LR-4200
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RESEARCH ARTICLE Legume Research - An International Journal

Study of Chlorophyll and Macro mutations Induced by Physical Mutagens in Black Gram [*Vigna mungo* (L.) Hepper]

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10.18805/LR-4200

ABSTRACT

Induction of mutation played a vital role in the crop improvement among methods of plant breeding. Genetic improvement through induced mutation was found to be very effective for induction of variability in the quantitative and qualitative characters. The mutagenic effect of gamma rays (200Gy, 300Gy and 400Gy) and Electron beam (200 Gy, 300Gy and 400 Gy) alone or in combination (200+200Gy, 300+300 Gy and 400+400 Gy) on frequency and spectrum of chlorophyll and micromutations in cultivar, ADT 3 and CO 6 of blackgram was studied. Micro and macro-mutants play an important role to assess the effectiveness of the mutagens. The individual treatment of electron beam was found to be more efficient than gamma rays and combined treatment to induce chlorophyll mutants. Five different types of chlorophyll mutants such as, chlorina, albino, xantha, viridis and xanthoviridis in M_2 generation were observed. The chlorophyll mutants were scored from 7 to 10 days after sowing. Among the chlorophyll mutations, xanthoviridis was the most frequent mutant in both the varieties. The highest frequency of chlorophyll mutants was reported at dose of 200 Gyelectron beam. Thus, it is concluded that the lower doses of mutagens are more useful to induce different type of chlorophyll mutants.

Key words: Blackgram, Chlorophyll mutations, Electron beam, Gamma rays.

INTRODUCTION

Mutation breeding is relatively a quicker method for improvements of crops. It has been observed that induced mutations can increase yield as well as other quantitative traits in plants. Black gram is an important kharif crop in India grown on about 2.7 lakh hectares. The seeds are mostly consumed by the people owing to its high protein content (Akhaury, 1991). The natural productivity of black gram is only 480 kg/ha (Chaturvedi and Ali, 2002). This low yield may be due to narrow genetic base and non availability of high yielding varieties with disease resistance. Natural variability is an essential pre-requisite for any successful breeding programme. Mutation breeding is a supplementary breeding programme to identify the mutants with high yield potential, early maturity, disease and pest resistance (Singh, 1981). The choice of mutagen holds great importance in changing the frequency and spectrum of chlorophyll mutations in a predictable manner.

Chlorophyll mutations although not useful for plant breeding purpose, may be used to assess the efficiency and effectiveness of mutagens in order to select suitable mutagen at appropriate concentration so as to use them in applied mutagenesis programme. Physical mutagens such as gamma rays and electron beam is used in induction of variability. Present investigations were undertaken to study the comparative effectiveness of these mutagens under similar treatment conditions. In the present study, the effect of gamma rays, electron beam and combined treatment (Electron beam + Gamma rays) employed singly or in combinations was studied on frequency and spectrum of chlorophyll mutations and viable macro mutations in M_2 generation of blackgram.

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How to cite this article: Lavanya, S.A., Vanniarajan, C. and Souframanien, J. (2020). Study of Chlorophyll and Macro mutations Induced by Physical Mutagens in Black Gram [*Vigna mungo* (L.) Hepper]. Legume Research.

Submitted: 22-07-2019 **Accepted**: 02-06-2020 **Published**:

MATERIALS AND METHODS

The dried seeds of the blackgram varieties ADT 3 and CO 6 were treated with 200Gy, 300Gy and 400 Gy doses of gamma irradiation using ^{60}CO gamma sourcefor appropriate time at the Bhabha Atomic Research Mumbai, India. For each treatment, well filled 500 seeds with uniform moisture content was used. For electron beam, the seeds of ADT3 and Co6 were treated with 200Gy, 300Gy and 400 Gy doses using 10 MeV electron beam from electron accelerator facility at Electron Beam Centre, Bhabha Atomic Research Centre, Kharghar, Navi Mumbai, India.

Dry, healthy and uniform sized seeds of blackgram variety ADT 3 and CO 6 were exposed with Electron beam and then same seeds were treated with gamma rays for combined treatment. The treated seeds with control were sown in germination trays at germination room, Plant Breeding and Genetics department, Agricultural College and

Research Institute, Madurai. Lethal dose (50%) was calculated byprobit analysis using the germination data.

