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Induksi Mutasi Pisang Tanduk secara In vitro dan Deteksi Awal Ketahanan Tunas Varian terhadap *Fusarium oxysporum* f.sp *cubense*

In vitro Induce Mutation of Pisang Tanduk and Early Detection of Shoots Variant Resistances to Fusarium oxysporum f.sp. cubense

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ABSTRACT

Fusarium oxysporum f.sp. cubense (Foc) that causes Fusarium wilt disease is a significant problem in banana breeding. Control strategies against these pathogens can be carried out through mutation induction and in vitro selection to obtain banana cultivars resistant to disease. The objective of this study was to induce mutation of Pisang Tanduk and identify in vitro variants that are resistant to Foc. The explants of Pisang Tanduk were grown in Murashige and Skoog's media with the addition of 6.5 mg L^{-1} BAP, 1.175 mg L^{-1} IAA, and 0.22 mg L^{-1} TDZ for three months. The banana shoots were irradiated with gamma-ray at 0, 20, 30, 40, 50, and 60 Gy (Co-60). The experiment using CurveExpert 1.4 showed that a reduction of in vitro shoots growth of Pisang Tanduk of 20-50% (LD_{20-50}) is in the range of 30.64 - 68.66 Gy. After shoot proliferation and multiplication for six months, it showed that the highest number of in vitro shoots and leaves was made by 0 Gy. The highest number of meristem nodules was made by 40 Gy. The test results using the dual culture technique indicated that most of the shoots variants tested were susceptible to Foc. However, the putative mutant shoots from 30 Gy gamma irradiation were mildly resistant to Foc.

Keywords: dual culture, gamma irradiation, lethal dose, mutant putative

ABSTRAK

Fusarium oxysporum f.sp. *cubense (Foc)* penyebab penyakit layu Fusarium merupakan masalah utama dalam pemuliaan pisang. Strategi pengendalian terhadap patogen dapat dilakukan melalui teknik mutasi induksi dan seleksi *in vitro* untuk mendapatkan kultivar pisang resisten terhadap penyakit. Tujuan penelitian untuk menginduksi mutasi pisang Tanduk dan mengidentifikasi klon pisang yang resisten terhadap infeksi *F. oxysporum* f.sp *cubense* (*Foc*). Pisang Tanduk ditanam pada media MS dengan penambahan 6.5 mg L^{-1} BAP, 1.175 mg L^{-1} IAA, dan 0.22 mg L^{-1} TDZ selama tiga bulan. Biakan tunas pisang kemudian di iradiasi gamma pada 0, 20, 30, 40, 50, dan 60 Gy (Co-60). Hasil analisis dengan CurveExpert 1.4 diketahui bahwa reduksi pertumbuhan tunas pisang sebesar 20-50% (LD_{20-50}) berada pada kisaran 30.64-68.66 Gy. Hasil multiplikasi tunas setelah 6 bulan menunjukkan bahwa jumlah tunas dan daun terbanyak dihasilkan oleh eksplan yang tidak di iradiasi (0 Gy). Jumlah nodul meristem dan tunas terendah dihasilkan oleh eksplan hasil iradiasi gamma 60 Gy. Skrining awal ketahanan tunas melalui teknik kultur ganda secara *in vitro* menunjukkan bahwa sebagian besar tunas bersifat rentan terhadap cendawan *Foc*. Namun, ditemukan tunas mutan putatif dari hasil iradiasi gamma 30 Gy yang agak tahan terhadap *Foc*.

Kata kunci: dosis letal, iradiasi gamma, kultur ganda, mutan putatif.

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INTRODUCTION

Banana and plantain are fruit plants that are widely consumed in Indonesia. The potential for banana development in Indonesia is enormous, supported by the high genetic diversity of bananas because Indonesia is one of the centers of origin and source of banana evolution (Valmayor *et al.,* 2000). This potential allows Indonesia to breed and or select new cultivars according to market demand, and the potential for banana commodities as a substitute for staple food (Edison and Hermanto, 2016). Pisang Tanduk or Horn Plantain (AAB) (Valmayor *et al.* 2000) is a cooking type and seedless, so ripening fruit is often processed as a snack food, and unripped green fruit is used as a supplement for the primary raw material in making chips or bread. Pisang Tanduk has other names, i.e., Tinduk (Philippines), Tanduk (Malaysia), Byar (Indonesia), and Kluai Klain (Thailand) (Valmayor *et al.,* 2000). The characteristics of Pisang Tanduk include (1) long self-life of fruits, (2) it has low regeneration shoots, and (3) less fruit yield because one bunch only produces 1-3 hands. The number of hands of Pisang Tanduk is less compared to Kepok, which has 4-7 hands per bunch (Ambarita *et al.,* 2015).

