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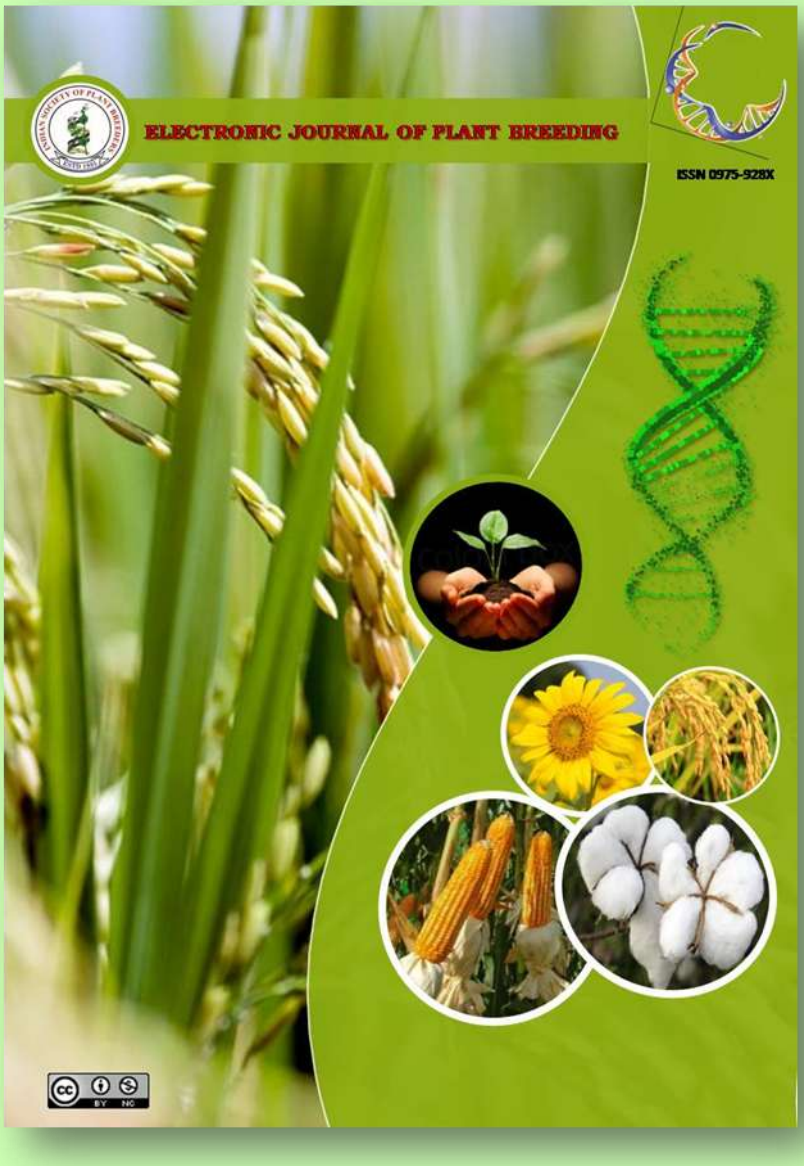

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Research Article

Determination of lethal dose and effect of EMS and gamma ray on germination percentage and seedling parameters in barnyard millet variety Co(Kv)2

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Abstract
An experiment was conducted to estimate the lethal dose of the chemical mutagen EMS and physical mutagen gamma ray in barnyard millet variety Co(Kv) 2. Genetically pure seeds were treated with different doses of EMS viz. 30mM, 40mM, 50mM, 60mM, 70mM, 80mM, 90mM and 100mM and gamma rays *viz.*, 500 Gy, 600 Gy, 700 Gy, 800 Gy, 900Gy and 1000 Gy. The LD_{50} values were observed based on growth reduction of seedlings after EMS and gamma ray treatment. The LD_{50} dose for EMS and gamma ray under *in vitro* and *in vivo* condition were fixed at 70mM and 800 Gy respectively, based on probit analysis. As the doses of applied EMS and 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.

Introduction

Barnyard millet (*Echinochloa* sp.) is one of the oldest domesticated minor millets in the semi-arid tropics of Asia and Africa. The genus *Echinochloa* includes some 20 species that are distributed widely in the warmer parts of the world. Two of the main species, *E. crus-galli* and *E. frumentacea* are grown as cereals(Clayton and Renvoize, 1986). Its wild ancestor is the tropical grass Echinochloa. It is grown in India, China, Japan and Malaysia and to some extent in Africa and United States of America. In India, it is cultivated over a wide array of environmental conditions and poor soils and is mainly confined to tribal belts of Orissa, Maharashtra, Gujarat, Madhya Pradesh, Tamil Nadu and Bihar besides hills of Uttar Pradesh (Channappagoudar *et al.*, 2008). The area under barnyard millet in India is about 1.95 lakh hectares and production of 1.67 million tonnes with the productivity of 8.57 q/ha. (Rashmi Yadav and Vijay KumarYadav, 2011). The yield level of barnyard millet is as high as 10 t/ha in Japan, where as in India it is 1.5 to 2 t/ha.So there is a greater scope for exploiting its potential in Indian condition (Channappagoudar *et al.*, 2008). The development of genotypes with high and stable productivity especially under semi arid agro-ecological situations of Tamil Nadu is very necessary.

