Original Research Article

Determination of radio sensitivity (LD_{50}) of embryo cultured banana (*Musa balbisiana*, BB) cv. Bhimkol

ABSTRACT

Banana is parthenocarpic and vegetatively propagated crop due to which *in vitro* mutagenesis approaches have substantial scope to create variations for crop improvement. Bhimkol cultivar is a wild seeded type and it set seeds naturally. The immature seeds can be used for the *in vitro* mutation to create variability. Prerequisite in an *in vitro* mutation study, is to determine the appropriate dosages to produce larger recovery of mutants with diminutive population loss. It is necessary to fix the lethal dose (LD_{50}) for optimizing the radiation doses for immature seeds of banana. In the present investigation, in order to fix the LD_{50} value immature seeds of banana were subjected to five different doses of gamma irradiation ranged from 5-25 Gy with control (untreated). The present findings observed that as gamma radiation dose increased, delayed germination and reduction in the germination percentage of immature embryos. And also, seedling height and root length reduced significantly. The probit analysis based on the germination percentage showed that, LD_{50} dose of gamma radiation to be 15 - 20 Gy for higher gemination percentage of immature seeds in banana cv. Bhimkol.

KEYWORDS: *In vitro*, Gamma rays, *Musa*, Zygotic Embryo, LD_{50}, Bhimkol

INTRODUCTION

Banana and plantains (*Musa* spp., Musaceae) are cultivated in the most of the countries of tropical and sub-tropical regions of the globe. Banana is considered fourth most important food commodity after rice, wheat and milk and also serves as vital food for millions in the world. India is the major producer of bananas in the world, with production of 31.5 million tonnes from an area of 0.878 million hectare with an average productivity of 35.8 tonnes per hectare (FAOSTAT, 2020). It is popular because of its year-round availability, high production and high consumer preference for its flavour and aroma. Banana (*Musa balbisiana*, BB) cv. Bhimkol is a wild diploid seeded cultivar which sets seeds naturally. The two seeded diploid cultivars of *Musa balbisiana* are Athiykol and Bhimkol

(2x=2n=22) from North-east region of India (Simmonds, 1962), which are not grown for commercial purpose as any other banana in the globe. They are vigorous in growth, very hardy species, highly tolerant to biotic and abiotic stresses (Uma *et al.*, 2011).

Bhimkhol is an important backyard banana variety spread mainly in Assam and some extent to neighbour states such as Arunachal Pradesh, Nagaland, Meghalaya and West Bengal. The Bhimkol fruits are consumed as dietary supplement by North-east people of India because of its rich source of carbohydrates, vitamins and proteins. Baby products like Bhimvita and Bhimsakti are prepared from the immature fruits of Bhimkol. Present day, market demand is increasing day by day for its nutritional value and health benefits. In addition, the various parts of the plant are used as food, in religious rites and also as medication to treat diseases like jaundice and dysentery. Therefore, this plant has incredible commercial value in North-east India (Borborah *et al.*, 2016). There is abiding need for the banana growers of North-east regions for seedless Bhimkol. However, till date no systemic research effort was initiated to make Bhimkol seedless. The consumers preference and industrial demand of Bhimkol shall be increased many folds, if Bhimkol is made seedless (Northeast News, 30th April, 2014).

Mutation breeding is well known to be appropriate method for changing a specific trait without changing much of the genetic constitution, but in practise, chromosomal aberrations are more common. As a result, selecting the appropriate dose is critical for inducing a higher mutation rate in a target trait with minute effect on the enduring genetic background (Coban, 1998).

In vitro mutagenesis is powerful tool and advantageous approach to create higher variations in the population with shorter span of time and space requirements. Chemical and physical mutagens can be used for inducing mutations. In physical mutation gamma rays as mutagen are most significantly used for mutation studies. The plants retort differently to the mutagens depending on the species, even diverges among the same genotype (Kwon and Im, 1973). The selection of potent mutagen and the determination of LD_{50} are the two most important steps involved in induced mutagenesis since they depend on the mutagen and genotype (Kodym and Afza, 2003).