After completion of the treatment with gamma rays, electron beam and combined treatment (Electron beam + Gamma rays) and their respective control seeds were sown immediately to raise the M_1 generation in a randomized block design (RBD) with two replications. Different biological parameters like germination, survival of plant, pollen fertility, plant height on 30th day, seed fertility was recorded in randomly selected plants in each treatment in M_1 generation and harvested on single plant basis.

For raising of M_2 generations, the seeds of M_1 generation were space planted in the field in two replications. M_2 generation was screened for lethal chlorophyll mutations during the first four weeks, after germination. Whereas, viable chlorophyll and macro mutants were scored throughout the crop duration. The population was screened for chlorophyll and macro mutations according to the procedure given by Gustafesson (1947) with suitable modifications. The spectrum was recorded as *xantha, chlorina, viridis*and *albina*. The *xantha* mutants displayed a bright yellow to deep golden yellow colour. *Chlorina* mutants were yellowish green in colour, the *viridis* mutants displayed light green colour and *albina*shows white colour. The chlorophyll mutants like *xantha, chlorine* and *viridis.* Mutations frequency was calculated by the following methods given by Gaul, (1957).

$$\text{Mutation Frequency based on } M_1 (\%) = \frac{\text{Number of Chl. mutant } M_1}{\text{Total number of } M_1} \times 100$$

$$\text{Mutation Frequency based on } M_2 (\%) = \frac{\text{Number of Chl. mutant } M_2}{\text{Total number of } M_2} \times 100$$

RESULTS AND DISCUSSION

Frequency of chlorophyll mutations observed in the M_2 generation of the varieties ADT 3 and CO 6 is provided in Table 1. The chlorophyll mutation frequency on the M_2 plant basis increased with the increase in the dose of gamma rays, electron beam and their combination (electron beam + gamma rays). The increased cholorophyll mutation frequency at higher doses may be attributed to the chromosomal aberrations or saturation in the mutational events which may result in the elimination of mutant cells during growth (Brock., 1965). Electron beam is most effective compared to other treatment.

In ADT 3, the chlorophyll mutants occurred in all the treatments. In gamma irradiated population, 200 Gy treatment recorded maximum frequency of chlorophyll mutation on M_1 plant basis (38.67 per cent) and M_2 plant basis (1.56 per cent). In electron beam treatment, 400 Gy treatment exhibited the maximum frequency of chlorophyll mutation on M_1 plant basis (48.15 per cent) while 200 Gy (1.70 per cent) registered higher frequency on M_2 seedling basis. In combined treatment (electron beam + gamma rays), the chlorophyll mutation frequencyin treatment 300 Gy + 300 Gy and 200 Gy + 200 Gy recorded maximum frequency of chlorophyll mutation on M_1 plant basis (40.51 per cent) and M_2 plant basis (1.66 per cent). In CO 6, the gamma irradiated population, 200 Gytreatment recorded maximum frequency of chlorophyll mutation on M_1 plant basis

Table 1: Chlorophyll mutation frequencies in M_2 generation in ADT 3 and CO 6.

Mutagen dose	No. of M_1 plants				No. of M_2 seedlings				Mutation frequency (%)			
	Plants forwarded		Segregating		Studied		Chlorophyll mutants		M_1 plant basis		M_2 seedling basis	
Gamma rays												
	ADT 3	CO 6	ADT 3	CO 6	ADT 3	CO 6	ADT 3	CO 6	ADT 3	CO 6	ADT 3	CO 6
Control	50	50			1450	1450						
200 Gy	225	212	87	74	7300	6782	114	107	38.67	34.91	1.56	1.58
300 Gy	198	186	68	61	6240	6177	89	78	34.34	32.80	1.43	1.26
400 Gy	143	152	53	51	5290	5345	68	62	37.06	33.55	1.29	1.16
Electron beam												
	ADT 3	CO 6	ADT 3	CO 6	ADT 3	CO 6	ADT 3	CO 6	ADT 3	CO 6	ADT 3	CO 6
Control	50	50			1450	1450						
200 Gy	252	232	104	88	7960	7605	135	112	41.27	37.93	1.70	1.47
300 Gy	204	197	96	83	6973	6510	92	85	47.06	42.13	1.32	1.31
400 Gy	162	156	78	68	5749	5468	71	67	48.15	43.59	1.23	1.23
Electron beam + Gamma rays												
	ADT 3	CO 6	ADT 3	CO 6	ADT 3	CO 6	ADT 3	CO 6	ADT 3	CO 6	ADT 3	CO 6
Control	50	50			1450	1450						
200 Gy + 200 Gy	202	193	75	71	6460	6457	107	93	37.13	36.79	1.66	1.44
300 Gy + 300 Gy	158	142	64	60	5470	5260	86	71	40.51	42.25	1.57	1.35
400 Gy + 400 Gy	128	112	51	49	4840	4833	67	56	39.84	43.75	1.38	1.16

