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Studies on Mutagenic Effectiveness and Mutagenic Efficiency of Gamma Rays in Cluster bean (Cyamopsis tetragonoloba (L) Taub) Var. NCB-12

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Abstract: *In the present investigation, the mutagenic effectiveness and efficiency of a physical mutagen gamma rays was studied in the cluster bean (Cyamopsis tetragonoloba (L) Taub) variety NCB-12. The seeds of cluster bean variety NCB-12 were mutagenised with the increasing doses of gamma rays 10kR, 20kR, 30kR, 40kR and 50kR. The effectiveness and efficiency was determined by accounting lethality, injury and sterility in M_1 generation of mutagenised seeds and frequency and spectrum of chlorophyll mutations in M_2 generation. The increasing doses of gamma rays decreased plant survival, seedling height and increased pollen sterility. Four types of chlorophyll mutants albina, xantha, chlorina and striata were screened in M_2 progeny. It was observed that the frequency of mutations increased with increasing doses of mutagen. The highest mutation frequency was noticed in 50kR dose of gamma rays. The mutagenic effectiveness and efficiency was decreased with increased doses of gamma rays. The highest mutagenic effectiveness was recorded 0.187 in 10kR and lowest 0.084 in 50kR dose of gamma rays. Whereas maximum mutagenic efficiency was recorded 0.231 in 10kR and minimum 0.132 in 50kR dose of gamma rays. Thus the lower doses of mutagen like gamma rays were effective and efficient than the higher doses of gamma rays in cluster bean variety NCB-12.*

Keywords: *Cluster bean, mutagen, mutagenic effectiveness, mutagenic efficiency, mutation frequency .*

I. Introduction

Guar or cluster bean (Cyamopsis tetragonoloba (L) Taub) 2n=14, is drought tolerant annual multipurpose legume crop, grown for feed, green fodder, vegetable & grain purpose. Guar is a very important legume & industrial crop in arid areas of India. Unlike the seeds of other legumes the guar bean has large endosperm contains galactomannan gum, which has several diversified industrial and pharmaceutical uses. It is an excellent soil improving crop with respect to available nitrogen.

Mutation breeding has been found very useful tool in crop improvement as it inducing genetic variability necessary in plant breeding programmes. Many desirable varieties of crops have been developed through mutation breeding (Gabriyal et al., 2009). In recent years, induction of mutations employing various mutagens has widely been accepted as an excellent tool for creating genetic variability and as supplementary approach in the crop improvement programmes in several crops (Singh and Pal, 1998). Induced mutations have been utilized to achieved success in improving plant yield. A number of chemical and physical mutagens are widely used to induce genetic variability in plants. Gamma radiation is most widely used physical mutagen in crop improvement (Gual H. 1964). Mutagens are specific to their action but varietals variations are present with respect to formation of mutations.

The mutagenic effectiveness and efficiency of mutagens and their doses are prerequisites for induction and utilization of mutations (Sharma et al., 2005). Mutagenic effectiveness means the rate of mutations as related to dose while efficiency refers to the mutation rate in relation to biological damage such as seedling injury, pollen sterility and lethality in M_1 generation (Nilan et al., 1965). Mutagenic effectiveness and efficiency gives an idea to evaluate a mutagen. Hence these two parameters were used in the present investigation in cluster bean variety NCB-12. The parameters of M_1 generation are the best indicator in measuring efficiency of mutagens and helped in comparing the effectiveness and efficiency of a physical mutagen gamma rays.

Most of the studies on mutagenic effectiveness and efficiency have been carried out in various legume crops like black gram (Gautam et al., 1992, Ahmed, 1995, Sharma et al., 2005), mungbean (Singh A. K. and Singh R.M., 2001), lentil (Solanki and Sharma 1994, Vandana et al., 1994), French bean (Manrique et al., 1988), Chick pea (Ayaz Weni 2009), Lathyrus (Nerkar 1977, Prasad A.B. and Das A.K. 1980), moth bean (Kothekar V.S. 1989), Soybean (Khan M.H. and Tyagi, 2010), But very little work was carried out regarding mutagenic effectiveness and efficiency in clusterbean (Bhosle and Kothekar 2010, Velu S. et al., 2007, Dubey K. G. et al., 2011, Reddy et al., 1991).

In the present investigation, attempt was made to analyze the mutagenic effectiveness and efficiency of a physical mutagen gamma rays in cluster bean variety NCB-12 by using biological damages like seedling injury, pollen sterility and lethality observed in M_1 generation in relation with the frequency and spectrum of chlorophyll mutations in M_2 generation.

