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STUDIES ON EFFECTIVENESS AND EFFICIENCY OF GAMMA RAYS IN GREEN GRAM (*Vigna radiata* (L.) Wilczek)

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SUMMARY

The aim of this study was undertaken to assess the effectiveness and efficiency of gamma rays on 2 different varieties of green gram. Genetically pure, uniform and dry seeds of green gram [*Vigna radiata* (L.) Wilczek] varieties Sujata and TARM-1 were treated with 5 different doses (20, 30, 40, 50 and 60 kR) of gamma rays. The biological damage based on lethality and injury was 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 doses of mutagenic treatment in induction of macromutation in M_2 generation was observed in both varieties. The degree of effectiveness and efficiency varied among different mutagenic doses and between 2 varieties. Higher degree of effectiveness and efficiency at lower and intermediate doses of mutagens were observed in both the varieties which may be due to less biological damage (lethality and sterility).

Key words: Effectiveness, efficiency, gamma rays, induced mutations, green gram.

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INTRODUCTION

Induced mutagenesis plays an important role in improvement of crops like green 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. Further, hybridization in this crop is difficult due to its small cleistogamous flower. Induction of mutation offers the scope of inducing desired traits in these crops.

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. Ahloowalia *et al*. (2004) and Tah (2006) 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; Konzak *et al.*, 1965 and Wagner and Foster, 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 2 varieties of green gram.

MATERIALS AND METHODS

Genetically pure, uniform and dry seeds (10% moisture content) of 2 green gram varieties Sujata and TARM-1 were taken for induction of mutation using gamma irradiation. Seeds of both varieties were irradiated with 5 different doses (20, 30, 40, 50 and 60 kR) of gamma rays at the Bhaba Atomic Research Centre, Trombay, India. Treated seeds along with controls of both varieties were sown in 2 series of experiments to raise M_1 generation. One experiment was conducted in earthen pots to observe the seedling response to mutagenic treatments and the other was conducted in the Instructional Farm field of Orissa University of Agriculture and Technology at Jajpur to study the adult plant response. Observations on germination, epicotyl length, hypocotyl length and root length were recorded on all surviving seedlings on the 7^{th} day after sowing of seeds in earthen pots and the means were expressed as a percentage of the control. Bulked seeds of all the M_1 plants from each treatment were used to raise the M_2 generation in RCBD with 3 replications 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;

$$\text{Effectiveness} = \frac{M_{sd}}{kR}$$

where, M_{sd} = percentage of viable mutation frequency in M_2,

kR = dose of mutagenic treatment in kilo rad,

Mutagenic efficiency was calculated in 2 different ways and then average was calculated.

$$\text{Mutagenic efficiency} = M_{sd} / I \quad \text{(a)}$$

$$\text{Mutagenic efficiency} = M_{sd} / L \quad \text{(b)}$$

$$\text{Average mutagenic efficiency} = (a+b)/2$$

where, M_{sd} = Percent of viable mutation frequency in M_2, I (injury) = percent reduction in seedling height in M_1, L (lethality) = percentage reduction in germination in M_1.

RESULTS AND DISCUSSIONS

Chlorophyll mutation

Macromutations are generally used to evaluate the genetic effects of various mutagens. 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 both varieties in M_2 generation which included albino, xantha, chlorina, viridis and sectorial. No chlorophyll mutations were observed in controls (untreated population) of both varieties. The frequency of chlorophyll mutations in different mutagenic-treated populations varied from 2.2% to 4.0% in Sujata and 2.1% to 4.9% in variety TARM-1 (Table 1 and Figure 1). Among different types of chlorophyll mutations, chlorina was the most frequent (2.15% in Sujata and 2.28% in TARM-1) in both cultivars suggesting high mutability of the gene controlling the phenotype. The sectorial type of chlorophyll mutation was the least frequent in the treated populations of Sujata and TARM-1. The average frequency of chlorophyll mutations in the variety Sujata was lower (3.30%) in comparison to that of TARM-1 (3.61%) suggesting differences between varieties to gamma treatment. The lowest frequency of chlorophyll mutations (2.2%) was observed using the 40 kR mutagenic treatment in the case of the variety Sujata and 60 kR dose for the variety TARM-1 (2.1%). The declining trend in the frequency of mutations with increasing dose of the mutagen was not linear. This was consistent with the findings of Khan and Tyagi (2010) in soybean. The occurrence of lower frequency of mutations in higher doses could be due to induction of greater lethality at higher doses. Similar dose dependant reduction in chlorophyll mutation frequency have been reported by many workers in different crops including green gram (Sharma and Haque, 1997; Singh and Singh, 2001; Singh and Singh, 2007; Goyal *et al.*, 2009 and Vasline and Sabesan, 2011). Khan and Tyagi (2010) also reported both reduction and increase in chlorophyll mutation frequency in 2 different varieties treated with same mutagen and dose.

