DOI : 10.58993/ijh/2023.80.4.2

Indian J. Hortic. 80(4), December 2023: 313-318

Assessment of radiation sensitivity and regeneration in shoot tip culture of banana cv. Grand Naine

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ABSTRACT

An investigation was carried out to initiate the *in vitro* mutagenesis in banana cv. Grand Naine using shoot tip culture. Based on the sensitivity of explants, the lethal dose was estimated using probit analysis. As a result of the adverse effects of higher irradiation doses, it was decided that determining the lethal dose can be very beneficial in reducing population loss. The LD50 dose of gamma irradiation of the banana shoot tip culture was determined to be 39.5 Gy. The study also discovered that 13 DAS explants when treated with 10 Gy recovered the highest number of shoots. The optimization of gamma irradiation with subculture days in explants of banana may be effective in developing significant variations with a particular trait of interest.

Key words: *Musa acuminata*, radiation, mutation, *in vitro*, LD_{50}

INTRODUCTION

The banana is a large Musaceae family herb cultivated primarily in tropical and subtropical parts of the world. Crop improvement requires a genetic variation of valuable traits in plant breeding. Mutation induction may serve as the exclusive viable approach for enhancing cultivar diversity in crops that lack seed production, such as the edible banana. The discovery of individuals possessing a desired mutation is a crucial element in mutation breeding, involving two basic steps: mutant screening and mutant confirmation (Forster *et al*., 6). According to the FAO/IAEA database, 3222 mutant types with enhanced characteristics have been formally released in crop plants. *In-vitro* mutation induction techniques enable the generation of mutations in a large number of propagules within a limited area, and several subculture cycles may be efficiently carried out in a limited duration to distinguish mutant sectors from non-mutated sectors (Ahloowalia *et al*., 2). Radiation-induced mutagenesis with *in-vitro* culture effectively induces genetic diversity, selection, and proliferation of mutant clones (Jain *et al*., 9; Suprasanna *et al*., 22). The most frequently used sources of gamma radiation are 60Co and 137Cs. In the context of in vitro mutagenesis, the explant is subjected to several dosages to ascertain the optimal dose, namely the LD50, at which 50% of the treated material survives and growth is halved (Mahajan *et al*., 15). This process is employed to identify an appropriate mutagenic treatment for *Musa*. Therefore, the present study aimed to address a fundamental research gap about the optimization of the stage of explant with respect to subculture duration, which has been identified as a crucial factor contributing to substantial variations. The present study aimed to examine the adverse effects of radiation and ascertain the optimal dosage range and duration of culturing required to induce successful mutation.

MATERIALS AND METHODS

Radiation sensitivity and regeneration in shoot tip culture of banana cv. Grand Naine were investigated during 2018-2020 in the Department of Horticulture (Fruit and Fruit Technology), BAU, Sabour, India. The explants required for *in vitro* production of plantlets of banana cv. Grand Naine was obtained following the micro-propagation protocol in the tissue culture lab (Razdan *et al*., 21). Murasaige and Skoog (MS) media was used as nutrient media. Shoot tip explants were incubated in autoclavable culture tubes (150× 25 mm) containing MS culture media supplemented with 2 mg/l BAP and 75 mg/l adenine sulphate for 2 weeks maintaining standard culture condition. The multiple shoot cultures cultured in jam bottles containing thirty shoot tip explants were subjected to gamma-ray irradiation at RNARC (Regional Nuclear Agricultural Research Center), BCKV, India. Cobalt 60 was used as the source of gamma-rays with a dose rate 20 Gy/min. In each treatment, thirty shoot tip explants of banana cv. Grand Naine were irradiated thrice. Each jam bottle that was parafilm-sealed and included plant material in the desired dose was labelled. Because significant radiation may be emitted, the irradiation device is stored in a highly protected facility with

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restricted access. It was made up of a radiation cell, and the cell was rotated to ensure that the dosage rates were consistent throughout. The gamma irradiation dosages utilized were 0, 10, 20, 30, 40, and 50 Grays, which had to be adjusted based on the time of exposure of the plantlets to the ionizing radiation released by 137 Cs (Caesium) for 10 Gy and 60 Co for other doses (Table 1). The corresponding time-lapse was determined after a series of computations because the irradiation varies with time due to the change in half-life and the capacity of the device. After the inoculation process, all cultures were placed in an environment with a temperature of 25±2 °C, relative humidity of 60%, and a photoperiod cycle of 16 h of light and 8 h of darkness. The light intensity of the photoperiod was consistently maintained within the range of 1500-3000 lux. The regeneration details of *in vitro* banana plantlet from M1V1 to M1V4 is depicted in Fig. 2.

