Induced Mutations Using Gamma Ray and Multiplication of Plantlet through Micro Cross Section Culture of Banana (Musa acuminata cv. Berangan)

A A Hasim1, A Shamsiah1,2, S Hussein3

1 Faculty of Plantation and Agrotechnology, Universiti Teknologi MARA Cawangan Melaka, Kampus Jasin, 77300 Merlimau, Malacca, Malaysia.
2 Agricultural Biotechnology Research Group, Faculty of Plantation and Agrotechnology Universiti Teknologi MARA, 40150 Shah Alam, Selangor.
3 Research Agrotechnology and Biosciences Division, Malaysian Nuclear Agency, 43000 Kajang, Selangor, Malaysia.

Corresponding author’s email address: aisyahahirah95@yahoo.com

Abstract. The conventional breeding approach in banana was slow predominantly because of the limited genetic diversity, infertility and polyploid condition of the species. Induced mutation is one of the promising tools to create new varieties for the improvement of banana. While vitro propagation technique ensures a rapid and in an organized manner for production of banana. Thus, induced mutation using gamma ray in combination with in vitro culture micro cross section culture was conducted to evaluate the effects of gamma radiation on growth and development of plantlet of Musa acuminata cv. Berangan. Tissue cultured banana plantlet was used as the source of explant. The plantlet was cut into halved and radiated using gamma ray with the dose of 0 (control), 20, 25, 30, 35, 40, 45, 50, 55, 60 and 80 Gy. The highest survival rate (23.33%) was recorded in treatment 10 Gy while the lowest survival (0.33%) was in treatment 80 Gy. The lethal dose (LD50) which had caused 50% mortality to the irradiated material was found to be 37 Gy. The morphological study on the growth of in vitro irradiated plantlets showed that 25 Gy induced a stimulation effect on the number of shoot produced (5.56), root number per explant (8.5) and root length (37.36 cm) compared to untreated banana explants (0 Gy). Hence, this study introduces the in vitro multiplication of gamma irradiated culture system for future breeding of a new varieties of banana.

Keywords: Banana, Gamma Radiation, Induced Mutation, In Vitro, LD50.

1. Introduction
Banana (Musa sp.) which belongs to Musaceae family and genus of Musa is one of the most popular fruit crops in Malaysia. In 2019, Malaysia has produced 345,850 metric tons of banana in 30,684 hectares of land [1] which Johor, Pahang, and Sarawak are the main producers of banana. The crop was cultivated as a single cropping and mixed or intercropping with the commercial crops [2]. Berangan and Cavendish banana are the most popular and mainly cultivated cultivars in which covered fifty percent of the bananas’ planting area in Malaysia while the other cultivar grown by the farmers are Mas, Rastali, Raja, Awak, Abu, Nipah, Nangka and Tanduk [3].
Bananas have a vast potential as a sustainable crop with many products and yet their production were limited as the result of climate change [4]. One of the major constraints of the supply inconsistency were due to scattered and non-staggered banana cultivation as most of the banana production were managed by the small-scale growers. Thus, a feasible supply of quality crop and extensive production are needed to support the industry and also to meet the local market demand. Limited success was reported when practicing conventional breeding method as it was hampered by narrow genetic variability of the cultivar, slow growth rate and also vulnerable to disease infestation. Furthermore, most of the banana cultivar are propagated vegetatively so typical cross breeding method is not possible because of its low fertility rate and polyplodiy nature of the banana cultivars and hence slowing down genetic enhancement of the crop [5].

The implementation of numerous biotechnological perspectives such as tissue culture has positive impacts to the banana plantation industry nowadays. In vitro tissue culture was proven as the promising way to increase the production of new planting material of banana in shorter time, smaller area, diseases-free planting material and ample germplasm conservation. In addition, mutation induction can be done for the genetic improvement of banana instead of conventional method [6, 7]. Mutation breeding is a promising tool to improve banana varieties using mutagenic treatment to generate a novel variety of banana [8, 9]. As compared to the traditional propagation method, the tissue cultured plantlets were generated rapidly, have vigorous growth, the development cycle is shorter and much more uniformed as well as able boost more income. A great variation was arising in terms of establishment, proliferation and regeneration of the shoots and roots produced by in vitro micropropagation techniques through a shoot tip culture due to some factors like genotype, type of explant used, composition of the culture media, plant growth regulators as well as the culture condition [10].