II. Materials And Method

The dry, genetically pure, non dormant viable and good quality seeds of cluster bean variety NCB-12 were irradiated with 10, 20, 30, 40 and 50 KR doses of gamma rays by using 60CO as a source of Mutation Breeding Centre, Department of Biotechnology, B.A.R.C., Mumbai. Five batches of 500 seeds each were irradiated. 50 seeds from each treatment were dried between the folds of filter paper and germinated in Petri plates to record germination percentage. Remaining 450 seeds from each treatment were sown in the field immediately following randomized block design (R.B.D.), with three replications along with control to raise M1 generation. The seeds were sown at the distance of 30 cm between the plants and 60 cm between the rows. All the cultural practices recommended by M.P.K.V., Rahuri including irrigation, mannuring, weeding and plant protection methods were followed during the growth period. In M1 generation, the reduction in seedling height, plant survival and pollen sterility were recorded and considered as injury (I), lethality (L), sterility(S) and expressed as percent of control (Sharma S.K. 1990, Sharma S.K. 2005, Singh A. K. and Singh R. M. 2001). At maturity all fertile M1 plants were harvested separately and the seeds were sown in plant to row method to raise M2 generation, in three replications. In M2 generation, the treated as well as control progenies were screened for chlorophyll mutations up to first four weeks after germination, whereas, viable and morphological mutations were scored throughout the crop duration. Four types of chlorophyll mutants viz., albina, xantha, chlorina and striata were scored till the age of four weeks (Kharakwal, 1998) in M2 generation. Mutagenic frequency was estimated as percentage for both chlorophyll and morphological mutations in each treatment (Gaul 1964). The mutagenic effectiveness and efficiency were determined by Konzak et al., (1965).

$$\textbf{(1) Mutagenic effectiveness} = \frac{\text{Mutation frequency (MF)}}{\text{Time X Concentration / Dose}}$$

$$\textbf{(2) Mutagenic efficiency} = \frac{\text{Mutation frequency (MF)}}{\text{Biological Damage}}$$

Where, MF = % chlorophyll mutations, in M2 generation.
L = % of lethality in M1 generation.
I = % of seedling injury In M2 generation.
S = % pollen sterility in M1 generation.

III. Results and Discussions

3.1 Biological Damages in M1 Generation :

In the present study, the biological damages like seedling injury, pollen sterility and lethality were recorded in M_1 generation. All these parameters were found increased with increasing doses of gamma rays. The seedling injury was highest 26.33% at 50KR and lowest 6.19% at 10 KR dose of gamma rays. The maximum sterility was 39.28% at 50 KR and minimum 6.19% at 10 KR dose whereas higher lethality was recorded 32.66% at 50 KR and lower 9.9% at 10 KR dose. Thus these biological damages were lower at lower doses of gamma rays and higher at higher doses of gamma rays in cluster bean variety NCB-12 (Table 1 and Fig. 1).

The increased injury, sterility and lethality with increasing doses of mutagens also reported by several investigators Bhosle and Kothekar (2010), S. Velu et al., (2007), Reddy et al., (1991) in cluster bean, The results of present investigations are in confirmity with these results. Similar results were also obtained by Mathur et al., (2001), Sonone et al., (2008) in ground nut and Upadhyaya et al., (1984), Khan M. F. and Tyagi S.D. in Soybean. They proved that most of the higher doses of mutagens like EMS and gamma rays showed increased pollen sterility. These results also supported the result of present study. The probable reason for increased pollen sterility might be meiotic irregularities such as translocations.

3.2 Mutation Frequency in M2 Generation :

Mutation frequency is frequency of chlorophyll mutations calculated on M_2 plant basis. In the present investigation, it was recorded that the mutation frequency increased with increasing doses of gamma rays, in cluster bean variety NCB-12. Chlorophyll mutants were found in almost all the mutagenic doses of gamma rays. The highest chlorophyll mutation frequency 4.22% was reported in 50KR while the lowest 1.87% found in 10KR treatments. In the segregating M_2 generation, spectrum of chlorophyll mutations indicated the presence of four types of chlorophyll mutants viz…, albina, xantha, chlorina and striata. All the four types of mutants were observed in 30,40 & 50 KR treatments, whereas 10 and 20 KR treatments induced only three types of mutants. The highest frequency of albina mutant was recorded 1.40% in 40 KR and lowest 0.40% I 10 KR treatments. The maximum frequency of xantha mutants was found. 1.15% in 40 KR while minimum 0.77% in 20 KR treatment. The higher frequency of chlorina mutant was reported 1.83% in 40 KR and lower 0.83% in 30 KR treatment, whereas striata mutants were found maximum 0.78% in 30 KR and minimum 0.45% in 50KR treatments (Table 2 and Fig. 2).

