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Research Article

Ascertaining gamma ray dosage sensitivity of *in vitro* cultures in banana cv. Ney Poovan (*Musa* AB)

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Abstract
In a vegetatively propagated and parthenocarpic crop like banana, the mutation breeding method offers considerable scope to create genetic variability for crop improvement. It is necessary to determine the LD_{50} dose for optimizing the radiation dosages when gamma rays are used for mutation induction. In the present study, in order to fix the LD_{50} dose for explants cultured *in vitro* in cv. Ney Poovan, *in vitro* shoot tips were subjected to five different doses of gamma rays ranged from 5 to 25 Gy. Proliferating multiple shoots were subjected to five different doses of gamma rays ranged from 2 to 10 Gy. The results revealed a linear and significant reduction in the survival percentage, shoot length and number of shoots with increasing levels of gamma irradiation doses in both the category of explants. The probit curve based analysis on mortality of treated explants revealed that LD_{50} dose of gamma rays to be 18.77 Gy for cultured shoot tips and 6.97 Gy for proliferating multiple shoots under *in vitro*.

Key words: Gamma irradiation-Shoot tip cultures-Lethal dosage-Banana.

INTRODUCTION

Banana (*Musa*spp., Musaceae) is considered not only as a fruit crop but also as the fourth most economically important food crop after wheat, rice and maize in terms of production and quantity of consumption, Bananas are seriously affected by many fungal, bacterial, viral and nematode pathogens and as well serious pests like stem weevil and corm weevil. Genetic improvement of bananas through conventional breeding for yield improvement or for pest and disease resistance is difficult due to several reasons such as inherent polyploidy nature, heterozygosity, interspecific hybridity, parthenocarpic fruit development and low levels of male and female fertility. Mutation breeding is often resorted to creating genetic variability to screen and select useful variants in bananas (Novak *et al*., 1993; Pillay and Tripathi, 2007). Determination of lethal dose of the mutagen is a prerequisite to optimizing the doses for recovery of a large number of population in mutation breeding (Leitao, 2012).

Among the different mutagenic agents, gamma rays are frequently employed for mutation induction in bananas. Earlier some induced mutation attempts have also resulted in improved varieties. Novak *et al*. (1990) used gamma radiation to induce an early flowering mutant of Grand Naine called GN-60Gy. In Malaysia, a further

selection of GN-60Gy resulted in the release of 'Novaria,' an early-fruiting Cavendish banana (Mak *et al*., 1996). Another mutant 'Klue Hom Thong KU1 with improved bunch weight was also reported through gamma irradiation, Further , tolerance to Fusarium wilt, height reduction, earliness, large fruit size have been listed in some of the putative mutants (Roux, 2004).

In earlier mutation breeding attempts, the suckers of bananas were subjected to mutation but due to poor availability, systemic infection, the large size of propagule and long duration required to identify chimeric mutants, success in such mutation attempts were far and few. Later use of gamma radiation technique with *in vitro* propagation has been found to be effective in terms of acquiring variation; rapid proliferation of mutants and obtaining disease free mutants (Lamo *et al.,* 2017). However, during such attempts optimizing the level of dose becomes crucial especially for *in vitro* culture derived plants as they are highly sensitive than conventional suckers. The lethal doses were also found to vary with the genomic constitution of the variety *in vitro* (Roux, 2004). It has been emphasized that during mutation attempts, it is essential to subject the *in vitro* cultures to series of subcultures irrespective of the explants after being mutagenized in order to screen and separate chimeras, since chimeric mutation remains undetectable in the first vegetative generation (Banerjee *et al*., 2015).

Among the various banana cultivars grown in India, the cv. Ney Poovan (*Musa* AB; syn: Elakki Bale) is one of the choicest in south India and fetches premium prices in the market. It is a diploid with both *Musa acuminata* and *Musa balbisiana* genomic constitution (AB) and it has poor female fertility and fruits are parthenocarpic but it is highly susceptible to fusarium wilt, nematodes and sigatoka leaf spot. Considering the scope of mutation breeding for improving this variety for yield and identifying variants with resistance to diseases, the present study was taken up to ascertain the lethal dose of gamma irradiation for the two different types of explants (shoot tips and proliferating multiple shoots).