Probit analysis was employed to determine the LD50 values associated with gamma radiation. The probit function is the inverse cumulative distribution function of the probability related to the standard normal distribution. Variance analysis was used to determine the lethal dose, and data were treated using the conventional analysis of variance approach using the SAS statistical program (LD50). The LD50 was determined through a linear regression model, where the response variable (per cent survival) was represented by the equation y = a + bx. In this equation, the independent variable (irradiation dosage) was denoted as x, while the slope and constant were represented by a and b, correspondingly.

Gamma irradiation was used for *in vitro* mutagenesis of shoot tip cultures of banana. The shoot materials were subcultured, and bottles containing shoot tip culture were exposed to gamma irradiation after 4, 7, 10, 13 and 16 DAS (Days of the subculture). Subculturing was practised on the growing *in vitro* cultures for chimera dissolution. Four subcultures were carried out at 4-week intervals from M1V1 generation (1st subculture) to M1V4 generation (4th subculture) and were taken to rooting (6 weeks) as suggested by (Novak *et al*., 17).

Table 1. The doses of gamma irradiation used for inducing mutation

Treatment	Doses Applied	Source	Time of radioactive decay
1	10 Gy	137Cs (BI-2000)	41 seconds
2	20 Gy	60Co (GC-5000)	12 seconds
3	30 Gy	60Co (GC-5000)	18 seconds
4	40 Gy	60Co (GC-5000)	25 seconds
5	50 Gy	60Co (GC-5000)	28 seconds
6	0 Gy	Control	-

The experimental design chosen was FCRD with five replications, using subculture days as one factor and irradiation doses as the second factor. The statistical analyses of data were conducted using the standard procedures of (Gomez and Gomez, 7). The least significant differences were calculated at a five per cent probability level, where the treatment differences were significant following the estimation of the analysis of variance.

RESULTS AND DISCUSSION

In the present study, the probit analysis for different doses of gamma rays for shoot tips of bananas was carried out. The LD50 value based on lethality when irradiated with different doses of gamma irradiation was recorded at 39.7 Gy. An incremental rise in the lethality of shoot tip culture was noted when the irradiation dosage increased from 10 Gy to 50 Gy, compared to the untreated control. After treating shoot tips of Grand Naine, a probit of kill curve was constructed, and other component estimations were calculated. The mortality rate had a positive correlation with the dosage increment. The highest mortality rate of shoot tips was reported in explants, treated with a radiation dose of 50 Gy (83%). The mortality rate in explants subjected to doses of up to 40 Gy was less than 50% (Table 2 - 3).

Gamma rays, with energy from 10 keV to several hundred keV, are the most powerful form of electromagnetic radiation. The radiation it emits penetrates matter more deeply than other radioactive materials like UV B or C on plant cells (Kovacs and Keresztes, 12). Free radicals are created when ionizing radiation like gamma rays interacts with atoms or molecules in a cell. It has been established that plants' morphology, anatomy, biochemistry, and physiology are affected differently by the irradiation level, and free radicals can damage or disrupt critical components of plant cells. Exposure of plant systems to ionizing radiation has been the subject of numerous cytogenetic and mutational research (Ludovici , 13).

