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STUDIES ON EFFECTIVENESS AND EFFICIENCY OF GAMMA RAYS IN BLACK GRAM (*Vigna mungo* (L.) HEPPER)

M. DHASARATHAN[1]*, S. GEETHA[2], N. MEENAKSHIGANESAN[3] AND D. SASSIKUMAR[4]
[1]Centre for Plant Breeding and Genetics,
Tamil Nadu Agricultural University (TNAU), Coimbatore - 641 003, Tamil Nadu
e-mail: maadhasarathan@gmail.com

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***Corresponding author**

ABSTRACT
The study was undertaken to assess the effectiveness and efficiency of gamma rays on three different varieties of black gram. Genetically pure, uniform and dry seeds of black gram varieties *viz*., ADT 3, CO 6 and TU 17-9 were treated with four different doses (200, 300, 400, and 500 Gy) of gamma rays. The biological damage based on lethality, injury and sterility were estimated in the M_1 generation. Both chlorophyll and morphological mutations were studied in the M_2 generation to calculate the effectiveness and efficiency of gamma rays based on biological damage in M_1. Differential response of varieties and varied doses of mutagenic treatment in induction of macromutation in M_2 generation was observed among the varieties. The degree of effectiveness and efficiency varied among different mutagenic doses and the varieties. Higher degree of effectiveness and efficiency at lower and intermediate doses of mutagen and optimum dose was found at 200 Gy, were observed among three varieties which may be due to less biological damage.

INTRODUCTION

Induced mutagenesis plays an important role in improvement of crops like black gram, where a large part of genetic variability has been eroded due to its continuous cultivation in marginal and sub-marginal land and its adaption to survival fitness rather than yield (Mishra and Singh, 2014). Further, hybridization in this crop is difficult due to its small cleistogamous flower. Induction of mutation provides the scope of creating desired traits in this crop.

The usefulness of any mutagenic agent in plant breading not only depends on the ability to induce mutations but also on its potentiality to induce larger proportion of desirable changes as compared to the undesirable ones (Dhasarathan *et al*., 2015). Ahloowalia *et al*. (2004), Tah (2006), Kumari *et al*. (2013) and Adhirwar *et al*. (2014) reported that physical mutagens namely X-rays, gamma rays, fast neutrons, thermal neutrons, ultraviolet and beta radiations are frequently used for induced mutagenesis. They also reported that hereditary changes of desirable type in crop plants were higher with the use of gamma rays (64%) than by X-rays (22%).

Mutagenic effectiveness is a measure of the frequency of mutations induced with application of unit dose of a mutagen, while mutagenic efficiency refers to the proportion of mutations in relation to other associated undesirable biological effects such as gross chromosomal aberrations, lethality and sterility induced by the mutagen (Ehrenberg, 1960 and Konzak *et al*., 1965). According to Gregory (1961) "efficacy" relates to the potency of a particular mutagen to produce useful mutations. Therefore, mutagenic effectiveness and efficiency depend upon type of mutagen; its effect on the genotypes where it is applied (Sparrow, 1961; Wani, 2009; Makeen and Babu, 2010). The observation of non-random pattern of variation in mutagenic effectiveness and efficiency demonstrates that the genotypic response to different mutagens is of genetic origin and depends upon the physical and chemical properties of the mutagen (Khan, 1981). Therefore, this study was undertaken to gather information on effectiveness and efficiency of different doses of gamma rays in three varieties of black gram.

MATERIALS AND METHODS

Genetically pure, uniform and dry seeds of three black gram varieties ADT 3, CO 6 and TU 17-9 were taken for induction of mutation using gamma irradiation. Seeds of three varieties were irradiated with 4 different doses (200, 300, 400 and 500 Gy) of gamma rays at the Tamil Nadu Agricultural University. Treated seeds along with controls of three varieties were sown in field under randomized block design with two replication (M_1 generation) at National Pulses Research Centre, Tamil Nadu Agricultural University. Observations on germination percentage, plant height and pollen sterility were recorded at appropriate time.

About 50 M_1 plants from each treatment were used to raise the M_2 generation in non-replicated trail under normal package and practices. Observations on macromutations *i.e.* chlorophyll and morphological (viable) mutations were recorded from the day of emergence till the plants attained physiological maturity. The spectrum and frequency of macromutations observed in various doses of mutagen treated populations were estimated following standard methods (Konzak *et al.*, 1965) and the mutagenic efficiency and effectiveness were calculated using the formula indicated below;

Mutagenic effectiveness (%)

$$\text{Gamma rays} = \frac{M}{Gy} \times 100$$

Where,

M - Chlorophyll or viable mutation frequency on M_2 plant basis

Gy - Dose of gamma radiation

Mutagenic efficiency (%)