Bhosle S.S. and Kothekar V.S. (2010) recorded increase in the mutation frequency with increasing doses of gamma rays, EMS and SA in M2 progenies of cluster bean varities GE-36 and HR. Among the gamma rays treatment, the highest mutation frequency was recorded 2.6% in 15KR and lowest 1.66% in 5 KR treatment in the variety GE-36 while it was higher 3.33% I 15KR and 1.61% in 5 KR treatment of gamma rays in the variety HR. These results are in agreement with the results of present investigation. These results were also supported by previous results recorded in various crops; Khan and Tyagi (2010) in soybean, S. K. Sharma and B. Sharma (1979) in lentil, Sonone et al.,(2008) in ground nut, Kothekar V.S. (1989) in moth bean , Mehta et al.,(1998) in french bean. All the investigators have recorded increased mutation values with increasing doses of physical mutagens like gamma rays, neutrons and chemical mutagens EMS, SA and NMU.

However, S. Velu et al., (2007) calculated mutation frequency in cluster bean variety Pusa-Navbahar on M2 plant basis showed a dose dependency, where highest mutation frequency was recorded 1.69% at lower doses of gamma rays 20KR and 1.92% in 0.2% EMS and showed lowest mutation frequency at higher doses of gamma rays 0.89% at 100KR and 1.46% in 1.0% EMS respectively. Thus they recorded decreased mutation frequency with increased doses of gamma ray and EMS. These results were in accordance to the results obtained by Gautam et al.,(1998) in Rajmah and Kumar D. et al., (2003) in lima bean. The high frequency of chlorophyll mutations obtained with gamma rays is due to preferential action of this mutagen on genes controlling the chlorophyll formation in the leaves (Chopra 2005).

3.3 Mutagenic Effectiveness

The mutagenic effectiveness is the ratio of mutation frequency in M_2 generation to the doses of gamma rays used. In the present investigation, it was recorded that mutagenic effectiveness decreased with increasing doses of gamma rays in cluster bean variety NCB-12. The highest value of mutagenic effectiveness 0.187 was recorded in 10KR treatment and lowest 0.084 in 50KR treatment. Thus the lower doses of gamma rays were most effective (Table 3 and Fig. 3).

These results were supported by the results obtained in cluster bean by Velu S. et al.,(2007). They reported higher mutagenic effect variety Pusa-Navbahar at lower doses of gamma rays and EMS. However EMS and it's doses were found to be more effective mutagen than the gamma rays. Bhosle S.S. and Kothekar V.S. (2010) also reported reduction in the value of mutagenic effectiveness with the increased doses or concentration of mutagens gamma rays, EMS and SA respectively in the cluster bean varieties GE-36 and HR. SA proved to be more effective than gamma rays and EMS in both the varieties. The mutagenic effectiveness was also reported by K.G. Dube et al.,(2011) in cluster bean variety Sharada by using variable doses of Gamma rays, EMS and their combination. The mutagenic effectiveness was calculated on the basis of chromosomal aberrations rather than chlorophyll mutation frequency in M2 plants. The mutagenic effectiveness was decreased with increasing doses and concentration of gamma rays and EMS as well as their combination treatment. These results also confirmed results of present investigation. However, the mutagenic effectiveness recorded was more in EMS treatment alone than gamma rays or their combination treatments.

Several investigators have also studied mutagenic effectiveness in various crops such as A. K. Singh and R. M. Singh (2001) in Mung bean, Nerkar (1977) in Lathyrus sativus, A.K. Mehta and R. P. Pandey (1998) in french bean, D. Sassi Kumar et al., (2003) in lima bean, Jabaraj S. and Marappan P.V. (1981) in green gram, S.K. Sharma et al.,(2005) in urd bean, using mutagens Gamma rays, and EMS and reported higher mutagenic effectiveness at lower doses. These results are in accordance to results of present investigation.

The effectiveness of any mutagen depends upon its dose or concentration and specificity to act on genes and genetic makeup of the cultivar (Blixt, 1968).

