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ORIGINAL ARTICLE

Selection of banana mutants for Fusarium wilt resistance using *in vitro* and *in vivo* techniques with pathogen filtrate and conidia of *Fusarium*

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Abstract

Fusarium oxysporum f. sp. *cubense* (*Foc*) is the most destructive fungus that causes Fusarium wilt disease, which has led to the loss of some banana germplasm. This research aims to identify banana mutants (*Musa acuminata*, AAA) resistant to Fusarium wilt using both *in vitro* selection with culture fluid filtrate (CFF) and *in vivo* selection methods with conidia of *Foc Tropical Race 4* (*TR4*). Explants from in vitro shoot mutants, derived from gamma irradiation, were cultivated on Murashige and Skoog's media supplemented with increasing concentrations of Culture Fluid Filtrate of *Foc*, ranging from 40% to 60% (v/v). This study demonstrates that the technique effectively selected banana shoots that were insensitive to CFF from *Foc*. The presence of CFF as a selective agent reduced the growth of the banana shoots by 13.88% to 48.99%, depending on the gamma irradiation dose from which the variants originated. A correlation analysis revealed a strong negative relationship (R^2 = 0.70) between CFF concentration and the shoots and nodule-like meristems produced. In vivo selection using a conidial suspension of *Foc* TR4 at a concentration of 2.5×10^7 ml^{-1} resulted in 118 plants (23.7%) surviving. Evaluation of banana plants in the greenhouse 24 weeks after infection identified 28 plants (34.6%) from the selected mutans as potentially resistant to Fusarium wilt; most of these plants originated from gamma-irradiated explants at doses of 30 and 50 Gy. This study demonstrates that developing a banana mutant cultivar, 'Ampyang', which is resistant to Fusarium wilt, through gamma irradiation and both in vitro and in vivo selection, is a promising method for identifying resistant plants. However, further assessments via molecular characterization and field evaluations in disease-endemic areas are necessary to confirm the resistance of the obtained mutants.

Keywords: conidial suspension, culture fluid filtrate, *Foc*, induced mutation, *Musa acuminata* (AAA)

Introduction

Bananas and plantains (*Musa* spp.) are among the world's most important cash crops and staple foods, alongside rice, wheat, and maize (Tripathi *et al.* 2008; Chong and Santos-Ordóñez 2020). The familiar eating banana is a naturally occurring hybrid among the nine subspecies of *M. acuminata* (A genome), and an interspecific hybrid between *M. acuminata* (A genome) and *M. balbisiana* (B genome) (Valmayor *et al.* 2001; Ploetz *et al.* 2007). In Indonesia, banana production was 9.686.599,45 tons in 2024 (BPS-SI 2025), representing a substantial increase of 3.7% compared to the previous year. This crop is a nationally important commodity. However, the vegetative propagation method using suckers is complicated for producing high-quality seeds and increases susceptibility to diseases such as Fusarium and bacterial wilt (FAO 2022). Banana crops are significantly affected by several diseases, with notable threats including Fusarium wilt (Tropical Race 4),

Sigatoka leaf diseases, and Xanthomonas wilt. These diseases pose serious risks to global food security (Ploetz 2015; Carlier *et al.* 2024; Munhoz *et al.* 2024).

The soil fungus *Fusarium oxysporum* Schlecht causes Fusarium wilt. Its forma specialis *cubense* (E.F. Smith) Snyder and Hansen (*Foc*) presents a significant challenge in banana breeding (Moore *et al.* 2001; Ploetz 2015). This plant pathogen attacks the vascular tissue and is one of the most destructive threats to bananas and plantains. The fungus was first reported in Indonesia in 1916 on a banana plantation in Java (Nasir *et al.* 2003). There is a concern that Fusarium wilt may become epidemic across plantations in Sumatra and Indonesia (Moore *et al.* 2001), with a spread rate of 32.2% within four years (Lee *et al.* 2001). Scheerer *et al.* (2016) have projected that by 2028, banana production areas will decline across several countries, with Indonesia expected to experience an 8% reduction. Recent studies in Asia have indicated that *Foc* Tropical Race 4 is the predominant strain in tropical and subtropical regions, affecting Cavendish and many other banana cultivars (Dita *et al.* 2020).

Control of Fusarium wilt in bananas can be achieved through chemical fumigants, crop rotation, or the addition of organic substances to enhance soil chemical properties. However, these methods often prove to be neither efficient nor cost-effective without significant changes in strategy (Li *et al.* 2013; Ordonez *et al.* 2015), primarily due to the persistence of the *Foc* pathogen in the soil (Ploetz and Churchill 2011). Research has shown that certain banana cultivars are resistant to the *Foc* Tropical Race 4, and has identified important pathogenesis-related proteins that induce plant defense mechanisms (Dale *et al.* 2017; Huo *et al.* 2025). Transferring resistance genes from these resistant cultivars into susceptible varieties remains a challenge. Furthermore, evaluations are needed to assess the potential negative impacts of transgenes on banana quality, environmental effects, and the biosafety of transgenic bananas (Poon and Teo 2019).