Before identifying the suitable dosage for in vitro mutagenesis of a particular cultivar, it is necessary to conduct radiosensitivity tests. According to Broertjes and Van Harten (3), the optimal dosage for generating mutations is often below the LD50 threshold and is commonly employed for bulk irradiation. In a study conducted by Predieri and Zimmerman (18), it was emphasized that one of the initial stages of mutagenesis treatment involves the identification of the optimal dosage. The current investigation aimed

Table 2. LD_{50} for gamma irradiated shoot tip culture of banana cv. Grand Naine

Doses	Tot No.	No. Kills	Mort. (%)	Log (Dose)	Exp. Prop	Emp Probit	Exp Probit	Work. probit	Weight	wx	wy	wx^2	wy^2	wxy	Y'
10 Gy	30.000	0.000	0.025	1.000	0.011	-1.960	-2.307	-1.788	2.237	2.237	-3.998	2.237	7.148	-3.998	-2.307
20 Gy	30.000	5.000	0.167	1.301	0.126	-0.967	-1.148	-0.949	11.650	15.157	-11.050	19.720	10.482	-14.377	-1.148
30 Gy	30.000	7.000	0.233	1.477	0.319	-0.728	-0.470	-0.710	17.620	26.027	-12.515	38.445	8.889	-18.486	-0.470
40 Gy	30.000	10.000	0.333	1.602	0.505	-0.431	0.012	-0.418	19.098	30.596	-7.979	49.016	3.334	-12.783	0.012
50 Gy	30.000	25.000	0.833	1.699	0.650	0.967	0.385	0.880	18.094	30.740	15.926	52.227	14.018	27.058	0.385

Component	Mean X	Mean Y	Intercept	Beta	LD_{50}	LL (Lower Limit)	UL (Upper Limit)	Log (LC_{50})	Log LL	Log UL	Chi-Square ML	Sign (Chi-Square
Estimate	33.488	-0.286	-6.159	3.825	39.721	31.575	49.969	1.599	1.499	1.699	10.048	0.018

wx, wy, wx^2, wxy, Y' are variables of the components X and Y

Table 3. Working probit regression line

Parameter	Estimate	Std. Error	t-cal	p-value
Intercept	-6.159	1.112	-5.538	0.005
Beta	3.852	0.725	5.312	0.006

to ascertain the LD50 value of the shoot tip subjected to gamma irradiation. The LD50 value was found to be 39.7 Gy. Similar LD50 values of 20-40 Gy were substantiated by Novak *et al.* (17) on the Grand Naine cultivar. Yang *et al.* (23) investigated the LD50 value of 38.6 Gy in the China Tinabao banana. Elagamawy *et al.* (4) examined the LD50 value of 20-40 Gy in banana, while Mishra *et al.* (16) reported an LD50 value of 35 Gy in the Grand Naine cultivar. According to Mishra *et al.* (16), employing irradiation therapy that results in a survival rate drop of less than 50% to induce mutation is preferable. This phenomenon may be attributed to the administration of doses lower than the LD50, which is recommended to promote post-treatment plant recovery. However, it should be noted that greater doses have the potential to induce excessive mutations, which may predominantly hinder plant recovery (Predieri and Di Virgilio, 19). The assessment of radiosensitivity revealed the importance of LD50 in several mutational research in fig, grapes, and citrus, as proposed by Ferreira *et al.* (5), Rayan *et al.* (20), and Kaur *et al.* (11).

The relationship between subculture days and irradiation doses differed significantly between M1V1 and M1V4 generations. The highest number of the shoots were discovered to be under control at 13 DAS. Applying a radiation dose of 40 Gy on 16 DAS explants resulted in the highest shoot count (22.0) in the M1V1 stage, surpassing lower doses. The most favorable outcome at M1V2 was achieved by subjecting the plants to a radiation dose of 30 Gy at 16 DAS, resulting in a shoot growth of 55.8. This was followed by the control group, which was treated at 13 DAS and had a growth rate 45.2. At M1V3, the doses of 20 Gy at 16 DAS showed a maximum number of shoots (39.0) and 40 Gy at 16 DAS (9.0) had the lowest values. At M1V4, explants treated with a radiation dose of 10 Gy exposure at 13 DAS explants resulted in the highest shoot production. Conversely, explants treated with radiation doses of 40 Gy at 16 DAS and 4 DAS yielded the lowest number of shoots. During the initial stages of M1V1 and M1V2, larger dosages of DAS resulted in the highest number of new shoots. However, in the later phases of M1V3 and M1V4, 13 DAS at all doses showed superior performance compared to other DAS. (Fig 1).

