International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
IJCS 2017; 5(2): 166-169

Received: 24-01-2017
Accepted: 25-02-2017

J Poornima Jency
Ph. D, Scholar, Tamil Nadu Agricultural University, Coimbatore - 641 003, Tamil Nadu, India

Ravikesavan R
Professor, Tamil Nadu Agricultural University, Coimbatore - 641 003, Tamil Nadu, India

Sumathi P
Professor & Head, Department of Millets, Centre for Plant Breeding and Genetics, Tamil Nadu Agricultural University, Coimbatore - 641 003, Tamil Nadu, India

Raveendran M
Professor, Centre for Plant Molecular Biology and Biotechnology, Tamil Nadu Agricultural University, Coimbatore - 641 003, Tamil Nadu, India

Correspondence
J Poornima Jency
Ph. D, Scholar, Tamil Nadu Agricultural University, Coimbatore - 641 003, Tamil Nadu, India

Effect of chemical mutagen on germination percentage and seedling parameters in Kodomillet variety Co 3

J Poornima Jency, Ravikesavan R, Sumathi P and Raveendran M

Abstract
An experiment was conducted to estimate the lethal dose of the chemical mutagen Ethyl methyl sulphonate (EMS) in Kodomillet variety CO 3. Genetically pure seeds were treated with different doses of EMS *viz.*, 0.2%, 0.3%, 0.4% and 0.5%. Untreated seeds were used as check for comparison. The LD_{50} values were observed based on growth reduction of seedlings after EMS treatment. The LD_{50} dose for EMS under *in vitro* 0.42*%* and *in vivo* condition was fixed 0.49% based on probit analysis. As the concentration of EMS 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.

Keywords: Kodomillet, EMS, Lethal Dose $_{50}$ (LD $_{50}$).

1. Introduction
India is endowed with a great wealth of agro-biodiversity and a large number of species are cultivated in India. For strengthening food and nutrition security in India, in a climate changing scenario, production of small millets need to be appreciated, enhanced and promoted (Padulosi *et al.*, 2009) [7]. Small millets in India are represented by six crops, namely, finger millet, Kodomillet, foxtail millet, little millet, prosomillet and banyard millet. Among small millets cultivated in India Kodomillet is the second important crop, next to finger millet. As genetic variability is essential for any crop improvement programme, 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. Among the approaches to create genetic variability, induced mutation is an important approach. Unlike hybridization and selection, mutation breeding has the advantage of improving a defect in an otherwise elite cultivar, without losing its agronomic and quality characteristics. It is the only straight forward alternative for crop improvement in plants having small size florets which is very difficult for emasculation and hybridization.
Alkylating agents were the first class of chemical mutagens to be discovered when Auerbach and Robson, 1946 [1] *found* the mutagenic effects of mustard gas and related compounds during World War II. Alkylating agents such as mustard gas, methyl methane sulfonate (MMS), ethyl methane sulfonate (EMS), and nitrosoguanidine have several effects on DNA. Because of its potency and ease with which it can be used, EMS is the most commonly used chemical mutagen in plants. EMS alkylates guanine bases and leads to mispairing, alkylated G pairs with T instead of C, resulting in primarily G/C- to-A/T transitions (Bhat *et. al.*, 2007) [2]. The present mutation breeding project, aims to report the effect of, ethyl methane sulphonate on the lethality, germination percentage and seedling parameters in an established variety of Kodomillet millet CO 3.

2. Materials and Methods
The present investigation aims at fixation of LD_{50} dose for EMS in Kodomillet variety CO3. The seeds were obtained from, Department of Millets, Tamil Nadu Agricultural University (TNAU), Coimbatore. Pre-soaking seeds before treatment enhances sensitivity to many chemical mutagens as reported by Singh and Sinha. The seeds were presoaked for 24 hours in distilled water initially and then the seeds after removal from the water were placed between