An event of unpredictable heritable trait modification that happens within the gene of an organism which are not caused by the genetic segregation or recombination, yet were introduced either by the physical, chemical, or even a biological agent was referred as mutagenesis [11]. Induced mutation using gamma rays through in vitro mutagenesis technique was done by the previous studies as an alternative for crop improvement. This is due to some advantages of gamma irradiation in terms of modification of physiology and morphology of mutated plant material. Besides, the radiation doses of absorbance are depending on the sensitivity of Musa species. Radiation by gamma ray could also modify the plant cell and metabolism including expansion of thylakoid membranes, alteration in photosynthesis, improvement of the anti-oxidative form and formation of secondary metabolite [12, 13, 14]. The main step to exploit the mutagenic treatment is by determining the mutagen sensitivity level (LD50) of the Musa species [15]. Mutation induction also were used in crops cultivation to develop certain attractive traits which influence the plant size, flowering time and fruit maturation, color of the fruit, self-compatibility, self-thinning, and resistance against pathogens. The success of any in vitro mutation programmed were relies on the establishment of reproducible in vitro plant regeneration procedures, maximized the used of mutagenic treatments, and a potent screening technique of the mutagenized populations for the desired variations [16, 17]. Thus, this research was conducted to study the effects of gamma radiation on the growth and development of the banana in vitro plantlet.

2. Materials and Methods

2.1 Establishment of micro-cross-section culture system and gamma irradiation treatment

Pseudostems of tissue cultured plantlets plantlet cv. Berangan (AAA) were used to make micro-cross-sections according to a previously reported procedure and modified from the existing method [18,19]. A pseudostem piece (2.0 cm × 6 mm) was cut off just above the first root of plantlet and placed on filter paper wetted with a few drops of sterilized distilled water in a sterile petri dish. Then the samples were exposed with nine different gamma doses from 0 Gy (control), 20 Gy, 25 Gy, 30 Gy, 35 Gy, 40 Gy, 45 Gy, 50 Gy, 55 Gy, 60 Gy and 80 Gy with a dose rate of 11.7 Gy/min. After the irradiation treatment, the treated sample were rinsed three times with sterile water and dissected into 1-mm-thick sections, termed micro-cross-sections and these micro-cross-section explants. The radiated explants were maintained in shoot-induction medium consisting of basic MS medium [20] supplemented with 10 µM 6-
benzylaminopurine (BAP), 10μM IAA, 50 μM kinetin, 30 g L⁻¹ sucrose and 2 g of gelrite (pH 5.8). The rooting media contained MS medium supplemented with 1 μM NAA, 30 g L⁻¹ glucose and 2 g gelrite with the pH of 5.8 and autoclaved at 121ºC. The culture was incubated in the culture room at 24±1ºC with 24-hour photoperiod.

Figure 2. Micro-cross Section Culture Method of Banana cv. Berangan

2.2 Hardening of the plantlet
After four weeks on rooting medium, well grown plantlets were carefully detached from the culture vessel and their roots were washed carefully under running tap water to remove the traces of nutrients and transplanted in polybags with a commercial soil mix potting medium.

2.3 Data Collection and Statistical Analysis
The lethal dose (LD₅₀) values were recorded based on the 50% decrease in the survival rate percentage of the explants. The survival percentages were recorded for every week interval for a month after gamma exposure. Post-irradiation responses of shooting and rooting in vitro culture were evaluated by recording number of leaves, seedlings height, days to root initiation, root number and root length (cm) during and after 30 days of treatment. Consequently, in the hardened plants, plant height (cm), number of leaves, diameter of the leaves (cm), leaves length (cm), pseudostem diameter (cm) were measured. The experiment was arranged in a completely randomized design (CRD) with 5 replications. The data were analyzed by one-way analysis of variance (ANOVA) and the differences between separated means was made using least significant difference (LSD) at P ≤ 0.05 level while the LD₅₀ were calculated using the survival rate percentage (%) and the data was analyzed by using linear regression [21]. The survival percentage of the treated explants was calculated based on the following formula:

\[
\text{Survival Percentage (\%)} = \frac{\text{No. of explants survived after gamma exposure}}{\text{Total no. of explants irradiated with gamma ray}} \times 100
\]

