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Effect of gamma radiation on germination and seedling parameter of finger millet (*Eleusine coracana* L. Gaertn.)

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Abstract
In the present study, irradiation techniques were used to investigate the effect of gamma irradiation on germination and seedling parameter of finger millet. Finger millet (Var. Dapoli-1 and Dapoli Safed) seeds were irradiated with using Co^{60} gamma radiation (400, 500, 600, 700 and 750 Gy) at BARC, Mumbai. To find out LD_{50} dose for the seed, influence on germination, mean daily germination (MDG), root length, shoot length and other seedling parameters were investigated. The results laboratory studies showed that irrespective of doses the germination percentage was reduced in treated seed. It was higher in control (98.20 % and 91.40 % in Dapoli-1 and Dapoli Safed, respectively) and lowest in 750 Gy dose of gamma rays (70.40 % and 66.60 % in Dapoli-1 and Dapoli Safed, respectively). Shoot and root length decreased with increasing radiation doses. On field level, the percent of germination had decreased gradually after irradiation and the effect become stronger with an addition of gamma dose. It was high at control (89.33 % and 82.0 %) and low at 750 Gy (50.25 per cent and 48.92 per cent in Dapoli-1 and Dapoli Safed, respectively). Survival percentage was high at control and low at 750 Gy dose. The result also indicates that, in both the varieties, 500 Gy dose had near about 50 per cent reduction in root and shot length in laboratory study and 50 per cent reduction in percent survival during field studies. The study clearly indicated an increase in the deleterious effects of gamma irradiation at regular intervals and the LD_{50} dose for the both varieties located near to the dose of 500 Gy for both varieties of finger millet.

Keywords: finger millet, gamma irradiation, ld_{50}, germination percentage, survival percentage

1. Introduction

Among the major food grains, finger millet (*Eleucina coracana* L. Gaertn) is one of the most nutritious crops. It is an important food crop in South Asia and Africa. The grain of finger millet has a fine aroma when cooked or roasted and it is known to have many healthy promising qualities. It is a rich source of calcium and has a good amount of magnesium, phosphorous and iron. Finger millet has a favourable amino acid spectrum that includes cysteine, tyrosine, tryptophan and methionine (Rachie, 1975) [20].

The seedling stage is a convenient phase in the plant's life cycle for use in radiological studies to determine relative radiosensitivity of species and its effects of various factors plants. Earlier experiments in this field have indicated that ionising radiation could cause permanent genetical effects, lethal or beneficial mutations, morphological modifications and other effects in plants. Several factors may be involved in the inhibition of germination and the growth of the plants from seeds following their exposure to high irradiation doses. A number of radiobiological parameters are commonly used in an early assessment of the effectiveness of radiation doses (Aparna *et al*., 2013) [18].

Gamma rays, the physical mutagen are non-particulate ionising radiations, having high energy and penetrable capacity in biological tissues and make changes in the base, disruptions of hydrogen bonds between complementary strands of DNA. A large majority of mutant varieties was developed by the use of gamma rays (Ahlowalia *et al*., 2004) [2].

This study was conducted to study the effects of gamma radiation of different doses from Co^{60} on the germination and seedling parameters of finger millet (*Eleusine coracana* L. Gaertn.). Such a study is needful to unveil any desirable features for agricultural and agronomic benefits and to determine the appropriate dose of irradiations before the actual commencement of field experiment.

2. Materials and methods
2.1 Plant material
The material for this study comprised of two varieties of finger millet, Dapoli-1 (mid-tall, fully open ear heads with brown seeds) and Dapoli Safed (mid-tall, partially open ear heads with white colour seeds).

2.2 Gamma irradiation
Irradiation of dry seed of both varieties was done using a Co^{60} (Cobalt 60) gamma source at the Bhabha Atomic Research Centre, Mumbai. The doses of exposure were 400 Gy, 500 Gy, 600 Gy, 700 Gy and 750 Gy. The control seeds were not enlightened to gamma rays.