Gamma rays = M × 100 / L

M × 100 / I

M × 100/ S

Where,

M - Chlorophyll or viable mutation frequency on M_2 plant basis

L - Percentage of lethality *i.e.,* per cent reduction in survival of seedlings on 30th day in M_1

I - Percentage of injury *i.e.,* per cent height reduction of seedlings on 30th day in M_1

S - Percentage of sterility *i.e.,* per cent pollen fertility reduction of seedlings in M_1

RESULTS AND DISCUSSION

Chlorophyll mutation

Macromutations are generally helpful to evaluate the genetic effects of mutagen used. Gaul (1964) reported that chlorophyll mutations are employed as markers for the evaluation of gene action of mutagenic factors in inducing mutation. He also reported that the appearance of more number of viridis type mutations could be attributed to the involvement of polygenes in chlorophyll formation.

In this study, a wide spectrum of chlorophyll mutations were observed in mutagen treated populations of three varieties in M_2 generation which included albina, xantha, chlorina and viridis. No chlorophyll mutations were observed in controls (untreated population). The frequency of chlorophyll mutations in different mutagenic-treated populations varied from 0.89 % to 1.87 % in ADT 3, 1.10 % to 2.19 % in variety CO 6 and 1.36 % to 2.05 % in TU 17-9 (Table 1). Among different types of chlorophyll mutations, chlorina was the most frequent (0.65 % in ADT 3, 0.39 % in CO 6 and 1.84 % in TU 17-9) in three cultivars suggesting high mutability of the gene controlling the phenotype. The Albina type of chlorophyll mutation was the least frequent in the treated populations of ADT 3, CO 6 and TU 17-9. The average frequency of chlorophyll mutations in the variety ADT 3 was lower (5.25 %) in comparison to that of CO 6 (6.33 %) and TU 17-9 (7.06 %) suggesting differences between varieties to gamma treatment. The lowest frequency of chlorophyll mutations (0.89 %) was observed using the 200 Gy mutagenic treatment in the case of the variety ADT 3, CO 6 (1.10 %) and TU 17-9 (1.36 %) respectively. The declining trend in the frequency of mutations with increasing dose of the mutagen was not linear because they occur at random. This was consistent with the findings of Khan and Tyagi (2010) in soybean, they reported that both reduction and increase in chlorophyll mutation frequency in 2 different varieties treated with same mutagen and dose. In contrast, Singh and Singh, 2007; Goyal *et al.*, 2009 and Vaseline and Sabesan, 2011 reported the occurrence of lower frequency of mutations in higher doses, it could be due to induction of greater lethality at higher doses.

With respect to spectrum of chlorophyll mutations in three varieties, quantitative variation in the mutation frequency of

Table 1: Spectrum and frequency of different chlorophyll mutations in M_2 generation

Treatments	No of M_2 plants scored	*Albina*	*Xantha*	*Chlorina*	*Viridis*	Total	Frequency (%)
ADT 3							
200 Gy	1565	0	8	6	0	14	0.89
300 Gy	1549	0	12	14	3	29	1.87
400 Gy	1246	0	7	10	0	17	1.36
500 Gy	1332	0	5	7	3	15	1.13
Total	5692	0	32	37	6	75	5.25
Percentage		0.00	0.56	0.65	0.11	1.32	
CO 6							
200 Gy	1451	0	4	5	0	9	1.10
300 Gy	1314	0	5	8	0	13	1.67
400 Gy	1279	0	4	4	2	10	2.19
500 Gy	1023	0	5	3	0	8	1.37
Total	5067	0	18	20	2	40	6.33
Percentage		0.00	0.36	0.39	0.04	0.79	
TU 17-9							
200 Gy	662	0	9	7	0	16	1.36
300 Gy	650	0	8	14	0	22	2.00
400 Gy	488	2	10	13	3	28	2.05
500 Gy	485	0	6	8	0	14	1.65
Total	2285	2	33	42	3	80	7.06
Percentage		0.09	1.44	1.84	0.13	3.50	

Table 2: Spectrum and frequency of different morphological mutations in M_2 generation