The two key factors *viz.*, rate of mutation and mutation efficiency, have a significant impact on the success of mutation breeding. The total dose of the mutagen affects the mutation rate, which can be altered by physical and biological factors. The efficacy of a

mutagenic action in encouraging genetic variations in crop plants depends on the genetic composition of test varieties and dosage of mutagen (Van Harten, 1998; Mba *et al.*, 2010).

The term "radio sensitivity" refers to a comparative assessment of the quantity of perceptible effects on the irradiated material (Owoseni *et al.*, 2007) and to fix the optimal dosages, it is critical to perceive the growth responses after irradiation. The optimal mutagenic dose can accomplish a higher mutation frequency (Mba *et al.*, 2010).

The present investigation was taken up as the primary step for the mutation studies in banana cv. Bhimkol, which is intensively cultivated for its nourishing health benefits in North-east regions of India. The current study was performed to fix the lethal dose (LD_{50}) of gamma irradiation for immature seeds of banana cv. Bhimkol.

MATERIALS AND METHODS

Explant source

The immature fruits were harvested at 70-80 days after shooting which correspondent to 60 – 65% maturity of the fruits. These immature seeds (immature zygotic embryos) were used as explant for the present experiment.

Extraction of seeds from immature fruits

The seeds were manually extracted from the pulp and the seeds were rubbed of between the fingers with continuous running tap water. The extracted seeds were collected in beaker containing distilled water and allowed to settle for 15 minutes. Most of the floating seeds which do not have embryos were discarded and only the sunken seeds were used for seed morphology studies and as an explant source for the present study.

Surface sterilization and gamma irradiation of explants

Surface sterilization of seeds was done at sterile condition in laminar air flow cabinet. Seeds were surface sterilized with 0.1% mercuric chloride ($HgCl_2$, SRL, Pvt. Ltd., India) for 10 minutes followed by rinsing the seeds 4-5 times with sterile distilled water. Then seeds were transferred to the sterile disposable petri plate (90 mm × 15 mm, Hi-media, India) containing MS medium without sucrose and growth regulators. These immature seeds were subjected to the gamma irradiation with different doses (5, 10, 15, 20 and 25 Gy). Gamma irradiation was given using Cobalt 60 (^{60}Co) as gamma source (Low dose irradiator 2000, Board of Radiation and Isotope Technology (BRIT), Mumbai, India) established at National

Research Centre on Banana, Tiruchirappalli. Exposure time (minute or second) was estimated based on the dose (Gy/s or Gy/min) of the gamma source.

Inoculation of immature zygotic embryos

Embryos from the gamma irradiated seeds were extracted in the laminar air flow cabinet. Embryos were excised manually by making a diagonal cut with a sharp scalpel blade near the micropylar plug of the seed to break it open in two unequal halves. The mushroom shaped embryos were removed gently and cultured on MS medium enhanced with 0.1 mgL^{-1} GA_3 of 15 mL of semi-solid medium held in autoclaved test tubes. The cultures were incubated in 16/8 hours (light/dark) photoperiod and temperature at 25±2 ºC for further observations. Cultures were examined daily for the first 15 days and then at weekly intervals to record contamination (if any), germination percentage and plantlet development, till six weeks after inoculation.

Observations and statistical analysis

The days taken for response, days taken for germination, germination percentage, shoot length and number of leaves were recorded at 40-45 days after inoculation. The experiment was laid in a completely randomized design (CRD), with four replications comprising 10 embryos per treatment. The data were analysed using SPSS statistics version 22.0 and the means were compared with DMRT (Duncan's Multiple Range Test) at 0.05% critical difference.