The development of bananas naturally with superior agronomic characteristics and disease resistance has constraints because the commercial bananas are commonly triploid and sterile. Increasing plant diversity can be achieved by applying mutagenic agents where mutations occur as a result of ionizing radiation and non-ionizing radiation (Oladosua *et al.,* 2016; Spencer-Lopez *et al.,* 2018). The combination of mutation induction and *in vitro* culture techniques has improved the diversity of the vegetatively propagated plants (Jankowicz-Cieslak and Till, 2017). With vegetative propagation, recombination of various plant characters cannot be obtained, so mutation induction is a prospective way to obtain novelty in increasing plant diversity (Datta *et al.,* 2018).

The biological effects of gamma rays are closely related to the interactions between atoms and molecules in cells to produce free radicals (Spencer-Lopes *et al.,* 2018). Free radicals resulting from the breakdown of water molecules can damage or modify essential components of plant cells so that they can affect the nature and characteristics of plants (Mba *et al.,* 2010). *In vitro* mutation induction to obtain variants is an alternative method used in several banana types, including Cavendish (Ganapathi *et al.,* 2016), Rajabulu (Due *et al.,* 2019), Barangan (Indrayanti *et al.,* 2013; Hasim *et al*., 2020), Kepok (Masykuroh *et al.,* 2016), and Tanduk (Abdul Hafiz *et al.,* 2018). Furthermore, mutation induction in explants through *in vitro* techniques is a strategy to reduce chimeras' effect and obtain the correct protocol for producing the desired mutant plants (Jankowicz-Cieslak *et al.,* 2017), including plant resistant to pathogens.

The development of plants resistant to diseases can be carried out based on *in vitro* techniques and the selection of variants resulting from induced mutations: a specific pathogen, phytotoxins, or culture filtrate as a selective agent (Chandra *et al,* 2010; Jankowicz-Cieslak *et al.,* 2017; Reboucas *et al*., 2021). The objectives of this experiment were to determine the radiosensitivity of *in vitro* shoots of Pisang Tanduk against gamma irradiation and to initial screening of the shoot variants that are resistant to *F. oxysporum* f.sp *cubense* (*Foc*) through *in vitro* dual culture techniques.

MATERIALS AND METHODS

The explants of Pisang Tanduk were obtained from the Center for Seed Development and Plant Protection of DKI Jakarta. Aseptic banana shoots were cultured in nutrient mediums for three months to obtain sufficient banana shoots for mutation induction. The nutrient medium used for shoots multiplication was Murashige and Skoog (MS) salts supplemented with a 6-benzyl amino purine (BAP), 4.5 mg L^{-1}, indole3-acetic acid (IAA) 0.175 mg L^{-1}, thidiazuron (TDZ) 0.22 mg L^{-1}, myo-inositol 100 mg L^{-1}, nicotinic acid 0.5 mg L^{-1}, Pyridoxine HCl 0.5 mg L^{-1}, thiamine HCl 0.5 mg L^{-1}, glycine 2.0 mg L^{-1}. Sugar 30 g L-1 and commercial agar 7.8 g L^{-1}. The shoots resulting from the multiplication stage were separated and used as materials for mutation induction.

Mutation Induction and Shoot Multiplication of Pisang Tanduk

Induce mutation was carried out at the Isotope and Radiation Technology Application Center - BATAN, Jakarta. Shoot explants of Pisang Tanduk were irradiated at gamma rays (Cobalt-60): 0, 20, 30, 40, 50, and 60 Gy. This experiment used a Fully Randomized Design (CRD), with six treatments and five replications. Each replication consisted of 10-15 shoots. The irradiated shoots were grown in Murashige and Skoog medium supplemented with BAP, IAA, and TDZ. Data were observed on the number of shoots, nodules like-meristem, and leaves after gamma irradiation treatment.