3. Results and Discussions

3.1 The Radiosensitivity Test and Determination of Lethal Dose (LD₅₀)
The radiosensitivity effects of gamma irradiation on regeneration and morphological characteristics of the Musa acuminata cv. Berangan explants was observed. Gamma radiation from radioactive cobalt (⁶⁰Co) is widely used as it has high penetrating potential into the layer of tissue although it is hazardous. Radiosensitivity test was essential when using gamma irradiation as it was the main step which emphasized on the early selection of a variant that had genetic changes [22, 23]. Based on the results obtained, lethal dose (LD₅₀) leads to 50% of mortality towards the irradiated samples and were estimated using the published protocol [24].

The results displayed significant difference in survival rate between the non-radiated, low dosage and high dosage of gamma radiation exposure towards the explants (Table 1). The results also shown a significant reduction trend in the percentage of the survived explant when treated with gamma ray as the regeneration of the irradiated explants were influenced greatly by the dose of gamma exposure. High radiation dosage prohibits the regeneration of explants. The explants showed a very visible changes like browning to blackening, retardation and eventually dead after exposure even in the lowest dosage of 20 Gy.
Figure 3. Radiosensitivity test curve (LD$_{50}$) demonstrating the effects of different dose of gamma rays on survival rate of individual in vitro shoot after 30 days of exposure.

The highest survival percentage among explants recorded was 25.78 % at 0 Gy (control treatment) followed by 23.33% at 20 Gy, 18.78% at 25 Gy and the lowest survival rate was 0.33% at 80 Gy. The percentage of survived explants at the highest dose (80 Gy) displayed a remarkable reduction of 97.32 % whereas for non-radiated explants (0 Gy) showed the highest survival percentage of 69.67 %.

The LD$_{50}$ was found to be at 37 Gy based on its survival percentage. Reduction in survival percentage and growth occurred when explants being exposed to a higher dosage of gamma ray which might leads to higher biological effects that resulting in lower chances of survival [25, 26]. In parallel to previous findings, they proposed that dosage rate that lower than the lethal dose (LD$_{50}$) helps in plant rejuvenation after radiation treatment whereas higher dosage of radiation increased the likelihood of too many mutations incident which eventually brings adverse effects to the radiated sample [23, 6].

Next, utilization of gamma ray lengthens the days for the first shoot to arise from two weeks in the control to 4-12 weeks for the irradiated sample. Based on the observations, there were visible changes occurs in the regenerative shoot where it starts with the proliferation of bud on the base of the treated explants then its slowly grown into a shoot and eventually a true leaf. Generally, the most constructive dosage for mutagenesis were assumed to be the lower dosage of gamma exposure than the LD$_{50}$ of the cultivar. Also, it is important to note that the nearest gamma dose which is lower and upper to the LD$_{50}$ is much more favorable to develop a desirable type of mutants [27]. Thereby, the low dose treatment at 20, 25 and 30 Gy of gamma radiation produced a great amount of regenerated shoot and then the treated explant were transferred into rooting medium.

3.2 The Effects of Gamma Exposure on Growth of Irradiated In Vitro Individual Shoots Under Aseptic Condition.

3.2.1 Effects of Gamma Ray on Shoot Rejuvenation.

From the gained data, gamma irradiation showed no remarkable effect on leaf formation in all radiated explant except for 20 Gy and 60 Gy. The data showed reduction trend in leaf number and were identified to be detrimental as the gamma dose increases. As compared to the control treatment, the number of shoots produced by explant were varies between treatment. The highest number of shoots produced was found in 45 Gy with 6.8 shoot per explant followed by 25 Gy with 5.6, 30 Gy with 5.5 and the lowest shoot produced was in 60 with 2.9 shoot per explant.

At 20 Gy, a significance stimulatory effect on shoot growth was noticed, conversely, reduction in shoot growth were observed at 35 Gy onwards. In opposed to past results, it was found that very large effect on the number of leaves in Cv. Decurrens at low dosage of gamma exposure [28]. Conversely,
our findings are in agreement with studies that mentioned higher levels of gamma rays diminished the number of leaves in the *Torenia fournieri* plant [29]. Lesser mitotic activity in meristematic tissues and declined in moisture contents of the explants used leads to the deduction in regeneration capability of a shoot and root development when exposed to higher doses of radiation [30]. Appropriate amount of cytokinin not just suppressed the apical dominance yet their existence also helps in regulating organogenesis. Thus, it can be concluded that the number of leaves tends to be lower with the increase of gamma dose radiation.