After 20-25 days, the germination percentage was calculated using the formula,

$$\text{Germination percentage (\%)} = \frac{\text{No.of embryos germinated after irradiation}}{\text{Total no.of seeds exposed to gamma irradiation}} \times 100$$

Based on the probit analysis and Finney's table (Finney 1971), the lethal dose for gamma irradiation was determined. The probit function associated with the standard normal distribution is the inverse cumulative distribution function (CDF). The corrected mortality was worked out the formula,

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

To fix the lethal dose (LD_{50}), the data were determined by the Probit analysis (Finney, 1978) and standard analysis of variance procedure using SPSS version 22.0. The LD_{50} was intended by fitting the straight-line equation y= a + bx (simple linear regression model), where y represents the response variable (mortality percentage), x represents the independent

variable (dosage of gamma rays), while a and b represent the slope and constant, respectively. The number regenerated plantlets from embryos were recorded. After 40-45 days, the plantlets were transferred to the rooting medium containing half strength MS medium supplemented with 0.5 mgL^{-1} IBA. The well-developed *in vitro* plantlets were hardened in cocopeat media.

RESULTS

One of the crucial parameters for determining the dose levels for a specific mutagen is the survival or germination percentage of irradiated material. The diploid bananas were most subtle to gamma radiations (Novak, 1990). In the present investigation a gradually reduces the germination percentage of immature zygotic embryos with increase in dose of gamma radiations (Table 1). Among the different treatments of gamma radiation for *in vitro* germination of immature zygotic embryos, the germination percentage was ranged from 33.33 per cent in 25 Gy (with 54.54 per cent reduction over control) to 63.33 per cent in 5 Gy (with 13.63 per cent reduction over control) and the highest germination percentage was 73.33 per cent in untreated (control) in Table 1.

The lethal dose (LD_{50}) was intent based on the germination percentage. The probit assay was carried out based on the germination percentage of zygotic embryos (Table 2). In the present study, LD_{50} value for gamma radiation as assessed from the probit curve analysis was 22.39 Gy for *in vitro* germination of immature zygotic embryos (Fig.1)

Similar to the germination percentage there was decline in seedling height (5.66 cm) and number of leaves (2.47) with increase in gamma irradiation dose as compare to control, whereas it took higher number of days taken for response (8.93 days) and germination (28.53 days) with increase in gamma irradiation dose as compare to control. The less number of days taken for response (6.53 days), days taken for germination (23 days), highest germination percentage, number of leaves, seedling length and root length (73.33%, 3.20, 6.12 cm and 7.27 cm respectively) was observed in control and the root length (7.53 cm) was on par with the treatment 25 Gy (Table 3, 4 and Fig. 2, 3).

DISCUSSION

The optimal dosage of gamma rays is crucial aspect in mutation induction and its consequence has been studied by estimating various responses such as lethality (Sripichtt *et al.*, 1988) and growth (Bottino *et al.*, 1975). Gamma radiation was reported to have an inhibitory effect at higher doses, although stimulatory effects may ensue at lower doses

(Radhadevi and Nayar, 1996; Kumari and Singh,1996). The increased germination, cell growth, cell proliferation, enzyme activity, stress resistance and crop yields at lesser dosage of gamma radiations have been reported by several researchers (Charbaji and Nabulsi, 1999; Baek *et al.,* 2005; Chakravarty and Sen, 2001; Kim *et al.,* 2000, 2005).

The current study found a linear decrease in germination percentage with increasing gamma irradiation doses, which is similar to previous mutation experiments in banana reported by many researchers (Hase *et al.,* 2002; Mishra *et al*., 2007; Taheri *et al.,* 2014; Abdulhafiz *et al*., 2018). Sharma *et al.* (2013) reported that seed germination percentage gradually declined with increasing doses of gamma rays in citrus (*Citrus jambhiri*). The germination percentage gradually decreased with increase in dosage of gamma radiation in okra (*Abelmoschus esculentus*) reported by Kumar and Mishra, 2004. The similar results were found in Avocado cv. Duke and Hass (Fuentes *et al.,* 2004). Decreased germination percentage with increasing in gamma radiation doses has also been reported in Pinus (Thapa, 2004), Rye (Akgun and Tosum, 2004) and Chickpea (Khan *et al.,* 2005; Toker *et al.,* 2005). This might be due the effect of mutagens on seed meristematic tissues may cause a per cent reduction or stimulation in seed germination. The decline in seed germination at higher mutagen doses might be attributed to cellular disruptions. It is possible that molecular damage to cell components or altered enzyme function is the cause of the reduced seed germination caused by mutagenic treatments (Goyal and Khan, 2010). Decreasing in germination percentage at higher dose of gamma rays may be primarily due to the killing of cells and ionization in the nuclei. However, a high dosage would kill the plant tissues because mutagens have a direct and lethal effect on plant tissue (Bodele, 2013).

