

# Effect of gamma irradiation on the morphological and physiological variation from *In vitro* individual shoot of banana cv. Tanduk (*Musa spp.*)

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**Abstract** Inducing genetic and morphological variation through conventional method is very difficult. Therefore, mutation induction through *in vitro* technology brings numerous advantages over the conventional breeding. Thus, the individual shoots (1 ~ 2 cm) were irradiated with gamma rays (10 ~ 70 Gy). The result revealed that the explants treated with higher doses (40, 50, 60, and 70 Gy) showed deleterious effects of ionizing radiation. The highest survival rate among  $\gamma$  treated explants recorded was 71% in 10 Gy treatments while the lowest survivality was 15% in 70 Gy. Lethal dose 50% (LD<sub>50</sub>) dose was found to be 33 Gy. In the *in vitro* condition, rooting reponse showed that increase in gamma irradiation dose resulted in the inhibition of root growth. Meanwhile, non-treated explants had the best rooting ability with the maximum number of root per explant (20) within a short period of time (6 days), with the highest root length of (15.1 cm). The longer period in rooting (12 days) and lowest number of root per explant (8) with shortest root length (10.1 cm) were recorded at 30 Gy treatment. The highest shoot length (13.6 cm) was observed at control treatment and the shortest shoot length (10.4 cm) was observed at 30 Gy. In the nursery, lowest leaf number (5) was observed at 30 Gy compared with other treatments. The highest chlorophyll content (49.8) was recorded at 10 Gy treated seedling. Irradiated explants with 10 Gy found to be superior over the control treatment and had positive effects in main growth parameters such as chlorophyll content.

**Keywords** Tanduk, LD<sub>50</sub>, mutation breeding, In vitro

## Introduction

Banana and plantains (*Musa spp.*) are important staple food crops for many people throughout the developing world and major source of nutrition for many people. Cultivar Tanduk is one of the popular banana varieties in Malaysia and widely cultivated by smallholders for production of banana used for the purpose of industrial processing. In banana production industry, the necessity to develop new commercially acceptable banana is still in a concern. Most of existing banana cultivars needs to be improved for some agronomical characteristics such as high yield, early flowering, better physical attributes and quality of fruit. However, it is not easy to fulfill the demands of both domestic and international markets without employing new approach. The conventional breeding of most commercially demanded banana is handicapped due to a complex genetic background, lack of seed formation, parthenocarpic nature and triploidy factors that hamper the improvement of this crop with the desired quality (Kumar, 2006). However, 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 conventional 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 (Javed et al. 2004). Through this technology a large number of improved mutant varieties have been released for commercial cultivation in many crops demonstrating the economic value of the mutation breeding technology (Kharkwal and Shu 2009; Jain and Suprasanna 2011).

Gamma rays are the most commonly used mutagenic agent in an *in vitro* mutagenesis experiment (Roux et al. 2004) since it has higher degree of accuracy, sufficient reproducibility and deep penetrating power into a biological matter that can cause a greater number of variation in physio-

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chemical composition (Yamaguchi, 2003). Biological effect of gamma radiation depends on atoms or molecules interaction inside the cell, especially water, to produce free radicals (Kovacs and Keresztes 2002). These radicals could harm or adjust main components of plant cells and disturb the physiochemical and biochemical process of plants depending on the radiation level (Ashraf et al., 2004). Gamma rays could restructure plant cell and metabolism include thylakoid membranes dilation, modification in photosynthesis, adjustment of the anti-oxidative structure, and accumulation of secondary metabolite (Ashraf et al. 2004; Kovacs and Keresztes 2002; Mbaye, 2017).