Data standardization was carried out by counting the number of banana shoots that survived at each irradiation compared to the control number. Data was then processed using CurveExpert 1.4 to determine the radiosensitivity of Pisang Tanduk against gamma irradiation (Tabel 1). Finally, the surviving banana shoots and nodules were multiplied in the multiplication medium until 25 weeks after irradiation with five subcultures.

Isolation of *Foc* and early identification of *Foc* resistant using Dual Culture Method

Isolation of *F. oxysporum* f.sp *cubense* (*Foc*) was carried out from banana plants cv. Ampyang is showing Fusarium wilt symptoms. The banana pseudostem with approximately 5-10 cm of diameter was thinly sliced so that the yellow-brown or reddish-colored vessel threads were visible. The incision of the vessel thread was then covered by tissue paper until it was dry. Dry vessel threads were cut to a size of 0.5-1 cm in as many as 4-6 pieces and then grown into Potato Dextrose Agar (PDA) media, then incubated at room temperature for 2-3 days (Jumjunidang, 2012). The fungi colonies were then purified 4 (four) times to obtain a pure culture of *Foc* isolates.

RESULTS AND DISCUSSION

Mutation Induction of Pisang Tanduk with Gamma Irradiation

The effect of gamma irradiation dose on the explants was evaluated by determining the explants radiosensitivity. Radiosensitivity is a relative measure indicating the effect of gamma irradiation on irradiated plant explants such as shoots, embryos, and seeds (Mba *et al.* 2010; Kodym *et al.* 2012). In this experiment, the number of *in vitro* shoots of Pisang Tanduk that grew five weeks after irradiation (M_1V_1) varied from 51 to 96 (Table 1). Plant damage in M_1 generation indicates the mutagen effect in plants, which can be measured by reducing germination, growth, sterility, and mutated plant mortality (Shu *et al.* 2012). The analysis of the number of shoots that can survive at M_1V_1 obtained a linear equation $y = a + bx$ with data coefficient $a = 103.64$, $b = -0.78$. The equation obtained is $y = 103.64 - 0.78x$. It can be determined that the dose of gamma irradiation causes a reduction in the growth of shoots of Pisang Tanduk of 20-50% (LD_{20-50}) was in the range of 30.64 - 68.66 Gy, with 94.76 % ($r = 0.94$) of shoot mortality (Figure 1).

The lethal dose of gamma irradiation was higher than that of other banana plants. However, the range obtained was within the range of irradiation doses used for mutation induction in cultivated bananas. The results on other cultivar bananas found that LD_{50} of Pisang Tanduk was at an irradiation dose of 37 Gy (Abdulhafiz *et al.* (2018). Kepok was at an irradiation dose of 50.7 Gy (Masykuroh *et al.* 2016), Barangan at a dose of 46.1 Gy (Indrayanti *et al.* 2013) and 37 Gy (Hasim *et al*. 2020), Lakatan at a dose of 50 Gy (Sales *et al.* 2013).

The difference in plant tissue response to the dose of gamma irradiation is influenced by the genotype and type of plant, size and physiological conditions of the explants, growth phase, and plant morphology when irradiated (Mba *et al.* 2010; Kodym *et al.* 2012). Therefore, to obtain variants in a plant, the determination of lethal dose can be a factor that supports the success of the mutation induction treatment (Oladesua *et al.* 2016; Datta *et al.* 2018). This dose is the gamma irradiation dose that will produce the highest mutation frequency with maximum diversity and the minimum number of unexpected mutants (Albokari *et al.* 2012).

Higher doses will cause DNA damage (Kodym *et al.* 2012), while gamma irradiation lower than the LD_{50} can be used to induce plant variability (Jankowicz-Cieslak *et al.* 2017).

