Electronic Journal of Plant Breeding, 7(4): 1122-1126 (December 2016)
ISSN 0975-928X
DOI: 10.5958/0975-928X.2016.00155.1

Research Note

Determination of lethal dose and effect of physical mutagen on germination percentage and seedling parameters in kodomillet variety CO 3

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(Received: 29 Nov 2016; Accepted: 26 Dec 2016)

Abstract
An experiment was conducted to estimate the lethal dose of the physical mutagen gamma ray in Kodomillet variety CO 3. Genetically pure seeds were treated with different doses of gamma rays *viz.*, 100 Gy, 200 Gy, 300 Gy, 400 Gy and 500 Gy. Untreated seeds were used as check for comparison. The LD_{50} values were observed based on growth reduction of seedlings after gamma ray treatment. The LD_{50} dose for gamma ray under *in vitro* and *in vivo* condition was fixed 300 Gy based on probit analysis. As the doses of applied gamma ray increased, there was a decrease in germination, survival rate of seedlings, root length, shoot length, seedling height, vigour index under *in vitro* conditions and emergence and survival under field (*in vivo*) conditions in M_1 generation as compared to the control.

Key words
Kodomillet, Gamma rays, Lethal Dose $_{50}$ (LD_{50})

Kodomillet (*Paspalum scrobiculatum* L.), a member of the family Poaceae, was domesticated in India some 3,000 years ago (Malleshi and Hadimani, 1994) and is cultivated as agricultural crop in parts of Madhya Pradesh, Maharashtra, Uttar Pradesh, Gujarat, Rajasthan and Tamil Nadu (de Wet *et al*., 1983). The creation and management of genetic variability becomes central base to crop breeding in any crop and more so in crops like Kodomillet, in which the available genetic variability is very limited owing to complete self-pollination in this crop due to its cleistogamous nature (Harinarayana, 1989).

The induced mutations are of considerable value for comprehension, evaluation and accelerating the process of plant improvement. Induced mutation by use of either physical or chemical mutagen is one way of creating variation in crop plants. The physical mutagens comprise of ionising radiation *viz.*, particulate (alpha rays, beta rays, fast neutrons and thermal neutrons) and non-particulate also called as electromagnetic radiation (X rays and gamma rays). The mutagenic action of X-ray was discovered by Muller (1927) in *Drosophila* and of gamma rays and X-rays in 1928 by Stadler in barley and maize, which opened up a new field of science in genetics. According to Kovacs and Keresztesa (2002), gamma rays are considered as the most penetrating in comparison to other radiation such as alpha and beta rays. Mutagenic effectiveness and efficiency in Kodomillet have been documented (Subramanian *et al.,* 2011). The identification of most effective mutagenic treatment and efficient mutagens is very essential to recover a high frequency and spectrum of useful mutations.

The present investigation aims at fixation of LD_{50} dose for gamma rays in Kodomillet variety CO 3. The seeds of Kodomillet variety CO 3 was obtained from, Department of Millets, Tamil Nadu Agricultural University (TNAU), Coimbatore. Well filled, healthy and uniform sized seeds handpicked from the seed lot and equilibrated to the moisture content of 8 per cent were packed in butter paper covers (500 seeds per treatment). The gamma chamber installed at Indra Gandhi Centre for Atomic Research, Kalpakkam where, Cobalt 60 serves as source of gamma rays was used for treatment. Seeds were placed in the Gamma chamber and exposed to gamma irradiation of five doses *viz.*, 100 Gy, 200 Gy, 300 Gy, 400 Gy and 500 Gy for appropriate time in each dose based on the half life of the source. Non-irradiated dry seeds were taken as control. The irradiated seeds were sown within 24 hours (M_1 generation). The treated seeds were placed in roll paper towels for germination test under *in vitro* condition with two replications. In another set of treatment seeds were sown in raised beds in the field (*in vivo*) along with the control. Germination %, survival % (14 DAT), shoot length, root length and vigour index were observed for both *in vitro* and *in vivo* conditions. Probit analysis (Finney 1971, 1978) was carried out to determine the lethal dose (LD_{50}) of gamma rays under *in vitro* and *in vivo* conditions.