Type of mutants	ADT 3				Total	Percentage	CO 6				Total	Percentage	TU 17-9				Total	Percentage
Treatments	200 Gy	300 Gy	400 Gy	500 Gy			200 Gy	300 Gy	400 Gy	500 Gy			200 Gy	300 Gy	400 Gy	500 Gy		
M_2 plant examined	1440	1363	1034	1026	4863		1371	1200	1124	871	4566		608	579	410	399	1996	
Tall	-	4	-	1	5	0.10	-	-	1	1	2	0.04	-	2	2	-	4	0.20
Dwarf	-	-	-	1	1	0.02	1	-	2	-	3	0.07	-	-	-	-	0	0.00
Erect stem	1	2	-	-	3	0.06	-	-	-	-	0	0.00	6	4	5	-	15	0.75
Spreading stem	-	-	-	1	1	0.02	-	1	-	-	1	0.02	-	-	-	-	0	0.00
Open type stem	-	1	1	-	2	0.04	-	-	-	-	0	0.00	-	-	-	-	0	0.00
Green stem	-	-	1	-	1	0.02	-	-	-	-	0	0.00	-	-	-	1	1	0.05
Pink stem	1	-	-	-	1	0.02	-	-	-	-	0	0.00	-	-	-	-	0	0.00
Branching pattern changed	-	-	-	-	0	0.00	-	-	3	-	3	0.07	-	-	-	-	0	0.00
Crinkled & leathery leaf	-	1	2	1	4	0.08	-	-	-	-	0	0.00	-	-	-	-	0	0.00
Narrow leaf	-	3	-	-	3	0.06	-	1	-	-	1	0.02	1	1	-	-	2	0.10
Ovate leaf	-	2	-	-	2	0.04	-	-	-	-	0	0.00	-	-	-	-	0	0.00
Pink margin leaf	-	-	1	1	2	0.04	-	-	-	-	0	0.00	-	-	3	-	3	0.15
Single foliate leaf	-	-	1	-	1	0.02	-	-	-	-	0	0.00	-	-	-	-	0	0.00
Early maturity	-	3	1	2	6	0.12	2	4	-	-	6	0.13	1	1	-	4	6	0.30
Glaborous	-	-	1	-	1	0.02	-	-	-	-	0	0.00	-	-	-	-	0	0.00
Hairy pods	-	-	-	-	0	0.00	-	-	-	-	0	0.00	-	-	-	-	0	0.00
increased pods numbers	14	11	6	9	40	0.82	10	6	7	5	28	0.61	3	4	3	4	14	0.70
Increased pod length	1	1	2	-	4	0.08	-	-	2	1	3	0.07	1	-	-	-	1	0.05
Short pods	1	2	1	-	4	0.08	1	-	1	1	3	0.07	-	-	-	-	0	0.00
Pod colour changed	-	1	1	1	3	0.06	-	-	-	3	3	0.07	-	-	-	-	0	0.00
Bold seeds	1	-	-	-	1	0.02	2	3	2	-	7	0.15	-	-	-	-	0	0.00
Small seeds	2	-	-	-	2	0.04	-	-	-	-	0	0.00	-	-	-	-	0	0.00
Brown seeds	-	1	1	-	2	0.04	-	-	-	1	1	0.02	-	-	-	1	1	0.05
Sterile plant	1	-	-	-	1	0.02	-	-	-	-	0	0.00	-	-	1	-	1	0.05
Leafy plant	-	-	-	1	1	0.02	-	-	1	-	1	0.02	-	-	-	-	0	0.00
Total	22	32	19	18	91	1.87	16	15	19	12	62	1.36	12	12	14	10	48	2.40
Frequency (%)	1.53	2.35	1.84	1.76	7.48		1.17	1.25	1.69	1.38	5.49		1.97	2.07	3.41	2.51	9.96	

Table 3: Mutagenic effectiveness and efficiency based on viable mutation on different varieties

Treatments	Per cent survival reduction on 30th day (Lethality)	Per cent height reduction on 30th day (Injury)	Per cent pollen fertility reduction (Sterility)	Viable mutant (M) per 100 M_2 seedlings	Effectiveness (M × 100)/ Gy	Mutagenic efficiency (M ×100)/ Lethality	(M ×100)/ Injury	(M ×100)/ Sterility	Average
ADT 3									
200 Gy	19.45	2.55	4.24	1.53	0.77	7.87	60.00	36.08	34.65
300 Gy	30.67	4.08	6.69	2.35	0.78	7.66	57.60	35.13	33.46
400 Gy	47.00	16.13	11.41	1.84	0.46	3.91	11.41	16.13	10.48
500 Gy	56.54	24.96	25.22	1.76	0.35	3.11	7.05	6.98	5.71
Average	38.42	11.93	11.89	1.87	0.59	5.64	34.02	23.58	21.08
CO 6									
200 Gy	16.27	3.18	3.45	1.97	0.99	12.11	61.95	57.10	43.72
300 Gy	32.26	5.87	4.24	2.07	0.69	6.42	35.26	48.82	30.17
400 Gy	49.79	16.96	12.86	3.41	0.85	6.85	20.11	26.52	17.83
500 Gy	55.16	25.51	31.46	2.51	0.50	4.55	9.84	7.98	7.46
Average	38.37	12.88	13.00	2.49	0.76	7.48	31.79	35.11	24.79
TU 17-9									
200 Gy	23.26	10.37	8.67	1.17	0.59	5.03	11.28	13.49	9.93
300 Gy	33.38	16.79	16.91	1.25	0.42	3.74	7.44	7.39	6.19
400 Gy	46.70	18.57	30.43	1.69	0.42	3.62	9.10	5.55	6.09
500 Gy	50.85	23.97	33.21	1.38	0.28	2.71	5.76	4.16	4.21
Average	38.55	17.43	22.31	1.37	0.43	3.78	8.40	7.65	6.61