The frequency of mutations can increase with the increase in irradiation dose, but the ability to survive and regenerate will decrease. The correct irradiation dose range will result in the optimum mutation frequency with minimum damage (Mba *et al.* 2010; Albokari *et al.* 2012). At the optimum irradiation dose, it will produce mutants that can be identified as superior genotypes. The irradiation dose lower than the LD_{20} (30.46 Gy), resulted in rapid shoot growth and green color leaves. Increasing gamma irradiation dose tends to inhibit shoot growth. At high gamma irradiation doses (50 – 60 Gy), banana shoots also turned into browning and death (Figure 2).

The main target of gamma irradiation physiologically is water molecules present in plant tissues. The energy absorption from ionizing radiation induces changes in plants at the molecular level (Spencer-Lopez *et al.* 2018). The effects of irradiation involve two mechanisms: 1) direct (physical) effects, which affect molecular damage, and 2) indirectly (chemically) by the formation of free radicals originating from the ionization of water molecules. Free radicals can form hydrogen peroxide (H_2O_2), which can destroy cells and cause plant death. Irradiation in tissues with a high water content can increase the frequency of forming variants in plants (Mba *et al.* 2010).

a. Effect of gamma irradiation on nodules-likes meristem growth of Pisang Tanduk.

Mutations are genetic material changes that are not caused by recombinations and segregations. These genetic material changes could be observed in the plant phenotype (Forster and Shu, 2011). After being multiplied, this experiment showed that the irradiated explants of bananas develop the small round nodules (nodule-like meristem), which result from cell division. The number of nodules formed from unirradiated explants (0 Gy) to 50 Gy gamma irradiation significantly different from 60 Gy (Table 2). These results suggested that a high dose of gamma irradiation could inhibit cell growth activity.

The highest percentage of nodules growth (54.76%) was regenerated from unirradiated explants (0 Gy). The number of nodules-like meristems increased due to plant growth regulators added to the medium and the possibility of damaged cell recovery. Cytokinins and auxins in the culture medium enhance meristem cells' cell division and development. It causes tissue swelling and forms small meristematic protrusions on the explants produced by irradiation (Figure 3).

In this research, we carried out a phenotypic evaluation at 25 weeks after irradiation (M_1V_5) because the variations that occurred at five weeks after irradiation (M_1V_1) did not represent inherited DNA mutations yet. However, the evaluation of M_1V_1 should be carried out because it was faster and cheaper (Jankowicz-Cieslak *et al.* 2017). Still, the chances of recovery were higher, and mutations were lower (Sarsu *et al.* 2018). According to Datta *et al.* (2018) the genome sequence of Cavendish cv. Novaria showed that the putative mutant irradiated by 20 and 40 Gy experienced a recovery of 70% and 60%.

b. Effect of gamma irradiation on the growth of in vitro shoots of Pisang Tanduk

The effect of gamma irradiation significantly inhibited the growth of *in vitro* banana shoots. Un-irradiated explants (0 Gy) produced the largest average number of shoots (24.60 ± 1.12), while the lowest number of shoots were made from 60 Gy (Table 3).

In this experiment, a higher irradiation dose decreased the growth of shoots and the percentage of survival plants and resulted in slower shoot growth. Increasing the dose of gamma irradiation can also increase plant sensitivity and reduce the activity of endogenous hormones (Kiong *et al.* 2008). High sensitivity causes cell damage or disruption of hormone balance and enzymatic activity. These changes include changes in the accumulation of phenolic compounds and endogenous hormone activity inhibition, which plays a role in cell division and elongation (Jankowicz-Cieslak *et al.* 2017).

c. Effect of Gamma Irradiation on Banana cv. Tanduk Leaves

In this experiment, applying gamma irradiation caused leaf growth inhibition. As a result, the growing shoots and nodules take a long time to develop the new leaves. Un-irradiated (0 Gy) explants produced the average number of leaves (8.20 ± 2.01), and it was significantly different from other treatments (Table 4). Leaf growth from 50 Gy

was reduced (-25.0%) compared to the initial observation (M_1V_1). This result indicates that the irradiation causes damage to the shoot apical meristem tissue (SAM) so that the formed nodules cannot develop into shoots and leaves. The average number of *in vitro* leaves of Pisang Tanduk varied from other banana cultivars. The nodule-like meristem and shoots that have formed mainly did not grow to form leaves, especially explants that were obtained from irradiation above 40 Gy. According to Abdulhafiz *et al*. (2018), the highest number of *in vitro* leaves of Pisang Tanduk produced from an irradiation dose of 10 Gy, Kepok was at 60 Gy (Masykuroh *et al.* 2016), and Barangan was at un-irradiated explant (0 Gy) (Indrayanti *et al.* 2013).