The success of mutation breeding greatly depends on the rate of mutation, the number of screened plants and the mutation efficiency. Lethal dose, the percentage of test material that is killed by a specific dosage of radiation in which half will die, is the optimum dose that causes high frequency of favorable mutations with minimum damage to the plant. Before the start of an experiment in induced

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mutations, fixation of LD_{50} is very important, it varies with biological materials, nature of treatment and subsequent environmental conditions. In the present investigation, seeds of Kodomillet variety CO 3 were irradiated form 100 Gy to 500 Gy with an interval of 100 Gy. The LD_{50} value for gamma irradiation under in *vitro* is 320 Gy and *in vivo* condition was arrived as 311 Gy respectively (Fig. 1a, 1b). Subramanian *et al.*, (2009) reported the dose of 500 Gy as the LD_{50} dose for kodomillet crop. Subramanian *et al.*, (2011) investigated two varieties of Kodomillet namely, TNAU 51 and CO 3 and concluded that for optimum recovery of viable mutants in kodomillet, the dose 500 Gray would be suitable. Horn and Shimelis (2013) found that the effect of different doses of gamma radiation depends on genotypes. Therefore, LD_{50} dose is the optimum dosage for mutagenizing the seeds of different varieties to induce mutations to produce viable mutants and maintenance of population for mutation breeding.

For gamma rays, under in *in vitro* condition, seedling mortality per cent showed variation over the treated population at each dose (Table 1) which was 80 per cent at 500 Gy, 52 per cent at 400 Gy, 44 per cent at 300 Gy, 36 per cent at 200 Gy and 12 per cent at 100 Gy. Similar trend of variation was observed under *in vivo* condition, with a mortality percent of 85 at 500 Gy, 53 at 400 Gy, 48 at 300 Gy, 40 at 200 Gy and 10 at 100 Gy (Table 2). Under *in vitro* and *in vivo* condition, the percent reduction in germination over control and doses of gamma rays followed a linear trend. Similar observations of general decrease in effectiveness with increasing gamma ray irradiation were reported by Ganapathy *et al.*, 2008. The maximum values of percent reduction in survival over control were observed at 500 Gy dose.

The observation on shoot length, root length, total seedling length and vigour index on gamma ray induced mutants showed a significant effect on all the traits as compared to the control (Fig. 2, 3). The maximum values for shoot length, root length and total seedling value were observed in 100 Gy and the least values were recorded in 500 Gy. Vigour index also followed the same pattern exhibiting the maximum value at 100Gy and the minimum value at 500 Gy. The percent reduction in germination over control increased with the dose of the mutagen and it ranged from (12 %) 100 Gy to (80 %) 500 Gy under *in vitro* and (10 %) 100 Gy to (85 %) 500 Gy under *in vivo* conditions (Table1,2). A comparison of treatments both under *in vitro* and *in vivo* revealed that the survival reduction per cent was more pronounced at higher doses of 500 Gy followed by 400 Gy. The Higher dose 500 Gy expressed a reduction in all the character, which was in contrast to the lower dose 100Gy, exhibited the highest valves for all the parameters. The greater sensitivity at higher doses of mutagens has been attributed to various factors such as changes in metabolic activity of cells and disturbances of balance between promoters and inhibitors of growth regulators.

The present study revealed that the LD_{50} value for Kodomillet variety CO 3 under *in vitro* as 320 Gy and *in vivo* condition as 311 Gy through assessment of traits such as seed germination, survival rate of seedlings, shoot length, root length and vigour index. Based on the results of the study, it is concluded that, for optimum recovery of viable mutants in kodomillet, the dose 300 Gray would be suitable.

Acknowledgement
The first author would like to acknowledge the University Grants Commission for their financial assistances under the scheme of Maulana Azad National fellowship for Minority students, to carry out the research.

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Table 1. Germination, survival percentage and seedling parameters following gamma mutagenesis under *in vitro* condition in Kodomillet

Treatment	Germination (%)	Survival % (Reduction) in 14 DAT	Shoot length (cm)	Root length (cm)	Total seedling length (cm)	Vigour Index
Control	97	96	9.9	6.7	16.6	1610.2
100 Gy	90	88	9.0	5.2	14.2	1278.0
200 Gy	67	64	8.5	4.7	13.2	884.4
300 Gy	60	56	7.9	4.9	14.8	888.0
400 Gy	52	48	7.1	4.2	11.3	587.6
500 Gy	25	20	5.0	3.6	8.6	215.0

Table 2. Germination, survival percentage and seedling parameters following gamma mutagenesis under *in vivo* condition in Kodomillet