The Co (Kv) 2 variety was released in the year 2009, which is a pure line selection from the local areas surrounding coimbatore district. So far, very limited work has been done for the genetic improvement of barnyard millet crop and continued to be a neglected and underutilized crop. So, there is an urgent need to develop a high yielding, nutritionally superior culture which is best suitable to the Southern districts of Tamil Nadu. Realizing the importance and need, the present investigation was, therefore, contemplated.

The creation and management of genetic variability becomes central base to crop breeding in any crop and more so in crops like barnyard millet, in which the available genetic variability is very limited owing to complete self-pollination in this crop due to its cleistogamous nature. The induced mutations are of considerable value for comprehension, evaluation and accelerating the process of plant improvement. The prime strategy in induced mutation breeding was to upgrade the well-adapted plant varieties by altering one or two major agronomic traits which limit their productivity or enhance their quality and potential source of creating variability (Novak and Brunner, 1992).However, mutation is regarded as random and success of obtaining desired mutant trait

depend on three factors such as efficiency of mutagenesis, the starting plant material and mutant screening (Hase *et al*., 2012).It has been clearly shown in a number of plant species that the effect induced varies with the varying mutagens and with variation in mutagen dose (Goyal and Khan, 2010). Induced mutagenesis has been successfully used to generate wider variability, portioning for isolating mutants with desirable characters of economic importance such as superior dwarf plant types for non-lodging, synchronous maturity, high tillers, high grain yield, larger seed size and desirable seed colour etc. (Ganapathy.et al., 2008). Induced mutation by use of either physical or chemical mutagen or both, is one way of creating variation in crop plants. The identification of most effective mutagenic treatment and efficient mutagens is very essential to recover a high frequency and spectrum of useful mutations (Jency et al, 2016). The physical mutagens comprise of ionising radiation *viz.*, particulate (alpha rays, beta rays, fast neutronsand thermal neutrons) and non-particulate also called as electromagnetic radiation (X rays and gamma rays). 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. Gamma-rays penetrate deeply into target tissues than other radiations (Mba et al., 2012) and it is less destructive, whereas other radioactive rays cause translocations, chromosome losses and large deletions (Sikora et al., 2011).

Alkylating agents such as mustard gas, methyl methane sulphonate (MMS), ethyl methane sulphonate (EMS), and nitroso guanidine have several effects on DNA. Among the alkylating agents, EMS is the most commonly used chemical mutagen in plants which 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). Therefore, EMS may likely be the mutagen of choice for TILLING (Target Induce Local Lesion in Genome) in plants. The dose assessment for chemicals is determined by varying the concentration and duration of treatment, solvent used or pH of the solution (Jain, 2010). Among the chemical mutagens, EMS has clastogenic (chromosome damaging) effects on plants via reactive oxygen-derived radicals (Yuan and Zhang 1993). These effects can occur both spontaneously and artificially following induction by mutagens. (Girija et al.,2013). However, the toxicity of EMS may vary de-pending on the species and pre or post treatments with other mutagens (Henikoff and Comai, 2003). The present investigation aims at fixation of LD_{50} dose for EMS and gamma ray in barnyard millet, variety CO(Kv) 2.

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. 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.

Materials and Methods

The seeds of barnyard millet, variety CO(Kv) 2 was obtained from, Department of Millets, Tamil Nadu Agricultural University (TNAU), coimbatore. For EMS mutagenesis, well filled, mature seeds (350 seeds each for each treatment) dried to 8% moisture content were selected and soaked in distilled water for 8 hours. Water was decanted and dried in shade for 6 hours. Fresh solution of Ethyl Methane Sulphonate (Hi-media, Mumbai) was prepared in phosphate buffer at pH 7.0 in different concentrations (30, 40, 50, 60, 70, 80, 90, and 100mM). Seeds were soaked for 4 hours at room temperature followed by decanting of the EMS and rinsing with 0.1 M sodium thiosulphate. Finally seeds were rinsed with running tap water for 1 hour to wash out the chemical residues. Thus treated seeds along with untreated water soaked seeds as control were used for the study.