(34.91per cent) and M_2 plant basis (1.58 per cent). In electron beam treatment, 400 Gy treatment exhibited the maximum frequency of chlorophyll mutation on M_1 plant basis (43.59per cent) while 200 Gy (1.47per cent) registered higher frequency on M_2 seedling basis. In combined treatment (electron beam + gamma rays), the treatment 400 Gy + 400 Gy and 200 Gy + 200 Gy recorded maximum frequency of chlorophyll mutation on M_1 plant basis (43.75per cent) and M_2 plant basis (1.44per cent).

In the present study, chlorophyll mutants were scored on M_1 plant and M_2 seedling bases. Of the two methods of estimating the frequency of mutations, M_2 seedling basis was considered as the best index (Gaul, 1960). The concept was reflected in present investigation wherein the high frequency of chlorophyll mutants was found at lower doses in ADT 3and CO 6 on M_1 plant basis and M_2 seedling basis in both gamma rays,electron beam and combined treatment (electron beam + gamma rays). Electron beam treatments produced maximum chlorophyll mutants than gammarays and combined treatment (electron beam + gamma rays) in both the varieties. Similar results were obtained in blackgram (Deepalakshmi and Ananda Kumar 2004; Thilagavathi and Mullinathan 2009) and in greengram (Sing *et al.* 2005; Awnindra and Sing 2007; Vairam *et al.* 2014).

The spectrum of chlorophyll mutants induced by gamma rays,electronbeam and their combination included albrina, chlorina, Xanthaviridis, virids and xanthan (Table 2). In gamma rays irradiated population of ADT 3, the occurrence of albino was very high followed by xantha and chlorina than the other types. The order of relative percentage of different chlorophyll mutants occurrence was Xanthaviridis (from 26.47 in 400 Gy to 29.21 per cent in 300Gy) > albino (from 8.99 in 300 Gy to 25.00 per cent in 400 Gy) >xantha (from 22.06 in 400 Gy to 23.68 per cent in 200 Gy) >chlorina (from 16.18 in 400 Gy to 20.22 per cent in 300 Gy) >viridis (from 10.29 in 400 Gy to 17.98 per cent in 300 Gy). Albino mutants were more in higher doses, whereas xanthaviridis, xantha, chlorina and viridis were higher at lower doses. In electron beam irradiated population, the order of relative percentage of different chlorophyll mutants occurrence was xanthaviridis (from 33.33 in 200 Gy to 35.21 per cent in 400Gy) >xantha (from 18.31 in 400 Gy to 22.83 per cent in 300 Gy) >chlorina (from 14.08 in 400 Gy to 20.65 per cent in 300 Gy) >viridis (from 14.13 in 300 Gy to 17.78 per cent in 200 Gy) > albino (from 8.70 in 300 Gy to 15.49 per cent in 400 Gy). Albino mutants were higher in higher doses. In combined treatment (electron beam +gamma ray) irradiated population, the order of relative percentage of different chlorophyll mutants occurrence was xanthaviridis (from 26.87 in 400 Gy +400 Gy to 33.72 per cent in 300Gy + 300 Gy) >xantha (from 22.09 in 300 Gy + 300 Gy to 23.36 per cent in 200 Gy + 200 Gy) >viridis (from 19.63 in 200 Gy + 200 Gy to 20.90 per cent in 400 Gy +400 Gy) > albino (from 8.14 in 300 Gy+ 300 Gy to 14.93 per cent in 400 Gy+ 400 Gy).

