plants that segregated for chlorophyll deficiency on the basis of M_2 seedlings and presented in Table 1 (Figure 1). The gamma rays induced chlorophyll mutant frequency in VBN 4 ranged from 1.60 in 50kR to 3.10 per cent in 60kR. In case of EMS treated population, the frequency of chlorophyll mutations varied between 1.97 per cent in 60mM to 3.61 per cent in 50mM. In combination treatments, the frequency of chlorophyll mutations varied from 0.82 per cent in 50kR+70mM to 4.97 per cent in 60kR+70mM. The highest mutation frequency (4.97) was observed at 60kR+70mM combination treatment. The higher effectiveness of combined treatments in inducing greatest frequency of chlorophyll mutations has been observed by several workers in barley[20], Gautam et al.[21] and Singh et al.[22] in blackgram and Singh and Singh[23] in greengram. The frequency of chlorophyll mutations recorded in the current investigation was very low and also fluctuating u nder various treatments. Similar kind of results was observed by Ignacimuthu and Babu[24] in cultivated bean and Khan and Tyagi[25] in soybean. The frequency of induced chlorophyll mutations in M_2 generation has been considered as an important index for noting the potency of mutagens due to greater accuracy in their scoring[7,26]. The chlorophyll mutation acts not only as a scale for evaluating effectiveness and efficiency of mutagens, but also as indicators to predict the size of vital factor mutations.

3.2 Spectrum of Chlorophyll Mutants

Chlorophyll mutations spectrum are computed on M_2 plant basis and the results are presented in Table 2 (Figure 2). Different types of chlorophyll mutations *viz*., Albina, Chlorina, Xantha and Viridis were observed in the M_2 generation. Almost all the mutagenic treatments showed different degree of mutants with respective dose.

Table 1. Frequency of chlorophyll mutants in M_2 generation

Treatment	Number of M_2 seedlings		Mutation frequency
	Examined	Showing chlorophyll mutants	
		Gamma rays (kR)	
Control	342	--	--
40kR	287	5	1.74
50kR	249	4	1.60
60kR	193	6	3.10
		EMS (mM)	
Control	328	--	--
50mM	249	9	3.61
60mM	203	4	1.97
70mM	182	6	3.29
		Gamma rays + EMS	
Control	328	--	--
40kR+50mM	302	6	1.98
40kR+60mM	254	9	3.54
40kR+70mM	223	3	1.34
50kR+50mM	312	14	4.48
50kR+60mM	276	7	2.53
50kR+70mM	243	2	0.82
60kR+50mM	300	5	1.66
60kR+60mM	272	4	1.47
60kR+70mM	241	12	4.97

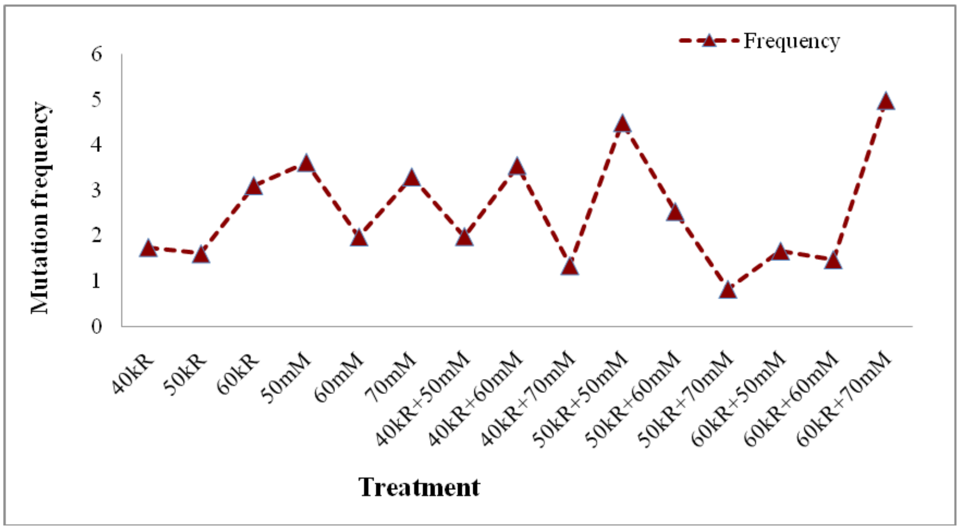

Figure 1. Frequency of chlorophyll mutants.