MATERIALS AND METHODS

Two to three months old sword suckers of banana cv. Ney Poovan were collected from Orchard, Horticultural College and Research Institute, Tamil Nadu Agricultural University, Coimbatore. The suckers were detopped to a size of 7 to 8 cm in length having the terminal meristematic bud, along with a small portion of the rhizome. The explants were taken to the laboratory and washed with running tap water for 30 minutes before soaking in a pre-treatment solution (Carbendazim 1.0 perncent WP + Teepol [1-2 drops/ 100 ml]) for an hour to reduce the microbial load. The explants were then washed and trimmed, by removing a layer of sheathing leaves, before soaking for one hour in streptocycline (0.05%). The explants were then washed with distilled water and trimmed to 3 x 2.5 cm size before soaking for 30 minutes in a 300 mg/l citric and 150 mg/l ascorbic acid solution to reduce explant browning caused by excessive polyphenol exudation. Then, the shoot tips were washed again and treated with 0.05% cetrimide solution for 30 minutes. Further, the explants were taken to a laminar airflow chamber and surface sterilized with 0.1% mercuric chloride for 10 minutes followed by rinsing with double distilled water thrice.

After sterilization, the explants were inoculated into the MS medium fortified with BAP 5 mg/l + Adenine Sulphate 75 mg/l +Ascorbic acid 175 mg/l +Citric acid 50 mg/l and gelled with 0.2% gelrite. The cultures were incubated in a culture room with a light intensity of 2500 to 3000 Lux, 25 ± 2 °C, relative humidity of 60–70% and 16 h / 8 h day / night cycle.

After a week, the shoot tips turned green indicating the regeneration process. At this stage, the shoot tips were subjected to five different doses of gamma radiation (5, 10, 15, 20, 25 Gy). Gamma irradiation was given using Co^{60}as gamma source (Gamma Chamber 5000, Board of Radiation and Isotope Technology (BRIT), Mumbai, India) at Indira Gandhi Centre for Atomic Research, Kalpakkam, Chennai. Exposure time (minutes/seconds) was calculated based on the dose rate (Gy/s or Gy/min) of the gamma source available in the gamma chamber on the day of carrying out the irradiation, based on dosimetry data.

After irradiation, the shoot tips were sub-cultured on to MS media supplemented with BAP 5mg/l + Adenine Sulphate 75 mg/l + Ascorbic acid 75 mg/l + Citric acid 50 mg/l gelled with 0.2% gelrite within 24 to 48 hrs.

The shoot tips were inoculated in MS medium fortified with BAP 5 mg/l + Adenine Sulphate 75 mg/l + Ascorbic acid 75 mg/l + Citric acid 50 mg/l and gelled with 0.2% gelrite. The cultures were sub-cultured 4 times at every 2 weeks interval to produce multiple shoots. After four sub-cultures the proliferating multiple shoots were subjected to five different doses of gamma radiation (2, 4, 6, 8, 10 Gy) including control. After irradiation, the proliferating multiple shoots were sub-cultured to MS media supplemented with BAP 5mg/l + Adenine Sulphate 75 mg/l + Ascorbic acid 75 mg/l + Citric acid 50 mg/l gelled with 0.2% gelrite within 24 hrs.

After one month, the survival percentage was calculated using the formula

Survival percentage (%) =

$$\frac{\text{Number of survived explants after gamma irradiation}}{\text{Total number of explants exposed to gamma irradiadion}} \times 100$$

The lethality for gamma radiation was obtained based on the probit analysis using Finney's table (Finney, 1971). The inverse cumulative distribution function (CDF) or

quantile function is the probit function associated with the standard normal distribution. The corrected mortality was worked out the formula:

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

Where,
M observed- Mortality in treatment,
M control – Mortality in control

To determine the lethal dose (LD_{50}), the data were subjected to a standard analysis of variance procedure using SPSS statistical package version 16. The LD_{50} for each explant was calculated 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 (gamma irradiation dose), while a and b represent the slope and constant, respectively. The shoot length of individual plants in each treatment was measured from the point of the base of shoot to the tip of the shoot and mean height was expressed in centimetres at the end of four subcultures *i.e.* M_1V_4 generation for both the explants. The number of adventitious shoots (microshoots) regenerated from an explant was recorded at subsequent generation for *in vitro* shoot tip cultures. All the mutated plants after 4 cycles of subculture were transferred to rooting media consist of ½ MS medium supplemented with 1.0mg/L IBA and 2.0 g/L activated charcoal. The well rooted plants were hardened in media containing pot mixture + cocopeat (1:1) under shade net and transferred to the field.

The experiment was implemented in a completely randomized design (CRD) with three replications and the analysis of treatment differences were computed based on variance estimates and working out critical differences at 5 % probability.