3.4 Mutagenic Efficiency

Mutagenic efficiency is the ratio of frequency of chlorophyll mutations induced in M2 generation to various biological damages such as seedling injury, pollen sterility and lethality observed in M1 generation. In the present study, the mutagenic efficiency decreased with the increasing doses of gamma rays with respect to seedling injury (I), pollen sterility (S) and lethality (L). Highest value of mutagenic efficiency was recorded 0.302 at 10KR and lowest 0.160 at 50KR dose with respect to seedling injury. The maximum value of mutagenic efficiency with respect to pollen sterility was 0.204 at 10 KR dose and lowest 0.107 at 50 KR dose where as with respect to lethality higher efficiency was 0.188 at 10KR and lowest 0.129 at 50 KR. On the basis of mean values, the highest mutagenic efficiency was recorded 0.210 in relation to seedling injury and lowest 0.146 in relation to pollen sterility (Table 4 and Fig. 4).

Similar results were also reported by S. Velu et al., (2007) in cluster bean variety Pusa-Navbahar and K. G. Dube et al., (2011) in cluster bean variety Sharada. They proved that lower doses of gamma rays were more efficient than the higher doses. These results were also supported by Manrique et al., (1998) in french bean, M. Khan and Tyagi (2010) in Soybean, S. K. Sharma et al.,(2005) in Urd bean, S. K. Sharma and B. Sharma (1979) in Lentil. They concluded that mutagenic efficiency was higher at lower doses of mutagens. These results are in agreement with the results of present investigation. However, Bhosle S.S. and Kothekar V.S. (2010) concluded that the mutagenic efficiency was highest at higher doses of mutagens like EMS, SA and gamma rays in cluster bean varieties GE-36 and HR. A.K. Singh and R.M. Singh (2001) have reported 40 KR dose of gamma rays was most efficient than lower doses in mung bean.

These findings are in accordance to the observations of several workers Konzak et al., (1965), in barley, Nerkar (1977) in Lathyrus sativus, Gautam et al., (1992) in black gram. Aijaz A Weni (2009) studied mutagenic efficiency of EMS and gamma rays the varieties (two) of chick pea and showed that intermediate treatments were found more efficient. According to Konzak et al., (1965) efficiency with an increase in concentration or doses of mutagenic agent is due to biological damage like seedling injury, lethality and sterility in M1 generation which increases with increase in dose or concentration.

IV. Figures and tables

Table 1 : Effect of Gamma rays on Biological damages in M_1 generation of cluster bean var. NCB-12 due to effect of Gamma rays.

MUTAGEN	DOSE	% SEEDLING INJURY (I)	% POLLEN STERILITY (S)	% LETHALITY (L)
GAMMA RAYS	10 KR	6.19	9.15	9.9
	20 KR	11.20	15.70	13.65
	30 KR	16.80	22.40	21.00
	40 KR	21.45	31.50	27.50
	50 KR	26.33	39.28	32.66

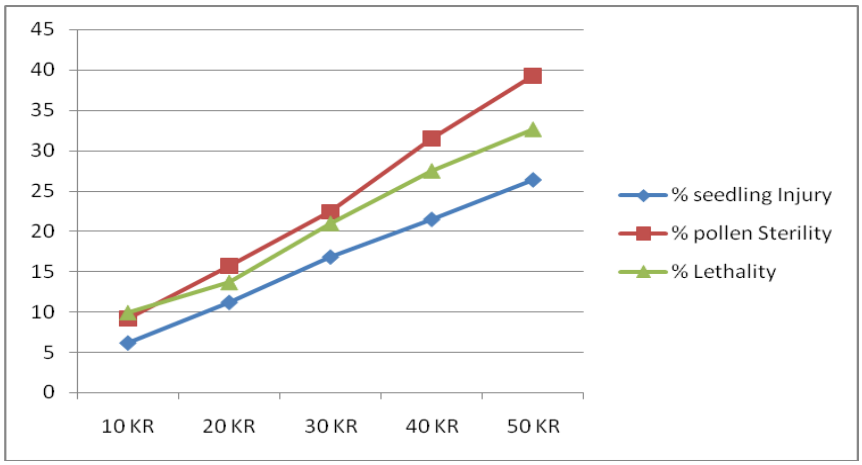

Fig.1 Effect of Gamma rays on Biological damages in M_1 generation of cluster bean var. NCB-12 due to effect of Gamma rays.

Table 2 : Effect of Gamma rays on the frequency of the chlorophyll mutations in M_2 generation of cluster bean var. NCB-12.