In vitro Screening of Variant Resistance of Pisang Tanduk against *Foc*

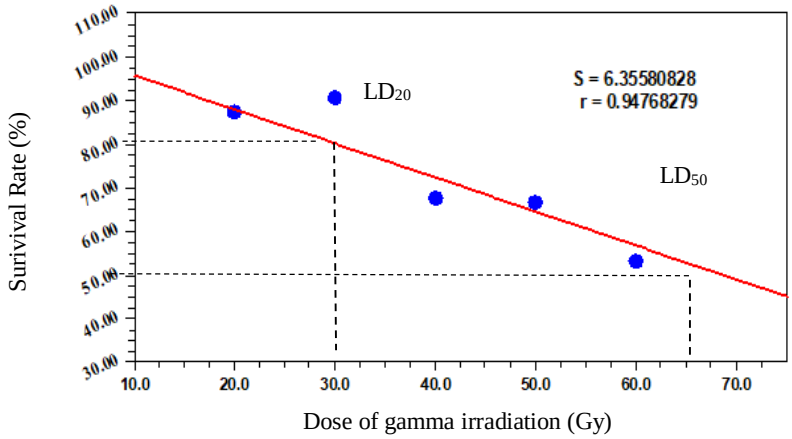
Banana and plantain are specific hosts of *F. oxysporum* f.sp *cubense* (*Foc*) and are known as one of the most destructive diseases (Ploetz, 2015; Rebouças *et al*. 2021). We carried out *Foc* isolation by growing a piece of pseudostem from a banana plant showing Fusarium wilt symptoms on a PDA medium. A characteristic yellowish-white fungi colony was identified as *Foc* fungi colonies, and microscopic identification showed that the conidia were crescent-shaped.

In vitro screening of banana variants using the dual culture technique showed that most banana shoot variants could survive seven days after infection. However, by 21 days, most of the infected shoots had died (Table 5). The period from the onset of banana shoots dead symptoms on Pisang Tanduk was faster than in Pisang Ampyang, which showed signs of necrosis and shoot death on average at 37.11 days after inoculation (Indrayanti and Sudarsono, 2013).

Table 1. An average number of shoots of Pisang Ampyang before irradiation (M_0V_0) and after being irradiated and proliferated for five weeks (M_1V_1).

The number of shoots	Dose of Gamma irradiation (Gy)					
	0	20	30	40	50	60
M_0V_0 (before irradiation)	76.00	74.00	70.00	76.00	76.00	70.00
M_1V_1 (after irradiation)	96.00	84.00	87.00	65.00	64.00	51.00
Standardization of shoot growth (%)	100.00	87.50	90.62	67.77	66.66	53.13

Noted: M0V0 = The number of shoots before irradiation. M1V1 =The number of shoots after irradiation in the first vegetative cycle.



Liniar Equations:
y = a + bx
Data Coefficient
a = 103.64
b = -0.78

Figure 1. Range of gamma irradiation dose that reduced the growth of banana shoots cv. Tanduk by 20-50% (LD_{20-50})

