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Determination of Lethal Dose for Gamma Rays Induced Mutagenesis in Butter Bean (*Phaseolus lunatus L*) Variety KKL-1

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A B S T R A C T

In the present studies treated the seeds using gamma rays different does 10 kR to 100 kR each treatment using 50 seeds. The percentage of seed germination ranges from 0 per cent to 52 per cent with different does of gamma rays in butter bean variety KKL-1 as compared to 86 per cent control. The different in seed germination are statistically significant. The LD50 value was found at 10 kR for seed germination. The percentage of survival was 81 per cent in control while it are significant range from 0 per cent 100 kR to 48 per cent 10 kR of gamma rays in butter bean variety KKL-1. The LD50 value for seedling survival has to fixed at 10 kR.

Keywords

Gamma ray, Lethal dose, Butter bean, KKL-1.

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Introduction

Induced mutation plays a significant role in the crop improvement of horticultural crops. It is an important tool for induction of variation in quantitative and qualitative characters. It can be a supplement to conventional breeding methods when it is desired to improve one or two characters in a well adapted variety.

Much progress has been made in generating superior genotype with favourable attributes through induced mutations. It is much more useful in crop where cross incompatibility mechanisms exist. Creation of variability in highly self pollinated crops like peas and beans is very difficult by heterosis breeding due to high crossing barrier and poor seed setting.

Butter beans (*Phaselous lunatus* L) belongs to the family Leguminaceae. KKL1 butter beans is a selection from a local type collected of Vilpatti. It is a pole type and bean growing upto a height of 2.42 m. The pods are cluster 11.6 cm long and beans are 5 to 6 numbers per pod. The pods are green when immature, turning creamy yellow with brownish purple streaks on the surface at maturity. The seeds are bold, globular snow white in color with excellent cooking quality. The variety is suited for hilly regions of Tamil Nadu with attitude longest from 1200 m to 2200 m above MSL. The crop will be ready for first harvest from 100 days after sowing and the harvest continues upto 140 days with a potential yield of 3.47 tonnes of mature pods per hectare in three or four pickings. The present investigation is to study the effect of gamma irradiation in KKL-1 in evolving a mutant with some specific for desirable traits.

Materials and Methods

The present investigation was carried out during 2012-13 on the "Studies on induced mutation in butter bean (*Phaseolus lunatus* L) var. KKL-1 through gamma rays at the Department of Vegetable crop, Horticultural College and Research Institute, Tamil Nadu Agricultural University, Coimbatore.

Field trial are conducted in the poly house and open field at Horticultural Research Station, Kodaikanal, which is geographically situated between 10°24' N latitude and 77°48' E longitude at an altitude of 2225 m above mean sea level. The mean minimum and maximum temperature during the study period between 2.5° C and 30°C respectively and the relative humidity was 40 to 100 per cent. The soil of the experimental field is loamy silt with a pH of about 5.85

Characters	KKL-1 Butter bean
Pedigree	Selection from a local type
Organisation of release	HRS, TNAU, Kodaikanal
Habit	Pole type (2.42 m)
Maturity	140 days
Pod yield	3.47 tonnes ha^{-1}

Physical mutagen viz. gamma rays (ionizing radiation) the gamma treatments were given by using the 1000 curie Cobalt-60 Gamma cell 900, located at the Centre for Plant Breeding and Genetics (CPBG), Tamil Nadu Agricultural University, Coimbatore, where cobalt- 60 serves as source of gamma rays.