different types of chlorophyll mutations was observed for different doses of gamma rays. This suggests that there are different varietal responses to the dose of mutagenic treatment. A similar differential response of varieties to mutagens and variation in frequency of chlorophyll as well as viable mutation frequency was also observed by Wani (2009) and Khan and Tyagi (2010).

Morphological Mutation

A wide spectrum of morphological mutations *i.e.* Tall, Dwarf, Erect stem, Spreading stem, Open type stem, Green stem, Pink stem, Branching pattern changed, Crinkled & leathery leaf, Narrow leaf, Ovate leaf, Pink margin leaf, Single foliate leaf, Early maturity, Glaborous, Hairy pods, increased pods numbers, Increased pod length, Short pods, Pod colour changed, Bold seeds, Small seeds, Brown seeds, Sterile plant, Leafy plant, were observed in M_2 generation of gamma treated population of all three varieties. No morphological mutations were observed in untreated controls of three varieties.

All the three varieties differed regarding to the quantity and spectrum of morphological mutations induced. The frequency of morphological mutations in different treatments varied from 1.53 % to 2.35 % in variety ADT 3, 1.17 % to 1.69 % in variety CO 6 and 1.97 % to 3.41 % (Table 2). Among different types of morphological mutations, the most frequent type was increased pod numbers in all the three varieties used. The frequency of morphological mutations like dwarf, spreading stem, green stem, pink stem, single foliate leaf, glaborous, bold seed and sterile plant was lower in the variety ADT 3, while spreading stem, narrow leaf and brown seeds was lower in variety CO 6 and green stem, increased pod length, brown seeds and sterile plant was lower in the variety TU 17-9. Considering the three varieties, the frequency of morphological mutations was higher in variety TU 17-9 (2.40 %) compared to other varieties ADT 3 (1.87 %) and CO 6 (1.36 %). This could be due to higher sensitivity of the variety TU 17-9 to gamma irradiation. The frequency of morphological mutations did not increased with higher doses of gamma irradiation in all the varieties indicating a nonlinear relationship between dose of mutagenic treatment and frequency of morphological mutations.

Effectiveness of Mutagen Treatments

The relative effectiveness and efficiency of different mutagenic treatments were evaluated by using the formula suggested by Konzak *et al.* (1965) and the results are presented in Table 3. The mutagenic efficiency varied depending on the criteria selected. The efficiency estimated on the basis of seedling injury was generally higher compared with sterility and lethality in both the varieties *viz*., ADT 3 and TU 17-9, while in CO 6 it was observed that efficiency estimated on the basis of sterility was higher compared with injury and lethality. Similar findings were reported by Wani (2009) and Khan and Tyagi (2010). Estimates of effectiveness were higher in CO 6. In all the three varieties, efficiency was found more in 200 and 300 Gy dose treatments in comparison to higher doses of mutagen treatments (400 and 500 Gy). The injury, lethality and sterility were highest when higher doses of mutagenic treatments were used. The efficiency of gamma ray treatments declined considerably with the increase in the dose of mutagens in cultivars used in this study. The decrease in efficiency at higher doses may be attributed to the failure in recovery of viable mutations proportionate to the dose of mutagens. This was consistent with the findings of Dixit and Dubey (1986); Wani (2009) and Khan and Tyagi (2010).

A common observation in this study revealed that the degree of effectiveness and efficiency varied among different mutagenic doses and between three varieties. Similar differences in mutagenic response have also been reported by many earlier workers (Bhat *et al.*, 2007; Dhanavel *et al.*, 2008 and Wani, 2009).

Gamma treatment 200 Gy was found to be most effective in all the varieties. Higher effectiveness and efficiency in lower and intermediate doses of mutagen have been reported by many workers including Bhat *et al*. (2007); Singh and Singh (2007); Dhanavel *et al*. (2008), Wani (2009) and Bolbhat *et al.*, (2012). However, Deepalakshmi and Kumar (2003) reported that medium dose of gamma ray was the most effective and efficient dose for induction of mutation with positive effects on direct yield component traits in green gram. Higher efficiency at lower and intermediate doses of mutagens may be due to reduction in biological damage (injury, lethality and sterility).

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