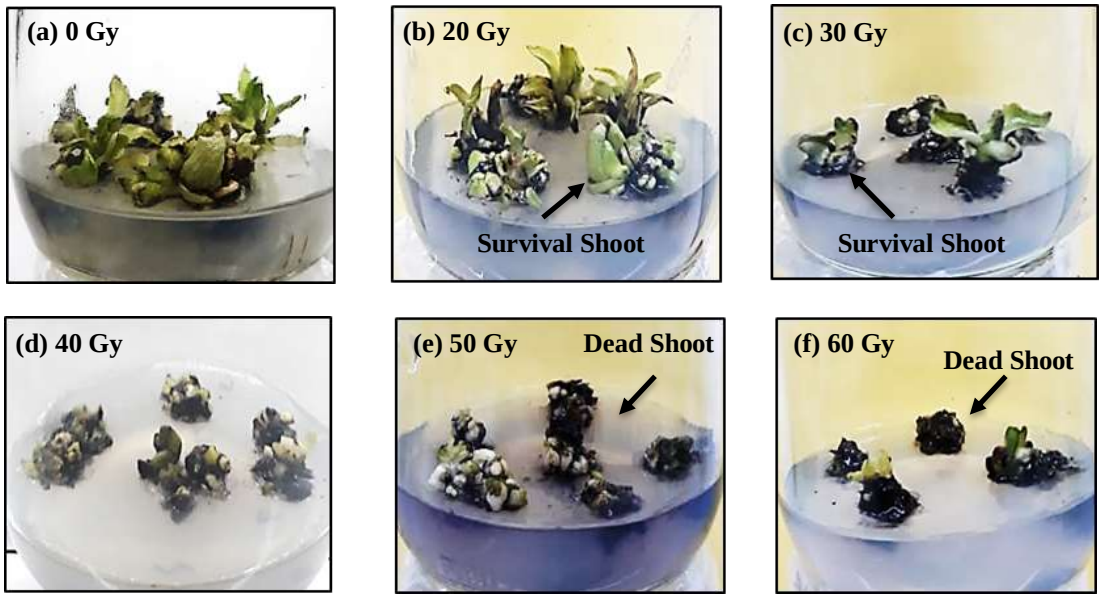


Figure 2. Representation of in vitro shoots of Pisang Tanduk regenerated from irradiated gamma dose 0, 20, 30, 40, 50, and 60 Gy (a-f) at 5 weeks after irradiation

Table 2. The average number of in vitro nodules-like meristem of Pisang Tanduk at 5 (M_1V_1) to 25 (M_1V_5) weeks after gamma irradiation.

Dose of irradiation (Gy)	5 weeks (M_1V_1)		10 weeks (M_1V_2)		15 weeks (M_1V_3)		20 weeks (M_1V_4)		25 weeks (M_1V_5)		Growth M_1V over M_1V_1 (%)
	Average	±SE	Average	±SE	Average	±SE	Average	±SE	Average	±SE	
0	8.40[a]	0.74	7.80[a]	0.58	12.60[a]	1.12	12.60[a]	0.97	13.00[a]	1.04	54.76
20	12.20[a]	2.35	11.40[a]	2.20	11.40[a]	2.21	11.80[a]	2.28	12.00[a]	2.16	-1.64
30	9.10[a]	2.50	8.40[a]	1.66	9.20[a]	1.42	9.80[a]	1.46	10.60[a]	1.32	16.48
40	9.60[a]	2.08	10.20[a]	2.08	12.01[a]	3.27	12.80[a]	3.39	13.20[a]	3.35	37.50
50	8.40[a]	3.18	9.40[a]	2.78	9.01[a]	3.14	11.10[a]	2.58	11.40[a]	2.46	35.71
60	3.40[b]	1.20	4.60[b]	1.20	4.20[b]	0.73	4.40[b]	0.97	4.60[b]	1.02	35.30

Note: * The percentage of nodule growth is calculated based on the average number of nodules in M1V5 - the average number of nodules in M1V1/ M1V1 x 100%. The numbers followed by the same letter in the same column are not significantly different based on the LSD test at the 5% level.

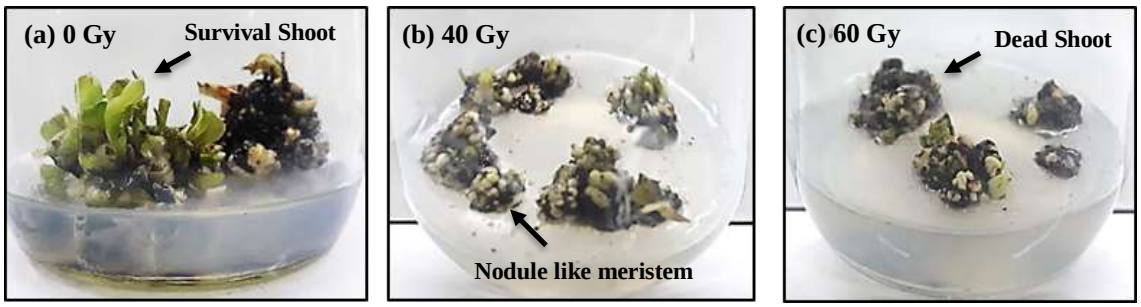


Figure 3. Representation of *in vitro* nodules-like meristem of Pisang Tanduk regenerated from irradiated gamma dose 0, 40 and 60 Gy (a-c) at 25 weeks after irradiation