Results and Discussions

Determination of LD_{50} value

For any induced mutagenesis programmed, it is necessary to fix the LD_{50} value based on which further doses will be fixed for the treatment and study of larger population. The LD_{50} value varies according to crop species, varieties, seeds or other planting materials, nature of treatment, method of raising, climate, cultural practices and other parameters (Singh, 1994). The LD_{50} value was found to be 10 kR in germination and seedling survival. Whereas, a slight variation is noticed between different varieties of same spices depends on size, maturity, hardiness and moisture content at the time of treatment (Alikhan *et al*., 1975 in redgram and Krishnaswami *et al*., 1977 in greengram). In the present study, based on the germination on 8th day and survival on 30th day, LD_{50} values of gamma rays have been arrived for KKL-1 butter bean The effect of various doses of gamma rays varied from 0 to 100 kR on seed germination and seedling survival are presented in Table 1 and illustrated in Figure1and Figure2. The percentage of seed germination ranged from 0 per cent to 52.00 per cent with different doses of gamma rays in butter bean variety KKL-1 as compared to 86.00 per cent in control. The differences in seed germination are statistically significant. The LD_{50} value was fixed as 10 kR for seed germination in butter bean variety KKL-1. The percentage of seedling survival was 81.00 per cent in control while it are significant ranged from 0 per cent 100 kR to 48.00 10 kR of gamma rays in butter bean variety KKl-1. The LD_{50} value for seedling survival has been fixed as 10 kR. Slightly higher doses of gamma rays at 55 kR for germination and 50-55 kR for seedling survival were reported in cowpea variety CO-VU-623 by Thirugnanakumar (1986). Gunasekaran (1992) reported that the LD_{50} value for germination was found to be 50-55 kR and for survival 35-40 kR for the cowpea variety CO 4 The lower dose of LD_{50} butter bean showed the sensitivity of the crops to physical mutagen i.e., gamma rays. Drastic reduction in mean survival per cent after 10 kR were pruned that the butter bean variety KKL-1 was highly sensitive to gamma rays and chance of creating variability is enormous.

Gaul (1970) reported that the physical and chemical mutagens induced physiological damages causing primary injury and factor mutations with chromosomal aberrations in the biological material in M_1 generation. A quantitative determination of M_1 altered configurations will help to predict the efficiency of mutagens. In the present investigation, variability in M_1 and M_2 generations were estimated under field conditions. There was a reduction in the seed germination with increase in doses of gamma rays in M_1 and M_2 generations. These are in conformity with the results obtained from earlier studies in lima bean (Mensah and Eruotor, 1993 and Deepthi Nair, 1996) and in soybean (Balakrishnan, 1991 and Geetha 1994 in cowpea (Thirugnanakumar, 1986; Packiaraj, 1988; Rangaswamy 1989 and Gunasekaran, 1992), in green gram (Ignacimuthu and Babu, 1988; Subramanian, 1981 and Mehetere *et al.*, 1990) and in blackgram (Ramaswamy, 1973).

The mutagenic sensitivity of a biological material can be attributed to the level of differentiation and development of embryo at the time of treatment and also the extent of damage to the growth processes like rate of cell division, cell elongation, various stages of hormone and biosynthetic pathways as observed by Scholz and Lehman (1962). The reduction in germination percentage due to gamma rays may be attributed to either a drop in the auxin level (Gordon and Webber, 1955) or chromosomal aberrations as reported by Reed (1959) and Sparrow (1961). In the present study, a delayed seed germination was observed due to mutagenesis. A characteristic effect of irradiation in dicots seeds to be that, the affected seedlings, after the emergence of cotyledonary leaves, remain alive in the critical stage for a considerably long time (Dubinin, 1964). During this phase, there is some type of repairs or compromise that enables the seedlings to recover and carryout the regular mitotic process (Gaul, 1958).

Alternatively the seedlings are not able to overcome the radiation damage and hence they die without formation of side shoots. The seedling mortality was reported to be due to the decline of assimilation mechanism (Quastler and Baer, 1950), inhibition of auxin synthesis (Skoog, 1935), inhibition of mitosis and chromosome damage (Gunkel and Sparrow, 1961). The survival of the seedlings was also seen to reduce with increase in does of gamma rays. An inverse relationship exists between survival and doses of mutagen. Many workers have reported such a dose dependant decrease in different leguminous crops *viz.*,) limabean (Mensah and Eruotor, 1993 and Deepthi Nair, 1996) soybean (Balakrishnan, 1991 and Geetha, 1994) Cowpea (Palanisamy, 1975) and Rangaswamy, 1989), greengram (Krishnaswami *et al.*, 1977; Subramanian, 1980; Ignacimuthu and Babu, 1988 and Mehetere *et al.*, 1990) and blackgram

(Ramaswamy, 1973). But the sudden decrease in survival after 10 kR indicate the week tolerance limit of butter bean gamma irradiation.