MUTAGEN	DOSE	TOTAL MUTATION FRQUENCY (%)	SPECTRUM OF MUTATIONS			
			ALBINA	XANTHA	CHLORINA	STRIATA
GAMMA RAYS	10 KR	1.87	0.40	--	0.87	0.60
	20 KR	2.45	--	0.77	1.17	0.51
	30 KR	3.20	0.64	0.95	0.83	0.78
	40 KR	3.89	0.80	0.90	1.45	0.57
	50 KR	4.22	1.22	1.15	1.40	0.45

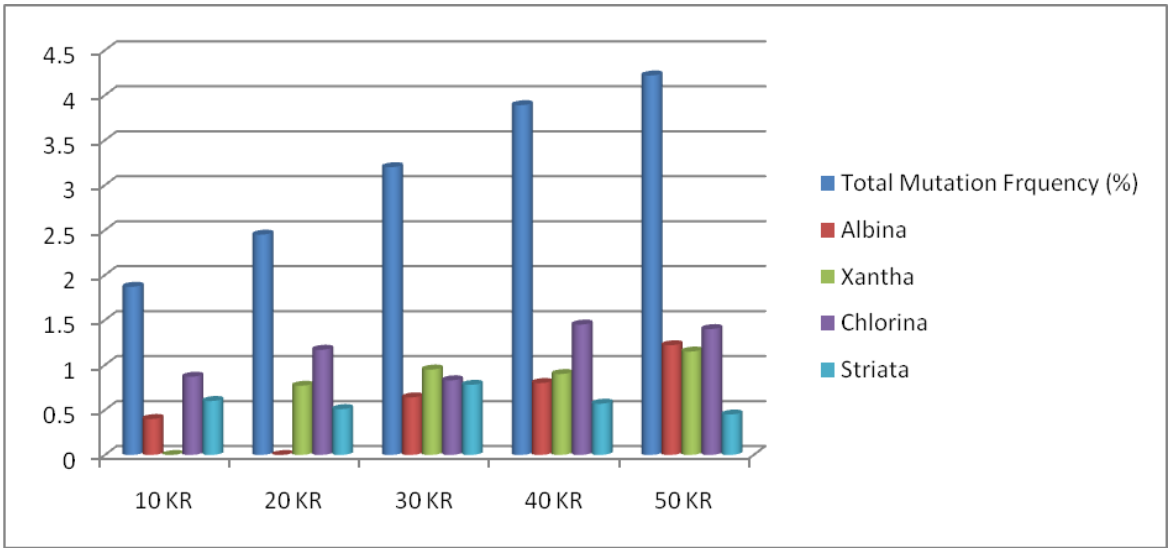

Fig.2 Effect of Gamma rays on the frequency of the chlorophyll mutations in M_2 generation of cluster bean var. NCB-12.

Table 3 : The effectiveness of Gamma rays in M_2 generation of cluster bean var. NCB-12.

MUTAGEN	DOSE	TOTAL MUTAGEN FREQUENCY MF (%)	EFFECTIVENESS MF/DOSE
GAMMA RAYS	10 KR	1.87	0.187
	20 KR	2.45	0.122
	30 KR	3.20	0.106
	40 KR	3.89	0.097
	50 KR	4.22	0.084
	MEAN	**3.126**	**0.119**

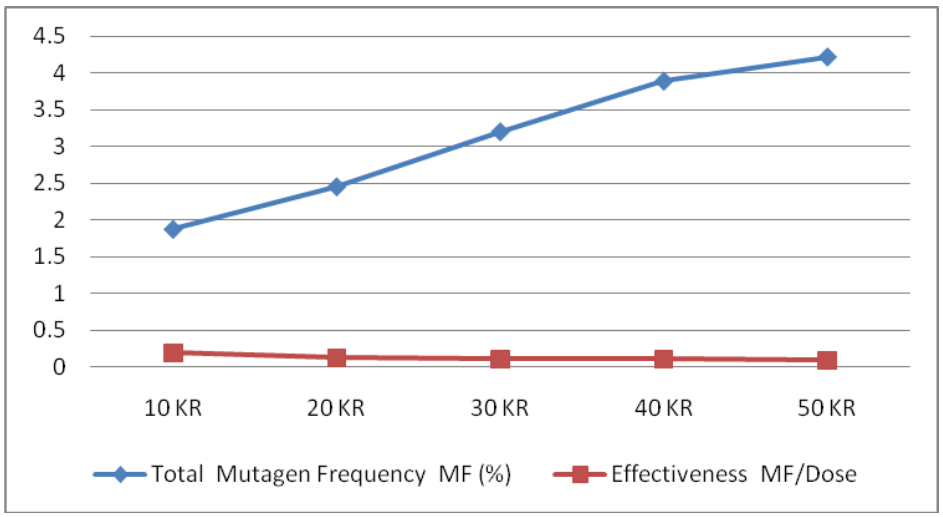

Fig.3 The effectiveness of Gamma rays in M_2 generation of cluster bean var. NCB-12.

