AJAB

Asian J Agri & Biol. 2018;6(2):135-142.

Original Research Article

Gamma irradiation effect on the growth of *Musa* cv. Tanduk (AAB)

Ferid Abdulhafiz, Fatimah Kayat, Suhana Zakaria

Faculty of Agro-Based Industry, University Malaysia Kelantan, 17600, Jeli, Kelantan, Malaysia

Received:
September 25, 2017

Accepted:
April 02, 2018

Published:
June 30, 2018

**Corresponding author email:*
feridabdul24@gmail.com

Abstract

Improvement of banana through conventional method have been very difficult due to ploidy and sterility of most popular cultivars. Hence, given the difficulties in conventional breeding, mutation breeding was attempted to induce variability and obtain improved banana varieties. Therefore, banana cv. Tanduk shoots meristem (1.5 x 1.0 cm) were exposed to γ rays at doses ranging from 10-70 Gy. Subculture was conducted up to four cycle followed by induction of rooting. The radio-sensitivity of *in vitro* shoots towards radiation was assessed through the percentage of the explants that survived the treatment. The highest survival rate (74%) among γ treated explants recorded was in 10 Gy treatment and the lowest survivality (20%) was in 70 Gy. The lethal dose (LD_{50}) which had caused 50% mortality to the irradiated material was found to be 37 Gy. The result on shoot growth showed that the highest shoot number (5) per explant recorded at radiation dosage of 20 Gy, followed by 3 shoots per explant at a dosage of 10 Gy and highest shoot length (3.43 cm) was observed at 10 Gy followed by 20 Gy and 30 Gy treatment caused significant reduction in shoot length. Similarly, 10 Gy induced maximum root length, whereas explants irradiated with 20 Gy and 30 Gy caused the reduction in root length significantly. Gamma rays at the lower dosage of 10 and 20 Gy imposed a significant impact on shoot growth and 30 Gy caused reduction in growth. Hence, the present research was conducted to study the effect of γ radiation on banana plant, while observing growth traits in γ irradiated explants.

Keywords: Mutation breeding, *In vitro* mutagenesis, LD_{50}

Introduction

Banana is one of the most important genus in the family of *Musaceae* and mainly found in Africa and Asia, and it is believed to be originated in South and Southeast Asia. World production of bananas, estimated about 106 million tons (Lescot, 2006), ranks fourth in agricultural production. Bananas make up the largest production of fruits and the largest international trade, more than apple, orange, grape and melon. Bananas are cultivated in more than 120 countries in tropical and subtropical zones on five continents and have a major contribution towards world's food production, especially in many African countries and throughout the developing countries where it is a primary food source. Banana products represent an essential food resource and have an important socioeconomic and ecological role. It is known as perennial plant with a quicker relative growth rate compared to other fruit crops. (Bakry et al., 2009; Manzo-Sanchez, 2015).

Currently, cultivated banana throughout the world are threatened by several disease and pest, which need to be taken into account for banana improvement. Therefore, altering morphological and genetic variation to building-up resistant crop towards these biotic factors is urgently needed (Siddiqui et al., 1995). However, creating genetic variability and