Conventional breeding strategies for bananas encounter significant challenges due to the triploid nature of most cultivars, which results in high levels of male and female sterility (Hwang and Ko 2004; Dale *et al.* 2017). This reproductive limitation has hindered efforts to develop improved cultivars through traditional methods, highlighting the urgent need for disease-resistant banana varieties (Ordonez *et al.* 2015; Ploetz 2015; Dale *et al.* 2017; Poon and Teo 2019). Biotechnological approaches, particularly *in vitro* mutation breeding and in vitro selection, are effective alternatives for producing plants resistant to Fusarium wilt. This process involves using pathogen culture filtrates (Mak *et al.* 2004; Saraswathi *et al.* 2016; García-Velasco *et al.* 2020; Izurdiaga *et al.* 2024), fusaric acid, a nonspecific toxins produced by various species within the genus Fusarium (Rebouças *et al.* 2021). Other methods include the application of Fusarium-derived elicitors to stimulate the production of defense-related enzymes in bananas (Thakker *et al.* 2011), in vitro dual technique involving pathogen mycelium (Nawrot-Chorabik 2013; Indrayanti *et al.* 2021), and conidial suspension infections (Ferreira *et al.* 2024; Indrayanti *et al.* 2024).

Culture fluid filtrates of *F. oxysporum* have been widely used to assess resistance in various banana cultivars, including 'Rastali' and 'Barangan' (Mak *et al.* 2004), 'Cavendish' (Hwang and Ko 2004), 'Rasthali' (Saraswathi *et al.* 2016.), and 'Gros Michel' and 'Grande Naine' (Portal *et al.* 2018). 'Pisang Lilin' and 'Paka' (García-Velasco *et al.* 2020). Additionally, culture filtrates of *F. oxysporum* of other formae speciales have been applied to screen for Fusarium wilt resistance in other plant species, such as carnation (Thakur *et al.* 2002), vanilla (Ramírez-Mosqueda *et al.* 2015), and brassica vegetables (Abeyawardana and Koudela 2019).

The potential mutants obtained from *in vitro* selection need to be reassessed for their resistance by transferring the plantlets to a greenhouse that contains various biotic and abiotic interactions (Soumare *et al.* 2021). Pathogens can enter the cell either through physical wounds on the plant or without any such injuries (Pegg *et al.* 2019; Duong *et al.* 2024; Santos *et al.* 2024). Methods that injure plant tissue facilitate spore entry through pinprick techniques with pins or microinjections (Kumari *et al.* 2017; Indrayanti *et al.* 2024) and inoculation by dipping the roots (Singh *et al.* 2018). Employing spores or conidia ensures that plants are exposed directly to the pathogen's infectious structures, making it possible to observe the actual host-pathogen interactions and resistance mechanisms.

The present study aims to identify banana variants resistant to Fusarium wilt using a combination of *in vitro* and *in vivo* selection methods involving *Foc* culture filtrate and conidial suspensions.

Material and Methods

Plant materials and fungal strains

Banana shoot variants of the cultivar 'Ampyang' (*M. acuminata*, AAA) regenerated from induced mutations 20–60 Gy collected from the Plant Tissue Culture Laboratory, UNJ, were used as a source of explants. The fungal pathogen *F. oxysporum* f. sp. *cubense* (*Foc*) isolate from Banyuwangi (Bw) was obtained from the Indonesian Agricultural Assemblies and Testing Institute for Sweeteners and Fiber Crops, Malang. *Foc* isolates from Medan Vegetative Compatibility Group (VCG) 01213/16 – Tropical Race 4 (TR4) were obtained from

the Indonesian Agricultural Assemblies and Testing Institute for Tropical Fruits, Solok, Indonesia.

Preparation of culture fluid filtrates and conidial suspension of *Foc*

Fusarium oxysporum f. sp. cubense (Foc) isolates from Banyuwangi and Medan VCG 01213/16 TR4 were cultured on Potato Dextrose Agar (PDA) for 7 to 14 days at an incubation temperature of 28–30°C. The crude extract of the culture fluid filtrate (CFF) was prepared by placing three pieces of well-grown mycelium of *Foc* (with a diameter of 0.5 cm) on the surface of 100 ml of Potato Dextrose Broth. The culture filtrates were then incubated at 28°C ± 2°C on a shaker set to 60 rpm. After 21 days of incubation, the mycelium and spores that floated to the surface were removed by filtering through a 0.22 µm filter paper. The resulting CFF was sterilized at 121°C for 20 minutes and stored in the dark at 4°C until use as a selective agent. For in vivo selection, the *Foc* VCG 01213/16 CFF without sterilization was adjusted to 2.5×10^7 conidia · ml^{-1} using a hemocytometer Leavy.