For gamma ray mutagenesis, well filled, healthy and uniform sized seeds handpicked from the seed lot (350 seeds each for each treatment) and equilibrated to the moisture content of 8 per cent were packed in butter paper covers. The gamma radiation from ^{60}CO source gamma chamber installed at Tamil Nadu Agricultural University, coimbatore was used for treatment. Seeds were placed in the Gamma chamber and exposed to gamma irradiation of six doses *viz.*, 500 Gy, 600 Gy, 700 Gy, 800 Gy, 900 Gyand 1000 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 EMS and Gamma ray treated seeds of 25 each in each treatment were placed in roll paper towels for germination test under *in vitro* condition in two

replications. In another set of treatment,150seeds each in 2 replications were sown in raised beds in the field (*in vivo*) along with the control. Germination % (7 DAT), survival % (30 DAT), shoot length and root length (15 DAT) were observed for both *in vitro* and *in vivo* conditions and compared with the control. Pollen sterility was tested for each treatment by using two per cent freshly prepared potassium iodide solution and examined under stereomicroscope. Dark stained and normal size pollen grains were considered as fertile and those of irregular shape and size with light or no stain were considered as sterile. 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.

Probit analysis: The probit function is the inverse cumulativedistribution function (CDF) or quartile function associated with the standard normal distribution. The procedure for determination of LD_{50} using probit analysis is as follows.

1. Mutagen dose values were transformed into $\log_{10}$ value.
2. Mortality percentage of seeds due to treatment doses were worked out and rounded to the nearest whole number.
3. Corrected mortality percentage was calculated by using Abbott's formula given below and rounded to the nearest whole number.

$$\text{Corrected mortality (\%)} = \frac{M_{\text{observed}} - M_{\text{control}}}{100 - M_{\text{control}}} \times 100$$

4. The corrected values were converted to the probit transformation.
5. Probit values were graphed (Y-axis) against Log_{10} concentration (X-axis) and a straight line passing through most of the plotted points were drawn to estimate the Log_{10} concentration associated with a probit of 5.
6. Antilog to the Log_{10} value corresponding to the probit 5 was calculated to find out the LD_{50} for the particular mutagen under study.

Results and Discussion

Barnyard millet, variety Co(Kv)2 was chosen to study the effect of different doses of EMS and gamma ray to determine LD_{50}values. LD_{50} for EMS and gamma ray were determined with the help of probit analysis based on their germination of both varieties. Optimum dose is the dose that cause maximum of mutation with minimum of damage to the plant. In the present investigation, the LD_{50} value for EMS is 70.81 mM and for gamma irradiation is808.40 Gy under *invitro* conditions. Like this different results have been reported by different researchers, such as Jensy et al., 2016 as 320 Gy for Kodo millet, variety Co.3; Subramanian *et al.,* (2011) as 500 Gy for kodomillet, variety TNAU.51; Veni et al., 2016 as 300 Gy for MDU,1 Black gram and Ramchander et al., 2015 as 350 Gy in Paddy, variety white Ponni.

Gaul (1970) reported that the biological damage caused by mutagens in M_1 generation on seed germination and plant survival, may be considered as an indication for the mutagenic effects. In this present investigation, impact ofdifferent doses of EMS and Gamma rays on biological parameters such as germination%, shoot length and root length (15DAT), survival% and plant height (30DAT), pollen fertility and seed fertility at maturity were recorded and presented in Table.2.

In case of Germination % over control, proportionate reduction was noticed in both EMS (from 71.16 to 30.37) and gamma ray (from 62.14 to 29.47). Likewise, survival % also decreased from 62.37 to 24.62 due to EMS treatment and from 54.38 to 33.15 due to gamma ray. This clearly indicated that both the mutagens caused significant influence on germinationand survival that displayed a dose dependent negative linear relationship between dose and germination percentage and survival percentage. This reduction may be attributed due to disturbances at cellular level (caused either at physiological level or at physical level) including chromosomal damages or due to the combined effect of both (Khan and Tyagi, 2010).The same relationship was also reported by Talebi et al., 2012 in Rice and Pavadai *et al.,* 2010 in soybean.

Effect of mutagenson pollen fertility of in M_1 generation was studied and presented in Table 2. Pollen fertility reduction due to EMS treatment ranged from 59.31 per cent (30 mM) to 12.32per cent (100 mM) and gamma ray treatment ranged from 42.77 per cent (500 Gy) to 19.32 per cent (1000 Gy). The reduction in seed fertility due to EMS treatment ranged from 89.75 per cent (30 mM) to 45.52 per cent (100 mM) and gamma ray treatment ranged from 84.47 per cent (500 Gy) to 63.77 per cent (1000 Gy). These results are in agreement with those of Ramchander et al., 2015 in paddy. The present investigation envisaged that, pollen fertility percentage was decreased in higher rate with the increase in concentration or dose of both EMS and gamma ray. Similar observations were previously reported in blackgram (Surender and Vanniarajan, 2014). In most cases, meiotic abnormalities are responsible for pollen fertility reduction. One of the factors responsible for poor seed setting was due to sterility of pollen grains caused by mutagens. The decrease in pollen fertility after irradiation is considered to be due to chromosomal aberrations (Matsuo and Onozawa, 1961). Mutagens have been effective in decreasing

the mitotic index (Savakan and Toker, 1991) or increasing micronuclei number and pollen abnormalities (Savakan and Atila, 1991).