improvement of banana through conventional breeding is hampered by several reasons such as very low clonal multiplication rates, parthenocarpic fruit development, polyploidy with extremely poor seed production and low levels of female fertility. These barriers impede sexual hybridization and consequently slow down genetic improvement of the crop (Lopez, et al., 2017). The difficulties associated with conventional breeding method led to the consideration of other methods for introducing useful characteristics into otherwise reliable clones. Many morphological, growth and agronomic variation have been appeared in plants obtained by micro-propagation and induced mutation to increase the variability of bananas by focusing on somaclonal variations. Therefore, the development of plant cell, tissue culture and mutation breeding using *in vitro* mutagenesis technique over the last 20 years has made it possible to transfer part of the breeding work from field to laboratory conditions. *In vitro* mutation breeding can support in overwhelming hurdles in the progress of new and superior banana cultivars for sustainable and continuous fruit production without disease spread (Jain and Maluszynski, et al., 2004). Induction of mutation and choice of desired characters in together with *in vitro* technology brings numerous returns over conventional breeding techniques include, the ability mutagenizing small plant part, having huge number of samples in short time span, quick production of large populations to eliminate chimeras, uniform mutagen treatment and assisting *in vitro* choice. Mutation induction has become a recognized way of creating dissimilarity within a crop variety using different types of mutagenic agents to widen the range of genetic variations, several research groups have worked to induce artificial mutations by chemical or physical treatments. The physical mutagens are categorized as ionization and non-ionization radiation. Ionizing electromagnetic radiation includes gamma rays, x-rays and cosmic rays (Mba et al., 2010; Liu et al., 2009; Van Harten, 1998). The advantage of using physical (gamma radiation) as compared to chemical mutagenesis is degree of accuracy, sufficient reproducibility, and uniform penetrating power in the tissue. Mutagens causes a greater number of variation in physiochemical composition by producing purine or pyrimidine dimers, resulting several kinds of mutations in the DNA sequence. However, the effects of ionization radiation are proportional to the dose applied and higher doses causes biological injures (Yamaguchi, 2003; Lagoda, 2012; Hasbullah et al., 2012). Biological effect of gamma radiation is depends on atoms or molecules interaction inside the cell, especially water to produce free radicals (Kovacs and Keresztes, 2002). Explants treated with mutagens could have a number of modifications in plant height, leaf shape, shoot growth and bunch features (Novak et al. 1990). Study conducted by Dwimahyani and Widiarsih, (2011), showed that irradiation at low γ dose (10 Gy) had promoted 10% multiplication rate of *in vitro* Chrysanthemum plant. Similarly, Qamar et al., (2016) reported that exposing shoot meristem to lower γ dose (10 Gy) showed higher shoot multiplication in three giant banana Cavendish tissue culture variant GCTCV-215, Yangambi KM-5 and FHIA-23 varieties and higher γ dosage was found to be inhibitory to the shoot growth and decline in the shoot multiplication Hence, the present study was conducted to determine the optimum dose of γ radiation for banana cultivar Tanduk meristematic shoot and study gamma irradiation effect on the growth of treated explants under *in vitro* conditions.

Material and Methods

Plant material, Explant preparation and γ irradiation procedure

The sword sucker of banana cv. Tanduk about 25-35 cm in height were collected from banana farm in Pengkalan Hulu, Perak Malaysia (5.7064° N, 100.9998° E). Shoot meristem (Figure 1) were prepared following the protocol described by Predieri, and Di Virgilio, (2007). The sucker were peeled and tissue block (3 cm x 4 cm) containing shoots meristem were isolated and soaked in 1.2 g/l of ascorbic acid for one hour to prevent browning. The explants were then surface sterilized with commercial available bleach (5.75% NaOCl) added with a few drops of Tween- 20 and placed on rotary shaker at 100 rpm for one hour. After sterilization process the sodium hypochlorite solution were decanted and dead tissue was cut off and then washed thoroughly with sterile water and then trimmed to the final size of (1.0 cm x 1.5 cm) to obtain the meristem tissue with two or more pairs of leaf primordia under sterile condition. γ irradiation was carried out at the Malaysian Nuclear Agency in Bangi, Selangor using BIOBEAM γ irradiation device using ^{137}Cs source. Shoot meristem were placed in the sterile Petri dish wetted with a few drops of sterilized distilled water and sealed to protect shoots from dehydration during γ exposure. Before the final irradiation treatment, preliminary investigations (radiosensitivity

test) was performed to determine suitable dose to be used. A total of 350 shoot meristem were subjected to seven doses (10, 20, 30, 30, 40, 50, 60, and 70 Gy) of γ radiations using Cesium-137 radiation source at a dose rate of 5.5 Gy/minute and non-treated samples served as control treatment. Radiosensitivity of explant to γ was assessed by the number of explant that survived after γ irradiation. After one month the survival percentage was calculated using the formula illustrated in (Equation 1).

$$\text{Survival Percentage (\%)} = \frac{\text{No. of survived explants after gamma exposure}}{\text{Total No. of explants exposed to gamma rays}} \times 100$$

Equation 1: Survival percentage formula

Based on the results from preliminary investigations, the lethal dose (LD_{50}) causing 50% mortality to gamma treated material was estimated, following the protocol described by Predieri and Di Virgilio, (2007). Therefore, γ dose lower than LD_{50} was selected (10 Gy, 20 Gy and 30 Gy) for the second round of γ irradiation.