Although there was no significant difference in the spectrum of chlorophyll mutations in both varieties, quantitative variation in the mutation frequency of different types of chlorophyll mutations was observed using 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 Sharma and Haque (1997); Singh (2007); Wani (2009) and Khan and Tyagi (2010).

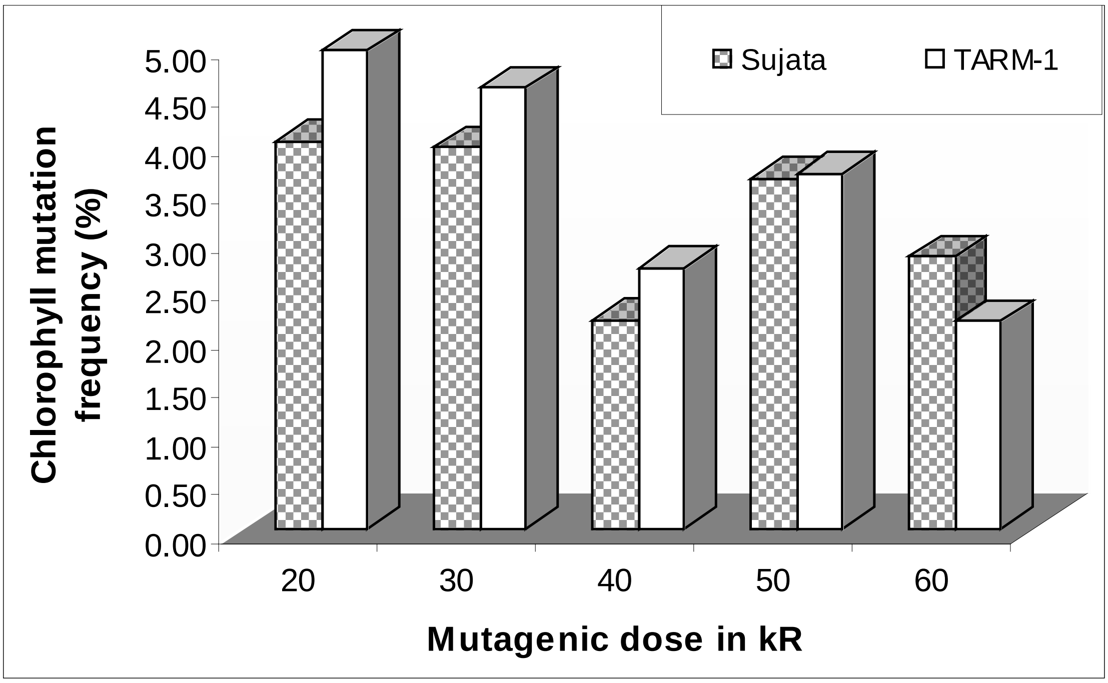

Figure 1. Chlorophyll mutation frequency (%) in variety Sujata and TARM-1.

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

Treatment	No. of M_2 Plants scored	Chlorophyll mutations					Total	Frequency (%)
		Chlorina	Xantha	Albina	Viridis	Sectorial		
Sujata (Control)	1280						-	-
Sujata (20 kR)	1250	38	4	2	5	1	50	4.0
Sujata (30 kR)	1120	32	3	2	6	1	44	3.9
Sujata (40 kR)	1254	16	2	1	6	2	27	2.2
Sujata (50 kR)	1080	24	2	4	8	1	39	3.6
Sujata (60 kR)	964	12	3	2	8	2	27	2.8
Total		122	14	11	33	7	187	16.5
Percentage		2.15	0.25	0.19	0.58	0.12	3.30	
TARM-1 (Control)	1320						-	-
TARM-1 (20 kR)	1256	42	4	2	12	2	62	4.9
TARM-1 (30 kR)	1145	37	2	4	7	2	52	4.5
TARM-1 (40 kR)	1262	22	2	0	9	1	34	2.7
TARM-1 (50 kR)	1010	18	3	1	13	2	37	3.7
TARM-1 (60 kR)	1124	13	1	1	8	1	24	2.1
Total		132	12	8	49	8	209	17.9
Percentage		2.28	0.21	0.14	0.85	0.14	3.61	

Morphological Mutation

A wide spectrum of morphological mutations *i.e.* unifoliate, bifolioate, quadrifoliate, pentafoliate, tall, dwarf, trailing type, early, late, profusely branched, more poded, erect bunch type, serrated leaf, modified inflorescence and simple leaf type were observed in M_2 generation of gamma treated population of both varieties. No morphological mutations were observed in untreated controls of both varieties.