The reduction in seedling height in higher gamma irradiation doses may be due to the delayed and inhibition gemination. Also, its might be due to modification in physio-biochemical mechanisms related to gibberellic acid activity, which defeat the plant cell activity that prevents or reduces mitotic cell division and cell elongation affects the plant growth habit at a higher dose thus killing or destructive meristematic cells (Datta and Banerjee, 1995; Fereolt *et al.,* 1996; Jain, 2010; Hasbullah *et al.*,2012).

CONCLUSION

The determination LD_{50} value in mutation experimentations is crucial, as excess dosage leads to higher mortality. For consecration of desired mutations by irradiation treatment being through accidental, it is harmless, less damage and higher germination

percentage and survival rates and also produce some advantageous putative mutants, which might be not probable by lower dose of radiations. In the current study, based on the germination percentage, LD_{50} dose for gamma rays have been fixed as 15-20 Gy. Lower doses of gamma radiation had a positive influence while higher dosage had contrary impact on *in vitro* germination of zygotic embryos in banana cv. Bhimkol, led to a higher mortality.

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Table 1. Effect of gamma radiations on *in vitro* germination of immature zygotic embryos of banana cv. Bhimkol

Treatments	Dose (Gy)	Germination	Per cent survival	Per cent reduction

		(%)	over control	over control
G_0	0	73.33	100	-
G_1	5	63.33	86.37	13.63
G_2	10	56.67	77.28	22.72
G_3	15	50.00	68.19	31.81
G_4	20	40.00	54.55	45.45
G_5	25	33.33	45.56	54.54
SEd		**3.84**	-	-
CD = 0.05		**8.39**	-	-
CV (%)		**8.93**	-	-

Table 2. Probit analysis for determining LD_{50} value for gamma irradiation in banana cv. Bhimkol

Dose (Gy)	Log_{10} of doses	Observed mortality (%)	Corrected mortality (%)	Empiric probit unit	LD_{50} value
G_0 - 0	0.00	23	-	-	
G_1 - 5	0.70	37	17	4.05	
G_2 - 10	1.00	43	26	4.36	
G_3 - 15	1.18	50	35	4.61	**22.39 Gy**
G_4 - 20	1.30	60	48	4.95	
G_5 - 25	1.40	67	57	5.18	

Table 3. Effect of gamma radiations on days for response, days for germination and germination frequency (%) of immature embryos in banana cv. Bhimkol

Dose (Gy)	Days for response	Days for germination	Germination frequency (%)
G_0 - 0	6.53^{e}	23.00^{d}	73.33^{a}
G_1 - 5	7.53^{d}	25.87^{c}	63.33^{b}
G_2 - 10	8.20^{cd}	26.80^{b}	56.67^{bc}
G_3 - 25	8.40^{bc}	27.40^{b}	50.00^{c}
G_4 - 20	8.67^{ab}	27.53^{b}	40.00^{d}
G_5 - 25	8.93^{a}	28.53^{a}	33.33^{d}
SEd	**0.17**	**0.33**	**3.85**
CD = 0.05	**0.37**	**0.75**	**8.39**
CV (%)	**2.55**	**1.60**	**8.93**