Establishment of shoots meristem cultures and plant regeneration

Immediately after irradiation, treated explants were washed with sterilized distilled water thoroughly and transferred to initiation media, consisted macro, micro nutrients, iron, organic constituent, 30 g/l sucrose and 2.0 mg/l N6-benzylaminopurine (BAP) (Dagnew et al., 2012). The initiation period were lasted for 30 days and the shoot meristem were transferred to shoot multiplication (MS media supplemented with 5 mg/l of BAP). Sub-culturing was carried out successively every 30 days up to four sub-culture cycles using the same proliferation medium. After fourth sub-culture cycle shoots were then transferred to rooting media (2 g/l activated charcoal) and cultured for one month. All cultures were incubated at 25±2 °C with 55 to 65% relative humidity at 16 hours photoperiod under fluorescent light (1000 lux) and 8 hours in the dark.

Data collection and Statistical analysis

The survival percentages were measured at one week interval for a period of one month after gamma irradiation. The data on shoot multiplication includes, the number of shoot per explants and shoot length was recorded. The root number and root length was measured at the end of 4th sub-culture cycle. The experiment was arranged in a completely randomized design (CRD) with five experimental replications. All data were subjected to one way ANOVA. The lethal dose (LD_{50}) for mutagenesis of banana multiple shoot with gamma irradiation were estimated through linear regression equation and Minitab version 15 statistical software were used to carry out regression analysis.

Results and Discussion

Gamma radiation sensitivity and determination of lethal dose (LD_{50})

The sensitivity of shoot meristems to γ irradiation was evaluated by computing the survival percentage of irradiated and non-irradiated explants. The result showed significant difference in the survival percentage among low doses, higher doses and non-treated explants (Table 1). The survival (%) reduction were observed in γ treated population with a corresponding increase in γ dose.

Table.1: Data on the survival percentage of explant at 4 weeks after γ irradiation

γ dose (Gy)	Survival percentage (%)
Control	100.00±0.00^{a}
10	74.00±5.09^{b}
20	62.00±4.89^{bc}
30	58.00±3.74^{c}
40	44.00±2.44^{d}
50	40.00±4.47^{d}
60	32.00±4.89^{de}
70	20.00±6.32^{e}

*Means with the same letter has no significant difference at *P<0.05* after determined by a Duncan test.

Table 2: Number of shoots per inoculated shoot measured in M_1V_1 - M_1V_4 generation after irradiation

γ doses (Gy)	Multiplication rate (Shoot/explant) in different sub-culture stages			
	M_1V_1	M_1V_2	M_1V_3	M_1V_4
0	2.2 ± 0.20^{c}	3.6 ± 0.24^{ab}	4.1 ± 0.02^{b}	4.6 ± 0.41^{b}
10	3.2 ± 0.37^{b}	3.6 ± 0.36^{ab}	4.6 ± 0.24^{ab}	5.1 ± 0.40^{ab}
20	4.6 ± 0.40^{a}	5.1 ± 0.89^{a}	5.6 ± 0.92^{a}	6.2 ± 1.60^{a}
30	1.6 ± 0.24^{c}	3.1 ± 0.30^{b}	3.2 ± 0.11^{c}	3.1 ± 0.12^{c}

*Means with the same letter has no significant difference at *P<0.05* after determined by a Duncan test.

Table 3: Effect of γ rays on shoot length at 12th weeks after γ irradiation

γ doses (Gy)	Shoot length (cm)
0	3.06 ± 0.16[a]
10	3.43 ± 0.15[a]
20	3.34 ± 0.23[a]
30	2.52 ± 0.12[b]

*Means with the same letter has no significant difference at *P<0.05* after determined by a Duncan test.

Table 4: Effect of γ rays on root length and root number at 12th weeks after γ irradiation

γ doses (Gy)	Root length (cm)	Root number
0	16.13±0.69[b]	20.16±1.48[a]
10	20.53±0.95[a]	18.33±1.33[a]
20	13.36±0.52[c]	12.26±1.33[b]
30	11.26±0.90[c]	8.16±0.44[c]

*Means with the same letter has no significant difference at *P<0.05* after determined by a Duncan test.