In vitro selection of banana shoot variant using CFF of *Foc*

The *in vitro* selection of banana shoot variants was conducted by culturing these variants in Murashige and Skoog (MS) nutrient solution containing 30% (v/v) of Culture Fluid Filtrate (CFF) from *F. oxysporum* f. sp. *cubense* (*Foc*). This experiment used a completely randomized design (CRD), with seven-dose irradiation treatments; each treatment included 10 replicates. The total number of shoots per treatment ranged from 180 to 300. Surviving shoots were subcultured every 5 to 6 weeks, with the concentration of the culture filtrate gradually increased to 40%, 50%, and finally 60% (v/v). Shoots were maintained in a culture room at 25 ± 2°C, under a 16-hour photoperiod with 1000 lux LED light intensity.

The shoots that survived in the selective media containing 60% *Foc* CFF were identified as banana variants exhibiting insensitivity to the filtrate. These variants were then regenerated for 8 weeks on a rooting medium composed of MS medium, supplemented with 6-benzyladenine (2.25 mgl^{-1}) and indole-3-acetic acid (0.175 mgl^{-1}), before being transplanted into soil. Observations included the average number of roots, root length, and survival rate. The plantlets were acclimatized in a greenhouse for in vivo selection. The percentage of surviving banana shoots insensitive to 60% of CFF *Foc* was calculated as:

Survival (%) = the number of plants alive after acclimatization at irradiation treatment/number of plants acclimatized × 100%.

Acclimatization and *in vivo* selection using conidial suspension of *Foc*

Banana plantlets were transplanted and grown in a greenhouse, where only those that survived eight weeks post-acclimatization were selected for resistance screening against Fusarium wilt. The surviving variants were carefully uprooted, rinsed under running tap water, and surface-sterilized using a 5% sodium hypochlorite (NaOCl) solution for 1–2 minutes. Root tissues were then cut into 2–3 cm segments from the base and inoculated by immersing them in a conidial suspension of *Foc* VCG 01213/16 (2.5×10^7 conidia · ml^{-1}) from the Medan isolate. After a two-hour soaking period, the clones were transferred into 300 ml plastic cups containing a nutrient-rich soil medium for fruit production. The inoculated plants were maintained under greenhouse conditions for an additional eight weeks, after which they were transferred to polybags (15 × 20 cm) for continued observation and evaluation.

Observations of banana plants focused on several parameters, including the percentage of dead plants, root length (in cm), leaf symptoms, and rhizome discoloration. Disease severity was evaluated using the Leaf Symptom Index (LSI) and the Rhizome Discoloration Index (RDI), which together provided a comprehensive assessment of Fusarium wilt damage. The LSI measures foliar disease severity on a scale from 0 to 4, based on the degree of yellowing and wilting observed in the foliage (Epp 1987; Mak *et al.* 2004). The Rhizome Discoloration Index (RDI) is based on the extent of internal tissue damage and was assessed by transversely slicing the rhizome and observing the degree of discoloration (Carlier *et al.* 2003).

Disease incidence and severity were used to calculate the Disease Intensity Index (DII), quantifying the average disease severity across the plant population. The DII was calculated using the formula:

$$DII = [\Sigma(Si \times Ni) / (S \times Nt)] \times 100\%,$$

where: Si – symptoms severity; Ni – number of plants with Si symptom severity; S – the maximum possible severity score and Nt – total number of plants assessed. Thus, DII indicated the average level of disease intensity at a specific time as a ratio of the highest potential disease level (Hervás *et al.* 1997). Plants that survived the in vivo screening were monitored for their growth and development for up to 24 weeks after infection under greenhouse conditions. The survival percentage was calculated by the number of survived plants at a given radiation dose / the number of infected plants × 100%.

Results

In vitro selection of banana shoot variants using CFF of *Foc*

The growth performance of banana shoot variants was assessed over 24 weeks. The effect of increasing the concentration on in vitro selection of banana mutants in media containing culture fluid filtrate (CFF) of *Foc* as a selective agent showed that shoots exposed to 50% and 60% culture fluid filtrate (CFF) experienced inhibited growth (Table 1). Banana shoot variants growing at 60% CFF, shoot growth decreased by 13.88% to 48.99% compared to the 30% CFF treatment. Variants from 40 Gy and 50 Gy irradiation showed the greatest suppression in the 60% CFF medium, averaging only 8.68 to 10.35 shoots and nodule-like meristems per explant (Table 1). The surviving variants were identified as insensitive to *Foc* CFF, with a correlation analysis showing a strong negative relationship ($R^2 = 0.70$) between CFF concentration and shoot and nodule-like meristem production during subculturing. Images of both insensitive and sensitive variants are shown in Figure 1.