RESULTS AND DISCUSSION

Survival per cent of irradiated material is considered as one of the important criteria to estimate the dose levels for the particular mutagen. Novak (1990) reported that banana diploids were most sensitive to gamma radiations. In the present study, a gradual reduction in the survival rate of *in vitro* shoot tips and proliferating multiple shoots with an increase in the dose of gamma rays was observed. Among the various irradiated treatments for *in vitro* cultured shoot tips, the survival per cent ranged from 33.33 per cent in 25 Gy (with 63.89 per cent reduction over control) to 91.67 per cent in 5Gy (with 5.55 per cent reduction over control) as compared to the highest survival per cent of 97.22 in non-irradiated explants **(Table 1)**. Survival rates for proliferating multiple shoots ranged from 28.33 per cent in 10 Gy (with a 66.67 per cent reduction over control) to 86.67 per cent in 2 Gy (with an 8.33 per cent reduction over control) among the various irradiation levels, while explants that were not irradiated had the highest survival rate of 95.00 per cent. **(Table 2)**. Linear decrease of survival percentage with an increase in doses of gamma rays was observed in the present study similar to earlier mutation experiments reported by many workers (Hase *et al.,* 2002; Mishra *et al*., 2007; Taheri *et al.,* 2014; Abdulhafiz *et al*., 2018). At higher doses, the mitotic cell division and cell elongation were also reported to be inhibited due to the decrease or inactivation of auxin resulting in poor establishment and survival (Mahure *et al*., 2010). It was reported that the interaction of gamma rays with cellular molecules produces free radicals, and these free radicals may form toxic substances such as hydrogen peroxide, which contribute to the destruction of cells and plant death (Dehgahi and Joniyasa, 2017).

Table 1. Effect of gamma irradiation dosage on per cent survival of in vitro cultured shoot tips of Banana cv. Ney Poovan

Treatment	Dose (Gy)	Per cent survival	Per cent reduction over control
T_1	Control	97.22 (83.96)*[a]	-
T_2	5 Gy	91.67 (76.15)[a]	5.55
T_3	10 Gy	77.78 (61.97)[b]	19.44
T_4	15 Gy	58.33 (49.84)[bc]	38.89
T_5	20 Gy	47.22 (43.40)[cd]	50.00
T_6	25 Gy	33.33 (35.16)[d]	63.89
	SEd	6.52	
	CD (0.05%)	13.03	

*The values given in parenthesis are arc sine transformed values

Table 2. Effect of gamma irradiation dosage on per cent survival of proliferating multiple shoots of Banana cv. Ney Poovan

Treatment	Dose (Gy)	Per cent survival	Per cent reduction over control
T_1	Control	95.00 (79.32)*[a]	-
T_2	2 Gy	86.67 (68.66)[b]	8.33
T_3	4 Gy	76.67 (61.15)[c]	18.33
T_4	6 Gy	51.67 (45.96)[d]	43.33
T_5	8 Gy	43.33 (41.16)[d]	51.67
T_6	10 Gy	28.33 (32.14)[e]	66.67
	SEd	3.48	
	CD (0.05%)	7.33	

*The values given in parenthesis are arc sine transformed values

LD_{50} for gamma radiation was fixed based on the survival percentage of *in vitro* shoot tips and proliferating multiple shoots. Probit analysis was carried out based on the mortality rate of the *in vitro* shoot tips and proliferating multiple shoots after treatment with different doses of gamma rays compared with that in the untreated control

(Tables 3 & 4). In the present study, the LD_{50} value for gamma rays as assessed from the probit curve analysis **(Fig. 1 and Fig. 2)** was 18.77 Gy for *in vitro* shoot tips and 6.97 Gy for proliferating multiple shoots, respectively. According to Sparrow *et al.* (1968), radio sensitivity varies between plant species and depends on nuclear volume, number of chromosomes and ploidy level. Based on the probit curve analysis, 50 per cent mortality was observed at 15-20 Gy for shoot tips and 6-8 Gy in case of proliferating multiple shoots for the diploid banana cv. Ney Poovan (AB) in the present study. The current findings are close to the lethal dose of 10-20 Gy suggested by Roux (2004) for diploid cultivars Calcutta 4 (AA) and Tani (BB). (Novak *et al*., 1990). It was indicated that, lower dose than the LD_{50}favors higher plant recovery after gamma irradiation, while higher doses increase the probability of inducing too many mutations which could mostly have a negative impact (Mishra *et al.,* 2007). LD_{50} dose is the optimum dose for mutagenizing of mutagens with minimum injury and maximum viable mutants (Veni *et al*., 2017)