Table 4. Effect of gamma radiations on seedling length (cm), number of leaves and root length of immature embryos of banana cv. Bhimkol

Irradiation Dose (Gy)	Seedling length (cm)	Number of leaves	Root length (cm)
G_0 - 0	6.12^{a}	3.20^{a}	7.27^{a}
G_1 - 5	5.67^{b}	2.33^{c}	4.27^{c}
G_2 - 10	5.81^{b}	2.73^{b}	3.47^{c}
G_3 - 25	5.81^{b}	2.60^{bc}	5.63^{b}
G_4 - 20	5.66^{b}	2.47^{bc}	5.73^{b}
G_5 - 25	5.74^{b}	2.67^{bc}	7.53^{a}
SEd	**0.10**	**0.16**	**0.44**
CD = 0.05	**0.21**	**0.34**	**0.96**
CV (%)	**2.01**	**7.07**	**9.58**

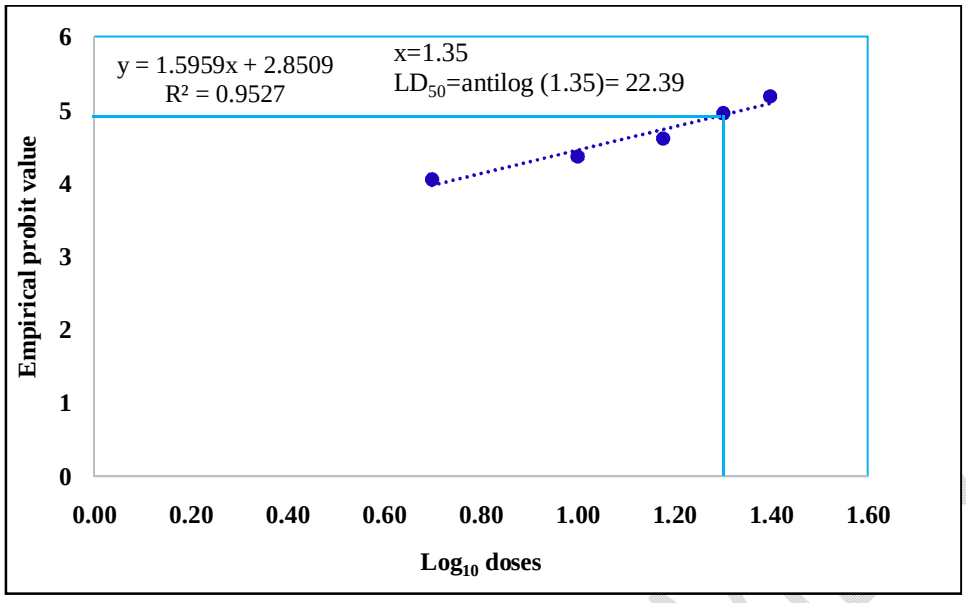

Fig.1. Probit curve for gamma irradiation based on corrected mortality rate of *in vitro* germination of embryos in banana cv. Bhimkol