On the other hand, most of the treated explant had attained the ability to produce more shoots in the second subculture. This is because of the lower physical impact in the following subculture cycle and the seedlings able to recover back and produced shoots normally [31]. Identically, in earlier studies did not discover any positive performance that could have been attributed to genetic variability [32]. Another study also identified that shoot formation depends on the proportion of cytokinin in the growth media, apart from the genetic factors [33, 34].

The morphological characteristic of the treated explants can be seen in (Figure 4) as it showed various type of morphology differences between treatment such as undifferentiated tissue, abnormal formation of shoot cluster and undifferentiated callus. Very poor rejuvenation reaction obtained in this study may be related to the detrimental impact of gamma irradiation [35]. As the plant tissues absorbed gamma radiation, it combines with molecules or atoms (especially water) to generate free radical damage in cells that strike nearby cells. Those radicals can alter various essential compounds of the plant cell which leads to the destruction of the plant cells. The above consequence of irradiation is crucial for plant cells due to the fact that cytoplasm comprises about 80% water [13]. Next, earlier findings discovered that the inhibitory impact of excessive doses of gamma irradiation on shoot development owing to the disturbance in physiochemical process associated with gibberellic acid activity, which generally promotes cell division, elongation, and higher dosage of radiation also killed the meristematic cells or destroy the less progeny cells which contributes to the inability of radiated tissue to consume the appropriate nutrient which eventually suppressed the growth of the treated tissue [36, 37].

![Figure 4. Morphologies of the micropropagated cultures after 4 weeks of cultures; (a) undifferentiated tissue, (b, c and d): abnormal shoot clusters, and f: undifferentiated callus.](image)

### 3.2.2 Effects of Gamma Ray on Fresh Weight of In Vitro Culture.

Analysis of variance revealed that there was statistically significance difference (p < 0.05) found in fresh weight of explants between the untreated and gamma irradiated treatment at 25 Gy, 45 Gy and 60 Gy. The results tend to show inconsistent trend in the fresh weight between treated and non-treated sample after 42 days of irradiation. The fresh weight of the sample was depended on the dose of the gamma ray exposure. The higher exposure of gamma ray leads to lower reading of fresh weight. Observations on the weight of the shoot tips found that the highest values recorded was 13.63 g at 25 Gy followed by 10.02 g at 35 Gy and 9.52 g at 45 Gy respectively while the lowest reading was 1.42 g at 60 Gy. Likewise, previous investigation stated that the reaction of micropropagated banana plants to gamma rays at 0, 10, 20, 30, 40 and 60 Gy and found that greater exposure doses had resulted in higher plant susceptibility towards survival rate, length of shoot, leaf count, root count, fresh and dry weight [38]. Moreover, high dose of irradiation will trigger the germination enzyme to become inactive thus the plant development is hindered [39].
3.2.3 Effects of Gamma Ray on In Vitro Seedlings Height.
Based on the analyzed results, there was no significant effect of gamma exposure on the in vitro seedlings height (p > 0.05) except for 35 Gy, 45 Gy and 60 Gy. The highest height of seedlings recorded at 25 Gy with 6.34 cm followed by 35 Gy (6.12 cm) and 45 Gy (6.03 cm) while the shortest was 2.84 cm at 60 Gy. The plantlet height differs between the treated and non-treated explants after 12 weeks of irradiation (Figure. 5). The average seedling height showed unsteady declined trend from the control treatment to maximum dose of gamma irradiation. The fluctuation trend may be due to the differences in the form of plant tissue used and the location of the plant tissue at the time of gamma radiation [40]. Younger tissue is more likely susceptible to gamma irradiation. A plant's capabilities to respond to gamma exposure varies. Radiation will influence the properties of plants, both positively and negatively. The 20 Gy and 25 Gy radiation doses had a positive influence as compared to controls, by increasing the mean of plantlet heights.

According to the other findings, an increased in dose of gamma radiation may curb the plant cells action and slightly alter the morphology of the plant [41]. In addition, previous study categorized the difference in banana plant morphology occurred from induced mutation by their agronomic attribute like height of the plant, the shape of the leaf formed and they also discovered dwarf type of banana raised from in vitro mutation technique [42]. Other studies found that there was substantial reduction in plant height of Xanthosoma produced in vitro as a result of increased sensitivity towards gamma rays [43, 44].