The number of new shoots has increased with an increase in subculture days. Notwithstanding

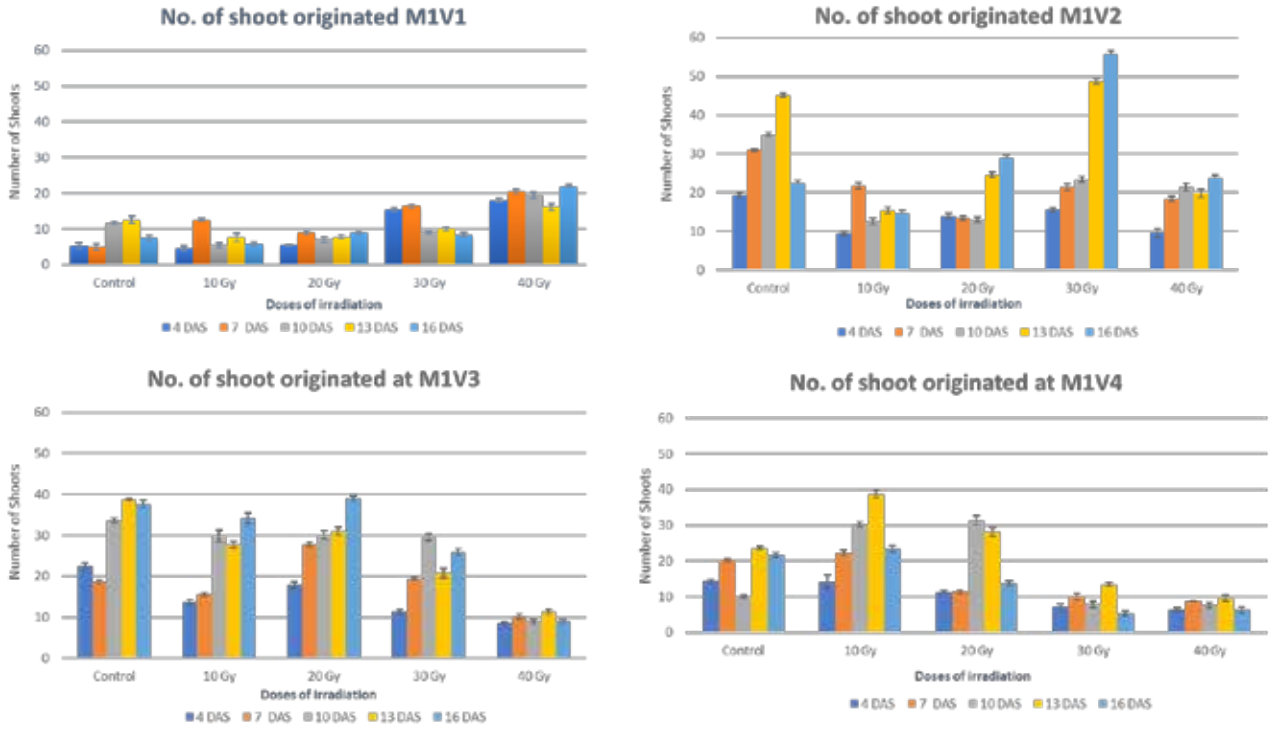

Fig. 1. Effect of different days of subculture and doses of gamma irradiation on number of new shoots originated from M1V1 to M1V4.

Vertical bars indicate the mean value of three replicates ± standard error. Significant differences were at LSD (P ≤ 0.05) (Tukey's Honest Significant Difference Test) for S×D interaction; DAS: days of subculture.