Both varieties differed regarding to the quantity and spectrum of morphological mutations induced. The frequency of morphological mutations in different treatments varied from 3.84% to 6.22% in variety Sujata and 1.83% to 4.36% in variety TARM-1 (Table 2 and Figure 2). Among different types of morphological mutations, the most frequent type was quadrifoliate in the variety Sujata and more poded-type plants in the variety TARM-1. The frequency of morphological mutations like trailing type, modified inflorescence and simple leaf was lower in the variety Sujata, while deeply serrated leaf and simple leaf mutants were the least frequent in variety TARM-1. Considering the 2 varieties, the frequency of morphological mutations was higher in variety Sujata (4.88%) compared to variety TARM-1 (3.27%). This could be due to higher sensitivity of the variety Sujata to gamma irradiation. The frequency of morphological mutations increased with higher doses of gamma irradiation in both the varieties indicating a positive relationship between dose of mutagenic treatment and frequency of morphological mutations. Similar differential induction of morphological mutation in different doses of mutagen as well as in different varieties have been reported by Kharkwal (2000); Gaur and Gour (2001); Mittal *et al*., (2001); Kartika and Subbalakshmi (2006); Singh (2007); Singh and Rao (2007); Wani (2009); Khan and Tyagi (2010); Shah *et al.*, (2011) and Vasline and Sabesan (2011).

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 lethality in both the varieties. This was consistent with the findings of Wani (2009) and Khan and Tyagi (2010). Estimates of effectiveness were higher in Sujata. In both the varieties, efficiency was found more in 20 and 30 kR dose treatments in comparison to higher doses of mutagen treatments (50 and 60 kR). The injury and lethality was 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 both cultivars. 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 2 varieties. Similar differences in mutagenic response have also been reported by many earlier workers (Kharkwal, 1998; Bhat *et al*., 2007; Dhanavel *et al*., 2008 and Wani, 2009).

Gamma treatment 20 kR was found to be most effective in variety Sujata followed by its 30 kR and 40 kR treated dose. The effectiveness in highest dose (60 kR) was low in both varieties. In TARM-1, the 30 kR treatment was the most effective followed by 20 kR, 40 kR and 50 kR dose. Higher effectiveness and efficiency in lower and intermediate doses of mutagen have been reported by many workers including Thakur and Sethi (1995); Kharkwal (1998); Khan *et al.* (2005); Bhat *et al.* (2007); Singh and Singh (2007); Dhanavel *et al.* (2008) and Wani (2009). Sharma and Haque (1997); Deepalakshmi and Kumar (2003); Das and Misra (2005) 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. Khan (1981); Tah (2006 and 2008); Goyal *et al*., (2009) and Bolbhat *et al*., (2012) reported higher mutagenic efficiency and effectiveness at lower doses of mutagens. Higher efficiency at lower and intermediate doses of mutagens may be due to reduction in biological damage (lethality and sterility).