The highest survival percentage among γ treated explants recorded was 74% in 10 Gy treatment, followed by 62% in 20 Gy, 58% in 30 Gy, 44% in 40 Gy, 40% in 50 Gy, 32% in 60 Gy and 20% in 70 Gy. The survival percentage of explants irradiated at the highest dosage (70 Gy) showed a remarkable reduction of 80%. Meanwhile, non-irradiated explants (0 Gy) recorded the highest survival percentage at 100%. Dehgahi and Joniyasa, (2017) stated that, γ rays interaction with cellular molecules, like water molecules causes production of free radicals, which these free radicles could form some toxic substance such as Hydrogen peroxide (H_2O_2), which contribute to the cells destruction and causes plant death. As evidence by Hase et al. (2002) and Taheri et al., (2014) who reported that, higher doses of gamma causes reduction in survivality percentage and growth which may have higher biological effects causing reduction of survival rate. Ikram et al., (2010) stated that, the γ irradiation dose to explants could be a critical factor affecting the explants survivality, as interaction with the γ rays with cells could also have a negative consequences to the biological materials. The LD_{50} for banana shoot meristems with γ radiation was determined based on the survival percentage of explants after treatments (Figure 2). Based on the calculation, 37 Gy was estimated as the lethal dose (LD_{50}) which had caused 50% mortality to the irradiated material. In principle, the most effective dosage for mutation induction are lower than the LD_{50} value and usually used for bulk irradiation (Broertjes and Van Harten, 2013). Mishra et al., (2007) suggested that γ treatment with less than 50% reduction in the survival rate is desirable for inducing mutation. Another study by Mishra et al., (2007) reported that the lethal dose for shoot meristems of banana cv. Grande Naine was found to be 35 Gy and they suggested that the γ doses lower than the LD_{50} value can be used for inducing variability. Similarly, Predieri and Di Virgilio, (2007); Novak et al., (1990) reported that, lower dosage than the LD_{50} is suggested to favor plant recovery after treatment, while higher doses increase the probability of inducing too many mutations which could have mostly negative impact. Therefore, the most effective dose selected from this study were 10, 20 and 30 Gy. The optimum dose for γ rays irradiation for banana cv. Tanduk shoot meristems and LD_{50} is indicated in the graph (Figure 2).