2.3 Radiation sensitivity test
Five replicates of 100 seeds for each replication were sowed for each dose on moistened filter paper and then germinated at 20 ℃ with an 8 hrs. photophase, in seed germinator under laboratory conditions. Seeds were considered germinated after initiation of radical and plumule elongation. Counts of germinated seeds were made for every day up to the seventh day of seed sowing to determine the final germination.

2.3.1 Determination of germination parameters
a. The final germination percentage (FGP) was worked out according to Anjum and Bajwa (2005) [7] as follows

$$FGP = \frac{NT \times 100}{N}$$

Where, NT = The proportion of germinated seeds in each treatment for the final measurement and N = Number of seeds sown on germination paper.

b. The Germination Index (GI) was computed as described in the Association of Official Seeds Analysts (AOSA).

Germination Index (GI) = $\sum(\frac{N_1}{D_1} + \frac{N_2}{D_2} + -------+\frac{N_n}{D_n})$

Where, N_1 = Number of germinated seed on first day
D_1 = Day of first count
N_n = Number of germinated seed on last count
D_n = Day of last count

c. Mean daily germination (MDG) was calculated by following formula (Edwards, 1934) [13].

$$MDG = \frac{\text{Total number of germinated seeds}}{\text{Total number of days}}$$

2.4 Root and shoot length
Effect on root and shoot was measured in terms of length on the seventh day. Shoot and root length were measured using ten seedlings collected at random from each dose per replication on the seventh day from the seeds subjected to germination test. The shoot and root length were measured in centimeters (cm) using a scale and root/shoot length ratio was calculated using the estimates of seedling length. Vigour index (VI) was estimated as per the formula given by Abdul-Baki and Anderson (1972) [14] as follows,

$$VI = (MRL + MSL) \times PG$$

Where, VI = Vigour index; MRL=Mean root length; MSL= Mean shoots length; PG = Per cent germination.

2.5 Field study
Five replicates of 100 seeds each treatment was sown in a plastic tray and all trays were kept in an open environment to understand the environmental effect on germination percentage. The numbers of plant survivals after 30 days was recorded to calculate the survival percentage.

2.6 Statistical analysis
Observation data were subjected to one-way analysis of variance (one-way ANOVA), at a 1 per cent and 5 per cent level of probability for laboratory and field study, respectively, to determine the differences in an average of all tested parameters between irradiated and non-irradiated plantlets. Statistical analysis was performed using Statistical Analysis System software (SASs) V. 9.1 (June 2006), SAS Institute.

2.7 Determination of LD_{50}
For determination of LD_{50}, observations on germination parameters and other seedling parameters were recorded. By considering cumulative averages of all parameters *viz*., germination (lab test and field level germination) and seedling parameters (root and shoot length), LD_{50} of gamma irradiation for both varieties were determined.

3. Result and discussion
3.1 Effect on germination (Lab experiment)
It was noted that irradiation with gamma rays resulted in to decrease in germination and other seedling parameters. Dapoli-1 showed the highest germination percentage in the control (98.20 %) followed by 400 Gy dose (96.00 %). A substantial decrease in germination percentage was observed at higher doses of gamma rays and maximum reduction was noted at 750 Gy dose (Table 1).

In variety Dapoli Safed, the maximum germination percentage was recorded in the control (91.40 %). The lowest germination percentage (66.60 %) was recorded at 750 Gy dose (Table 1). From the results, it indicates that both varieties *viz*., Dapoli-1 and Dapoli Safed, responded differently at similar doses of gamma irradiation. The different response of both varieties on the same level of irradiation was may be due to a different genetic constituent of both varieties. Similar outcomes were reported by Ambavane *et al*. (2014) [4] in finger millet, Ahmad and Qureshi in *Zea mays* L. and Din *et al*. (2003) [12] in *Triticum aestivum* L.

Noticeable variations were observed in germination percentage after gamma irradiation in lab germination test, but the variation was neither proportional to the increase in dosages nor definite pattern was found in both varieties. The irregular response of the different doses on germination might be due to the irradiation of gamma rays sometimes inhibit synthesis of protein or sometimes it enhances the protein synthesis and other metabolic activity. Similar results have been reported in finger millet by Ambavane *et al*. (2014) [4], in rice by Cheema and Atta (2003) [11], Pathak and Patel (1988) [19], Ando (1970) [6].