folds of blotting paper to remove water adhering on the surface. Then the seeds were immersed for 4 hours in the requisite concentration of mutagen with intermittent shaking. To ensure uniform absorption of the mutagen, the volume of mutagen solution was maintained at proportion of ten times to that of the seed volume. The whole treatment was carried out at a room temperature of 28 ± 1 °C. A sample of 100 seeds was soaked in distilled water for the respective duration to utilize it as control. Immediately after the completion of treatment duration, the treated seeds were thoroughly washed in running tap water for half an hour to eliminate the residual effect of the chemical and the excess moisture in the seed coat was removed by using folds of blotting paper and immediately sown. The treated seeds were placed in roll paper towels for germination test under *in vitro* condition with two replications. In another set of treatment the seeds were sown in raised beds in the field (*in vivo*) along with 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) [4, 5] was carried out to determine the lethal dose (LD_{50}) of EMS under *in vitro* and *in vivo* conditions.

3. Results and discussion

3.1 Determination of LD_{50} value

The success of mutation breeding greatly depends on the rate of mutation, the number of screened plants and the mutation efficiency. To avoid excessive loss of actual experimental materials, fixation of LD_{50} is very important; it varies with biological materials, nature of treatment and subsequent environmental condition. In the present investigation the seeds were treated with an EMS concentration of 0.2%, 0.3%, 0.4% and 0.5% to study the effect on various parameters *viz.*, survival, shoot length, root length, vigour index. LD_{50} values were determined with the help of probit analysis based on their survival rate after treatment with different concentration of EMS compared with untreated control. Optimum dose is the dose that cause maximum of mutation with minimum of damage to the plant. From the probit curve analysis the LD_{50} value for EMS under *in vitro* is 0.45% and *in vivo* condition was arrived as 0.47% respectively (Figure 1a, 1b). Therefore, LD_{50} dose is the optimum dosage for mutagenizing the seeds of different varieties to indue mutations to produce viable mutants and maintenance of population for mutation breeding.

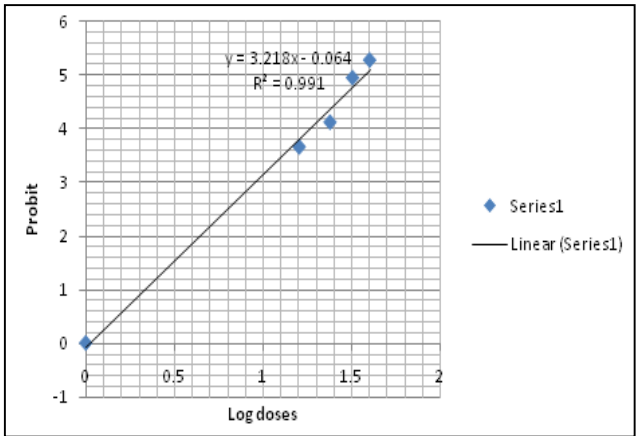

Fig 1a: Calculation of LD_{50} of EMS in Kodomillet under *in vitro* condition

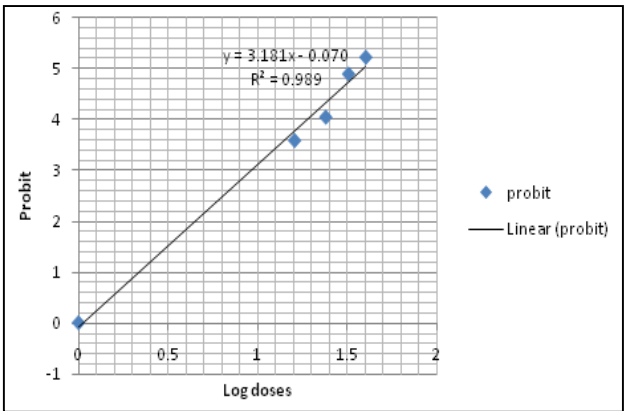

Fig 1b: Calculation of LD_{50} of EMS in Kodomillet under *in vivo* condition

3.2 Effect of EMS mutagenesis on Germination

Germination percentage of Kodomillet CO3 under different dose concentrations were calculated based on the germinated seeds after treatment and compared with control (non-treated). The study on germination percentage reported that there was a decrease in germination of seeds with related to increase in dose concentration of EMS under both conditions of *in vitro* and *in vivo*. In this study, the highest germination and survival percentage was recorded by the treatment 0.2%. (Table 1). Seedling mortality per cent showed variation over the treated population at each dose which was 60 per cent at 0.5%, 40 per cent at 0.4%, 26 per cent at 0.3% and 20 per cent at 0.2% similar trends of variation was observed under *in vivo* conditions, with a mortality percent cent of 55 per cent at 0.5%, 29 per cent at 0.4% and 17 per cent at 0.3% as well as in 0.2% (Table 2). Under *in vitro* and *in vivo* condition, the per cent reduction in germination over control and concentration of EMS followed a linear trend.