Acclimatization and *in vivo* selection using conidial suspension of *Foc*

The acclimatization of banana plantlets insensitive to *Foc* resulted in a survival rate of between 59.7% and 75.3% at three weeks after acclimatization (Table 2). However, this rate dropped significantly to 3.0% and 56.2% after eight weeks. Plantlets treated with gamma irradiation at 20 Gy and 35 Gy exhibited very low survival rates of 3.0% and 3.9%, respectively. In contrast, the 30 Gy irradiation treatment showed the highest survival rate and the longest roots, measuring 16.0 cm (Table 2). Only 118 out of 499 acclimatized plantlets (23.7%) survived after eight weeks. Additionally, a sensitive plant regenerated from 25 Gy gamma irradiation displayed severely wilted leaves and Fusarium fungal colonies in the soil, as shown in Fig. 2A. Furthermore, for in vitro screening, we only assessed 77 banana plants insensitive to the CFF by dipping roots in a conidial suspension of *Foc* isolates Medan VCG 01213/16 and growing them under greenhouse conditions.

Characteristics of banana plants after eight weeks of in vivo selection revealed different disease responses based on the radiation doses applied (Table 3). The

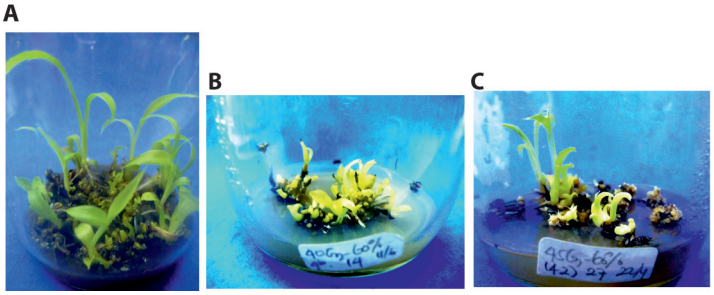

Fig. 1. *In vitro* selection of banana mutants in media containing culture filtrate. The banana shoots mutants that showed insensitivity to the CFF of *Foc* up to 60% derived from doses of (A) 30 Gy, (B) 40 Gy, (C) 45 Gy

Table 1. The effect of increasing the concentration on *in vitro* selection of banana mutants in media containing culture fluid filtrate (CFF) of Foc as a selective agent on inhibiting the number of shoots and nodule like-meristem per explant

Variant regenerated from [Gy]	N	Average No. of shoots and nodule like-meristem growing on CFF Foc at concentration				R Square	Growth 60% over 30% [%]
		30%	40%	50%	60%		
20	270	17.87	21.87	16.29	12.87	0.506	(–27.97)
25	250	13.91	17.23	16.06	11.31	0.249	(–18.69)
30	300	10.72	15.11	10.78	6.68	0.380	(–37.68)
35	180	7.92	8.76	7.41	6.82	0.533	(–13.88)
40	300	16.61	16.84	15.71	9.57	0.696	(–42.38)
45	270	16.96	15.36	12.00	8.68	0.977	(–48.99)
50	304	19.63	15.10	14.39	10.35	0.939	(–47.27)
Average		14.54	15.75	13.23	9.35	0.707	

Note: N – the number of shoots selected for each treatment

Table 2. Acclimatization of banana variants insensitive to the CFF of *Foc* at 3 and 8 weeks after in a greenhouset

Plantlet regenerated from [Gy]	No. of plantlets acclimatized	Avg. No. of root	Avg. root length [cm]	No. and % of surviving mutants Insensitive to CFF *Foc* at	
				3 weeks	8 weeks
20	67	9.2 bc	8.2 bc	40 (59.7)*	2 (3.0)**
25	99	10.2 ab	11.1 ab	60 (60.6)	13 (13.1)
30	73	9.5 a	16.0 a	55 (75.3)	41 (56.2)
35	26	7.5 c	7.96 c	16 (61.5)	1 (3.9)
40	87	8.1 a	15.70 a	55 (63.2)	16 (18.4)
45	73	9.5 a	13.53 a	50 (68.5)	23 (31.5)
50	74	9.7 b	10.11 b	45 (60.8)	22 (29.7)
Total	499 plantlet			321 plants	118 plants