Germination Index (GI) and Mean daily germination (MDG) was maximum in the control of both varieties, whereas, it 750 Gy dose GI and MDG both were minimum (Table 1). Both, GI and MDG decreased linearly with increase in the strength of gamma rays. From this result, it was observed that irradiation resulted into delay in the germination process. As the strength of irradiation increased linearly, time require for germination increased. Similar kind of results were reported by Cheema and Atta (2003) [11], Pathak and Patel (1988) [19], Ando (1970) [6] in rice.

Table 1: Germination parameters affected by gamma rays in two finger millet varieties (Laboratory Test) (data represents mean ± SE)

Doses	Variety	Germination %	% Reduction	Germination index	Mean daily germination
Control	Dapoli-1	98.20 ± 0.37	-	19.73	14.03
	Dapoli Safed	91.40 ± 0.60	-	18.95	13.06
400 Gy	Dapoli-1	96.00 ± 1.05	2.24	17.52	13.71
	Dapoli Safed	85.60 ± 0.75	6.35	15.82	12.23
500 Gy	Dapoli-1	87.00 ± 1.10	11.41	16.06	12.43
	Dapoli Safed	81.00 ± 0.71	11.38	14.72	11.57
600 Gy	Dapoli-1	91.00 ± 0.58	7.33	15.68	13.00
	Dapoli Safed	85.40 ± 0.96	6.56	14.43	12.20
700 Gy	Dapoli-1	93.00 ± 1.00	5.30	15.17	13.29
	Dapoli Safed	86.00 ± 0.71	5.91	14.21	12.29
750 Gy	Dapoli-1	70.40 ± 0.51	28.31	10.84	10.06
	Dapoli Safed	66.60 ± 1.33	27.13	10.77	9.51

3.2 Effect on seedling parameters

Seedling height is widely used as an index in determining the biological effects of various physical and chemical mutagens (Konzak *et al.*, 1972) [17]. The data on root and shoot length, seedling vigour (Vigour Index), per cent reduction (root and shoot length) and Root/Shoot length ratio of treated seed is given in Table 2. Maximum root length (5 cm) was observed in the untreated seed of Dapoli-1 and it was lowest (0.80 cm) in treated seed of 750 Gy. Similarly, in the shoot length was maximum (4.64 cm) in untreated seed whereas it was minimum (0.50 cm) in treated seed of 750 Gy.

In Dapoli Safed, the maximum (7.06 cm) root length was observed in untreated seed and minimum (2.50 cm) was recorded in 750 Gy treated seed. The maximum shoot length (3.75 cm) was observed in untreated seed, whereas it was minimum in 750 Gy (1.15 cm).

Root and shoot length decreased after doses of irradiation as compared to non-irradiated control in both varieties. The maximum reduction in root and shoot length was observed in 750 Gy dose. The reduction in root and shoot length occurred with each corresponding increase in the strength of gamma rays dose. It was also observed that the root and shoot length decreased with increase in gamma rays dose on approximately linear mode. A similar result was reported by Ambavane *et al.* (2014) [4] in finger millet, Talebi and Talebi (2012) in rice.

Seedling vigour (Vigour Index) were decreased progressively with increasing radiation doses (Table 2) in both varieties. It was higher at a lower dose and lower at a higher dose. The same results were also observed earlier by Ambavane *et al.* (2014) [4] in finger millet, in sesame by Anbarasan *et al.* 2013 [5] and Boranayaka *et al.* 2010 [10].