Table 3. The average number of *in vitro* shoots of Pisang Tanduk at 5 (M_1V_1) to 25 (M_1V_5) weeks after gamma irradiation.

Dose of irradiation (Gy)	Average number of shoot	5 weeks (M_1V_1)		10 weeks (M_1V_2)		15 weeks (M_1V_3)		20 weeks (M_1V_4)		25 weeks (M_1V_5)	
		Average	±SE	Average	±SE	Average	±SE	Average	±SE	Average	±SE
0	15.20	19.20[a]	0.58	22.44[a]	0.80	23.60[a]	0.97	24.40[a]	1.20	24.60[a]	1.12
20	14.80	16.80[a]	1.24	18.10[b]	0.94	18.00[a]	1.22	18.20[a]	1.28	18.80[a]	1.46
30	14.00	17.40[a]	1.85	16.60[b]	1.85	15.20[a]	1.46	14.20[a]	1.39	14.60[a]	1.20
40	15.20	13.00[b]	1.35	10.80[b]	1.52	10.20[b]	1.46	10.60[b]	1.36	11.20[a]	1.31
50	15.20	12.80[b]	1.15	5.60[c]	0.87	5.40[c]	0.74	5.40[c]	0.74	6.00[b]	0.83
60	15.20	10.20[c]	0.87	3.10[c]	1.04	3.20[c]	1.20	3.40[c]	1.12	2.80[c]	1.01

Note: The numbers followed by the same letter in the same column are not significantly different based on the LSD test at the 5% level

Table 4. Growth of the average number of *in vitro* leaves of Pisang Tanduk Tanduk at 5 weeks after irradiation (M_1V_1) to 25 weeks after gamma irradiation (M_1V_5)

Dose of irradiation (Gy)	5 weeks (M_1V_1)		10 weeks (M_1V_2)		15 weeks (M_1V_3)		20 weeks (M_1V_4)		25 weeks (M_1V_5)		Growth M_1V_5 over M_1V_1 (%)
	Average	±SE	Average	±SE	Average	±SE	Average	±SE	Average	±SE	
0	4.40[a]	0.51	4.80[a]	0.58	5.20[a]	1.06	7.00[a]	1.34	8.20[a]	2.01	86.36
20	1.60[b]	0.51	0.60[b]	1.20	1.80[b]	0.58	2.00[b]	0.70	2.20[b]	0.66	37.50
30	1.40[b]	0.74	1.20[b]	0.37	1.20[bc]	0.37	1.20[b]	0.37	1.40[b]	0.40	0.00
40	0.60[b]	0.41	0.40[b]	0.20	0.60[c]	0.40	0.60[c]	0.40	0.60[c]	0.40	0.00
50	0.80[b]	0.48	0.60[b]	0.40	0.60[c]	0.40	0.60[c]	0.40	0.60[c]	0.40	-25.00
60	0.60	0.21	0.60[c]	0.20	0.60[c]	0.40	0.60[c]	0.58	0.60[c]	0.58	33.00

Note: * The percentage of nodule growth is calculated based on the average number of nodules in M_1V_5 - the average number of nodules in M_1V_1 / M_1V_1 x 100%. The numbers followed by the same letter in the same column are not significantly different based on the LSD test at the 5% level.

Table 5. The number and percentage of banana survival shoots. The scoring of damage symptoms to *in vitro* shoot variants of Pisang Tanduk on the medium containing the *Foc* fungus at 7-28 days after infection (DAI).