3.3 Effect of EMS on shoot length, Root length and vigour index

According to results obtained, shoot length and root length decreased in the proportion with increase in EMS concentrations. The shoot and root length decreased after increasing concentrations of EMS as compared to non-treatment control (Fig 3 & 4). The higher values for shoot length, root length and total seedling values were observed in 0.2% and the lowest values were recorded in 0.5%. Vigour index also followed the same pattern exhibiting the maximum value at 0.2% and the minimum value at 0.5%. Similar results were recorded by Eswari *et al*., (2014) [3] in finger millet. The percent reduction in germination over control increased with the dose of the mutagen and it ranged from (20 per cent) 0.2% to (60 per cent) 0.5% Gy under *in vitro* and (17 per cent) 0.2% to (55 per cent) 0.5% 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 0.5% followed by 0.4%. The Higher dose 0.5% expressed a reduction in all the character, which was in contrast to the lower dose 0.2%, exhibited the highest valves for all the characters. Therefore higher efficiency at lower concentration of the mutagen concentration was observed.

Table 1: Germination, survival reduction percentage and seedling parameters following EMS mutagenesis under *in vitro* condition in Kodomillet variety CO 3

Treatment	Germination (%) GP	Survival % (Reduction) in 14 DAT (SR)	Shoot length (cm) (SL)	Root length (cm) (RL)	Total seedling length (cm) TSL	Vigour Index (VI)
Control	96	94	15.3	7.4	22.7	2133.8
0.2% 16.10mM*	84	80	10.2	5.7	16.9	1419.6
0.3% 24.15mM*	76	74	7.4	3.2	10.6	805.6
0.4% 32.2mM*	60	60	4.6	2.4	7.0	420.0
0.5% 40.25mM*	40	40	5.1	2.1	7.2	288.0

*mM- millimolar

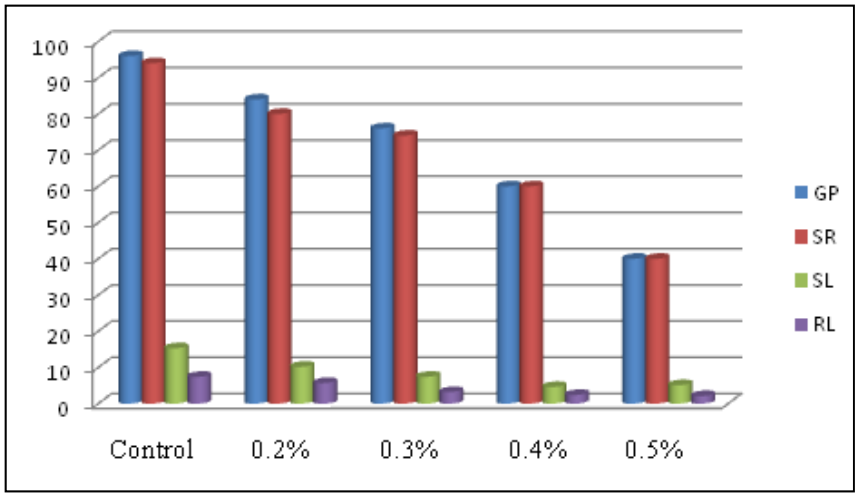

Fig 3: Effect of EMS concentration on germination % and seedling parameter under *in vitro* condition

Table 2: Germination, survival reduction percentage and seedling parameters following EMS mutagenesis under *in vivo* condition in Kodomillet variety CO 3