Table 3. Probit analysis for gamma irradiation on survival of shoot tips of banana cv. Ney Poovan

Treatment	Dose (Gy)	Log_{10} of doses	Observed mortality (%)	Corrected mortality (%)	Probit units
T_1	Control	0	3	0	18.77Gy
T_2	5	0.70	8	6	
T_3	10	1.00	22	20	
T_4	15	1.18	42	40	
T_5	20	1.30	53	51	
T_6	25	1.40	67	66	

Table 4. Probit analysis for gamma irradiation on survival of proliferating multiple shoots of banana cv. Ney Poovan

Treatment	Dose (Gy)	Log_{10} of doses	Observed mortality (%)	Corrected mortality (%)	Probit units
T_1	Control	0	5	0	6.97 Gy
T_2	2	0.30	13	9	
T_3	4	0.60	23	19	
T_4	6	0.78	48	46	
T_5	8	0.90	57	54	
T_6	10	1.00	72	70	

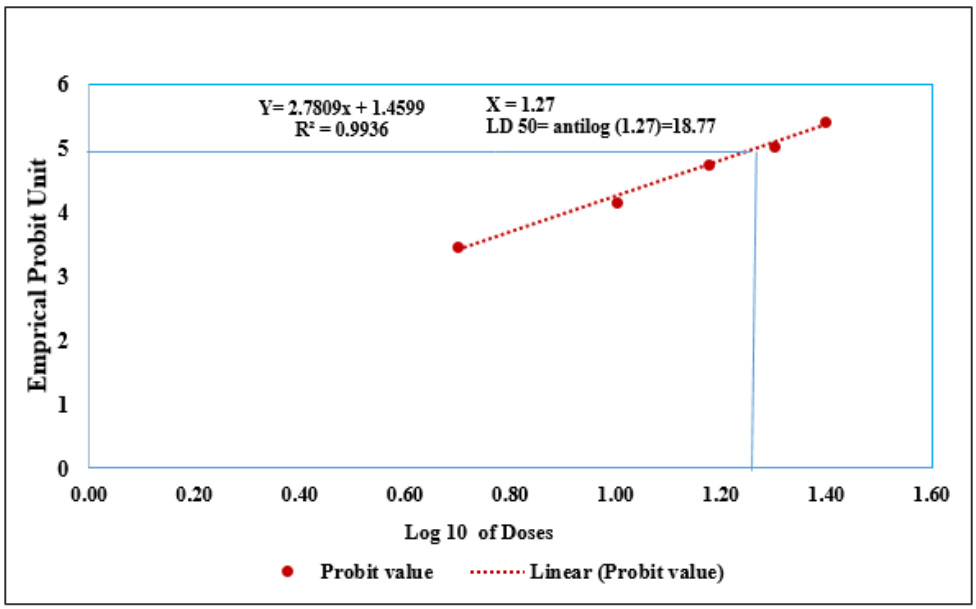

Fig.1. Probit curve for gamma irradiation based on corrected mortality rates of *in vitro* cultured shoot tips of Banana cv. Ney Poovan