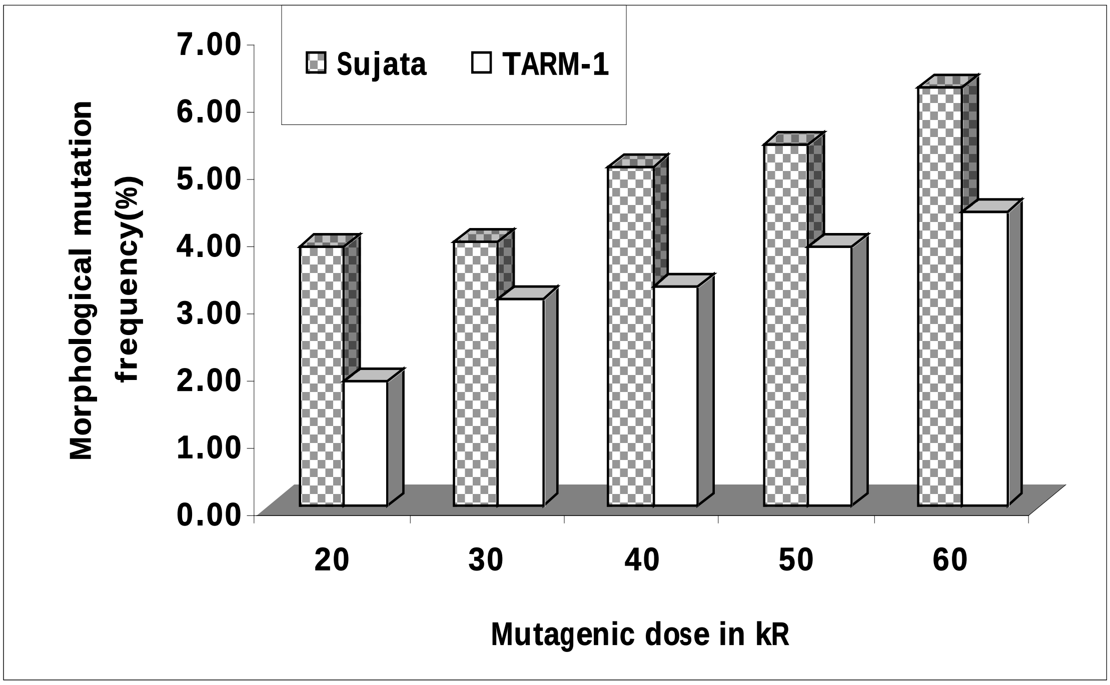

Figure 2. Morphological mutation frequency (%) in variety Sujata and TARM-1.

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

Treatment	No. of M_2 plants	Unifoliate	Bifoliate	Quadrifoliate	Pentafoliate	Tall	Dwarf	Trailing	Early	Late	Profuse branches	More podded	Erect bunch	Deeply serrated	Slightly serrated leaf	Modified infloresce	Simple leaf	Total	Frequency (%)
Sujata (Control)	1280																	-	-
Sujata (20 kR)	1250	1		13	2		11		5	7		9						48	3.84
Sujata (30 kR)	1120	2		11	3		6		6	3	2	11						44	3.93
Sujata (40 kR)	1254	0	1	18	7	3	9		13	2		9	1					63	5.02
Sujata (50 kR)	1080	1	2	14	5	6	4	1	8	5	4	7		1				58	5.37
Sujata (60 kR)	964	1		9	3	8	6		5	9	3	13	1		1	1		60	6.22
Total		5	3	65	20	17	36	1	37	26	9	49	2	1	1	1	0	273	24.4
Percentage		0.09	0.05	1.15	0.35	0.30	0.64	0.02	0.65	0.46	0.16	0.86	0.04	0.02	0.02	0.02	0.0	4.82	
TARM-1 (Control)	1320																		
TARM-1 (20 kR)	1256	1	1	2	2				2	3		10				2		23	1.83
TARM-1 (30 kR)	1145	1		6	4	2				9	2	8	1		1	1		35	3.06
TARM-1 (40 kR)	1262	3	2	5		5	4		4	4	4	9				1		41	3.25
TARM-1 (50 kR)	1010	2		12	5		3	2	2	6		4	2		1			39	3.86
TARM-1 (60 kR)	1124	1		2	3	9	7		4	8	3	11	1					49	4.36
Total		8	3	27	14	16	14	2	12	30	9	42	4	0	2	4	0	187	16.36
Percentage		0.14	0.05	0.47	0.24	0.28	0.24	0.03	0.21	0.52	0.16	0.72	0.07	0.0	0.03	0.07	0.0	3.23	

Table 3. Effectiveness and efficiency of different mutagenic treatments in Sujata and TARM-1.

Treatment	Viable mutation frequency (M_{sd})	Injury (I)	Lethality (L)	Mutagenic efficiency			Mutagenic effectiveness
				M_{sd} / I	M_{sd}/ L	Average	
Sujata 20 kR	3.84	0.38	3.26	10.11	1.18	5.65	0.19
Sujata 30 kR	3.93	2.50	12.60	1.57	0.31	0.94	0.13
Sujata 40 kR	5.02	5.87	20.00	0.86	0.25	0.55	0.13
Sujata 50 kR	5.37	26.00	38.70	0.21	0.14	0.17	0.11
Sujata 60 kR	6.22	48.75	47.42	0.13	0.13	0.13	0.10
Average	4.88	16.70	24.42	2.60	0.40	1.49	0.13
TARM-1 20 kR	1.83	0.37	2.82	4.90	0.65	2.77	0.09
TARM-1 30 kR	3.06	1.00	11.52	3.07	0.27	1.67	0.10
TARM-1 40 kR	3.25	1.87	18.72	1.74	0.17	0.96	0.08
TARM-1 50 kR	3.86	16.69	39.16	0.23	0.10	0.17	0.08
TARM-1 60 kR	4.36	43.71	46.90	0.10	0.09	0.10	0.07
Average	3.27	12.73	23.82	2.01	0.26	1.13	0.08

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