Treatment	Germination (%) GP	Survival % (Reduction) in 14 DAT (SR)	Shoot length (cm) (SL)	Root length (cm) (RL)	Total seedling length (cm) TSL	Vigour Index (VI)
Control	96	94	11.3	5.8	17.1	1607.4
0.2% 16.10mM*	87	83	10.9	5.3	16.2	1409.4
0.3% 24.15mM*	90	83	9.2	5.1	14.3	1344.2
0.4% 32.2mM*	75	71	9.5	4.2	13.7	972.7
0.5% 40.25mM*	50	45	7.2	4.5	11.7	526.5

*mM- millimolar

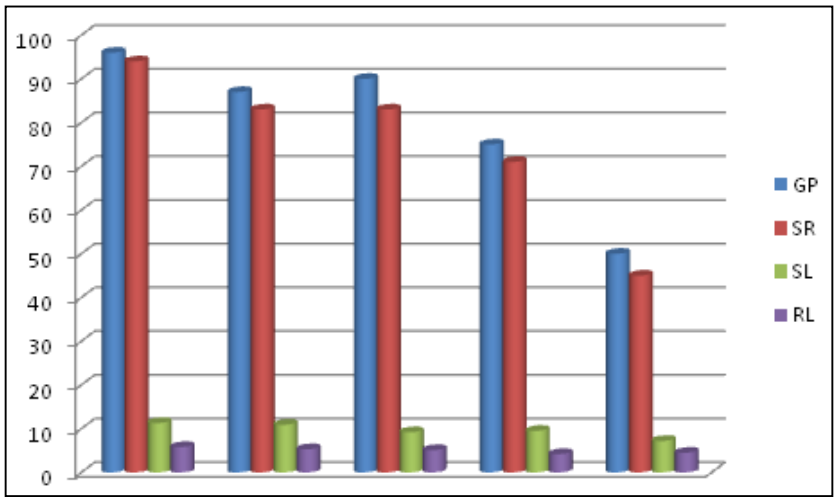

Fig 4: Effect of EMS concentration on germination % and seedling parameter under *in vivo* condition

4. Conclusion

Determination of LD_{50} dose is an essential pre-requisite in mutation studies, as excess dosage increases unwanted mutations, leading to sterility or even lethality. For induction of desired mutations by chemical being by chance, it is safer to choose the doses that can cause less damage and give higher multiplication and survival rates and also produce some useful mutations. On the whole, differences between concentration of EMS treatments significantly affected seedling height, root length and germination In the present study, based on the survival and growth rates, LD_{50} dose for Kodomillet variety CO3 have been fixed as 0.45% under *in vivo* and 0.47% under *in vitro* conditions. It is concluded that, for optimum recovery of viable mutants in Kodomillet, a dose of 0.4% would be suitable.

5. References

1. Auerbach C, Robson JM. Chemical Production of Mutations, *Nature*, 1946; 157(3984):302.
2. Bhat R, Upadhyaya N, Chaudhury A, Raghavan C, Qiu F, Wang H et al. Chemical- and Irradiation-Induced Mutants and Tilling. Rice Functional Genomics: Challenges, Progress and Prospects, Springer, New York, 2007.
3. Eswari K, Gogulan G, Hari Prathab KA, Sakila M. Development of early maturing mutants in finger millet, Res. J Agriculture and Forestry Sci. 2014.
4. Finney DJ. Probit analysis (3rd edition) Cambridge University Press, Cambridge, UK, 1971.
5. Finney DJ. Statistical method in biological assay. Charles Griffin and Co.edn, 1978.
6. Konzak CP, Nilan RA, Wagner J, Foster RJ. Efficient chemical mutagenesis. Radiat. Bot. 1956; 5:49-70.
7. Padulosi S, Bhag Mal, Bala Ravi S. Food security and climate change: role of plant genetic resources of minor millets. Indian J. of Plant Genetic Resources. 2009; 22(1):1-16.
8. Singh B, Sinha SK. Enhanced Sensitivity to Mutagens by Pre-Soaking - A Taxometric Study Using M_1 Seedling Traits in Finger Millet, Indian J. of Agric. Res, 2010.