Dose of Irradiation (Gy)	N	7 DAI		14 DAI		21 DAI		28 DAI	
		the number and % of survival shoot	the score for symptoms	the number and % of survival shoot	the score for symptoms	the number and % of survival shoot	the score for symptoms	the number and % of survival shoot	the score for symptoms
0	24	6 (25.0)	3.1	5 (20.8)	3.4	4(16.6)	3.8	0 (0.0)	4.0
20	21	21 (100.0)	0.0	0 (0.0)	4.0	0 (0.0)	4.0	0 (0.0)	4.0
30	22	9 (40.9)	2.2	4 (18.1)	2.8	4(18.1)	3.2	1 (4.5)	3.2
40	23	5 (21.7)	3.3	2 (8.6)	3.9	0 (0.0)	4.0	0 (0.0)	4.0

Note : N = number of infected shoots. Scoring is base on symptoms of a disease. A score of 0 - healthy seeds and no signs of wilt; score 1 - the lower leaves turn slightly yellow and dry out; score 2 - an increase in the number of yellowing leaves and seedlings starting to wither; score 3 - all seedlings are dry except for new or unopened leaves; score 4 - dead seeds (Epp, 1987).

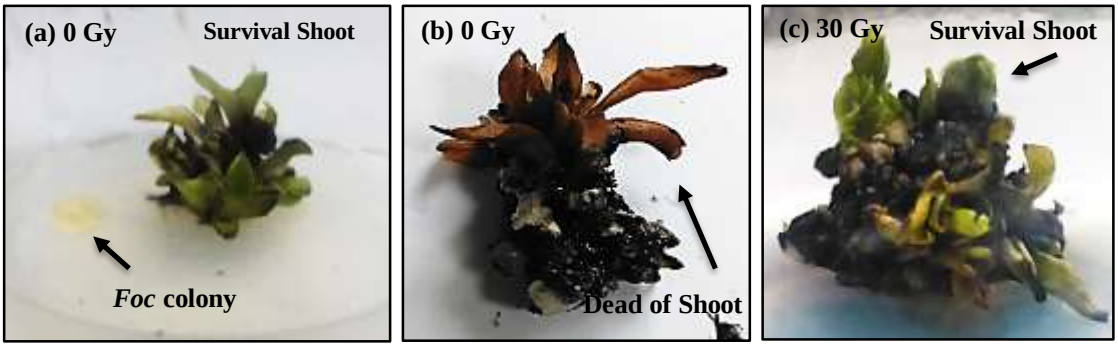


Figure 4. Representation of *in vitro* shoot variants of Pisang Tanduk regenerated from un-irradiated gamma (0 Gy) at one day and 28 days after inoculation (a-b) and regenerated from irradiated-gamma 30 Gy (c) at 21 days after infection.

In this study, initially, the *in vitro* shoots and meristem nodules variants of Pisang Tanduk were obtained from gamma irradiation of 0, 20, 30, 40, 50, and 60 Gy treatments. Then, after being multiplied for 25 weeks, the variant was identified for resistance to the *Foc*. This research showed that all variant shoots were susceptible to *Foc*, and the population of banana variants regenerated from the 30 Gy gamma irradiation treatment had lower symptoms than other treatments (Figure 4). Therefore, the shoot of 30 Gy irradiated variants identified as putative resistant to *Foc* infection has the potential to be re-tested for their resistance in the greenhouse to ensure the stability of their resistant properties. According to Ploetz (2015), the prospect of banana disease control depends on the availability of resistant cultivars.

CONCLUSION

The dose of gamma irradiation that can reduce shoot growth by 20-50% (LD_{20-50}) in Pisang Tanduk (AAB) was 30.64 - 68.66 Gy. The gamma irradiation dose resulted in various phenotypic characters in the number of nodules-like meristems, shoots, and leaves. The un-irradiated shoot explants (0 Gy) produced the highest number of *in vitro* shoots and leaves. *In vitro* shoot variants of Pisang Tanduk tested against *Foc* inoculum showed that one shoot of variants (4.5%) obtained from 30 Gy gamma irradiation treatment was putative resistant to *Foc* infection and had the potential to be re-tested for its resistance in greenhouses and on the field. This result also indicates that *in vitro* plant resistance screening can use dual culture methods for initial identification and screening of resistance of plant variants against pathogens. Furthermore, it is a simple method of identifying the putative mutant.

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