Fig. 2. Banana mutants regenerated from gamma irradiation and *in vitro* selection from (A) 25 Gy irradiation, (B) 30 Gy, and (C) 25 Gy, observed 8 weeks after acclimatization in a greenhouse

Table 3. Characteristics of banana plants after eight weeks of *in vivo* selection using a conidial suspension of *Foc* isolates Medan VCG 01213/16 (TR4) in a greenhouse

Plant reg. from [Gy]	No. of i-infected plants	No. dead plants [%]	Root length [cm]	LDI	Leaf DII [%]	RDI	Rhizome DII [%]
20	1	1 (100.0)*	14.00 ± 0.00	4.00	100.0	5.00	100.0
25	9	8 (88.8)	8.67 ± 3.84	3.56	88.9	4.11	77.8
30	27	6 (22.2)	23.59 ± 2.37	1.56	38.9	2.59	64.8
35	1	1 (100.0)	19.50 ± 0.00	4.00	100.0	5.00	100.0
40	11	8 (72.7)	11.59 ± 2.70	3.36	84.1	3.82	76.4
45	12	7 (58.3)	15.06 ± 3.22	2.75	68.8	3.75	75.0
50	16	6 (37.5)	17.13 ± 3.08	1.75	43.8	2.75	55.0

Note: *the number of plants that survived eight weeks after inoculating / the number of plants infected of *Foc* × 100%. LDI – Leaf Symptom Index, RDI – Rhizome Symptom Index, DII – Disease Intensity Index

highest mortality rates of 100% were observed in the 20 Gy and 35 Gy treatments, with severe symptoms indicated by high Leaf Symptom Index (LSI) and Rhizome Discoloration Index (RDI) values, both at their maximum severity (Table 3; Fig. 3D-E and 3J-K). Other doses, such as 25 Gy and 40 Gy, also exhibited susceptibility, with mortality rates of 88.8% and 72.7% for leaf and rhizome, respectively. On the other hand, plants treated with 30 Gy exhibited the lowest mortality at 22.2%, the longest average root length of 23.59 cm, and relatively low LSI and RDI values, indicating some degree of disease resistance. These plants displayed fewer wilting symptoms and healthier root growth. The 50 Gy plants showed moderate resistance, with a 37.5% mortality rate and moderate disease symptoms; however, their average root length was shorter than that of the 30 Gy-treated plants, as shown in Table 3.

Microscopic observations of banana root cross-sections revealed differences between healthy and infected plants. Healthy roots were green with numerous fibrous or hair-like roots (Fig. 4A), while infected roots exhibited brown discoloration in the epidermis and xylem, lacking the hairy roots (Fig. 4B). The xylem tissue of infected roots appeared maroon under fluorescence microscopy (Fig. 4C), which corresponds to the coloration of the *Foc* culture filtrate, as illustrated by the maroon color of the *Foc* TR4 CFF (Fig. 4D).

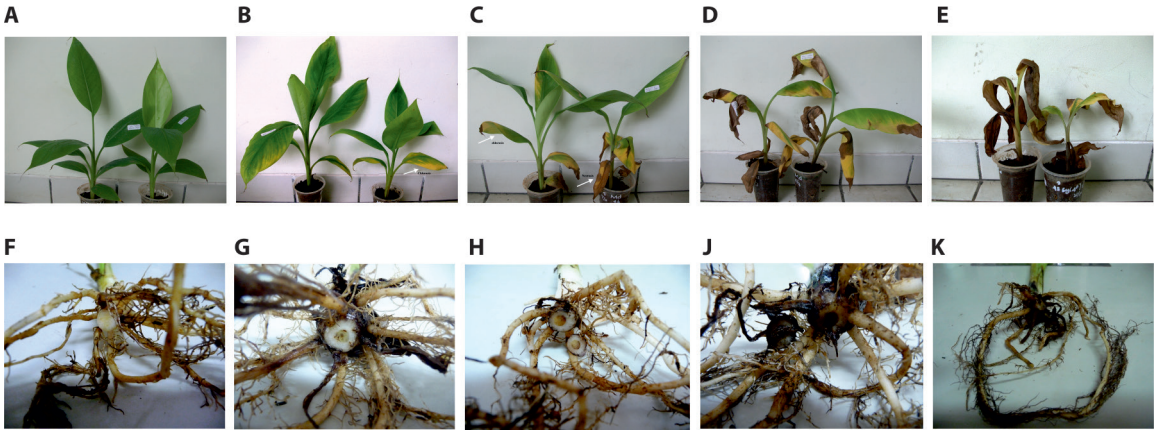

Fig. 3. *In vivo* selection using conidia of *Foc* TR4. Banana leaves showing yellowing and wilting leaf symptom (score 0–4) (A–E), Rhizome and root discoloration of banana at 4–5 weeks after infection with a conidial suspension of *Foc* isolates Medan VCG 01213/16 (Score 0–5) (F–K)