In CO 6, Xanthaviridis and xantha occurred in higher proportion followed by viridis, albino and chlorinafor gamma rays, electron beam and combined treatments. The frequency of xanthaviridis ranged from 30.65 per cent (400 Gy) to 35.90 per cent (300 Gy). In electron beam, the frequency of xanthaviridis ranged from 28.24 per cent (300 Gy) to 34.82 per cent (200 Gy). In combined treatment (electron beam + gamma ray), the frequency of xanthaviridis ranged from 30.36 per cent (400 Gy + 400 Gy) to 31.18 per cent (200 Gy + 200 Gy)

In ADT 3, the chlorophyll mutants occurred in all the treatments. Gamma rays, electron beam and combined treatment (electron beam + gamma rays) exhibited

Table 2: Frequency of different types of chlorophyll mutants in M_2 generation in ADT 3 and CO 6.

Mutagen	Total chlorophyll mutants in M_2 generation		Relative percentage of chlorophyll mutants (%)									
Gamma rays			Albino		Xantha		Chlorina		Viridis		Xanthaviridis	
	ADT 3	CO 6	ADT 3	CO 6	ADT 3	CO 6	ADT 3	CO 6	ADT 3	CO 6	ADT 3	CO 6
200 Gy	114	107	10.53	8.41	23.68	22.43	20.18	14.95	16.67	19.63	28.95	34.58
300 Gy	89	78	8.99	15.38	23.60	15.38	20.22	15.38	17.98	17.95	29.21	35.90
400 Gy	68	62	25.00	6.45	22.06	14.52	16.18	30.65	10.29	17.74	26.47	30.65
Electron beam												
	ADT 3	CO 6	ADT 3	CO 6	ADT 3	CO 6	ADT 3	CO 6	ADT 3	CO 6	ADT 3	CO 6
200 Gy	135	112	11.11	11.61	20.74	21.43	17.04	15.18	17.78	16.96	33.33	34.82
300 Gy	92	85	8.70	17.65	22.83	22.35	20.65	15.29	14.13	16.47	33.70	28.24
400 Gy	71	67	15.49	14.93	18.31	22.39	14.08	17.91	16.90	13.43	35.21	31.34
Electron beam + Gamma rays												
	ADT 3	CO 6	ADT 3	CO 6	ADT 3	CO 6	ADT 3	CO 6	ADT 3	CO 6	ADT 3	CO 6
200 Gy + 200 Gy	107	93	8.41	7.53	23.36	23.66	16.82	12.90	19.63	24.73	31.78	31.18
300 Gy + 300 Gy	86	71	8.14	7.04	22.09	23.94	16.28	14.08	19.77	23.94	33.72	30.99
400 Gy + 400 Gy	67	56	14.93	7.14	22.39	23.21	14.93	16.07	20.90	23.21	26.87	30.36

maximum number of xanthaviridis mutants while xanthaand viridis were intermediate in its occurrence. Chlorina and albino occurred at least proportion. Similar finding was reported in cowpea (Devmani Bind *et al.* 2016). The occurrence of chlorophyll mutants was higher in electron beam while compared to gamma ray treatments. Similar results were reported in blackgram (Souframanien *et al*. 2016; Loyavar Ram chander *et al.* 2017) and in greengram(Singh *et al.* 2005; Singh, 2007). In Co 6,xanthaviridis and xantha occurred in higher proportion followed by viridis, chlorina and albino in all the treatments of gamma rays, electron beam treatments and combined treatment (electron beam + gamma rays). Similar finding was observed by Gautam and Mittal, 1998 in blackgram; Devmani *et al*., 2016 in cowpea. To increase the mutation frequency and mutagenic rate of specific mutagen, appropriate concentrations will be effective (Auti, 2005). In the present study, chlorophyll mutants were high in electron beam treatment than Gamma rays and combined treatment. The efficiency was found to be highest at lower and intermediate doses of mutagenic treatments. These results suggest that high mutation rates could be obtained with moderate dose of mutagen in blackgram.

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