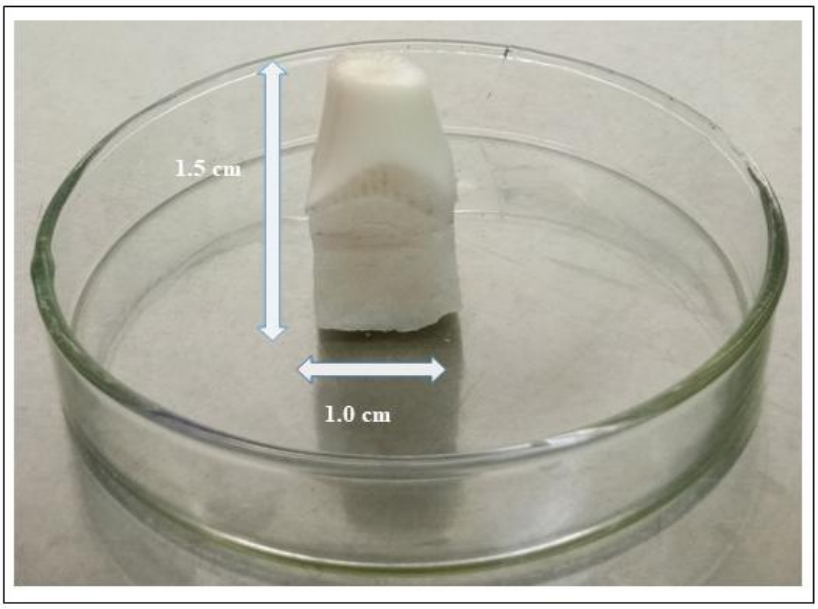

Figure 1: Shoot meristem used for γ radiation

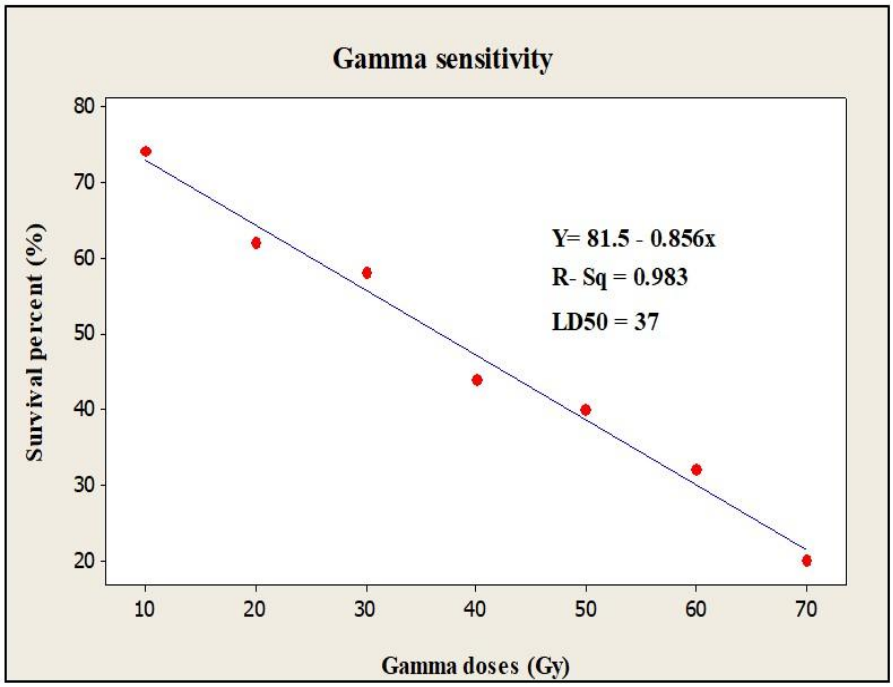

Figure 2: LD_{50} calculation on the survival rate of shoot meristems exposed to different γ ray dosage.The γ dose that resulted in 50% reduction of survival rate (LD_{50}) was assessed based on the survival rate recorded after one month of culture. The γ dose inducing LD_{50} was calculated with the linear regression equation by substituting (y) with the value of 50% of control (0 Gy). The linear regression equation result is Y= 81.5 – 0.856x with an R^2 = 0.9833 and LD_{50} is 37 Gy.

Figure 3: Effect of different doses of γ rays on the number of shoots of banana cultivar Tanduk in 2nd sub-culture generation (A: Control), (B: 10 Gy), (C: 20 Gy) and (D: 30 Gy).

Effect of gamma irradiation on shoot regeneration

Analysis of variance showed significant difference in the number of shoot per explant produced between irradiated and non-irradiated shoot meristems. As compare to control, variations were observed in shoot meristems treated with 20 Gy which accounts for 4.6±0.40 shoots per explant, followed by 10 Gy 3.2±0.37 and 1.6±0.24 at 30 Gy treatment in 1st sub-culture (M_1V_1) generation (Table 2). In this study, after irradiation with 10 and 20 Gy of γ irradiation, significance stimulatory effect on shoot growth was noticed, conversely, reduction in shoot growth were observed at 30 Gy of γ irradiation. Similar results had been obtained by Abdul et al., (2010) with 10 and 20 Gy of γ radiation, enhanced shoot multiplication were observed, whereas higher dosage had resulted in growth reduction. As reported by Janick (2008), an interesting phenomenon of growth stimulation can be observed where the treated samples shows higher survival rates and better growth than non-treated samples and concluded that the growth stimulation observed depends on gamma dosage. Enhancement in shoot multiplication at a lower dosage (10 and 20 Gy) was also reported by (Siddiqui et al., 1995). In this experiment, 30 Gy treatment had reduced the shoot multiplication rate into 76% as compared to control. Similar result had been recorded by Qamar et al., (2016) with 30 Gy produce lower shoot multiplication compared to other treatments. The mutagenesis effect on the shoot multiplication rate was also studied in the 2nd to the 4th generation. Result showed that the number of shoot increased gradually at the lower γ doses (10 Gy and 20 Gy) with the highest shoot produced at 6.2±1.6 in the 4th generation. The number of shoot produced increased gradually and showed progress in the 3rd and 4th sub-culture. Explants treated with 30 Gy had regained the ability to produce more shoots in the 2nd sub-culture. This might be due to the reduction of physical damage in the later sub-culture and plantlets regain the capacity to recover and start producing shoots normally (Prabakaran, 2001). The highest shoot number recorded in 20 Gy (T2) followed by 10 Gy (T1) and control (T0) in 3rd and 4th sub-culture stage. The number of shoots increased gradually in 30 Gy (T3) treatment starting from the 3rd sub-culture. This finding is in line with Qamar et al., (2016) who reported that the shoot multiplication decreases with the increase of γ irradiation dose (> 20 Gy).