![Figure 5](image_url) In vitro growth of irradiated seedlings for every treatment after 12 weeks of culture a) 0 Gy (control), b) 20 Gy, c) 25 Gy, d) 30 Gy, e) 35 Gy, f) 40 Gy, g) 45 Gy, h) 50 Gy, i) 55 Gy, j) 60 Gy.

3.2.4 Effects of Gamma Ray on In Vitro Number of Root.
Significant difference was observed on rooting ability of the explants in higher exposure of gamma ray of 60 Gy (Table 1). Increased dose of gamma radiation has been observed to inhibit root development of the explants. The maximum number of rooting explants was obtained at 45 Gy with 9.5 root per explant followed by 25 Gy with 8.5 root per explant and 8.33 total root at 40 Gy. In general, fluctuation number of rooting responses was observed in treated and non-treated explants. The number of roots produced at 50 Gy, 55 Gy and 60 Gy of irradiated plant was reduced correspondent to the increased in gamma irradiation treatment. Owing to the disruption of hormone regulation within the explant and the hormonal synthesis action and carried from the tip of the targeted site or to the exogenous source of auxin used in the rooting.

Similarly, earlier study claimed that the factor may be attributed to the interference of hormonal control and enzymatic behavior [45]. The sensitivity of the plants towards gamma rays increased corresponding to the high dosage of gamma ray and lesser the amount of endogenous growth regulators.
produced, especially the auxin and cytokinin [45, 46, 47]. Previously [48] found that explants of banana FHIA-23 and Yangambi KM-5 treated with 10 Gy showed greatest rooting ability with a substantial reduction in number of root and length of root when radiation dose increase. According to [49] plants able to restore themselves (self-repair) from cells damaged caused by radiation disturbances and that is why plants cannot maintain their normal growth and development. Yet, the presence observed in normal development following restoration of defective cells does not generally mean that genetic modifications do not exist unless they cannot be reflected in their development.

3.2.5 Effects of Gamma Ray on In Vitro Root Length.
Significant difference was observed on the root length of explant treated with 35, 40, 45 and 60 Gy and it was observed that higher gamma dose inhibits the root growth. The highest root length was observed in explants from treatment 45 Gy (49.83 cm) followed by 40 Gy (43.00 cm) while the lowest was observed at 60 Gy (9.6 cm). The result indicates that treatment with low gamma dose was found to be stimulatory for the root length of irradiated plantlets. Previously, similar result was obtained in banana cultivar GCTCV-215, where 10 Gy of radiation dosage showed the highest root length, while seedlings exposed to higher than 10 Gy obtained remarkable reduction on the root length [48].

A certain irregularity like thin roots was observed in high radiation dosage that beyond 35 Gy. The oddities could be due to genetic expression and disturbances in the biochemical synthesis as stated by [50]. Similarly, previous study proposed that irradiation dosage also modifies the ploidy level hence formed irregularities and build up abnormal vegetative growth [51]. Only slight differences in rooting quality were observed in lower dosage of gamma radiation of 20 Gy. Meanwhile, in higher doses treatment, the root quality was poor in which the root was thin and easily deteriorated and could not withstand the plants during the hardening stage.

Table 1. Effects of gamma irradiation on in vitro individual shoot of banana cv. Berangan after 2 months of exposure.