Table 2: Average of root and shoot length affected by gamma rays in two finger millet varieties (data represents mean ± SE)

Doses	Variety	Root length (cm)	Shoot length (cm)	Vigour index	% Reduction		Root/shoot length ratio
					Root length	Shoot length	
Control	Dapoli-1	5.00 ± 0.07	4.64 ± 0.23	946.64	-	-	1.08
	Dapoli Safed	7.06 ± 0.15	3.75 ± 0.04	988.03	-	-	1.88
400 Gy	Dapoli-1	3.80 ± 0.11	3.10 ± 0.10	662.40	24.00	33.19	1.23
	Dapoli Safed	4.75 ± 0.22	2.43 ± 0.06	614.60	32.72	35.20	1.95
500 Gy	Dapoli-1	2.60 ± 0.04	2.25 ± 0.09	421.95	48.00	51.51	1.16
	Dapoli Safed	3.58 ± 0.37	1.95 ± 0.10	447.93	49.29	48.00	1.84
600 Gy	Dapoli-1	1.35 ± 0.12	1.60 ± 0.11	268.45	73.00	65.52	0.84
	Dapoli Safed	3.45 ± 0.35	1.95 ± 0.09	461.16	51.33	48.00	1.77
700 Gy	Dapoli-1	1.20 ± 0.05	1.45 ± 0.09	246.45	76.00	68.75	0.83
	Dapoli Safed	3.25 ± 0.13	1.48 ± 0.08	406.78	53.96	60.53	2.20
750 Gy	Dapoli-1	0.80 ± 0.10	0.50 ± 0.13	155.58	84.00	89.22	1.60
	Dapoli Safed	2.50 ± 0.14	1.15 ± 0.33	243.09	64.58	69.33	2.17

3.3 Effect on germination (Field level experiment)

On field level, germination percentage decreased with increase in strength of gamma rays in both varieties (Table 3). It was maximum in control *i.e.* 89.33 per cent and 82.00 per cent in Dapoli-1 and Dapoli Safed, respectively. Lower germination reported in 750 Gy dose in both varieties. Percentage of reduction in germination linearly increased with increase in strength of gamma rays (Table 3). Similar results have been reported by Ambavane *et al.* (2014) [4] in finger millet, Talebi and Talebi (2012), Harding *et al.* (2012) [14], Cheema and Atta (2003) [11], Singh *et al.* (1998), Pathak and Patel (1988) [19], Ando (1970) [6] in rice. This might be due to the irradiation of gamma rays interrupt or slow down metabolic activity of seedling and inhibit synthesis of protein during field level germination.

In Dapoli-1, maximum survival percentage was recorded in control (98.00 %). In treated seed maximum survival percentage was recorded in 400 Gy dose (78.55 %) whereas it was minimum at 750 Gy dose (32.22 %).

In Dapoli Safed, maximum survival percentage was recorded in control (96.00 %). In treated seed, maximum survival percentage was recorded in 400 Gy dose (74.22 %) and it was minimum at 750 Gy dose (30.10 %). Survival percentage linearly decreased with increase in strength of gamma rays in both varieties (Table 3).

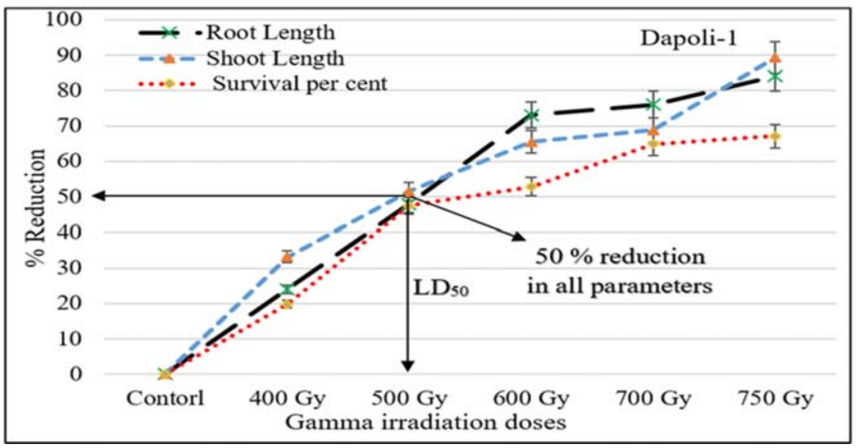
The result showed that increasing dosage from 400 Gy to 750 Gy reduced field survival and mean value for field and survival were decreased with increase in the dose of gamma rays. The present results are in conformity with earlier reports in rice by Harding *et al*. (2012) [14]; Horn *et al*. (2010) [18] in pearl millet; Cheema and Atta (2003) [11] in Basmati rice variety. The results of Koing *et al*., (2008) have shown that survival of plants to maturity depends on the nature and extent of the chromosomal damage. Increasing frequency of chromosomal damage with increasing radiation dose may be responsible for less germinability and reduction in plant growth and survival (Borzouei *et al*., 2010) [9].