Albina was white in colour, due to absence of all pigment and relatively smaller than the normal seedlings. This was leaded to the death of the plants at 10-15 days after germination. Viridis was seedlings with whitish tips of leaves, leads to lethal were observed. Xantha was straw yellow seedling with normal growth in the beginning but it was started withering after 12-15 days. Chlorina was light green coloured seedlings, lethal (10-12 days). In all the three treatments, xantha and chlorina occurred in greater

Table 2. Spectrum of chlorophyll mutants in M_2 generation

Treatment	Total number of mutants	Spectrum (Relative %) of Chlorophyll Mutants			
		Albina	Xantha	Chlorina	Viridis
Gamma rays (kR)					
40kR	5	--	33.33	44.45	22.22
50kR	4	--	58.33	33.33	8.33
60kR	6	--	40.00	60.00	--
EMS (mM)					
50mM	9	--	33.33	66.67	--
60mM	4	25.00	--	75.00	--
70mM	6	--	16.67	72.13	11.20
Gamma rays + EMS					
40kR+50mM	6	33.33	16.67	33.33	16.67
40kR+60mM	9	--	54.55	36.00	9.09
40kR+70mM	3	--	33.33	66.66	--
50kR+50mM	14	--	28.57	57.14	14.29
50kR+60mM	7	--	25.00	50.00	25.00
50kR+70mM	2	--	50.00	50.00	--
60kR+50mM	5	--	40.00	60.00	--
60kR+60mM	4	--	28.57	57.14	14.29
60kR+70mM	12	6.66	33.33	43.33	16.68

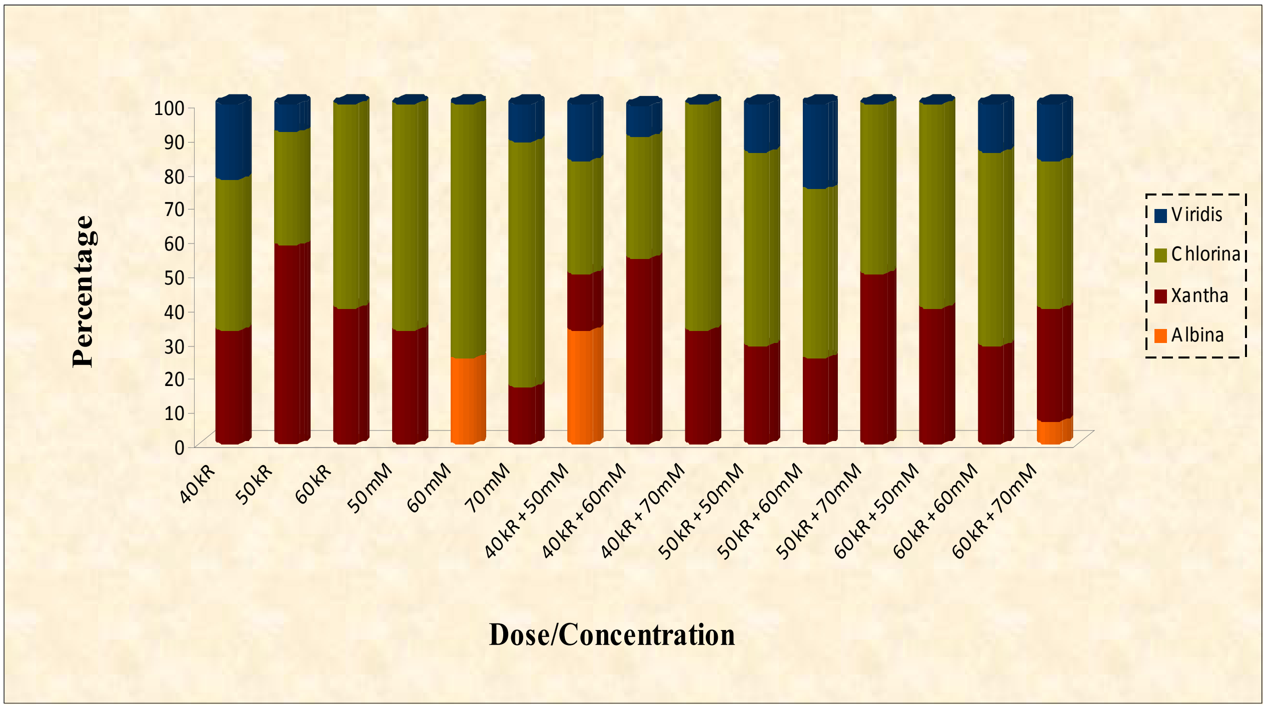

Figure 2. Spectrum of chlorophyll mutants.