| Treatment (Gy) | Fresh weight (g) | Seeding height (cm) | Number of shoots | Number of roots | Length of root (cm) | Survival rate (%) |
|---------------|------------------|---------------------|------------------|-----------------|-------------------|------------------|
| 0             | 4.97±1.08a       | 4.29±0.63abc       | 4.61±0.63b       | 7.11±1.14a      | 31.74±7.27bc      | 25.78±2.98a      |
| 20            | 6.17±1.50bcde    | 5.10±0.61abc       | 5.13±0.68bc      | 6.70±0.79b      | 34.58±8.00abc     | 23.33±7.05b      |
| 25            | 13.63±4.77b      | 6.34±0.24ac        | 5.62±0.98bc      | 8.50±1.84b      | 37.36±10.46abc    | 18.78±3.39b      |
| 30            | 7.05±2.09bcd     | 4.85±0.55abc       | 5.50±0.92bc      | 8.08±1.71b      | 30.68±8.09abcd    | 14.56±4.43c      |
| 35            | 10.02±3.83c      | 6.12±0.42c         | 5.25±0.70bc      | 7.33±1.94bc     | 40.28±16.69b      | 11.00±1.00b      |
| 40            | 5.86±2.22bcd     | 4.5±0.66bc         | 4.82±0.70bc      | 8.33±1.85bcd    | 43.00±16.31b      | 12.67±1.39b      |
| 45            | 9.52±1.87bcd     | 6.03±0.37bc        | 6.33±0.31abc     | 9.50±0.67bcd    | 49.83±6.58c       | 12.00±2.52bc     |
| 50            | 2.57±1.02abcde   | 5.10±0.60ac        | 3.50±1.50abc     | 8.00±2.00bcd    | 24.68±8.84bcd     | 6.89±1.16c       |
| 55            | 6.99±2.09bcd     | 4.90±1.10abc       | 3.75±0.25abc     | 7.00±1.78bcd    | 40.10±11.28abcd   | 3.11±1.02c       |
| 60            | 1.42±0.34ab      | 2.84±0.31c         | 2.93±0.33c       | 3.90±0.61f      | 9.60±2.97c        | 1.78±1.35c       |
| 80            | -                | -                   | -                | -               | -                 | 0.33±0.33d       |

Note: Different letters indicate values are significantly different (P ≤ 0.05) by LSD multiple range test; Values are mean ± standard error based on five replications.

4. Conclusion
In conclusion, the outcome of the present study indicated that the gamma treatment induced physiological and morphological variations on banana cv. Berangan. Lower dose of 25 Gy had a positive effect on the growth of in vitro plantlets during shoot regeneration and also rooting response whereas greater gamma dose gave negative effect on the growth rate and increase the mortality rate. Mutation induction seemed not just to enhance the variability of these two quantitative attributes but also displayed a much greater incidence for plants with shorter plant size and root length development. Even
though in vitro mutation induction may contribute to genetic variations as well as various unfavorable variants, it is highly necessary to incorporate in vitro mutations into a selection method that can be tested for large mutagen-treated populations.

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References
[1] Department of Agriculture Malaysia 2019. Statistik tanaman (sub-sektor tanaman makanan). Retrieved from http://www.doa.gov.my/
[2] Mokhtaruddin H and William R 2011. Status of banana cultivation and disease in Malaysia. Paper presented during the workshop Integrated approaches in banana disease management, 22 March 2011, Serdang. Organizer: Department of Agriculture Malaysia and MARDI.
[3] Rafidah B, Norliza T A B and Jameah B 2018. Banana blood disease: background and analysis of genome blood disease bacterium (BDB). Bul.Tek MARDI. 13 27-37.
[4] Ranjitkar S, Sujakhu N M, Merz J, Kindt R, Xu J and Matin M A 2016. Suitability analysis and projected climate change impact on banana and coffee production zones in Nepal. PLoS ONE 11 9, 1-18.
[5] 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 Jankowicz-Cieslak J, Tai T, Kumlehn J, Till B. (eds) Biotechnologies for Plant Mutation Breeding. (Springer: Cham) pp 55 – 71.
[6] Novak F J, Afza R, Van D M and Omar M S 1990. Mutation induction by gamma irradiation of in vitro-cultured shoot-tips of banana and plantain (Musa cvs). Trop. Agric. (Trinidad) 67 21–8.
[7] Jankowicz C J, Huynh O A, Brozynska M, Nakitandwe J and Till B J 2012. Induction, rapid fixation and retention of mutations in vegetatively propagated banana. Plant Biotechnol. J. 10 1056–1066.
[8] Govindaraj M, Vetriventhan M and Srinivasan M 2015. Importance of genetic diversity assessment in crop plants and its recent advances: an overview of its analytical perspectives. Genet. Res. Int. 1 – 14.
[9] Kalwar K and Dahot M U 2017. Effect of induced mutation by uv radiation on cotton growth, seeds and protease activity. Pak. J. Biotechnol. 14 1 105 – 107.
[10] Vuylsteke D 1998. Shoot-tip culture for the propagation, conservation and exchange of Musa Germplasm. In: Practical Manuals for Handling Crop Germplasm In Vitro, 2 International Institute of Tropical Agriculture, Ibadan, Nigeria pp 82.
[11] Roychowdhury R and Tah J 2013. Mutagenesis: a potential approach for crop improvement. In: Hakeem K R, Ahmad P, Ozturk M, editors. Crop improvement: new approaches and modern techniques. (New York: Springer) pp 149- 187.
[12] Ashraf M, Cheema A A, Rashid M, Qamar Z 2004. Effect of gamma rays on MI generation in basmati rice. Pak J Bot. 35 791–796.
[13] Kovacs E and Keresztes A 2002. Effect of gamma and UV-B/C radiation on plant cells. Micron. 33 199–210.
[14] Mbaye G, Soumboundou M, Diouf L A D, Ndong B, Djiboune A R, Sy P M, Diarra M 2017. Evaluation of the effects of irradiation of peanut grain by a gamma-ray beam on culture. Ope J Biophy. 7 94.
[15] Jain S M 2010. Mutagenesis in crop improvement under the climate change. Roma. Biotech. Lette. 15 2 88-106.
[16] Jain S M 2007. Recent advances in plant tissue culture and mutagenesis. Acta Hort 736 205–211.
[17] Xu L, Najeeb U, Naem M S, Wan G L, Jin Z L, Khan F and Zhou W J 2012. In vitro mutagenesis and genetic improvement. In: SK G (ed) Technological innovations in major world oil crops, vol 2 (Springer: Heidelberg) pp 151–173.