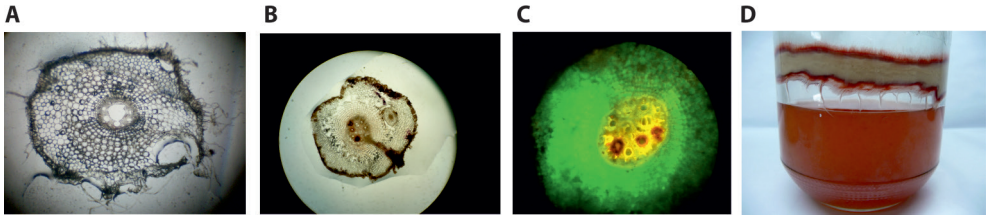

Fig. 4. Cross-section of banana root (A) healthy roots and (B) infected root, as observed under a light microscope, (C) the xylem tissue of infected roots is maroon-red, as seen using a fluorescent microscope at 40x magnification, (D) the maroon-red colour of CFF *Foc* TR4

Evaluation of survival plant at greenhouse

The evaluation of banana plant regeneration after 24 weeks of infection showed variable survival and root growth responses among plantlets derived from different gamma irradiation doses (see Table 4). Plantlets derived from gamma irradiation with 30 Gy exhibited the highest survival percentage (15.58%) with 12 surviving plants and an average root length ranging from 18.5 to 42.0 cm. Several of these plantlets (codes 3.31 and 3.35) showed recovery by producing new leaves and no longer exhibited signs of wilting, suggesting partial resistance and adaptive regeneration under infection stress. The 50 Gy treatment produced a relatively high number of survivors (10 plants, 12.98%), with root lengths ranging from 14.0 to 40.0 cm, indicating stress-induced variability. On the other hand, lower irradiation doses (25 Gy and 40–45 Gy) resulted in fewer survivors, with only 2 to 3 plants per treatment. Banana mutants regenerated from 40 Gy showed moderate survival (3.89%). The 25 Gy treatment resulted in the fewest survivors (1.59%), with only one plant surviving out of nine.

Further observation of the banana variants after infection with the conidial suspension of *Foc* from the Medan isolate VCG 01213/16 showed promising results: 28 out of 77 selected plants (36.4%) were predicted as putative mutants resistant to Fusarium wilt. This resistance was achieved through strategic regeneration from various doses of gamma irradiation, in vitro selection, and in vivo selection, as follows: 30 Gy of gamma irradiation (12 plants), 50 Gy (10 plants), 40 Gy (3 plants), 45 Gy (2 plants), 25 Gy (1 plant) (see Table 4). Representative figures of banana resistance to *Foc* VCG 01213/16 are seen in Figure 5.

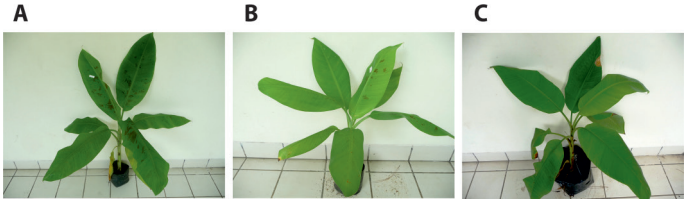

Fig. 5. Representative figures of banana variant resistance to *Foc* VCG 01213/16 from Medan isolates at 24 weeks after infection: (A) 25 Gy. 4.31. and (B) 30 Gy 3.32 an (C) 50 Gy 3.01

Table 4. Evaluation of banana plant growth in the greenhouse at 24 weeks after infection

Plant regenerated from [Gy]	No. of infected plants	No of surviving plants	Surviving plant code	Root length [cm]	LSI	RDI	% of Plant survived
25	9	1	4.31	37.5	0	1	(1.29)*
30	27	12	3.08	39.0	0	0	(15.58)
			3.16	28.5	0	3	
			3.17	38.5	0	2	
			3.18	24.0	1	1	
			3.19	27.5	0	2	
			3.20	20.0	0	1	
			3.23	18.5	1	1	
			3.24	42.0	1	2	
			3.28	26.0	0	1	
			3.31 (*recov.*)	18.5	3	2	
			3.35 *(recov.)*	32.5	2	2	
			3.36	33.0	1	1	
40	11	3	4.15 (*recov.*)	13.0	4	5	(3.89)
			4.16	21.5	1	2	
			4.32	32.0	1	2	
45	12	2	4.20	12.0	4	4	(2.59)
			4.35	19.5	1	2	
50	16	10	1.18	37.0	1	2	(12.98)
			1.19	22.0	0	1	
			3.01	21.0	1	2	
			3.04	16.5	1	1	
			3.05	14.0	2	3	
			3.08	20.0	0	1	
			3.09	19.5	1	1	
			3.12	40.0	0	1	
			3.14	31.0	1	3	
			3.24	20.5	0	2	
Total	77	28	(36.4%)				