*In vitro* mutagenesis refers to mutations induced by treating explants or *in vitro* cultures with a mutagen, followed by mutant screening and characterization. This process involves a sequence of essential steps: choice of proper target plant material (explants or cultures); choice of a mutagen and determination of a suitable dose, post-treatment management and a series of subculture, and regeneration of plants for mutant selection. Mutagenic treatments can be applied to explants or *in vitro* cultures such as multiple shoot cultures, callus, cell suspensions, protoplasts and microspores and a variety of explants can be also used such as apical meristems, axillary buds, roots and tubers. In a principle, all kind of plant material used for mutagenic treatment, whether from explants or *in vitro* cultures are needed to undergo a series of subcultures after being mutagenized to separate chimeras and produce plants for selection of desired mutants since mutation remains undetectable by chimeras in the first generation (Banerjee et al. 2015).

However, in the recent studies by Karmarkar et al. (2001), who had made an attempt to induce mutation using *in vitro* individual shoot, the researcher had exposed *in vitro* individual shoot to gamma radiation followed by sub culturing on rooting medium without undertaking sub-culture processes to examine the post-irradiation rooting response in the laboratory condition. However, he could not go further and observe the explants response to gamma irradiation in the nursery stage. The present study was conducted to fill this gap. Thus, the individual shoot was singled out from multiple shoot culture and mutagenized with gamma radiation and then immediately transferred to rooting medium without undertaking sub-culture. The post-radiation rooting response and growth performance were studied in the laboratory condition and nursery stage. Hence, the present study was conducted to study the effect of different dose of gamma rays on the *in vitro* individual shoot growth performance under *in vitro* and nursery condition.

## Material and Methods

### Gamma exposure, culture condition and acclimatization of mutated plantlets

The sword sucker of banana cultivar Tanduk (25 ~ 35 cm) in height approximately 3 ~ 4 months old were used. Shoot tip culture were established and maintained until fourth ( $M_1V_4$ ) sub-culture cycle using MS medium supplemented with 5 mg/l BAP. Individual shoot (1 ~ 2 cm in length) were isolated and used as explant (Fig. 1). The roots, necrotic tissue and traces of solidifying agent were removed from the explant and then placed in the sterile Petri dish wetted with distilled water. The individual shoot in the Petri dishes were irradiated with different doses of gamma ( $\gamma$ ) rays (10, 20, 30, 40, 50, 60 and 70 Gy) using Cesium-137 source at a dose rate of 5.5 Grey/minute to study the radiosensitivity. The total samples of (140) individual shoot were irradiated and the final treatment were selected after this test. The treated explants were then washed thoroughly with sterilized distilled water prior to culture and then cultured aseptically on MS medium fortified with 2 mg/l activated charcoal to regenerate into complete plantlets. The basal medium contained inorganic salts of MS (Murashige and Skoog, 1962) plus Myo-inositol 100 mg/L, Nicotinic Acid 0.5 mg/L, Pyridoxine HCL 0.5mg/L, Thiamine HCl 0.5 mg/L, Glycine 2.00 mg/L and sucrose 30 g/L. The pH was adjusted to 5.7 prior to adding Agar (7 g/L). All cultures were incubated in the culture room at  $25 \pm 2^\circ\text{C}$  and 55 ~ 65% relative humidity with photoperiod of 16 hours light under fluorescent light (1000 lux) and 8 hours dark.



**Fig. 1** Individual shoot explant.

After 4 weeks, well grown rooted plantlets (7~9 cm in length) were carefully removed from the culture vessel and then the roots were washed thoroughly in tap water to remove the traces of nutrients and were transplanted in the greenhouse in polybags containing a ratio 2:1 mixture of peat moss and coco peat.