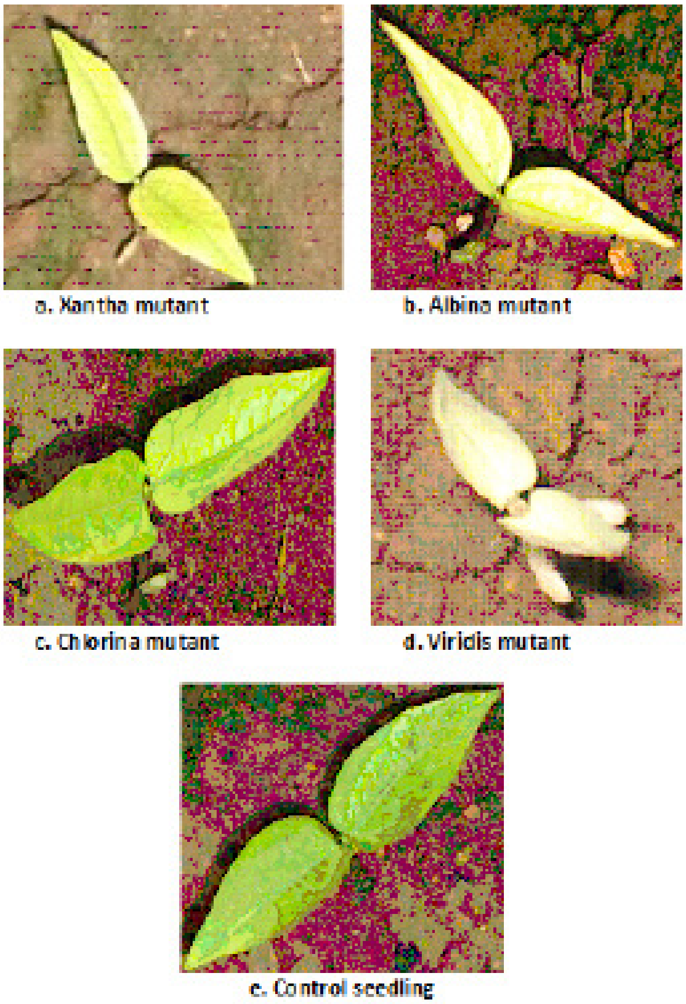

Figure 3. Chlorophyll mutants in M_2 generation of VBN 4 blackgram.

proportion among the different types of chlorophyll mutants. The higher proportion of chlorina was in agreement with findings of Singh et al.[17] and Singh and Tejeswar Rao[27] in greengram and Senapati et al.[4] in blackgram. The albina type of chlorophyll mutants occurred only at 60mM of EMS and 40kR+50mM, 60kR+70mM of combination treatments and it was not found to be present in any one of the gamma ray treatments. The occurrence of Albina was very rare and could be clarified with the report of Shah[28] who suggested that the occurrence of Albina was a rare sequence in Leguminosae. Hemavathy and Ravindran[12] also reported about the rare occurrence of albina in blackgram. The albina seedling itself has no practical value; however, such seedlings may be used as genetic markers for estimation of natural selfing. The phenomenon of albinism is rarely exhibited by plants which characteristic deficiency of chlorophyll and subsequent whitish-yellow colour of entire selling[5]. The occurrence of Viridis was high at 50kR+60mM of combination treatment. It can be represented as Chlorina > Xantha > Viridis > Albina (Figure 3). Similar results have also been observed in an earlier study[29]. Albina, chlorine and xantha mutants were recorded in lentil with effect of chemical mutagen treatment[30]. Different type of the chlorophyll mutants *viz.*, Albina, Chlorine, Viriscence and Xantha in the segregating M_2 plants based on the intensity of color pigmentation at the seedling stage in the varieties in cowpea was observed[3]. These classes of chlorophyll mutations were studied in mungbean[31], chickpea[32] and in grasspea[33].

In soybean, Khan and Tyagi[25] reported four types of chlorophyll mutants. Elmer[34] suggested that the mitochondrial malate dehydrogenase enzyme activity due to irradiation restricts the production of ἀ-keto glutaric acid, a precursor of glutamic acid which is an essential intermediary in chlorophyll biosynthesis. Xing et al.[35] opined that chloroplasts in albina leaves were normal but the inner structure was simpler and lacked a rich and highly organized internal thylakoid system. Only stroma and DNA-like fibrils were observed. The development of chloroplast is thus obviously delayed, leading to chlorophyll deficient mutants.

Induced mutations have been recognized as an important tool for crop improvement, and have sufficient scope in pulses. Chlorophyll mutants inferred in this investigation, how chlorophyll gene response to mutagen. Mutations in these chlorophyll genes are expressed in the M_2 and coming after generations in the form of various types of mutants.

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