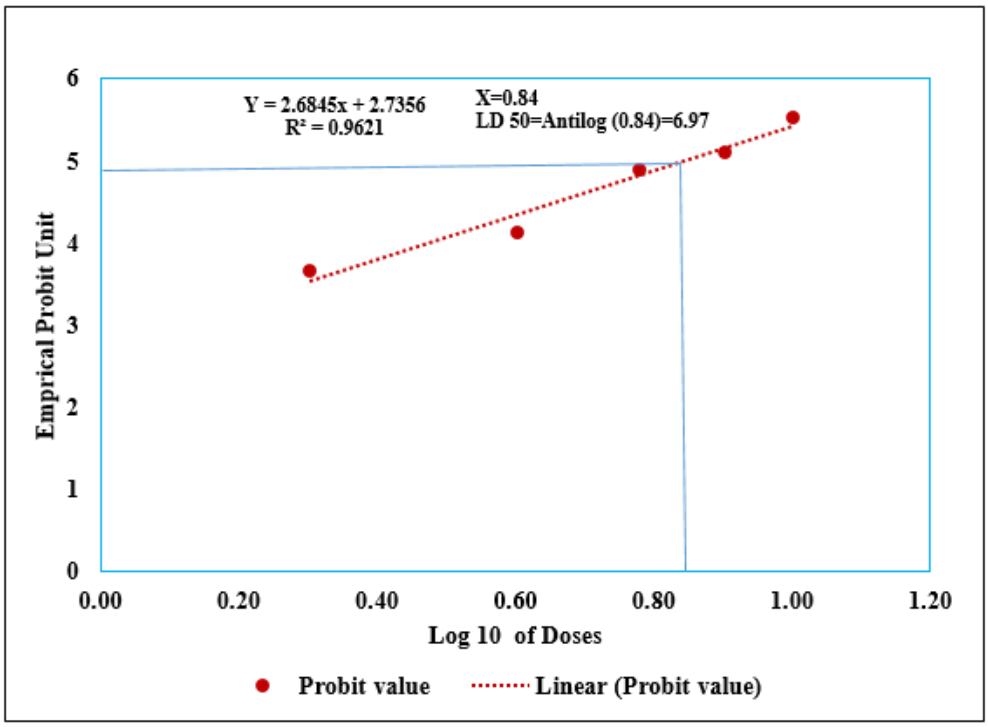

Fig.2. Probit curve for gamma irradiation based on corrected mortality rates of *in vitro* proliferating multiple shoots of Banana cv. Ney Poovan

The number of shoots formed per explant decreased with an increase in different doses of gamma irradiation (**Table 5, Plate 1 & 2**). Among the treatments, 5 Gy (T_2) recorded a significant and higher number of shoots (2.00, 1.60, 1.40 and 1.20) at M_1V_1, M_1V_2, M_1V_3 and M_1V_4 generation, respectively followed by treatment T_3 (10 Gy) recording (1.73, 1.40, 1.13 and 1.00) number of shoots at M_1V_1, M_1V_2, M_1V_3 and M_1V_4 generation, respectively. The non-irradiated *in vitro* shoot tips recorded the higher number of shoots per explant (2.20, 2.60, 2.67, and 2.87) at M_1V_1, M_1V_2, M_1V_3 and M_1V_4 generation, respectively as compared to gamma ray treated explants. In many vegetatively propagated crops, the limiting effects of a higher dose of gamma radiation on shoot growth and development have been documented (Broertjes and Van Harten, 1988). The pronounced effect of radiation in shoot multiplication can be attributed to either cellular disturbances causing imbalances in hormonal balance as reported in earlier studies in red pepper (Kim *et al*, 2004) or possibly a high degree of apical dominance in certain shoots that resist multiplication of *in vitro* shoots Kulkarni *et al*. (2007). Similar results were reported by Qamar *et al*. (2016) and Abdulhafiz *et al*. (2018).

The shoot length of *in vitro* shoot tip cultures was significantly influenced by different doses of gamma irradiations **(Table 6)**. Among the treatments, 5 Gy (T_2) recorded significant and the highest shoot length of

Table 5. Effect of gamma irradiation doses on the number of shoots from shoot tip culture grown *in vitro* of Banana cv. Ney Poovan

Treatment	Mean number of shoots at each generation			
	M_1V_1	M_1V_2	M_1V_3	M_1V_4
Control	2.20±0.00^a	2.60±0.12^a	2.67±0.18^a	2.87±0.07^a
5 Gy	2.00±0.00^a	1.60±0.12^b	1.40±0.12^b	1.20±0.00^b
10 Gy	1.73±0.07^b	1.40±0.00^{bc}	1.13±0.07^b	1.00±0.00^{bc}
15 Gy	1.53±0.13^b	1.27±0.07^c	1.07±0.07^b	0.93±0.07^c
20 Gy	1.13±0.07^c	0.73±0.07^d	0.40±0.00^c	0.27±0.13^d
25 Gy	1.07±0.07^c	0.33±0.07^e	0.13±0.13^c	0.07±0.07^d
SEd	0.10	0.12	0.15	0.10
CD (0.05%)	0.22	0.25	0.34	0.22

