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

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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 LD50 values were observed based on growth reduction of seedlings after gamma ray treatment. The LD50 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 and vigour index under in vitro conditions and emergence and survival under field (in vivo) conditions in M1 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 LD50 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 (M1 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 (LD50) 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
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 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 (Table 1, 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 values 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.

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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       |
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