[18] Huang X, Huang X L, Wang H H and Li X J 2001. Studies on the plant regeneration from the micro-cross sections of banana. *Acta Hort Sinica* **28** 19–24.

[19] Okole B N and Schulz F A 1996. Micro-cross sections of banana and plantains (*Musa* spp.) morphogenesis and regeneration of callus and shoot buds. *Plant Science* **116** 185–95.

[20] Murashige T and Skoog F 1962. A revised medium for rapid growth and bioassays with tobacco tissue. *Physiologia Plantarum* **15** 473–97.

[21] Singh H P 1990. *Report on banana and plantain - India*. In: R. V. Valmayor (Ed.), Banana and plantain Research and development in Asia and the Pacific: proceedings of a regional consultation on banana and plantain R & D networking, Manila and Davao, 20-24 November 1989. INIBAP, France pp 161-185.

[22] Abdul Muhaimein A K, Maheran A A, Noor Camellia N A and Mansor H 2020. In vitro mutagenesis using bio-beam irradiation on in vitro culture of Cavendish banana cultivar (*Musa acuminata* Colla) explants. *Res. On Crops* **21** 1 99-105.

[23] Abdullah, S, Kamaruddin, N Y, Harun, A R 2018. The effect of gamma radiation on plant morphological characteristics of *Zingiber officinale* Roscoe. *International Journal on Advanced Science, Engineering and Information Technology* **8** 5 2085 – 2091

[24] Predieri S and Di Virgilio N 2007. In *vitro* mutagenesis and mutant multiplication. In Protocols for micropropagation of woody trees and fruits. (Springer: Netherland) pp 323-333.

[25] 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.

[26] Taheri S, Abdullah L T, 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. *BioMed Research. Intern.* 14-19.

[27] Kulkarni V M, Ganapathi T R, Suprasanna P and Bapat V A 2007. In vitro mutagenesis in banana (*Musa* spp.) using gamma irradiation. In: SM J, Ha’ggman H (eds) Protocols for micropropagation of woody trees and fruits. (Springer: Dordrecht) pp 543–559.

[28] Yadav V 2016. Effect of gamma radiation on various growth parameters and biomass of *Canscora decurrens* Dalz, *Intern. J Herbal Medic.* **4** 109-115

[29] Jala A 2011. Morphological change due to effects of acute gamma ray on wishbone flower (*Torenia fournieri*) in vitro. *Inter Transac J Enginee Manage App Sci Tech.* **2** 375-384.

[30] Majeed A, Muhammad Z, Ahmad H and Khan A U R 2009. Gamma irradiation effects on some growth parameters of *Lepidium Sativum* L. *American-Eurasian J Sustain Agri.* **3** 424-427.

[31] 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).