Treatment	Germination (%)	Survival % (Reduction) in 14 DAT	Shoot length (cm)	Root length (cm)	Total seedling length (cm)	Vigor Index
Control	96	94	15.3	7.2	22.5	2160.0
100 Gy	92	90	15.4	7.4	22.8	2097.6
200 Gy	64	60	12.3	7.2	19.5	1248.0
300 Gy	56	52	12.2	6.3	18.5	1036.0
400 Gy	48	47	12.4	6.4	18.8	902.4
500 Gy	20	15	9.3	6.2	15.5	310.0

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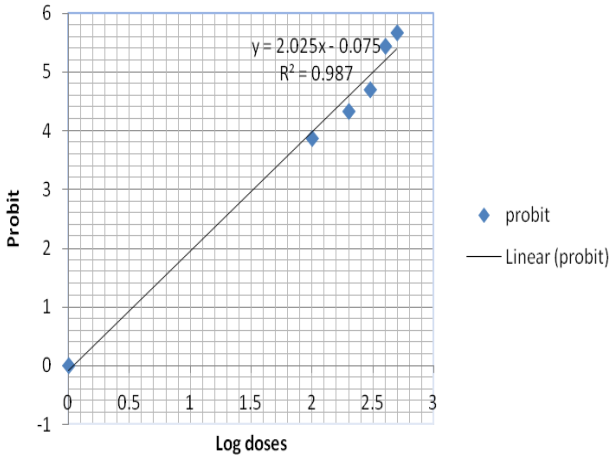

Fig. 1a. Calculation of LD_{50} of gamma irradiation in Kodomillet under *in vitro* condition

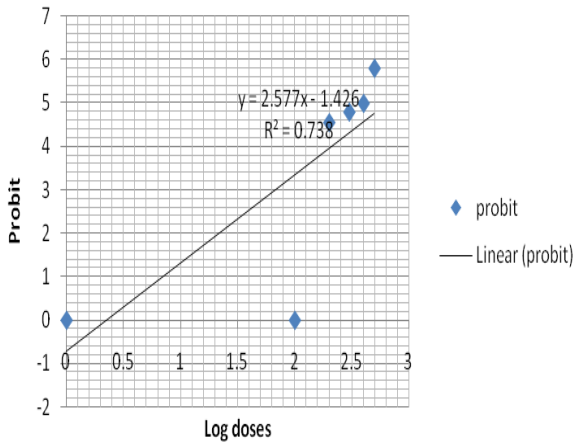

Fig. 1b. Calculation of LD_{50} of gamma irradiation in Kodomillet under *in vivo* condition

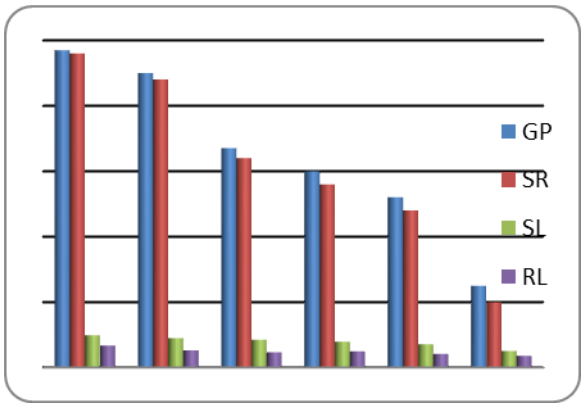

Fig. 2. Effect of gamma irradiation on germination % and seedling parameters under *in vitro* condition

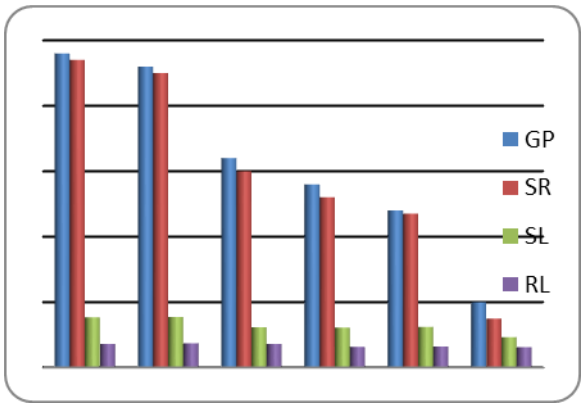

Fig. 3. Effect of gamma irradiation on germination % and seedling parameters under *in vivo* condition