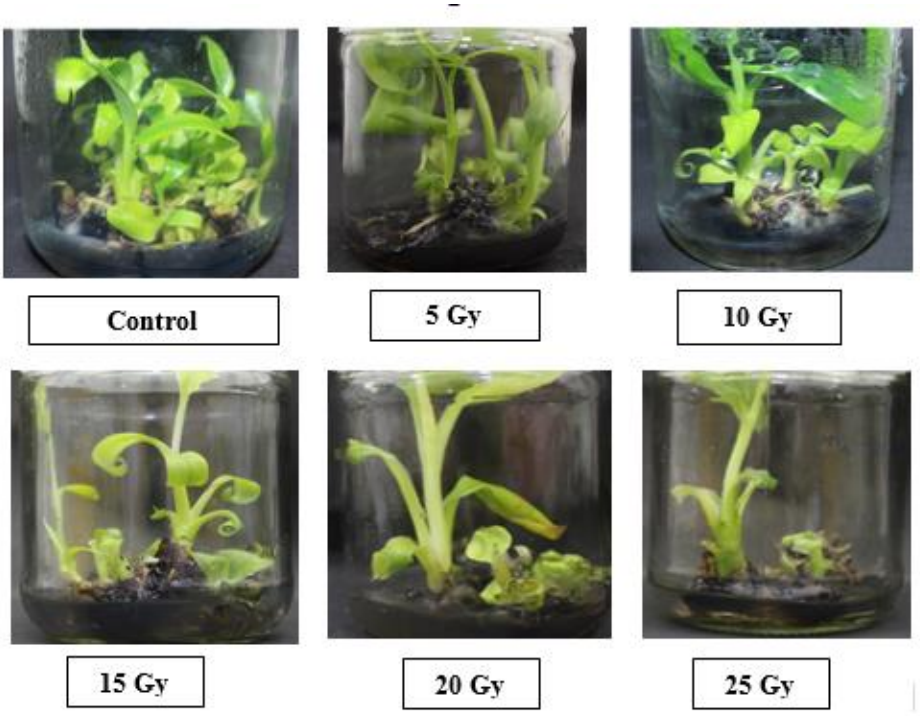

Plate 1. Effect of Gamma irradiation on *in vitro* cultured shoot tips of banana cv. Ney Poovan at M_1V_4 generation

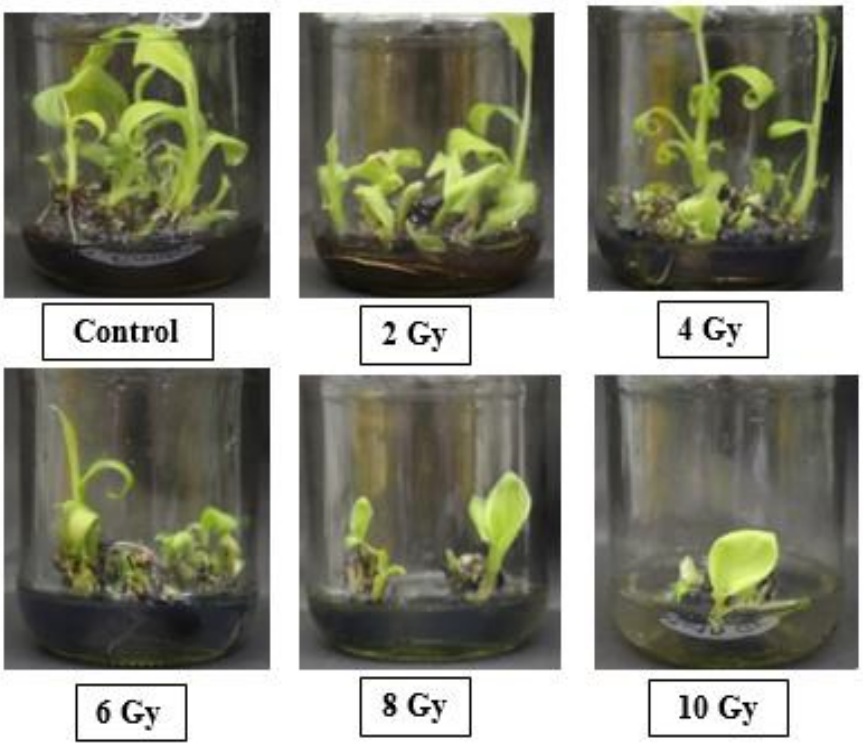

Plate 2. Effect of Gamma irradiation on proliferating multiple shoots of banana cv. Ney Poovan at M_1V_4 generation

6.00 cm (with a decrease of 7.12% over control) at the end of M_1V_4 generation followed by treatment T_3 (10 Gy) recording 5.49 cm. The lowest shoot length (4.27 cm) (with a decrease of 33.85 % over control) was recorded in treatment T_6 (25 Gy). The non-irradiated *in vitro* shoot tips recorded the highest shoot length (6.46 cm).