3.4 Determination of LD_{50}

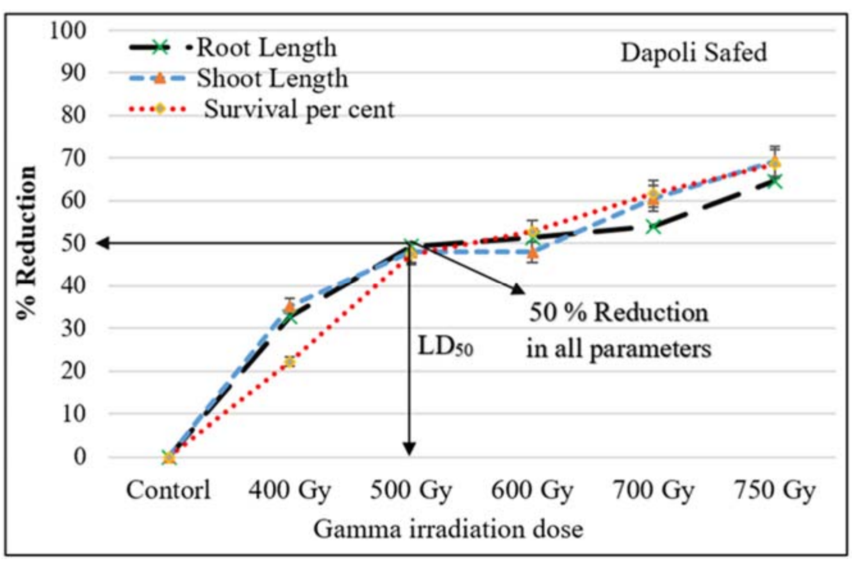
From the result obtain in this study reviled that, reduction in root and shoot length in 500 Gy dose was about 50 per cent in both varieties (Table 2, Fig. 1). Also, near about 50 per cent reduction in per cent survival was observed in 500 Gy dose in both the varieties (Table 3, Fig. 1). This results reviled that, LD_{50} for the both verities were located around the 500 Gy dose of gamma rays for the both varieties of finger millet use for this study.

Table 3: Germination and survival percentage affected by gamma rays in two finger millet varieties (Field Trial) (data represents mean ± SE)

Doses	Variety	Germination %	Survival %	% Reduction in germination	% Reduction in survival
Control	Dapoli-1	89.33 ± 1.05	98.00	-	-
	Dapoli Safed	82.00 ± 2.28	96.00	-	-
400 Gy	Dapoli-1	83.00 ± 2.59	78.55	7.09	19.84
	Dapoli Safed	76.54 ± 1.08	74.22	6.66	22.22
500 Gy	Dapoli-1	79.26 ± 1.34	51.42	11.27	47.53
	Dapoli Safed	73.32 ± 1.60	49.54	16.59	47.40
600 Gy	Dapoli-1	75.42 ± 2.52	46.25	15.57	52.80
	Dapoli Safed	70.91 ± 1.21	44.23	13.52	52.85
700 Gy	Dapoli-1	68.38 ± 4.78	34.41	23.45	64.88
	Dapoli Safed	67.12 ± 1.41	35.55	18.15	61.68
750 Gy	Dapoli-1	50.25 ± 2.75	32.22	43.75	67.12
	Dapoli Safed	48.92 ± 2.82	30.10	40.34	68.65



A



B

Fig 1: Per cent reduction in survival per cent, root shoot length as of control of two varieties (A-Dapoli-1, B-Dapoli Safed) subjected to five levels of gamma irradiation

4. Conclusion

The results of this work show that higher doses of gamma rays produce a detrimental effect on germination and other seedling parameters. From both, laboratory and field studies, it revealed that LD_{50} for the finger millet is located around the 500 Gy dose and treating finger millet with dosages around LD_{50} will be more awardable in order to get number of desirable mutants.

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