Anincreasedreduction on shoot length and root length was noticed in EMS compared to gamma rays. In both mutagens, all the treatments showed significant differences for shoot and root length reduction per cent over control. (Table,2). Shoot length showed reduction in both EMS and gamma ray as 89.16 per cent to 59.98 per cent and 91.16 per cent to 69.12 per cent respectively. Likewise, root length also decreased from 87.76 per cent to 58.39 per cent due to EMS treatment and 89.21 per cent to 67.55 per cent due to gamma ray.Plant height reduction due to EMS treatment ranged from 89.31 per cent (30 mM) to 65.59 per cent (100 mM) and gamma ray treatment ranged from 88.65 per cent (500 Gy) to 74.15 per cent (1000 Gy).The study exhibited gradual reduction of plant height in all the treatments of EMS and gamma rays compared to control and this exhibited linear fashion and dose dependant. Plant height was decreasing by increasing the dose of EMS and gamma ray (Tabasum *et al*. 2011).Seedling injury represented by reduction in shoot length, root length, plant height is broadly used as an index of determining biological effects of various physical mutagens in M_1 generation, which may be due to the metabolic processes affected at embryonic level. Sparrow (1961), while working on cytological effect of radiation, concluded that the decrease in vegetative growth is a result of radiation induced cytological changes such as chromosomal damages, inhibited mitotic division, degeneration of nuclei, cell enlargement, *etc*.

Based on the results of the study, it is concluded that, the optimum dosage for mutagenizing barnyard millet, Co (Kv) 2, for optimum recovery of viable mutants, the dose of 70 mM for EMS and the dose 800 Gray for gamma ray would be suitable, to produce viable mutants and maintain population for mutation breeding.

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Table 1. Determination of lethal doses by EMS and Gamma ray in Barnyard millet, Co (Kv) 2 Probit Analysis

Group	Dosage mM	Log dose	% Germination	% Corrected	Probits
			EMS		
1.	30.00	1.48	83.32	43.57	4.85
2.	40.00	1.60	78.66	48.13	4.95
3.	50.00	1.70	73.32	52.84	5.08
4.	60.00	1.78	68.66	56.64	5.18
5.	70.00	1.85	64.66	59.71	5.25
6.	80.00	1.90	57.32	65.07	5.39
7.	90.00	1.95	47.32	71.76	5.58
8.	100.00	2.00	34.66	79.72	5.84
			gamma ray		
1.	500 Gy	2.70	20.68	47.51	4.95
2.	600 Gy	2.78	26.68	52.84	5.08
3.	700 Gy	2.85	32.00	57.16	5.18
4.	800 Gy	2.90	38.00	61.68	5.31
5.	900 Gy	2.95	44.68	66.41	5.41
6.	1000 Gy	3.00	51.34	70.88	5.55

EMS LD50: 70.81mM, gamma ray - LD50 : 808.40 Gy

Table 2. Impact of EMS and gamma ray on biological parameters of barnyard millet, Co (Kv)2 in M_1 generation

Treatment	Germination (%) over control	Shoot length (%) over Control	Root length (%) Over Control	Plant height on 30th DAS (%) over control	Survival on 30th DAS (%) over control	Pollen Fertility Over Control (%)	Seed Fertility over control (%)
EMS							
30.00	71.16	89.16	87.76	89.31	62.37	59.31	89.75
40.00	64.97	87.65	86.33	88.67	58.74	52.67	87.99
50.00	58.66	86.24	84.92	88.12	55.32	48.68	86.31
60.00	53.73	85.89	83.05	87.85	51.28	45.9	84.0
70.00	49.93	83.43	78.60	84.88	44.36	37.3	76.2
80.00	43.29	78.52	73.44	82.14	39.10	29.9	70.2
90.00	35.86	71.16	64.75	76.35	32.38	19.16	61.19
100.00	30.37	59.98	58.39	65.59	24.62	12.32	45.52
Gamma ray							
500 Gy	62.14	91.16	89.21	88.65	54.38	42.77	84.47
600 Gy	59.21	89.82	86.54	87.12	51.65	41.36	81.35
700 Gy	55.66	86.59	84.34	85.64	48.29	38.0	78.9
800 Gy	49.86	80.53	79.83	83.52	43.01	26.8	73.3
900 Gy	41.90	74.98	72.51	78.88	37.42	24.0	69.6
1000 Gy	29.47	69.12	67.55	74.15	33.15	19.32	63.77

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