Effect of gamma radiation on *in vitro* shoot length

Analysis of variance for the shoot length showed no significance difference among the control, 10 Gy (T1) and 20 Gy (T2). However, there was a significant

difference between the control and 30 Gy (T3) of γ treatment (Table 3). The highest shoot length observed was 3.43±0.15 cm in 10 Gy, followed by 3.34±0.23 cm in 20 Gy, 3.06±0.16 cm in control while 2.52±0.12 cm in 30 Gy treatment. Result shows that, the shoot meristems irradiated higher γ radiation dose (30 Gy) had lowest shoot length as compared to other treatment. Hasbullah et al., (2012) reported that increase of γ irradiation dose could suppress the activity of plant cells and affect morphological characteristics of the plant. As indicated by Wi et al., (2007) the reduction in shoot length of γ treated explants with 30 Gy probably due to the adverse effect of γ irradiation on the explant tissue which disrupt hormonal balance and enzymatic activities. This is due to the fact that, main injuries are retardation or inhibition of cell division and this affects the growth habit and alterations in plant morphology (Jain, 2010).

Effect of gamma radiation on *in vitro* root length

Analysis of variance showed, there were statistically significance difference found in root length between control and gamma treatments (Table 4). Whereby, maximum root length was recorded in 10 Gy treatment, followed by 16.13±0.69 cm in the control, 13.36±0.52 in 20 Gy and 11.26±0.90 cm in the 30 Gy was observed. The result indicated that, gamma treatment at lower dose of γ rays was found to be stimulatory for the root length of irradiated plantlets. In other way, higher γ dose (30 Gy) treatment had induced shortest root length at 11.26±0.90 cm. Similar result was obtained by Qamar et al., (2016) in banana cultivar GCTCV-215, (10 Gy) showed greatest root length, while plantlets irradiated with higher than 10 Gy induced a significant reduction in the root length. Data on root number showed that, significance difference were observed in root number of shoot meristem subjected to the higher γ dose 20 Gy (T2) and 30 Gy (T3) treatments. However, no significance difference were observed between the control and 10 Gy (T1) treatments. The root number per explant was lower in γ treated explants, whereby the highest root number observed was 20.16±1.48 in the control (0 Gy) explants followed by 18.33±1.33 in 10 Gy, 12.26±1.33 in 20 Gy and 8.16±0.44 in 30 Gy plantlets. The root number in γ irradiated plant had drastically reduced in corresponding increase in γ rays treatment. The present work was supported by Karmarkar et al., (2001) and Qamar et al., (2016), who reported that, explants subjected to 10 Gy treatment on FHIA-23 variety gave maximum rooting response with a significant reduction in root number and length with increase in γ doses of Yangambi KM-5 variety.

Conclusion

Result from the current experiment indicated that γ treatment induced growth stimulation and variation on banana cultivar Tanduk. Lower dose of γ irradiation had a positive impact on the growth of *in vitro* plants during multiplication and rooting stage, whereas higher γ irradiation had caused a negative impact on the growth, in terms of higher mortality rate and reduction in growth. Among the γ treatment applied, 10 Gy and 20 Gy was found to be the best treatment in the induction of variability. This was demonstrated in terms of higher survivality, leaf growth and greater root growth. Therefore, the most appropriate dose of γ irradiation for inducing growth and morphological variation was 10 Gy and 20 Gy, While higher γ irradiation (> 30 Gy), showed lower survivality and reduction in growth. Additionally, 20 Gy had significant impact in shoot multiplication and gave profound stimulation effect on shoot multiplication rate under *in vitro* condition.

Acknowledgement

The author would like to acknowledge Islamic Development Bank (IDB) for funding of this research.

References

Abdul M, Khan AUR, Habib A and Zahir M, 2010. Gamma irradiation effects on some growth parameters of *Lepidium sativum* L. J. Agri Bio Sci. 5(1): 39-42.

Bakry F, Carreel F, Jenny C, and Horry JP, 2009. Genetic improvement of banana. In breeding plantation tree crops: tropical species. Springer, New York. 3-50.

Broertjes C and Van Harten AM, 2013. Applied mutation breeding for vegetatively propagated crops. Elsevier. 12(1): 17-20.

Dagnew A, Shibru S, Debebe A, Lemma A, Dessalegn L, Berhanu, B and Sierra YM, 2012. Micropropagation of banana varieties (*Musa* spp.) using shoot-tip culture. Eth. J. Agri. Sci. 22(1): 14-25.

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.

Dwimahyani I and Widiarsih S, 2011. The effects of gamma irradiation on the growth and propagation of in-vitro chrysanthemum shoot explants (cv. Yellow Puma). Atom. Indo. 36(2): 45-49.