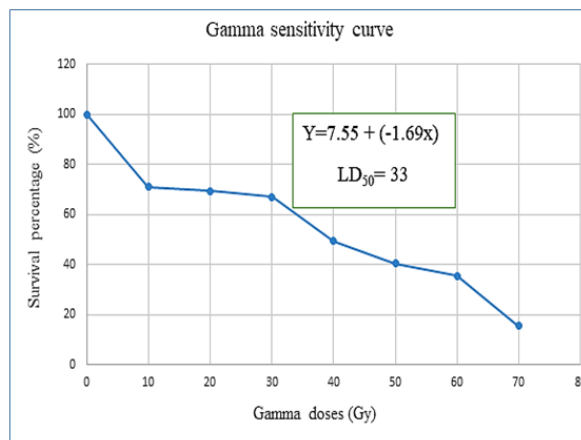
#### Experimental observation and statistical analysis

The LD<sub>50</sub> values were determined based on the percentage of surviving of explants. Post-irradiation rooting responses of *in vitro* individual shoots was determined by recording days to root initiation, root number, root length (cm) and shoot length (cm), during and after 30 days of treatment. In the case of hardened plants; plant height (cm), number of leaves and chlorophyll content by using (Minolta chlorophyll meter SPAD-502) (Spectrum Technologies, Inc., Plainfield, IL, U.S.) were measured. The experiment was laid out in a completely randomized design (CRD) with a 5 replication. The data were analyzed by one-way analysis of variance (ANOVA) with Duncan Multiple Range Test ( $p < 0.05$ ). The LD<sub>50</sub> calculation using survival rates (%) were analyzed by linear regression (Singh, 1990).

## Results and Discussion

#### Radiosensitivity and determination of lethal dose (LD<sub>50</sub>)

In the present study, the survival rate of irradiated explants was found to decrease linearly with the increasing of the gamma doses (Fig. 2). A remarkable decrease in survival rate was observed at 40, 50, 60 and 70 Gy of gamma dosage. Meanwhile, among the treated explants maximum survival rate of 71% was recorded at 10 Gy, followed by 69.5% at a dose of 20 Gy, 67 % at 30 Gy and minimum of survival rate was observed following the T7 (70 Gy) treatment, where remarkably dropped from 100% to 42% at the first week of culture and 15.5% at the fourth weeks



**Fig. 2** Radiosensitivity test curve illustrating the effect of increasing dose of gamma rays on survival rate of individual *in-vitro* shoot.

of culture resulting in a total reduction of 74.5%. The non-irradiated explant recorded the highest survival percentage of 100%. The lethal dose (LD<sub>50</sub>) was found to be 33 Gy based on the survival percentage, the estimation was carried out using linear regression analysis. Karmarkar et al. 2001 had made similar observations in cv. Basrai (AAA) which corroborate our finding. In principle, the most effective doses for mutation induction are thought to be the gamma doses lower than the LD<sub>50</sub>. As a result of these measurements, a large quantity of individual shoot was finally treated with 10, 20 and 30 Gy doses of gamma radiation and then the explants were transferred on rooting medium.

Effect of gamma irradiation on the growth of irradiated *in vitro* individual shoots under laboratory condition

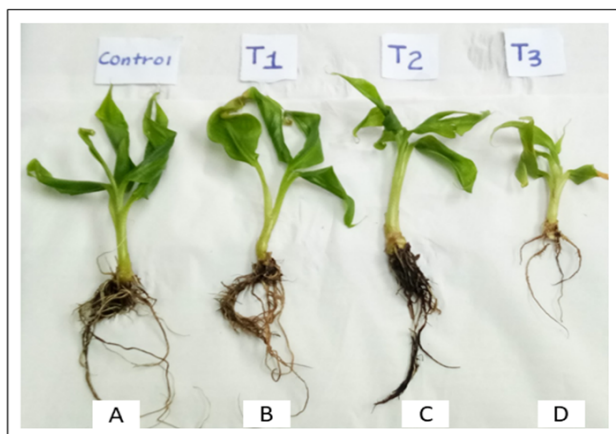
#### Effect on shoot elongation

The shoot length reduced prominently with increase in gamma irradiation doses (Table 1). The shoot length was significantly lower at higher dose (30 Gy), however lower gamma doses at 10 and 20 Gy showed no significance