Discussion

In vitro selection using the culture fluid filtrate (CFF) from *Foc* allowed effective selection of banana shoot variants insensitive to this filtrate. Banana variants at doses of 20, 25, and 35 Gy showed a moderate decrease in growth and better survival rates. In contrast, higher doses of gamma irradiation, specifically 30, 40, and 50 Gy, significantly reduced growth and increased mortality rates (see Table 1 and Fig. 1). This indicates that CFF at concentrations of 30% to 60% could select banana shoots that were insensitive to culture filtrate. However, further evaluation is necessary to confirm that the resulting mutants are stable. The survival of shoots suggests that mutagenic treatment at this dose may be sufficient to induce resistance to *Foc*. The varying effects of gamma radiation doses on growth reduction and survival are associated with dose-dependent biological responses (Mba *et al*. 2010; Oladesu *et al*. 2015). Plantlet mortality may result from various forms of biotic stress, genetic damage, oxidative stress, and disruptions in hormonal regulation (Kodym *et al*. 2012; Jankowicz-Cieslak *et al*. 2017).

The *in vitro* selection method uses secondary metabolites as selectors to create plant varieties resistant to disease. Specific genes are activated when plants face intense stress, allowing them to remain resistant even under challenging conditions (Chandra *et al*. 2010). This method, which uses culture fluid filtrate, more closely resembles natural host-pathogen interactions than traditional toxic pathogen selection methods

(Moore 2001; Perichery *et a*l. 2019). The culture fluid contains various proteins and toxins (Companioni *et al*. 2005; Portal Gonzalez et al. 2021). Izurdiaga *et al.* (2024) emphasized its effectiveness in identifying pathogen-resistant plant varieties. Additionally, microbial cell-free filtrates (CFFs) offer benefits similar to traditional microbe-based products, promoting defensive responses against pathogens and improving the quality of resistant plants (Prusky and Romanazzi 2023). The cross-sectional observation on banana roots showed that infected plants had brownish roots due to toxins damaging the vascular tissue, suggesting that these toxins can lead to plant death (Fig. 4).

Some types of bananas have shown better resistance to pathogens when grown with culture fluid from *F. oxysporum* f. sp. *cubense* (*Foc*) (Chandra *et al*. 2010). Research on the 'Rasthali' banana cultivar found that using 8% *Foc* culture filtrate resulted in only an 18% plantlet survival rate (Saraswathi *et al*. 2016). Vanilla plants also suffered damage when exposed to 15% and 30% of *F. oxysporum* f. sp. *vanilla* (Ramírez-Mosqueda *et al.* 2015). A study by Abeyawardana and Koudela (2019) reported survival rates of 0% to 86.7% in shoot tips of a Brassicaceae cultivar when exposed to *F. oxysporum* f. sp. *conglutinans* at inoculum concentrations of 15% and 20%, suggesting some level of resistance. In carnation flowers, calli grown with 15% of *F. oxysporum* f. sp. *dianthii* had very few survivors (Thakur *et al*. 2002). According to García-Velasco *et al*. (2020), resistance or susceptibility to banana Fusarium wilt can be identified using filtrates from various *F. oxysporum* f. sp. *cubense* populations.

In vivo selection showed that most selected plant variants had a high disease intensity (DII), with values between 88.9% and 100% in leaves and 55% to 100% in rhizomes. Disease intensity refers to the severity of the disease in a plant, typically based on the number of affected plants or the severity of their symptoms. Plants become susceptible to Fusarium wilt when the DII exceeds 50% (Carlier *et al.* 2003; Mak *et al.* 2004). In this study, plants exposed to 20, 25, 35, 40, and 45 Gy doses were identified as highly sensitive (Table 3). Symptoms observed included yellowing of most leaves, except for new and unopened ones, and discoloration in more than two-thirds of the rhizomes (see Fig. 3). These results highlight the strong virulence of the *Foc* isolate Medan VCG against the banana cultivar 'Ampyang', indicating a significant threat to banana production. Some tested variants may exhibit resistance, as the severity of symptoms and disease progression varied among the plants.