[32] Yang P, Li C, Wei F, Huang W, Zheng J, Huang D, He R, Guo J, Huang X, Chen B and Lin W 1995. Radiation mutation induction in ‘China Tianbao’ banana. In: *In vitro mutation breeding of bananas and plantains*, Final reports of an FAO/IAEA co-ordinated research programme organized by the joint FAO/IAEA division of nuclear techniques in food and agriculture from 1988 to 1993, FAO/IAEA, Vienna, Austria pp 17–30.

[33] Gübbük H and Pekmezci M 2004. In vitro propagation of some new banana types (*Musa* spp.). *Turkish J Agric* **28** 355 – 361.

[34] Ngomuo M, Mnoney E and Ndakidemi P 2013. The effects of auxins and cytokinin on growth and development of (*Musa* sp.) var “Yangambi” explants in tissue culture. *Am J Plant Sci* **4** 174 – 2180.

[35] Suprasanna P, Sidha M, Ganapathi T R 2008. Characterization of radiation induced and tissue culture derived dwarf types in banana by using SCAR marker. *Aust J Crop Sci* **1** 2 47 – 52.

[36] Datta S K and Banerji B K 1995. Improvement of garden chrysanthemum through induced mutation. *Flora and Fauna.* **1** 1–4.
[37] Fereol L, Louis S and Luce A 2015. Effects of gamma radiation on in vitro plantlets of Alpinia purpurata. Int. J. Hortic. Sci. 71 2 243-247.
[38] Harb E M Z, Ahmed A H H, El-Shihy O M and Bayerly R M S 2005. Effect of gamma irradiation on increasing salinity tolerance of micropropagated banana plants. Bull. Faculty of Agriculture, Cairo Univ. 56 55–88.
[39] Suwarno A, Noor A H, Lina H 2013. Respon pertumbuhan plantlet anggrek Phalaeonopsis amabilis L. var. Jawa candiochid akibat radiasi sinar gamma. Unnes J Life Sci Indonesia 2 78 – 84.
[40] Predieri S 2001. Mutation induction and tissue culture in improving fruits. Plant Cell Tiss Org Cult 64 185 – 210.
[41] Hasbullah N A, Taha R M, Saleh A, Mahmad N 2012. Irradiation effect on in vitro organogenesis, callus growth and plantlet development of Gerbera jamesonii. Hortic. Bras. 30 1 – 6.
[42] Suprasanna P, Sidha M and Bapat V A 2009. Integrated approaches of mutagenesis and in vitro selection for crop improvement. In: Plant Tissue Culture and Molecular Markers: Their Roles in Improving Crop Productivity. Int. Pub. Hous, India pp 73-91.
[43] Ndzana X, Zok S, Sama A E 2008. Preliminary study on radiation sensitivity of in vitro cultures of Xanthosoma (macabo) in Cameroon. Plant Mutation Reports 2 10-12.
[44] Barakat M N, Abdel Fattah R S, Badr M and El-Torky M G 2010. In vitro mutagenesis and identification of new variants via RAPD markers for improving Chrysanthemum morifolium. Afr. J. Agric. Res. 5 748-757.
[45] Wi S G, Chung B Y and Kim J S 2007. Effects of gamma irradiation on morphological changes and biological responses in plants. Micron. 38 553-564.
[46] Karmarkar V M, Kulkarni V M, Suprasanna P, Bapat V A and Rao P S 2001. Radio-sensitivity of in vivo and in vitro cultures of banana cv. Basrai (AAA). Fruits 56 67 – 74.
[47] Kiong A, Ling A P, Grace Lai S H and Harun A R. 2008. Physiological responses of Orthosiphon stamineus plantlets to gamma irradiation. Am-Eurasian J. Sustain. Agric. 2 135-149.
[48] 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. 13 237-242.
[49] Astutik 2009. Peningkatan kualitas bibit pisang kapok melalui radiasi sinar gamma secara in vitro. Buana Sains 9 69 – 75.
[50] Murti R H, Kim H Y and Yeoung Y R 2013. Effectiveness of gamma ray irradiation and ethyl methane sulphonate on in vitro mutagenesis of strawberry. African J Biotech 12 4803-12.
[51] Kuksova V B, Piven N M and Gleba Y Y 1997. Somaclonal variation and in vitro induced mutagenesis in grapevine. Plant Cell. Tiss. Org. Cult. 49 17-27.