**Table 1** Effect of gamma irradiation on the *in vitro* individual shoot of cultivar Tanduk after gamma irradiation

Gamma treatment	Growth parameter						
	Shoot length	Leaf number	Days to root initiation	Root number	Root length	Plant height	Chlorophyll 1 (SPAD)
T0: Control	13.6±0.6 <sup>a</sup>	7.01±0.5 <sup>a</sup>	7.2±0.3 <sup>a</sup>	20.02±1.4 <sup>a</sup>	15.1±0.5 <sup>a</sup>	27.8±1.7 <sup>a</sup>	44.2±2.1 <sup>b</sup>
T1: 10 Gy	12.7±0.1 <sup>ab</sup>	7.02±0.3 <sup>a</sup>	11.1±1.1 <sup>b</sup>	18.3±1.3 <sup>a</sup>	13.2±0.3 <sup>b</sup>	25.5±0.5 <sup>a</sup>	49.8±3.2 <sup>a</sup>
T2: 20 Gy	11.6±0.2 <sup>bc</sup>	5.00±0.6 <sup>ab</sup>	12.5±1.2 <sup>b</sup>	12.1±1.3 <sup>b</sup>	12.5±0.2 <sup>b</sup>	24.4±0.3 <sup>a</sup>	42.4±3.2 <sup>bc</sup>
T3: 30 Gy	10.4±0.2 <sup>c</sup>	4.99±0.4 <sup>c</sup>	12.3±2.5 <sup>b</sup>	8.2±0.40 <sup>c</sup>	10.1±0.4 <sup>c</sup>	21.2±0.4 <sup>b</sup>	39.6±3.0 <sup>c</sup>

Means sharing the same letters within a column are not significantly different from each other at  $P < 0.05$  after testing Duncan test.



**Fig. 3** Irradiated and rooted *in vitro* individual shoot explants 30 days after irradiation (A): Control, (B): 10 Gy, (C): 20 Gy and (D): 30 Gy

difference over the control. The highest shoot length (13.6 cm) was observed at control treatment, followed by 10 Gy (12.7 cm), 20 Gy (11.6 cm), and the shortest shoot length (10.4 cm) was observed at 30 Gy (Fig. 3). As reported by Majeed et al. (2009) and Khalil et al. (1986), reduction of a shoot and root growth at higher doses of gamma rays is due to reduced mitotic activity in meristematic tissues and reduced moisture contents of explants. In agreement with the present investigations, Al-Salhi et al. (2004) observed that decrease in shoot length is in proportion with increasing gamma dose in corn *Nahrung*. Similar conclusion by Wi et al. (2007) stated that the reason could be due to disruption of hormonal balance and enzymatic activities. A similar results have been reported by several researchers (Datta and Banerjee 1995; Fereol, 1996) on the inhibitory effects of high doses of gamma irradiation on shoot growth, reported that the inhibition might be due to turbulences in physio-biochemical processes related to the action of gibberellic acid, which typically stimulates cell division and cell elongation and higher dose kills meristematic cells, or damage cells producing fewer progeny cells and causing inability of irradiated tissues cells to absorb available nutrient, which leads to reduced growth.

#### *Effects on rooting*

Significant difference was observed in rooting of explants subjected to the higher dose of  $\gamma$  (30 Gy) (Table 1). It was observed that increase in gamma irradiation dose resulted inhibition of root growth. Non-treated explants had the best rooting ability with the maximum number of root per explant (20) within a short period of time (6 d), with the highest length of a root (15.1 cm). The longer period in



**Fig. 4** Acclimatizaion of irradiated plantlets

rooting (12 d) and lowest number of root per explant (8) with shortest root length (10.1cm) were recorded at 30 Gy treatment. Generally, the non-treated explants exhibited better rooting performance in all root growth parameter. This could be due to perturbations of hormones balance inside the explant and the activity of hormone synthesized and transported from tip to the site of action or exogenous supply of auxin used for rooting. The inhibition of rooting at higher gamma doses have been reported by several researchers (Karmarkar et al. 2001, Wi et al. 2007 and Kiong et al. 2008) who described that rise of radiation dose increases plants sensitivity to gamma rays effect and reduces the amount of endogenous growth regulators, particularly the auxin and cytokines. This would resulted breakdown, or lack of synthesis and or disruption of hormonal balance and enzymatic activities. In contrast, Muthusamy and Jayabalan, (2014) reported that the days to shoot and root initiation was faster when the explants exposed to gamma radiation at a lower dose in cotton seed.