Hasbullah NA, 2010. Morphogenesis and tissue culture studies of Gerbera jamesonii Bolus Ex. Hook F. Diss. Universiti Malaya.

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.

Ikram N, Dawar S, Abbas Z and Zaki MJ, 2010. Effect of (60 cobalt) gamma rays on growth and root rot diseases in mungbean (*Vigna radiata* L.). Pak. J. Bio. Sci. 42: 2165-2170.

Jain SM and Maluszynski M, 2004. Induced mutations and biotechnology in improving crops. In vitro. 170-202.

Jain SM, 2010. Mutagenesis in crop improvement under the climate change. Roma. Biotech. Lette. 15(2): 88-106.

Janick J, 2008. Plant breeding reviews (Vol. 30). John Wiley and Sons. Penjuru, Singapore. 61-65.

Kovacs E and Keresztes A, 2002. Effect of gamma and UV-B/C radiation on plant cells. Micron. 33(2):199-210.

Katungi EM, 2007. Social capital and technology adoption on small farms: The case of banana production technology in Uganda (Doctoral dissertation).

Lagoda PJL, 2012. Effects of radiation on living cells and plants. Plant Muta. Breedi. Biotech. 123-134.

Lescot T, 2006. The banana in figures: the favourite fruit of the planet. Fruit crop. 140: 5-9.

Liu H, Wang Y, Xu J, Su T, Liu G and Ren D, 2008. Ethylene signaling is required for the acceleration of cell death induced by the activation of AtMEK5 in Arabidopsis. Cell. Res. 18(3): 422-432.

Lopez J, Rayas A, Santos A, Medero V, Beovides Y and Basail M, 2017. Mutation Induction Using Gamma Irradiation and Embryogenic Cell Suspensions in Plantain (Musa spp.). In Biotechnologies for Plant Mutation Breeding, Spri. Intern. Pub. 55-71.

Manzo-Sánchez G, Buenrostro-Nava MT, Guzmán-González S, Orozco-Santos M, Youssef M and Medrano R.MEG, 2015. Genetic Diversity in Bananas and Plantains (*Musa spp*.). In Molecular Approaches to Genetic Diversity. In Tech. 47-62.

Mba C, Afza R, Bado S, and Jain SM, 2010. Induced mutagenesis in plants using physical and chemical agents. Plant cell culture. 20: 111-130.

Mishra PJ, Ganapathi TR, Suprasanna P and Bapat VA, 2007. Effect of single and recurrent gamma irradiation on in vitro shoot cultures of banana. Inter. J. Fru. Sci. 7(1):47-57.

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

Prabakaran G, 2001. Studies On In Vitro Mutation Breeding In Banana (Musa Spp.) For Resistance to Fusarium Wilt Disease (Doctoral dissertation, Tamil Nadu Agricultural University; Coimbatore).

Predieri S, and Di Virgilio N, 2007. *In vitro* mutagenesis and mutant multiplication. In Protocols for micro propagation of woody trees and fruits, Spri. Netherl. 323-333.

Qamar M, Qureshi ST, Khan IA, Memon SA, Bano Z and Solangi SK, 2016. Influence of gamma radiation on the physiochemical properties of in vitro triploid and tetraploid banana species. Pak. J. Biotechnol. 13(4): 237-244.

Siddiqui SH, Khatri A, Khan IA, Nizamani G and Khan R, 1995. Improvement of banana (*Musa*) through in vitro culture techniques and induced mutations (No. IAEA-TECDOC--800).

Taheri S, Abdullah TL, Ahmad Z and Abdullah NAP, 2014. Effect of acute gamma irradiation on Curcuma alismatifolia varieties and detection of DNA polymorphism through SSR Marker. BioMed Research. Intern. 14-19.

Van Harten AM, 1998. Mutation breeding: theory and practical applications. Cambridge University Press. 274-282.

Wi SG, Chung BY and Kim JH, 2005. Ultrastructural changes of cell organelles in Arabidopsis stems after gamma irradiation. *Journal of Plant Biology*. 48(2). 195–200.

Yamaguchi H, Nagatomi S, Morishita T, Degi K, Tanaka A, Shikazono N and Hase Y, 2003. Mutation induced with ion beam irradiation in rose. Nuclear Instruments and Methods in Physics Research: Beam Interactions with Materials and Atoms. 206: 561-564.