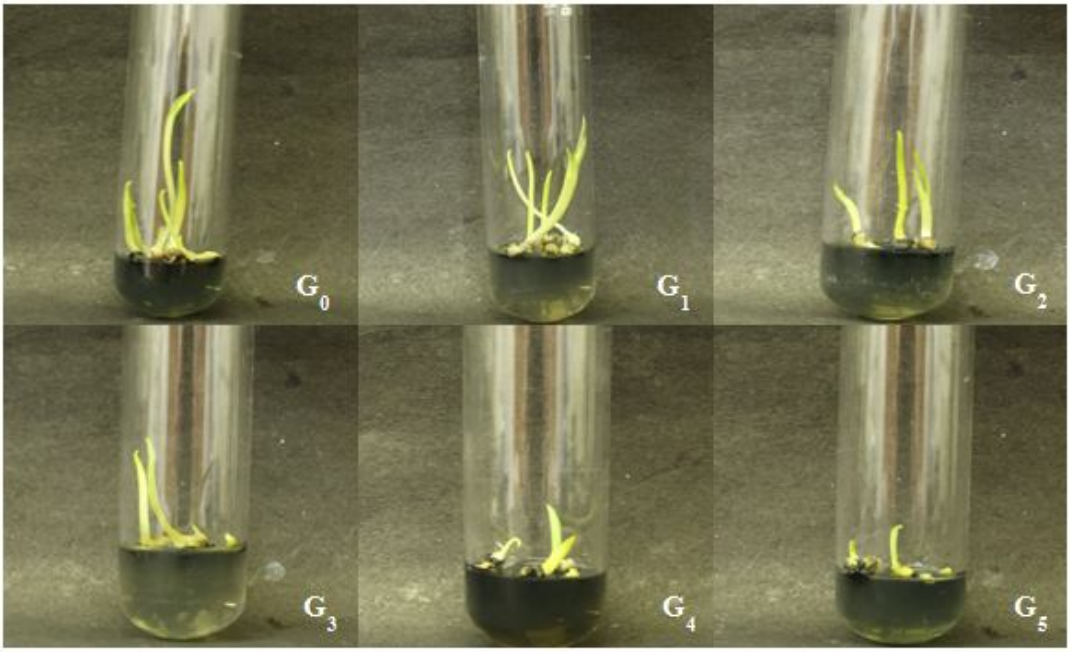

Fig. 2. Effect of different doses of gamma irradiation on *in vitro* germination of immature zygotic embryos of banana cv. Bhimkol

G_0 : 0 Gy (Control) **G_1** : 5 Gy **G_2** : 10 Gy
G_3 : 15 Gy **G_4** : 20 Gy **G_5** : 25 Gy

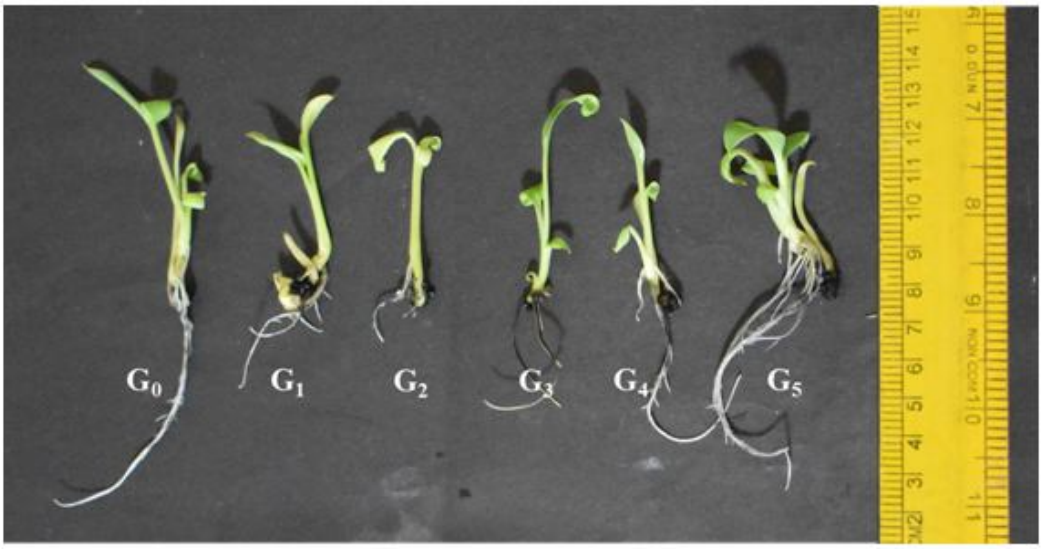

Fig. 3. Influence of gamma radiations on seedling height and rooting of immature zygotic embryo culture derived plants in banana cv. Bhimkol

G_0 : 0 Gy (Control) **G_1** : 5 Gy **G_2** : 10 Gy

G_3 : 15 Gy **G_4** : 20 Gy **G_5** : 25 Gy