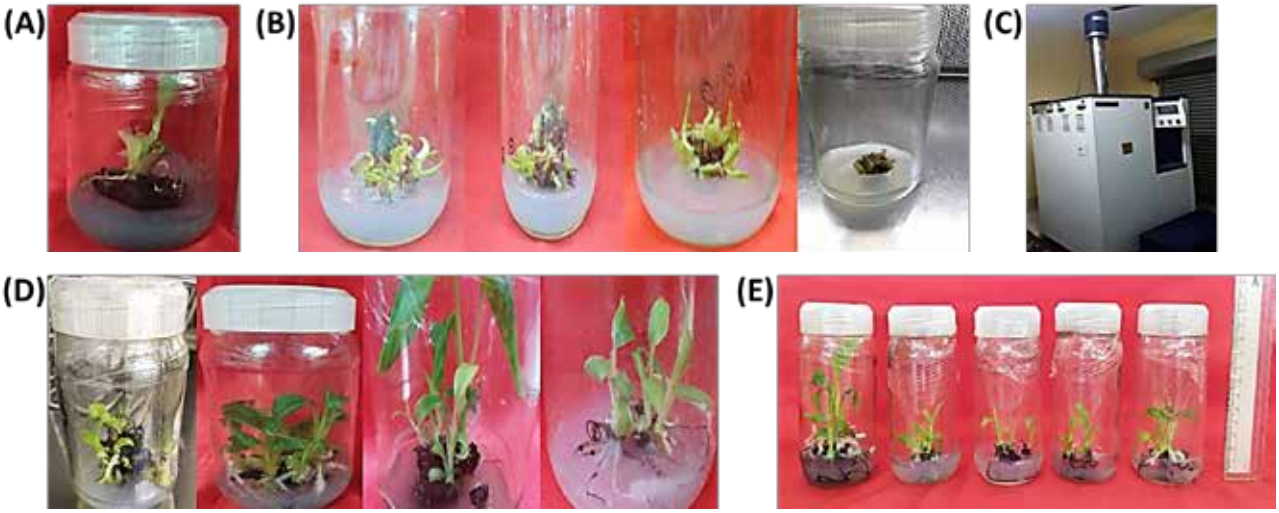

Fig. 2. Regeneration details of in vitro banana plantlet from M1V1 to M1V4. (a, b) Initiation and growth of banana shoot tip culture from sucker; (b) Subculture of cultures at different days; (c) Treatment with gamma irradiation (d) Identification of visible mutants and rapid chimera dissolution up to M1V4; (e) Separation of mutants into replication after regeneration.

the days of subculture, new shoots were found to be substantially higher at higher irradiation doses in M1V1 and M1V2. However, new shoots declined at higher doses in M1V3 and M1V4. At M1V4, the maximum number of shots was recorded at 10 Gy at 13 DAS, while the minimum shots were recorded at 40 Gy at 16 DAS. Hence, the findings unveiled an intriguing anomaly in growth stimulation, wherein the treated samples exhibited higher survival rates and superior growth compared to the untreated samples. This leads to the conclusion that the observed growth stimulation is contingent upon the gamma dose, thereby corroborating the findings reported by Janick *et al.* (9). The enhanced shoot development was observed at lower doses, whereas higher doses had resulted in growth reduction as have also been reported by other researchers (Majeed *et al.*, 14; Abdulhafiz *et al.*, 1). Also, the gradual increase in the number of shoots in M1V3 and M1V4 generation was similar to the findings of Abdulhafiz *et al.* (1) in bananas. More disturbances in the plant's genome can affect the production of new shoots during the initial stages of subculture. However, in the next generation, the ability has been reduced. Explants treated with lower doses have regained the ability to produce more M1V3 phase shoots. This might be because the resulting subculture decreases physical damage, and plantlets regain the ability to regenerate and start producing shoots normally in bananas (Hasim *et al.*, 8).

Overall, it can be concluded that for conducting in vitro mutagenesis in banana cv. Grand Naine, the doses should be fixed to obtain variation for targeted traits not less than 39.7 Gy. Therefore, it may be inferred that higher doses resulted in the most shoot recovery during the initial stages. In contrast, lower doses promoted the development of new shoots during later stages of subculture. It was also inferred that 13 DAS explants treated with 10 Gy could recover the highest number of shoots, which may be further utilized for screening for the desired trait.

AUTHORS' CONTRIBUTION

The conceptualization and design of the research work (AKP and MFA), execution of field and laboratory studies, as well as data gathering (RR), analysis and interpretation of collected data (RR and KP), preparation of the manuscript (RR).

DECLARATION

The authors of this article assert that they possess no potential conflict of interest.

ACKNOWLEDGMENTS

The authors are grateful to RNARC (Regional Nuclear Agricultural Research Center), BCKV, India to provide a gamma irradiation facility for research work.

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Received: August 2023, Revised: December 2023, Accepted: December 2023