Growth assessment of irradiated *in vitro* individual plantlets in the nursery condition

#### *Effect on leaf formation and plant height*

From the data obtained the leaf formation of irradiated plantlets with 10 Gy and 20 Gy was not significantly different over the control but treated explants with 30 Gy showed significant difference with that of control. The maximum number of leaves (7) was recorded at control plants. The lowest leaf number (5) was observed at 30 Gy compared with all treatments. Leaves number decreased in proportion with increasing gamma dose. All explants exposed to gamma dose showed a reducing trend in leaves

number and found to be inhibitory. From the data obtained, gamma radiation had no significant impact on leaf number in all treated explant rather it showed reduction trend in leaf number as the gamma dose increases. In contradiction with earlier findings, Yadav, (2016) had observed highly significant impact on leaf number in Cv. *decurrens* in a lower dose of gamma. However, our experiments are consistent with recent studies by Jala, (2011), who reported that higher concentration of gamma rays decreased the number of leaves in *Torenia fourmieri* plant. The data collected on plant height showed significance differences in plant height of banana seedlings subjected to higher  $\gamma$  dose (20 and 30 Gy). The average seedling height declined significantly from the control to the highest dose of  $\gamma$ . The tallest seedling observed was  $27.8 \pm 1.7$  cm in control, followed by  $25.5 \pm 0.5$  in 10 Gy,  $25.4 \pm 0.3$  in 20 Gy and  $21.2 \pm 0.4$ . Furthermore, Suprasanna et al. (2009) identify morphological variation resulted from mutagenic treatment in banana plant by some agronomic characters such as plant height and leaf shape and they observed dwarf banana plants derived from *in vitro* mutagenesis.

#### Effects on chlorophyll content

The effect of gamma irradiation on chlorophyll content showed 10 Gy showed significant difference over the control treatment (Table 1). The highest chlorophyll content (49.8) was recorded at 10 Gy treated plantlets, followed by 44.6, 42.4 and 39.6 in treatment 20 Gy, 30 Gy, and control respectively. Our experiments are consistent with recent studies by Sakr et al. (2013) stated that exposure of *Dracaena surculosa* plant to 10 Gy of gamma radiation gave the highest content of chlorophylls. In another study by Atak et al. (2007) reported that an increase in chlorophyll was observed in *Paulownia tomentosa* plants that were exposed to 20 Gy gamma irradiation, about 20.6% of chlorophyll content increased. Similarly, in our investigation, the chlorophyll contents of irradiated plantlets of were displayed a gradual increment at a low dose of gamma rays. The reason as reported by Ferreira-Castro et al. (2007) indicated that the chlorophyll level increases when the explants exposed to low levels of radiation as a result of an activated enzyme system. This would have enhanced photosynthetic rate and therefore gave larger yield in the field.

#### Conclusion

In conclusion, the result from the current experiment

indicated that  $\gamma$  treatments induced morphological and physiological variations on banana cultivar Pisang Tanduk. Lower dose of  $\gamma$  irradiation induced positive impact on the growth of *in vitro* plantlets during the multiplication phase and rooting stage, whereas higher  $\gamma$  irradiation had caused a negative impact on the growth, in terms of higher mortality rate and reduction in growth.

Therefore, we suggested that  $\gamma$  radiation can be usefully employed in banana for induction of desirable mutations and further, work on field performance of mutants, in terms of early flowering, yield quantity, quality and other agronomical traits need to be looking next.

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