Foc is categorized into vegetative compatibility groups (VCGs) based on their ability to fuse and form stable hybrids (Fourie *et al*. 2011; Somrith *et al.* 2011). Each group shows various levels of aggressiveness and virulence toward different banana cultivars, influenced by genetic, molecular, and environmental factors (Fourie *et al.* 2011; Ordonez *et al.* 2018). VCG 01213/16 poses the most serious threat to banana farming in Indonesia, due to its destructive impact and persistence in the soil. Isolates from Medan are part of *Foc* race 4, or ‚tropical' (TR4) (Hermanto *et al*. 2011; Ordonez *et al.* 2015). TR4 is widespread in Indonesia. It affects local banana cultivars, including ‚Barangan' (AAA), ‚Raja' (AAB), ‚Raja Nangka' (AAB), ‚Kepok' (ABB), and ‚Ambon Hijau' (AAA), that exhibit different ploidy and genomic characteristics (Hermanto *et al*. 2011; Wibowo *et al.* 2011).

The evaluation of greenhouse plants revealed that some sensitive and highly sensitive plants initially wilted; however, they later recovered and produced new leaves, particularly those treated with gamma radiation doses of 30 Gy and 40 Gy (see Table 4). The responses of these plants to pathogens are predicted to develop slowly, as observed in a banana variety eight weeks after infection (see Table 3). After infection, conidia may trigger resistance genes, helping the plants target and block pathogens in their root tissues. In contrast, susceptible banana plants exhibit a steady disease progression caused by these pathogens. Transferring the surviving plantlets was crucial because host plants respond differently to the culture filtrate at the cellular level (*in vitro*) than to their overall response (*in vivo*) in a greenhouse. Furthermore, this step aimed to predict potential epigenetic variations in banana plants.

Plants have developed an integrated defense mechanism to combat diseases caused by fungi. This preformed resistance is based on the inherent characteristics of normally uninfected plants, which include a thickened cuticle, trichomes, and the production of antimicrobial compounds (Moerschbacher and Mendgen 2000; Huang 2001). When pathogens attack, plants can activate an additional defense response called Systemic Acquired Resistance (SAR), which enhances their ability to produce more antimicrobial compounds (Andersen *et al*. 2018; Reglinsky *et al*. 2023). Banana root cross-sections showed anatomical differences between healthy and *Foc*-infected plants (Fig. 4). Infected roots exhibited brown discoloration in the epidermis and xylem, accompanied by a complete absence of hairy roots. Furthermore, the xylem roots showed a maroon-red color under fluorescence microscopy, similar to the color of CFF in Potato Destrose Broth (PDB) medium (Fig. 4 C-D). This color change in root tissue indicates colonization of the vascular tissue and tissue degradation by the pathogen. These findings confirm that dipping plant roots in a suspension of *Foc* conidia causes severe vascular damage and can lead to the death of susceptible plants (Table 3; Fig. 4).

In this study, banana plantlets grown in the presence of pathogens became stiffer, which likely helped them better resist stress (see Fig. 1). This stiffness may result from changes in their root system, such as the hardening of cell walls. Additionally, studies have shown that some banana plants can survive after facing selection pressure, suggesting that they possess an induced resistance mechanism. Different doses of gamma irradiation are important for developing plant varieties. Research showed that 28 banana mutants were putatively resistant to Fusarium wilt and were found across various treatments, mainly from 30 and 50 Gy (see Fig. 5). The high survival percentage may be due to an adaptive stress response, which involves increased antioxidant enzyme activity and root system plasticity (Datta *et al*. 2018). Previous reports indicate that moderate levels of mutagenic stress can activate genes related to plant defense, thereby increasing plant survival after infection (Sharma *et al.* 2012). However, irradiation at doses of 25 and 45 Gy is likely to cause permanent damage to cellular structures and DNA, limiting root regeneration and overall plant vitality. This study aligns with findings by Ferreira *et al*. (2024), who reported that inducing variation in the ‚Prata' banana cultivar led to the selection of resistant plants.

These results offer hope for managing the Medan isolate VCG 01213/16 TR4 and emphasize the effectiveness of mutagenesis followed by in vitro and in vivo selection to obtain disease-resistant banana plants. Biotechnological approaches, such as in vitro mutation breeding and selection programs, have shown significant potential for creating disease-resistant plants (Bhoi *et al*. 2022). These approaches not only facilitate the identification of resistant lines but also contribute to expanding genetic diversity within the banana germplasm (Hwang and Ko 2004; Chandra *et al*. 2010; Bidabadi and Sijun 2018; Siamak and Zheng 2018). However, identifying genes that enhance resistance, particularly chitinases and β-1,3-glucanases, is also critical (Balasubramanian *et al*. 2012; Kumar *et al*. 2018). Hence, future research should prioritize the long-term stability of resistance traits in these plant varieties by screening in Fusarium wilt endemic field and identifying resistance genes.

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