With respect to proliferating multiple shoots, 2 Gy (T_2) recorded significant and the highest shoot length of 6.91 cm (with 9.04 % increase over control) at the end of M_1V_4 generation followed by treatment T_3 (4 Gy) recorded 6.78 cm. The lowest shoot length of proliferating multiple shoots was recorded in treatment T_6 (10 Gy – 2.04 cm) (with 67.82 % in decrease over control) (**Table 7**). The non-irradiated proliferating multiple shoots recorded a mean shoot length of 6.49 cm. Several researchers have also observed inhibitory effects of high doses of gamma irradiation on shoot development, suggesting that the inhibition may be due to alteration in physio-biochemical processes related to the action of gibberellic acid, which suppress the plant cell activity leading to inhibition/reduced mitotic cell division and cell elongation affecting the growth habit at a higher dose thereby killing or damaging meristematic cells (Datta and Banerjee (1995); Fereolt *et al.* (1996); Jain (2010) and Hasbullah *et al.,*2012).

Table 6. Effect of gamma irradiation doses on shoot length of *in vitro* shoot tip cultures of banana cv. Ney Poovan

Treatment	Dose (Gy)	Shoot length (cm)	Per cent decrease over control
T_1	Control	6.46±0.04^a	-
T_2	5 Gy	6.00±0.03^b	7.12
T_3	10 Gy	5.49±0.04^c	14.96
T_4	15 Gy	5.08±0.01^d	21.36
T_5	20 Gy	4.60±0.07^e	28.79
T_6	25 Gy	4.27±0.06^f	33.85
	SEd	0.07	
	CD (0.05%)	0.15	

Table 7. Effect of gamma irradiation doses on shoot length of proliferating multiple shoots cultures in banana cv. Ney Poovan

Treatment	Dose (Gy)	Shoot length (cm)	Per cent difference over control
T_1	Control	6.34±0.05^c	-
T_2	2 Gy	6.91±0.02^a	9.04
T_3	4 Gy	6.78±0.05^b	6.94
T_4	6 Gy	5.68±0.01^d	-10.41
T_5	8 Gy	3.14±0.03^e	-50.47
T_6	10 Gy	2.04±0.02^f	-67.82
	SEd	0.05	
	CD (0.05%)	0.12	

The study taken up on banana cv. Ney Poovan indicated that the lethal dose of gamma radiation or *in vitro* shoot tip cultures was 18.77 Gy and for proliferating multiple shoots was 6.97 Gy. Lower doses of gamma radiation had a positive impact on the growth of *in vitro* plants, whereas higher doses had an adverse impact on the growth, leading to a higher mortality rate and reduction in growth.

REFERENCES

Abdulhafiz, F., Kayat, F. and Zakaria, S. 2018. Gamma irradiation effect on the growth of *Musa* cv. Tanduk (AAB). *Asian J. Agri. & Biol.,* **6(2):**135-142.

Banerjee, A., Suprasanna, P., Variyar, P.S. and Sharma, A. 2015. Gamma irradiation inhibits wound induced browning in shredded cabbage. *Food Chemi.,* **173:**38-44. [Cross Ref]

Broertjes, C. and Van Harten, A. M. 1988. Applied *mutation breeding* for *vegetatively propagated crops*. *Developments in crop science*:**12.**

Datta, S. K and Banerjee, B. K. 1995. Improvement of garden chrysanthemum through induced mutation. *Flora and Fauna*., **1:**1-4.

Dehgahi, R. and Joniyasa, A. 2017. Gamma irradiation induced variation in *Dendrobium* Sonia-28 Orchid Protocom- Like bodies (PLBs). *Fung. Gen. Bio.,* **7 (2):** 151. [Cross Ref]

Fereolt, L., Louis, S. and Lucez, A. 1996. Effects of gamma radiation on *in vitro* plantlets of *Alpiniapurpurata*. *J. Hort. Sci.,* **71 (2):** 243-247. [Cross Ref]

Finney, D. J.1971. Probit Analysis, 3rd ed. Cambridge. University Press, Pp. 333.

Hasbullah, N.A., Taha, R.M., Saleh A. and Mahmad. N. 2012. Irradiation effect on *in vitro* organogenesis, callus growth, and plantlet development of *Gerbera jamesonii*. *Hortic. Bras.,* **30:** 252- 257. [Cross Ref]

Hase, Y., Yamaguchi, M., Inoue M. and Tanaka, A. 2002. Reduction of survival and induction of chromosome aberrations in tobacco irradiated by carbon ions with different linear energy transfers. *Int. J. Rad. Bio.,* **78(9):** 799-806. [Cross Ref]

Jain, S. M. 2010. Mutagenesis in crop improvement under the climate change. *Rom. Biotechnol. Lett.,* **15(2):** 88-106.