Table.1 Fixing up LD 50 for gamma rays

Treatment	Doses	Germination		Survival	
		Mean percentage	Percentage to control	Mean percentage	Percentage to control
Control		86.0	100	81.0	100
Gamma rays	**10 kR**	**52.0**	**60.46**	**48.0**	**59.25**
	20 kR	12.0	13.95	8.0	9.87
	30 kR	6.0	6.97	4.0	4.93
	40 kR	0.0	0.0	0.0	0.0
	50 kR	0.0	0.0	0.0	0.0
	60 kR	0.0	0.0	0.0	0.0
	70 kR	0.0	0.0	0.0	0.0
	80 kR	0.0	0.0	0.0	0.0
	90 kR	0.0	0.0	0.0	0.0
	100 kR	0.0	0.0	0.0	0.0

Fig.1 Sensitivity studies –Effect of gamma rays on seed germination per cent

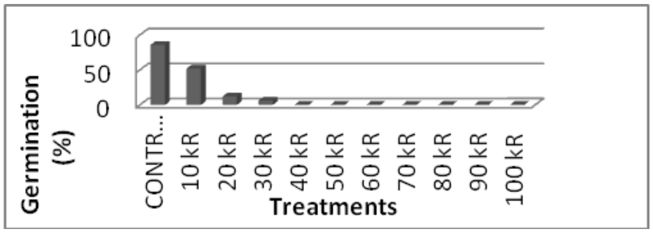

Fig.2 Sensitivity studies –Effect of gamma rays on seedling survival per cent

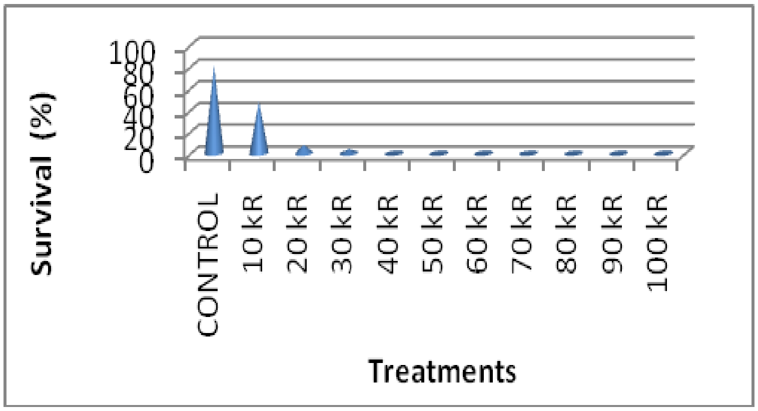

Fig.3 Plates showing germination percentage and survival percentage treated gamma rays

In conclusion, lethal dose for any mutagen is essential to generate highest practicable mutants with lowest damage to the plant. The LD50 dose based on survival percentage of the seedlings, after treatment with diverse doses of gamma rays for the butter bean cultivar kkl-1 were 100 kR to 10 kR respectively. In addition, the optimum dose based on the reduction in germination and survival was 10 kR of gamma rays to generate maximum variability with least number of unwanted mutants. Increasing gamma rays dose decreased the germination percentage and survival percentage Generally, higher gamma rays (20 kR,30 kR,40 kR, 50 kR 60 kR, 70 kR, 80 kR, 90 kR and 100 kR) doses higher concentration of had prominent/lethal effect on the morphological and growth characteristics of butter bean seedlings. These optimal mutagen doses determined for the butter bean genotype could be useful while formulating mutation breeding programme for enrichment of meticulous traits in butter bean.

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