Table 4 : The mutagenic efficiency of Gamma rays in M_2 generation of cluster bean var. NCB-12.

MUTAGEN	DOSE	TOTAL FREQUENCY MF(%)	% SEEDLING INJURY(I)	EFFICI-ENCY MF/I	% STERILITY (S)	EFFICI-ENCY MF/S	% LETHA-LITY (L)	EFFICI-ENCY MF/L
GAMMA RAYS	10 KR	1.87	6.19	0.302	9.15	0.204	9.9	0.188
	20 KR	2.45	11.20	0.218	15.70	0.156	13.65	0.179
	30 KR	3.20	16.80	0.190	22.40	0.142	21.00	0.152
	40 KR	3.89	21.45	0.181	31.50	0.123	27.50	0.141
	50 KR	4.22	26.33	0.160	39.80	0.107	32.66	0.129
	MEAN	**3.126**	**16.39**	**0.210**	**23.60**	**0.146**	**20.94**	**0.157**

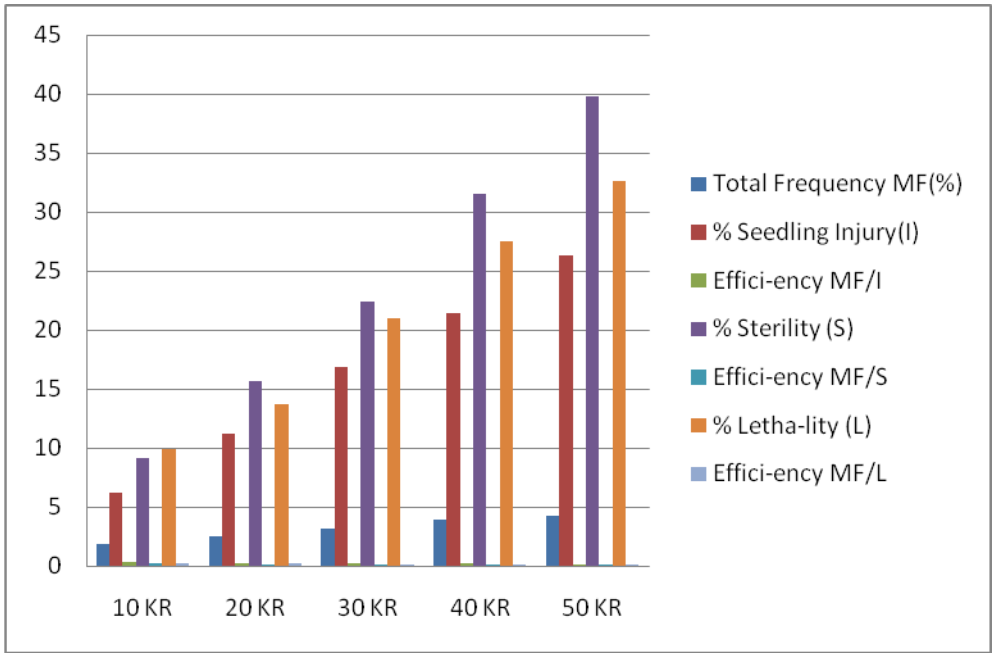

Fig.4 The mutagenic efficiency of Gamma rays in M_2 generation of cluster bean var. NCB-12.

V. Conclusions

From the present investigation, it was concluded that mutagenic effectiveness and mutagenic efficiency were highest at lower dose of gamma rays in the cluster bean variety NCB-12. The chlorophyll frequency increased with increasing dose of gamma rays and it was maximum at higher doses and minimum at lower doses of gamma rays. The increased mutagenic dose causes the decrease in the mutagenic effectiveness at higher doses. The mutagenic efficiency was higher at lower doses due to the increased injury, sterility and lethality with increasing doses of gamma rays. Thus gamma rays is effective and efficient mutagen in cluster bean which may create genetic variability useful in crop improvement.

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