Kim, J. H., Beak, M., Chung, H., Wiand, S. G. and Kim, J. S. 2004. Alterations in the photosynthetic pigments and antioxidant machineries of red pepper (*Capsicum annuum* L.) plants from control and gamma-irradiated seeds. *J. Plant Biol.,* **47:**314–321. [Cross Ref]

Kulkarni, V. M., Ganapathi, T.R., Suprasanna P. and Bapat, V.A. 2007. *In vitro* mutagenesis in banana (*Musa* spp.) using gamma irradiation. In: Jain, S.M. and H. Haggman. (Eds.) Protocolsfor micropropagation of woody trees and fruits. 543-559. [Cross Ref]

Lamo, K., Bhat, D., Kour, J. K. and Solanki, S. P. S. 2017. Mutation Studies in Fruit Crops: A Review, *Int. J. Curr. Microbiol. App. Sci.,* **6(12):** 3620-3633. [Cross Ref]

Leitao, J. 2012. Chemical mutagenesis. CABI Publishing, Q Y Shu-Plant mutation breeding and biotechnology - CABI., 140-163. [Cross Ref]

Mahure, H.R., Choudhary, M. L., Prasad K. V. and Singh S. K. 2010. Mutation in chrysanthemum through gamma irradiation. *Indian J. Hort.,* **67**:356-358.

Mak, C., Ho, Y. W., Tan, Y. P. and Rusli, I.1996. 'Novaria' - a new banana mutant induced by gamma irradiation. *Info. Musa.,* **5**: 35-36.

Mishra, P.J., Ganapathi, T.R., Suprasanna, P. and Bapat, V.A. 2007. Effect of single and recurrent gamma irradiation on *in vitro* shoot cultures of banana. *Int. J. Fruit. Sci.,* **7(1):**47-57. [Cross Ref]

Novak, F.J. 1990. Micropropagation and plant tissue culture in developing countries of Africa. In *Micropropagation*, 205-213. Springer. [Cross Ref]

Novak, F. J., Brunner, H., Afza, R. and Van Duren, M. 1993. Mutation Breeding of *Musa sp*. (Banana, Plantain). *Mutation Breeding Newsletter*, **40:** 2- 4.

Novak, F. J., Afza, R., Duren, M.V. and Omar, M.S. 1990. Mutation induction by gamma irradiation of *in vitro* cultured shoot-tips of banana and plantain (*Musa*). *Tropic. Agri.,* **67(1):** 21-28.

Pillay, M. and Tripathi, L. 2007. Banana breeding. In: Breeding major food staples, M.S. Kang and P.M. Priyadarshan, eds., 393–428. New York: Blackwell Science. [Cross Ref]

Qamar, M., Qureshi, S. T., Khan, I. A., Memon, S. A., Bano, Z. and Solangi, S. K. 2016. Influence of gamma radiation on the physiochemical properties of *in vitro* triploid and tetraploid banana species. *Pak. J. Biotechnol.,* **13(4):** 237-244.

Roux, N.S. 2004. Mutation induction in *Musa* -review. In: Jain, S.M. and Swennen. R (Eds.) Banana improvement: cellular, molecular biology and induced mutations. *R. Sci. Pub*. Inc. Enfield. NH. USA, 23-32.

Sparrow, A. H., Rogers, A. F. and Schwemmer, S. S. 1968. Radio sensitivity studies in woody plants acute gamma irradiation survival data for 28 species and prediction for 190 species. *Rad. Bot.,* **8:** 149-186. [Cross Ref]

Taheri, S., Abdullah, T. L.. Ahmad, Z. and Abdullah, N.A.P. 2014. Effect of acute gamma irradiation on *Curcuma alismatifolia* varieties and detection of DNA polymorphism through SSR Marker. *Bio. Med Res. Int.* 14-1

Venkatachalam, L., Sreedhar, R. V. and Bhagyalakshmi, N. 2007. Genetic analyses of micropropagated and regenerated plantlets of banana as assessed by RAPD and ISSR markers. *In Vitro Cell. Dev. Biol. Plant.,* **43**: 267-274. [Cross Ref]

Veni, K., Vanniarajan, C. and Souframanien, J. 2017. Probit analysis and effect of electron beam and gamma rays in blacgram (*Vigna mungo* (L.) Hepper). *Electronic Journal of Plant Breeding*, **8(3):** 950-955. [Cross Ref]